OSTEOSARCOPENIC OBESITY: THE DEVELOPMENT OF SCREENING TEST CRITERIA AND THE ASSOCIATION WITH BIOAVAILABLE 25(OH)D

by

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Abstract

Concurrent presence of low bone density (osteopenia/osteoporosis), low muscle mass (sarcopenia), and high adiposity (obesity) in the elderly has led to the recognition of Osteosarcopenic Obesity (OSO) as a singular entity. Currently, no established criteria exist to identify OSO, particularly in Malaysian population. Therefore, the main aim of this study was to develop simple screening test to identify obese Malaysian postmenopausal women at high risk for osteosarcopenia using portable equipment; quantitative ultrasound (QUS) and bio-electrical impedance analysis (BIA). Additionally, the relationships between OSO and 25(OH)D indices were also explored. One hundred and forty-one (n=141) functionally independent, community-dwelling postmenopausal Malaysian women (aged 45 to 88 years) were recruited from the area of Klang Valley, Kuala Lumpur, Malaysia. Body composition was assessed using BIA and bone density was assessed using QUS. Serum total 25(OH)D was measured using chemiluminescent microparticle immunoassay (CMIA). Serum vitamin D binding protein (VDBP) was measured using a monoclonal enzyme-linked immunosorbent assay (ELISA) and bioavailable 25(OH)D was calculated using modified Vermuelen formula. After selecting the best variables using receiver operating characteristic (ROC) curve, the final model to estimate the risk of osteosarcopenia in obese women comprised of five variables: handgrip strength (HGS, ≤16.5kg), skeletal muscle mass index (SMMI, ≤8.2 kg/m^2), fat-free mass index (FFMI, ≤15.2kg/m^2), broadband ultrasonic attenuation

(BUA, ≤52.85 dB/MHz) and speed of sound (SOS, ≤1492.15 m/s). Mean and SD of total, bioavailable 25(OH)D, and VDBP was, 52.4(17.7) nmol/L, 6.9(3.0) nmol/L, and 224.7(44.8) ug/mL, respectively. A significant and positive correlation was found between total 25(OH)D and bioavailable 25(OH)D ($r=0.883$, $p<0.01$). Both total and bioavailable 25(OH)D were negatively correlated with body fat percent and positively correlated with muscle mass ($p<0.05$). Although both forms of 25(OH)D were positively correlated with bone density (BUA), the correlation of bioavailable 25(OH)D was marginally stronger compared to total 25(OH)D ($r=0.234$, $p=0.012$ and $r=0.199$, $p=0.030$, respectively). However, the small differences in the R values and effect size does not warrant the conclusion that bioavailable 25(OH)D is superior to total 25(OH)D in its association with the BUA. While no significant correlation was found between OSO and any index of 25(OH)D, participants with severe obesity [Body fat %: Mean (SD) 44.9 (4.7) %] and concurrent presence of low bone density (Osteopenic Obesity) were likely to be Vitamin D deficient (total 25(OH)D <30nmol/L) compared to participants without any musculoskeletal health disorders, obese or otherwise ($p=0.070$). OSO is a progressive disorder that could lead to functional impairment. The screening test developed from this study could help identify asymptomatic obese women with osteosarcopenia who may be good candidates for early intervention. Severely obese people are prone to hypovitaminosis D, which could lead to the manifestation of musculoskeletal health disorders. Intervention measures should include an effort to increase Vitamin D levels.

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LIST OF TERMINOLOGIES

ASM (appendicular skeletal muscle mass)	Lean body mass from limbs, a surrogate measure of skeletal muscle mass
AppSMMI (appendicular skeletal muscle mass index)	Appendicular skeletal muscle mass adjusted for height in squared meters
LBM (lean body mass)	The sum of lean compartments of the body, excluding bone mineral content
FFM (fat-free mass)	The sum of LBM plus bone mineral content
FFMI (fat free mass index)	Fat free mass adjusted for height in squared meters
SMM (skeletal muscle mass)	The mass of muscle that powers movement of the skeleton, as in walking and lifting
SMMI (skeletal muscle mass index)	Skeletal muscle mass adjusted for height in squared meters
Obesity (based on body fat percentage)	Fat mass > 32%, as per recommendation for women by the American Society of Bariatric Physicians
BFP (body fat percentage)	The difference between fatty and muscular mass in the body, measured in percentage.
Osteopenic/ Osteoporotic	Low bone mass, defined as T-score ≤-1.0 (osteopenia) and T-score ≤-2.5 (osteoporosis) (according to the World Health Organisation)
BUA (broadband ultrasonic attenuation)	The slope between attenuation of sound signals and its frequency, and the unit used is dB/MHz. Attenuation occurs because the energy is absorbed by the soft tissue and bone when the sound waves travel through them. Therefore, the higher the BUA, the denser the bone.

Sarcopenic	Low skeletal muscle mass or muscle wasting, defined as appSSMI $\leq 5.7 kg/m^2$ for women by Asian Working Group on Sarcopenia
Osteosarcopenic Obese	Concurrent presence of osteoporosis, low muscle mass and obesity
Osteopenic Obese	Concurrent presence of obesity and osteopenic/osteoporosis
Sarcopenic Obese	Concurrent presence of obesity and low muscle mass
Total 25-hydroxyvitamin D	Hydroxylated version of VitaminD2 and D3 in the liver, with half-life of approximately 3 weeks
Bioavailable 25-hydroxyvitamin D	The unbounded and the loosely-bound 25(OH)D (the fraction that is bounded to Albumin)
Vitamin D Binding Protein	A 58-kDa protein, produced in the liver and a major carrier protein of Vitamin D

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Osteosarcopenic obesity (OSO) is a term used to describe concurrent presence of obesity, low bone mass (osteoporosis) and low muscle mass (sarcopenia) in an individual (Ma, Zhang, Han, Kohzuki, & Guo, 2020; Ilich, Inglis, & Kelly, 2016; Ilich et al., 2014; Ormsbee et al., 2014; Cooper et al., 2013). OSO is an age-related disorder and considered to be the most advanced functional impairment related to bone, muscle, and adiposity (JafariNasabian, Inglis, Kelly, & Ilich, 2017). Other possible manifestation of this syndrome include osteopenic obesity (OO) and sarcopenic obesity (SO) , where 'obesity' is not defined only by its clinical diagnosis, but also depends on infiltration of fat in muscle tissues and its impact on the skeleton. With time, both conditions (OO and SO) may eventually result in OSO.

Currently, information about the etiology, prevalence and long-term effect of OSO on older adults is sparse. One of the hallmarks of OSO is the interconnected nature of the syndrome—from its cellular connections to the deterioration of musculoskeletal tissues. Previously, osteoporosis, sarcopenia and obesity were considered as separate disorders and were rarely studied together. However, multiple studies suggest that bone, muscle and fat are strongly linked (Vaidya, 2014; Migliaccio, Greco, Aversa, & Lenzi, 2014). There are increasing evidence showing pathophysiological overlapping of these disorders (Ormsbee, et al., 2014). For example, studies have found that sarcopenia and osteoporosis share risk factors that include genetics,

endocrine and mechanical function. In addition, bone and muscle have been found to closely interact with each other not only mechanically but also metabolically. These findings have led to the recognition of a term 'osteosarcopenia' as a single entity (Hirschfeld, Kinsella, & Duque, 2017). However, studies are still in its infancy and it is still a long way to go to establish causal relationships. As the body ages, in addition to hormonal changes (i.e. a sharp decrease in the levels of anabolic hormones), an increase in total and/or abdominal adipose tissue leads to an increase in pro-inflammatory cytokines. These reactions leads to the losses of both muscle and bone tissues through a variety of mechanisms which ultimately affect clinical outcomes, such as an increase in risk for falls and fractures (Ormsbee, et al., 2014). Therefore, the recognition of OSO as a single entity is thought to be more physiologically relevant and may help guide a comprehensive treatment plan due to the interconnectedness of the disorder.

Separately, each component of the syndrome (osteoporosis, sarcopenia and obesity) had been found to be associated with multiple factors related to cellular and endocrine levels, and also lifestyle. Studies have shown that low estrogen level, in addition to low vitamin D and sedentary life, to be some of the most significant risk factors shared by all three disorders (Clegg, 2012; Messier et al., 2011; Finkelstein et al., 2002). Therefore, the current hypothesis is that women after reaching menopause would have a higher risk of developing OSO due to their sudden drop in estrogen level, age-related increased in adiposity and low physical activity.

Osteopenia/osteoporosis and sarcopenia are age-related disorders and have been found to share common pathophysiology (Ormsbee et al., 2014). The

addition of obesity to these conditions was thought to worsen existing metabolic abnormalities, leading to impaired quality of life, higher risk of morbidity and mortality (Corica et al., 2015). Currently, there are a number of hypothesis on possible mechanisms underlying OSO. Cooper et al. (2013) suggests that an increase in pro-inflammatory cytokines induced by an increase in total and/or abdominal adipose tissue, in conjunction with some hormonal disturbances, lead to bone and muscle tissue loss through a variety of mechanisms, which ultimately result in various clinical outcomes such as increase risk of falls and fracture (Ormsbee et al., 2014; Binkley & Buehring, 2009). Individuals presenting all three conditions concurrently are expected to suffer poorer clinical outcomes compared to individuals with either one of the conditions alone. A study in China showed that older women with OSO had a lower quality of blood lipid profile (Mo et al., 2018) compared to those without OSO, while a Mexican study found that women with OSO had a significantly lower physical performance and poorer frailty scores compared to those without the syndrome (Szlejf, Parra-Rodriguez, & Rosas-Carrasco, 2017). Further, Ilich et al. (2015) found a strong correlation between OSO and a weaker handgrip strength, and an inferior balance and walking abilities in older women compared to similar age obese-only women. Metabolic abnormalities such as dyslipidemia and insulin resistance (IR) were also found to be worse when compounded with the co-existence of all three disorders (Kalinkovich & Livshits, 2016).

The importance of body composition measurement

As an individual ages, changes in body composition is inevitable. Accurate analysis of body composition is important in clinical situations where there

are perceived or expected abnormalities. For example, in an elderly individual with normal body weight and BMI, presence of dynapenia (low muscle strength) and sarcopenia (low muscle mass) may go undetected if no measurement of muscle strength or muscle mass were made. These individuals may also have osteopenia/osteoporosis, which may be undetected without screening for bone density (Ilich & Brownbill, 2008; Ilich-Ernst, Brownbill, Ludemann, & Fu, 2002).

Studies of OSO as a single entity is still in infancy (Ma, Zhang, Han, Kohzuki, & Guo, 2020; JafariNasabian et al., 2017; Ilich, Inglis, & Kelly, 2016; Ilich et al., 2014; Ormsbee et al., 2014; Cooper et al., 2013). The reason for why osteopenia/osteoporosis, sarcopenia and obesity were rarely studied together was likely due to various studies showing favourable impact of obesity on bone density (Radak, 2004; Guney et al., 2003; Douchi et al., 2000). Previously, it was widely believed that obese people have lower risk of fracture due to higher bone density typically found in these individuals. It was also theorized that due to high soft-tissue padding, obese people would be less impacted by falls and less likely to suffer bone fracture (Radak, 2004; Guney et al., 2003; Douchi et al., 2000). Mechanical loading exert by excess body weight on cortical bone was suggested to have a positive effect on bone formation despite being a risk factor for various chronic health disorders (Cao, 2011). Nevertheless, despite various studies showing positive correlations between body weight/obesity and bone mineral density (Qiao et al., 2020; Lins Vieira, Da Silva Nascimento, Do Nascimento, Barros Neto, , & Oliveira Dos Santos, 2020; Reid, 2002; Felson, Zhang, Hannan, & Anderson, 1993), there are numerous data from epidemiological and animal

studies showing otherwise (Palermo et al., 2016; Greco et al., 2010; Rosen, & Kawai, 2010; Cao, 2011; Zhao et al., 2008; Rosen & Klibanski, 2009; Lecka-Czernik), although some of the the relevances was highly dependent on fat deposition (Gilsanz et al., 2009; Bredella et al., 2011). Studies by Gilsanz et al. (2009) and Bredella et al. (2011) found that visceral or abdominal fat was more harmful to the body, particularly to the bones, than subcutaneous fat (subcutaneous fat was actually found to have protective effect on the bones). Gilsanz et al (2009), for example, found that subcutaneous and visceral fat have strong, albeit opposing relations with bone structure and strength. They found that although subcutaneous fat had positive correlations with cross-sectional area (CSA), cortical bone area (CBA), visceral fat had negative associations with all bone phenotypes.

Diagnostic criteria for OSO

Due to its relatively recent recognition, currently, there are no standard definitions for OSO, leading to a challenge in official diagnosis. In 2016, Ilich, Kelly and Inglis had introduced a set of diagnostic criteria for OSO in older women which involved physical and functional assessment. The diagnostic criteria for the physical assessment are as follows: 1) T-score for BMD ≤-1.0 SD at the femoral neck, proximal femur, or lumbar spine, 2) 20th percentile of appendicular lean mass (ALM) for women, with the equation: ALM = -17.4 +18.3 x height (m) + 0.16 x body fat (kg) (Ilich et al., 2015), and 3) fat mass ≥ 32% of body weight for women. All three criteria were required to be assessed using dual energy X-ray absorptiometry (DXA). The functional assessment include handgrip strength (≤20 kg for women), and modified components of short physical performance battery test (SPPB): one

leg stance: ≤16 sec, gait speed: ≤0.8 m/sec, sit-to-stand chair test: ≤20 times. It was clear that these diagnostic criteria were meant to identify OSO when it has reached clinical stages for bone loss, muscle loss and excessive body fat. In addition, these criteria may not be applicable to all population due to factors such as differences in ethnicity and/or instrumental feasibility in large scale epidemiological studies. Due to progressive nature of OSO, early diagnosis of the syndrome is important for effective intervention. The awareness and acknowledgement of OSO as potential threat to public health will help curb the incidence before it becomes an epidemic. Ultimately, the early awareness might also reduce the cost of public health by preventing the need for behavioral, nutritional or pharmacological interventions in the future. At the individual level, currently, there is little evidence available on the multiplicative health impact of osteoporosis in a distinct overlap with obesity and sarcopenia. However, health outcomes associated with each component of OSO such as increased risk of fractures, impaired functional status (Curtis, Litwic, Cooper, & Dennison, 2015, Wang, et al., 2015), physical disability, frailty, insulin resistance, increased risk of infections, and increased length of hospital stay may be avoided in the future.

Therefore, one of the aims of the current study was to develop screening criteria for OSO that are feasible for epidemiological study and screening purposes, while still accurately predictive of the development of the syndrome by using portable qualitative ultrasound (QUS) machine and other tests that are feasible to be conducted in a community at large.

1.1.1 The role of Vitamin D in the Manifestation of OSO and Testing the Free Hormone Hypothesis

Studies have found that people with bone and/or muscle wasting tend to have low Vitamin D level (Bruyère, Cavalier & Reginstera, 2017). Vitamin D is a fat-soluble vitamin that plays critical role in calcium absorption and bone metabolism. The metabolism of Vitamin D occurred in the skin, the liver, and the kidney. First, Vitamin D_3 (cholecalciferol) is produced in the skin from 7-dehydrocholesterol. However, this version itself is not biologically active. It needs to be hydroxylated further in the liver and kidney into its biologically active form. From the skin, it is transported in the blood by Vitamin D binding protein (VDBP) to the liver. In the liver, Vitamin D is activated into 25-hydroxyvitamin D [25(OH)D], and then converted into 1,25-dihydroxyvitamin D (1,25(OH)2D), the hormonally active form of Vitamin D, in the kidney. This version is responsible for most biologic action of Vitamin D. Low Vitamin D has been found to affect calcium absorption which then triggers the loss of muscle and bone mass (Gunton, Girgis, Baldock, & Lips, 2015). To date, very few studies were designed to assess Vitamin D status in osteosarcopenic individuals. The close interrelationship between muscle and bone led to the hypothesis that an improvement in one tissue may be beneficial to the other. Therefore, it is worth to explore the association between Vitamin D and OSO, which may lead to a more comprehensive and effective treatment plan.

Free hormone hypothesis: total vs. bioavailable 25(OH)D

Currently, 'total' 25-hydroxyvitamin D (25(OH)D) is considered to be the indicator of Vitamin D status due to its long half-life in the body. This form of Vitamin D reflects the overall body storage of Vitamin D precursor that is hydroxylated to active form of Vitamin D (1,25-dihydroxyvitamin D). In the

body, ‘total’ 25(OH)D is bound to VDBP and albumin, which get converted to active 1,25-dihydroxyvitamin D (1,25(OH)2D) in the kidney, colon and several other tissues (Deeb, et al., 2007). Higher concentrations of both 25(OH)D and 1,25(OH)2D are bound to VDBP, approximately 10–15% to Albumin, and less than 1% is ‘free’ (Bikle, et al., 1986). Since the affinity of 25(OH)D or 1,25(OH)2D to Albumin is weaker than to VDBP, the loosely bound fraction and the ‘free’ fraction together make up the ‘bioavailable’ 25(OH)D (Brown & Coyne, 2012). Recently, emerging evidence showed that bioavailable 25(OH)D (the fraction that’s not bound to Vitamin D binding protein), may be the better indicator of Vitamin D status compared to the ‘total’ 25(OH)D (Yousefzadeh, et al., 2014). Vitamin D receptor (VDR), which is a key nuclear receptor, can only be activated by the free form of 1,25(OH)2D. The ligand-receptor binding then regulates the transcription of numerous genes that are responsible for cell proliferation, cell differentiation, angiogenesis, etc. (Ying, et al., 2015). Therefore, free and bioavailable 25(OH)D was hypothesized to be a better biomarker of Vitamin D status in comparison to ‘total’ 25(OH)D.

A study examining the relationship between total and bioavailable 25(OH)D levels and BMD involving 49 healthy young adults, found that free and bioavailable 25(OH)D were more strongly correlated with BMD than total 25(OH)D (Powe et al., 2011). Bhan et al. (2012) reported that bioavailable Vitamin D had stronger correlation to bone mineral metabolism than total Vitamin D in hemodialysis patients. Theses findings suggest that bioavailable 25(OH)D may be the better indicator of Vitamin D status in individuals than total 25(OH)D. In addition, a study on the association of Vitamin D indices

and risk of colorectal cancer (CRC) by Ying, et al. in 2015 found that high levels of free and bioavailable 25(OH)D were associated significantly lower risk of CRC among participants with lower than average VDBP. Further, it has been reported that in addition to bone health, free and bioavailable 25(OH)D was associated with other health outcomes as well (Yu et al., 2018; Jorde, 2019).

However, the levels of bioavailable 25(OH)D was dependent on the levels of VDBP, and VDBP is influenced by variations in VDBP-binding affinity for specific vitamin D metabolites and the role of 1-alpha hydroxylase enzymes. 25-hydroxyvitamin D-1α-hydroxylase (1-α hydroxylase) is a vitamin D-activating enzyme and the impact of Vitamin D is dependent on its expression, in addition to the VDR (Chun, et al., 2014). Since VDBP has stronger affinity to 25(OH)D compared to 1,25(OH)2D, it has been theorised that VDBP could have a much greater impact on 25(OH)D mediated intracrine responses. Further, due to its dependent on the levels of VDBP and the differences in binding-affinity, the level of bioavailable 25(OH)D differs between individuals. Presence of higher circulating levels of VDBP may sequester higher concentrations of 25(OH)D leading to less free and bioavailable 25(OH)D). A review stated that the concentration of VDBP and 25(OH)D levels were also affected by age, genetic, race, ethnicity, pregnancy, and obesity (Yousefzadeh et al., 2014). In addition, the concentration of VDBP and 25(OH)D levels were also affected by some pathological factors such as liver disease, renal disease, some cancers, inflammation and HIV (Yousefzadeh et al., 2014). More information on the degree of correlation is needed. Factors affecting bioavailability of 25(OH)D

with health outcomes may have implications on recommendations of Vitamin D to humans at all life stages. Currently, there is still an ongoing investigation on whether VDBP can replace total 25(OH) D for health outcomes.

In 2013, a study which examined the effect of race on the concentration of VDBP and bioavailable 25(OH)D found that although African American participants had lower total serum 25(OH)D levels and lower VDBP than the Caucasians, they have higher BMD (which consequently, known as a paradox) (Powe et al., 2013). The study further states that African Americans typically lack the accompanying characteristic commonly resulted from Vitamin D deficiency. African Americans were found to have higher BMD, higher calcium levels, and only slightly higher parathyroid hormone levels than the Caucasians (Powe et al., 2013). Interestingly, both groups were found to have similar concentrations of bioavailable 25(OH)D (Powe et al., 2013). Therefore, the authors theorized that the level of VDBP and bioavailable 25(OH)D may be responsible for observed racial differences in total serum 25(OH)D levels and manifestations of Vitamin D deficiency symptoms. In addition, a study by Lowe et al. (2010) which investigated the differences in Vitamin D status in postmenopausal South Asian and Caucasian women in the UK and its relationship to parathyroid hormone (PTH) concentration, biochemical markers of bone turnover and bone quality, found that although the South Asian women had significantly lower 25(OH)D concentrations and higher serum parathyroid hormone (PTH) than the Caucasian women, they were not associated with significantly higher markers of bone resorption, nor reduced bone quality. These findings suggest that factors other than just the levels of total 25(OH)D may play a role in bone

quality and health, which, based on various studies, are likely to be the VDBP and the bioavailable fraction of 25(OH)D. Perhaps the measurement of VDBP and bioavailable 25(OH)D may need to be incorporated into the routine assessment to improve the determination of Vitamin D status in diverse populations.

Although the association between low circulating 25(OH)D concentrations and obesity is well established (Drincic, Armas, Van Diest, & Heaney, 2012), it is unclear whether the VDBP and bioavailable 25(OH)D in the body are affected by adiposity. In a study comparing the concentration of VDBP and bioavailable 25(OH)D between obese and normal weight adolescent, it was shown that VDBP was lower in children with obesity (Ashraf et al., 2014). However, in a study involving obese women of reproductive age, it was shown that obese women had higher VDBP concentrations and lower free 25(OH)D compared with normal-weight women (Karlsson et al., 2014). Further study is needed to achieve a consensus, whether the concentration of VDBP and bioavailable 25(OH)D is related to obesity and how it affects bone and muscle mass in an obese individual. It may be, similar to what was found in people of African American descent, that lower total serum 25(OH)D level is not necessarily associated with lower bioavailable 25(OH)D levels. If studies found that BMD is high in obese people, the reason could be due to the activity of bioavailable 25(OH)D. Further study is needed to confirm this hypothesis. The added knowledge would have a great implication on the assessments and how we treat obese people with symptoms of Vitamin D deficiency.

1.2 Problem statements

Studies on osteoporosis and sarcopenia in obese postmenopausal Malaysian women are sparse. The present study began by determining the current prevalence of OSO, SO and OO in Malaysian postmenopausal women. Progressive degeneration of both muscle and bone in this vulnerable group exposes them to risk of falls and subsequent fractures which can be life threatening. Therefore, this study also discussed how bone and muscle mass are affected in this vulnerable group.

In addition, the present study also developed cut-off values for the screening of OSO using portable equipment of quantitative ultrasound (QUS) and bio-electrical impedance analysis (BIA). Using DXA is not feasible for a large scale/epidemiological study. DXA is normally used when the presence of a disease (i.e. osteoporosis) is highly suspected (i.e. presence of fracture) and is used as a basis for treatment decisions. Therefore, a large number of people with high risk factor for developing OSO might not be aware of the risk and will only find out when it is too late. Thus, a formulation of screening test criteria using portable instruments to detect early sign or advanced stage but yet-to-show symptoms for OSO in large numbers of apparently healthy individuals is needed. The current study determined cut-off values for low muscle mass and low bone mass in obese postmenopausal women using bioelectrical impedance analyser (BIA) and quantitative ultrasound (QUS) machine.

Moreover, due to their higher weight load, cut-off values for low muscle mass and low bone mass for obese people may be different from the standard

values. The current study also determined cut-off values for low muscle mass and low bone mass in postmenopausal women using 3 different statistical modelling methods, and compare the results with the standard cut-offs.

Finally, the present study explored the free hormone hypothesis in relation to Vitamin D. Currently, there are contradicting results on the correlation of total 25(OH)D with bone density and muscle mass. Studies suggests that bioavailable fraction of 25(OH)D may be a better indicator for Vitamin D status than total 25(OH)D in relation to musculoskeletal health. To the best of our knowledge serum levels of bioavailable 25(OH)D and its association with bone density and muscle mass has not been studied so far in community-dwelling, postmenopausal Malaysian women. The current study determined if bioavailable fraction of 25(OH)D is the better biochemical indicator in relation to OSO than total 25(OH)D. The finding could revolutionised the testing for Vitamin D among people with high risk for OSO in the general population.

1.3 Hypothesis

1) There will be 5-25% of postmenopausal women with OSO in the population.
2) Cut-off values for low bone density and low muscle mass in obese postmenopausal women are different from the standard cut-off values.
3) There is a positive and significant association between Vitamin D and Osteosarcopenic Obesity.

4) Bioavailable 25-hydroxyvitamin D is a better biochemical indicator of Vitamin D status than total 25-hydroxyvitamin D.

1.4 Objectives of study

1) To assess body composition, bone density and physical performance of postmenopausal Malaysian women and determine the prevalence of Osteosarcopenic Obesity and its variations in the population.
2) To study the interrelationship between fat, bone, muscle and biochemical indices of Vitamin D.
3) To develop screening test criteria for easy identification of Osteosarcopenic obesity, Osteopenic obesity, and Sarcopenic obesity using portable equipment.
4) To test the free hormone hypothesis by determining if the bioavailable fraction of 25(OH)D has a stronger correlation to musculoskeletal health compared to total 25(OH)D.

1.5 Significance of the study

This study determined the prevalence of Osteosarcopenic obesity, Osteopenic obesity, and Sarcopenic obesity in postmenopausal Malaysian women, proposing suitable and feasible screening test criteria for the above-mentioned groups and testing the free hormone hypothesis related to 25(OH)D. This study also determined a potentially better biochemical indicator for the assessment of Vitamin D status in postmenopausal women.

The screening of Osteosarcopenia in asymptomatic obese women may reduce their risk of adverse health outcomes at a later age. The acknowledgment of

Osteosarcopenic obesity as potential public health problem will increase scientific and public awareness for the diagnosis, public health costs, and ultimately the development of behavioral, nutritional, and possibly pharmacological interventions to prevent or reverse this syndrome.

Impact on society

The screening for Osteosarcopenia in older obese women will raise public awareness of the condition. Clinical outcomes that would result from having concurrent syndromes of obesity, osteoporosis/osteopenia and sarcopenia can be avoided in the future.

Potential application

1) Screening test criteria for Osteosarcopenia in obese postmenopausal women using portable equipment.
2) The current status of Vitamin D levels in multi-ethnic postmenopausal Malaysian women.
3) Improvement over current practices in analysing total 25-hydroxyvitamin D as the biomarker for Vitamin D status.

Evidence of free hormone hypothesis with regards to the association of 25(OH)D indices with bone and muscle mass in obese postmenopausal women is not known. If association is found, it may revolutionize the assessment of Vitamin D and will give implications on how we treat obese individuals with symptoms of Vitamin D deficiency. Further, prevalence for OSO in older women in Malaysia will also be identified. Additionally, suitable screening test criteria for OSO among postmenopausal Malaysian women will be determined.

CHAPTER 2

LITERATURE REVIEW

2.1 Osteopenia/Osteoporosis

Osteoporosis is a bone disease described by an impairment in bone strength and/or quality. Osteoporosis is typically diagnosed when the bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (DXA) to be below a certain score value (T-score ≤ -2.5 at either the femoral neck or the lumbar spine) (Cosman et al., 2014). The disorder is also diagnosed when an individual was found to have weak bones in terms of strength, impairment in osteoblast and osteoclast activity, and/or an increased risk of fracture, additional to the low BMD (Cosman et al., 2014). Currently, BMD, which determines the quantity of the calcified bone, is the gold standard technique in diagnosing osteopenia and osteoporosis. However, BMD alone is unable to discriminate between osteomalacia and osteoporosis, increasing the risk of misdiagnosis, and thus mismanagement. Osteoporosis was described as a lack of osteoid (bone matrix) on which calcium hydroxyapatite could be deposited, and osteomalacia was described as a lack of calcium hydroxyapatite deposition on the bony matrix. Basically, osteomalacia is a result of Vitamin D or phosphate deficiency, characterized by lack of calcium reabsorption in the intestine to mineralize bones. Therefore, in order to differentiate between the two conditions, it was advised to test for serum 25(OH)D and calcium level, in addition to BMD. The difference in the management between the two disorders is that osteomalacia is easier to treat than osteoporosis. It was found that prescribing higher doses of calcium and

Vitamin D were enough improve the condition of osteomalacic patients in a few months time (Saghafi, Azarian, Hashemzadeh, Sahebari, & Rezaieyazdi, 2013), whereas for osteoporosis, different/additional course of treatments are needed (i.e. reduce future fractures, in addition to improvement of BMD).

Generally, osteoporosis goes undiagnosed until there is a trauma from a fall that results in a fracture. Diagnosis of a fracture typically involves a full BMD assessment and this is usually when the disease is discovered. While osteoporosis-related fracture is not site-specific and can occur almost anywhere in the skeleton, it was commonly found that the wrist, the vertebrae, the head or the hip (femoral neck) are the sites that suffer the greatest stress from accidental fall (Ong, Sahota, Tan, & Marshall, 2014; Kanis, Johnell, Oden, Johansson, & McCloskey, 2008; WHO Scientific Group on the Assessment of Osteoporosis at Primary Health Care Level, 2004). The World Health Organisation (WHO) introduced a standardized score, called T-score that compares BMD at the femoral neck to average values for young healthy women. The categories for diagnosis were as the following; 1) normal (T-score -1.0 and above), 2) low bone mass, diagnosed as osteopenia (T-score between -1.0 and -2.5), 3) osteoporosis (T-score -2.5 and below) and 4) severe osteoporosis (T-score -2.5 and below, with history of a fracture) (Siris et al., 2014). The measurement of BMD is the most common tool used to diagnose osteopenia/osteoporosis and risk of fracture. BMD test is often considered in guiding decisions for treatments of osteopenia/osteoporosis. The National Osteoporosis Foundation (NOF) recommends that all women aged 65 and older, and men age 70 and older to

be tested, even without any clinical risk factors. Other factors warranted for BMD testing include (Cosman et al., 2014):

1) Postmenopausal women and men aged 50 to 70 years, with risk factors.
2) Women in the menopausal transition with risk factors for fracture such as low body weight, previous low-trauma fracture, or taking high risk medication.
3) People who have experienced a fracture after the age of 50.
4) People with a certain condition (e.g., rheumatoid arthritis) or taking a certain medication (e.g., glucocorticoids, ≥5 mg/day for ≥ 3 months) associated with low bone mass.
5) Anyone being considered for osteoporosis medication.
6) Anyone being treated for osteoporosis (as a mean to monitor the effect of treatment).
7) Postmenopausal women who want to discontinue estrogen therapy.

Malaysian Clinical Guidance on the management of postmenopausal and male osteoporosis (Yeap et al., 2016) states that the traditional risk factors, such as advanced age, being Asian & Caucasian, female, premature menopause (<45 years) including surgical menopause, family history, and personal history of fracture as an adult are some of the non-modifiable risk factors for subjects at risk of osteoporosis and fracture. Modifiable risk factors include; low calcium and/or Vitamin D intake, sedentary lifestyle, cigarette smoking, alcohol intake of more than 3 units daily, low body weight (BMI<19 kg/m^2) and estrogen deficiency (Yeap et al., 2016). The best

method of assessing BMD is still using dual-energy X-ray absorptiometry (DXA) at the lumbar spine and hip. According to the guidelines, when an individual have had a low trauma fracture, osteoporosis is often presumed, and further testing of BMD measurement with DXA is advised. However, in the absence of DXA, treatment should still be initiated. In the case of quantitative ultrasound (QUS) at the heel, it can still be used to predict fragility fracture in postmenopausal women (hip, vertebral and global fracture risk) and men over the age of 65 (hip and all non-vertebral fractures). However, it should not be used for the diagnosis of osteoporosis or for monitoring treatment effects. Instead, individuals with low QUS results should be referred for BMD measurement using DXA. In addition, according to the guidelines, bone turnover markers cannot be used for the diagnosis of osteoporosis. However, they can provide additional information on fracture risk and can be used to assess compliance with treatments.

In 2008, The University of Sheffield, UK introduced an algorithm on absolute fracture risk, called FRAX®, and was included in the NOF guidelines (Watts et al., 2008; Kanis et al., 2008). At the time, the University hosted the The World Health Organisation (WHO) Collaborating Centre for Metabolic Bone Diseases (1991-2010), and the FRAX® tool was based on data generated from that centre. In The FRAX® algorithm, in addition to the BMD at the hip, nine specific clinical risk factors for osteoporosis and related fractures were also considered. This algorithm estimates 10-year probability of hip fracture (≥3%) and major osteoporosis-related fracture (20%) for asymptomatic or undiagnosed patients between the ages 40 to 90 years. FRAX® uses clinical risk factors which include an individual's age, gender,

height, weight, fracture history, parental history of hip fracture, smoking, long-term use of glucocorticoids, rheumatoid arthritis and alcohol consumption (Kanis et al., 2008). One advantage of FRAX® is that BMD assessment is not necessary for calculation of fracture probability. However, if a BMD is available, only the femoral neck BMD is to be used. At present, it is not recommended to use BMD scores from other sites because they have not been validated with FRAX® (Kanis et al., 2008).

Currently, the country-specific FRAX® prediction algorithms are unavailable in Malaysia. For Malaysians, the NOF recommended the use of ethnic-specific algorithms from neighboring countries such as Singapore Chinese or Hong Kong, Singapore Malay, and Singapore Indian until local data is available. Due to the high cost of assessment, general screening for osteopenia/osteoporosis (i.e. DXA) is not recommended. Therefore, only three major high risk groups will be considered for treatments. They include: 1) individuals with history of fracture of the hip or spine, 2) individuals with BMD in the osteoporosis range (T-score of -2.5 or lower), and 3) individuals with BMD in the low bone mass or osteopenia range with a higher risk of fracture defined by FRAX® scores (Siris et al., 2014). In Malaysia, the NOF suggests that treatment in premenopausal women should only be considered if they match certain criteria which includes previous low trauma hip, vertebral or wrist fracture, or a T-score -2.5 measured using DXA (provided they do not have secondary causes for osteoporosis). For osteopenic patients, treatment should only be initiated if they have a fracture probability of ≥3% at 10 years for hip, or 20% at 10 years for major osteoporosis-related fracture

(National Osteoporosis Foundation Clinician's Guide to Prevention and Treatment of Osteoporosis, 2010).

2.1.1 Quantitative Ultrasound (QUS)

Other than DXA, calcaneal (heel) quantitative ultrasound (QUS) can also be used to predict fragility fracture in postmenopausal women and men over the age of 65 (ISCD Official Positions-Adult, 2015). However, QUS was not recommended to be use as a mean of diagnosis or for monitoring the effect of treatment of osteoporosis. Instead, individuals with low QUS results should be referred for further BMD assessment using DXA. Currently, there is no consensus on what cut-off values to use with QUS to diagnose osteoporosis. To date, seveal studies have proven the predictive power of QUS in fracture risk (Johansen, Evans & Stone, 1999; Hans et al., 1996). For example, in a large prospective study involving 6189 postmenopausal women over age 65, it was found that quantitative calcaneal ultrasound predicted hip fracture as accurately as bone densitometry (Bauer et al., 1997). This finding is supported by a larger study involving 14,824 patients (men and women) aged between 42 to 82 years, which found that quantitative calcaneal ultrasound was also a good predictor of total and hip fracture risk (Khaw et al., 2004). In addition to being a good predictor for hip fracture risk, heel QUS was also found to be as good as central DXA in identifying women at high risk of vertebral osteoporotic fracture (Glüer et al., 2004). Further, other studies have also found that quantitative ultrasound also works well in predicting women at risk for osteoporosis (Hodson & Marsh, 2003, Stewart & Reid, 2000). Dane et al. (2008) reported that all three QUS indices, BUA, SOS and SI were significantly correlated to BMD at lumbar spine and femur

in postmenopausal women, but only SOS correlated significantly to BMD at lumbar spine and femur in premenopausal women. While ultrasound parameters do not directly measure BMD, BUA and SOS results are correlated (R=0.82 - 0.85) with heel BMD results obtained by the standard dual energy X-ray absorptiometry (DXA) technique, as are results for the combined QUI parameter (R=0.85).

One of the major limitations in using heel QUS as a screening tool is that there are no established criteria exist for diagnosing osteoporosis (Díez-Pérez et al., 2003). Furthermore, peripheral sites have slower rates of change in bone mass, thus cannot be reliably used to follow treatment effects of osteoporosis. Therefore, high risk patients indicated by ultrasound results (i.e. T-score $\leq$-2.5) will need to use DXA to determine the need for treatment based upon well-established guidelines, and as a baseline for monitoring therapy (Hashmi & Elfandi, 2016).

In addition to QUS, there are several other assessment of the bones available for the diagnosis of osteoporosis or the evaluation of an increased risk of fracture, namely; dual-energy x-ray absorptiometry (DXA), peripheral DXA (pDXA), quantitative computed tomography (QCT), and radiographic absorptiometry (RA).

2.1.2 Osteopenia/osteoporosis among postmenopausal women

Osteopenia and osteoporosis typically affect women more than men (75% vs. 25%) (Barling, 2013; Pietschmann, Rauner, Sipos, & Schindl, 2009). This is because overall, women reached lower peak BMD than men, resulting in lower bone quality. Also, the onset of menopause triggers a rapid decline of

bone density in women making them more prone to be diagnosed with osteoporosis after reaching menopause (Ohta et al., 1996). Furthermore, the longer life expectancy in women increases the risk of them having age-related diseases more than men (Barling, 2013).

More studies should be done to better understand the etiology and pathways involves in the process of menopause and its interaction with estrogen hormone. In menopausal transition, the production of hormones estrogen and progesterone is slowly decreased (Grady, 2006). A woman is described to reach menopause when the estrogen and progesterone production decreases permanently and the ovaries stopped producing eggs. Menopause, according to the World Health Organization (WHO) is defined as permanent cessation of menstruation resulting from physiological changes involving the loss of ovarian follicular activity. Postmenopausal phase begins when a woman has not had a period for 12 months in a row (amenorrhea) and no other reasons are attributed to this change (Grady, 2006). Menopause may occur within a wide range of age, as early as 40s to late 50s. Globally, the average menopausal age is around 51 years old and in Malaysia, the mean menopausal age is 50.7 years old (Ismael, 1994). One of the common symptoms of menopause is low BMD due to decreased production of estrogen (estrogen helps regulates osteoclast and osteoblast activity) (Krassas & Papadopoulou, 2001). In healthy women, bone mass normally peaked at the age of 30. This is followed by a gradual bone loss averaging at 0.6% every year before and after menopause. During the first 5 to 10 years after menopause, the rate of bone loss is significantly higher due to low estrogen level causing increased activity of bone resorption by osteoclasts. At the beginning of menopause,

the decline of trabecular bone mass in the vertebral column is reportedly between 1.8% and 2.3%, and the pelvic bones, 1.0-1.4%. After 5 years of menopause the average decline of bone density in the vertebral column is reportedly between 7 to 10% in the vertebral column and 5 to 7% in the pelvic bones, resulting in increased fracture risk (Finkelstein et al., 2002). Although rapid bone loss was found to occur in women at the beginning of or a bit after menopause, few women suffers from fractures before the age of 65. Due to the high cost of BMD assessment, it is not feasible to do a public screening. Therefore, osteoporosis or osteopenia tend to be diagnosed only when fractures happened. In 2002, the NOF of the United States reported that approximately 10 million US adults of 50 years of age and older had osteoporosis and an additional 33 million had low bone mass [based on the data collection from the National Health and Nutrition Examination Survey (NHANES) – III (1988–1994)] (National Osteoporosis Foundation, 2002). A study by Wright et al. (2014) estimated that 10.3% or 10.2 million adults age 50 years and older in the United States had osteoporosis and 43.9% or 43.4 million had low bone mass. When combined, the estimated number of adults with osteoporosis and low bone mass was 54% (Wright et al., 2014).

The prevalence of osteoporosis increase as the population ages, and differed by gender, race and ethnicity. For Caucasians, it was estimated that about one in two women will experience an osteoporosis-related fracture at some point in her lifetime, as will approximately one in five men (Office of the Surgeon General (US), 2004). A study in the U.S found that African Americans had the lowest prevalence of osteoporosis and low bone mass compared to non-Hispanic white and Mexican American (Wright et al., 2014). Although

African Americans are less predisposed to osteoporosis, those with the disease have the same elevated fracture risk as Caucasians and Asians (National Osteoporosis Foundation, 2014). In 2010, a study in Indonesia found that the prevalence of osteoporosis in postmenopausal women aged 47 to 60 years was 20.2% for the lumbar spine, and and 30% for the distal radius (Meiyanti, 2010). By comparison, a study in Thailand, which had stratified the age groups, reported that the prevalence of osteoporosis in the lumbar spine of Thai postmenopausal women aged 50 to 54 years was 9.4%, and 55 to 59 years, was 22.6%.

In Malaysia, as the aging population increases, it was expected that the prevalence of age-related diseases such as osteoporosis to also increase. Although the current prevalence is not known, data from a previous epidemiological study conducted from the year 1996 to 1997 found that the rates for hip fracture in the Malaysian population aged over 50 (per 100,000) are stated to be 88 for men, and 218 for women (Lau et al., 2001). These figures means that a woman living to the age of 74, which is the average female life expectancy in Malaysia, have a lifetime risk of 5.2% of having the disease. Therefore, from these statistics, it was projected that osteoporosis-related fracture was likely to be a primary cause of mortality for at least 2.6% of the female population of Malaysia. This will result in a significant increase in the healthcare cost. It was estimated that the inpatient hospital cost for hip fractures in 1997 was 6.8 million USD (RM22 million), which did not include rehabilitation or nursing home care costs. It was projected that incidence of hip fractures and costs of treatments to increase along with the age of the population (Yeap et al., 2013).

A hip fracture projection in Asia by Cheung et al. (2018) shows that the total number of hip fractures in Asia (China, Hong Kong, India, Japan, Korea, Malaysia, Singapore, Taiwan, and Thailand) will increase from 1.12 million in 2018 to 2.56 million in 2050, and the direct medical cost was projected to increase from USD 9.5 billion in 2018 to USD 15 billion in 2050. Surprisingly, the study projected that Malaysia will have 3.55 fold increase in hip fracture, the highest among the studied countries (Cheung et al., 2018). Asia has long been regarded as a 'high risk' region with the highest increase in hip fracture number. Therefore, now, more than ever, there needs to be an improvement in the diagnosis, medications, fracture prevention programmes, and research, to reduce the incidence of fracture worldwide. With the change in demographics and the increase of life expectancy worldwide, the total number of hip fracture may be continue to rise.

The underlying mechanisms of age-related osteoporosis are different than those associated with low estrogen level. In age-related osteoporosis, BMD will decline gradually and progressively over the years (seen in both men and women). Bone has a process of self-regeneration which serves to maintain bone mass and strength. During the process of self-regeneration, old bones are removed and replaced with new ones (Riggs, Khosla, & Melton, 2002). This process of removal and replacement of bones are regulated by two types of bone cells; osteoblast, which is responsible for the bone formation, and osteoclast, which is responsible for bone resorption. However, with aging, the delicate balance between osteoblast and osteoclast activity is shifted, favoring more in bone resorption and less in bone formation. The resulting loss in bone mass and strength ultimately leads to osteoporosis and fractures.

In estrogen-derived osteoporosis, the rate of self-regeneration of bone increases, leading to increased impact of osteoblast and osteoclast imbalance. The loss of BMD disrupted skeletal architecture and increase in fracture risk. Studies found that hormone therapy are effective in decreasing the incidence of osteoporosis and minimize the risk of fracture in postmenopausal women (Gambacciani & Vacca, 2004). However, since many women have opted to stop or avoid hormone therapy altogether after reaching menopause, it is important for healthcare providers and clinicians to actively screen women at risk for osteoporosis and fracture. During the peri-menopausal transition, it is especially important to encourage the type of exercises that involves weight-bearing and also increased calcium/vitamin D intake as a mean of precaution.

The pathophysiology of age-related bone loss is very complex involving various factors such as genetic, hormonal, biochemical, and environment. These, in combination with intrinsic and extrinsic factors of aging accelerates the decline in bone mass that predisposes to fractures (Demontiero, Vidal, & Duque, 2012). Despite various clinical and animal studies which shed significant lights on the mechanisms involving these factors, more research is needed to determine the relative involvements of each of these factors in order to improve preventative, intervention, and therapeutic measures (Demontiero, Vidal, & Duque, 2012).

2.1.3 Osteopenia/osteoporosis and Vitamin D

Various studies have shown the association between osteoporosis and low level of Vitamin D (Sadat-Ali et al., 2011; Lukert, Higgins, & Stoskopf,

1992; Villareal et al., 1991). The primary function of vitamin D is to maintain serum calcium concentrations within the physiologically acceptable range. It was discovered that when the body is deficient of Vitamin D, trans-cellular absorption of calcium in the small intestine will decrease. This will kick-start the homeostatic negative-feedback mechanism in the body which ultimately leads to the release of calcium from skeleton reserves in order to maintain circulating calcium concentrations (Lips, 2001). Essentially, when low calcium absorption is detected in the small intestine, this will cause an increase in osteoclast production which will break down more calcium from the bone into the bloodstream.

Vitamin D supplements have traditionally been recommended for older adults to treat or prevent osteoporosis. A study shows that the serum $25(OH)D_3$ concentrations must be at least 78 nmol/L (30 ng/mL) in order to achieve optimal intestinal calcium absorption (Heaney et al., 2003). When the body has sufficient level of vitamin D, up to 30% of calcium is typically absorbed in the small intestine, and as much as 60-80% is absorbed during growth period and during pregnancy or lactation (due to higher demand of calcium). However, when the body is deficient of vitamin D, only 10-15% of calcium is absorbed from diet (Holick, 2004; Holick, 1994). The continuous process of bone turnover and resorption weakens the architecture of bones and increases the risk of fracture via secondary hyperparathyroidism, (Lips, 2001) which ultimately lead to the development of osteomalacia and osteoporosis. Overall, there is evidence that Vitamin D deficiency (<20 ng/ml) is associated with a greater risk of fracture in frail institutionalized elderly men and women, whereas it is unlikely that those individuals with

25(OH)D levels between 20-30 ng/ml are at a greater risk (Kahwati et al., 2018). Nevertheless, the recently completed VITAL trial demonstrated that 2000 IU of Vitamin D to healthy men and women did not prevent bone loss or alter bone turnover (LeBoff et al., 2020). A recent reports suggests that a better approach to determine an optimal 25(OH)D is through cut-offs below and above which deleterious effects may occur, as well as the the levels of 25(OH)D associated with beneficial effects, based on the available interventional studies (Souberbielle et al., 2020).

Vitamin D has been shown to have direct effect on the skeletal system. Historically, Vitamin D is known to play an important role in calcium homeostasis and bone metabolism. However, there have also been studies showing otherwise. For example, a recent study published in January 2020 involving a cohort of 771 individuals (46.7% women) living in Boston, Massachusetts found no evidence on the benefits of Vitamin D supplementation on bone density or strength in older adults after daily vitamin D3 supplementation for 2 years (LeBoff, Chou, Murata, et al., 2020). The same holds true for other non-skeletal benefits of vitamin D, such as diabetes and cardiovascular, respiratory, neurological, renal, and liver diseases. Studies have found both benefits as well as lack of benefits (Stokes & Lammert, 2016). However, there are still some justification to use Vitamin D supplements to maintain or improve musculoskeletal health. For one, daily supplementation is a good way to prevent rare conditions such as rickets and osteomalacia in high-risk groups, which can occur due to Vitamin D deficiency after a prolonged lack of exposure to sunshine.

2.2 Sarcopenia

Sarcopenia is described as an age-related decline in muscle mass, strength and function, which typically results in immobility, falls, disability and even death in the elderly (Morley & Cao, 2015). After the age of 30, women tend to lose muscle mass gradually and will experience an accelerated decline after the age of 50 (Rolland, Perry, Patrick, Banks, & Morley, 2007; Gallagher et al., 1997). The development of sarcopenia has been found to be correlated to various factors. For example, the decline in muscle mass in sarcopenia was thought to occur due to a combination of the loss and the atrophy of muscle fibers. Another hypothesis states that the denervation of motor units which are then re-innervated with slower motor units was thought to cause muscle to become more fatigued (Erim, Beg, Burke, & de Luca, 1999). At present, the etiology and mechanism of sarcopenia is not yet fully understood. However, observational studies have shown that the number of satellite cells, which are involved in muscle regeneration were found in a much lower quantity in older people and, thus, could potentially play a role in the development of sarcopenia (Thornell, Lindström, Renault, Mouly, & Butler-Browne, 2003). Other factors that have been found to be associated with the development of sarcopenia include reduced level of growth hormone (GH), insulin-like growth factor (IGF-1) and androgens, which are related to the development of skeletal muscle (Bian et al., 2020). Further, studies have also implied the involvement of renin-angiotensin system in muscle function modulation. The theory goes that circulating angiotensin II is associated with muscle wasting, reduced IGF-1 levels, and insulin resistance and therefore, could potentially contribute to the development of sarcopenia (Bian et al.,

2020; Brink, Wellen, & Delafontaine, 1996). Chronic inflammation has also been found to be associated with sarcopenia, and observational studies have shown increased levels of pro-inflammatory cytokines, tumor necrosis factor-α, and interleukin-6 in aging muscle in people with sarcopenia (Schaap, Pluijm, Deeg, & Visser, 2006).

Due to the complex nature of the disease, no global consensus exist on the definition of sarcopenia, which makes it harder to be diagnosed. Currently, there are two diagnostic methods exist for the European and the Asian population. The European Working Group on Sarcopenia in Older People (EWGSOP) has defined sarcopenia as 'low muscle mass with low muscle strength OR with low gait speed'. However, the Asian Working Group of Sarcopenia (AWGS) (which was modified from the European Union Geriatric Medicine Society (EUGMS) and the European Working Group on Sarcopenia in Older People (EWGSOP)) has slightly different measurements. For AWGS, the diagnosis requires measurements of muscle mass, muscle strength, AND physical performance (Chen et al., 2014). It is different from the EWGSOP in measurement of both muscle strength and physical performance in addition to muscle mass (3 criterion instead of 2) and uses different cut-off values of each parameter based on the existing studies in Asian societies. For EWGSOP, the diagnosis requires measurement of muscle mass plus muscle strength OR physical performance. For both sets of criteria (AWGS and EWGSOP), severities of sarcopenia are classified into 3 groups; pre-sarcopenia, sarcopenia and severe sarcopenia. Pre-sarcopenia is defined as a presence of low muscle mass only, sarcopenia is diagnosed when there is a presence of low muscle mass and either low muscle strength or low

physical performance, and severe sarcopenia is defined when low muscle mass, low muscle strength and low physical performance are present at the same time (Limpawattanaa, Kotruchinb, & Pongchaiyakulc, 2015; Cruz-Jentoft et al., 2010). A study by Arimi Fitri, et al. (2015) found a dramatic difference in prevalence of sarcopenia in Malaysia by utilizing the two different criteria. The study found that the prevalence of sarcopenia in Malaysian elders ranged from 7% to 44% dependent upon which guidelines used to define the disorder. It was found that the prevalence is higher when the EWGSOP guideline was used (Arimi Fitri et al., 2015). This finding shows that diagnostic criteria for Western population is not comparable to Asians. Therefore, when Ilich, Kelly and Inglis (2016) proposed a new diagnostic criteria for OSO, question arise if the same criteria can be applied to Asians and whether a separate set of criteria is needed to diagnose OSO in people of Asian ethnicities (Ilich, Inglis, Kelly, & McGee, 2015).

Sarcopenia also tended to be more predominant in women than in men. Based on the findings of six cross-sectional studies from United States, United Kingdom, Brazil, Japan, South Korea and Taiwan, the prevalence of sarcopenia in women was higher than men in 5 out of the 6 studies; United States = 10.0% in women vs. 7.0% in men, United Kingdom = 7.9% in women vs. 4.6% in men, Brazil = 16.1% in women vs. 14.4% in men, Japan = 22.1% in women vs. 21.8% in men, South Korea = 11.8% in women vs. 9.7% in men. However in Taiwan, the prevalence of sarcopenia was found to be higher in men (Taiwan = 5.4% in men vs. 2.5% in women) (Diz et al., 2015). Similarly, in Malaysia, a study by Norshafarina et al. (2013) reported that the prevalence of sarcopenia among elderly Malaysians was significantly

higher in men than women (Norshafarina et al., 2013). The study found that the prevalence of sarcopenia was 89.0% and 40.3% in men and women, respectively, with the overall prevalence of 59.8%. This is significantly higher than those documented in the west and other Asians countries. Nevertheless, it must be noted that the discrepancy and the differences of the prevalence of sarcopenia between the studies may not only be due to differences in body composition across ethnic groups and the techniques utilized, but also depends on the diagnostic criteria. Most studies used the cut-off points proposed by Baumgartner et al. (1998), which described sarcopenia as having a relative skeletal muscle mass (RSMI) of less than 2.0 standard deviation (SD) below the young references group; or less than 7.26 kg/m^2 in men and less than 5.45 kg/m^2 in women, while others, such as the one conducted in Malaysia, used cut-off points proposed by Janssen et al. (2002) which described moderate to high degree of sarcopenia as that within the SMI values of <10.75 kg/m^2 in men and <6.75 kg/m^2 in women (Janssen, Heymsfield, & Ross, 2002). These differences give different findings in prevalence studies, which disallow comparisons to be made between studies. Basic therapy for sarcopenia includes resistance exercise and protein and Vitamin D supplementation (Morley, Anker & Haehling, 2014).

Currently, several methods are used in the assessment of muscle mass, strength, and physical performance. The commonly used and feasible tools for the assessment of muscle mass are dual energy X-ray absorptiometry (DXA) and bio-electrical impedance analysis (BIA) (Janssen, Heymsfield, Baumgartner, & Ross, 2000), although other more complicated tools such as computerized tomography (CT scan) and magnetic resonance tomography

(MRI) have also been used (Rolland et al., 2008; Chien, Huang, & Wu, 2008; Visser et al., 2005). Muscle mass below 2 SD of the mean appendicular muscle of young healthy adults is a commonly used diagnostic criteria for sarcopenia (Gallagher et al., 1997). Muscle strength may be measured by variety of methods, namely handgrip strength (HGS), knee flexion/extension (quadriceps strength), and peak expiratory flow (PEF). Grip strength is the commonly used method for assessment of muscle strength (Snih, Markides, Ottenbacher, & Raji, 2004; Rantanen et al., 2003). For the testing of physical performance, the international working group has recommended a battery of tasks, entitled short physical performance battery (SPPB) as the standard evaluation in research and clinical setting (Guralnik et al., 1994). However, other physical performance tests have also been routinely used among the elderly such as gait speed (Working Group on Functional Outcome Measures for Clinical Trials, 2008), timed get-up-and-go test (TGUG) (Mathias, Nayak, & Isaacs, 1986), and stair climb power test (SCPT) (Bean, Kiely, LaRose, Alian, & Frontera, 2007). Table 2.1 shows the recommended tools and their cut-off values in Asia according to the consensus of the AWGS and existing studies (Chen et al., 2014; Assantachai, Muangpaisan, Intalapaporn, Sitthichai, & Udompunturak, 2014; Arai, Akishita, & Chen, 2014; Cruz-Jentoft et al., 2010).

Table 2.1 AWGS sarcopenia diagnosis criteria: recommended tools and their cut-off values in research and clinical practice

Measurement	Tools	Cut-off values	
		Men	Women
Muscle mass [a]	Dual energy X-ray absorptiometry (DXA)	7 kg/m^2	5.4 kg/m^2
	Bioimpedance analysis (BIA) (defined by appendicular skeletal muscle mass/height^2)	7 kg/m^2	5.7 kg/m^2
Muscle strength	Handgrip strength	<26 kg	<18 kg
	Knee flexion/extension	<18 kg	<16 kg
Physical performance	6-m usual gait speed	<0.8 m/s	<0.8 m/s

[a] Relative appendicular skeletal mass/height (Chen et al., 2014)

2.2.1 Sarcopenia among postmenopausal women

After achieving peak muscle strength in the early 40s (Grimby & Saltin, 1983), women tend to lose muscle mass gradually and will experience an accelerated decline after 50 years old (Rolland, Perry, Patrick, Banks, & Morley, 2007; Gallagher et al., 1997). However, the decrement in muscle strength and mass is not linear (Goodpaster et al., 2006) and the rate and age at which it occur is different between sexes. In fact, women might experience it earlier due to the onset of menopause (Phillips, Rook, Siddle, Bruce, & Woledge, 1993). After the age of 50, muscle strength have been reported to decrease 1.5% to 3% per year (Vandervoort, 2002; Roubenoff & Hughes, 2000) and muscle mass, 1% to 2% per year (Hughes, Frontera, Roubenoff, Evans, & Singh, 2002). During the first 3 years after menopause, total body potassium, which is a marker for lean body mass has also been found to decrease significantly in older women (Kyle et al., 2001; Aloia, McGowan,

Vaswani, Ross, & Cohn, 1991). Further, it was found that compared to their younger counterparts, women aged 65 to 80 years old have twice the amount of non-contractile muscle tissue per unit of muscle cross-sectional area (CSA) (Jubrias, Odderson, Esselman, & Conley, 1997). Intramuscular fat, which is an example of non-contractile muscle tissue has been found to increase after menopause (Forsberg, Nilsson, Werneman, Bergstrom, & Hultman, 1991). Brown (2008) explained that women tended to store fat in the muscle because compared to men, they uses more fat than glycogen as fuel (Brown, 2008). Lipoprotein lipase (LPL), which is an enzyme responsible for utilizing triglycerides in muscle, decrease with aging and this likely to lead to an increase in intramuscular fat storage (Hamilton, Areiqat, Hamilton, & Bey, 2001). Basically, it means that after menopause, women tend to lose their ability to oxidize fat stored in muscle, leading to an excess accumulation of intramuscular fat (Maltais, Desroches, & Dionne, 2009). Animal studies have also shown compelling evidence on the role of estrogen on muscle contractile properties. Wohlers, et al. (2009) found that mice with removed ovaries have reduced capability of activating adenosine monophosphate kinase (AMPK) phosphorylation (Wohlers, Sweeney, Ward, Lovering, & Spangenburg, 2009). The AMPK protein plays an important role in glucose uptake (Jørgensen, Richter, & Wojtaszewski, 2006) and lipid oxidation in muscle (Osler & Zierath, 2008), hence closely related in the production of energy that is needed to produce muscle contractions.

Sarcopenia is highly prevalent in postmenopausal women, from which, leads to restriction in movement, functional impairment, physical disability and fractures in this cohort. The prevalence of sarcopenia in postmenopausal

women was reported to be from 10% to 40%, contingent on the diagnostic method used and the population (Diz et al., 2015; Abellan Van Kan, 2009). It has been hypothesised that menopause accounts for 10–15% the loss of muscle muscle strength in women, in addition to the age itself (Phillips, Rook, Siddle, Bruce, & Woledge, 1993). Moreover, this hypothesis was supported by the findings that hormone replacement therapy responded favourably in maintaining muscle strength and performance (Elliott-Sale, 2014; Chen et al., 2005; Sipilä, Taaffe, Cheng, Puolakka, Toivanen, & Suominen, 2001; Sørensen, Rosenfalck, Højgaard, & Ottesen, 2001), although this effect has not been found consistently across studies (Hansen, Raja, Baber, Lieberman, & Allen, 2003; Kenny, Dawson, Kleppinger, Iannuzzi-Sucich, & Judge, 2003). In addition to muscle strength, a good body of evidence have also shown correlation between low muscle mass and low estrogen level in postmenopausal women (Van Geel, Geusen, Winkens, Sels, & Dinant, 2009; Iannuzzi-Sucich, Prestwood, & Kenny, 2002). Estrogen depletion has been hypothesized to be one of the reasons for the loss of muscle mass in postmenopausal women (Messier et al., 2011) and the reason was explained through the action or lack thereof of plasma estrone and estradiol (Iannuzzi-Sucich, Prestwood, & Kenny, 2002) on the estrogen receptors in the skeletal muscle (Messier et al., 2011; Brown, 2008). The decrease of estrogen has been found to contribute not only to the loss of muscle mass and strength, but as previously mentioned, also to the loss of BMD, the increased in visceral fat, the increased risk of cardiovascular disease and the decrease in quality of life (Carr, 2003). Although the mechanisms underlying the relationship between low estrogen level and

detrimental effect on muscles are not yet understood, various findings have shown compelling evidence on the correlations, specifically between muscle sub-characteristics and estrogen metabolism (Sakuma & Yamaguchi, 2012; Messier et al., 2011; Maltais, Desroches, & Dionne, 2009). In addition to low estrogen level, other factors such as Vitamin D deficiency, low physical activity, low protein intakes, increased oxidative stress, increased inflammation (Roubenoff, 2003) and decrease testosterone level (Maggio, Lauretani, & Ceda, 2013) were also found to be some of the best contributors to sarcopenia in postmenopausal women (Sirola & Kröger, 2011).

2.2.2 Sarcopenia and Vitamin D

There are increasing evidence suggesting a strong relationship between Vitamin D deficiency and sarcopenia (Anagnostis, Dimopoulou, Karras, Lambrinoudaki, & Goulis, 2015). A review by Anagnostic et al. (2015) discussed updated data on the beneficial effect of vitamin D supplementation on muscle strength, physical performance and prevention of falls and fractures in older women. Although it is still inconclusive if and to what extent mode of treatments, such as dosage, mode of administration and duration of supplementation, could influence the treatment outcome, increasing evidence suggest that low vitamin D is associated with low muscle mass in elderly women, independent of body composition, diet and hormonal status (Anagnostis et al, 2015). With the identification of the Vitamin D receptor (VDR) in muscle cells and neuronal cells, the results of cell culture and experimental animal studies have led the hypothesis that Vitamin D affects muscle growth, development, and contraction (Abiri & Vafa, 2017). Recent animal study found that the effect of Vitamin D on muscle strength

and physical performance depends on physical activity level in aged mice (Yang et al., 2020). Since muscle atrophy is exacerbated by physical inactivity and Vitamin D deficiency, the biological mechanism may involve synergistic effects of Vitamin D and physical activity on the promotion of muscle protein ubiquitination and degradation (Yang et al., 2020). Various studies have also shown that Vitamin D supplementation was able to reduce the risk of falls (Ringe, 2012; Sato, Iwamoto, Kanoko, & Satoh, 2005). It was hypothesized that Vitamin D reduced the risk of falls by its combined effects on bone and muscle. In a randomized controlled study involving 96 elderly women with post-stroke hemiplegia, daily supplementation of vitamin D (1000 IU) for 2 years resulted in 59% reduction in falls (95% CI, 28-81%; p=0.003), improved muscle strength and increased in number and size of type II muscle fibers (reduced of type II muscle fibers is one of the histopathological signs in sarcopenia) (Sato et al., 2005). In addition, studies also found that proximal myopathy induced by low Vitamin D can be reversed with Vitamin D supplementation (Rasheed, Sethi, & Bixby, 2013; Skaria, Katiyar, Srivastava, & Dube, 1975; Prineas, Mason, & Henson, 1965). Further, in a comparison study between older adults who had high serum 25(OH)D_3 levels (>94 nmol/l) versus lower serum 25(OH)D_3 levels (<60 nmol/l), it was found that the former showed better performance in 8-foot walk test and chair stand than the latter (Bischoff-Ferrari et al., 2004). Previous study has also found a positive correlation between hand grip strength and serum 25(OH)D_3 levels (Visser, Deeg, & Lips, 2003). In a multicenter cross-sectional study involving 976 older people aged 65–102 years, Vitamin D status was demonstrated to be positively associated with

handgrip strength and short physical performance battery (SPPB) test. Participants with low levels of $25(OH)D_3$ were found to have lower SPPB test scores and handgrip strength compared to participants with higher $25(OH)D_3$ level. The study reported that both female and male participants with serum $25(OH)D_3$ < 25 nmol/L had significantly lower SPPB scores compared to those with serum $25(OH)D_3$ ≥25 nmol/L, ($p<0.05$). Similarly, those with serum $25(OH)D_3$ <50 nmol/L had significantly lower handgrip strength than those with serum ≥50 nmol/L, ($p<0.05$) (Houston et al., 2007). Similar finding has also been reported in the Longitudinal Aging Study Amsterdam where it was found that risk of falling is inversely associated with levels of $25(OH)D_3$ (Snijder et al., 2006). Further, in a retrospective study by Iolascon et al. (2015), postmenopausal women with Vitamin D insufficiency ($25(OH)D_3$ <30 ng/mL) was found to be associated with worse upper and lower-extremity muscle strength and physical performance based on hand grip strength, knee extension strength, SPPB, and 4-meter gait speed compared to women with normal level of $25(OH)D_3$ (Iolascon et al., 2015). These findings suggest that low Vitamin D level could be one of the major factors in the development of sarcopenia and could potentially cause adverse effects in muscle functions. The hypothesized mechanism to explain the association between Vitamin D and muscle performance appears to be at the muscular cell level and structure. A study found that low Vitamin D level was associated with increased infiltration of fat between muscle fibers and enlarged interfibrillar spaces (Visser, Deeg, & Lips, 2003). Due to this finding, it was theorized that Vitamin D plays a crucial role in the maintenance of type II fibers and the prevention of falls. Consequently, it was

recommended that postmenopausal women to increase the intake Vitamin D with calcium supplementation to improve their body sway and gait performance and reduce or prevent the incidence of falls (Bruyère et al., 2014).

On the flipside, although, the biological role of Vitamin D on skeletal muscles has been widely investigated (Agergaard et al., 2015, Granic et al., 2017), the expression of Vitamin D receptor (VDR) on skeletal muscle cells has been questioned. In fact, some studies, using specific and sensitive immunohistochemical assays, showed that the Vitamin D receptor was undetectable in skeletal, cardiac and smooth muscle (Wang & DeLuca, 2011). Furthermore, there have also been evidence showing that VDR expression changes over the life span; more expressed in satellite cells than in mature muscle fibers, and being less expressed with increasing age. This suggest that Vitamin D plays important role in the early-stage muscle development, but a less important role in muscles of advanced age (Olsson et al., 2016). However, biological, experimental and epidemiological studies have shown evidence in supporting the benefits of Vitamin D supplementation in preventing and treating sarcopenia in older adults. Nevertheless, because of the high heterogeneity of the observational study and the conflicting results of randomized controlled trials, the exact role of Vitamin D supplementation to prevent and treat sarcopenia is still inconclusive and requires further investigation. Furthermroe, intervention studies are needed to determine its optimal serum levels to maintain a good physical function in older age.

2.3 Obesity

Based on a global study, obesity among women is prevalent worldwide (Swinburn et al., 2011). In 2007, Rampal et al. reported that the prevalence of obesity among older women in Malaysia was at 13.2% compared to only 8.8% in older men. Although no recent data can be found on the older cohort, a study published by the British Medical Journal, The Lancet (2014), reported that 49% of Malaysian women and 44% of Malaysian men were either obese or overweight, which was the highest percentage found in Asia. The study reported that overall, Malaysia was rated the heaviest with almost half of its population (45.3%) being obese or overweight. Second in line was South Korea with 33.2% of its population being overweight, followed by Pakistan with 30.7% and China with 28.3% (The Star Online, 2014). Both developing and developed countries are having smilar issues regarding high prevalency of overweight and obesity (Ellulu, Abed, Rahmat, Ranneh, & Ali, 2014). In any study, the prevalence of obesity was found to be higher in women than in men (Hedley et al., 2004). In Malaysia, for example, obesity were more predominant in Malay and Indian women compared to Chinese women while in men, the Chinese were found to have the highest obesity prevalence followed by the Malays and Indians (Ismail et al., 2002). It was hypothesized that the reason for the high prevalence of obesity in Malaysia is because of the population's lifestyle which typically includes high energy and fat consumption combined with lack of exercise (The Star Online, 2014; World Health Organization. Obesity: Preventing and Managing the Global Epidemic, 2000). Although the problem of obesity has only been recently highlighted in the past 5 years, the issue has been existing in the country for

quite a while (Lim et al., 2000). It is highly likely that the problem will only escalate if no countermeasures made to curb the incidence. It is important to note that in Asian adults, health risk associated with obesity occur at a lower body mass index (BMI) compared with Caucasians/European population (Misra, 2015; He, Tan, Li, & Kung, 2001; Deurenberg-Yap, Schmidt, Staveren, & Deurenberg, 2000;). For Asian population, the World Health Organisation (WHO) has redefined the cut-off point for overweight and obesity with BMI value $\geq 23 kg/m^2$ and $\geq 27.5 kg/m^2$, respectively. Therefore the obesity prevalence estimates in Asia are likely to underestimate the burden of public health in Asian countries.

In 2004, the committee of Clinical Practice Guidelines on Management of Obesity in Malaysia had proposed a new criteria in defining overweight and obesity for Asian population. This is due to compelling evidence which found that health risks associated with obesity such as type 2 diabetes mellitus and cardiovascular disease occur at a lower BMI (below $25 kg/m^2$) in Asians than their Caucasian counterpart (Ismail, Bebakar, & Kamaruddin, 2004). However, in 2009, waist circumference (WC) was proposed to be used instead to define overweight and obesity in obesity-related cardiovascular risk factors studies (i.e. dyslipidaemia, hypertension, diabetes mellitus) in men and women (≥ 83 cm in both men and women) as it was found to be the better indicator (Zaher et al., 2009). Various studies have found that central or abdominal adiposity may be more detrimental to health than fat deposited in other parts of the body (Lee, Huxley, Wildman, & Woodward, 2008; Schneider et al., 2007; Wang et al., 2005). High level of central adiposity in adults have been found to be associated with increased risk of obesity-related

conditions including type 2 diabetes, hypertension and heart disease (Lee et al., 2008; Wang et al., 2005). Although measures of central adiposity are closely correlated with BMI, they have been shown to predict future ill health independently of BMI (National Obesity Observatory, 2009). In addition, studies also found strong relationship between abdominal fat mass with bone and muscle health (Wannenes et al., 2014; Bredella et al., 2011; Cao, 2011; Gilsanz et al., 2009; Blüher, 2009; Das, 2001). A study by Gilsanz, et al. (2009) found that an increased production by visceral fat of adiponectin and pro-inflammatory cytokines may result in adverse effects on bone. Subcutaneous fat, on the other hand, was found to give an opposite effect. Another study by Bredella, et al. (2011) also found an inverse relationship between visceral fat mass and BMD in postmenopausal women. It appears that fat accumulation around abdominal and internal organs pose a stronger threat to BMD than fat found under the skin. Due to various evidences which showed significant relationship between fat deposition and adverse health effect, diagnostic criteria proposed by Ilich, Kelly and Inglis (2016) which used overall fat mass to define obesity to diagnose OSO is critically questioned. Perhaps, WC would be a better parameter to determine risk factors for OSO. Therefore, in this study, we aim to find associations, if any, between WC and Osteosarcopenic obesity, Osteopenic obesity and Sarcopenic obesity.

2.3.1 Obesity among postmenopausal women

Obesity is a complex, multifactorial condition defined by excess body fat. Studies found that adiposity has a strong correlation with important physiological parameters such as hormone regulation, blood pressure, serum

triglyceride and leptin concentrations and systemic insulin sensitivity (Weisberg et al., 2003; Liuzzi et al., 1999). However, some evidences indicate that the location where the fat is distributed plays an important role on how it affects the body. Visceral fat mass, as previously mentioned, was found to be more closely correlated with obesity-associated pathology than overall adiposity (Bredella et al., 2011; Stolk, Meijer, Mali, Grobbee, & van der Graaf, 2003). The prevalence of obesity is worrisome, especially in women due to wide-ranging effects it has on a variety of women's health issues. Overweight or obese women have a significantly increase risk of diabetes, coronary artery disease, low back pain, knee osteoarthritis, depression and several types of cancer including endometrial cancer, cervical cancer, breast cancer, ovarian cancer (Kulie et al., 2011) and osteoporosis (Kindler et al., 2017). Kindler et al. (2017) who conducted advanced three-dimensional bone scans on 24 women ages 18 and 19 with the cohort divided into 2 groups; 12 normal weight (NW< 32 percent) and 12 obese (OB> 32 percent) participants, found that the obese group had lower apparent trabecular thickness (radius and tibia), higher apparent trabecular separation (radius), and lower apparent bone volume to total volume compared to the normal weight group, suggesting adverse effect of obesity to bone. Although the relationships between menopausal transition, weight gain, and obesity are well established, (Reynolds & Obermeyer, 2005; Rodstrom et al., 2003) the mechanism involves is still unclear. Some researchers claimed that the absence of estrogens may be an important obesity-triggering factor (Clegg, 2012), while others claimed that obesity increases the production of estrogen (Neuhouser et al., 2015).

There is a seemingly biological paradox found in postmenopausal women who are obese. Obesity has been found to be positively associated with the release of estrogens in postmenopausal women because estrogen was found to be made by fat tissue (Neuhouser et al., 2015). Therefore, in theory, obesity should help mitigate the symptoms of menopause especially in bone loss through the increase production of estrogen. However, the hypothesized benefit of obesity in postmenopausal women might be hampered by a compelling evidence which suggest a strong link between obesity and a type of cancer, namely, breast cancer. In a data collected from more than 67,000 postmenopausal women in 1993 to 1998, (as part of a study called the Women's Health Initiative), it was found that highly obese women were at risk for estrogen- and progesterone-driven breast cancer. In addition, they were also more likely to have large tumors and cancer that spread beyond the breast and into the lymph nodes (Neuhouser et al., 2015). The study explained that the increased risk for breast cancer might be due to an increase in estrogen, although it cautioned that the study did not prove a cause-and-effect link. The implication begs further questions on the relationship between adiposity and estrogen in postmenopausal women. Questions arise on whether obesity is protective or detrimental to older women's health. As described above, a body of literature showed conflicting results. A study found that adiposity increases the level of estrogen in the body, which is a good sign in the estrogen-depleted body of postmenopausal women (Lizcano & Guzmán, 2014). However, the adiposity-induced estrogen is found to have strong association to the development of breast cancer in the cohort (Kuhl et al., 2005). It was hypothesized that this is because of an increase in the serum

concentration of bioavailable estradiol (Collaborative Group on Hormonal Factors in Breast Cancer, 1997). Perhaps studies by Gilsanz et al. (2009) and Bredella et al. (2011) offered some explanations on this conundrum. The studies found that fat disposition played important role in the beneficial or detrimental effect of adiposity in older women. Visceral or abdominal fat was found to be more harmful to the body, particularly to the bones, than subcutaneous fat (subcutaneous fat was actually found to have protective effect on the bones). Perhaps if an older woman who is obese has minimal amount of visceral fat, obesity offer no danger to her body. It is not known whether visceral fat is associated with the development of breast cancer in postmenopausal women. Perhaps further studies are warranted to prove these hypotheses.

2.3.2 Obesity and Vitamin D

Obesity has consistently been linked with low Vitamin D level (Wakayo, Whiting, & Belachew, 2016; Cheng et al., 2010; Moschonis et al., 2009; Snijder et al., 2005; Parikh, Edelman, & Uwaifo, 2004; Arunabh, Pollack, Yeh, & Aloia, 2003; Wortsman, Matsuoka, Chen, Lu, & Holick, 2000; Bell, Epstein, & Greene, 1985; Liel, Ulmer, Shary, Hollis, & Bell, 1988). Although the mechanism or the explanation for the subnormal concentrations of $25(OH)D_3$ in obesity have not reached a consensus, it has been postulated that the deficiency might be due to several reasons; 1) active avoidance of UV radiation by obese individuals which significantly affect the cutaneous synthesis of Vitamin D_3 (Compston et al., 1981), 2) enhanced production of active Vitamin D metabolite 1,25-dihydroxyvitamin D3 [$1,25(OH)_2D_3$], causing negative feedback control on the synthesis of $25(OH)D_3$ in the liver

(Bell et al., 1985), or 3) the fat-soluble Vitamin D is sequestered in the larger body pool of fat of obese individuals (Wortsman et al., 2000). Animal, human models as well as multiple epidemiological studies have shown inverse association between Vitamin D status and body mass across all subpopulations, ages and ethnicities (Wakayo, Whiting, & Belachew, 2016; Melnyk, Evans, Korourian, & Hakkak, 2014; Drincic, Armas, van Diest, & Heaney, 2012; Bell et al., 1985). Obese individuals in general requires higher dosage of Vitamin D than what was recommended for general population. Furthermore, recent systematic review and meta-analysis of randomized controlled trials suggest that obesity and overweight may decrease the effect of Vitamin D supplementation in adults (de Oliveira et al., 2020). The data gathered from 18 descriptive studies found that after supplementation, individuals with obesity presented increased serum Vitamin D at 39.83 nmol/L (95% CI: 34.06–45.61) compared to the control/placebo group. However, the obese state decreased serum Vitamin D concentration by -38.17 nmol/L (95% CI: -59.90/-16.44) compared to the normal weight group. Additionally, increasing the dose of Vitamin D supplementation does not appear to contribute significantly to increased serum Vitamin D levels (de Oliveira et al., 2020). The study suggest that there should be an optimal dose of vitamin D supplementation for people with obesity. Understanding the mechanism involve behind the relationship between low Vitamin D status and obesity has important therapeutic implications, particularly in prescribing appropriate dosage of Vitamin D for obese individuals, and potential effects of treatment of Vitamin D deficiency.

2.4 Osteoporosis, sarcopenia and obesity (Osteosarcopenic obesity)

Osteosarcopenic obesity (OSO) is characterized by concurrent manifestation of three distinct musculoskeletal disorders comprised of osteopenia/osteoporosis, sarcopenia, and obesity. OSO is a highly complex disorder that involves a number of interconnecting pathways. Currently, the etiology and mechanism of the condition can only be hypothesized. Osteopenia/osteoporosis and sarcopenia are age-related disorders and have been found to share common pathophysiology (Ormsbee et al., 2014). For example, both muscles and bones originates from the same mesenchymal cell populations (stromal cells in the bone marrow) and their growth trajectories also display similar patterns (Hamrick, McGee-Lawrence, & Frechette, 2016). However, the addition of obesity to combined manifestation of osteopenia/osteoporosis and sarcopenia was projected to worsen any type of abnormalities present, leading to low quality of life, and higher risk of morbidity and mortality (Corica et al., 2015).

The interrelationship between muscle, fat, bone has been described by Ilich et al. (2014). A major problem of OSO is due to the age-related fat redistribution and subsequent infiltration into bone and muscle. Normally, bone, muscle, and fat progenitor cells differentiate in parallel form to enable normal development of tissue. However, in OSO, some impairments in the differentiation pattern occured, and fat production predominates, leading to infiltration of fat into bone and muscle (Ilich et al., 2014). Fat infiltration is one of the hallmarks of sarcopenia and osteoporosis (Saedi et al., 2019; Hardouin, Rharass, & Lucas, 2016; Ji, Han, & Won, 2015). High levels of marrow adipose tissue are associated with low bone density and osteoporosis

(Hardouin, Rharass, & Lucas, 2016). Marrow adipose tissue secretes adipocytokines and fatty acids, which are toxic to the cells in the vicinity of adipocytes, decreasing osteoblast activity (bone formation) and increasing osteoclast activity (bone resorption) (Singh et al., 2018). Similarly, fat infiltration in muscle fibers is associated with cell dysfunction (Schaap, Koster, & Visser, 2013). In 2013, Cooper et al. hypothesized possible mechanisms underlying OSO. The authors suggest that high amount of pro-inflammatory cytokines may play a role due to increased abdominal fat tissue. In 2016, Hamrick, et al., suggested another mechanism that linked osteoporosis, sarcopenia and obesity, which involves age-related fat infiltration into bone and muscle (Hamrick et al., 2016, Kirkland, Tchkonia, Pirtskhalava, Han, & Karagiannides, 2002). For example, in bone, fat accumulates in the marrow cavities of long bones and this condition is directly correlated with low estrogen level and medications that includes glucocorticoids. In muscle, although the mechanism for myosteatosis (i.e. the accumulation of fat in between and/or within the muscle tissue) is not yet known, various evidence suggests that myosteatosis are associated with sedentary lifestyle, disruption in leptin signaling, and glucocorticoid medication. Interestingly, all of these conditions also occur from fat accumulation in the bone marrow. Fat infiltration in bone and skeletal muscle have been found to be directly correlated to low muscle strength, reduced insulin sensitivity, and increased mortality in older adults (Hamrick, McGee-Lawrence, & Frechette, 2016).

Individuals who manifested all three conditions at the same time are expected to experience poorer clinical outcomes compared to individuals with only one

of the conditions. For example, sarcopenia is associated with dyslipidemia and insulin resistance (IR), which could be made worse in the presence of obesity and osteoporosis (Kalinkovich & Livshits, 2016). It has been hypothesized that one of the major mechanisms that cause IR is the accumulation of lipid metabolism byproducts such as diacylglycerol and ceramides in myocytes. In addition, IR was also found to be correlated with impaired lipid oxidation in mitochondria in obesity (Hafizi Abu Bakar et al., 2015; Dela & Helge, 2013) and in aging (Hepple, 2016; Romanello & Sandri, 2016; Carter et al., 2015; Leduc-Gaudet et al., 2015; Konopka & Sreekumaran, 2013; Marzetti et al., 2013). Further, high oxidative stress due to lipotoxicity and impaired mitochondrial function also contribute to the development of IR.

Various studies have shown OSO to be related to functional impairments; low handgrip strength, slow normal and brisk walking speed, and limited balance ability (Ilich, Inglis, Kelly, & McGee, 2015). Although no large scale prevalence study has been done for OSO, Ilich, Inglis, Kelly, and McGee (2015) found that 12% (32 out of 258 postmenopausal women) of its study population had the syndrome. In addition, Hong et al. (2015) found that the prevalence of OSO in Chinese men with vertebral fracture, hip fracture and ankle fracture were high, compared to the non-fractured group, and the prevalence of OSO was also high in women with ankle fracture compared to the non-fractured group, which implied strong correlation of osteoporosis with sarcopenia and obesity. Further, Inglis, et al. (2013) reported that in a post-hoc analysis involving over 500 overweight/obese women across the life span, it was found that 25% of the women had OSO (Inglis, Panton, Ormsbee,

Kelly, & Ilich, 2013). Interestingly, the manifestation of OSO is not exclusive to older adults. A study, which involved over 2,500 young women and men (18–21 years), found that those who were overweight/obese showed poorer body composition (in terms of muscle and bone) and higher pro-inflammatory markers, compared to their normal-weight counterparts (Stefanaki, Peppa, Boschiero, & Chrousos, 2016). Perhaps, early intervention is required to prevent the development of the disorder at a later age.

To date, the culprits for OSO were suggested to be due to poor diet and/or physical inactivity (Villareal et al., 2011). There is a chicken-and-egg situation when it comes to physical inactivity. Physical inactivity could happen due to bone and muscle loss. Once the loss hit below a threshold, limited physical movement will occur, leading to vicious circle of progressive loss of muscle and bone accompanied by obesity due to high energy intake and low expenditure (Figure 2.1) (Ormsbee et al., 2014). Consequently, obesity leads to accumulation of pro-inflammatory cytokines that leads to impairment in musculoskeletal function (Issa & Griffin, 2012; Meyer, Clegg, Prossnitz, & Barton, 2011). Nevertheless, physical inactivity could also be the cause for bone and muscle loss. It is likely that both poor diet (Ormsbee et al., 2014; Kopelman, 2000) and physical inactivity (Li & Heber, 2012) contributes to OSO, which leads to poor mobility, and reduced quality of life.

In addition to poor diet and sedentary lifestyle, another culprit had been suggested to be a contributor to the development of OSO. It has been suggested that Vitamin D may play a role in the development of OSO (Bruyère, Cavalier & Reginstera, 2017). Obese individuals tend to have low 25(OH)D, which is associated with increased parathyroid hormone, which

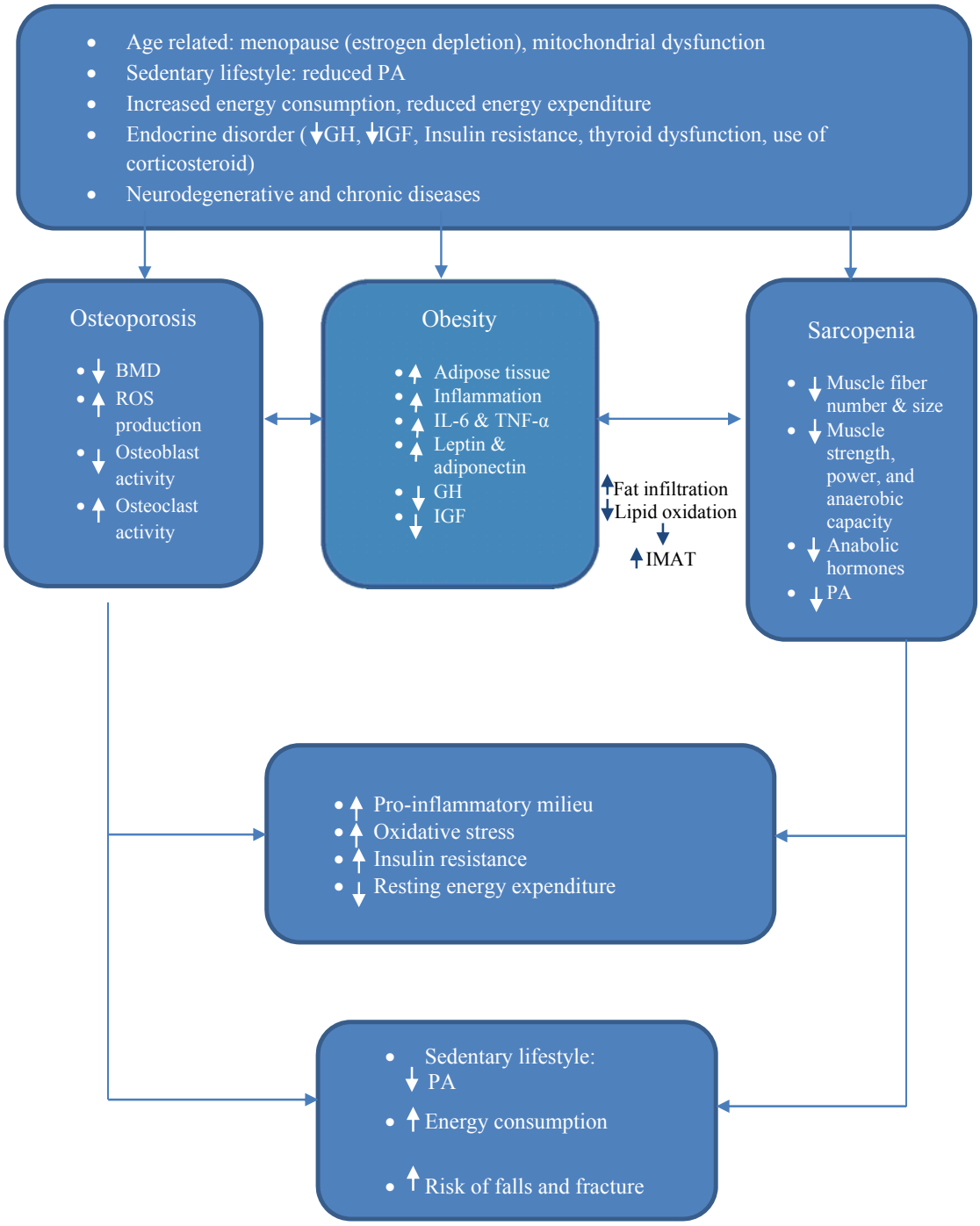
contribute to the catabolism of bones (Earthman, Beckman, Masodkar, & Sibley, 2012; Grethen et al., 2011; Cheng et al., 2010). Hypovitaminosis D has been known to have adverse effects on the absorption of calcium, bone remodeling and consequently, bone density (Sadat-Ali, Al Elq, Al-Turki, Al-Mulhim, & Al Ali, 2011; Lukert, Higgins, & Stoskopf, 1992). In addition, studies have also shown significant correlation between Vitamin D receptor polymorphisms and reduced muscle mass and function in older adults (Roth, Zmuda, Cauley, Shea, & Ferrell, 2004), suggesting the role of Vitamin D in sarcopenia (Scott et al., 2010). Studies on human subjects also showed hypovitaminosis D to be associated with a low muscle strength and function (Visser, Deeg, & Lips, 2003) and an increased risk of falling (Snijder et al., 2006).

Several tools could be used to screen for OSO in the general population based on 2 diagnostic principles; 1) through physical measurements of bone and body composition (i.e. QUS and BIA) and/or 2) via functional performance measures as a proxy for bone and body composition data (i.e. SPPB) (Kelley et al., 2019). Other measures include anthropometric variables, such as BMI, waist circumference, waist-to-hip ratio, waist-to-thigh ratio or skinfold thickness. It is important to note that these methods are characterized by limited accuracy and/or are subject to large intra- and inter-observer variability and may be suitable for screening purposes only (as opposed to diagnostic purposes). Other methods, such as underwater weighing and deuterium oxide (D2O) dilution, have been proposed for body composition measurement, but are expensive or time-consuming, are not patient-friendly and are restricted to research settings (Peppa et al., 2017). Dual-energy X-ray

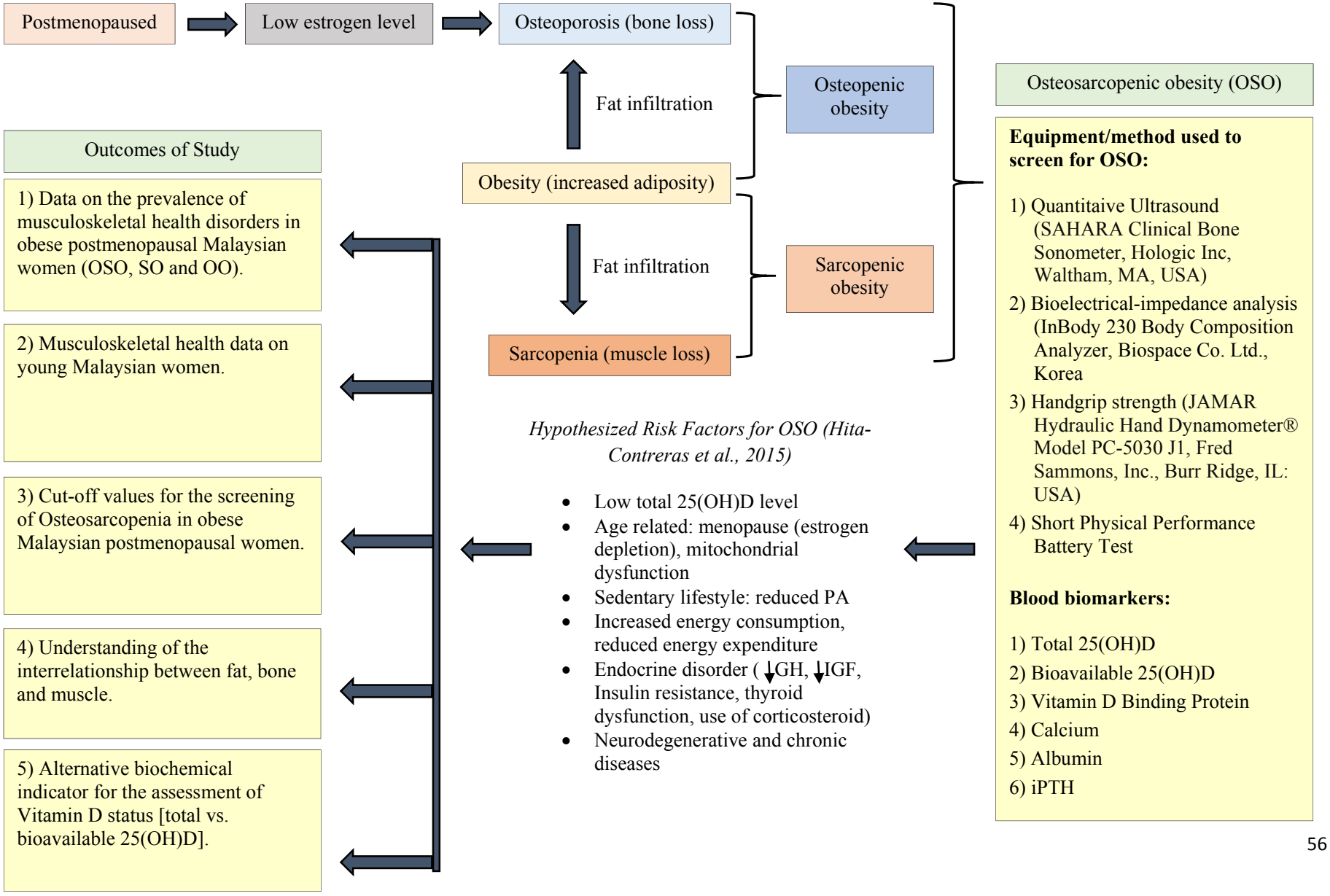
absorptiometry (DXA), computed tomography (CT) and magnetic resonance imaging (MRI) are currently the 'gold standard' methods for the direct measurements of body composition, including bone density. However, their use is limited because of inaccessibility to equipment, relatively high cost and, in the case of computed tomography, exposure to ionizing radiation.

Figure 2.1 shows the pathogenic hypothesis of OSO which described possible interrelationship between bone, muscle and adipose tissue.

Figure 2.1 The pathogenic hypothesis of OSO: hypothesized interrelationship between bone, muscle and adipose tissue. IMAT=intramuscular adipose tissue; GH=growth hormone; IGF=insulin-like growth factor I; ROS=reactive oxygen species, PA=physical activity. Adapted and modified from Zamboni, et al. (2008), Ezzat-Zadeh, et al. (2017), and Roubenoff, (2000) and Hita-Contrerasa, Martínez-Amata, Cruz-Díaza, Pérez-López, 2015)



2.5 Conceptual Framework of the Current Study



CHAPTER 3

METHODOLOGY

3.1 Study design

Cross-sectional

3.1.1 Sample size

Sample size calculator for cross sectional studies with the formula as the following (Charan & Biswas, 2013; Pourhoseingholi, Vahedi & Rahimzadeh, 2013) was used to calculate the sample size:

Sample size formula: $\frac{Z_{1-\alpha/2}^2\, p\,(1-p)}{d^2}$

Sample size: $\frac{1.96^2 \times 0.71(1-0.71)}{0.10^2} = 79$ participants

$Z_{1-\alpha/2}^2$ = Is standard normal variate (at 5% type 1 error ($P<0.05$) it is 1.96). As in majority of studies P values are considered significant below 0.05 hence 1.96 is used in formula.

p = 71% (Expected proportion in population based on previous studies or pilot studies).

d = 0.10 (Precision).

Calculations was based on the following: Level of confidence=95%, prevalence of Vitamin D insufficiency =71% (with levels in the range of 25 – 50 nmol/L). The prevalence was obtained from a previous study by Rahman et al. (2004) based on the level of 25(OH)D in postmenopausal Malay women. This index was used to ensure enough blood samples were collected since 25(OH)D is the main biomarker of interest. From the sample calculation, the sample size of the study yield to 79 participants. With the anticipation of 15% attrition rate, 91 participants were needed for this study (plus matched number for young reference group).

3.1.2 Selection and recruitment of participants

One hundred and forty-one (n=141) postmenopausal Malaysian women (45 to 88 years) and one hundred and eighteen (n=118) young Malaysian women (18 to 32 years) were recruited from various places around the area of Semenyih and Klang Valley, Kuala Lumpur, Malaysia (i.e. Malaysia Menopause Society, senior citizens clubs, residential areas, religious centers and The University of Nottingham Malaysia). Young adult females were recruited as a control group. In addition, their data were required in one of the statistical modelling methods used in Study 2 (two SD below the mean value of a young reference group). Postmenopausal was defined as having no menstrual period, bleeding, or spotting during the 12 consecutive months prior to enrollment. Before enrollment, details about the study covering the objectives, procedures, benefits, risks, and possible discomforts from the study were briefed to interested participants.

3.2 Screening

Apparently healthy and interested participants were screened for eligibility with the following inclusion criteria:

Postmenopausal women

Inclusion criteria:

i) A woman

ii) Citizen of Malaysia (of Malay, Indian or Chinese ethnicity)

iii) Postmenopausal (no menstrual period, bleeding, or spotting 12 consecutive months prior to enrolment)

Young women

Inclusion criteria:

i) A woman

ii) Citizen of Malaysia (of Malay, Indian or Chinese ethnicity)

iii) Aged between 18-32 years

Exclusion criteria:

i) Inability to stand for height, weight and gait speed assessments

ii) Presence of artificial limbs and/or metal implants

iii) Severe cardiac, pulmonary, or musculoskeletal disorders

iv) Severe cognitive impairment or any disability that makes communication impossible

v) Presence of terminal illness

vi) Co-morbidities associated with high risk of falls (e.g. Parkinson's disease) or that may directly affect gait speed.

Eligible participants were explained further on the purpose and procedure of the study and were asked to give a written consent.

3.3 Assessments

3.3.1 Demographic status

Demographic information were collected using a structured and validated questionnaire with items including age, sex, level of education, history of diseases, and self-rated level of physical activity. Questions on menopause were taken from the Menopause Health Questionnaire (The North American Menopause Society, 2005) and questions related to Vitamin D status were taken from Bolek-Berquist et al., 2009.

3.3.2 Anthropometric measurements

3.3.2.1 Height

Height was measured to the nearest 0.1cm using a portable stadiometer (SECA 217, Vogel & Halke GmbH & Co., Germany). Participants were asked to stand with their shoulders, buttocks and heels resting against the stadiometer, toe tips forming a 45° angle, heels touching each other, head held straight and neck in a natural position.

3.3.2.2 Body fat percentage

Body fat percentage was assessed using a segmental bio-electrical impedance analyzer (InBody 230 Body Composition Analyzer, Biospace Co. Ltd., Korea). While on this machine, weight of the participant was automatically generated.

3.3.2.3 Waist circumference

A measuring tape (SECA 203, GmbH & Co. Kg., Hamburg, Germany) was used to measure waist circumference. Waist circumference (cm) was measured at the mid-point between the last rib and the anterior superior iliac spine with subjects standing upright.

3.3.3 Quantitative ultrasound (QUS) bone assessments

Bone density was assessed using calcaneal ultrasound bone densitometer (SAHARA® Clinical Bone Sonometer, Hologic Inc, Waltham, MA, USA). For calibration purposes, bone assessment of the Principal Investigator was assessed before every data collection. The coefficient variation (CV%) was as the following: T-score (Right heel)=4.1%, T-score (Left heel)=6.1%,

BMD (Right heel)=1.9%, BMD (Left heel)=5.0%, BUA (Right heel)=2.2%, BUA (Left heel)=5.6%, SOS (Right heel)=0.2%, SOS (Left heel)=0.3%, QUI (Right heel)=6.3%, QUI (Left heel)=6.1%. The speed of sound (SOS) and broadband ultrasonic attenuation (BUA) were measured in m/s and dB/Mhz, respectively. The system software estimated the quantitative ultrasound index (QUI), bone mineral density (BMD), and T-Scores (the number of SD units that your bone density is above or below the average i.e young adults. In the current study, the database of Hong Kong young females were used). Z-scores were calculated based on the BMD results of the study cohort. Z-score is the number of SD away from the average value of the reference group. This reference group usually consists of people of the same age and gender.

$$\text{T-score} = \frac{\text{Patient's measured BMD} - \text{mean BMD of young normal population}}{\text{SD of BMD of young normal population}}$$

$$\text{Z-score} = \frac{\text{Patient's measured BMD} - \text{mean BMD of age-matched group}}{\text{SD of BMD of age-matched group}}$$

SAHARA® densitometer measures the speed of sound (SOS, in m/s) and broadband ultrasonic attenuation (BUA, in dB/MHz) of an ultrasound beam passed through the calcaneus (heel), and combines these results linearly to obtain the Quantitative Ultrasound Index (QUI), and an estimate of a patient's heel BMD. While the BMD was not directly measured by ultrasound parameters, BUA and SOS results are significantly correlated (R=0.82 - 0.85) with heel BMD results obtained by the standard dual energy X-ray absorptiometry (DXA) technique, as are results for the combined QUI parameter (R=0.85) (Grampp, et al., 1997). Therefore, the heel BMD results by SAHARA® is estimated by a simple linear re-scaling of the QUI parameter into heel BMD units (in g/cm^2). The level of correlation (R=0.85)

between SAHARA® and DXA heel BMD results is similar to that seen between other acceptable methods that uses heel as the site of assessment (Grampp, et al., 1997).

Broadband Ultrasound Attenuation (BUA)

Prior studies using quantitative ultrasound (QUS) found that high frequency sound waves were attenuated easier by bone compared to low frequency sound waves. Ultrasonic sound waves in the frequency range of 0.2 to 0.6 MHz were found to be linearly correlated with the level of attenuation. The slope of the linear regression of these two parameters (attenuation versus sound waves in the frequency range) was defined as broadband ultrasound attenuation (BUA), and is measured in dB/MHz. On the SAHARA® system, the BUA and SOS are measured simultaneously. In order to determine the sound attenuation of the heel alone, without any bias arising from the transducers and/or transducer pads, a comparison measurement must be made through a reference medium. This reference medium was made using the SAHARA® QC Phantom (supplied with the SAHARA® unit) when the unit was calibrated at the factory. The range of BUA observed with SAHARA® in a typical population is approximately 30-130 dB/MHz, with young/healthy subjects having higher BUA results than older or osteoporotic subjects (SAHARA® Clinical Bone Sonometer User's Guide, 1998).

Speed of Sound (SOS)

SOS is defined as the speed of sound that went through the heel. SOS is determined by the width of the heel, and the time it takes between the initial transmission of the sound waves (by one transducer) and the receipt of the

sound waves (by the second transducer). Before each measurement, the SAHARA® system will automatically do a calibration by carrying out the measurement without the heel (i.e., with the two pads touching one another). This way, the time it takes for the soundwaves to travel throught the heel alone was determined and corrected for measurement. The time (t) the ultrasound signal takes to go through the heel alone is the propagation time of the ultrasound going through the heel and the transducer pads minus the propagation time measured with the pads touching and with no heel interposed. The width of the heel (w) was automatically measured by SAHARA® using a micrometer attached to the transducers. The SOS value is then equal to w/t and is measured in meter per second (m/s). The range observed with SAHARA® in a typical population is approximately 1450-1700 m/s, with young/healthy subjects having higher SOS values than older or osteoporotic subjects.

3.3.4 FRAX® (a fracture risk assessment score)

In combination with QUS assessment, a fracture risk assessment tool by The University of Sheffield, in collaboration with WHO (FRAX®) was used to estimate the probability of a fracture in participant. Since currently, there are no reference algorithms for Malaysians available, the ethnic specific algorithms based on Singaporeans was used (e.g. Singapore Chinese, Singapore Malay, and Singapore Indian) and is available free online at https://www.shef.ac.uk/FRAX/tool.aspx?country=36.

3.3.5 Appendicular skeletal muscle mass index (appSMMI)

Body composition was assessed using segmental bio-electrical impedance analyzer (BIA, InBody 230 Body Composition Analyzer, Biospace Co. Ltd., Seoul, Korea). Appendicular skeletal muscle mass was calculated by adding the sum of the muscle masses of the four limbs. Appendicular Skeletal Muscle Mass Index (appSMMI) was defined as the sum of the muscle masses of the four limbs, adjusted for height in squared meters (kg)/height^2. The cut-off criteria for appSMMI, when BIA was used is 5.7 kg/m^2 for women (Chen, et al., 2014), as recommended by the Asian Working Group on Sarcopenia (AWGS). AppSMMI was first suggested by Baumgartner et al. (1998) in the New Mexico Elder Health Survey. This index provided significant associations with physical disability or frailty.

3.3.6 Muscle strength

Muscle strength was assessed by handgrip strength and was measured in each hand using a hand dynamometer (JAMAR Hydraulic Hand Dynamometer® Model PC-5030 J1, Fred Sammons, Inc., Burr Ridge, IL: USA). Grip strength was measured twice for each hand, and the higher of the two values was used in the analysis. Standardised positioning recommended by The American Society of Hand Therapists (ASHT) was used: subject seated, shoulders adducted and neutrally rotated, elbow flexed at 90°, forearm in neutral and wrist between 0 and 30° of dorsiflexion (Fess, 1992).

3.3.7 Functional performance

3.3.7.1 Short physical performance battery (SPPB) test

Functional performance was assessed using modified components of short physical performance battery (SPPB) test. Based on the recommendation by Ilich, Kelly and Inglis (2016), the following tests were conducted under the SPPB: one-leg stance for balance, gait speed for endurance, and sit-to-stand chair test for lower extremity strength. Each test has its own cut-off value (See Table 3.1). The SPPB has internal consistency of 0.76 and has predictive validity for the risk of mortality, nursing home admission, and disability (Guralnik et al., 1994).

3.3.7.1.1 One-Leg Stance

For one-leg stance, the participant was asked to stand on one leg while lifting the other limb, for up to 30 seconds, performed on both the right and left legs. The test stopped when the participant touches any surface or lowers the other limb to the ground or, ultimately, at the end of 30 seconds (Ilich, Kelly, & Inglis, 2016). An average score or cut-off for healthy older adults is 16 seconds (Shin, Liu, Panton, & Ilich, 2014).

3.3.7.1.2 Gait Speed

Gait speed was measured by timing a 6-meter normal walk. The 6-meter course was marked by two cones or pieces of tape measured using a roll-up, self-retracting construction measuring tape. The participant starts at one end of the course, walking at a normal pace and walking past the other end of the course. The timing starts on the command “begin” and stops when one of the

participant's feet is all the way across the 6- meter marker. Participants were allowed to use a cane or any other walking device they normally use when walking. The cut-off value is <0.8 m/s based on the Asian Working Group for Sarcopenia (AWGS) criteria (Chen, et al., 2014).

3.3.7.1.3 Sit-to-Stand Chair Test

At the beginning of this test, the participant was seated in an armless chair, arms crossed over chest, back straight, and feet flat on the floor. The participant was then asked to rise from the chair and sit down again as many times as possible in a 30-second period. The number of consecutive chair sit-to-stand tests completed was recorded, with the last time the participant sat down in the chair being the final count. A "fit" paticipant, with good lower extremity strength, is defined as the person who completes 20 or more sit-to-stands in a 30-second period (Ilich, Kelly, & Inglis, 2016; Shin, Liu, Panton, & Ilich, 2014; Jones, Rikli, & Beam, 1999).

Table 3.1 Assessment and scoring of the functional performance and corresponding cut-off values (modified from Ilich, Kelly & Inglis, 2016 diagnostic criteria).

Functional status	Handgrip strength [f] (<18 kg)	One-leg stance (≤16 sec)	Gait speed [f] (<0.8 m/sec)	STS chair test (≤20 times)	Total score
Severe functional decline	0	0	0	0	0
Major functional decline∗	0	1	0	0	1
Moderate functional decline∗∗	0	0	1	1	2
Minor functional decline∗∗∗	0	1	1	1	3
No functional decline	1	1	1	1	4

The score of "0" is assigned to each test performed at or below the given cut-off and the score of "1" to each test performed above the cut-off value.
∗Any one performance could be scored as "1," if it is above the cut-off for a given functionality.
∗∗Any two performances could be scored as "1," if they are above the cut-off for given functionality.
∗∗∗Any three performances could be scored as "1," if they are above the cut-off for given functionality.
A total score of 0 indicates a state of severe functional decline. A total score of 1 indicates a state of major functional decline. A total score of 2 indicates moderate functional decline. A total score of 3 indicates minor functional decline. A total score of 4 indicates no functional decline.
[f] criteria proposed by the Asian Working Group for Sarcopenia (AWGS), STS=Sit-to-stand

3.3.8 Blood samples (Total 25(OH)D, VDBP, iPTH, Calcium, Albumin)

Blood samples were collected by a trained phlebotomist, processed and stored at -80 °C until measurement. Blood serum were collected using yellow-topped plasma tubes (SST) and plasma was collected using lavender-topped plasma tubes (EDTA as additive). All blood samples were centrifuged at 1,200 × *g* for 15 min, aliquoted and stored at −80°C until measurement. Serum VDBP was measured using ELISA technique [Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA)] that employs the quantitative sandwich enzyme immunoassay using monoclonal antibody. The intra-assay CV was 5.4%. The % recovery was 100.13% (MyAssays.com). Total 25(OH)D concentrations were measured using chemiluminescent microparticle immunoassay CMIA on Siemens® platforms. Serum albumin levels were measured by using BCG Dye bonding on ADVIA® 2400 Clinical Chemistry System, intact parathyroid hormone level was measured using 2-site sandwich microparticle immunoassay on Siemens® ADVIA® Centaur XP immunoassay system and serum calcium level was measured using Arsenazo III Method on ADVIA® 2400 Clinical Chemistry System. Free and bioavailable 25(OH)D were calculated from total 25(OH)D, VDBP, and serum albumin concentrations using Vermuelen method for free testosterone estimation (Kim et al., 2017, Heijboer et al., 2012, Powe et al., 2011; Bikle et al., 1986):

Bioavailable 25(OH)D = *free* 25(OH)D + *albumin bound* 25(OH)D

Free 25(OH)D= $[-b + \sqrt{b^2-4ac}] \div 2a$

Bioavailable 25(OH)D= [Free 25(OH)D] + [DAlb]= (K_{alb} x [Alb] +1) x Free 25(OH)D

$a = K_{VDBP} \times K_{alb} \times [Alb] + K_{VDBP}$

$b = (K_{VDBP} \times [VDBP]) - (K_{VDBP} \times [Total\ 25(OH)D]) + (K_{alb} \times [Alb]) + 1$

$c = -[Total\ 25(OH)D]$

K_{alb} = affinity constant between 25(OH)D and albumin = 6 x 10^5 M^{-1}

K_{VDBP} = affinity constant between 25(OH)D and VDBP = 7 x 10^8 M^{-1}

[Alb] = concentration of albumin

[Total 25(OH)D] = concentration of total 25(OH)D

[VDBP] = concentration of VDBP

[DAlb] = Albumin-bound 25(OH)D= Bioavailable 25(OH)D-Free 25(OH)D

Calculations of all forms of 25(OH)D were done in moles per liter (mol/L), using Vermeulen method as it provides separately for free and bioavailable 25(OH)D. Subsequently, bioavailable 25(OH)D was converted to nanomoles per litre (nmol/L) while free 25(OH)D was expressed as nmol/L and picomoles per litre (pmol/L).

3.4 Statistical analyses

Statistical analyses were performed using the statistical program SPSS (version 24 for Windows; SPSS, Inc., Chicago, IL, USA). The variables were checked for normality (Shapiro–Wilk test) and presented as mean ± standard deviation, unless noted otherwise. The characteristics of the study

participants were presented as mean and standard deviations (SD) or the number of participants and the corresponding proportion. Where possible, analyses were stratified by ethnicity. Frequency and percentages were reported for categorical variables. The chi-square test and/or the Exact Fisher's test (2x2) were used to analyse categorical data. Outliers were detected using 'outlier labeling rule', which is based on multiplying the Interquartile Range (IQR) by a factor of 2.2 to get the higher or lower range of the data (Hoaglin & Iglewicz, 1987). Receiver operating characteristic (ROC) curve was used to define optimal cut-off values based on the point closest to 0,1 corner in the ROC plane, which defines the optimal cut-point as the point minimizing the Euclidean distance between the ROC curve and the (0,1) point (Perkins & Schisterman, 2006). Whenever there is a trade-off between sensitivity and specificity, sensitivity was prioritise over specificity to detect screening test criteria (Habibzadeh, Habibzadeh & Yadollahie, 2016). The sensitivity (true positive) represents the proportion of subjects actually presenting with osteosarcopenia (OS), having been correctly identified as OS. The specificity is the proportion of subjects who do not actually have OS, which were incorrectly identified as OS using the screening equipment (false positive). Area under each respective ROC curve (AUC) closest to 1 (>0.6) is considered as good predictor (high screening power). A comparison of the distributions of various parameters between groups was performed using an analysis of variance (ANOVA). When significant differences were found with ANOVA, the post-hoc Tukey's honestly significant difference (HSD) test was applied to correct for use of multiple comparisons. Pearson's correlation analysis was used to assess the

correlation of each of the characteristics with the target outcomes. Univariate and multivariate linear regression analysis were used to assess the association between target parameters, adjusted for potential confounders. Two-tailed p-value<0.05 was recognized as statistically significant.

3.5 Ethics

This study was reviewed and approved by the Science and Engineering Research Ethics Committee of the University of Nottingham Malaysia [SEREC- NZA051016]. In accordance with the Helsinki Declaration, before entering the study, each subject gave informed written consent.

CHAPTER 4: RESULTS PART I

4.1 Participants' characteristics

This section describes the characteristic, anthropometric and body composition of the participants. Participants with low musle mass (sarcopenic) were categorized based on appendicular skeletal muscle mass index (appSMMI, kg/m^2) and participants with low bone mass (osteopenic/osteoporosis) were categorized based on broadband ultrasonic attenuation (BUA, dB/MHz). All participants were Malaysian women comprised of Malay, Chinese and Indian ethnicity, recruited on volunteer basis from Semenyih and Klang Valley area, Malaysia. Postmenopausal women have stopped menstruation for at least 12 consecutive months prior to enrollment.

4.1.1 Participants' characteristics, anthropometrics and body composition results

One hundred and forty-one postmenopausal women (N=141, age range=45 to 88) and one hundred and eighteen young Malaysian women (N=118, age range=18 to 32) participated in the study and were included in the analysis. The ethnic distribution of the participants were 36.0% Malays (n=51), 30.0% Chinese (n=42) and 34.0% Indians (n=48) for postmenopausal women, and 25.0% Malays (n=30), 48.0% Chinese (n=56) and 27.0% Indians (n=32) for the young women. Categorization based on BMI shows that prevalence of 'Obesity' (BMI≥27.5kg/m^2) was higher in postmenopausal women compared to young women (48.0%, n=68 vs. 14.0%, n=17, respectively). Similar pattern was also seen in 'Overweight' (BMI=23-27.49kg/m^2) category (28.0%, n=40 vs. 19.0%, n=23, respectively). Conversely, the percentage for

'Normal' (BMI=18.5-22.99 kg/m^2) was higher in young women compared to postmenopausal women (49.0%, n=58 vs. 21.0%, n=29, respectively) (Table 4.1 and Table 4.2). Similarly, the percentage for 'Underweight' (BMI<18.5kg/m^2) was also higher in young women than postmenopausal women (18.0%, n=21 vs. 2.8%, n=4, respectively). Alternatively, if categorization was based on waist circumference (WC) and body fat percent (BFP), the proportion for Obese/Overweight reflects the BMI results (WC; postmenopausal women 63.0%, n=87 vs. young women 18.0%, n=19 and BFP; postmenopausal women 86.0%, n=121 vs. young women 48.0%, n=57) (Table 4.1 and Table 4.2).

Additionally, the mean for all three markers for obesity (BMI, WC, BFP) in postmenopausal women were significantly higher than their respective standard cut-off values ($p<0.01$, Table 4.1). Conversely, in young women, significant difference was only found in WC, which was lower than the standard cut-off value (71.9[SD 9.3] cm < 80.0 cm [standard cut-off]), $p<0.001$, Table 4.2).

When looking at muscle mass, appendicular skeletal muscle mass index (appSMMI) for both age groups (postmenopausal women and young women) were found to be significantly higher than the standard cut-off values (postmenopausal women= 6.1[SD 0.9] kg/m^2, young women= 5.9[SD 0.7] kg/m^2, $p<0.001$), indicating good level of muscle mass (Table 4.1 and Table 4.2). Interestingly, there was a higher prevalence of 'low muscle mass' in the younger cohort compared to their older counterparts (young women 40.0% (n=48) vs. postmenopausal women 31.2% (n=44)) (Table 4.1 and Table 4.2). However, due to their young age, the low muscle mass was not necessarily

indicative of 'sarcopenia', but rather inadequate level of muscle mass compared to the standard cut-off (<5.7kg/m^2).

Based on the results for broadband ultrasonic attenuation (BUA, dB/MHz), it was revealed that the majority of participants in both age groups had a healthy level of bone density (≥70.0 dB/MHz). Only 18.0% (n=25) of postmenopausal women and <1.0% of younger women, were categorized as 'osteopenic/osteoporotic' based on the standard cut-off (<54 dB/MHz, Table 4.1 and Table 4.2).

Due to logistical reasons, background information such as education level, smoking habit, alcohol-drinking habit, physical activity level and co-morbities were only collected from postmenopausal women. The current sudy found that majority of the participants were educated at secondary school level or higher (77.0%), non-cigarrete smoking (98.6%), non-alcohol drinking (97.8%) and non-milk drinking (70.4%) individuals. Half of the cohort (50.4%) reported to be 'inactive' during the week while the other half reported to have some amount of physical activity (other than regular type of activity such as household chores) at least 10 minutes per day. Forty-three percent (43.2%) of participants reported to not have or ever have been diagnosed with any type of disorders listed in the questionnaire (Table 4.1).

Table 4.1 Characteristics and body composition measurements of postmenopausal women, *N* = 141 (Malay, n=51; Chinese, n=42; Indian, n=48)

Variables		*N*	Mean (SD)	Minimum-Maximum	Normal Range and Standard Cut-off	P-value	Mean difference	95% CI of difference
Age (years)		141	60.4(7.5)	45.0-88.0	na			
Age at menarche (years)		125	13.3(1.5)	9.0-17.0	na			
Age at menopause [β] (years)		121	50.5(4.1)	36.0-59.0	na			
Years since menopause		121	9.5(7.3)	1.0-35.0	na			
Height (cm)		141	153.1(6.2)	137.5-169.0	na			
Weight (kg)		141	63.4(12.6)	31.9-100.9	na			
BMI (kg/m^2)	All	141	27.1(5.3)	15.4-43.0	23.0	**0.000***	+4.1	3.2 to 4.9
	Normal	29	20.8(1.3)	18.5-22.8	18.5-22.99[ł]			
	Overweight	40	25.2(1.4)	23.1-27.4	23.0-27.49[ł]			
	Obese Type 1 and 2	68	31.4(3.7)	27.5-43.0	≥27.5[ł]			
Waist circ. (cm)	All	139	84.2(12.6)	55.1-121.0	80.0	**0.000***	+4.2	2.1 to 6.3
	Overweight/Obese	87	91.6(9.3)	80.0-121.0	≥80.0[δ]			
Body fat (%)	All	141	40.5(7.8)	20.7-54.0	32.0	**0.000***	+8.5	7.2 to 9.8
	Obese	121	42.8(5.7)	32.3-54.0	≥32.0[γ]			
FFMI (kg/m^2)		141	15.8(1.7)	11.8-21.8				
SMMI (kg/m^2)		141	8.4(1.1)	6.0-12.8				
AppSMMI (kg/m^2)	All	140	6.1(0.9)	4.0-10.7	5.7	**0.000***	+0.43	0.3 to 0.6
	Sarcopenic	44	5.2(0.4)	4.01-5.69	≤5.7[*f*]			
BUA (dB/MHz)	All	139	70.0(16.8)	35.9-122.2	54.0	**0.000***	+15.9	13.1 to 18.7
	Osteopenic	25	47.5(5.3)	35.9-53.8	<54.0[μ]			

N.B: All participants reported no menstrual bleeding or spotting for at least 1 year prior to enrollment, β Only 121 participants remember the exact age they reached menopause, BMI= body mass index, four participants (n=4) were underweight BMI<18.5 kg/m^2, FFMI=fat free mass index, SMMI=skeletal muscle mass index, SD=standard deviation, CI=confidence interval, * analysed using one-sample T-test, (overall mean vs. respective cut-points; BMI=23kg/m^2, WC=80cm, BFP=32%, appendicular SMMI=5.7 kg/m^2, p≤0.01), γ Ilich et al., 2016, *f* AWGS (Asian Working Group for Sarcopenia),δ World Health Organization, 2008, ł Asian BMI cut points by WHO expert consultation, 2004, μ Johansen, Evans & Stone, 1999

Table 4.1 (continued)

Variables	*N Total*			*n*	Percentage (%)
Highest Education Level	140	No Formal Education		10	7.1
		Primary School		22	15.7
		Secondary School		55	39.3
		Certificate/Diploma		29	20.7
		University Degree		19	13.6
		Postgraduate Degree		5	3.6
Cigarette Smoking Status	139	Non-smoker		137	98.6
		Current smoker		2	1.4
Alcohol Drinking	139	Non-drinker		136	97.8
		Current drinker		2	2.2
Milk Drinking	135	Non-drinker		95	70.4
		Drinker	1 serving/day	33	24.4
			2 or more servings/day	7	5.2
Self-rated PA Status	137				
		Inactive		69	50.4
		Active (at least 10mins per day)		68	49.6
Disease(s)/disorder(s)	139				
		None		60	43.2
		Hypertension		53	
		Diabetes Type 2		31	
		Heart problems		11	
		Osteoarthritis		12	
		Rheumatoid Arthritis		7	
		Osteoporosis		8	
		Have had stroke		4	
		Depression/anxiety		6	

Table 4.2 Characteristics and body composition measurements of young women, *N* = 118 (Malay, n=30; Chinese, n=56; Indian, n=32)

Variables		*N*	Mean (SD)	Minimum-Maximum	Normal Range and Standard Cut-off	P-value	Mean difference	95% CI of difference
Age (years)		118	22.1(2.2)	18.0-32.0	na			
Height (cm)		118	159.3(5.5)	142.6-173.0	na			
Weight (kg)		118	57.0(11.6)	39.0-100.8	na			
BMI (kg/m^2)	All	118	22.4(4.5)	16.3-40.3	23.0	0.346	-0.40	-1.3 to 0.4
	Normal	58	20.5(1.4)	18.5-22.8	18.5-22.99[ł]			
	Overweight	23	25.1(1.2)	23.1-27.2	23.0-27.49[ł]			
	Obese Type 1 and 2	17	31.3(3.7)	27.5-40.3	≥27.5[ł]			
Waist circ. (cm)	All	106	71.9(9.3)	59.0-99.5	80.0	**0.000***	-7.70	-9.5 to -5.9
	Overweight/Obese	19	88.1(6.2)	80.0-99.5	≥80.0[δ]			
Body fat (%)	All	118	32.4(7.7)	17.4-53.3	32.0	0.312	+0.73	-0.7 to 2.2
	Obese	57	39.2(5.9)	32.1-53.3	≥32.0[γ]			
FFMI (kg/m^2)		118	14.8(1.5)	10.8-18.8				
SMMI (kg/m^2)		118	8.0(0.9)	5.5-10.3				
AppSMMI (kg/m^2)	All	118	5.9(0.7)	4.1-7.6	5.7	**0.000***	+0.25	0.1 to 0.4
	Low muscle mass	48	5.2(0.3)	4.01-5.69	≤5.7[*f*]			
BUA (dB/MHz)	All	118	86.5(16.3)	52.7-132.2	54.0	**0.000***	+32.5	29.6 to 35.3
	Low bone mass	1	52.7	-	<54.0[μ]			

N.B: BMI= body mass index, twenty participants (n=20) were underweight BMI<18.5 kg/m^2, FFMI=fat free mass index, SMMI=skeletal muscle mass index, SD=standard deviation, CI=confidence interval, * analysed using one-sample T-test, (overall mean vs. respective cut-points; BMI=23kg/m^2, WC=80cm, BFP=32%, appendicular SMMI=5.7kg/m^2, p≤0.01), γ Ilich et al., 2016, *ƒ* AWGS (Asian Working Group for Sarcopenia),δ World Health Organization, 2008, ł Asian BMI cut points by WHO expert consultation, 2004, μ Johansen, Evans & Stone, 1999

Table 4.3 shows the results of fracture risk assessment (FRAX®) scores for overall participants and differences between ethnicities. FRAX® scores, which determines a 10-year probability fracture risk for 'major osteoporotic related fracture' and 'hip fracture', showed that the study cohort had low risk for major osteoporosis-related fracture (mean [SD] 5.2±3.7% vs. standard threshold 20%, $p<0.001$) and hip fracture (1.3±1.6% vs. standard threshold 3%, $p<0.001$) in 10-years time.

Results also showed that the Chinese had significantly higher risk for 'hip fracture' in 10-years time, followed by Indians and Malays (Chinese>Indian>Malay, $p<0.01$, Table 4.3). No differences in ethnicities were found for the risk of 'major osteoporotic fracture'.

Table 4.3a shows the frequency results for the FRAX® components. The study found that all participants have <20% chance of having 'major osteoporosis-related fractures' in 10 years time. Conversely, for 'hip fracture', 14.2% of participants have ≥3% chance of having hip fracture in 10-years time.

Table 4.3 Fracture risk assessment tool (FRAX®) scores (postmenopausal women)

Variables	Ethnicity	N	Mean (SD)	Minimum-Maximum	Standard cut-off	P-value
FRAX® Score (Major osteoporotic fracture) (%)	Overall	113	5.2(3.7)	0.60-18.00	20.0[γ]	**0.000 [δ*]**
	Malay	44	4.8(3.5)	0.60-16.00		0.121 [f]
	Chinese	31	6.3(4.4)	0.90-18.0		
	Indian	38	4.7(3.1)	0.8-11.0		
FRAX® Score (Hip fracture) (%)	Overall	103	1.3(1.6)	0.00-9.3	3.0[γ]	**0.000 [δ*]**
	Malay	44	0.8(1.0)[a]	0.00-4.70		**0.001[f*]**
	Chinese	31	2.1(2.2)[b]	0.10-9.3		
	Indian	38	1.2(1.2)[c]	0.10-4.30		

N.B: *f* Differences between ethnicities were analysed using one-way ANOVA with Tukey's HSD Post-hoc test, *p<0.05, a and b = statistically different from each other, b and c = statistically different from each other, δ = analysed using one-sample T-test (overall mean vs. standard cut-off), γ Kanis et al., 2008

Table 4.3a Fracture probability based on FRAX ® scores (postmenopausal women)

FRAX ® Component	Frequency (n)	Percentage (%)
10-year probability of major osteoporosis-related fracture		
≥20%	0	0
<20%	113	100
10-year probability of hip fracture		
≥3%	16	14.2
<3%	97	85.8

N.B: questions include; previous fractures, parent history of fractures, smoking habits, use of glucocorticoids, presence of rheumatoid arthritis, presence of secondary osteoporosis and daily alcohol intake (Kanis et al., 2008)

Table 4.4 shows differences in characteristics between groups with 'no sarcopenia' and different stages of sarcopenia (pre-sarcopenia, sarcopenia, severe sarcopenia). 'Pre-sarcopenia' was defined as low muscle mass without reduced muscle strength (handgrip strength) or physical performance (gait speed), 'sarcopenia' was defined as low muscle mass, plus low muscle strength or low physical performance, and 'severe sarcopenia' was defined as low muscle mass, plus low muscle strength and low physical performance. The current study found that the overall prevalence of 'sarcopenia' among this community-dwelling, postmenopausal Malaysian women was 29.4% (40/136), among which 6.6% (9/136), had pre-sarcopenia and 5.9% (8/136) had severe sarcopenia (Table 4.4). Figure 4.1 shows the percentage of participants with low muscle mass, low muscle strength (dynapenia) and low gait speed (31.4%, 30.0% and 38.6% respectively).

One-way ANOVA analysis revealed significant differences in age, adiposity (BMI, WC, BFP), muscle mass (FFMI, SMMI, appSMMI), gait speed and bone density (BUA) between 'no sarcopenic' group and the group with 'severe sarcopenia' (Table 4.4). There were no significant differences found in age, years since menopause, adiposity (BMI, WC, BFP), and whole-body muscle mass (FFMI, SMMI) across all groups. Results also showed no significant differences in terms of handgrip strength (HGS) and gait speed between people with 'sarcopenia' and 'severe sarcopenia' (Table 4.4).

Table 4.4 Characteristics of participants across sarcopenia stages

Variables	*N*	No sarcopenia, n=96 Mean (SD)	Pre-sarcopenia, n=9 Mean (SD)	Sarcopenia, n=23 Mean (SD)	Severe sarcopenia, n=8 Mean (SD)	P-value
Age (years)	136	59.1(6.7) [ł]	63.0(9.0)	62.84(7.4)	65.88(11.0)	**0.010***
Years since menopause (years)	115	8.6(6.5)	9.8(6.6)	12.7(8.7)	13.50(11.2)	0.077*
Height (cm)	136	154.1(5.8)[ł]	151.1(6.7)	152.0(6.8)	147.8(6.17)	**0.017***
Weight (kg)	136	68.9(10.5)[δ, β, ł]	50.0(8.2)	53.4(6.4)	47.16(8.48)	**0.000***
Body Mass Index (kg/m^2)	136	29.1(4.8) [δ, β, ł]	21.8(2.8)	23.3(3.6)	21.63(4.22)	**0.000***
WC (cm)	133	88.6(11.3) [δ, β, ł]	73.8(13.0)	75.3(8.7)	72.86(8.54)	**0.000***
Body fat percent (%)	136	42.4(7.2) [δ, β, ł]	34.3(9.1)	37.9(7.4)	35.35(7.9)	**0.000***
FFMI (kg/m^2)	136	16.5(1.3) [δ, β, ł]	14.1(0.7)	14.2(1.0)	13.70(1.14)	**0.000***
SMMI (kg/m^2)	136	8.9(0.8) [δ, β, ł]	7.4(0.4)	7.4(0.5)	7.05(0.6)	**0.000***
AppSMMI (kg/m^2)	136	6.5(0.7) [δ, β, ł]	5.3(0.2) [ł]	5.3(0.4)[ł]	4.8(0.58)	**0.000***
Handgrip strength (kg)	129	20.7(5.0)[ł]	22.6(3.4)[ł]	17.9(4.4)	14.9(1.5)	**0.001***
Gait speed (m/s)	127	0.9(0.3)[ł]	1.1(0.1)[ł]	0.8(0.2)	0.7(0.1)	**0.005***
BUA (dB/MHz)	134	72.2(16.0)[ł]	73.7(16.4)[ł]	65.9(15.9)[ł]	47.9(8.86)	**0.001***

N.B: SD=standard deviation, Pre-sarcopenia= low muscle mass without impact on muscle strength or physical performance, sarcopenia= low muscle mass, plus low muscle strength or low physical performance, severe sarcopenia= low muscle mass, plus low muscle strength and low physical performance, BUA=broadband ultrasonic attenuation, WC=waist circumference, SMMI=skeletal muscle mass index, FFMI=fat-free mass index, AppSMMI=appendicular skeletal muscle mass index, *analysed using one-way ANOVA with Tukey's HSD Post-hoc test, $p<0.05$, β different from sarcopenia $p<0.05$, δ different from pre-sarcopenia, ł different from severe sarcopenia $p<0.05$

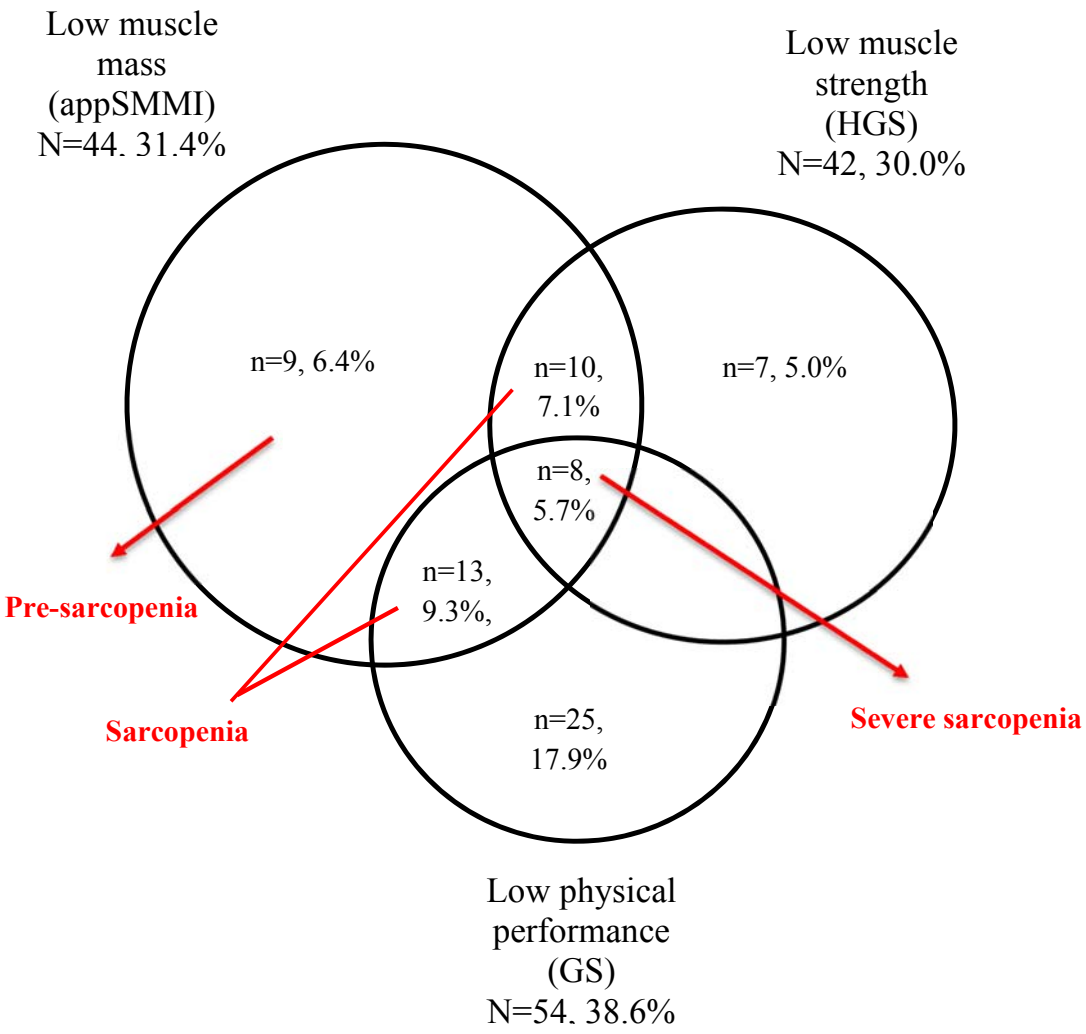


Figure 4.1 Venn diagram showing proportions of participants with low muscle mass, low muscle strength and low physical performance. N.B: AppSMMI=appendicular skeletal muscle mass index, kg/m^2, 4 participants (n=4) had missing values for handgrip strength (HGS) or gait speed (GS) and were not included in the categorization for 'sarcopenia' and 'severe sarcopenia'

4.1.2 Ethnic disparities in characteristics

This sub-section shows the differences in characteristics between Malays (36.0%, n=51), Chinese (30.0%, n=42) and Indians (34.0%, n=48) among postmenopausal women (Table 4.5). The Malays were significantly younger (mean [standard deviation] 57.9[6.4] years) compared to Chinese (61.7[6.4] years) and Indian (61.9[8.9] years, $p<0.05$). Similarly, the number of 'years since menopause' for the Malays was also the lowest compared to Chinese and Indians ($p<0.01$). The Chinese, however, were significantly taller (cm), while their body weight (kg), BMI (kg/m^2), and body fat percentage (BFP, %) were significantly lower compared to Malays and Indians, ($p<0.001$).

For whole-body muscle mass (fat-free muscle mass index [FFMI] and skeletal muscle mass index [SMMI]), the Malays were found to have significantly higher muscle mass compared to the Chinese ($p<0.01$ and $p<0.05$, respectively). No significant difference, however, was found for appendicular skeletal muscle mass index (appSMMI) across all ethnic groups (Malays, Chinese, Indians).

With regards to functional performance, the Chinese had stronger grip strength (HGS) compared to the Indians ($p<0.01$), wherease no differences were found with Malays, nor between Malays and Indians. The Chinese also had stronger lower extremity strength (sit-to-stand test) compared to their Malays and Indians counterparts, ($p<0.001$). Additionally, when looking at endurance (gait speed) and balance, the Indians performed the least compared to Malays and Chinese ($p<0.01$ and $p<0.05$, respectively).

No significant differences were found for any of the bone density indices (BUA, Est. BMD, SOS, QUI, T-score and Z-score) accross the ethnic groups. Differences in blood biomarkers will be discussed in Part III.

Table 4.5 Differences in characteristics according to ethnicity (postmenopausal women)

Variables	*N*	Overall Mean (SD)	Malay, n=51 Mean (SD)	Chinese, n=42 Mean (SD)	Indian, n=48 Mean (SD)	*P-value
Age (years)	141	60.4(7.5)	57.9(6.4)$^{+}$	61.7(6.4)	61.9(8.9)	**0.011**
Age at menarche (years)	125	13.3(1.5)	13.2(1.4)	12.9(1.4)**	13.9(1.5)	**0.008**
Age at menopause (years)	121	50.5(4.1)	50.9(3.5)	50.1(4.9)	50.2(4.1)	0.629
Years since menopause (years)	121	9.5(7.3)	6.8(5.9)$^{+}$	11.7(7.5)	10.8(7.9)	**0.004**
Height (cm)	141	153.1(6.2)	152.1(5.7)$^{\#}$	156.9(5.3)**	150.9(6.0)	**0.000**
Weight (kg)	141	63.4(12.6)	67.1(11.9)$^{\#}$	57.2(10.6)**	64.9(13.1)	**0.000**
BMI (kg/m^2)	141	27.1(5.3)	29.0(5.0)$^{\#}$	23.2(3.8)**	28.4(5.2)	**0.000**
WC (cm)	139	84.2(12.6)	88.7(12.3)$^{\#}$	76.5(10.4)**	86.3(11.7)	**0.000**
BFP (%)	141	40.5(7.8)	43.2(6.9)$^{\#}$	34.2(6.5)**	43.1(6.8)	**0.000**
FFMI (kg/m^2)	141	15.8(1.7)	16.2(1.5)$^{\#}$	15.1(1.5)**	15.9(1.9)	**0.004**
SMMI (kg/m^2)	141	8.4(1.1)	8.7(0.9)$^{\#}$	8.0(0.9)	8.5(1.3)	**0.018**
AppSMMI (kg/m^2)	140	6.1(0.9)	6.3(0.7)	6.0(0.8)	6.1(1.1)	0.275

N.B: SD=standard deviation, BMI= body mass index, WC=waist circumference, BFP=body fat percent, FFMI=fat free mass index, SMMI=skeletal muscle mass index, AppSMMI=appendicular skeletal muscle mass index, *analysed using one-way ANOVA with Tukey's HSD Post-hoc test, $p<0.05$, +=different from Chinese and Indian, **=different from Indian, #=different from Chinese

Table 4.5 Continued

Variables	*N*	Overall Mean (SD)	Malay, n=51 Mean (SD)	Chinese, n=42 Mean (SD)	Indian, n=48 Mean (SD)	*P-value
HG Strength (kg)	134	19.9(5.0)	20.1(4.7)	21.9(4.5)**	18.0(5.1)	**0.001**
Sit-to stand test (times)	127	12.0(3.7)	10.5(3.1)#	14.2(3.4)**	11.7(3.6)	**0.000**
Gait speed (m/s)	128	0.9(0.3)	1.0(0.2)**	1.0(0.3)**	0.8(0.3)	**0.006**
Balance (sec)	125	19.6(10.7)	21.5(9.7)**	21.2(10.4)**	14.9(11.4)	**0.011**
BUA (dB/MHz)	140	69.9(16.8)	67.0(15.7)	73.3(18.3)	70.1(16.4)	0.198
Est. BMD (g/cm^2)	140	0.445(0.122)	0.422(0.115)	0.470(0.133)	0.447(0.116)	0.162
SOS (m/s)	140	1523.7(31.5)	1517.9(30.3)	1530.2(34.3)	1524.6(29.8)	0.174
QUI	140	81.8(20.4)	78.8(18.3)	86.4(21.0)	81.1(21.7)	0.198
T-score (HK Ref.)	140	-1.9(1.1)	-2.1(1.0)	-1.6(1.2)	-1.8(1.0)	0.195
T-score (MY Ref.)	140	-1.4(1.0)	-1.5(0.9)	-1.1(1.1)	-1.3(0.9)	0.161
Z-score	140	-0.0(1.0)	-0.2(0.9)	0.1(1.1)	0.0(0.9)	0.448
Total 25(OH)D (nmol/l)	120	51.68(17.7)	45.5(13.3)#	63.9(16.7)**	48.2(18.2)	**0.000**
Bioavailable 25(OH)D (nmol/l)	116	6.94(3.0)	5.5(1.5)#	9.6(3.3)**	6.2(2.4)	**0.000**
VDBP (ug/mL)	116	224.7(44.8)	242.8(42.4)#, **	205.8(42.5)	217.7(41.5)	**0.000**
Calcium (mmol/L)	120	2.39(0.1)	2.38(0.1)#	2.46(0.09)**	2.34(0.1)	**0.000**

N.B: SD=standard deviation, HG=handgrip, BUA=broadband ultrasonic attenuation, Est. BMD= estimated bone mineral density, SOS=speed of sound, QUI=quantitative ultrasonic index, HK= Hong Kong, MY= Malaysia, 25(OH)D= 25 hydroxyvitamin D, VDBP= Vitamin D Binding Protein, *analysed using one-way ANOVA with Tukey's HSD Post-hoc test, $p<0.05$, **=different from Indian, #=different from Chinese

4.1.3 Functional performance

This sub-section shows the scoring for functional performance of the participants. Table 4.6 and Figure 4.2 shows the differences in functional performance scores for overall participants, and Figure 4.3 shows the differences in functional performance scores according to ethnicity (Malays, Chinese and Indians). Overall scores shows that the majority of participants had 'minor functional decline' (40.6%), followed by moderate (28.1%), major (22.7%), severe (6.3%) and the least percentage for 'no decline' (2.3%).

When divided by ethnicity, the current study found that majority of Malays and Chinese had 'minor functional decline' (53% and 55%, respectively), followed by 'moderate decline', 'major decline', 'severe decline', and finally, 'no decline'. However, the same pattern was not seen for Indians. Majority of Indians were in the 'major decline' category (43.0%), followed by 'moderate decline' (37.1%), 'severe decline' (14.3%), and 'minor decline' (5.7%). There were no participants in the 'No decline' category among the Indians.

Table 4.6 Assessment and scoring of the functional performance (postmenopausal women)

Functional performance/ Ethnicity	Severe functional decline (Total Score=0)		Major functional decline (Total Score=1)		Moderate functional decline (Total Score=2)		Minor functional decline (Total Score=3)		No decline (Total Score=4)		P-value
	n	%	n	%	n	%	n	%	n	%	
Overall (N=128)	**8**	**6.3**	**29**	**22.7**	**36**	**28.1**	**52**	**40.6**	**3**	**2.3**	**0.000**[f]
Malay (N=51)	2	3.9	9	17.6	12	23.5	27	52.9	1	2.0	
Chinese (N=42)	1	2.4	5	11.9	11	26.2	23	54.8	2	4.8	
Indian (N=35)	5	14.3	15	42.9	13	37.1	2	5.7	0	0.0	

N.B:*f* analysed using Pearson Chi-square test (ethnicities * functional performance categories), p<0.05

Table 4.6a Scoring reference

Functional status	Handgrip strength[f] (<18 kg)	One-leg stance[l] (≤16 sec)	Gait speed[f] (<0.8 m/sec)	Sit-to-stand chair test[l] (≤20 times)	Total score
Severe functional decline	0	0	0	0	0
Major functional decline*	0	1	0	0	1
Moderate functional decline**	0	0	1	1	2
Minor functional decline***	0	1	1	1	3
No functional decline	1	1	1	1	4

The score of "0" is assigned to each test performed at or below the given cut-off and the score of "1" to each test performed above the cut-off value.
*Any one performance could be scored as "1," if it is above the cut-off for a given functionality.
**Any two performances could be scored as "1," if they are above the cut-off for given functionality.
***Any three performances could be scored as "1," if they are above the cut-off for given functionality.
A total score of 0 indicates a state of severe functional decline. A total score of 1 indicates a state of major functional decline. A total score of 2 indicates moderate functional decline. A total score of 3 indicates minor functional decline. A total score of 4 indicates no functional decline.
[f] criteria proposed by the Asian Working Group for Sarcopenia (AWGS), [l] Ilich, Kelly & Inglis, 2016

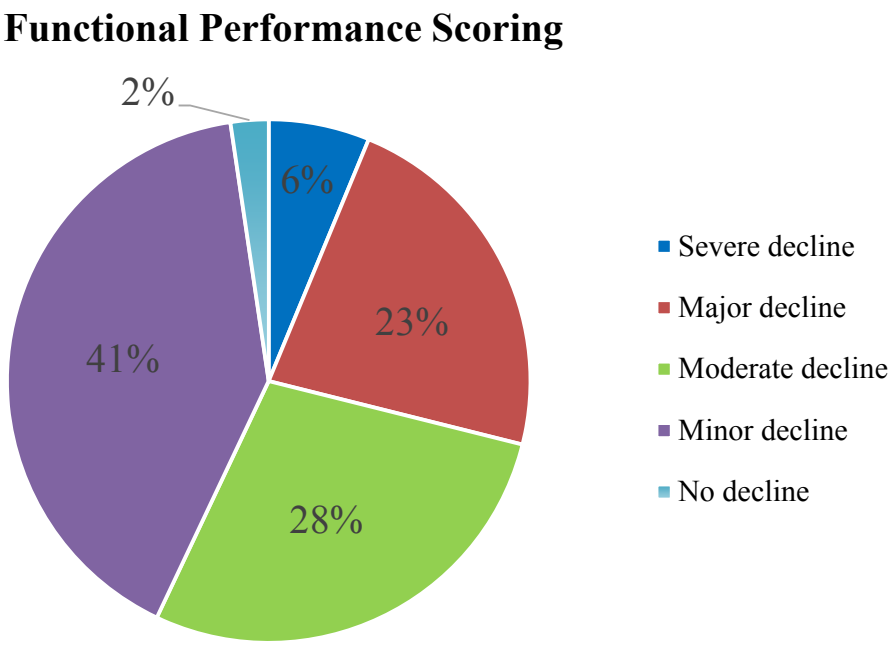


Figure 4.2 Functional performance scoring of participants; *proportions of participants with various degrees of functional decline* (overall, N=128)

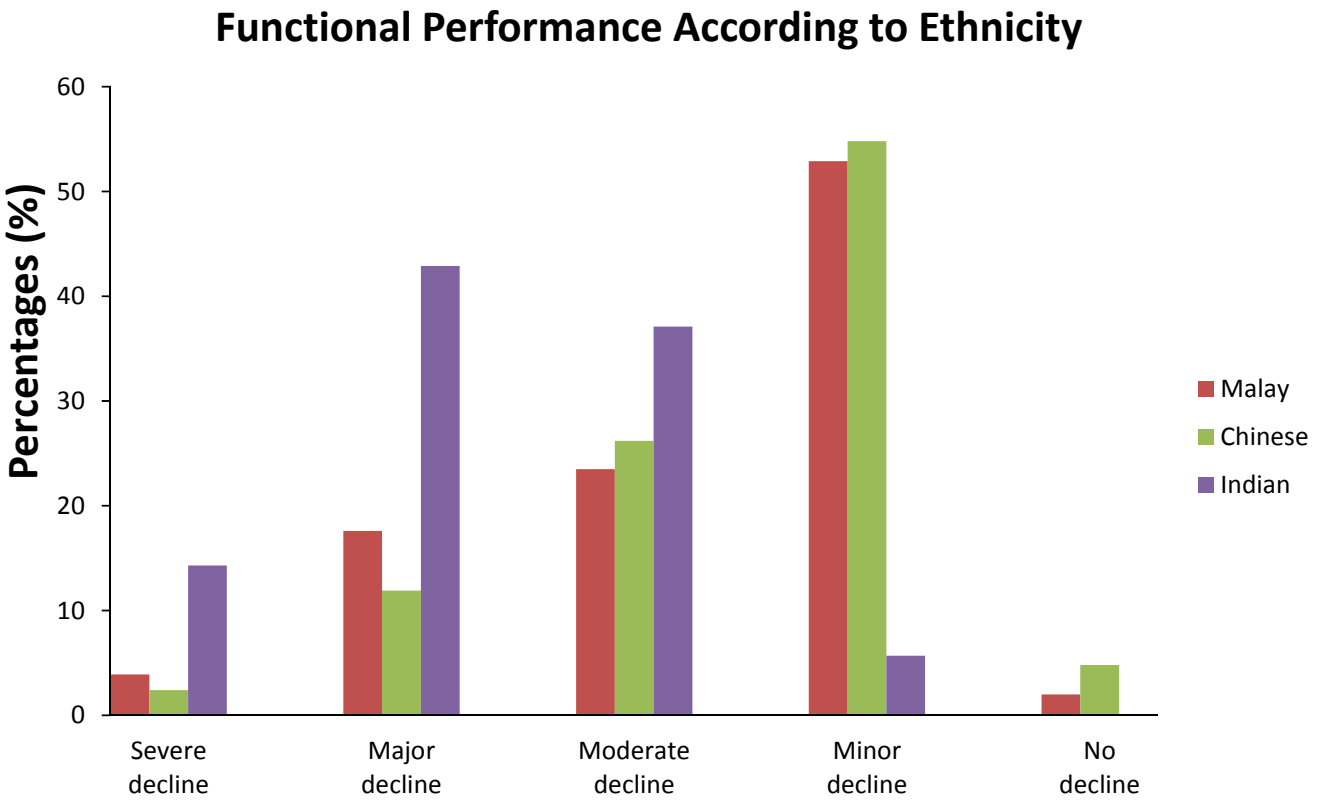


Figure 4.3 Functional performance of participants (by ethnicity)

4.1.4 Interrelationship between fat, bone and muscle indices

This sub-section describes the interrelationship between fat, bone and muscle indices. Correlation between various indices for fat, bone, and muscle are shown on Table 4.7. Pearson's analysis revealed strong and significant positive correlations between fat (BMI, WC, BFP) and muscle mass (FFMI, SMMI, appSMMI, $p<0.01$), while no correlation was found between fat and bone density (BUA). Additionally, lower extremity strength (sit-to-stand test) was found to be negatively correlated with all fat indices (BMI, WC, BFP, $r=-0.196$, $r=-0.299$, $r=-0.198$, respectively, $p<0.05$), whereas balance was found to be negatively correlated only with body fat percent (BFP), ($r=-0.211$, $p<0.05$).

Handgrip strength (HGS) showed significant positive correlations with each index of muscle mass (FFMI, SMMI, appSMMI), ($r=0.237$, $r=0.268$, $r=0.341$**,** respectively, $p<0.01$). Among the indices of muscle mass, only appSMMI showed significant and positive correlation with BUA ($r=0.192$, $p<0.05$) and endurance (gait speed, $r=0.248$, $p<0.01$).

Table 4.7 Pearson's correlation coefficient between fat, bone, muscle, and physical performance (postmenopausal women)

Parameters	BMI, (kg/m^2)	BFP (%)	WC (cm)	FFMI (kg/m^2)	SMMI (kg/m^2)	AppSMMI (kg/m^2)	BUA (dB/MHz)	HG Strength	STS	GS (m/s)	BLN (sec)
BMI, (kg/m^2)	1	**0.845****	**0.831****	**0.788****	**0.753****	**0.685****	0.087	0.044	**-0.196***	0.024	-0.148
BFP (%)	**0.845****	1	**0.725****	**0.370****	**0.325****	**0.359****	0.043	-0.141	**-0.198***	-0.046	**-0.211***
WC (cm)	**0.831****	**0.725****	1	**0.635****	**0.599****	**0.563****	0.038	0.034	**-0.299****	0.061	-0.128
FFMI (kg/m^2)	**0.788****	**0.370****	**0.635****	1	**0.992****	**0.864****	0.129	**0.237****	-0.084	0.140	0.006
SMMI (kg/m^2)	**0.753****	**0.325****	**0.599****	**0.992****	1	**0.907****	0.151	**0.268****	-0.049	0.161	0.037
AppSMMI (kg/m^2)	**0.685****	**0.359****	**0.563****	**0.864****	**0.907****	1	**0.192***	**0.341****	0.011	**0.248****	0.116
BUA (dB/MHz)	0.087	0.041	0.038	0.129	0.151	**0.192***	1	**0.192***	0.143	0.050	0.156
HG Strength (kg)	0.044	-0.141	0.034	**0.237****	**0.268****	**0.341****	**0.192***	1	**0.285****	**0.243****	**0.261****
STS	**-0.196***	**-0.198***	**-0.299****	-0.084	-0.049	0.011	0.143	**0.285****	1	**0.350****	**0.212***
GS (m/s)	0.024	-0.046	0.061	0.140	0.161	**0.248****	0.050	**0.243****	**0.350****	1	**0.296****
BLN (sec)	-0.148	**-0.211***	-0.128	0.006	0.037	0.116	0.156	**0.261****	**0.212***	**0.296****	1

N.B: BMI=body mass index, BFP=body fat percent, WC=waist circumference, FFMI=fat free mass index, SMMI=skeletal muscle mass index, appSMMI=appendicular skeletal muscle mass index, BUA=broadband ultrasonic attenuation, HG=handgrip, STS= sit-to-stand, GS=gait speed, BLN=balance, **p-value < 0.01, * p-value < 0.05.

4.1.5 The impact of age and 'years since menopause' on body composition and physical performance

The impact of age and years since menopause on body composition and physical performance are shown on Table 4.8. Pearson's correlation analysis shows that BFP, SMMI, appSMMI, endurance (gait speed) and balance, were negatively correlated with age ($r=-0.204$, $r=-0.169$, $r=-0.220$, $r=-0.411$, $r=-0.425$, respectively, $p<0.05$). Age was not correlated with FFMI, BUA, HGS, and lower extremity strength (sit-to-stand test).

Years since menopause was negatively correlated only with BFP, endurance (gait speed) and balance, ($r=-0.263$, $r=-0.357$, $r=-0.490$, respectively, $p<0.05$).

Table 4.8 Pearson's correlation coefficient between age, years since menopause, body composition and physical performance (postmenopausal women)

Parameters	Age (years)	YSM	BFP (%)	FFMI (kg/m^2)	SMMI (kg/m^2)	AppSMMI (kg/m^2)	BUA (dB/MHz)	HGS (kg)	STS	GS (m/s)	BLN (sec)
Age (years)	1	**0.846****	**-0.204***	-0.153	**-0.169***	**-0.220****	-0.148	-0.159	-0.143	**-0.411****	**-0.425****
YSM	**0.846****	1	**-0.263***	-0.077	-0.079	-0.120	-0.122	-0.061	-0.139	**-0.357****	**-0.490****

N.B: YSM= years since menopause, BFP=body fat percent, FFMI=fat free mass index, SMMI=skeletal muscle mass index, appSMMI=appendicular skeletal muscle mass index, BUA=broadband ultrasonic attenuation, HGS= handgrip strength, STS= sit-to-stand, GS=gait speed, BLN=balance, **p-value < 0.01, *p-value < 0.05.

4.1.6 The impact of body fat and muscle mass on bone density

To examine the combined contribution of independent and controlled variables to bone density (BUA), hierarchical regression analysis was performed. For each model, the dependent variable was BUA, and independent variables were indices for fat (BMI, WC and BFP) and muscle mass (appSMMI, SMMI).

Model 1 depicts the contribution of adiposity indices (BMI, WC, and BFP) to BUA while controlling for age, years since menopause and appendicular skeletal muscle mass index (appSMMI). The hierarchical regression analysis for Model 1 shows that there was no direct correlation between adiposity indices and variations in the BUA (Table 4.9a).

Table 4.9a Hierarchical Linear Regression Model For Bone Density (Broadband Ultrasonic Attenuation): Model 1 (Adiposity indices)

Variable	Step 1. Control Variables					Step 2. Adiposity indices				
	B	*SE*	*β*	*t*	*P*	*B*	*SE*	*β*	*t*	*P*
Age (Years)	-0.207	0.296	-0.094	-0.697	0.487	-0.174	0.300	-0.079	-0.579	0.563
Years since menopause	-0.052	0.324	-0.021	-0.162	0.871	-0.098	0.331	-0.040	-0.297	0.767
AppSMMI(kg/m^2)	3.168	1.637	0.166	1.935	0.055	5.390	2.731	0.282	1.974	0.050
BMI (kg/m^2)						-0.471	0.803	-0.151	-0.586	0.559
WC (cm)						-0.122	0.185	-0.091	-0.659	0.511
BFP (%)						0.226	0.415	0.106	0.544	0.587
R^2			0.047					0.057		
F (3,138)			2.282					0.472		

NB: There are no direct correlations between adiposity indices and bone density

Model 2 depicts the contribution of appendicular skeletal muscle mass index (appSMMI) to BUA while controlling for age, years since menopause and adiposity indices (BMI, WC, and BFP). The hierarchical regression analysis for Model 2 shows that appSMMI accounted for 6.2% of the variance and a significant predictor for BUA ($p<0.05$), even after controlling for adiposity indices (Table 4.9b).

Table 4.9b Hierarchical Linear Regression Model For Bone Density (Broadband Ultrasonic Attenuation): Model 2 (AppSMMI)

Variable	Step 1. Control Variables					Step 2. Muscle index				
	B	*SE*	*β*	*t*	*P*	*B*	*SE*	*β*	*t*	*P*
Age (Years)	-0.272	0.300	-0.123	-0.905	0.367	-0.172	0.300	-0.078	-0.574	0.567
Years since menopause	-0.037	0.335	-0.015	-0.110	0.913	-0.096	0.332	-0.039	-0.289	0.773
BMI (kg/m^2)	0.629	0.586	0.201	1.073	0.285	-0.650	0.834	-0.208	-0.779	0.437
WC (cm)	-0.085	0.187	-0.063	-0.451	0.653	-0.146	0.187	-0.110	-0.780	0.437
BFP (%)	-0.297	0.827	-0.139	-0.359	0.720	-0.346	0.816	-0.163	-0.424	0.672
Trunk Fat (%)	0.057	0.831	0.026	0.069	0.945	0.714	0.877	0.323	0.815	0.417
AppSMMI(kg/m^2)						6.226	2.920	0.325	2.132	**0.035***
R^2			0.030					0.062		
$F_{(6,135)}$			0.695					4.546*		

*NB: There is a direct correlation between appendicular skeletal muscle mass index (appSMMI) and bone density

Similarly, when tested with another muscle index (skeletal muscle mass index, SMMI) in Model 3, the hierarchical regression analysis shows that SMMI accounted for 6.7% of the variance and a significant predictor for BUA ($p<0.05$), even after controlling for adiposity indices (Table 4.9c).

It should be noted that in all of the regression models none of the control variables accounted for a significant amount of variance. There were also no signs of multicollinearity (tolerance values of 0.60 or higher) in significant as well as non-significant coefficients.

Table 4.9c Hierarchical Linear Regression Model For Bone Density (Broadband Ultrasonic Attenuation): Model 3 (SMMI)

Variable	Step 1. Control Variables					Step 2. Muscle index				
	B	*SE*	*β*	*t*	*P*	*B*	*SE*	*β*	*t*	*P*
Age (Years)	-0.272	0.300	-0.123	-0.905	0.367	-0.150	0.300	-0.068	-0.499	0.618
Years since menopause	-0.037	0.335	-0.015	-0.110	0.913	-0.133	0.332	-0.054	-0.401	0.689
BMI (kg/m^2)	0.629	0.586	0.201	1.073	0.285	-3.270	1.782	-1.046	-1.835	0.069
WC (cm)	-0.085	0.187	-0.063	-0.451	0.653	-0.131	0.186	-0.098	-0.707	0.481
BFP (%)	-0.297	0.827	-0.139	-0.359	0.720	2.628	1.504	1.234	1.748	0.083
Trunk Fat (%)	0.057	0.831	0.026	0.069	0.945	-1.176	0.977	-0.533	-1.204	0.231
SMMI(kg/m^2)						12.059	5.215	0.775	2.312	**0.022***
R^2			0.030				0.067			
$F_{(6,135)}$			0.695				5.348*			

*NB: There is a direct correlation between skeletal muscle mass index (SMMI) and bone density

CHAPTER 5: DISCUSSION PART I

5.1 Obesity, Osteoporosis and Sarcopenia: Insights into Fat, Bone and Muscle Relationships in Postmenopausal Women

Due to advancement in the healthcare system, the ageing population around the world is increasing. However, better technology and better transportation system come at a cost. In recent years, the advancement in technology have reduced people to rely more heavily on digital media devices to perform daily tasks. Significant use of such devices is closely related with reduced daily activities. Fast foods with low nutrients are also consumed daily due to fast deliveries and sedentary lifestyle is a norm. Consequently, high adiposity, with concurrent incidence of age-related low bone mass and low muscle mass are becoming a concern among people of advanced age. These disorders, which shared several interrelated mechanisms and metabolic pathways, if present at the same time in an individual, form a syndrome known as osteosarcopenic obesity (OSO). In Malaysia, published reports on the prevalence of OSO are still scarce among the population. To date, there are still no consensus on the interactions between fat, bone, and muscle, particularly on the definition of disorders, method of measurements, and treatments. These issues have become even more problematic among populations with extreme variances in body compositions such as people with high fat distribution and/or low muscle mass (Ong et al., 2014; Liu et al., 2014; Reina Armamento-Villareal et al., 2014). Therefore, before delving further on the identification and method of measurements for OSO, this study will first discuss the prevalence and predictors associated with obesity, osteoporosis (low bone mass) and sarcopenia (low muscle mass) among

young and postmenopausal women in Malaysia. The recruitment of young and postmenopausal women in this study allows for comparisons between the two age groups. Usually, osteoporosis study only focuses on postmenopausal women. However, it is important to include young adult as a reference group as peak bone mass is achieved during this phase of life (20-35 years-old) (Yahya et al., 2018). Findings from this study will show how bone density varies between these two age groups. In addition, the study will also show the current status of musculoskeletal health among Malaysian women. Studies have shown that South Asians tend to have lower muscle mass relative to stature and total body mass compared to Europeans (Rush, Freitas & Plank, 2009). The low muscle mass was theorized to be one of the reasons why they tend to develop non-communicable diseases (NCDs) such as Type-2 diabetes (T2D) at a lower BMI compared to other populations (Pomeroy et al., 2019). While it is known that obesity and lifestyle factors such as diet and sedentary lifestyle play important roles in the development of NCD, studies have shown that variations between populations is also related to the syndrome. For example, Tilin et al. (2015) found that South Asians living in London, UK, had twice the risk of developing T2D compared to those of European ancestry, with onset typically occurs 5 years earlier and at a lower body mass index (by 5 kg/m^2) (Tillin et al., 2015). It was theorized that the reason for this increased susceptibility is due to low lean mass in this population (Pomeroy et al., 2019). Therefore, in this study, we investigated age as well as ethnic disparities among Malaysian women and whether body composition and fat distribution may play a hand in the differences in bone density among Malaysian multiethnic population.

5.1.1 Prevalence of obesity in study cohort

Due to logistical reasons, background information such as education level, smoking habit, alcohol-drinking habit, physical activity level and co-morbidities were only collected from postmenopausal women. Postmenopausal women in this study were recruited from the area of Klang Valley and Semenyih, Malaysia and included in the analysis if their menstrual period have stopped for 12 consecutive months prior to enrollment. The young women in this study (aged 18-32 years) were recruited from The University of Nottingham Malaysia during term time (October to April 2017/2018) and comprised of undergraduate students and staff members.

Postmenopausal women in this study were educated at secondary school level or higher, non-cigarette smoking, non-alcohol drinking and non-milk drinking individuals (Table 4.1). Interestingly, half of the cohort (50.4%) reported to be 'inactive' during the week while another half reported to have some physical activity (other than regular type of activity such as household chores) at least 10 minutes per day. This finding is well reflected in their obesity and overweight statuses. Categorization based on BMI shows that the prevalence of obesity and overweight in postmenopausal women were 48.0% and 28.0%, respectively. Although the physical activity level of the young adult group is not known, the body composition revealed lower prevalence of obesity and overweight in this age group (14.0% and 19.0%, respectively, Table 4.2). Obesity rate tend to be higher among older population compared to their younger counterparts, implying obesity increases with age. Even when different adiposity indicator were used to define obesity status (waist circumference [WC] and body fat percent [BFP]), the trend persists.

Abdominal obesity was also lower in younger women compared to their older counterparts (Table 4.2). Conversely, there was a higher percentage of participants in the normal weight category in younger women compared to their older counterparts (49.0% [Table 4.2] vs. 21.0% [Table 4.1]). Although the National Health and Morbidity Survey Malaysia (NHMS) in 2011 and 2015 had reported reduced increment rate in obesity among Malaysian adults, prevalence of obesity in Malaysia is still among the highest in South East Asia. Malaysian adults have been reported to have the highest prevalence of overweight (44.2%) and obesity (14.0%), followed by Thailand (overweight: 32.2%; obesity: 8.8%) and Singapore (overweight: 30.2%; obesity: 7.1%) (WHO, 2014). Over the past decade, Malaysian has seen such growth in economic, industrialization, and globalization. Urbanization has also led to reduced daily activity and increased in sedentary lifestyle. On diet front, increased in fast food consumption had also been attributed to the increasing rate of overweight and obesity in Malaysia. A Malaysian study in 2012 using BMI status reported that among 125 postmenopausal women aged 50 to 65 years old, 80.0% were overweight and obese (Hasnah, Amin & Suzana, 2012), consistent with the finding of the current study (76.0%). This means that in more than 5 years, the rate remains the same. In the case of younger population, although the prevalence of overweight and obesity is lower than the older population, findings from the current study showed an increment in the prevalence compared to previous studies. Previous studies (2010-2011) on Malaysian undergraduates found that prevalence of overweight among female undergraduate students ranged from 6.11% (Huda & Ahmad, 2010) to 8.5% (Gan et al., 2011) and the

prevalence of obesity ranged from 0.56% (Huda & Ahmad, 2010) to 3.8% (Gan et al., 2011). These percentages were lower than the findings from the current study (19.0% of overweight and 14.0% of obesity). Interestingly, a Malaysian study published in 2019 also reported similar, if not higher proportion for overweight and obesity prevalence among Malaysian undergraduate students (23% of overweight and 17.6% of obesity, Radzi et al., 2019). Compared to other countries, the general prevalence of overweight and obesity among Malaysian university students is higher (Jiang et al., 2018; García-Hermoso et al., 2018; Du, Zhu & Jiao, 2017; Peltzer et al., 2014). This is presumably due to stress induced by adapting to a new lifestyle and environment at the university. Studies have found that weight gain is normal among university students during the course of their education (Pliner & Saunders, 2008, Economos, Hildebrandt & Hyatt, 2008). Stress was found to be a factor that was significantly and positively correlated with obesity and overweight in university students (Kim, 2016; Odlaug et al., 2015; Gupta, Ray, & Saha, 2009). Stress is characterised as a negative emotional experience normally occur in parallel with physiological, behavioural and sometimes, biochemical changes. Nevertheless, the increment in obesity rate among young Malaysian women shows that some action should be taken to prevent an escalation of incidence.

Apart from general obesity, information on the area of the body where the fat tend to accumulates is also important, as some diseases tend to be significantly correlated to fat distribution. For instance, independently of the BMI, a high waist circumference was found to be predictive of an increased risk of dyslipidemia, hypertension, cardiovascular diseases, and type 2

diabetes mellitus (Jean-Pierre Després, 2012). World Health Organization (WHO) guidelines state that alternative measures that reflect abdominal obesity such as WC had been found to be superior to BMI (World Health Organization, 2008). A study on Chinese population found that while BMI and WC were robust indices for obesity, WC was the better measurement of obesity (Yang et al., 2006). Similarly, according to Mamtani and Kulkani (2005), WC was also a more accurate predictor of type 2 diabetes mellitus than other indices such as BMI and WHR (Mamtani & Kulkarni, 2005*)*. In the current study, waist circumference (WC) data allowed us to see how fat distribution differ between women from the two age groups. According to World Health Organization/ International Association for the Study of Obesity/ International Obesity Task Force, WHO/ IASO/ IOTF (2000) (WHO/ IASO/ IOTF, 2000) and International Diabetes Federation, IDF (2006) (IDF, 2006), a WC of ≥ 80cm for female in the context of Asian population was classified as abdominal obesity. It is well known that abdominal obesity increases with age (Kowalkowska et al., 2016; Krzysztoszek, Wierzejska, & Zielińska, 2015; Wang et al., 2012). The present study found that older women had significantly larger waist circumference than the standard cut-off (Table 4.1), whereas younger women had significantly lower waist circumference than the standard cut-off (Table 4.2). Other studies have also found the prevalence of global and abdominal obesity was higher among older participants (López-Sobaler et al., 2016). In the Malaysian general population, the National Health and Morbidity Surveys (NHMS) in 2015 reported an increase in abdominal obesity. It was reported that abdominal obesity increased from 17.4% in 2006, to 20.9% in

2011, to 20.0% in 2014, and to 23.0% in 2015 (NHMS, 2015). However, the incidence of abdominal obesity is still lower in younger population compared to older adults. Previous studies have found that the prevalence of abdominal obesity ranged from 11.4% (Gan et al., 2011) to 18.1% (Chan et al., 2014) among Malaysian undergraduate students, although there was a higher prevalence among females (21.4%) than males (0%) (Haemamalar, Zalilah & Neng Azhanie, 2010).

5.1.2 Prevalence of osteopenic/osteoporotic conditions in study cohort

Osteoporosis is an age-related and asymptomatic condition characterized by low bone density and micro-architectural bone tissue deterioration, which typically result in bone fragility and susceptibility to fracture (WHO, 2000). Osteoporosis is not accompanied by dramatic clinical symptoms and typically remain undiagnosed until there is a fracture or a screening test is conducted. People with osteoporosis mainly experience fractures occurring at the spine, hip, and/or wrist. Osteoporotic fractures tend to cause substantial healthcare burden on individuals, families, and society. In Malaysia, the prevalence of osteoporosis is not well documented. A frequently cited study on the prevalence of osteoporosis in Malaysia was by Lee and Khir (2007) who reported that the incidence of hip fracture in Malaysian elderly aged 50 years and above was 90 per 100,000 individuals, with Chinese having the highest fracture rates (63%), followed by Malays (20%) and Indians (13%) (Lee and Khir, 2007). Available reports showed that compared to other Asian countries, the prevalence of osteoporosis in Malaysia was the highest (24.1%) (Lim et al., 2005) followed by the Philippines (19.8%) (Miura, Saavedra & Yamamoto, 2008), China (16.1%) (Li et al., 2002), Thailand (12.6%)

(Pongchaiyakul et al., 2008) and Taiwan (10.1%) (Yang et al., 2004). Few reports are available on the prevalence of osteoporosis is presumably due to low feasibility of assessments or screening of bone density at the population level. Clinically, the diagnosis of osteoporosis is determined using dual-energy X-ray absorptiometry (DXA) based on the T-score of the bone mineral density (BMD) assessment (Schuit et al., 2004). However, due to the high cost, specific requirement of facility, and invasive nature of the procedure, accessibility to DXA is limited especially in the developing countries (Mithal et al., 2014).

In the current study, proxy for bone density is determined by broadband ultrasonic attenuation (BUA). The current study found that the younger age group had higher bone density level (BUA, dB/MHz) compared to their older counterparts [mean (standard deviation): 86.5(16.3) dB/MHz, Table 4.2 vs. 70.0(16.8) dB/MHz, Table 4.1]. Nevertheless, majority of the participants in both age groups (young and postmenopausal women) had BUA higher than 54 dB/MHz (Johansen, Evans and Stone, 1999), which indicates healthy bone density level. Only 18.0% of postmenopausal women had BUA less than 54 dB/MHz (categorized as osteopenic/osteoporotic, Johansen, Evans & Stone, 1999), and only one person in the younger age group was categorized as such. Osteopenic/osteoporotic conditions was identified based on the BUA due to the equipment used to determine bone density in this study. Quantitative Ultrasound Scan (QUS) is a portable equipment that offers an alternative (or used alongside) to dual energy X-ray absorptiometry (DXA) for screening and assessing the status of peripheral skeleton. According to Johansen, Evans and Stone (1999) who had compared the quivalency of DXA and heel QUS

involving 73 women aged 29–86 (mean 65) years, subjects with BUA below the 54 dB/MHz threshold were found to have low femoral neck density (assessed by DXA). Although DXA is the gold standard method recommended by the World Health Organization in the diagnosis of osteoporosis, it is not feasible to be used in the screening of osteoporosis at the population level. Quantitative ultrasound (QUS) is an adequate alternative to DXA and offers wider accessibility to the public because its portability, user-friendly, cost-effective and does not emit ionizing radiation (Laugier, 2004). QUS can be used on population with various ages (children [Baroncelli, 2008] and infants [Mimura et al., 2008]) and gender (Miura, Saavedra & Yamamoto, 2008, Mészáros et al., 2007). Broadband ultrasonic attenuation (BUA) is one of the two parameters generated by QUS. The other is the speed of sound (SOS, m/s). BUA, which is measured in decibel per megahertz (dB/MHz), refers to the slope between the attenuation of sound signals and its frequency. When sound waves travel through the bone, attenuation occurs when the energy is absorbed by the soft tissue and bone. The speed of sound (which is measured by meter per second, m/s), refers to the time it takes for the sound waves to travel, divided by the length of the body part studied. Studies have shown that QUS indices are significantly correlated with BMD values at various body sites. For example, a study on postmenopausal women found that all three QUS indices, BUA, SOS and SI were significantly correlated to BMD at lumbar spine and femur (Dane et al., 2008). A longitudinal study involving 80 Swedish women aged 53-73 years found that BMD assessed by DXA at multiple skeletal sites were found to be significantly correlated to BUA and SOS at baseline and after 7

years. Similarly, any subsequent changes detected by DXA and QUS measurements during the follow-up period were also significantly correlated (Trimpou et al., 2010). In an Asian longitudinal study (5 year follow-up), Fujiwara et al. (2005) found that SOS, BUA and SI significantly predicted hip, wrist and non-spinal fractures in Japanese men and women (Fujiwara et al., 2005*)*. In a meta-analysis which analysed 21 independent studies, Moayyeri et al. concluded that SOS, BUA, SI and QUI were good predictors for fractures (Moayyeri et al., 2012*)*.

Previous Malaysian study on Malaysian female elderly residing in Kuala Lumpur, Malaysia (aged ≥50 years) using QUS as method of assessment found that 8.0% of the sample group was osteoporotic (Chin et al., 2016). Similarly, a study by Hasnah et al. (Hasnah, Amin & Suzana, 2012) reported that among the 125 postmenopausal Malay females (mean age: 59 years [SD: 4] years) assessed using a QUS device, 6.0% were osteoporotic. Going back a few years in 2000, Damodaran et al. (2000) found that among 164 perimenopausal and postmenopausal females attending a menopause clinic, only four (2.44%) were osteoporotic (Damodaran et al., 2000). Considering that the prevalence of osteoporotic condition in the current study was 18.0% among the postmenopausal women (Table 4.1), an upward trajectory can be seen. This warrants intervention strategies aimed at creating awareness on osteoporosis and its risk factors in Malaysian postmenopausal women.

5.1.3 Prevalence of sarcopenic conditions in study cohort

Sarcopenia is an age-related disorder defined by progressive loss of muscle mass and strength. The decrease of muscle mass and strength causes health outcomes such as functional limitations, physical disability and frailty,

leading to increased fall risk, poor quality of life, high burden of healthcare cost and in severe cases, mortality (Rizzoli et al., 2014; Beaudart et al., 2014; Cruz-Jentoft et al., 2010; Janssen, Shepard & Katzmarzyk., 2004). According to the diagnostic criteria proposed by the Asian Working Group for Sarcopenia (AWGS), the disorder is diagnosed when there is low muscle mass (defined as appendicular skeletal muscle index ≤ 7.0 kg/m^2 in males and ≤ 5.7 kg/m^2 in females), combined with either low muscle strength (defined as handgrip strength < 26 kg in males and < 18 kg in females) or low physical performance (defined as six-meter gait speed <0.8 m/s) or both (Chen et al., 2014). The European Working Group on Sarcopenia in Older People (EWGSOP) had stratified sarcopenia into three spectrum of severity; 'pre-sarcopenia' is defined as having only low muscle mass without impact on muscle strength or physical performance; 'sarcopenia' is defined as having two out of the three criteria: low muscle mass, plus low muscle strength or low physical performance; and 'severe sarcopenia' is diagnosed when all three criteria of the definition are present (Cruz-Jentoft et al., 2010). When there is a loss of muscle mass of more than 40%, severe reduction in physical activity and aerobic capacity is usually seen and this tends to lead to morbidity and reduced strength. Studies have shown that the prevalence of sarcopenia was higher in Asians compared to Caucasians (Chen et al., 2014; Cruz-Jentoft et al., 2010).

Studies in western countries found that the prevalence of sarcopenia ranged from 7.0% to 16.0% and more likely to occur in women compared to men. For example, a study in United Kingdom which examined 1787 elderly aged 60 years or more (67±2.6 years), of which 765 were men and 1022 were

women, found that the prevalence of sarcopenia was 4.6% in men and 7.9% in women (based on the diagnosis algorithm proposed by the European Working Group on Sarcopenia in Older People, EWGSOP). It is interesting to note that the indicator for low muscle mass in this study was based on the lower third of the gender-specific distribution of the fat-free mass index (FFMI) (Patel et al., 2013). A study in Brazil (Alexandre et al., 2014) which examined 1149 elderly of similar age group (69.6±0.6 years), living in an urban area of the municipality of São Paulo, found that the prevalence of sarcopenia in the whole population was 15.4%, with higher prevalence in women (16.1%) compared to men (14.4%). Similar to the UK study, the prevalence of the Brazilian study was also determined by the diagnosis algorithm proposed by EWGSOP. However, the indicator for muscle mass loss was based on appendicular skeletal muscle mass index (ASMMI) instead of FFMI and the cut-off points was determined based on the lowest 20% of the distribution of the population (8.90 kg/m² for men and 6.37 kg/m² for women).

In Asia, it is difficult to do a comparison study due to differences in study design, sample size, geographical boundaries, population backgrounds, definition of sarcopenia, diagnostic criteria, techniques and also body compositions across ethnic groups (Chen et al., 2016; Diz et al., 2015; Tanko´ et al., 2002). For example, a study in Japan which examined 1882 healthy elderly community-dwellers aged between 65 and 89 years (74.9 ±5.5 years), using BIA to assess muscle mass, found that prevalence of sarcopenia was 21.8% for men and 22.1% for women (Yamada et al., 2013). The diagnostic criteria used was based on European working group for sarcopenia

(EWGSOP) rather than Asian Working Group for Sarcopenia (AWGS) and the indicator for muscle mass loss was skeletal muscle mass index (SMMI). Similarly, a study in South Korea which analysed 2332 elderly aged 65 years or more found that the prevalence of sarcopenia was 9.7% for men and 11.8% for women (Kim et al., 2012). Although a study in Taiwan had used the same diagnostic criteria (EWGSOP), the study, which assessed 2867 individuals aged 65 years or more (74±6 years) found that the prevalence of sarcopenia was much lower (5.4% for men and 2.5% for women). It is worth mentioning that the study had used appendicular skeletal muscle mass index (appSMMI) as indicator of muscle loss rather than whole body muscle mass (Wu et al., 2014). A comparison study had found that the prevalence of sarcopenia ranged from 0% to around 10% when different diagnostic criteria were used (International Working Group on Sarcopenia (IGWS), EWGSOP or AGWS) (Wen et al., 2015). Furthermore, since ethnic disparities exist in body compositions, cut-off points of health risk may also differ across different populations (Tanko´ et al., 2002).

The current study, which had used diagnostic criteria proposed by AWGS to define sarcopenia, found that overall prevalence of sarcopenia among community-dwelling, postmenopausal Malaysian women was 29.4%, among which, 6.6%, had pre-sarcopenia and 5.9% had severe sarcopenia (Table 4.4 and Figure 4.1). This finding is similar to a study on 387 community-dwelling older adults (aged 68.3 ± 5.66 years) in Singapore (based on diagnostic criteria proposed by AWGS) which was 27.4% (Fung et al., 2019). These percentages, however, was lower than the percentage reported by Norshafarina et al. (2013) with a similar multi-ethnic Asian study population

(59.8%). Norshafarina et al. (2013) had defined sarcopenia using diagnostic criteria by EWGSOP and the indicator for muscle loss was from whole body muscle mass index without the combination with muscle strength and/or muscle performance. The reason was probably due to whole body muscle mass being easier to measure, which can be estimated using various techniques, from anthropometry to bioimpedance analysis, the counting of total body potassium and magnetic resonance imaging. Norshafarina et al. (2013) had also used different cut-off points. The researchers had used cut-points proposed by Janssen et al. (2002) for each spectrum of sarcopenia; total skeletal muscle mass index <6.75 kg/m^2 for sarcopenia, 5.76 to 6.75 kg/m^2 for moderate sarcopenia and <5.75 kg/m^2 for severe sarcopenia (Janssen, Heymsfield & Ross., 2002). Conversely, the current study had used the cut-off values recommended by AWGS which used appendicular skeletal muscle mass index (appSMMI) as indicator to define low muscle mass, in combination with either low muscle strength and/or low muscle performance. Appendicular skeletal muscle mass index (appSMMI) was used as an indicator for low muscle mass because approximately 75% of SMM is located in the appendicular region (Rathnayake et al., 2018). Evidence found that reduction of appSMMI leads to negative health consequences such as weakness, disability, impaired quality of life (QOL), and even mortality (Cruz-Jentoft et al., 2010). Norshafarina et al. (2013) also acknowledged that the prevalence of sarcopenia among their cohort was significantly higher than those documented in the west and also other Asians countries. Interestingly, although sarcopenia is age-related, studies have shown that the correlation is not seen before aged 40 years (Baier et al., 2009, Flakoll et al., 2004, Grimby

et al., 1982, Janssen et al., 2000). Young women, particularly Asian women tend to have low muscle mass, although in young adults, this condition may not be age-related, but rather, due to their low body fat percent. In the current study, when looking at disparities between the age groups, higher percentage of low muscle mass (sarcopenic) was found among the young adults compared to their older counterparts (40.3% in young women [Table 4.2] vs. 28.6% in older women [Table 4.1]). The reason was likely due to higher adiposity in older women, which will be discussed further in the later part of this thesis. Nevertheless, although the younger age group had lower muscle mass, they still had stronger handgrip strength compared to the older age group (Table 4.15), which showed that high quantity does not equal high quality.

When analyses were done across the severity spectrum of sarcopenia, significant differences were found in some variables between people with severe sarcopenia and those without the disorder (Table 4.4). Apart from having lower appendicular muscle mass, people with severe sarcopenia were also found to be significantly older, slimmer, with lower gait speed and lower bone density compared to their healthier counterparts (no sarcopenia) (Table 4.4). The current study also found no significant differences in age, years since menopause, adiposity indices (BMI, WC, BFP), and whole-body muscle mass indices (FFMI, SMMI) across the severity spectrum (pre-sarcopenia to severe sarcopenia, Table 4.4). In addition, people with sarcopenia had the same level of handgrip strength and gait speed as those with severe form of the disorder (no significant difference), implying that strength-wise and endurance-wise, people with sarcopenia and severe

sarcopenia are in the same category (Table 4.4). It is well-established that muscle mass and muscle strength decrease with ageing, although it is more prominent after the age of 40 years (Baier et al., 2009; Flakoll et al., 2004; Janssen et al., 2000; Grimby et al., 1982). Age-related loss of muscle mass may be due to the loss of anabolic factors such as neural growth factors, growth hormones, physical activity and cytokines-induced inflammation. Further, reduced protein synthesis and mitochondrial damage may lead to lower endurance capacity and possibly weaker strength in elderly related to aging. The current study found that adiposity indices (BMI, WC, BFP) were lower among the sarcopenic compared to normal subjects (No sarcopenia, Table 4.4). The loss of muscle mass in conjunction with weight loss in the elderly may cause serious functional disabilities and physical limitations in their daily lives.

In summary, the present study documents at high prevalence (29.4%) of sarcopenia among postmenopausal women in Malaysia compared to other Asian countries. Older age, lower adiposity, lower gait speed and lower bone density were observed among severely sarcopenic subjects (Table 4.4). This could imply that some interventions to build up muscle mass and muscle strength are needed to among Malaysian women. It is possible that interventions in a form of physical therapy may help improve muscle strength among this high risk group and geared towards the improvement of functional performance as well as their quality of life.

5.1.4 Ethnic disparities in the prevalence of obesity, osteoporosis and sarcopenia

5.1.4.1 Obesity

Aging is associated with changes in body composition, and studies have found that these changes in body composition vary by ethnic groups (Silva, Shen & Heo, 2010; Araujo, Chiu & Kupelian, 2010; Wagner & Heyward, 2000). While there was a biologically reasonable explanation for the differences of obesity prevalence between age groups (young vs. old), it is interesting to see ethnic disparities in the prevalence of obesity, osteoporosis and sarcopenia in Malaysia. Malaysia is a country with a multiethnic population comprising the Malays (69.3%), which make up the majority of Malaysian population, followed by Chinese (22.8%), Indian (6.9%) and others (1.0%) as the major groups within the total population of 32.6 million (Department of Statistics Malaysia, Current Population Estimates 2018-2019). Studies have shown that there was a significant health and economic disparities among Malaysia's major ethnic groups—Malay, Chinese, Indian. Findings from the current study showed that the Chinese were significantly taller, while their body weight (kg), body mass index (BMI), and body fat percentage (BFP) were significantly lower compared to Malays and Indians (Table 4.5). These results supports the findings of previous population surveys in Malaysia. Data from previous studies showed Indian women were equally as obese as Malay women, but the Chinese only had about half the prevalence rates (Azmi et al., 2009; Rampal et al., 2007; Fatimah et al., 1999). Across the national population surveys however (NHMS II, MANS 2003, NSCVDRF 2004, NHMS III, NHMS 2011), Indians were found to be more overweight than Chinese and Malays (18.6% vs. 16.5%, 31.0% vs. 27.2%, 33.2% vs. 29.8%, 30.8% vs. 31.1%, respectively) except perhaps in the NMHS 2011, where the Malays appeared to have moved ahead.

5.1.4.2 Osteopenia/osteoporosis

In the current study, no ethnic disparities were found for bone health indices (Table 4.5). Similarly, previous Malaysian studies on female elderly residing in Kuala Lumpur, Malaysia (aged ≥50 years) using similar method of assessment (QUS), also found no ethnic differences in bone health indices nor in the prevalence of osteoporosis/osteopenia (Chin et al., 2016). Older studies by Lim et al (2005) and Goh et al. (2004) also reported no ethnic differences in BMD in Malaysian females. Although no ethnic disparity in bone density were found, some disparities were found in fracture risk. Previous studies have shown that hip fractures were more likely to occur in Chinese compared to other ethnicities in Malaysia (Chan et al., 2014; Lee & Khir, 2007). Similarly, a study by Koh et al. in 2001 reported that Chinese women had an age-adjusted incidence of 410 of hip fractures per 100,000, followed by 264 per 100,000 in Malays, and 361 per 100,000 in Indians (Koh et al., 2001). FRAX© score from the present study also supported this finding (Table 4.3). FRAX© score, which determines a 10-year probability fracture risk for major osteoporotic related fracture and hip fracture, revealed that the Chinese had significantly higher risk for hip fracture in 10-years' time, compared to Indians and Malays ($p<0.01$). Goh, Low & Das (2004) theorized that the reason the Chinese had higher fracture rate than other ethnicities is due to them having faster age-related bone loss after reaching a certain age. Goh et al. (2004), who studied Malaysian women between 20 to 59 years of age, found that although there were no significant difference in peak bone mineral density (BMD) at lumbar spine and femoral neck among the ethnic groups, a significant bone loss was found between the age of 20 to 50 years

at the femoral neck in Chinese (and Malay). These findings suggest that age-related bone loss, rather than peak bone mass, may partially explain why the Chinese had higher fracture rate than other ethnicities. Other studies have attempted to explain this variation. A local study reported that parity, habitual tea consumption, and low body mass index (BMI) were some of the positive predictors of osteoporosis in Chinese women aged ≥50 years (Chan et al., 2014). The caffeine in tea, for example, (which accounts for 2% ~ 4% of the dry weight of the tea) had been found to cause an adverse effect on bone health through increased urinary calcium excretion thereby reducing the intestinal absorption of calcium (Namkung et al., 2010; Xiang, 2009; Heaney, 2002). Furthermore, the differences between hip fracture incidence and ethnic disparity in terms of suboptimal bone health could be attributed to several reasons. Firstly, other than bone density, falls and fragility fracture are attributed to many predictors such as muscle weakness, weak gait and balance ability, visual and cognitive impairments, and postural hypotension (Rubenstein, 2006). Therefore, variances in bone density alone could not explain the differences in the risk of fracture among the ethnic groups. Secondly, lifestyle changes among these ethnic groups during the period between the fracture incidence data first published (1997) (Lee & Khir, 2007) and the current study could have contributed to the discrepancy.

5.1.4.3 Sarcopenia

Muscle mass and physical performance are highly variable in older adults and strongly dependent on ethnicity and lifestyle (Du et al., 2018; Silva, Shen & Heo, 2010; Araujo, Chiu & Kupelian, 2010; Wagner & Heyward, 2000). It is worth addressing the impact of ethnic disparities, health disparities, and

genetic variations on phenotype (body composition, body mass index or BMI) when discussing sarcopenia and ageing (Du et al., 2018). Currently, very few studies have examined the prevalence of sarcopenia across different ethnic populations in Malaysia. So far, only one Malaysian study reported the prevalence of sarcopenia across different ethnic groups (Norshafarina et al., 2013). Norshafarina et al. (2013) reported that based on whole body skeletal muscle mass index (SMMI), the prevalence of sarcopenia among women aged 60 years and above, was the highest in Indians (68.8%, n=11/16), followed by Chinese (50.0%, n=25/50) and Malays (35.5%, n=58/164). Similarly, the current study found that whole body muscle mass index; skeletal (SMMI) and fat-free mass index (FFMI), were the highest in Malays (means low incidence of sarcopenia) (Table 4.5). However, contrary to the findings by Norshafarina et al. (2013), the Chinese were found to have the lowest whole-body muscle mass (SMMI and FFMI, Table 4.5), followed by Indians. This is likely due to them having the lowest adiposity compared to the other ethnic groups (Table 4.5). Nevertheless, as mentioned earlier, quantity does not equal quality. Although the Chinese had the lowest muscle mass, they have the strongest handgrip strength and lower extremity strength (Sit-to-stand test) compared to the Malays and Indians (Table 4.5). The Indians, however, performed the least in terms of endurance (gait speed) and balance (Table 4.5) compared to Chinese and Malays. These findings supported the theory high muscle mass induced by high adiposity does not have the same quality as high muscle mass coupled with low adiposity.

From the findings, it is clear that prevalence studies in special groups (i.e. the obese) are needed in order to have targeted and effective interventions.

Obesity, for example, is highly influenced by behavioural, social and environmental factors, which are closely related to cultural and ethnic backgrounds. Further studies on these factors are needed to identify techniques that culturally-resonates in order to motivate people to increase and maintain physical activity and nutritionally-sound diet. In addition, awareness studies to assess the insight of community in relation to their understanding and perceptions on weight maintenance is also important. For example, awareness campaigns could be in the form of the various language-specific media such as newspapers, magazines, television programs and radio channels. In addition, using influencers such as local celebrities or otherwise notable spokespersons from a particular ethnic group as a role model may also be a powerful technique to increase awareness. Policy makers in Malaysia should focus more into reducing inequality among citizens of different ethnic groups. A better understanding of how obesity incidence may vary by sociodemographic and lifestyle–related factors is important in order to monitor and ultimately reduce disparities in obesity–related health risks in Malaysia.

5.2 Musculoskeletal health status and functional performance of participants

Currently, data on musculoskeletal health in older Malaysian women is sparse. Studies have found that the prevalence of musculoskeletal disorders were higher among Asian women compared to Caucasian women (World Health Organization, 2015; Fuh, et al., 2001; Avis, et al., 2001). Once a woman reached menopause, the risk for musculoskeletal disorder increases. Reduced estrogen level has been known to cause muscle and bone wasting leading to musculoskeletal disorders such as sarcopenia and osteoporosis

(Khadilkar, 2019). Musculoskeletal disorders significantly affect the locomotor system (i.e. muscle, bones, joints), leading to reduced mobility, dexterity and inability to maintain economic, social, and functional independence. The disorders can be made worse when compounded with obesity, which has risen dramatically in the developing world (Kelly et al., 2008). In 2015, The World Health Organization (WHO) published a report describing the impact of impaired musculoskeletal health on healthy aging (World Health Organization, 2015). Impaired musculoskeletal health is associated with reduced physical capability (which include hand grip strength, walking speed, the ability to stand up from a chair, and standing balance times), chronic and persistent pain, impairments in mobility and function, reduced quality of life and mental well-being (Cooper et al., 2010). Frailty is usually one of the main clinical outcomes of the disorder.

In 2016, based on the algorithm provided by the European Working Group on Sarcopenia in Older People (EWGSOP) (Cruz-Jentoft et al., 2010), on the International Working Group on Sarcopenia (Fielding et al., 2011), and on the Foundation for the NIH Sarcopenia Project (Studenski et al., 2014), Ilich et al. (2016) proposed the measurements of handgrip strength and 3 modified components of short physical performance battery (SPPB) to assess the overall physical performance for the categorization and the supplemental diagnosis of OSO. The 3 tests comprised of gait speed (<0.8m/s), one-leg stance (≤16sec) and sit-to stand test (≤20 times), in addition to handgrip strength. Based on these criteria and scoring reference, we assessed the functional performance of our study cohort (Table 4.6). Table 4.6a outlines the assessment criteria and the corresponding scores. The cut-off for handgrip

strength was <18kg to suit the Asian population. The score of "0" was assigned to each test performed at or below the given cut-off and the score of "1" to each test performed above the cut-off value. Based on the scores, four levels of functionality status were assigned: *severe functional decline (total score of 0), major functional decline (total score of 1)*, *moderate functional decline (total score of 2)*, and *minor functional decline (total score of 3)*. Participants with total score of 4 (perfect score) were categorized as '*no functional decline*'. Overall, the majority of our study population only have minor functional decline (40.6%), followed by moderate (28.1%), major (22.7%), and severe functional decline (6.3%). Participants with 'no decline' was only 2.3% (Table 4.6 and Figure 4.2). The majority of Malays and Chinese had minor functional decline (53% and 55%, respectively), followed by moderate decline, major decline, severe decline, and finally, no decline (Table 4.6). However, the same pattern was not seen for Indians. The highest percentage of Indians are in the major decline category (43.0%), followed by moderate decline (37.1%), severe decline (14.3%), and minor decline (5.7%). There were no participants in the 'No decline' category among the Indians (Figure 4.3). It is interesting to note that although the Indians have higher muscle mass compared to Chinese (Table 4.5), they do not have better functional performance. This may be due to higher adiposity among the Indians which induce higher muscle mass. This type of muscle mass is not necessarily accompanied by higher quality of muscle.

Functional assessments may be more meaningful for clinicians as they represent the real life physical function for people, and are simple enough to be performed anywhere and anytime. For obese population, where high

quantity of muscle does not necessarily reflect high quality of muscle, functional performance assessment may be a good supplemental diagnostic criteria. By assessing strength and function, a clear picture of the entire musculoskeletal system which include muscles, tendons, ligaments, bones, and cartilage could be ascertain. This may explain the gap in knowledge of the relationship between muscle mass and strength/function (Hamerman, 1997), as well as the nervous system (Liu et al., 2014), lung capacity, and other functions such as blood flow, flexibility, and postural stability (Frames, Soangra, Lockhart, 2013). Although the classical definition of sarcopenia is the loss of muscle mass, the measurements of muscle strength and function give a better representation for body functioning. Therefore, the assessments of functional performance may improve the assessment of body composition status and lead to better overall diagnosis of osteosarcopenic obesity (OSO). Subsequently, dietary and/or physical activity measures could be included as part of the standard care.

5.3 Interrelationship between fat, bone and muscle

Due to conflicting results available on the association between body composition and bone density, we investigated the relationship between fat, muscle, and bone density in Malaysian postmenopausal women. We hypothesized that increased muscle mass is directly correlated with higher bone density (due to Wolff's Law on bone formation), while no direct correlation will be found between obesity and bone density. Earlier epidemiological studies investigating the relationship between obesity and osteoporosis had used BMI to define obesity, and provided the generally accepted view that increased mechanical loading correlates to increase in

bone mass (Skerry & Suva, 2003). However, no information was provided on potential confounders. Although prior studies have used fat mass to assess the correlation between obesity and bone mass (Reid, 2002), they generally did not adjust for muscle mass. Therefore, the conclusions from these studies may be confounded by the effect of muscle mass on the skeletal system. From this, a critical question arises: is there a direct correlation between obesity and osteoporosis? To investigate the relationship between these two parameters, it is necessary to control for the amount of muscle mass in the analyses.

A cross-sectional study was performed examining the associations between body composition, anthropometric measures, and bone health status in Malaysian women (Table 4.7). A total of 141 postmenopausal women aged between 45 and 88 years participated in the study. The current study found that muscle mass of the limbs (appSMMI) was significantly associated with bone density (BUA, $r=0.192$, $p<0.05$) (Table 4.7). No correlations, however, were observed between fat indices (BMI, WC, BFP) and bone density (BUA) (Table 4.7), even after controlling for confounders (Table 4.9a). Muscle mass of the limbs (appSMMI) and whole body skeletal muscle mass (SMMI) were also found to be strong predictors of BUA, even after age, years since menopause, and all indices for adiposity were kept constant [appSMMI ($\beta = 0.325$, $p<0.05$, Table 4.9b), SMMI ($\beta = 0.775$, $p < 0.05$, Table 4.9c)]. Age was negatively associated with skeletal muscle mass (appSMMI and SMMI, $p<0.05$) and physical performance (GS and BLN, $p<0.05$) (Table 4.8). AppSMMI was positively associated with HGS and GS ($p<0.05$) (Table

4.7), while fat indices (BMI, WC, BFP) were positively associated with muscle mass indices (FFMI, SMMI, appSMMI, $p<0.01$) (Table 4.7).

Muscle, fat and bone mass are the three components of body weight found to be associated with bone health (Ilesanmi-Oyelere et al., 2018; Siris et al., 2014). To date, it is still not clear which component, muscle or fat has greater influence on bone (Lim et al., 2004; Li et al., 2004; Ilich-Ernst et al., 2002). Bone (osteoblast), fat (adipocytes) and muscle (myocytes) cells originate from the same mesenchymal stem cells (Ilich et al., 2016; Khosla et al., 1996). There had been controversial reports on whether obesity is detrimental or protective of bones. For example, many studies have shown positive associations between fat and bone mass (Richards et al., 2007; Pesonen et al., 2005). The association was largely explained by the effect of gravitational loading and mechanical stimulation of bones by higher weight. In addition, adipocytes is an endocrine gland which produces estrogen and leptin. These hormones are known to increase bone mass by increasing osteoblast and reducing osteoclast activity (Reid, 2002*).* Therefore, it was a generally accepted concept that excess weight (comprising fat and muscle tissue) protects against osteoporosis. The protective effect of obesity on bone mass has been termed "obesity paradox" or "reverse epidemiology" (Zhao et al., 2007). Some studies have reported that muscle mass, not fat mass, is associated with bone mass (Casale et al., 2016; Sotunde et al., 2015; Li et al., 2004; Liu et al., 2004; Chen et al., 1997; Ilich-Ernst et al., 2002) whereas others (Rodrigues et al., 2016; Hsu et al., 2006) have found that fat mass, not muscle mass, is more determinant of bone density. Some studies have also suggested that both fat mass and muscle mass can equally serve as a

predicting factor for bone density (Harris & Dawson-Hughes, 1996). Furthermore, some studies have reported that the importance of fat mass and muscle mass on bone density is dependent of menopausal status. Mizuma et al. (2006) suggested that that muscle mass is more important than fat mass in premenopausal women, whereas Ijuin et al. (2002) suggested that fat mass is more significant than muscle mass in postmenopausal women. In the present study, no direct correlations were found between fat indices and bone density. However, significant and positive correlations were found between muscle mass and bone density. The significance remained even after controlling for fat indices. A strong association between muscle mass and bone density may be due to innervation and mechanical interactions with bone. Interestingly, muscle mass and fat mass were positively correlated. This suggest that 'obesity paradox' phenomena on bone health may be due to high muscle mass induced by increased adiposity in obese individuals. Pollock et al. (2007) studied the relationship between bone health indices and body fat percent (BFP), while adjusting for muscle mass indices in 115 late adolescent females (aged 18.2 ± 0.4 years). The study, using dual-energy X-ray absorptiometry (DXA), showed that the bones of those with high body fat were nine percent weaker than those of normal body fat participants, indicating a strong role of muscle mass. It is important to note that this study was done on late adolescent women where bones had stopped growing and age-related bone loss had not yet begun (Pollock et al., 2007). The authors briefly discussed Wolff's law of bone formation which states that bone growth is stimulated by the constant force applied by muscles. Since overweight people typically have more muscle surrounding their bones than

thinner people, many researchers arrived to the skewed conclusion that being overweight is good for bone health. However, when corrected for the muscle mass, the researchers found that BFP was inversely correlated to each index of bone (radial cortical bone area, total bone cross-sectional area (CSA), cortical bone mineral content (BMC), periosteal circumference, and strength-strain index (SSI) (20% site; all $p < 0.05$) (Pollock et al., 2007). Comparatively, the current study found inverse correlation between bone density (BUA), BMI and WC when the muscle mass was controlled, albeit non-significant (Table 4.9a). Lecka-Czernik et al. (2015) found that the beneficial effect of obesity on bone mass is short-lived and only occur at the initial stages. Over time, bone formation is unable to maintain the bone and it then causes a reduction in quality (Lecka-Czernik et al., 2015). Additionally, in a study of self-reported fractures over 10 years in sarcopenic obese men and women, the protective effect of fat mass on bone was reduced by the presence of sarcopenia (Scott et al., 2016), suggesting an interdependent link among fat, bone and muscle.

According to Wang et al., (1994) the conflicting results from the research on fat, bone and muscle could be attributed to the changes in body composition with age, ethnicities, and weight. Women after menopause experience drastic decrease in bone and muscle mass and significant increase in weight and fat mass (Cruz-Jentoft et al.. 2010). In the case of ethnic disparities, studies have reported that fat mass is positively associated with bone density in Caucasian women (Reid, Plank & Evans, 1992; Reid et al., 1992), whereas in a later study of a large cohort of Chinese women of different ages, it was found that the risks of osteoporosis, osteopenia, and non-spine fractures were

significantly higher in those with higher percentage of body fat (Hsu et al., 2006). These conflicting results suggest a complex relationship among bone, fat and muscle. It is also interesting to note that various studies have found that fracture sites play important roles when studying the relationship between BMI and fracture. For example, obese women have lower risk of hip fracture compares to men (Nielson, Srikanth & Orwoll, 2012), but not for other sites such as the ankle, leg, humerus, and vertebrae (Premaor, Comim & Compston, 2014, Ong et al., 2014). The difference in fracture sites in obese individuals may be due to higher fat padding surrounding some area that reduces the impact of falling while other areas are more exposed due to different patterns or forces subjected upon falling.

Currently, the relationship between fat, bone and muscle is still being studied and debated. One of the theories stated that due to the body's adaptation mechanism, muscle and bone will increase along with the increase of adipose tissue in order to support body's general movement and also the increased weight, hence why obese people are likely to have high bone mass and muscle mass. A study by Liu et al. (2014) found that obese women had significantly greater amount of muscle mass (kg) and higher BMD of total body, total femur, and radius (all p-value<0.05), compared to normal-weight women (Liu, Ilich, Brummel-Smith, & Ghosh, 2014). When only overweight and obese women were analyzed, the relationship between BMD of each skeletal site and muscle mass was significantly positive and linear (Liu et al., 2014). Similarly, the current study found positive correlations between bone density and muscle mass (Table 4.7). A direct explanation for this is likely due to higher muscle mass causes higher mechanical loading to the skeleton, which

leads to increased in density. Table 4.9a shows that after controlling for muscle mass, BMI and WC were negatively correlated to bone density. Similar finding was also found by Zhu et al. (2017) where it was reported that in women with a discordance between fat mass index (FMI) and body mass index (BMI), higher body fat mass for BMI was associated with lower BMD at the femoral neck, total hip and total body, suggesting that increased fat mass without a parallel increase in muscle mass may be detrimental to bones. These findings corroborated the findings by Zhao et al. in 2007. The study found that before mechanical loading was adjusted, significant and positive relationship was found between bone mass and fat mass. However, when mechanical loading was controlled, the relationship became inverse ($p<0.01$). To summarise, the current study supports the well-established beneficial effects of mechanical loading on bone, but challenge the theory that obesity protects against osteoporosis.

Additionally, with regards to muscle performance, the current study found that strength of lower extremity (measured using sit-to-stand test, STS) was negatively affected by high body weight (i.e BMI, WC, and BFP) (Table 4.7), suggested by the fact that the heavier the individual, the less number of sit-to-stand one can perform. This finding is logically sound, as the lower extremity of the body has to support more weight of the individual. As for endurance (measured by gait speed, GS), the current study found that it decreases with age and years since menopause (Table 4.8), and increases with the muscle mass of the limbs (appSMMI, Table 4.7). This finding is also logical since gait speed depends on the mass of the leg muscles. No correlation, however, was found between gait speed and whole body muscle

mass (FFMI and SMMI) (Table 4.7). Interestingly, for balance, which was tested by one-leg stance, was negatively affected by age, years since menopause (Table 4.8) and body fat percent (Table 4.7). Previous studies have found similar findings in which balance is significantly affected by body weight and age (Gomes et al., 2013; Urushihata et al., 2010).

These findings suggest that muscle mass is the stronger predictor of bone density. This is important when devising prevention, treatments or managements of osteoporosis. Maintaining or increasing muscle mass should be the target of improvements, in addition to reducing fat mass in postmenopausal women. High muscle mass that was induced by high fat mass do not produce quality muscle, and had minimal benefits on physical performance.

CHAPTER 4: RESULTS PART II

4.2 Differences in characteristics between Osteosarcopenic obese (OSO), Osteopenic obese (OO), Sarcopenic obese (SO), Obese-only (OB) and Normal (NR, non-obese, non-osteopenic, non-sarcopenic) participants

To test the hypothesis that OSO, OO, SO, Obese-only (OB) and women with normal weight, bone and muscle mass (NR) have equal means of body compositions and muscle performance, one-way ANOVA test was performed. In this cohort of postmenopausal women, participants were categorized into 'OSO', 'OO', 'SO', 'OB' and 'NR' group based on the criteria and standard cut-off proposed by previous studies; WHO (T-score ≤2.5), Ilich et al. (2016) (BFP ≥32%) and the Asian Working Group for Sarcopenia (AWGS) (appSMMI ≤5.7kg/m^2). The T-scores were derived based on QUS-generated Est. BMD of young Malaysian women aged 18-32 years. Differences in the characteristics are depicted on Table 4.10 and Table 4.11.

Based on the criteria, there were 6.3% (n=8) women with OSO, 4.7% (n=6) of women with OO, and 18.9% (n=24) of women with SO. Majority of participants were Obese-only (OB, 64.6%, n=82), and 5.5% (n=7) of women were normal weight, with healthy bone and muscle mass (NR, Table 4.10). Fourteen (n=14) participants were either sarcopenic-only or osteopenic/osteoporotic-only (normal weight) and were not included in the analysis.

The OSO group were significantly older, slimmer, with smaller waist circumference and lower muscle mass compared to OB participans ($p<0.05$) (Table 4.10). Years since menopause had no impact to any of the disorders.

Obese participants had significantly higher body fat percent and trunk fat percent, and significantly weaker handgrip strength than the normal weight participants (NR) ($p<0.001$) (Table 4.10). Interestingly, although OB and NR group have similar amount of muscle mass (no significant difference), OB group had significantly lower handgrip strength compared to NR group (Table 4.10). No significant difference was found for lower extremity strength (sit-to stand test), endurance (gait speed) and balance between any of the groups (Table 4.10).

Additionally, OSO group and SO group did not differ significantly in any of the variables (age, anthropometrics, body composition and physical performance) (Table 4.10). Conversely, OO group had significantly higher muscle mass (SMMI and appSMMI) compared to both OSO and SO group ($p<0.001$) (Table 4.10). Although non-significant, OO group also performed better than OSO and SO for each index of functional performance (HGS, Sit-to-stand test, gait speed and balance) (Table 4.10).

Table 4.10 Anthropometrics and body composition measurement of participants based on the classification of OSO, SO, OO, Obese-only and Normal

Variables	Osteosarcopenic Obese (n=8, 6.3%)	Osteopenic Obese (n=6, 4.7%)	Sarcopenic Obese (n=24, 18.9%)	Obese-only (n=82, 64.6%)	Normal (n=7, 5.5%)	P-value	Eta-squared
Age (year)	67.4(8.4)	57.0(4.1)	61.7(9.5)	59.2(6.7)$^{+}$	55.0(IQR 14.0)	**0.031***	0.083
Years since menopause (year)	10.5(6.6)	6.2(3.8)	10.0(IQR 18)	7.0(IQR 9.0)	12.00(8.8)	0.167	0.060
Height (cm)	145.3(6.0)	154.9(30)$^{+}$	151.2(5.2)	153.7(5.9)$^{+}$	156.5(IQR 6.5)$^{\delta}$	**0.000****	0.185
Weight (kg)	50.1(5.7)	70.9(10.8)$^{+}$	54.8(6.4)$^{\beta}$	69.5(IQR 11.4)$^{\delta}$	53.5(3.0)$^{\beta}$	**0.000****	0.448
BMI (kg/m^2)	23.8(2.9)	29.6(4.4)	24.0(2.9)$^{\beta}$	29.1(IQR 5.0)$^{\delta}$	21.1(0.7)$^{\beta}$	**0.000****	0.376
Body fat mass (kg)	19.6(4.4)	32.0(7.4)$^{\delta}$	22.6(5.0)$^{\beta}$	30.4(IQR 9.5)$^{\delta}$	14.8(2.6)$^{\beta}$	**0.000****	0.361
BFP (%)	38.7(4.9)	44.9(4.7)	40.8(4.7)	43.7(5.8)	27.5(4.2)$^{\beta,\ \delta}$	**0.000****	0.345
Trunk fat (%)	39.6(5.2)	45.5(3.4)	41.4(4.7)	44.5(IQR 7.3)	27.8(4.4)$^{\beta,\ \delta}$	**0.000****	0.388
FFMI (kg/m^2)	14.5(0.8)	16.2(1.9)	14.1(0.9)$^{\beta}$	16.2(IQR 1.7)$^{\delta}$	15.3(1.0)	**0.000****	0.439
SMMI (kg/m^2)	7.5(0.5)	8.1 (IQR 2.4)$^{\delta}$	7.4(0.5)$^{\beta}$	8.7(IQR 1.1)$^{\delta}$	8.3(0.6)^{f}	**0.000****	0.460
Appendicular SMMI (kg/m^2)	5.1(0.5)	6.1 (IQR 1.4)+	5.3(IQR 0.4)$^{\beta}$	6.5(IQR 0.8)$^{\delta}$	6.5(IQR 0.7)$^{\delta}$	**0.000****	0.482
HGS (kg)	17.3(3.5)	19.2(2.6)	18.2(4.6)	20.3(4.9)	26.4(4.6)$^{\beta,\ \delta}$	**0.001***	0.147
Sit-to-stand test in 30 sec (times)	11.0 (IQR 3.0)	12.7(4.6)	11.7(3.1)	11.8(4.0)	12.4(2.8)	0.974	0.004
Gait speed (m/s)	0.9(0.3)	0.9(0.2)	0.9(0.3)	0.9(IQR 0.4)	0.9(0.2)	0.855	0.012
Balance (sec)	13.6(12.5)	18.7(8.3)	14.5(IQR 21.0)	25.0(IQR 17.0)	30.0(IQR 7.0)	**0.046***	0.086

N.B: All results are presented in mean (standard deviation) unless stated otherwise. Interquartile range (IQR) are presented with median. WC=waist circumference, BMI=body mass index, BFP=body fat percent, FFMI=fat free mass index, SMMI=skeletal muscle mass index, appSMMI=appendicular skeletal muscle mass index, HGS=handgrip strength, OSO= T-score (≤-2.5), appendicular skeletal muscle mass index (≤5.7kg/m^2), and body fat percent (≥32%), Normal=non-obese (body fat percent<32%), non-sarcopenic (appSMMI>5.7kg/m^2) and non-osteoporotic (T-score>-2.5), *analysed using one-way ANOVA, with Tukey's HSD Post-hoc test, p<0.05, +=different from OSO, δ=different from SO and OSO, β=different from Obese-only and OO, ł=different from Obese-only, *f*=different from SO, Normality tested using Shapiro-Wilks test=P-value>0.05 is normally distributed , Eta-sqaured=n^2=Sum of squares (between)/Sum of squares (total)

Table 4.11 depicts the differences in bone density indices (Est. BMD, BUA, SOS, QUI, T-score and Z-score) between the groups. Groups with low bone density (OSO and OO) showed significantly lower results for each of the QUS index compared to the rest of the groups (SO, OB, and NR, $p<0.001$).

Table 4.11 Bone density indices of participants based on the classification of OSO, SO, OO, Obese-only and Normal

Variables	Osteosarcopenic Obese (n=8, 6.3%)	Osteopenic Obese (n=6, 4.7%)	Sarcopenic Obese (n=24, 18.9%)	Obese only (n=82, 64.6%)	Normal (n=7, 5.5%)	P-value	Eta-squared
Est. BMD heel (g/cm^2)	0.277(0.022)	0.269(0.023)	0.445(IQR 0.2)$^{\gamma,+}$	0.446(IQR 0.2)$^{\gamma,+}$	0.538(0.097)$^{\gamma,+}$	**0.000****	0.290
BUA dB/MHz	45.4(4.8)	49.0(5.3)	68.1(IQR 17.5)$^{\gamma,+}$	73.5(14.9)$^{\gamma,+}$	85.0(13.4)$^{\gamma,+}$	**0.000****	0.283
SOS m/s	1483.7(6.9)	1476.9(6.6)	1527.1(IQR 43.5)$^{\gamma,+}$	1523.0(IQR 34.6)$^{\gamma,+}$	1549.0(26.2)$^{\gamma,+}$	**0.000****	0.279
QUI/Stiffness	55.9(3.5)	54.6(3.7)	82.5(IQR 23.7)$^{\gamma,+}$	82.6(IQR 23.2)$^{\gamma,+}$	97.3(15.3)$^{\gamma,+}$	**0.000****	0.289
T-score (MY Ref.)	-2.8(0.2)	-2.8(0.2)	-1.4(IQR 1.3)$^{\gamma,+}$	-1.4(IQR 1.2)$^{\gamma,+}$	-0.6(0.8)$^{\gamma,+}$	**0.000****	0.291
Z-score	-1.3(0.2)	-1.4(0.2)	0.00(IQR 1.2)$^{\gamma,+}$	-0.03(IQR 1.2)$^{\gamma,+}$	0.8(0.8)$^{\gamma,+}$	**0.000****	0.281

N.B: Est. BMD=estimated bone mineral density, BUA=broadband ultrasonic attenuation, SOS=speed of sound, QUI=quantitative ultrasound index, MY=Malaysia, OSO= T-score (≤-2.5), appendicular skeletal muscle mass index (≤5.7kg/m2), and body fat percent (≥32%), Normal=non-obese (percent body fat<32%), non-sarcopenic (appSMMI>5.7kg/m^2) and non-osteoporotic (T-score>-2.5),*analysed using one-way ANOVA, with Tukey's HSD Post-hoc test, p<0.05, +=different from OSO, δ=different from SO and OSO, β=different from Obese-only and OO, ł=different from Obese-only, *f*=different from SO, γ=different from OO, Normality tested using Shapiro-Wilks test=P-value>0.05 is normally distributed , Eta-sqaured=n2=Sum of squares (between)/Sum of squares (total)

Table 4.12 shows the likelihood of obese people with bone and/or muscle disorders (OSO, OO, SO) to have results lower than the standard cut-off for various parameters, compared to participants without muscle and/or bone disorders (NR and OB). The findings showed that participants with OSO, OO and SO were more likely to have handgrip strength, bone density, and total 25(OH)D levels lower than the standard cut-off (HGS<18kg, BUA<54 db/MHz, total 25(OH)D<30nmol/L, respectively), compared to the NR group. In fact, no one in the NR group has the above-mentioned parameters lower than the standard cut-off.

Interestingly, when OSO, OO and SO groups were compared with their much healthier counterparts (OB and NR), differences were noted in several variables. The current study found that the prevalence of abdominal obesity (WC) were higher among OB participants compared to their counterparts with muscle disorders (OB versus OSO: 80.5% vs. 25.0%, OB versus SO: 80.5% vs. 45.8%). Additionally, obese participants with bone disorder (OSO and OO) were significantly more likely to have low BUA (dB/MHz) compared to their healthier counterparts (OB and NR, $p<0.05$, Table 4.12).

When looking at muscle strength, obese particiants with both muscle and bone disorder (OSO) were found to be significantly more likely to have low handgrip strength compared to their counterparts without any musculoskeletal health disorders (HGS<18kg, OSO=62.5% vs. OB=24.4%, $p\leq0.05$).

With regards to circulating Vitamin D (total 25(OH)D), OO group was more likely to be Vitamin D deficient compared to their OB counterparts

($p<0.001$). Additionally, the current study found that obese participants with low muscle mass (SO) were more likely to have low capability for balance (≤16sec) compared to their obese counterparts with healthy muscle mass (OB, $p<0.05$).

Table 4.12 Fisher's exact test results on OSO, OO, and SO, associated with various variables, compared to Normal and Obese-only participants

	OSO (n=8), n, %	vs.	Normal (n=7), n, %	P-value	OSO (n=8), n, %	vs.	Obese-only (n=82), n, %	P-value
WC ≥80cm	2, 25		0, 0	0.467	2, 25		66, 80.5	**0.001**
BMI ≥27.5kg/m^2	1, 12.5		0, 0	1.000	1, 12.5		60, 73.2	**0.001**
HGS <18kg	5, 62.5		0, 0	**0.026**	5, 62.5		20, 24.4	**0.050**
Sit-to-stand test ≤20 times	7, 87.5		7, 100	1.000	7, 87.5		69, 84.1	1.000
Gait speed <0.8m/s	2, 25		1, 14.3	1.000	2, 25		20, 24.4	0.100
Balance test ≤16sec	4, 50		1, 14.3	0.266	4, 50		19, 23.2	0.185
BUA <54dB/MHz	8, 100		0, 0	**0.000**	8, 100		7, 8.5	**0.000**
25(OH)D <30nmol/L	1, 12.5		0, 0	1.000	1, 12.5		4, 4.9	0.392
	OO (n=6), n, %	**vs.**	**Normal (n=7), n, %**	**P-value**	**OO (n=6), n, %**	**vs.**	**Obese-only (n=82), n, %**	**P-value**
WC ≥80cm	5, 83.3		0, 0	**0.005**	5, 83.3		66, 80.5	1.000
BMI ≥27.5kg/m^2	4, 66.7		0, 0	**0.021**	4, 66.7		60, 73.2	0.662
HGS <18kg	1, 16.7		0, 0	0.462	1, 16.7		20, 24.4	1.000
Sit-to-stand test ≤20 times	5, 83.3		7, 100	0.462	5, 83.3		69, 84.1	0.279
Gait speed <0.8m/s	1, 16.7		1, 14.3	1.000	1, 16.7		20, 24.4	1.000
Balance test ≤16sec	1, 16.7		1, 14.3	1.000	1, 16.7		19, 23.2	1.000
BUA <54dB/MHz	5, 83.3		0, 0	**0.015**	5, 83.3		7, 8.5	**0.000**
25(OH)D <30nmol/L	2, 33.3		0, 0	0.192	2, 33.3		4, 4.9	**0.070**

NB: OSO=osteosarcopenic obese, OO=osteopenic obese, SO=sarcopenic obese, Normal=non-obese (percent body fat<32%), non-sarcopenic (appSMMI>5.7kg/m^2) and non-osteoporotic (T-score>-2.5), WC=waist circumference, BMI=body mass index, HGS=hand grip strength, BUA=broadband ultrasonic attenuation, 25(OH)D=25 hydroxyvitamin

Table 4.12 Continued.

	SO (n=24), n, %	vs.	Normal (n=7), n, %	P-value	SO (n=24), n, %	vs.	Obese Only (n=82), n, %	P-value
WC ≥80cm	11, 45.8		0, 0	**0.033**	11, 45.8		66, 80.5	**0.001**
BMI ≥27.5kg/m^2	2, 8.3		0, 0	1.000	2, 8.3		60, 73.2	**0.000**
HGS <18kg	10, 41.7		0, 0	0.066	10, 41.7		20, 24.4	0.204
Sit-to-stand test ≤20 times	22, 91.7		7, 100	1.000	22, 91.7		69, 84.1	1.000
Gait speed <0.8m/s	7, 29.2		1, 14.3	0.635	7, 29.2		20, 24.4	0.788
Balance test ≤16sec	12, 50.0		1, 14.3	0.165	12, 50.0		19, 23.2	**0.016**
BUA <54dB/MHz	1, 4.2		0, 0	1.000	1, 4.2		7, 8.5	0.680
25(OH)D <30nmol/L	1, 4.2		0, 0	1.000	1, 4.2		4, 4.9	1.000
	Obese Only (n=82), n, %	**vs.**	**Normal (n=7), n, %**	**P-value**				
WC ≥80cm	66, 80.5		0, 0	**0.000**				
BMI ≥27.5kg/m^2	60, 73.2		0, 0	**0.000**				
HGS <18kg	20, 24.4		0, 0	0.186				
Sit-to-stand test ≤20 times	69, 84.1		7, 100	1.000				
Gait speed <0.8m/s	20, 24.4		1, 14.3	0.669				
Balance test ≤16sec	19, 23.2		1, 14.3	1.000				
BUA <54dB/MHz	7, 8.5		0, 0	1.000				
25(OH)D <30nmol/L	4, 4.9		0, 0	1.000				

NB: OSO=osteosarcopenic obese, OO=osteopenic obese, SO=sarcopenic obese, Normal=non-obese (percent body fat<32%), non-sarcopenic (appSMMI>5.7kg/m^2) and non-osteoporotic (T-score>-2.5), WC=waist circumference, BMI=body mass index, HGS=hand grip strength, BUA=broadband ultrasonic attenuation, 25(OH)D=25 hydroxyvitamin D

4.2.1 Cut-off values for the screening of osteosarcopenia in postmenopausal Malaysian women

In this section, the cut-off values of various variables were determined, in order to screen for Osteosarcopenia in postmenopausal women. The cut-off values were determined using 3 different statistical modelling techniques; 1) receiver operatic characteristic curve (ROC), 2) lowest quintile (20^{th}) of the study population, and 3) 2 SD below the mean value of a young reference group. Later, the results were compared with the values of standard cut-offs.

4.2.1.1 ROC Curve

Figure 4.3 depicts the ability of handgrip strength (HGS, kg) in predicting Osteosarcopenia (OS) in obese women using ROC curve plotted against healthy, obese-only (OB) counterparts (without Osteosarcopenia). The ROC curve shows that HGS is a good predictor for OS with AUC=0.698, p-value=0.066, 95%CI=0.544 to 0.853, true positive=0.625, false positive=0.240, threshold ≤16.5 kg.

It is interesting to note that the threshold for HGS in the current study was much lower than the threshold proposed by Asian Working Group for Sarcopenia (AWGS) (16.5 kg vs. 18.0 kg, respectively).

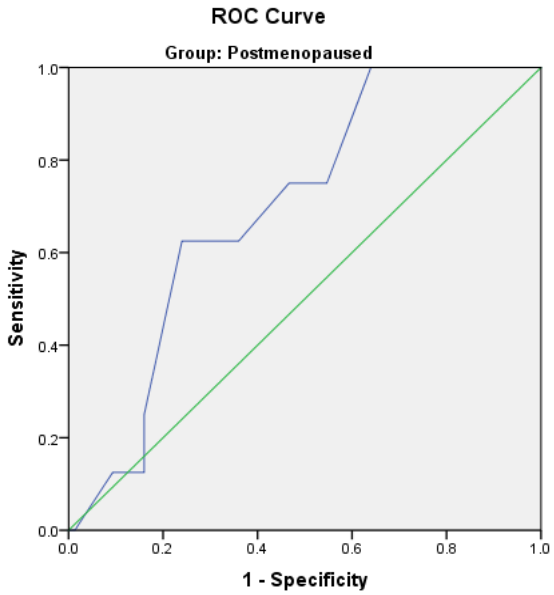


Figure 4.4 Receiver operating characteristic curve (ROC) showing the ability of handgrip strength (kg) in predicting Osteosarcopenia (T-score ≤-2.5, appendicular skeletal muscle mass index ≤5.7kg/m^2) in obese participant (BFP ≥32%), plotted against obese participants without Osteosarcopenia.

Area under curve

AUC	Std. error	P-value	95% CI
0.698	0.079	0.066	0.544 to 0.853

Coordinates of curve

Threshold (handgrip, kg)	Sensitivity	1-specificity
16.5[a]	**0.625**	**0.240**
17.5	0.625	0.267
18.5	0.625	0.360

a= presence of OSO if less than or equal to

Figure 4.4 shows the ability of fat-free mass index (FFMI) in predicting Osteosarcopenia (OS) in obese participants using ROC curve plotted against healthy, obese-only (OB) counterparts (without Osteosarcopenia). The ROC curve shows that FFMI is a good predictor for OS with AUC=0.946, p-value=0.000, 95%CI=0.887 to 1.000, true positive=0.750, false positive=0.085, threshold $\leq$15.2kg/m^2. Currently, there are no standard threshold for FFMI for the screening of sarcopenia in normal weight nor obese individuals.

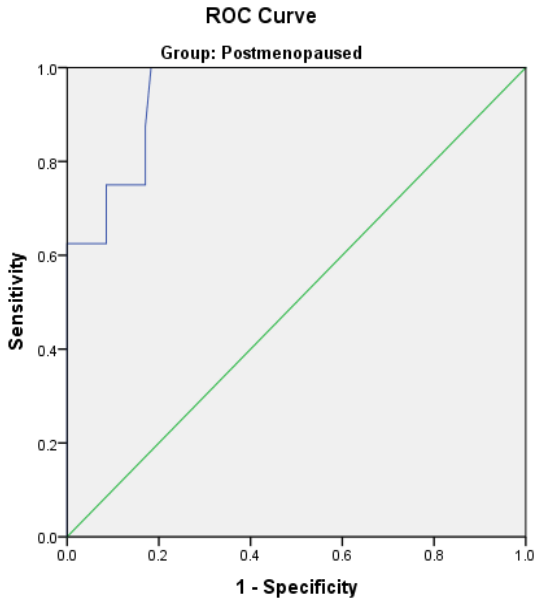


Figure 4.5 Receiver operating characteristic curve (ROC) showing the ability of fat-free mass index (kg/m^2) in predicting Osteosarcopenia (T-score ≤-2.5, Appendicular skeletal muscle mass index ≤5.7kg/m^2) in obese participant (BFP ≥32%), plotted against obese participants without Osteosarcopenia.

Area under curve

AUC	Std. error	P-value	95% CI
0.946	0.030	0.000	0.887 to 1.000

Coordinates of curve

Threshold (FFMI, kg/m^2)	Sensitivity	1-specificity
15.12	0.625	0.085
15.24[a]	**0.750**	**0.085**
15.33	0.750	0.110

a= presence of OSO if less than or equal to

Figure 4.5 shows the ability of skeletal muscle mass index (SMMI) in predicting Osteosarcopenia (OS) in obese participants using ROC curve plotted against healthy, obese-only (OB) counterparts (without Osteosarcopenia). The ROC curve shows that SMMI is also a good predictor for OS with AUC=0.966, p-value=0.000, 95%CI=0.923 to 1.000, true positive=0.875, false positive=0.098, threshold $\leq$8.2 kg/m^2. Currently, there are no standard threshold for SMMI for the screening of sarcopenia in normal weight nor obese individuals.

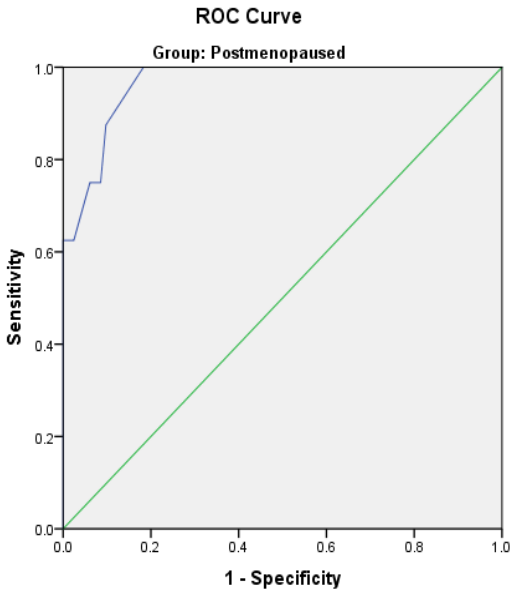


Figure 4.6 Receiver operating characteristic curve (ROC) showing the ability of skeletal muscle mass index (kg/m^2) in predicting Osteosarcopenia (T-score ≤-2.5, Appendicular skeletal muscle mass index ≤5.7kg/m^2) in obese participant (BFP ≥32%), plotted against obese participants without Osteosarcopenia.

Area under curve

AUC	Std. error	P-value	95% CI
0.966	0.022	0.000	0.923 to 1.000

Coordinates of curve

Threshold (SMMI, kg/m^2)	Sensitivity	1-specificity
8.05	0.750	0.085
8.15[a]	**0.875**	**0.098**
8.25	1.000	0.183

a= presence of OSO if less than or equal to

Figure 4.6 shows the ability of BUA (dB/MHz) in predicting Osteosarcopenia (OS) in obese participants using ROC curve plotted against healthy, obese-only (OB) counterparts (without Osteosarcopenia). The ROC curve shows that BUA is a good predictor for OS with AUC=0.987, p-value=0.000, 95%CI=0.966 to 1.000, true positive=0.875, false positive=0.049, threshold ≤52.85 dB/MHz. This threshold is marginally lower than than the cut-off for low bone density proposed by Johansen, Evans & Stone, 1999 (<54.0 dB/MHz).

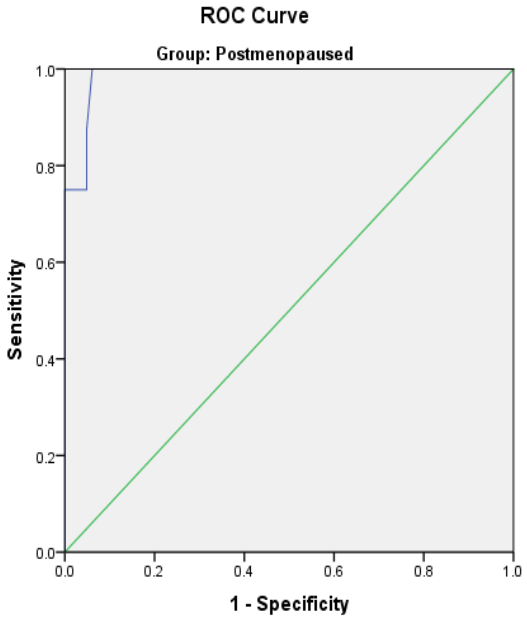


Figure 4.7 Receiver operating characteristic (ROC) curve showing the ability of broadband ultrasonic attenuation (BUA, dB/MHz) in predicting Osteosarcopenia (T-score ≤-2.5, Appendicular skeletal muscle mass index ≤5.7kg/m^2) in obese participant (BFP ≥32%), plotted against obese participants without Osteosarcopenia.

Area under curve

AUC	Std. error	P-value	95% CI
0.987	0.011	0.000	0.966 to 1.000

Coordinates of curve

Threshold (BUA dB/MHz)	Sensitivity	1-specificity
52.025	0.750	0.049
52.850[a]	**0.875**	**0.049**
53.375	1.000	0.061

a= presence of OSO if less than or equal to

Figure 4.7 shows the ability of speed of sound (SOS) in predicting Osteosarcopenia (OS) in obese participants using ROC curve plotted against healthy, obese-only (OB) counterparts (without Osteosarcopenia). The ROC curve shows that SOS is a good predictor for OS with AUC=0.991, p-value=0.000, 95%CI=0.975 to 1.000, true positive=0.875, false positive=0.037, threshold ≤1492.15 m/s. Currently, there is no standard threshold for SOS.

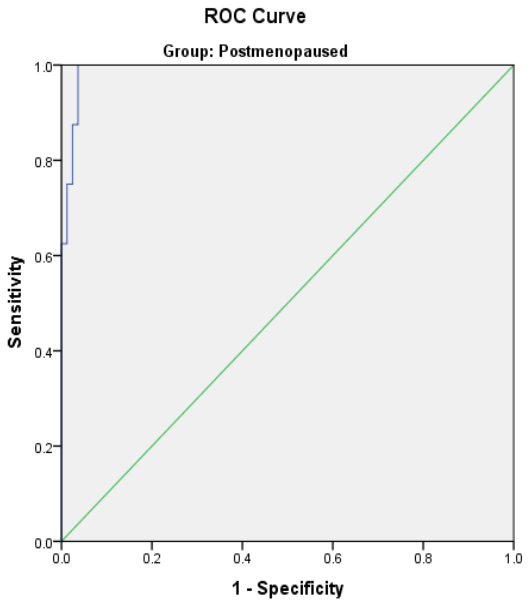


Figure 4.8 Receiver operating characteristic (ROC) curve showing the ability of speed of sound (m/sec) in predicting Osteosarcopenia (T-score ≤-2.5, Appendicular skeletal muscle mass index ≤5.7kg/m^2) in obese participant (BFP ≥32%), plotted against obese participants without Osteosarcopenia.

Area under curve

AUC	Std. error	P-value	95% CI
0.991	0.008	0.000	0.975 to 1.000

Coordinates of curve

Threshold (SOS, m/sec)	Sensitivity	1-specificity
1491.25	0.875	0.024
1492.15[a]	**0.875**	**0.037**
1492.90	1.000	0.037

a= presence of OSO if less than or equal to

Table 4.13 shows the differences in prevalence when the new cut-off values were used to define Osteosarcopenic obesity (OSO), Osteopenic obesity (OO), Sarcopenic obesity (SO), Obese-only (OB) and Normal (NR) participants.

It is interesting to note that the prevalence for OSO was close to two times higher when SMMI was used as indicator for muscle mass, compared to FFMI (9.4% vs. 5.5%, respectively). The prevalence was also the lowest when functional performance (HGS) was added to the criteria.

Table 4.13 Prevalence of OSO and its variations based on the new ROC-derived cut-off values

Criteria and cut-off values used to define OSO	OSO	OO	SO	OB	NR
	N (%)	N (%)	N (%)	N (%)	N (%)
Old					
T-score (≤-2.5), appSMMI (≤5.7kg/m^2), and body fat percent (≥32%)	8 (6.3)	6 (4.7)	24 (18.9)	82 (64.6)	7 (5.5)
New					
BUA ≤52.85 dB/MHz, SMMI ≤8.2 kg/m^2, and body fat percent (≥32%)	12 (9.4)	5 (3.9)	39 (30.7)	64 (50.4)	5 (3.9)
New					
BUA ≤52.85 dB/MHz, FFMI ≤15.2kg/m^2, and body fat percent (≥32%)	7 (5.5)	10 (7.9)	29 (22.8)	74 (58.3)	5 (3.9)
New					
BUA ≤52.85 dB/MHz, SMMI ≤8.2 kg/m^2, HGS ≤16.5 kg, and body fat percent (≥32%)	5 (3.9)	3 (2.4)	15 (11.8)	46 (36.2)	4 (3.1)

NB:ROC=receiver operating characteristic curve, OSO=osteosarcopenic obesity, OO=osteopenic obesity, SO=sarcopenic obesity, OB=obese-only, NR=normal, appSMMI=appendicular skeletal muscle mass, BUA=broadband ultrasonic attenuation, SMMI=skeletal muscle mass index, FFMI=fat-free mass index, HGS=handgrip strength

4.2.1.2 Lowest Quintile (20th)

In this section, the cut-off values for muscle and bone indices were determined to screen the risk of Osteosarcopenia (OS) in postmenopausal women using the lowest 20th percentile method. For this method, postmenopausal women who reported to had been diagnosed with musculoskeletal-related disorders were excluded from the analysis (i.e osteoarthritis, rheumatoid arthritis, osteoporosis, and people who had previously suffered stroke). Ultimately, data from one-hundred and eighteen postmenopausal women (n=118) were analysed (Table 4.14). For comparison purposes, data from young adults (n=118) were also described on the table.

Table 4.14 shows the cut-off values for muscle mass indices, handgrip strength (HGS) and QUS-generated bone density indices between young and postmenopausal women, derived using the lowest 20th percentile. Result shows that the cut-off values for muscle mass indices (FFMI, SMMI, appSMMI) for young women were slightly lower than those of older women. Conversely, the cut-off values for HGS and bone density indices (BUA, SOS, Est. BMD and T-score) were higher for young women compared to postmenopausal women.

Table 4.14 Cut-off values derived using lowest 20th percentile for the screening of low bone mass and low muscle mass

Variables	N	Young women Mean (SD)	Lowest 20th percentile cut-off	N	Postmenopausal women Mean(SD)	Lowest 20th percentile cut-off	Differences of mean between cut-offs (young - old)
FFMI (kg/m^2)	118	14.8(1.5)	13.5	118	15.8(1.7)	14.4	-0.9
SMMI (kg/m^2)	118	8.0(0.9)	7.1	118	8.5(1.1)	7.6	-0.5
AppSMMI (kg/m^2)	118	5.9(0.7)	5.3	118	6.1(0.9)	5.4	-0.1
Hand grip strength (kg)	117	24.7(4.2)	21.0	112	19.8(5.0)	16.0	+5.0
BUA (dB/MHz)	117	86.5(16.3)	72.9	118	70.3(17.2)	55.3	+17.6
SOS (ms/)	117	1570.9(33.1)	1540.8	118	1525.0(31.9)	1501.4	+39.4
Est. BMD (g/cm^2)	117	0.610(0.122)	0.503	118	0.449(0.124)	0.349	+0.154
T-score (MY Ref.)	117	-0.4(1.1)	-1.3	118	-1.3(1.0)	-2.1	+0.8

N.B:FFMI= fat –free mass index, SMMI= skeletal muscle mass index, appSMMI= appendicular skeletal muscle mass index, BUA= broadband ultrasonic attenuation, SOS=speed of sound, Est. BMD= estimated bone mineral density, MY=Malaysia.

4.2.1.3 Two standard deviation (SD) below the mean value of a young reference group

Table 4.15 shows comparison of characteristics between young and postmenopausal women. Similarly, for this method, postmenopausal women who reported to had been diagnosed with musculoskeletal-related disorders were excluded from the analysis (i.e osteoarthritis, rheumatoid arthritis, osteoporosis, and people who had previously suffered stroke).

Result shows that the younger age group was significantly younger, taller, have stronger handgrip strength and denser bone compared to their older counterparts ($p \leq 0.05$). Conversely, older women were found to be significantly heavier (body weight and BMI), with larger mid-section (WC) and higher body fat percent (BFP) compared to their younger counterparts ($p \leq 0.05$). Consequently, their muscle mass indices (FFMI, SMMI, appSMMI) were also significantly higher than their younger counterparts ($p \leq 0.05$).

Table 4.15a shows cut-off values derived using 2 SD below the mean value of young reference group.

Table 4.15 Differences in characteristics: young vs. postmenopausal women

Variables	*N*	Young women Mean (SD)	*N*	Postmenopausal women Mean (SD)	*P-value	Cohen's d (Effect size)
Age (years)	118	22.1(2.2)	118	60.0(7.8)	**0.00**	-6.574
Height (cm)	118	159.3(5.5)	118	152.8(6.2)	**0.00**	1.104
Weight (kg)	118	56.9(11.6)	118	63.9(12.6)	**0.00**	-0.586
BMI (kg/m^2)	118	22.4(4.5)	118	27.4(5.3)	**0.00**	-1.015
WC (cm)	106	71.9(9.3)	115	84.8(12.5)	**0.00**	-1.171
BFP (%)	118	32.4(7.7)	118	41.1(7.6)	**0.00**	-1.133
FFMI (kg/m^2)	118	14.8(1.5)	118	15.8(1.7)	**0.00**	-0.599
SMMI (kg/m^2)	118	8.0(0.9)	118	8.5(1.1)	**0.00**	-0.482
AppSMMI (kg/m^2)	118	5.9(0.7)	118	6.1(0.9)	**0.05**	-0.248
HGS (kg)	117	24.7(4.2)	112	19.8(5.0)	**0.00**	1.058
BUA (dB/MHz)	117	86.5(16.3)	116	70.3(17.2)	**0.00**	0.970
SOS (m/s)	117	1570.9(33.1)	116	1525.0(31.9)	**0.00**	1.415
Est. BMD (g/m^2)	117	0.610(0.122)	116	0.449(0.124)	**0.00**	1.310
QUI/Stiffness	117	108.1(19.9)	116	82.3(21.0)	**0.00**	1.268
T-score (MY Ref.)	117	-0.4(1.1)	116	-1.3(1.0)	**0.00**	1.323
Z-score	117	-0.1(1.0)	116	0.0(1.0)	0.34	-0.131

N.B: SD=standard deviation, CI=Confidence interval, BMI= body mass index, WC=waist circumference, BFP=body fat percent, FFMI=fat free mass index, SMMI=skeletal muscle mass index, AppSMMI=appendicular skeletal muscle mass index, HGS=hand grip strength, BUA=broadband ultrasonic attenuation, Est. BMD= estimated bone mineral density, SOS=speed of sound, QUI=quantitative ultrasonic index, MY=Malaysia, *analysed using Independent T-test, Formula for Cohen's d=t √(N1+N2/N1*N2), Small=0.2, Medium=0.5, Large=0.8

Table 4.15a Cut-off values derived using 2 SD below the mean value of a young reference group

Variables	*N*	Young women SD x 2	*N*	Postmenopausal women Mean-2 SD of young women
BMI (kg/m^2)	118	4.5*2 = 9	118	27.4 – 9 = **18.4**
WC (cm)	106	9.3*2 = 18.6	115	84.8 - 18.6 = **66.2**
BFP (%)	118	7.7*2 = 15.4	118	41.1 - 15.4 = **25.7**
FFMI (kg/m^2)	118	1.5*2 = 3	118	15.8 – 3 = **12.8**
SMMI (kg/m^2)	118	0.9*2 = 1.8	118	8.5 - 1.8 = **6.7**
AppSMMI (kg/m^2)	118	0.7*2 = 1.4	118	6.1 - 1.4 = **4.7**
HGS (kg)	117	4.2*2 = 8.4	112	19.8 - 8.4 = **11.4**
BUA (dB/MHz)	117	16.3*2 = 32.6	116	70.3 - 32.6 = **37.7**
SOS (m/s)	117	33.1*2 = 66.2	116	1525.0 - 66.2 = **1458.8**
Est. BMD (g/m^2)	117	0.122*2 = 0.244	116	0.449 - 0.244 = **0.205**
QUI/Stiffness	117	19.9*2 = 39.8	116	82.3 - 39.8 = **42.5**
T-score	117	1.1*2 = 2.2	116	-1.3 - 2.2 = **-3.5**
Z-score	117	1.0 * 2 = 2.0	116	0.0 – 2.0 = **-2.0**

N.B: SD=standard deviation, CI=Confidence interval, BMI= body mass index, WC=waist circumference, BFP=body fat percent, FFMI=fat free mass index, SMMI=skeletal muscle mass index, AppSMMI=appendicular skeletal muscle mass index, HGS=hand grip strength, BUA=broadband ultrasonic attenuation, Est. BMD= estimated bone mineral density, SOS=speed of sound, QUI=quantitative ultrasonic index

4.2.2 Comparison of cut-off values between different statistical modelling techniques

Table 4.16 shows differences in cut-off values using 3 different method of analyses. Comparatively, when using the ROC and the lowest 20th percentile method to derive the values, the cut-offs were similar to the standard cut-offs proposed by previous studies; AWGS (Chen, et al., 2014) and Johansen, Evans & Stone (1999).

Interestingly, the cut-off values derived using 2SD below the mean of young reference group were much lower than the standard cut-offs.

Table 4.16 Comparison of cut-off values according to different methods

Variables	ROC Curve	Lowest 20th percentile	2SD below young reference group	Standard cut-off
FFMI (kg/m^2)	15.2	14.4	12.8	-
SMMI (kg/m^2)	8.2	7.6	6.7	-
AppSMMI (kg/m^2)	-	5.4	4.7	5.7[ł]
Hand grip strength (kg)	16.5	16.0	11.4	18.0[ł]
BUA (dB/MHz)	53.0	55.3	37.7	54.0[*f*]
SOS (m/s)	1492.15	1501.4	1458.8	-
Est. BMD (g/cm^2)	-	0.349	0.205	-
T-score (MY Ref.)	-	-2.1	-3.5	-2.5[δ]

NB: ROC=receiver operating characteristic curve, SD=standard deviation, FFMI=fat-free mass index, SMMI=skeletal muscle mass index, appSMMI=appendicular skeletal muscle mass index, BUA=broadband ultrasonic attenuation, SOS=speed of sound, Est. BMD=estimated bone mineral density, MY= Malaysia, *f* Johansen, Evans & Stone, 1999, ł Chen, et al., 2014, δ World Health Organisation (WHO).

CHAPTER 5: DISCUSSION PART II

5.2 Test Criteria for the Screening of Osteosarcopenia in Obese Postmenopausal Malaysian Women

5.2.1 Differences in characteristics of Osteosarcopenic obese (OSO), Osteopenic/osteoporotic obese (OO), Sarcopenic obese (SO), Obese-only (OB) and Normal (NR, non-obese, non-osteopenic/osteoporotic, non-sarcopenic) participants

Osteosarcopenic obesity (OSO) is characterized by concurrent appearance of osteopenia/osteoporosis, sarcopenia, and adiposity. In this study, we identified obese participants with musculoskeletal health disorders (OSO, OO and SO) among community-dwelling, postmenopausal Malaysian women, evaluated their physical performance, and comparing them with Obese-only (OB) and Normal weight women without musculoskeletal health disorders (NR). We hypothesized that women with OSO will have the poorest outcome for each of the variable measured. Postmenopausal women in this study were categorized into OSO, OO, SO, and OB based on the standard criteria and cut-off proposed by WHO, the Asian Working Group for Sarcopenia (AWGS) and Ilich et al. (2016); T-scores, ≤-2.5 (WHO), appSMMI, ≤5.7kg/m^2 (AWGS) and BFP, ≥32% (Illich et al., 2016). Normal weight women without musculoskeletal health disorders (NR) was kept as the control group. Majority of studies (~90%) had used exclusively muscle mass for the definition of sarcopenia, while less than 10% of studies included mass, strength, and performance, as recommended by the European Sarcopenia Consensus (Pagotto and Silveira, 2014). For this reason, we decided to use only muscle mass for the definition of sarcopenia in the classification of OSO and its variants in this study.

After analyses, it was revealed that the majority of women in this sample population was OB at 65.0%, followed by SO at 19.0%, OSO and Normal at 6.0%, and finally, OO at 5.0% (Table 4.10). In contrast to the findings by Ilich et al. (2015), the proportion for SO in the current study was higher than OO. Various studies (including the current study), have found direct and positive correlations between muscle mass and bone density, implying that if one decreases, the other might follow. Muscle loss typically occurs first before bone loss (Kim et al., 2014), hence, in theory, the proportion of people with SO in the population should be higher than OO. People with SO are also more susceptible to progress into full OSO over time. Conversely, OO, where individuals were obese, with healthy volume of muscle mass but low bone mass, should, in theory be difficult to find in the population due to built-in remodeling function of bones. In the current study, there was only 5.0% of participants with this condition (Table 4.10).

OSO is a progressive disorder which can begin with any one of the three conditions: osteopenia/osteoporosis, sarcopenia or obesity. According to Ilich et al. (2016), OSO likely occurs due to deregulation of stem cell lineage, which leads to impedance in the cross talk between bone, muscle and fat through altered concentrations of osteokine (bone), myokine (muscle) and adipokine (fat) (Ilich et al., 2016). People with OSO tend to have a higher risk of falls, fractures, disability and reduced quality of life (Szlejf, Parra-Rodriguez & Rosas-Carrasco, 2017; Ilich et al., 2016; Ilich et la., 2015; Ilich et al., 2014). To date, little is known about the prevalence of OSO in the general population, in no small part

due to lack of consensus in the test criteria, definitions and cut-offs of the syndrome components. We had found that the prevalence of OSO in the current study was higher if the T-scores were generated using non-local young adults as population of reference (i.e. T-score generated using Hong Kong young females as population of reference=10.2% vs. T–score generated using Malaysian young females as population of reference=6.0%). Clearly, the cut-off values and test criteria for the disorder needs to be population-specific. A study by Ilich et al. on Caucasian postmenopausal women in 2015 found that the percentage of OSO in their study population was 12.0% (32 out of 258 postmenopausal women). Comparatively, a Mexican study involving 543 adults found that 16.6% of its study population had OSO (Szlejf, Parra-Rodriguez & Rosas-Carrasco, 2017). However, in a Korean study on postmenopausal women, the prevalence of OSO in their sample population was higher at 25% (Kim et al., 2017). Similarly, Inglis, et al. (2013) also reported the same percentage of women with OSO (25.0%) in a study involving over 500 overweight/obese Caucasian women across the life span (Inglis, Panton, Ormsbee, Kelly, & Ilich, 2013). The high margin in various prevalence studies was likely due to differences in the criteria used, ethnic and genetic background and equipment used, as well as differences in cut-off points, hence the need for a standardization in characterizing OSO in the general population.

It was hypothesized that individuals presenting all three conditions (obesity, sarcopenia and osteoporosis) concurrently were expected to suffer poorer clinical outcomes compared to individuals with either one of the conditions alone

(Kalinkovich & Livshits, 2016; Ilich, Inglis, Kelly, & McGee, 2015). In the current study, the participants in the OSO group were significantly older, slimmer, with smaller waist and lower muscle mass compared to OB participants ($p<0.05$), showing the phenotype of 'fat frail' (Table 4.10). Additionally, the OSO group demonstrated significantly lower handgrip strength, lower muscle mass (whole body and peripheral, Table 4.10) and bone mass (all indices generated by QUS, Table 4.11) compared to NR group. Although no significance achieved, the OSO group in the current study also had lower scores for gait speed, sit-to-stand test and one-leg stance compared to other groups (SO, OO, OB, NR, Table 4.10). This finding supported the finding by Ilich et al. (2015) which showed that OSO syndrome was correlated with low handgrip score, slow normal and brisk walking speed, and short time for leg stance (Ilich, Inglis, Kelly, & McGee, 2015). The current study also found that individuals with OSO were more likely to have HGS <18kg compared to NR group ($p<0.05$, Table 4.12). In fact, no one in the NR group had HGS <18kg (Table 4.12). It was clear that handgrip strength was affected the most among people with OSO, similar to what was found by Ilich et al. (2015). This finding indicates that HGS could be used as additional test criteria for identifying OSO in an individual. Additionally, Shin et al. (2014) reported that both handgrip strength and walking speed were positively associated with muscle mass, and negatively associated with fat mass in older women, similar to the findings of the current study. With regards to bone density, previous studies have found that femoral neck BMD was found to be positively correlated with walking speed and total BMD was positively correlated with HGS (Shin et al., 2014, Lindsey et al., 2005). Although no

significant correlation was found between bone density (BUA) and walking speed, the current study found significant and positive correlation between bone density (BUA) and HGS (Table 4.7).

These findings indicate that low muscle mass (sarcopenia), low bone mass (osteopenia/osteoporosis), and high fat mass (obesity), as seen in the OSO group, may be the reasons for inferior physical performance measured. Reduced grip strength has been shown to lead to a greater risk of fragility fractures and associated morbidity (Theou et al., 2011). Studies have shown that muscle strength is a stronger predictor of long-term functional decline than muscle mass (Schaap, Koster & Visser, 2013). Yang et al. (2015) found that low handgrip strength, combined with high BMI were strongly associated with an increased risk of functional decline (Yang et al., 2015). In an 11-year longitudinal study, low muscle strength and abdominal obesity was associated with increased mobility disability and risk of hospitalization (OR 2.10, 95% CI 1.14–3.88 and OR 1.36, 95% CI 1.04–1.78, respectively) (Jung et al., 2016). High BMI and low muscle strength were also related to limitation in mobility at 2-year follow-up (OR 3.88, 95% CI 1.08–13.91) (Jung et al., 2016). In addition, besides chronological age, gait speed and balance had been identified as the most reliable and accurate measures of frailty in older women. Therefore, low scores for these measures in OSO group is worrisome and in need of a proper attention.

To date, studies on Osteopenic obesity (OO) and Sarcopenic obesity (SO) are still in infancy as these two disorders, especially the former, are just beginning to gather interest by the scientific community due to its fairly recent recognition

(Kelly et al., 2019; Ilich et al., 2016; Ilich et al., 2014; Stenholm et al., 2008; Zamboni et al., 2008). Sarcopenic obesity (SO) is described as concurrent presence of sarcopenia and obesity. Sarcopenic obesity is a major health concern due to its correlation to reduced activities of daily living (ADL) and physical limitations. The combination of high fat and low peripheral muscle mass, leads SO to be recognized as 'fat frail'. This phenotype is known for intramuscular fat accumulation, which caused inflammation, mitochondrial dysfunction and insulin resistance within muscle and reduces the synthesis of muscle proteins (Schrauwen-Hinderling et al., 2006; Visser et al., 2005). Studies have reported that people with SO are more likely to have higher rates of impaired ADL and physical function limitations compared to normal weight individuals with or without sarcopenia (Rolland, Lauwers-Cances & Cristini, 2009; Baumgartner et al., 2004). Interestingly, the current study found no significant differences in age, anthropometrics, body composition and physical performance between OSO group and SO group (Table 4.10), indicating that people with SO have similar degree of physical impairments to OSO. A Korean study involving middle aged and elderly males and females (aged 50 years or older), found that SO was strongly associated with osteoporosis (Chung et al., 2016), suggesting close relation of SO and OSO. In general population, the prevalence of SO was hypothesized to be high among the elderly aged 65 years and older due to age-related increased in fat mass and reduced muscle mass. Currently, there is a difficulty in ascertaining accurate prevalence rates for SO due to lack of a consistent definition for either sarcopenia or obesity. For example, a prevalence study involving individuals with a BMI $\geq$35 kg/m^2 using DXA-defined body fat

in 120 predominantly female adults (46.9 ± 11.0 years) reported that the prevalence of SO ranged from 0–84.5% in women to 0–100% in males depending upon the definition applied (Johnson et al., 2017). In a population-based study using National Health and Nutrition Examination Survey (NHANES) data (appendicular lean mass was used to define sarcopenia), the prevalence of SO was 12.6% in men and 33.5% in women. The study also found that the rates of SO was positively correlated with age, reaching 48.0% in women and 27.5% in men, in those aged over 80 years (Batsis et al., 2017). In Asian studies, the prevalence was equally diverse. In a group of individuals (aged 20–80 years) from Korean Sarcopenic Obesity Study (South Korea), the prevalence of SO ranged from 1.3–15.4% in men to 0.8–22.3% in women (Kim et al., 2009). In Japan, the prevalence of SO among older Japanese women (mean age 72.5 years) was 16% (Takayama et al., 2018). Although it is difficult to compare the prevalence of SO due to differences in populations and the definitions of SO, the approximate average prevalence of SO in older adults was estimated to be about 5–10%, and the prevalence is significantly higher in people aged ≥80 years (Lee et al., 2016).

With regards to functional performance, the current study found that SO group was more likely to have low capability for maintaining balance (≤16sec) compared to their counterparts with healthy muscle mass (OB, $p<0.05$, Table 4.12). Balance is an important ability in many activities of daily living, from simple act such as quiet standing, to more complex tasks such as walking while talking or changing of directions. Low ability to maintain balance typically leads

to falls. In the elderly, falls are relatively common, with 20 to 30% of them experiencing one or more falls annually. A fraction of these events (at least 10%) result in very serious injury such as fractures, dislocation or head injury and these injuries could cost somewhere between $3,476 to $10,749 per faller (Nnodim & Yung, 2015; Davis et al., 2010). Although changes in body composition due to aging is inevitable, SO is a modifiable condition that could be treated and even prevented using various form of therapy. Physical activity, for example, has been recognized as a key lifestyle factor to prevent and delay age-related muscle loss and obesity in the elderly (Goisser et al., 2015). Currently, SO is not fully recognized and thus, are not routinely screened for in clinical practices. This leads to the disorder being unidentified and untreated in the general population. One of the reasons is likely due to lack of clinically viable tools to easily measure body composition and an understanding of how to use them. Without body composition measurements, decreasing skeletal muscle mass can be masked by excess body fat, making SO easy to be overlooked. It is impossible to see changes in body compositions from body weight and BMI measurements, making SO incredibly challenging to be clinically diagnosed. Current body composition measurement tools such as DXA, computed tomography (CT) scan, and magnetic resonance imaging (MRI) are feasible to be used only in clinical and research settings due to high cost and low portability. Further, data from such measurements may not be regularly published in population health studies, and popular measures of physical functionality such as handgrip strength and gait speed, were not readily available in many existing databases. It is therefore, critical to develop effective health screening tools to identify SO, as well as

formulate early intervention strategies to help prevent progression of this disorder. When developing the care management strategy for SO, increased physical activity and optimized diet are some of the approaches that should be included in the early intervention strategies to help limit the progression of disability and loss of independent living. Resistance exercise is a particularly effective method to preserve muscle mass and increase muscle strength in older adults.

There is a marginally lower prevalence of Osteopenic Obesity (OO) in the population, as obese people, in general, tend to have high bone mineral content and density. In the current study, only 5.0% of the study population had the disorder (Table 4.10). OO is a combined condition of low bone density and high body fat. Ilich et al. (2015) reported that obese women with osteopenia/osteoporosis (OO) had significantly lower physical performance (such as normal walking speed) than obese-only women (those with healthy muscle and bone mass), suggesting that bone plays important role in functional performance. In the current study, although no significance were detected, women with OO had weaker balance and handgrip strength compared to OB and NR women (Table 4.10). It is also fair to note that OO group had the highest BMI and body fat percent (BFP) among the groups (OSO, SO, OB and NR, Table 4.10). It is possible that the high adiposity of OO group was one of the reasons for the poor functional performance.

Earlier part of this study had shown positive correlations of fat indices with balance and HGS (Table 4.7). In the current study, in addition to identifying

differences in body composition and physical performance, differences in serum biomarkers were also analyzed, particularly, Vitamin D. Vitamin D is a type of secoteroid that is fat-soluble, which critical function is for increasing intestinal absorption of calcium, magnesium, and phosphate. Vitamin D is instrumental in the metabolism of bones and many cellular and immunological processes. Chronic illnesess such as rickets in children and osteoporosis in adults had been significantly linked to low levels of Vitamin D. Studies have shown that Vitamin D deficiency plays important role in the formation of osteopenic syndrome in obese women (Albrahim & Binobead, 2019, Walsh, Bowles & Evans, 2017). The current study found that OO group was more likely to be Vitamin D deficient (25(OH)D<30nmol/L) compared to their Obese-only (OB) counterparts ($p=0.070$, Table 4.12), suggesting close relationship between bone density and Vitamin D. Other study have reported lower incidence of fractures in individuals with 25(OH)D serum levels greater than 30nmol/L (van Schoor et al., 2008), supporting the possible correlation between Vitamin D and bone density.

Currently, there is a misleading term called 'healthy obese' being used in literature. This term is used due to various studies highlighting the benefits of obesity on health, also known as 'obesity paradox'. Obesity paradox is a medical hypothesis which states that obesity may counterintuitively be protective, rather than detrimental in certain type of conditions, such as osteoporosis and associated with higher survival chance in certain groups of people, such as the very elderly or those with certain chronic diseases. Some examples of obesity paradox include; 1) protective effect of obesity from osteoporosis, and 2) increasing evidence that elderly patients with several chronic diseases and

higher-than-normal BMI, may have lower all-cause and cardiovascular mortality compared to patients of normal weight (Lee & Dierickx, 2018). In the current study, majority of participants were obese with healthy muscle and bone mass (OB=65.0%, Table 4.10). These participants can generally be described as 'healthy obese'. It is interesting to note that although this group had significantly higher peripheral muscle mass (appSMMI) compared to their normal weight counterparts (NR), their handgrip strength was significantly lower compared to NR (Table 4.10). This supports the 'quantity versus quality' argument whereby fat-induced muscle mass has a lower quality compared to protein intake and/or resistance trainings-induced muscle (Lee & Dierickx, 2018). One of the reasons is likely due to intramuscular fat infiltration, reducing the muscle function. Some investigators have shown that an increase in fat mass is positively associated with muscle mass, and intramuscular fat increases by 35.5-74.6% in men and 16.8-50% in women with aging (Miljkovic & Zmuda, 2010). Muscle mass is only useful if it is beneficial to functional performance. Findings from the current study showed that the muscle mass of the 'healthy obese', while high, was not sufficient nor efficient in giving meaningful benefits to grip strength. This supports the theory that obese individuals may require alternative cut-offs, or at least, a different set of criteria from normal population for the diagnosis of muscle disorders. Perhaps higher cut-off is required for the obese population. Further, while OB and NR groups had similar amount of muscle mass, OB had significantly higher weight and body fat percent than NR group (Table 4.10). This shows that OB had significantly heavier weight to carry with similar amount of muscle mass to people with normal weight, fitting a 'moped pulling a

speedboat' analogy, aptly used by Dr. Neil Binkley from the University of Wisconsin-Madison to describe Sarcopenic obesity. This condition is dangerous as it can increase the risk of falls, causing serious muscle and bone-related injuries. As mentioned earlier, the current study had used standard cut-off to describe sarcopenia. Perhaps if alternative and BMI-specific cut-off was used, the prevalence for SO (consequently, OSO) would be higher. Domiciano et al. (2013) had provided some evidence in support of this theory. The researchers theorized that standard criteria for height-adjusted appendicular skeletal muscle mass (appSMMI, kg/m^2) to define sarcopenia may underestimate prevalence in overweight/obese people. As evidence, the study found that the frequency of sarcopenia was lower using the criteria of appSMM/height^2 (3.7%) than appSMM adjusted for fat (19.9%) ($p < 0.0001$). They also note that less than 5% (1/23) of sarcopenic women, according to appSMM/height^2, had overweight/obesity, whereas 60% (74/122) of sarcopenic women by fat-adjusted appSMM were overweight/obesity. Therefore, in the case of overweight/obese population, it may be more accurate to use fat-adjusted variables or population of similar body composition as the reference group (instead of using young adults).

5.2.2 Determining cut-off values for the screening of osteosarcopenia in obese postmenopausal women using bio-impedance analysis (BIA) and quantitative ultrasound (QUS).

The current definitions of osteosarcopenic obesity (OSO) are based on the individual definitions of osteoporosis, sarcopenia and obesity. However,

questions arise if OSO should be treated as a singular entity and derive cut-points accordingly. Currently, there are no established criteria to define and to properly diagnose OSO, although there have been some preliminary diagnostic criteria proposed (Ilich, Kelly, & Inglis, 2016; Ilich et al., 2014). The criteria, however, requires the use of sophisticated equipment available only in specialized laboratory and hospitals; i.e. DXA scan. According to Ilich, Kelly, & Inglis (2016), the following are required to diagnose OSO;

i) T-score of L1–L4 of the spine and/or total femur or femoral neck <−1.0, assessed by DXA

ii) Lowest 20th percentile of appendicular lean mass (ALM) for women, with the equation: ALM = -17.4 +18.3 x height (m) + 0.16 x body fat (kg), assessed by DXA, and

iii) Body fat percent ≥32%, also assessed by DXA.

Studies have shown that different definitions of sarcopenia (low muscle mass) is related to different clinical outcomes, especially in aging population. For example, Jang et al. (2018), who studied sex-specific distributions of muscle indices adjusted by height, weight, and BMI, found that height-adjusted muscle mass index showed significant association to major health outcomes only in women. Further, anthropometric parameters are affected by ethnic differences, causing researchers to establish population-specific definitions of the decreased muscle mass in various countries. Therefore, determining appropriate cut-off values for sarcopenia diagnosis in Asia is critical to ensure accurate diagnosis

and device proper treatments specific for Asian population. Several authoritative research groups have proposed varying definitions of sarcopenia. Persistent controversies exist in how to define reduced skeletal muscle mass. Different cut-off also exist for different methods of assessment. EWGSOP recommends DXA, computed tomography (CT), magnetic resonance imaging (MRI), and bio-impedance analysis (BIA) for sarcopenia studies. Although the precision of DXA, CT, and MRI had been well established, some limitations exist when using BIA in measuring muscle mass. BIA was developed to estimate the volume of body fat and whole body lean muscle mass. Very few models of BIA were created with a function to measure appendicular lean mass (appSMMI), rendering the use of current diagnostic criteria difficult to be used for screening purposes in the general population (appSMMI $\leq$5.7kg/m^2). In order to limit the use of non-portable and costly diagnostic devices, researchers have attempted to develop screening methods to allow the clinicians to identify only high-risk individuals to undergo a more demanding diagnostic instrument to determine the presence of sarcopenia. Early identification of sarcopenia will allow the implementation of preventive strategies, thus reducing the risk of fracture and hospitalization.

Osteoporosis and sarcopenia are asymptomatic. Normal procedure at most general practices requires a patient to be sent for scans only if a fracture occur, or if there are notably reduced function of the limbs. Therefore, most cases of muscle and bone-related disorders are detected only when it's too late for effective interventions. Since early diagnosis is critical in treating

osteosarcopenia, a comprehensive screening criteria is needed. Currently, the most logistically-friendly and cost-effective screening techniques available for osteoporosis, sarcopenia and obesity would be quantitative ultrasound-based devices (QUS) and bioelectrical impedance devises (BIA). These devices are portable and the time required for the assessments are short, making them the best devises to be used for screening methods in the general population. For example, calcaneus QUS machines such as the SAHARA® bone densitometer had been found to be reliable at predicting risk of fracture in postmenopausal women (hip, vertebral and global fracture risk) and men aged 65 years and above (hip and all non-vertebral fractures), independently of central DXA measurement of bone density (International Society of Clinical Densitometry (ISCD) Official Positions-Adult, 2015). However, it is important to note that QUS and BIA devices are for screening purposes only and unsuitable for diagnostic nor treatment monitoring effects. At many general practices, individuals with low QUS results are advised for further BMD measurement using DXA scan. In some instances, people with physical disability such as frail elderly may have difficulty to attend a DXA facility. In those cases, BIA and QUS may be a useful, low-cost alternative, which can be combined with grip strength to diagnose sarcopenia and osteoporosis.

Currently, the cut-off values for sarcopenia and osteoporosis using BIA and QUS have not yet been determined in the Malaysian population. One of the aims of this study was to determine cut-off values for low muscle quality (mass and strength) and low bone density, using portable equipment suitable for screening

purposes (i.e. BIA and QUS). In addition, the current study had compared and contrasted the cut-off values derived by 3 different statistical modelling methods and discussed the most suitable method to screen for the presence of OSO among Malaysian postmenopausal women.

Sarcopenia working groups such as AWGS (Asian) and EWGSOP (European) had proposed diagnostic classifications and cut-off point identification using various different methods. AWGS recommended 2 standard deviations (SD) below the mean value of a young reference group or lowest quintile (20%) of the study population as a cut-off, whereas EWGSOP recommends only the former. Often, population and gender-specific lowest quintile (predictive technique) was used as the cut-off value if population norms of young adults was not available. Other studies had used ROC curve to derive cut-off points for the diagnosis of sarcopenia (Kim et al., 2014; Yang et al., 2018). In the current study, we determined the cut-off values for Osteosarcopenia using all 3 statistical modelling techniques; 1) ROC curve, 2) lowest quintile of study population, 3) 2 SD below the mean value of a young reference group, and compared the values with standard cut-off values available from other studies. The variations in cut-off values for the estimation of muscle and bone mass are shown in Table 4.15.

5.2.2.1 ROC Curve

In order to develop cut-off values for the screening of Osteosarcopenia as a singular entity, receiver operating characteristic (ROC) analysis was the best and the most appropriate method to use. ROC analysis allowed us to determine the area under the curve (AUC). This type of analysis only furnishes dichotomous

results to the screening test; with and without the disease. One of the most important criteria of an ideal screening test is the ability to demonstrate accurate sensitivity and specificity. In order to determine whether the screening test is positive or negative, we used cut-off values proposed by previous studies to identify obese participants (BFP ≥32%) with osteosarcopenia (T-score ≤-2.5, appSMMI ≤5.7kg/m^2). The reference group were obese-only (OB) participant (BFP≥32%) without osteosarcopenia (T-score>-2.5, appSMMI>5.7kg/m^2). The 'Sensitivity' represents the proportion of participants actually presenting the specified criteria for Osteosarcopenia, and having been correctly identified as osteosarcopenic, using the screening tool (positive screening test). The 'Specificity' represents the proportion of participants who do not actually have Osteosarcopenia, and were correctly identified as non-osteosarcopenic, using the screening tool (negative screening test). The positive predicted values (PPV) is defined as the probability of presenting osteosarcopenia in case of a positive test result, and the negative predicted values (NPV) is the probability of not suffering from Osteosarcopenia in case of a negative test result. All of these proportions were presented with their exact 95% confidence interval (CI). An AUC value less than 0.5 reflects no discriminatory power, while an AUC between 0.5 and 1.0 has high predictive value for clinical testing (Park, Goo & Jo, 2004). As a rule of thumb, an AUC closer to 1 demonstrates a higher screening power and considered to perform better at distinguishing those at risk of osteosarcopenia compared to those not at risk. In the current study, we highlighted an excellent performance of the HGS, SMMI, FFMI, BUA and SOS to predict osteosarcopenia in obese postmenopausal women (AUC value up to 0.9). The

ROC curve showed that HGS (≤16.5kg, Figure 4.4), FFMI (≤15.2kg/m^2, Figure 4.5), SMMI (≤8.2 kg/m^2, Figure 4.6), BUA (≤52.85 dB/MHz, Figure 4.7) and SOS (≤1492.15 m/s, Figure 4.8) were the best markers for the screening of Osteosarcopenia. The cut-off values were identified by maximizing the sum of sensitivity and specificity derived on the basis of the persistent lower extremity limitation outcome. When these cut-off values were used to determine OSO (BFP≥32%, SMMI≤8.2 kg/m^2, BUA≤53 dB/MHz), the prevalence increased 1.5 times in the sample group (standard cut-off=8 people with OSO vs. New ROC Curve cut-off=12 people with OSO, Table 4.13). This finding corresponds to the finding obtained by Kim et al. (2014) who reported the increase of likelihood by 1.88 times when their ROC-derived cut-off values were used to predict the risk of osteoporosis in sarcopenic elderly.

Currently, no cut-off values exist for these parameters in relation to OSO as singular entity. The cut-off values for FFMI and SMMI, which represent whole body muscle mass, would be useful for types of BIA without the ability to assess appendicular lean mass (appSMMI). Further, the cut-off for BUA will be useful for the screening of bone density in Malaysian women, as currently available QUS devices do not generate T-scores based on this population. The advantage of this particular method (ROC Curve analysis) was the ability to determine cut-off values based on obese people presenting both osteoporosis and sarcopenia at the same time. The curve was plotted against obese people without the simultaneous form of disorder. This allowed us to see how much worse-off an individual would be without accounting obesity as a factor (obesity is the

common denominator). In the current study, it was revealed that obese people with Osteosarcopenia were likely to have HGS equal or less than 16.5 kg (Figure 4.4), which was marginally lower than the cut-off proposed by AWGS for Asian population (18.0 kg). Ilich et al. (2015) studying 258 white postmenopausal women with age of 61.6±7.4 years and $\geq$ 35% of body fat, found that obese women with Osteosarcopenia had significantly lower HGS compared to obese women without Osteosarcopenia. Weak handgrip strength has been shown to lead to a greater probability for fractures and associated morbidity (Theou et al., 2011). Therefore, women with OSO carries with it a higher risk for frailty and fracture risk. Further, the lower HGS strength found in the current study may indicate fat infiltration into both muscle and bone, impairing each tissue's physiology and functioning.

While whole body MRI had been established to provide the most accurate measurement of skeletal muscle mass, the use of the machine is limited by low access, high cost and invasive nature. These issues are also shared by the gold-standard dual-energy X-ray absorptiometry (DXA), which limit its utility in field studies. Therefore, the BIA is a more convenient method to be used in field studies. In the current study, whole-body skeletal muscle mass index (SMMI) of $\leq$8.2kg/m^2 was derived using ROC Curve (Figure 4.6). This value is greater than the cut-off set by several Asian and Western populations; SMMI <6.40 kg/m^2 (China, Lau et al., 2005), SMMI <6.20 kg/m^2 (France, Tichet et al., 2008), SMMI <6.50 kg/m^2 (Taiwan, Chien et al., 2008), SMMI <6.68 kg/m^2 (Spain, Mesanes et al., 2012), SMMI <6.75 kg/m^2 (USA, Janssen et al. 2002), SMMI <5.22 kg/m^2

(Mexico, Alemán and Ruiz, 2014). These cut-off values, however, were derived using two standard deviations (SD) below the mean value of young adults, rather than ROC Curve. Studies have shown that BMI and BFP are positively correlated with SMMI (Fukuoka et al., 2019). It is possible that the higher cut-off for SMMI derived from the ROC Curve is due to the high BFP of the group (≥32%).

Interestingly, the BUA cut-off derived by ROC Curve in the current study was similar to the cut-off proposed by Johansen, Evans & Stone (1999); 53.0 dB/MHz vs. 54.0 dB/MHz (Johansen, Evans & Stone, 1999). Johansen, Evans & Stone (1999) found that subjects with BUA below the 54 dB/MHz threshold value were shown to have low femoral neck BMD. The study, however, had used 2.5 SD below the young adult mean as the method to derive the cut-off value. Young adults tend to have higher bone density compared to older adults, which likely raised the cut-off. High bone density has been consistently found to be correlated with obesity. Therefore, it is possible that the similarity in cut-off values is due to the reference groups (young adults and obese population).

Findings from the current study suggest that individuals with high adiposity may require alternative cut-points to define low bone mass and low muscle mass. ROC Curve method, in particular, allowed us to derive cut-off values specifically for obese population by using the obese as the population of reference, instead of mixing people of normal weight into the reference group. Studies have found that the body has its own conserved regulatory mechanism or 'mechanical thermostat' (mechanostat) that senses mechanical strain and adapt accordingly. This adaptive mechanism optimize bone mass and architecture based on

prevailing mechanical strain. Therefore, any changes in weight, whether due to excessive caloric intake (obesity), weightlessness (spaceflight), and hypergravity (modeled by centrifugation), will induce an adaptive skeletal response (Lenzi et al., 2014). Therefore, as an 'adaptive' mechanism, a body with high adiposity will have stronger core structure to support the extra weight (high bone density and muscle mass). However, questions arised whether the adaptive response maintains the mechanical competence of the skeleton, which means it is difficult to establish if added quantity of the mass is accompanied by increased quality. The mechanostat support bone metabolism (i.e. mineral homeostasis, hormones and nutrient availability) by inputs sent by the regulatory mechanism. While the skeleton is efficient in adapting to any weight change, the mechanostat has limits. When the mechanostat reached its limits, extreme deviations from normal weight and body composition are associated with reduced bone strength to increasing body size. With regards to bone mass and muscle mass, what is considered as 'sufficient' for normal weight individuals may not be so for people with high adiposity, hence why they may require alternative cut-points. Lenzi et al. (2014) also raised the question in the accuracy of defining sarcopenia in obese individuals. The author states that in people with Sarcopenic obesity, the simultaneous increase of muscle mass in parallel with body fat mass serve as a protective mechanism for the obese individuals to sustain the additional fat mass. In other words, the increase of muscle mass in parallel of fat mass is a way for the body to protect itself by increasing the core structure to sustain the extra weight load. This is also describe by Wolff's law of bone formation (Wolff et al., 1999). Wolff's law, which was developed by a German anatomist and surgeon

Julius Wolff (1836–1902) in the 19th century, states that bone in a healthy person or animal will adapt to the loads under which it is placed (Wolff et al., 1999; Frost, 1994). Since muscle and bone grows in tandem, this law is also applied to muscle. However, this protective mechanism will only occur up to a point (mechanostat limit) until there is an imbalance between muscle mass, excess body fat and total body size, resulting in disproportionate ratios between muscle mass and excess fat mass, creating a situation where body weight exceeds that which the muscle mass can support. The authors hypothesised that despite the appearance of good muscle mass in obese people, it probably is not sufficient in proportion to the total body mass to prevent the onset of functional impairment and disability (Lenzi et al., 2014). A study by Norshafarina et al. in 2013, which categorised its study sample into sarcopenic group and non-sarcopenic group, found that overall skeletal muscle mass (SMMI) for the non-sarcopenic Malaysian women over the age of 60 was 8.24 ±3.74 kg/m^2 (Norshafarina et al., 2013). This value is similar to the current study (8.2 kg/m^2, Figure 4.6). This finding shows that the cut-off value of SMMI to define sarcopenia in obese individual is similar to the mean SMMI of women without sarcopenia, suggesting the need for alternative cut-off for the obese population.

5.2.2.2 Lowest quintile (20th)

Separating data into quintiles is another method used to create cut-off points for a given population. A quintile is a statistical value of a data set that represents 20% of a given population. The first quintile represents the lowest fifth of data and final quintile represents the final or last fifth of a data. This is a good method

to show distribution of data. For example, in order to determine the distribution of wealth in the society, a socio-economic study conferred by the government may use this method to quantify the maximum amount of money a family could possess in order to belong to the lowest quintile of society. This maximum amount can then be used as a pre-requisite for a family to receive a type of welfare or special government subsidy aimed to assist the people of less fortunate in the society. In the case of sarcopenia, researchers have used this method based on the same concept, and had been widely accepted by several working groups for sarcopenia (Chen et al., 2014; Fielding et al., 2011; Cruz-Jentoft et al., 2010). Using quintiles is a convenient way to represent data. However, it might not be the best way to categorize data when the exposure is not normally distributed. In the current study, SMMI and appSMMI were non-normally distributed data (although FFMI, HGS and BUA were all normally distributed). Using this method, data from a reference group is not required, which is one of the advantages of this method. Further, cut-off values can be determined for any desired parameters. A study by Jang et al. (2018) found that the cut-off points using the lowest quintile of Korean rural older adults was <5.2 kg/m^2 for women in height-adjusted appSMMI, comparable to reports from other countries. A Taiwanese study using the same method found the cut-off for decreased appSMMI was <5.5 kg/m^2 for women (Lee et al., 2013). A Korean study found that the cut-off values for appSMMI was <5.4 kg/m^2, HGS <9.1 kg, and gait speed <0.5 m/s using the same method (Moon et al., 2016). In the current study, the cut-off for appSMMI derived from this method was <5.4 kg/m^2 (Table 4.14), comparable to the study in Taiwan and Korea. The cut-off for handgrip strength,

however, was higher in the current study compared to a Korean study (HGS <16 kg [current study, Table 4.14] vs. HGS <9.1 kg [Moon et al., 2016]). The Korean study, which had compared their own cut-off values (derived using lowest quintile) with cut-off values recommended by the Foundation for the National Institutes of Health (FNIH), argued that using the lowest quintile method showed better predictive values in mortality than using the FNIH cut-offs. However, this argument is only based on one study.

5.2.2.3 Two SD below the mean value of a young reference group

Despite its limitations, the most used method to derive cut-off values is the 2 standard deviations (SD) below the mean for a young reference population. Working group such as EWGSOP, in particular, recommends the use of this method in defining sarcopenia. This recommendation is based on the understanding that body composition may be affected by race and environmental factors such as diet and physical activity. Therefore, the reference population should derive from the same population of interest, and represents people who are at peaked conditions, such as the young adults (Table 4.15). In the current study, the cut-off values derived using this method were lower than previously mentioned methods (ROC Curve and lowest quintile), including the standard cut-offs (Table 4.15a). Considering skeletal muscle mass, muscle strength and bone density, cut-off points defined as 2SD lower than healthy young adults in this study were the following; SMMI <6.7kg/m^2, appSMMI<4.7kg/m^2, HGS<11.4kg, and BUA<37.7dB/MHz (Table 4.15a). These cut-offs were also much lower than the cut-offs proposed by AWGS for the Asian population and

other studies (Table 4.16). Height-adjusted appendicular skeletal muscle mass, in particular, was much lower than other Asian and Western studies which had used the same methods to derive the cut-off value; appSMMI≤5.40 kg/m^2 (AWGS, Chen et al., 2014), appSMMI <5.8 kg/m^2 (Japan, Tanimoto et al., 2012), and appSMMI <5.07 kg/m^2 (Korea, Goodpaster et al., 2006), appSMMI≤5.50 kg/m^2 (EWGSOP, Cruz-Jentoft et al., 2010), appSMMI≤5.67 kg/m^2 (IWGS, Fielding et al., 2011), appSMMI≤5.18 kg/m^2 (Society of Sarcopenia, Cachexia and Wasting Disorders, Morley et al., 2011). However, three studies had produce similar findings; appSMMI <4.72 kg/m^2 (Mexico, Alemán and Ruiz, 2014) and appSMMI <4.82 kg/m^2 (China, Lau et al., 2005), appSMMI<4.4 kg/m^2 (Korea, Kwon, Ha & Park, 2015). Despite its popularity, this method is not without limitations. Visser (2009) argued that the current definition for sarcopenia refers to a state of deficiency in muscle mass and does not indicate muscle loss (Visser, 2009). Various studies had shown progressive loss of musculoskeletal health after the third decade of life, and continue to lose 1-2% of muscle mass after the fifth decade and becoming more evidenced after the sixth (Zacker, 2006). Therefore, cautioned was advised when using young adults as group of reference as they have not been exposed to the same factors that older people have experienced throughout their lives, in addition to the already natural progression of muscle loss due to aging. Perhaps, using healthy, community-dwelling elderly with high quality of life could reflect with greater precision the deficiency of muscle mass instead of the comparison with young population. Therefore, various studies on the causes of sarcopenia are taking into account factors beyond just the risk factors, such as physical inactivity, dietary

intake, hormonal influence, and cytokine levels, recognizing the disorder as a geriatric syndrome (Rolland, et al., 2008).

5.2.3 Comparisons of diagnostic criteria and corresponding cut-off values

This section compares the above-mentioned diagnostic methods and the corresponding cut-off values (Table 4.16), while highlighting the problems resulting from the lack of uniformity in diagnostic criteria. Often, screening tools are chosen based on the means and objective of the researchers. For example, T-scores requires time-consuming calculations, which may hinder its use. Therefore, in the case of QUS, direct cut-off value for indicator such as the BUA may be preferred. In addition, other efficiency criteria are also to be taken into account such as the rapidity, simplicity and easy administration of the screening tool use. This reflects the feasibility of these tools in clinical practice. In addition, creating specific cut-off points for high-risk group such as the obese population is important for accurate labelling and identification of low bone mass and low muscle mass so that appropriate intervention can be instigated and reducing the risk of having advanced musculoskeletal disorder in their later years. Uniformity in diagnostic criteria is also important to facilitate standardizations and comparisons of this disorder between countries. Lack of uniformity could adversely affect public health policies. For example, any over- or under estimation of the prevalence of a particular disorder could increase the risk of providing unnecessary treatment to a false-positive patient or depriving a false-negative patient from treatments. In the current study, we proposed cut-off values suitable for obese population, using portable and cost-effective screening tools.

However, it is important to note that these cut-offs are given as an indication, due to the study's limitations in its external validity. Although the BIA and QUS methods are reliable techniques for measuring body composition and bone mineral density, they are by no means proper diagnostic tools. The next step of the study will be to validate the cut-offs using dual-energy x-ray absorptiometry (DXA), and with a larger sample size. Although the study used statistical inference techniques, biases may have been present, largely due to the participant selection process which was mainly composed of voluntary subjects. These subjects could be more health-conscious and be willing to undertake a 1-hour interview, including blood collection and body composition assessments, than a random sample of the population. However, the current study's comparison of various diagnostic methods may bridge the gap in knowledge of Osteosarcopenia and contribute towards a more evidence-based and less theoretical definition of the syndrome, not only in epidemiological research but also in health services. This study may contribute to the effort in standardizing the diagnostic criteria for Osteosarcopenia and accurately determine the magnitude of the syndrome in the elderly. ROC Curve method may be the best statistical modelling method to be used for deriving cut-off values for Osteosarcopenia studies in overweight and/or obese populations. In addition to showing the differences in cut-off values derived from using different methods, the current study demonstrates that a low proportion of the Malaysian postmenopausal women had Osteosarcopenic Obesity (6.0%, Table 4.10). This rate, however, may rise without warning if no awareness was instilled in the population.

Osteosarcopenic obesity (OSO) is a prevalent musculoskeletal syndrome conferring an increased risk of falls, fractures, and hospitalizations. Findings from preliminary studies suggest that OSO could be a good target for translational research due to interconnected pathways illustrating a cross-talk among fat, bone and muscle tissues. Currently, resistance exercise, high protein, Vitamin D, calcium, and creatine intake are the only evidence-based strategies to reduce the progression of osteoporosis and sarcopenia. More research in OSO is needed as the recognition of this syndrome is currently in its infancy. Increased awareness among geriatrics and gerontology healthcare professionals is important and must be included in the context of public health policies.

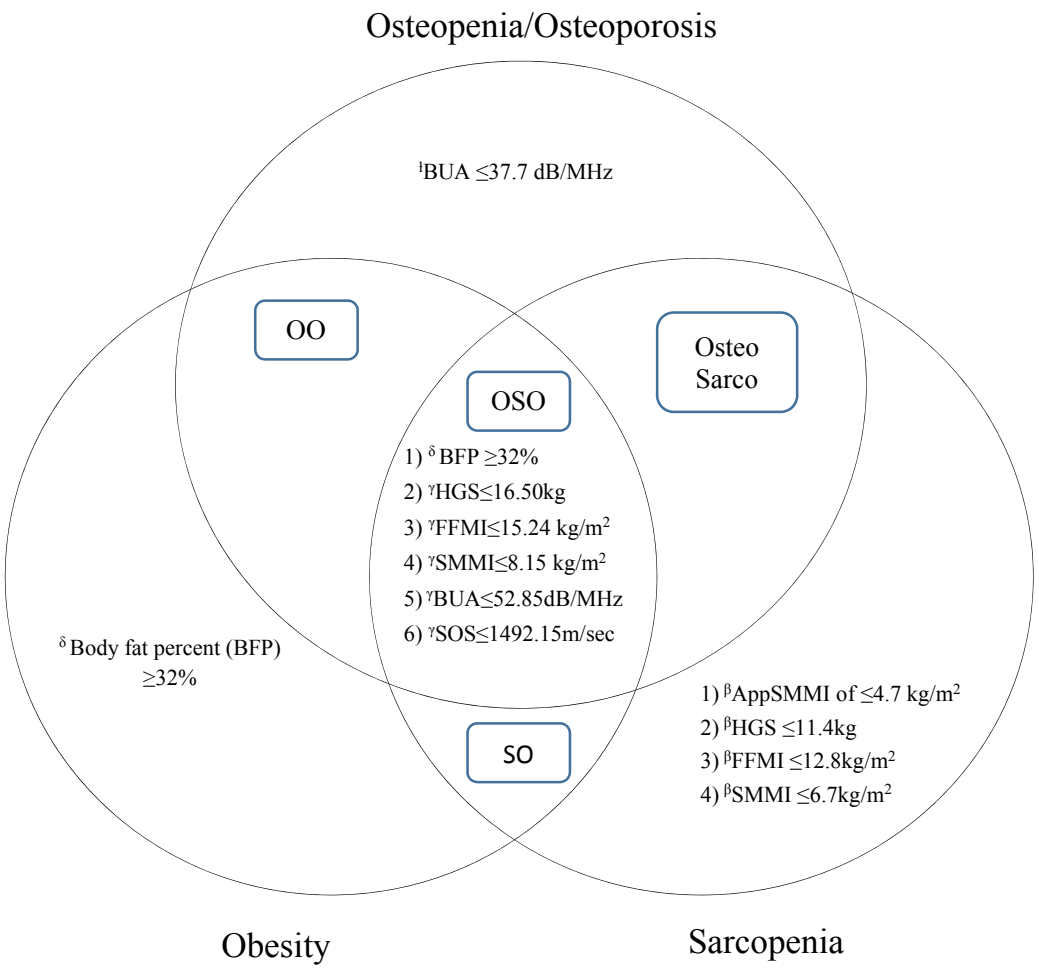


Figure 5.1 Hypothetical presentation and a tentative characterization of obesity and musculoskeletal health disorders based on QUS and BIA indices assessed among postmenopausal Malaysian women (δ proposed by Ilich et al., 2016, γ derived based on ROC Curve plotted against obese-only group, ł, β derived based on 2SD below young Malaysian reference group), OSO: osteosarcopenic obese, OO: osteopenic obese, SO: sarcopenic obese, BUA: boroadband ultrasonic attenuation, appSMMI: appendicular skeletal muscle mass index, SMMI: skeletal muscle mass index, FFMI: fat free mass index, HGS: hand grip strength)

CHAPTER 4: RESULTS PART III

4.3 Serum 25(OH)D and its association with bone, fat and muscle

This section describes the levels of 3 forms of vitamin D (total 25(OH)D, bioavailable 25(OH)D, free 25(OH)D) and their associations with bone, fat and muscle. One hundred and twenty (n=120) postmenopausal women consented to their blood being drawn and analysed for intact parathyroid hormone (iPTH), albumin, calcium, vitamin D binding protein (VDBP), and total 25(OH)D. One sample for iPTH was excluded from analysis due to being an outlier and 4 samples for VDBP were excluded due to high percentage of coefficient variations (% CV).

The results for blood parameters of the participants are shown on Table 4.17. The means of total, bioavailable and free 25(OH)D were 52.4(17.7) nmol/L, 6.9(3.0) nmol/L, and 17.3(7.2) pmol/L, respectively. The result for each blood parameter was all within the normal range for adults.

Table 4.17 Blood parameters of participants

Variables	n	Minimum-Maximum	Mean(SD)	Reference range for adults[ł]
Total 25(OH)D (ng/mL)	120	9.20-47.04	21.0(7.1)	
Total 25(OH)D (nmol/L)	120	23.0-117.6	52.4(17.7)	>20nmol/L[γ]
Serum Ca (mmol/L)	120	2.15-2.65	2.4(0.1)	2.10-2.55 mmol/L
Serum Ca (mmol/L) (corrected)	120	2.02-2.49	2.3(0.1)	
Plasma iPTH (pmol/L)	119	1.60-12.30	5.8(2.7)	1.5-7.6 pmol/L
Serum albumin (g/L)	120	38.0-50.0	44.6(2.8)	35-50 g/L
Bioavailable 25(OH)D (ng/mL)	116	1.13-6.80	2.8 (1.2)	1.92-8.82 ng/mL
Bioavailable 25(OH)D (nmol/L)	116	2.83-17.0	6.9(3.0)	
Free 25(OH)D (pmol/L)	116	6.87-43.2	17.3(7.2)	
VDBP (ng/mL)	116	123500.0-327700.0	224727.6(44831.5)	
VDBP (ug/mL)	116	123.5-327.7	224.7(44.8)	104 – 477 ug/ml

N.B: 25(OH)D= 25 hydroxyvitamin D, Ca= calcium, iPTH= intact parathyroid hormone, VDBP= vitamin D binding protein, ł Pan Laboratories, Irvine, USA reference range for adults, http://panlaboratories.com/bioavailable-vitamin-d-25-hydroxy/ which were based on previous studies (Bhan et al., 2012, Powe et al., 2011, Powe et al., 2010), γTuan Salwani Tuan Ismail, et al., 2017

Table 4.18 describes the characteristics of people with different statuses of Vitamin D (deficient, insufficient, and replete). Majority of participants in this cohort were in the 'Vitamin D replete' category (51.7%, >50nmol/L), followed by 'Vitamin D insufficient' (40.8%, 30-50nmol/L) and 'Vitamin D deficient' (7.5%,<30nmol/L).

Result shows that participants with lower levels of Vitamin D (deficient and insuffient groups) had significantly higher body fat percent (BFP) ($p<0.05$) and lower free and bioavailable 25(OH)D ($p<0.001$) compared to participants with higher levels of Vitamin D (replete group). Additionally, participants in the 'deficient' group had significantly lower bone density (BUA) and calcium level compared to the groups with higher levels of Vitamin D (insufficient and replete) ($p<0.05$).

With regard to functional performance, participants with lower levels of Vitamin D (deficient and insufficient) were found to have significantly lower endurance (walk speed) compared to participants with higher level of Vitamin D (replete, $p<0.01$). No signicant differences, however, were found for lower extremity strength (sit-to-stand test) and balance between the groups. There were also no significant differences found for any of the muscle indices, handgrip strength (HGS) and VDBP between the groups.

Table 4.18 Differences in characteristics for participants who were vitamin D deficient, insufficient, and replete, N=120

Variables	Deficient (<30nmol/L)^{f} Mean (SD), n=9	Insufficient (30-50nmol/L)^{f} Mean (SD), n=49	Replete (>50nmol/L)^{f} Mean (SD), n=62	P-value
HGS (kg)	20.7 (5.5)	18.7 (4.7)	20.8 (4.6)	0.069
AppSMMI (kg/m^2)	6.0 (1.3)	6.1 (0.8)	6.1 (0.7)	0.944
SMMI (kg)	8.7 (1.5)	8.4 (1.0)	8.4 (0.9)	0.691
FFMI (kg)	16.3 (2.3)	15.8 (1.6)	15.7 (1.5)	0.547
BFP (%)	43.6 (9.8) **	43.1 (6.7)**	38.8 (8.1)	**0.010***
WC (cm)	89.0 (14.8)	84.6 (12.6)	83.9 (12.4)	0.526
BUA (dB/MHz)	56.5 (16.6)**	68.5 (15.8)	72.2 (17.4)	**0.029***
Sit-to-stand test (times in 30 sec)	11.8 (2.6)	11.1 (3.7)	12.4 (3.6)	0.178
Walk speed (m/s)	0.7 (0.2)**	0.9 (0.2)**	1.0 (0.3)	**0.003***
Balance (sec)	17.9 (11.7)	17.3 (11.8)	21.3 (9.8)	0.163
Calcium (mmol/L)	2.3 (0.1)**	2.4 (0.1)	2.4 (0.1)	**0.035***
iPTH (pmol/L)	9.1 (3.1)**$^{\#}$	6.7 (2.5)**	4.7 (2.2)	**0.000***
Bioavailable 25(OH)D (nmol/L)	3.6(0.6)**	5.3(1.2)**	8.8(3.0)	**0.000***
Free 25(OH)D (pmol/L)	9.2(1.6)**	13.2(3.1)**	22.0(7.0)	**0.000***
VDBP (ug/mL)	206.1(36.8)	227.1(48.3)	225.6(43.0)	0.430

N.B: HGS= handgrip strength, 25(OH)D= 25 hydroxyvitamin D, BUA = Broadband ultrasonic attenuation, iPTH=intact parathyroid hormone, appSMMI=appendicular skeletal muscle mass index, SMMI=skeletal muscle mass index, FFMI=fat free mass index, BFP=body fat percent, WC=waist circumference, VDBP=vitamin D binding protein, SD = standard deviation, *f*= based on cut-points by IOM (Institute of Medicine) Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: The National Academies Press; 2011, *analysed using one-way ANOVA with Tukey's HSD Post-hoc test, p-value<0.05, **=different from Replete, #=different from Insufficient

Figure 4.9 shows ethnic disparities (Malays, Chinese and Indians) for various forms of 25(OH)D (total, free and bioavailable 25(OH)D).

Result shows that the Chinese had significantly higher level of total, free and bioavailable 25(OH)D compared to Malays and Indians ($p<0.05$). Although there were slight variations in the levels of Vitamin D indices between the Malays and Indians (Indians>Malays), no statistical significance were found.

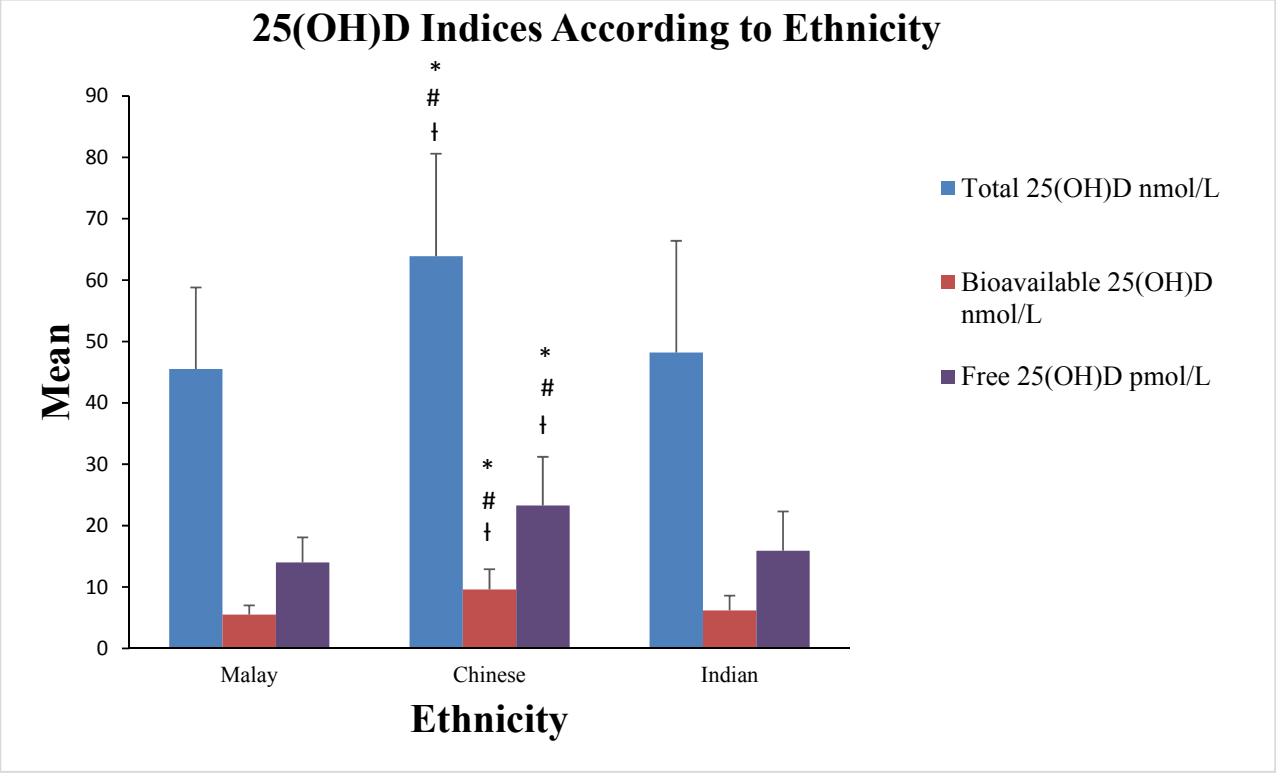


Figure 4.9 25(OH)D indices according to ethnicity; Error bars: + 1 Standard deviation, *analysed using one-way ANOVA, with Tukey's HSD Post-hoc test, $p<0.001$, ł=different from Indians, #=different from Malays

Figure 4.10 shows the differences in various forms of 25(OH)D (total, free and bioavailable 25(OH)D) between participants with bone and/or muscle disorders; Osteosarcopenic Obese (OSO), Osteopenic Obese (OO) and Sarcopenic Obese (SO).

Result shows that each form of 25(OH)D (total, free and bioavailable 25(OH)D) was affected by body fat percent (BFP %) where it was found that healthy participants with normal body fat percent (NR groups) had significantly higher levels of total, free and bioavailable 25(OH)D compared to Obese-only (OB) group ($p<0.05$).

Although both OSO and OO groups were obese with low bone density, OO group, which had the highest body fat percent [mean (standard deviation: 44.9(4.7)%], was found to have the lowest 25(OH)D levels (total, free and bioavailable 25(OH)D), compared to OSO and the rest of the groups (albeit non-significant).

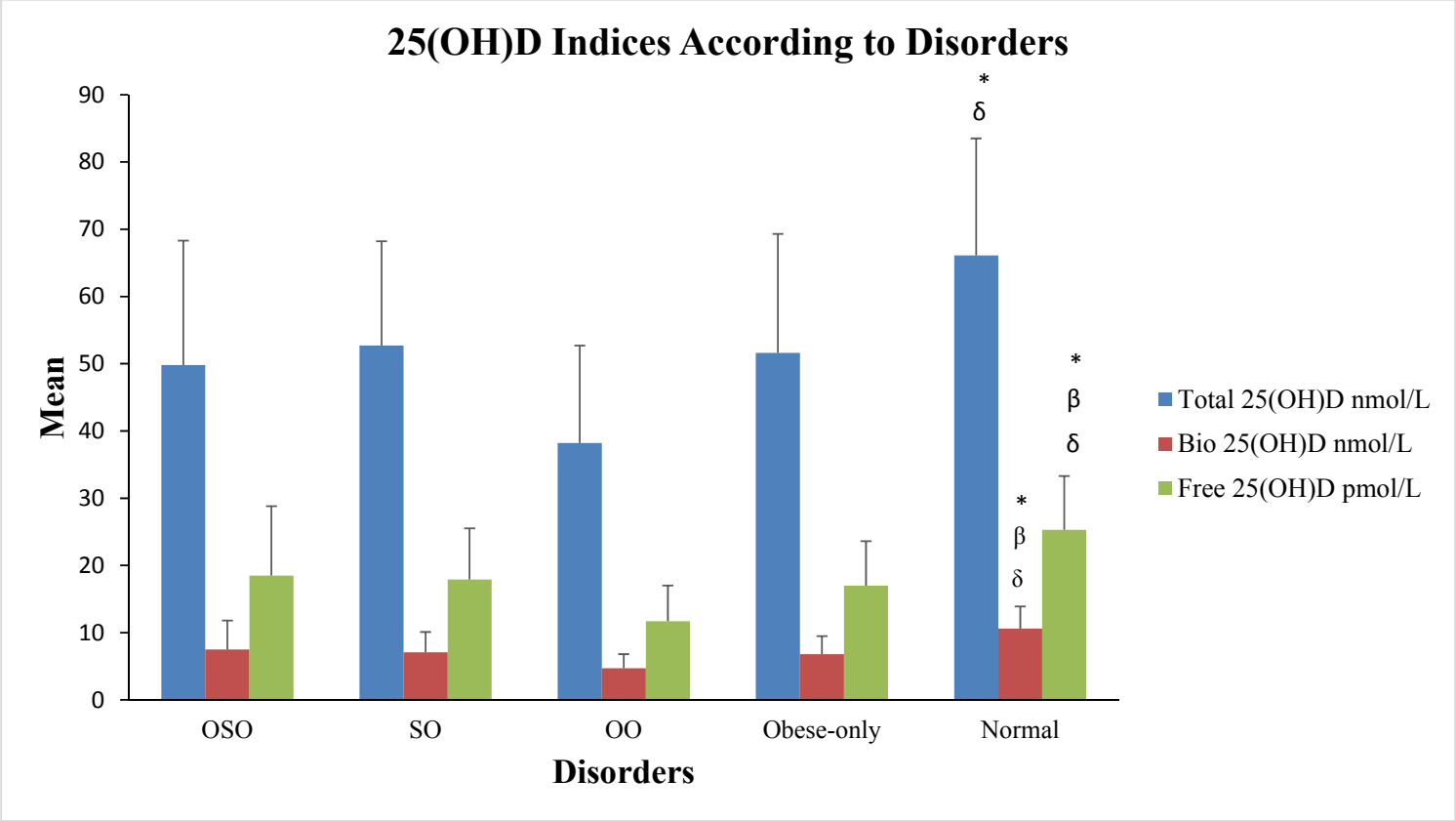


Figure 4.10 25(OH)D indices according to Normal, Osteosarcopenic Obese (OSO), Sarcopenic Obese (SO), Osteopenic Obese (OSO) and Obese-only participants; Error bars: + 1 Standard deviation, *analysed using one-way ANOVA, with Tukey's HSD Post-hoc test, $p<0.05$, β =different from Obese-only, δ =different from OO

Table 4.19a, Table 4.19b, and Table 4.19c show how the blood biomarkers are correlated with fat, bone and muscle indices, respectively.

The current study found that each form of 25(OH)D (total 25(OH)D, bioavailable 25(OH)D, free 25(OH)D) was negatively correlated with body fat percent (BFP) ($r=-0.298$, $r=-0.380$, $r=-0.335$, respectively, $p<0.05$, Table 4.19a) and positively correlated with bone density, BUA ($r=0.199$, $r=0.234$, $r=0.227$, respectively, $p<0.05$, Table 4.19b). No significant correlations, however, were found between 25(OH)D and muscle mass (Table 4.19c).

With regards to functional performance, handgrip strength (HGS) was found to be positively correlated with calcium ($r=0.242$, $p<0.01$), while lower extremity strength (sit-to-stand test) was found to be positively correlated with bioavailable 25(OH)D, ($r=0.202$, $p<0.05$, Table 4.19c). Endurance (gait speed), on the other hand, was found to be positively correlated with total 25(OH)D (Table 4.19c, $p<0.05$)

Table 4.19a Pearson's correlation coefficient of blood biomarkers with fat indices (postmenopausal women)

Parameters	Total 25OHD (nmol/L)	Free 25OHD (pmol/L)	Bio 25OHD (nmol/L)	VDBP (ug/mL)	Calcium (mmol/L)	iPTH (pmol/L)	BFP (%)	BMI (kg/m^2)	WC (cm)
Total 25(OH)D (nmol/L)	1	**0.883****	**0.883****	-0.045	**0.192***	**-0.394****	**-0.298****	**-0.240****	-0.121
Free 25(OH)D (pmol/L)	**0.883****	1	**0.988****	**-0.469****	0.151	**-0.395****	**-0.335****	**-0.255****	-0.167
Bio 25(OH)D (nmol/L)	**0.883****	**0.988****	1	**-0.446****	**0.238***	**-0.426****	**-0.380****	**-0.301****	**-0.221***
VDBP (ug/mL)	-0.045	**-0.469****	**-0.446****	1	0.123	0.071	0.149	0.019	0.048
Calcium (mmol/L)	**0.192***	0.151	**0.238***	0.123	1	**-0.497****	**-0.294****	**-0.357****	**-0.294****
iPTH (pmol/L)	**-0.394****	**-0.395****	**-0.426****	0.071	**-0.497****	1	**0.448****	**0.445****	**0.362****
BFP (%)	**-0.298****	**-0.335****	**-0.380****	0.149	**-0.294****	**0.448****	1	**0.845****	**0.725****
BMI (kg/m^2)	**-0.240****	**-0.255****	**-0.301****	0.019	**-0.357****	**0.445****	**0.845****	1	**0.831****
WC (cm)	-0.121	-0.167	**-0.221***	0.048	**-0.294****	**0.362****	**0.725****	**0.831****	1

N.B: 25(OH)D= 25 hydroxyvitamin D, VDBP= vitamin D binding protein, iPTH= intact parathyroid hormone, BFP=body fat percent, BMI=body mass index, WC=waist circumference, **P-value < 0.01, * P-value < 0.05.

Table 4.19b Pearson's correlation coefficient of blood biomarkers with bone indices (postmenopausal women)

Parameters	Total 25(OH)D (nmol/L)	Free 25(OH)D (pmol/L)	Bio 25(OH)D (nmol/L)	VDBP (ug/mL)	Calcium (mmol/L)	iPTH (pmol/L)	BUA (dB/MHz)	SOS (m/s)	Est. BMD (g/m^2)	T-score
Total 25(OH)D (nmol/L)	1	**0.883****	**0.883****	-0.045	**0.192***	**-0.394****	**0.199***	**0.217***	**0.216***	**0.215***
Free 25(OH)D (pmol/L)	**0.883****	1	**0.988****	**-0.469****	0.151	**-0.395****	**0.227***	**0.261****	**0.255****	**0.254****
Bio 25(OH)D (nmol/L)	**0.883****	**0.988****	1	**-0.446****	**0.238***	**-0.426****	**0.234***	**0.271****	**0.264****	**0.264****
VDBP (ug/mL)	-0.045	**-0.469****	**-0.446****	1	0.123	0.071	-0.080	**-0.211***	**-0.198***	**-0.197***
Calcium (mmol/L)	**0.192***	0.151	**0.238***	0.123	1	**-0.497****	0.072	0.057	0.061	0.061
iPTH (pmol/L)	**-0.394****	**-0.395****	**-0.426****	0.071	**-0.497****	1	-0.117	-0.162	-0.148	-0.144
BUA (dB/MHz)	**0.199***	**0.227***	**0.234***	-0.080	0.072	-0.117	1	**0.895****	**0.953****	**0.953****
SOS (m/s)	**0.217***	**0.261****	**0.271****	**-0.211***	0.057	-0.162	**0.895****	1	**0.988****	**0.988****
Est. BMD	**0.216***	**0.255****	**0.264****	**-0.198***	0.061	-0.148	**0.953****	**0.988****	1	**1.00****
T-score	**0.215***	**0.254****	**0.264****	**-0.197***	0.061	-0.144	**0.953****	**0.988****	**1.00****	1

N.B: 25(OH)D= 25 hydroxyvitamin D, VDBP= vitamin D binding protein, iPTH= intact parathyroid hormone, BMI=body mass index, WC=waist circumference, FFMI=fat free mass index, SMMI=skeletal muscle mass index, appSMMI=appendicular skeletal muscle mass index, BUA=broadband ultrasonic attenuation, SOS=speed of sound, Est. BMD= estimated bone mineral density, **P-value < 0.01, * P-value < 0.05.

Table 4.19c Pearson's correlation coefficient of blood biomarkers with muscle indices and physical performance (postmenopausal women)

Parameters	Total 25(OH)D (nmol/L)	Free 25(OH)D (pmol/L)	Bio 25(OH)D (nmol/L)	VDBP (ug/mL)	Calcium (mmol/L)	iPTH (pmol/L)	App SMMI (kg/m^2)	SMMI (kg/m^2)	HGS (kg)	STS	GS (m/s)	BLN (sec)
Total 25(OH)D	1	**0.883****	**0.883****	-0.045	**0.192***	**-0.394****	0.012	-0.043	0.113	0.129	**0.191***	0.133
Free 25(OH)D	**0.883****	1	**0.988****	**-0.469****	0.151	**-0.395****	0.010	-0.026	0.073	0.162	0.112	0.003
Bio 25(OH)D	**0.883****	**0.988****	1	**-0.446****	**0.238***	**-0.426****	-0.007	-0.054	0.104	**0.202***	0.134	0.031
VDBP	-0.045	**-0.469****	**-0.446****	1	0.123	0.071	0.124	-0.118	-0.160	0.002	-0.115	0.099
Calcium	**0.192***	0.151	**0.238***	0.123	1	**-0.497****	-0.170	**-0.262****	**0.242****	0.164	0.045	0.094
iPTH	**-0.394****	**-0.395****	**-0.426****	0.071	**-0.497****	1	**0.241****	**0.257****	-0.174	**-0.249****	-0.067	0.041
AppSMMI	0.012	0.010	-0.007	0.124	-0.170	**0.241****	1	**0.907****	**0.341****	0.011	**0.248****	0.116
SMMI	-0.043	-0.026	-0.054	-0.118	**-0.262****	**0.257****	**0.907****	1	0.268**	-0.049	0.161	0.037
HGS	0.113	0.073	0.104	-0.160	**0.242****	-0.174	**0.341****	**0.268****	1	**0.285****	**0.243****	**0.261****
STS	0.129	0.162	**0.202***	0.002	0.164	**-0.249****	0.011	-0.049	**0.285****	1	**0.350****	**0.212***
GS	**0.191***	0.112	0.134	-0.115	0.045	-0.067	**0.248****	0.161	**0.243****	**0.350****	1	**0.296****
BLN	0.133	0.003	0.031	0.099	0.094	0.041	0.116	0.037	**0.261****	**0.212***	**0.296****	1

N.B: 25(OH)D= 25 hydroxyvitamin D, VDBP= vitamin D binding protein, iPTH= intact parathyroid hormone, SMMI=skeletal muscle mass index, appSMMI=appendicular skeletal muscle mass index, HGS= handgrip strength, STS- sit-to-stand test, GS= gait speed, BLN= balance, **p < 0.01, *p < 0.05.

Figure 4.11 shows the correlations of total and bioavailable 25(OH)D with vitamin D binding protein (VDBP) (Figure 4.11A and 4.11B) and BUA (Figure 4.11C and 4.11D).

A significant and negative correlation was found between bioavailable 25(OH)D and VDBP, whereas no significant correlation was found for total 25(OH)D and VDBP ($r = -0.446$, $p<0.001$, $r = -0.045$, $p= 0.631$, respectively, Table 4.11A and 4.11B).

While both forms of 25(OH)D (total and bioavailable 25(OH)D) were found to be positively correlated with BUA, marginally stronger correlation was found with bioavailable 25(OH)D compared to total 25(OH)D (bioavailable; $r = 0.234$, $p = 0.012$ vs. total; $r = 0.199$, $p = 0.030$) (Figure 4.11C and 4.11D).

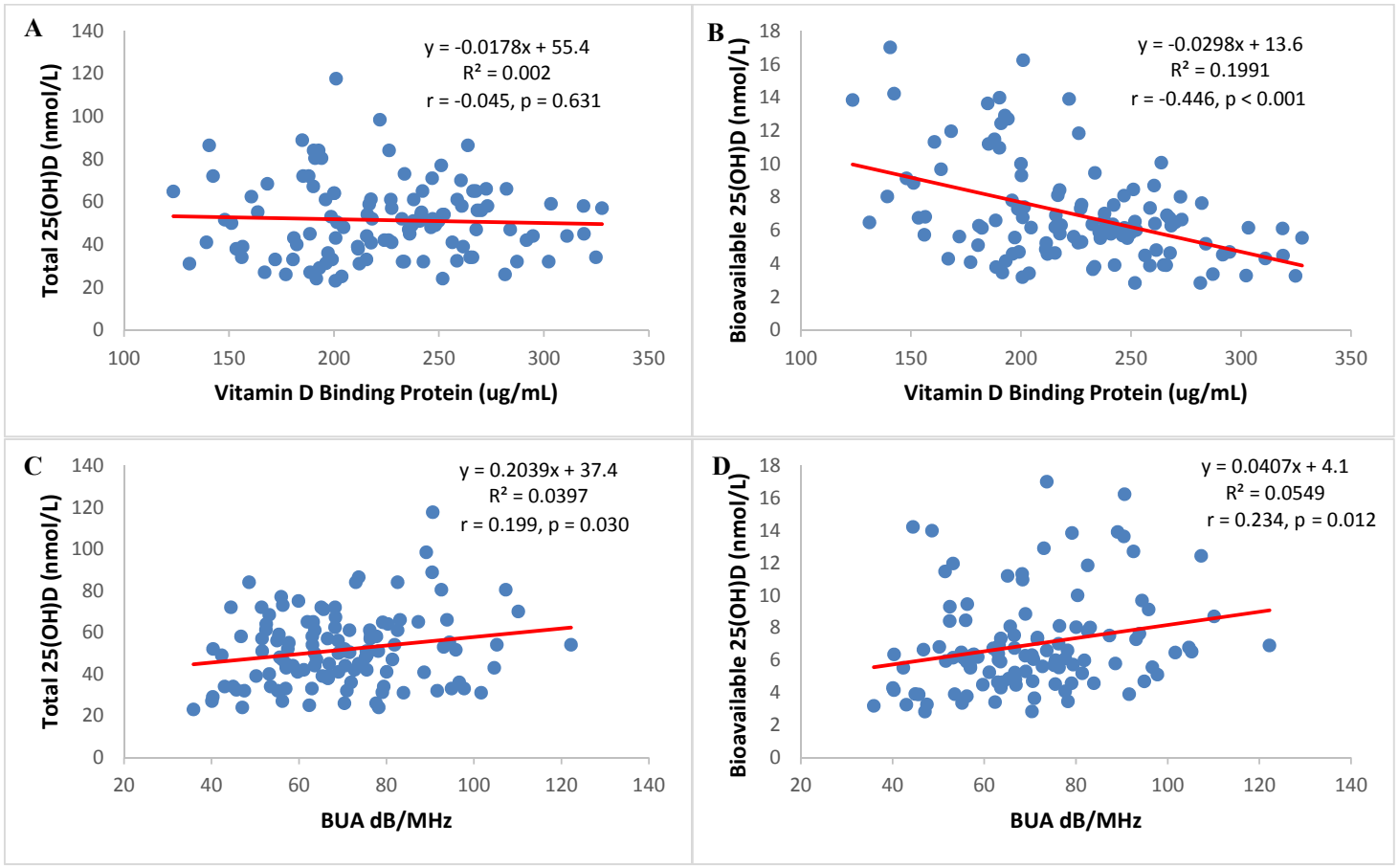


Figure 4.11 (A) The correlation between total 25(OH)D or the (B) bioavailable 25(OH)D levels and vitamin D binding protein (VDBP) concentrations are presented. (C) The correlations between the total 25(OH)D level or (D) bioavailable 25(OH)D level and the BUA are presented.

To determine the extent to which the 25(OH)D indices are responsible for the variations in the bone density (BUA) and muscle (appSMMI) values, standardized linearity regression analyses were conducted using univariate and multivariate models (Table 4.20 and Table 4.21, respectively).

Univariate analysis without any adjustment shows that total (standardized β=0.182, p=0.030), bioavailable (standardized β=0.214, p=0.011) and free 25(OH)D (standardized β=0.208, p=0.013) may be determinants of the BUA in postmenopausal women (Table 4.20). Even after adjusted for age, years since menopause, BFP and BMI, the significance remained (standardized β=0.212, p=0.016, standardized β=0.273, p=0.002, and standardized β=0.260, p=0.003, respectively, Table 4.20).

For the models depicting the relationship between 25(OH)D indices and muscle mass (appSMMI, Table 4.21), results found that age, BFP and BMI may be counfounding factors of the relationship. Univariate analysis shows that age (standardized β=-0.218, p=0.009), BFP (standardized β=0.355, p=0.000) and BMI (standardized β=0.684, p=0.000) were responsible for the variations in the appSMMI values. However, after these factors were controlled, multivariate regression analysis shows that each form of 25(OH)D (total, bioavailable and free) were determinants of the variations of appSMMI values (total 25(OH)D: standardized β=0.115, p=0.029, bioavailable 25)OH)D: standardized β=0.124,P=0.022, and free 25(OH)D: standardized β=0.110, p=0.038).

Table 4.20 Univariate and multivariate linear regression analyses of the Vitamin D indices and bone density (BUA)

Variables	Standardized β-coefficients (SE)[a]	P-value[a]	Standardized β-coefficients (SE)[b]	P-value[b]	Standardized β-coefficients (SE)[c]	P-value[c]	Standardized β-coefficients (SE)[d]	P-value[d]
Age (years)	-0.146(0.184)	0.082	-0.159(0.293)	0.233	-0.166(0.289)	0.208	-0.170(0.290)	0.198
Years since menopause	-0.112(0.206)	0.185	0.024(0.327)	0.858	0.007(0.322)	0.959	0.006(0.322)	0.966
BFP (%)	0.040(0.180)	0.636	-0.052(0.340)	0.746	-0.012(0.339)	0.940	-0.022(0.339)	0.888
BMI (kg/m^2)	0.085(0.263)	0.314	0.147(0.490)	0.351	0.137(0.484)	0.378	0.130(0.486)	0.405
Total 25(OH)D (nmol/L)	0.182(0.085)	**0.030***	0.212(0.089)	**0.016***				
Bioavailable 25(OH)D (nmol/L)	0.214(0.508)	**0.011***			0.273(0.540)	**0.002***		
Free 25(OH)D (pmol/L)	0.208(0.210)	**0.013***					0.260(0.221)	**0.003***

N.B: BFP=body fat percent; BMI=body mass index; 25(OH)D=25-hydroxy vitamin D; SE=standard error

[a]Univariate linear regression model without adjustment.

[b]Multivariate regression model 1 with adjustment for age, years since menopause, BFP, BMI, and the total 25(OH)D level as independent variables.

[c]Multivariate regression model 2 with adjustment for age, years since menopause, BFP, BMI, and the bioavailable 25(OH)D level as independent variables.

[d]Multivariate regression model 3 with adjustment for age, years since menopause, BFP, BMI, and the free 25(OH)D level as independent variables

*$p<0.05$

Table 4.21 Univariate and multivariate linear regression analyses of the Vitamin D indices and appendicular skeletal muscle mass index (appSMMI)

Variables	Standardized β-coefficients (SE)[a]	P-value[a]	Standardized β-coefficients (SE)[b]	P-value[b]	Standardized β-coefficients (SE)[c]	P-value[c]	Standardized β-coefficients (SE)[d]	P-value[d]
Age (years)	-0.218(0.010)	**0.009***	-0.164(0.009)	**0.041***	-0.164(0.009)	**0.041***	-0.165(0.009)	**0.041***
Years since menopause	-0.108(0.011)	0.199	0.098(0.010)	0.221	0.088(0.010)	0.273	0087(0.010)	0.280
BFP (%)	0.355(0.009)	**0.000****	-0.735(0.011)	**0.000****	-0.723(0.011)	**0.000****	-0.731(0.011)	**0.000****
BMI (kg/m^2)	0.684(0.010)	**0.000****	1.315(0.015)	**0.000****	1.311(0.015)	**0.000****	1.309(0.015)	**0.000****
Total 25(OH)D (nmol/L)	0.010(0.005)	0.903	0.115(0.003)	**0.029***				
Bioavailable 25(OH)D (nmol/L)	-0.006(0.027)	0.945			0.124(0.017)	**0.022***		
Free 25(OH)D (pmol/L)	0.008(0.011)	0.922					0.110(0.007)	**0.038***

N.B: BFP=body fat percent; BMI=body mass index; 25(OH)D=25-hydroxy vitamin D; SE=standard error

[a]Univariate linear regression model without adjustment.

[b]Multivariate regression model 1 with adjustment for age, years since menopause, BFP, BMI, and the total 25(OH)D level as independent variables.

[c]Multivariate regression model 2 with adjustment for age, years since menopause, BFP, BMI, and the bioavailable 25(OH)D level as independent variables.

[d]Multivariate regression model 3 with adjustment for age, years since menopause, BFP, BMI, and the free 25(OH)D level as independent variables

*p<0.05, **P<0.001

Figure 4.12 and Figure 4.13 describe bone density (BUA) of participants at each quartile (from lowest to highest) of total and bioavailable 25(OH)D, respectively.

Results for overall participants showed significant positive correlation between both forms of 25(OH)D and BUA (Figure 4.12A and Figure 4.13A). However, when looking at the differences between ethnicities (Figure 4.12B and Figure 4.13B), result shows that the Chinese reached peak bone density (BUA) at a lower quartile (2^{nd}) compared to the Malays and Indians, which peaked at a higher quartile (3^{rd}).

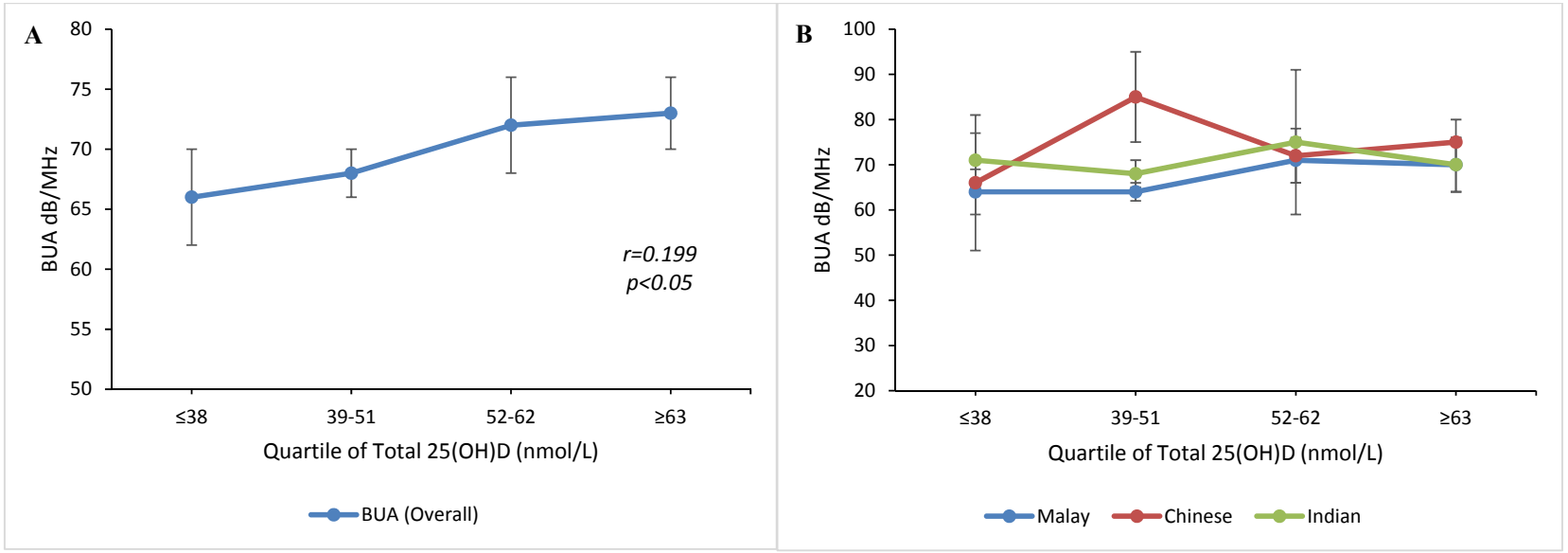


Figure 4.12 BUA of participants according to quartiles of Total 25 hydroxy vitamin D, A) Results for all participants, B) Results according to ethnicity; Malay, Chinese and Indian. Error bar: standard error of mean.

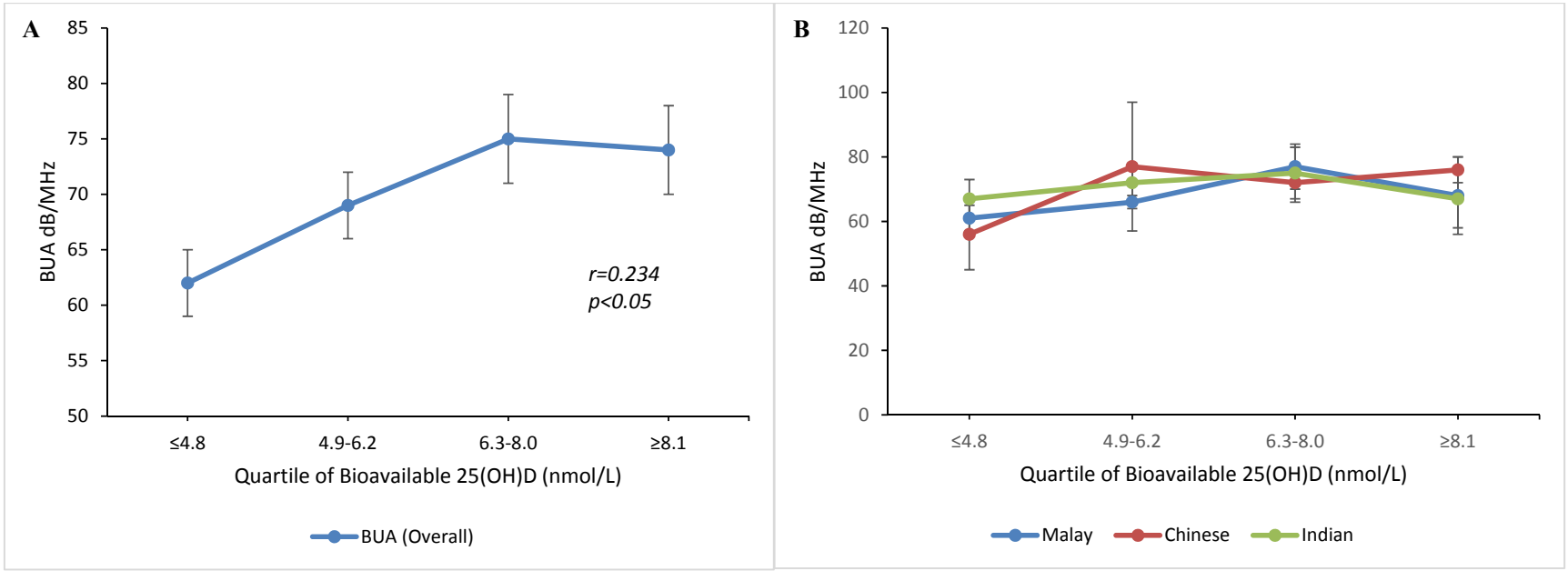


Figure 4.13 BUA of participants according to quartiles of Bioavailable 25 hydroxy vitamin D, A) Results for all participants, B) Results according to ethnicity; Malay, Chinese and Indian. Error bar: standard error of mean.

Figure 4.14 and Figure 4.15 describe differences of total and bioavailable 25(OH)D at each quartile (from lowest to highest) of body fat percent (BFP), respectively.

Results for overall participants (Figure 4.14A and 4.15A) reveal significant and negative correlation between both forms of 25(OH)D (total and bioavailable 25(OH)D, respectively) and BFP ($p \leq 0.001$). When looking at differences between ethnicities, at similar levels of BFP, the Chinese had significantly higher levels of 25(OH)D (total and bioavailable) compared to Malays and Indians (Figure 4.14B and 4.15B, respectively).

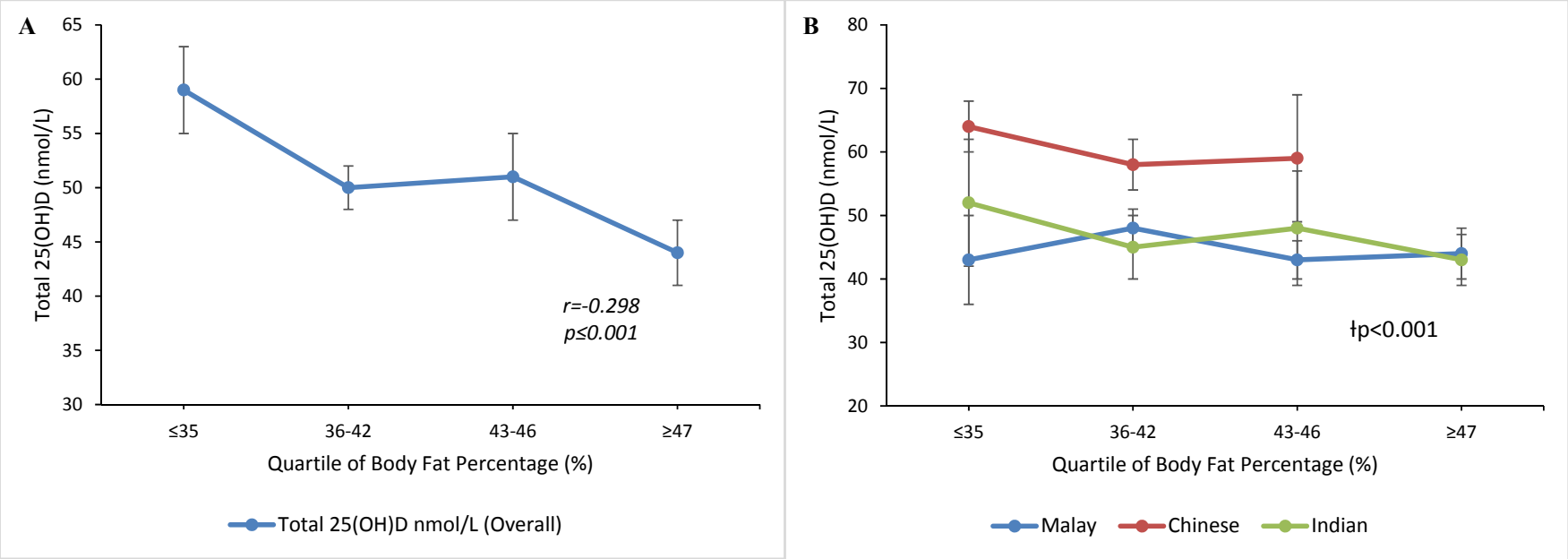


Figure 4.14 Total 25 hydroxyvitamin D of participants according to quartiles of body fat percentage, A) Results for all participants, B) Results according to ethnicity; Malay, Chinese and Indian. Error bar: standard error of mean, ł Analysed using ANOVA for differences between ethnicity

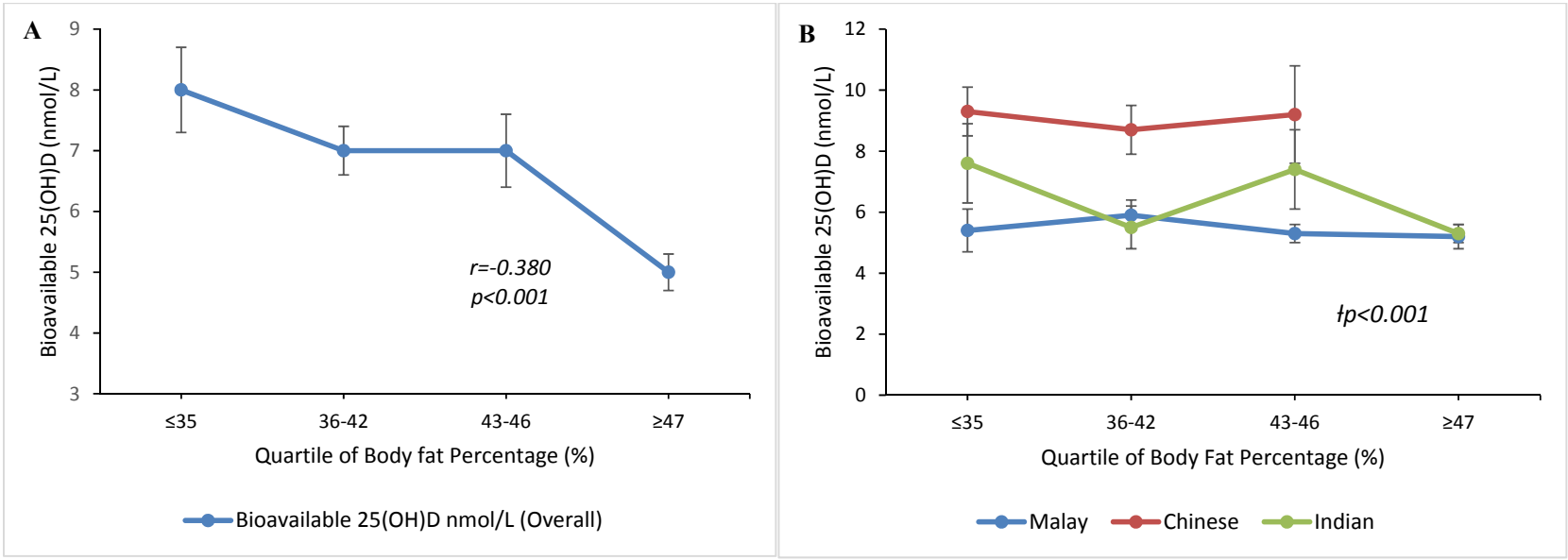


Figure 4.15 Bioavailable 25 hydroxyvitamin D of participants according to quartiles of body fat percentage, A) Results for all participants, B) Results according to ethnicity; Malay, Chinese and Indian. Error bar: standard error of mean, ŧ Analysed using ANOVA for differences between ethnicity

CHAPTER 5: DISCUSSION PART III

5.3 Free hormone hypothesis: the core activity of a hormone is due to the bioavailable fraction and its passive diffusion across the membrane

In this part of the discussion, the thesis will begin by describing the current status of total, bioavailable, and free 25(OH)D in postmenopausal Malaysian women, and the differences in characteristics between the groups with different levels of 25(OH)D indices. In the next section, it will discuss the free-hormone hypothesis and the findings on the relationship of total and bioavailable 25(OH)D with body composition (fat, bone and muscle). Finally, the findings on the status of total and bioavailable 25(OH)D among obese population with musculoskeletal disorder (OSO, OO, and SO) will be discussed.

5.3.1 Total, bioavailable and free 25(OH)D status of postmenopausal Malaysian women

Vitamin D is a fat-soluble hormone that is responsible for maintaining good musculoskeletal health (Anastasiou, Yannakoulia & Scarmeas, 2014). Free hormone hypothesis posits that the activity of a hormone is due to the bioavailable fraction and its passive diffusion across the tissue membrane. In the case of Vitamin D, majority of the vitamin in the blood is bounded to vitamin D binding protein (VDBP), rendering it unavailable for passive diffusion. Currently, total 25-hydroxyvitamin D [25(OH)D] is the recommended biomarker for the assessment of Vitamin D levels in the body (Holick, 2009). 25-hydroxyvitamin D is the major circulating form of Vitamin D present in the blood and has been shown to be linked to indices of

bone health (Jemielita et al., 2016). However, to date, the relationship between 25(OH)D and bone density had been inconclusive. Some studies found significant and positive relationship between the two, while others did not. Recent evidence suggest that the unbound or the bioavailable fraction of 25(OH)D may be a better indicator of Vitamin D status as it has been shown to correlate better with bone density (Li et al., 2017). With regards to Vitamin D, the free hormone hypothesis posits that the free and/or bioavailable fractions of 25(OH)D may correlate more strongly with its biological action than the total 25(OH)D due to their higher biological 'availability' (Johnsen et al., 2014). Currently, there are limited assessments of free and bioavailable 25(OH)D in the Asian population. So far, there was only one other study which had examined the free hormone hypothesis related to 25(OH)D in a cohort of Malaysian women (Thambiah et al., 2018). However, the study was conducted on women with rheumatoid arthritis (mean age 53.7 years) and the results may not be applicable to the general population. Regardless, the study had found no differences in VDBP and total 25(OH)D levels between the case and control group (without rheumatoid arthritis) and had analysed the dataset as a whole.

As mentioned, the total 25(OH)D level is currently used as the standard biomarker for the Vitamin D status in various populations, and according to the Institute of Medicine (2011), the threshold of <30 nmol/L was defined as 'deficient' (Institute of Medicine (IOM), 2011). This threshold was determined by markedly increased parathyroid hormone level or decreased calcium absorption in the body (Rosen et al., 2012; Institute of Medicine (IOM), 2011; Sai et al., 2011,). However, due to conflicting findings from

experimental and epidemiological studies, the optimal threshold for the Vitamin D level has not been conclusively defined (Matchar et al., 2016, Bischoff-Ferrari, 2008). The IOM committee suggest that 50 nmol/L was the level of serum 25(OH)D needed for good bone health in most individuals. According to the committee, majority of people in the United States and Canada had 25(OH)D level above that threshold. Further, the committee suggests that people with serum 25(OH)D levels less than 30 nmol/L are at risk of deficiency, and some, but not all, are potentially at risk for insufficiency at serum 25(OH)D levels between 30 nmol/L and 50 nmol/L (Institute of Medicine (IOM), 2011).

In the current study, a cohort of Malay, Chinese and Indian postmenopausal Malaysian women (120 participants), residing in Klang Valley and Semenyih, Malaysia, volunteered for their blood to be taken and measured for total 25(OH)D, VDBP, intact parathyroid hormone (iPTH), serum calcium and serum albumin. Free and bioavailable 25(OH)D were calculated using modified Vermuelen method for free testosterone estimation (Powe et al., 2011). The Vermeulen method was used because this method gives separate measurements of free and bioavailable 25(OH)D, rather than the Bikle method which only gives the free 25(OH)D (Thambiah et al., 2018, Powe et al., 2011). Nevertheless, studies have shown that the results of these two formulas are significantly correlated (Thambiah et al., 2018). Both forms of 25(OH)D (free and bioavailable) were calculated in moles per liter (mol/L). Subsequently, bioavailable 25(OH)D was converted to nanomole per litre (nmol/L) while free 25(OH)D was expressed as nmol/L and subsequently, picomole per litre (pmol/L). Total 25(OH)D, VDBP and

albumin measurements were incorporated into the calculations to derive the values for free and bioavailable 25(OH)D (Thambiah et al., 2018, Powe et al., 2011).

In this community-dwelling population of postmenopausal Malaysian women, Vitamin D deficiency was rare. In fact, few participants had iPTH levels outside the normal range (Table 4.17, mean (SD) 5.8(2.7) pmol/L). Parathyroid Hormone (PTH) is responsible for maintaining normal level of calcium and phosphate. The hormone is regulated through the levels of serum Vitamin D and calcium (Steingrimsdottir et al., 2005). When the level of Vitamin D level is high, the level of PTH is low (inverse correlation) (Shafinaz and Moy, 2016; Souberbielle et al., 2001). The finding from the current study also confirmed this correlation (Table 4.19a). In addition to Vitamin D deficiency, an elevated iPTH concentration is known to be associated with cardiometabolic diseases (Lavie et al., 2013). Therefore, it is important to monitor both levels of serum 25(OH)D and iPTH. The mean for total 25(OH)D in the current study participants was >50 nmol/L (Table 4.17). On the basis of the IOM guidelines, only 7.5% of the current study's participants would be classified as Vitamin D deficient (<30nmol/L, Table 4.18), while the majority of participants in this cohort were in the Vitamin D replete category (51.7%, >50nmol/L, Table 4.18). This finding is well reflected in the cohort's healthy bone density (BUA, Table 4.1), high serum calcium level, and normal range iPTH levels (Table 4.17). According to a report from the Ministry of Science Technology and Innovation (MOSTI) in 2011, Malaysians received at least 6 hours of sunshine daily. The high amount of sun exposure may be the reason for their above average Vitamin

D level. Lips (2007) also reported low prevalence of Vitamin D deficiency in Southeast Asia, particularly Malaysia and Singapore (Lips, 2007). Nevertheless, previous Malaysian studies had shown contrasting findings (Shafinaz and Moy, 2016, Moy and Bulgiba, 2011). For example, Shafinaz and Moy (2016) reported a high prevalence of Vitamin D deficiency among the Malaysian general population (<30nmol/L), while Moy and Bulgiba (2011) reported high prevalence of Vitamin D insufficiency among Malaysian women (<50 nmol/L). The reasons for the discrepancies may be due to differences in age groups, gender, and method of 25(OH)D assessment. The current study, for example, had used chemiluminescence immunoassay (CLIA) method to assess the total 25(OH)D, which had been proven to be highly sensitive and consistently used for clinical diagnosis (Valcour et al., 2016). On the other hand, some immunoassays, such as the ones that come in kit versions, may underestimate 25(OH)D metabolites due to the differences in the antibody affinity or Vitamin D binding protein employed. Nevertheless, ethnic disparity in 25(OH)D levels in the current study were consistent with findings of previous Malaysian studies (Shafinaz and Moy, 2016, Nurbazlin et al., 2013, Rahman, 2004). The current study found that women of Indian and Malay ethnicity had significantly lower Vitamin D level (<50nmol/L) compared to the Chinese (>50nmol/L, $p<0.05$, Figure 4.9). Comparatively, Rahman et al. (2004) also reported that the level of 25 (OH) D was significantly lower in the postmenopausal Malay women (44.4 [10.6] nmol/L) compared to the Chinese women (68.8 [15.7] nmol/L). Studies have consistently found ethnicity to be one of the strongest indicator of Vitamin D status (Shafinaz and Moy, 2016; Nurbazlin et al., 2013;

Rahman et al., 2004,). Theoretically, one of the reasons was due to melanin content in the skin. Indians and Malays normally have higher melanin content in the skin (Fitzpatrick skin type VI and types V and VI, respectively) compared to the Chinese (types III and IV) (Sng et al., 2009). Higher melanin content inhibits Vitamin D synthesis (Rahman, 2004). People with darker skin tone or higher melanin content generally required longer sun exposure compared to those with lighter skin tone to produce the same level of Vitamin D needed in the body (Tsiaras & Weinstock, 2011). Although Malaysia is a country with plenty of sunlight all year round, a number of findings showed that the women, particularly of Malay ethnicity have low Vitamin D status (Shafinaz & Moy, 2016; Moy and Bulgiba, 2011; Lips, 2007; Rahman, 2004). The Malays constituted about 60% of the country's population and they are Muslims by religion. Previous studies have shown that sun-avoidance behavior is prevalent among the Malays who tend to stay indoors especially during the mid-day (Rahman, 2004). Although 15 minutes of sun exposure during mid-day is all it takes for humans to obtain adequate Vitamin D level, the clothing habits of Malay women (i.e headscarf, long sleeves, long skirts) which only leaves the face and hands to sun exposure, as well as their generally darker skin tone, reduced the synthesis of Vitamin D (Lips, 2007, Rahman, 2004). In the case of free and bioavailable 25(OH)D, the levels of these two forms of 25(OH)D reflects the level of total 25(OH)D (Figure 4.9 and Table 4.19a). The mean value for bioavailable 25(OH)D for this study cohort were 2.8 ng/mL (Table 4.17), comparable to previous findings in healthy women in Seoul, Korea (2.6 ng/mL) (Kim et al., 2017) and Shanghai, China (2.9 ng/mL) (Li et al., 2017).

The concentration of VDBP in the body depends on genetic variants. There are 3 major electrophoretic variants of VDBP (Gc2, Gc1s, Gc1f) that differ by amino acid substitutions and extent of glycosylation. Studies have found distinct racial distribution patterns of these allelles, with Gc1f genotype being the most abundant among Africans and Asians, whereas Gc1s and Gc2 were prevalent in Caucasians (Thambiah et al., 2018; Aloia, 2008). Currently, there are no data on VDBP polymorphisms in the Malaysian population, which may be a confounding factor in this study. Nevertheless, it had been hypothesized that VDBP genotypes may be similar among Asians (Thambiah et al., 2018). In addition to genetic variants, type of assay used for the measurement of VDBP had been shown to affect the results on racial distribution pattern (Jemielita et al., 2016). Powe et al. (2011) who had used a monoclonal assay to measure the VDBP, reported that measured VDBP was lower in African Americans compared to Caucasians. However, other studies using polyclonal immunoassays or mass spectrometric assays to measure VDBP have not shown racial differences (Couchman & Moniz, 2017, Denburg et al., 2016, Henderson et al., 2016). Similar to Powe et al. (2011), the current study had used a monoclonal ELISA assay to measure VDBP and found distinct racial distribution pattern between Malays and both Chinese and Indians. The current study found that measured VDBP was significantly higher in Malay women (242.8 ug/mL) compared to Chinese (205.8 ug/mL) and Indians (217.7 ug/mL, Table 4.5 and Figure 4.9). Although no significance was found by Thambiah et al. (2018), similar trend was reported. Conversely, a Singaporean cross-sectional study on 295 older adults comprised of Malay, Chinese and Indian ethnicity, found the Indians to have

the highest levels of VDBP (220.1 ug/ml), followed by the Malays (188.8 ug/ml) and Chinese (169.6ug/ml) (Merchant et al, 2018).

VDBP concentration in blood plays significant role in the bioavailability of 25(OH)D in the body. VDBP act as a reservoir for 25(OH)D in the body, acting as a placeholder until the body needs 25(OH)D, then it will be released. In a high-estrogen state, such as pregnancy, VDBP levels had been found to increase by up to 50%, and in some disease states, such as severe hepatic disease, the level had been found to decrease (Bhan, 2014; Chun, 2012; Heijboer et al., 2012). Hyun-Jeong et al. (2017) found that the mean VDBP level of the group of intensive care unit (ICU) patients was significantly lower than that of the healthy control group (Kim et al., 2017). This finding suggests that the production of VDBP may be lowered during vulnerable states such as after an operation, even in the absence of hepatic disease, likely to increase the bio-availability of Vitamin D to be used by the body. Similar finding was also found by Heijboer et al. (2012) (Heijboer et al., 2012). In theory, bioavailable 25(OH)D concentrations may increase or decrease depending on the degree of VDBP down-regulation. In support of this theory, previous studies have shown negative association between VDBP and bioavailable 25(OH)D. For example, Hyun-Jeong et al. (2017) found that in pregnant women, the VDBP level (225.01 μg/mL, CI 130.24-422.92 μg/mL) was significantly higher than that of healthy controls (167.18 μg/mL, CI 105.99-257.70 μg/mL, p=0.008), and bioavailable 25(OH)D levels was significantly lower (1.93 ng/mL, CI 1.03-3.41 ng/mL, p=0.0027) than those in healthy controls (2.56 ng/mL, CI 1.95-4.22 ng/mL) (Kim et al., 2017). Therefore, the amount of bioavailable 25(OH)D depends on the down-regulation of VDBP

in the body, rather than sun exposure or diet. Currently, factors affecting the down-regulation of VDBP is still unclear, although studies have found that it increases in high-estrogen state and decreases in certain disease state (Chun, 2012, Bhan, 2014, Heijboer et al., 2012). In the current study, the Malays, which had the highest level of VDBP (Table 4.5), were found to have the lowest level of free and bioavailable 25(OH)D, compared to Indians and Chinese (Figure 4.9). Consequently, the Malays also had the lowest bone density (BUA 67.0 dB/MHz) compared to Indians (70.1 dB/MHz) and Chinese (73.3 dB/MHz, Table 4.5). Nevertheless, overall, the mean value for serum VDBP was well within the normal range for adults (Table 4.17, Bhan et al., 2012, Powe et al., 2011, Powe et al., 2010). In the current study, it was unclear why the Malays had high levels of VDBP. Based on the current study's design, it is impossible to know the cause. However, it is likely that it is related to genetic polymorphism and should be explored further in the future.

Current and other studies have shown that obesity was negatively associated with serum 25(OH)D levels (Li et al., 2017). However, it was unclear of which obesity index (BMI, WC or body fat percent) was relevant to Vitamin D status in the general population. The current study found no differences in WC between the Vitamin D deficient, insufficient, and the replete group (Table 4.18). Interestingly, findings from the current study suggest that there may be a certain degree of body fat percent (BFP) that may trigger the negative association with Vitamin D. For example, the current study found that the BFP of people in the Replete group (38.8%) was significantly lower ($p<0.05$) than the Deficient and Insufficient groups (43.6% and 43.1%,

respectively, Table 4.18). Similarly, as discussed in Discussion part II, the current study found that people who were Osteopenic obese had the highest BFP at 44.9% (Table 4.10) and were likely to be Vitamin D deficient (<30nmol/L), compared to obese-only (OB) group ($p<0.10$, Table 4.12). It is possible that people with ~40% of body fat (BFP) are more prone to having low Vitamin D level. Studies have shown that obese individuals may need higher than normal doses of Vitamin D. One of the reasons is mainly due to the fat-solubility of Vitamin D that cause it to 'sink' into the adipose tissue. Interestingly, obese individuals also triggers homeostatic response of iPTH at a lower level of Vitamin D. Shapses et al. (2013) found that obese people (BMI>30 kg/m^2) needs lower level of 25(OH)D concentration to maximally suppress iPTH, implying that the lower level of Vitamin D to be the optimal dose. However, the lower concentration may not have the same physiological significance as in the general population.

When compared with Vitamin D deficient group, people in the Vitamin D replete group had significantly lower iPTH level, and significantly higher bone density (BUA) and faster walking speed (Table 4.18, $p<0.05$). The Vitamin D replete group also had significantly higher serum calcium and bioavailable 25(OH)D concentration than Vitamin D deficient group (Table 4.18). Between the three groups of Vitamin D deficient, insufficient and replete, there were no significant differences in the HGS, muscle mass, lower extremity strength, balance, and VDBP (Table 4.18). Nevertheless, a study had found that U-shaped relationship may exist between Vitamin D and physical function, where plasma 25(OH)D levels higher than 120 nmol/L was found to be associated with a poorer physical performance (Bischoff-Ferrari

et al., 2004). Therefore, non-significant differences between the groups may not indicate lack of correlations. Perhaps a dose-dependent study could confirm the U-shaped relationship.

5.3.2 Total, bioavailable 25(OH)D and their associations with body composition (fat, bone and muscle)

Before delving further on the possible impact of vitamin D status on the manifestation of osteosarcopenia in obese population, the thesis will first discuss the correlation of Vitamin D with each component of OSO; fat, bone and muscle mass. It will also discuss how body fat percent (BFP) affects the level of total and bioavailable 25(OH)D, and whether it influence the relationships with bone and muscle mass.

5.3.2.1 Body fat indices (BMI, WC, BFP)

In addition to skin color, sun exposure, and age, another factor that could be affecting hypovitaminosis D is obesity. Available evidence showed that obese individuals may need higher than normal doses of Vitamin D (Shapses et al., 2013). Malaysia is a developing country where the prevalence of obesity is increasing along with its development. In a 2011 report, the National Health Morbidity Survey found that the prevalence of overweight and obesity among Malaysian adults aged 18 years and above was 51.2% (Chan et al., 2017). Vitamin D deficiency in obese individuals is most likely due to increased sequestration of Vitamin D in the adipose tissue, hence, decreasing its bioavailability (Wamberg et al., 2013, Marcotorchino, Tourniaire, & Landrier, 2013, Wortsman et al., 2000). Adipose tissues are active endocrine tissues that regulate various biochemical and physiological

processes involved in homeostasis, adipokine secretion, glucose and lipid metabolism, appetite control, vascular remodeling, and insulin reaction. Studies in both human and mice have found that adipose tissues have a high concentration of 25(OH)D and its metabolizing enzymes, likely due to the fat-solubility of 25(OH)D (Moukayed & Grant, 2019, Wamberg et al., 2013). Obese individuals commonly store more vitamin D in adipose tissue than lean individuals (Marcotorchino, Tourniaire, & Landrier, 2013).

Interestingly, the reverse may also be possible. Foss (2009) speculates that Vitamin D deficiency may be the cause of obesity. It was hypothesized that low level of calcidiol in the blood stimulates adipogenesis, hence, the manifestation of obesity and subsequent metabolic syndrome. It may be possible to reverse the condition of obesity by improving Vitamin D status (Foss, 2009). However, Foss (2009) acknowledged that due to the cross-sectional design of the study, causal relationship was impossible to prove.

A number of studies have shown that body mass index (BMI) ≥ 30 kg/m^2, is associated with Vitamin D deficiency (Shapses et al., 2013; Vanlint, 2013; González-Molero et al., 2013; Coin et al., 2008; Coin, et al., 2012,). Shapses et al. (2013) found that suppression of iPTH in obese people with BMI >30 kg/m^2 occurred at a 25(OH)D concentration of 11.1 ng/mL, which is much lower than the average population (21.7 ng/mL). The researchers argued that this concentration may not have the same physiological significance as in the general population. The obese may need a higher concentrations of Vitamin D, regardless at which level it suppresses the iPTH. Currently, it was unclear of which indicator of obesity, i.e., BMI, WC or BFP, that should be taken into consideration while assessing Vitamin D

status in the general population. Some studies argued that it was BFP, rather than BMI that influence serum 25(OH)D level (Arunabh et al., 2003). Another study had even claim abdominal obesity (WC) to be the better indicator for 25(OH)D deficiency (Mansouri et al., 2019). The current study found that BFP may be the superior indicator of obesity compared to BMI. We found that all forms of serum 25(OH)D (total, free and bioavailable) were more strongly associated with BFP (Table 4.19a, r=-0.298, r=-0.335, r=-0.380, respectively, $p<0.001$) compared to BMI, which suggest that adiposity, rather than body mass that influenced the serum 25(OH)D level. However, with regards to abdominal obesity (WC), significant correlation was only found with bioavailable 25(OH)D (Table 4.19a, r=-0.221**,** $p<0.05$). Questions arise whether low bioavailable 25(OH)D causes fat to be stored in the abdominal region, or does belly fat decreases the levels of bioavailable 25(OH)D. Further research is needed to determine the cause and effect relationship.

When BFP were divided into quartiles, it was revealed that total 25(OH)D (Figure 4.14A) and bioavailable 25(OH)D (Figure 4.15A) decreases when BFP is above 35.0%. People with body fat of 47.0% or higher tend to have <50nmol/L of total 25(OH)D (Figure 4.14A) and <5.0nmol/L of bioavailable 25(OH)D (Figure 4.15A). Interestingly, at similar levels of BFP (35.0%-46.0%), the Chinese had significantly higher levels of total 25(OH)D (Figure 4.14B) and bioavailable 25(OH)D (Figure 4.15B) than the Malays and Indians ($p<0.001$). The reason could be due to their lighter skin color that made it easy for them to naturally produce Vitamin D. In addition, the Chinese also known to be more health conscious and spend a lot more time

outdoors compared to Malays and Indians. This is also reflected in their body composition. No Chinese had BFP more than 46.0%. Due to the observational nature of this study, it was difficult to determine the causal relationship between obesity and Vitamin D levels. Further study is warranted to determine the causations.

5.3.2.2 Bone indices

Studies have consistently found significant and positive relationship between Vitamin D and bone health. Vitamin D increased the absorption of calcium in the gut, regulate the mineralization of bone tissue and may play an important role in muscle function (Holick & Chen, 2008). In the current cross-sectional study, it was found that both the bioavailable 25(OH)D and total 25(OH)D levels were positively correlated with the BUA (Table 4.19b, Figure 4.12(A) and Figure 4.13(A), $p<0.05$). When divided by ethnicity, the Chinese achieved peak (highest) BUA at a lower concentration of total 25(OH)D (Figure 4.12B) and bioavailable 25(OH)D (Figure 4.13B) compared to Malays and Indians (Second quartile vs. third quartile). The reason could be due to existing calcium concentration. A study has reported that calcium-sufficient people need less Vitamin D. Heaney (2002) found that Africans Americans had better renal conservation of calcium (more efficient calcium economy) compared to Caucasians (Heaney, 2002), which explains why, despite their low Vitamin D level, African Americans have a lower risk of osteoporotic fractures than do Caucasians (Aloia, 2008). Indeed, in the current study, the Chinese had significantly higher calcium concentration compared to Malays and Indians (Table 4.5, $p<0.001$), thus do not need as

much Vitamin D to aid calcium absorption in the gut for bone remodeling (i.e. higher BUA at a lower level of Vitamin D).

In the univariate and multivariate regression analyses, the current study found that total, free and bioavailable 25(OH)D were independent determinant of the BUA before and after adjustments for age, years since menopause, BMI, and BFP (Table 4.20). This indicate a strong relationship between Vitamin D and bone density. Further, similar to findings by Thambiah et al. (2018), the circulating VDBP level was significantly correlated with bioavailable 25(OH)D level ($r=-0.446$, $p<0.01$) but not with the total 25(OH)D level (Figure 4.11A and Figure 4.11B). VDBP act as a reservoir and aiding in the reabsorption of filtered Vitamin D in the kidneys (Safadi et al., 1999). Therefore, the lower the level of reservoir, the higher the amount of free and bioavailable 25(OH)D (Safadi et al., 1999). Nielson et al. (2016) also failed to find strong correlation between total 25(OH)D and VDBP (Spearman $r \leq 0.28$). Li et al. (2016), however, found significant and positive correlation between the two ($r = 0.356$, $p<0.001$). It is important to note, that in their study, total 25(OH)D was analysed using liquid chromatography mass spectrometry (LCMS), whereas in the current study, we had used immunoassay method. Hence, the contradictory results could be due to the discrepancies that exist among commercially available 25(OH)D assays. The current study also found that the level of free and bioavailable 25(OH)D reflects the level of total 25(OH)D (Table 4.19a and Figure 4.9), similar to findings by Thambiah et al. (2018). Furthermore, the iPTH level was found to be negatively correlated with the BUA value (albeit non-significant, Table 4.19b), which may be due to the fact that iPTH stimulates bone turnover in

the elderly. Additionally, a significant positive correlation was found between total 25(OH)D and bioavailable 25(OH) D (Figure 4.18a, r=0.883, p<0.01). Interestingly, although both forms of 25(OH)D were positively correlated with bone density (BUA), the correlation of bioavailable 25(OH)D was marginally stronger compared to total 25(OH)D (r=0.234, p=0.012 and r=0.199, p=0.030, respectively, Figure 4.11C and Figure 4.11D). The current study also found that there was a slightly stronger correlation between the iPTH concentrations and bioavailable 25(OH)D than with the total 25(OH)D level (Table 4.19a). Nevertheless, the small differences in the R value and the small effect size does not warrant the conclusion that bioavailable 25(OH)D is more correlated to the BUA than total 25(OH)D. More research is needed with a larger sample size to validate this finding.

Although some epidemiological studies have shown significant correlation between levels of different forms of 25(OH)D and bone density in healthy populations, others have shown otherwise. A study by Johnsen et al. (2014) on the correlations between different forms of 25(OH)D and BMD found that bioavailable or free 25(OH)D was the superior indicator for Vitamin D assessment compared to total 25(OH)D (Johnsen et al., 2014). Conversely, a study with 304 adults aged between 21 and 81 years found no significant association between any form of 25(OH)D and BMD (Jemielita et al., 2016). In addition to BUA, the current study also found significant and positive correlation of total and bioavailable 25(OH)D with other QUS indices (SOS, Est. BMD and T-score, Table 4.19b). After adjustment of confounders (age, years since menopause, BMI and BFP), a stronger correlations was noted for each 25(OH)D index, suggesting that all forms of 25(OH)D were

independent determinant for BUA (Table 4.20). Confounders were determined based on past research. Lim et al. (2005) found that increased age and years since menopause were positively associated with osteoporosis, while BMI was negatively associated with osteoporosis among healthy Malaysian women (≥45 years). In addition, Chan et al. (2020) also found that the predictors of suboptimal bone health and osteoporosis among 786 Malaysians aged > 40 years were increased age and higher fat mass. Interestingly, the current found no evidence for obesity paradox which hypothesized that obesity is correlated with higher bone density (Table 4.9a). No significant correlations were found between all indicators of obesity (BMI, BFP and WC) and BUA (Table 4.7 and Table 4.9a), suggesting that women with higher adiposity may not have higher bone density. This is consistent with previous studies reporting no correlations between obesity and high bone mineral density (BMD) (Chan et al., 2020). However, it is fair to note that in obese populations, U-shaped relationship had been described between BMI and BMD. Palermo et al. (2016) found that the protective effects of weight on bone only went up to a certain level, and decreased along with the increment of BMI (Palermo et al., 2016). So far, many studies exploring the relationships between the obesity and bone density in human subjects had been observational in nature. Further studies, with identifiable confounding factors, are needed in order to determine the impact of obesity on bone density. In the analyses of the current study, confounding factors were determined by the degree of change in Beta coefficient. The Beta coefficient for age, BFP, and BMI changed more than 10%, suggesting that

the variables may be confounders in the relationship between BUA and 25(OH)D indices (Table 4.20).

5.3.2.3 Muscle indices

Vitamin D has been found to play a role in muscle function in a number of studies (Girgis et al., 2013). For example, a Korean study involving adults aged 40 years and older showed that men with Vitamin D deficiency (defined as serum 25(OH)D < 20 ng/mL) had lower appendicular skeletal muscle mass (appSMMI) than those with higher level of Vitamin D. No correlation, however, was found in women (Ko et al., 2015). Interestingly, the current study found significant and positive correlation between each form of 25(OH)D (total, free and bioavailable) and appSMMI ($p<0.05$, Table 4.21). Multivariate linear regression revealed that total, free and bioavailable 25(OH)D were determinant of the appSMMI, but only after adjustments for age, years since menopause, BFP, and BMI (Table 4.21, $p<0.05$). Other studies have found similar findings. For example, Visser, Deeg & Lips (2003) found that after adjustment for physical activity level, season of data collection, serum creatinine concentration, chronic disease, smoking, and BMI, older adults with low baseline 25(OH)D levels (<25 nmol/L) were 2.14 times (0.73-6.33, based on appendicular skeletal muscle mass) more likely to experience sarcopenia, compared to those with high 25(OH)D levels (>50 nmol/L). Similar to BUA, bioavailable 25(OH)D also showed a stronger correlation with appSMMI than total 25(OH)D (Table 4.21). Nevertheless, the margin of difference is too small to draw a meaningful conclusion. Confounding factors were found in age, BFP and BMI (Table 4.21). Appendicular skeletal muscle mass index (appSMMI) was negatively

correlated with age ($p<0.05$, Table 4.21) and positively correlated with BFP and BMI (Table 4.21, $p<0.001$). It is interesting to note, however, that the correlation between BFP and appSMMI became negative after 25(OH)D was added into the analysis (Table 4.21), which implied that Vitamin D may be a confounder in this relationship. It is possible that Vitamin D may play a role in the parallel increase of muscle mass along with fat. Whilst Vitamin D is primarily stored in adipose tissue, there is evidence of Vitamin D uptake in skeletal muscle (Abboud, et al., 2013). Vitamin D uptake in skeletal muscle may help the muscle grow along with body fat as a part of body's adaptation to compensate for the increase in body mass (i.e. Wolff's Law). A significant and positive correlation between muscle mass and fat was also found in the current study.

Sarcopenia is an age-related disorder characterised by loss of skeletal muscle mass, decrease of muscle strength and/or physical performance in the elderly (Delmonico et al., 2009; Rosenberg, 1997). One of the clinical outcomes of sarcopenia is frailty. A number of observational studies have explored the relationship between Vitamin D status and frailty. For example, in an observational study involving 1659 community-dwelling men with 10% prevalence of frailty, Hirani et al (2014) described an independent link between hypovitaminosis D and frailty (Hirani et al., 2014). Tajar et al. (2013) also described similar results in another cohort of elderly men. According to Tajar et al. (2013), participants with Vitamin D level less than 50 nmol/L were twice likely of being classified into the "frail" phenotype than the "robust" phenotype (Tajar et al., 2013). The association may also be non-gender specific. Wilhelm-Leen et al. (2010) found that in a cohort that

include both elderly men and women, people with low Vitamin D status were 4 times more likely to be frail than people with higher Vitamin D levels (Wilhelm-Leen, et al., 2010). Further, rather than acute impact, low Vitamin D levels had been found to be associated with an increased risk to develop frailty over time. In a prospective study involving elderly women (aged > 69 years), non-frail women at baseline with low Vitamin D level (< 50 nmol/L) had a higher risk of becoming frail during the 4.5 years of follow-up, than women with a higher level of Vitamin D at baseline (Ensrud et al., 2010).

When examining factors that could affect muscle strength (assessed using hand grip strength (HGS) as proxy), the current study found that HGS was positively associated with whole body skeletal muscle mass (SMMI), appendicular skeletal muscle mass (appSMMI), serum calcium, and each component of SPPB (gait speed, sit-to-stand test and balance, p-value<0.05) (Table 4.19c). Low HGS has been found to be correlated with increased functional disability by a number of studies (Garcia et al., 2011, Bijlsma et al., 2014, Bohannon, 2008). In the case of muscle mass, Zheng et al., (2016) found significant correlation between HGS and appSMMI (Zeng et al., 2016). Albeit positive in nature, the current study failed to find any significant associations of HGS with any forms of 25(OH)D (Table 4.19c). A number of other studies have also failed to find any significant associations between HGS and Vitamin D (Wang et al., 2019, Sahin et al., 2013, Matheï et al., 2013, Dang et al., 2019). Some authors explained the absence of association by the decrease in Vitamin D receptors (VDR) expression observed in the elderly, especially the ones of advanced age (Matheï et al., 2013). Active form of vitamin D, ($1,25(OH)_2D_3$) relies on the presence of VDR to exert

genomic effects at target sites. VDR is a nuclear receptor that is found on numerous parts of the body, and when bound to $1,25(OH)_2D_3$, regulates a wide array of genes. In order to understand the clinical effects of Vitamin D deficiency on muscle function, various studies had been conducted to determine the presence of VDR in skeletal muscle (Matheï et al., 2013, Wang, Becklund & DeLuca., 2010). The expression of VDR in skeletal muscle may imply direct action of Vitamin D on this tissue. Conversely, if no VDR expression is found in skeletal muscle, the effect of Vitamin D on this tissue may also be indirect, and occur via systemic changes in calcium and phosphate levels. In vivo, however, the study of the presence of VDR in skeletal muscle has been confounded by several factors. For example, VDR may change during differentiation and development of muscle. In addition, the presence of VDR in skeletal muscle had been found to be affected by technical factors such as non-specific VDR antibodies, variations in muscle models and protein extraction methods (Wang, Becklund & DeLuca., 2010). Moreover, difficulty exist when establishing the presence of VDR in muscle or any of its components due to the complex nature of muscle that comprised of multinucleated, post-mitotic fibers, myofibroblasts, endothelial cells and satellite cells with myogenic potential. Current hypothesis posits that VDR is expressed in skeletal muscle but at very low levels. The expression may also be higher during the early stages of muscle development and during muscle repair and finally, modulates the uptake of Vitamin D into skeletal muscle fibers (Abboud et al., 2018; Makanae et al., 2015; Abboud et al., 2013; Srikuea et al., 2012).

In addition to a decrease in muscle mass, hypovitaminosis D has also been found to be significantly associated with a decrease in lower limb strength in older men and women (Scott, et al., 2010). A cross-sectional study conducted by Ahern et al. (2014) reported slower walking speed in 252 severely obese and Vitamin D deficient adults (Ahern et al., 2014). In the current study, we found that different forms of 25(OH)D is correlated to different component of functional performance (Table 4.19c). For example, total 25(OH)D was found to be significantly and positively correlated with walking speed (GS), and bioavailable 25(OH)D was found to be correlated with lower extremity strength (STS, Table 4.19c, $p<0.05$). These observed associations suggest that regardless of fractions of Vitamin D, older women with low Vitamin D level may be at a higher risk for future falls and fall-related injury due to weaker lower body strength. Other studies have reported significant associations between low Vitamin D status and subsequent falls risk, decline in physical performance and development of sarcopenia in the elderly (Wicherts et al., 2007; Snijder et al., 2006; Visser, Deeg & Lips, 2003).

In the Invecchiare in Chianti (InCHIANTI) study (involving 966 individuals, 435 men and 531 women) with a mean age of 75 years, a significant association was found between low level of vitamin D and poor physical performance, as assessed by HGS and a short physical performance battery test (SPPB) (Houston et al., 2007). In the study, subjects with serum vitamin D <25 nmol/L performed poorer than those with a level above 25 nmol/L. Muscle strength using a handgrip test was also significantly higher in subjects with Vitamin D levels >50 nmol/L than in those with levels below this threshold (Houston et al., 2007). Further, an American study on US women

over the age of 65 years, residing in long-term care demonstrated that subjects with 25(OH)D level of < 50 nmol/l at baseline exhibited a greater decline in physical function at 12 and 24 months, and higher number of falls, compared to women with sufficient Vitamin D level at baseline, despite daily supplementation of 800 IU of vitamin D in all participants (Kotlarczyk et al., 2017). Finally, a case-control study involving 55 veiled Arabic women with severe vitamin D deficiency (mean 25OHD 7 nmol/L) reported low results on all tested parameters of muscle function, compared to a control group of 22 Danish women with higher levels of Vitamin D (47 nmol/l) (Glerup et al., 2000). Consequently, increase in Vitamin D levels leads to better functional performance. The same study found that following frequent supplementation of Vitamin D (Vitamin D2: 100,000 IU per week for 1 month, then monthly for 5 months and 400–600 IU orally daily), significant improvements in muscle function and pain at 3 and 6 months were reported in the Arabic women. Additionally, a longitudinal study by Flicker et al (2003) involving 1600 institutionalized, older women found that by doubling the level of 25(OH)D over a 5 month period, a 20% reduction in falls risk was found (Flicker et al., 2003). Mastaglia et al. (2011) also reported that, in healthy women aged over 65 years (n=54), Vitamin D levels above 50 nmol/L were associated with a higher lower body strength compared to women with lower levels of Vitamin D (stronger knee extensor of 13.4 (2.7) versus 11.6 (2.5) kg, $p<0.03$) (Mastaglia et al., 2011). Finally, in a study involving 230 elderly men and 370 elderly women, Boye et al. (2013) found that a higher level of vitamin D was significantly associated with a 3 times faster time-and-get-up (TUG) test, with a five times faster sit-to-stand test in men, and

with a 2.5 times faster TUG test in women. However, whether gender differences exist in the effect of Vitamin D is still unclear (Boyé et al., 2013). In an Italian study involving 2694 community-dwelling elderly (1597 women and 1097 men, mean age of 74 years), Toffanello et al. (2012) reported that lower vitamin D levels (<50nmol/L) were associated with a slower 6-minute walking test and weaker strength, independently of gender (Toffanello et al., 2012).

The results of these studies may suggest strong interaction between Vitamin D and muscle. However, given the various design of observational and interventional studies, some controversies still exist in explaining the relationship between Vitamin D and musculoskeletal health. However, general consensus between the experts states that a minimum level of 50nmol/L of 25(OH)D is required in the general elderly population, and for people with high risk for falls and fracture, minimum level of 75nmol/L should be met (Rizzoli et al., 2014, Rizzoli et al., 2013). Vitamin D supplementation is generally safe and inexpensive. Therefore, it is highly recommended in patients at risk for falls, such as the elderly, institutionalized patients, frail patients, and patients with chronic diseases. These individuals tend to have low levels of Vitamin D and muscle disorders. Therefore, supplementations is justified regardless of any assumed effect on the prevention of falls.

5.3.3 Total, bioavailable and free 25(OH)D status of obese postmenopausal women with musculoskeletal health disorders

Osteosarcopenic obesity syndrome (OSO) is a condition characterized by low bone mass (osteopenia/osteoporosis), low muscle mass (sarcopenia) and high adiposity (obesity). Bone and muscle are integrated organs with shared principal functions in structure, strength, and motion. Current and other studies (Binkley & Buehring, 2009) have found significant and positive correlations between bone and muscle mass. This correlation may be due to muscle-induced skeletal strain, which helps increasing the density of the skeleton (Binkley & Buehring, 2009). However, whether low bone mass (osteoporosis) and low muscle mass (sarcopenia) should be considered as separate entity or combined into a single condition is still unclear. Previously, due to numerous studies describing positive relationship between bone and muscle, 'sarcoosteopenia' was suggested to describe the combined disorder of the loss of bone mass and muscle mass. Other terms, such as sarcoosteoporosis or osteosarcopenia, have also been suggested. However, no consensus has been reached. Additionally, there are currently no clear operational definition of the combination of osteoporosis and sarcopenia, which resulted in heterogeneity in all epidemiological-related studies. Regardless, various studies have emerged to give more insight into the OSO syndrome and its individual components (JafariNasabian et al., 2017).

Among the potential determinants of OSO, Vitamin D had been suggested to be one of the factors that may impact the manifestation of the syndrome (Bruyère et al., 2017). In muscle, active form of Vitamin D was theorized to stimulate the proliferation of muscle cell and growth by activating Vitamin D receptors (VDR). These receptors became the mediators for both gene transcription and rapid non-transcriptional signal transduction. This in turn,

will regulate the synthesis of protein and calcium handling involved in muscle cell development (Ceglia & Harris, 2013). In fat, Vitamin D may modulate VDR connected to energy metabolism (Wong et al., 2011) and also potentially modulate adipogenesis and preadipocyte differentiation (Ding et al., 2012). Vitamin D deficiency may induce adipogenesis, whereas higher Vitamin D levels could potentially attenuate the effect (Floss, 2009, Ryan et al., 2013). Conversely, obesity may also be a risk factor for Vitamin D deficiency.

The interconnection between muscle and bone led to the hypothesis that the improvement of one tissue will also benefit the other. Currently, few studies have examined the relationship between serum Vitamin D levels with combined abnormalities in body composition such as in OSO (Lee, 2013, Shantavasinkul et al, 2015). So far, there was only one study investigating 25(OH)D association with osteosarcopenia in obese population (Kim et al., 2017) and one review paper detailing the relationship between Vitamin D and osteosarcopenia (Bruyère, Cavalier & Reginster, 2017). In their report, Kim et al. (2017) concluded that a high serum Vitamin D levels in middle age to elderly men and women, was associated with reduced odds of the manifestation of OSO, suggesting that maintaining adequate levels Vitamin D is important in the prevention of OSO. The study also found that Vitamin D deficient women were also more likely to demonstrate OSO (OR = 1.99, 95% CI: 1.30, 3.05) compared with those having normal levels of serum Vitamin D. Conversely, in the current study, we found that people with OSO did not have a particularly low level of 25(OH)D (Figure 4.10). The current study found that healthy, normal weight participants (NR) have significantly

higher total, bioavailable and free 25(OH)D levels compared to OO ($p<0.05$), but not with OSO group (Figure 4.10). The OSO group in the current study appears to have healthy levels of total 25(OH)D (~50nmol/L, Figure 4.10). Inglis, Kelley and Ilich (2015) also reported similar findings. The study, which assessed nutritional and Vitamin D status of postmenopausal obese and osteosarcopenic obese women, found that the level of total 25(OH)D of women with OSO was 78.8±71.8 nmol/L (Inglis, Kelly & Ilich, 2015), which was higher than the threshold for 'Replete' level according to IOM (50 nmol/L). In the current study, the adequate level of 25(OH)D in the OSO group could be explained by their low percent of body fat (BFP). Although still categorized as obese, the OSO group had the lowest BFP (38.7(4.9)%) compared to OO [44.9(4.7)%], SO [40.8(4.7)%], and OB [43.7(5.8)%] (Table 4.10). Obesity and low Vitamin D levels are often linked together. Studies have found that independent of age and geographical location, overweight and obese adults have a higher prevalence of hypovitaminosis D compared to normal weight adults (Pereira-Santos et al., 2015). Low concentration of Vitamin D in obese people may be due to the sequestration of 25(OH)D into adipose tissue. In the current study, the group with the highest BFP was the OO group (44.9(4.7) %, Table 4.10). This group was found to have the lowest levels of 25(OH)D compared to OSO, SO, OB and NR group (Figure 4.10), likely due to the sequestration of 25(OH)D into adipose tissue. The low level of total 25(OH)D (<40nmol/L) may cause the manifestation of osteoporosis in this group (OO). In the case of OSO group, there may be other factors than 25(OH)D causing the manifestation of both osteoporosis and sarcopenia,

which may include genetic, diet, age and physical activity levels (Bauer et al., 2019).

Indeed, there have been studies showing no significant associations between Vitamin D indices and bone health (Saarnio et al., 2018, Walsh et al., 2016) nor with skeletal muscle mass (Ko et al., 2015) in middle-aged and older overweight and obese adults. Due to the complex nature of OSO, its manifestation may also involve complex mechanism that include multiple factors, such as endocrine regulation, thresholds variances, hormonal changes related to comorbidities, and cross-talk between endocrine, immune and neurological system (Kelly et al., 2019). Although interactions between hormonal changes and altered body composition are known, there are still debates regarding causalities and also the possibilities of reverse causalities, as the nature of the interactions could be bidirectional. While many potential and biologically plausible contributing mechanisms have been hypothesized, they are still inconclusive to propose a concrete description of pathophysiology, prevention, and treatment. Nevertheless, there are studies that have shown beneficial effects of Vitamin D on the interaction between bone and muscle (Tanaka et al., 2014, Souberbielle et al., 2010). In the current study, the OB and NR group had total 25(OH)D levels of ≥50nmol/L (Figure 4.10) with healthy bone and muscle mass, which may suggest beneficial effect of 25(OH)D on bone and muscle.

More studies is needed to determine the cause and effect of this relationship. Currently, no interventional studies have been performed to assess the effect of Vitamin D on osteosarcopenic population, obese or otherwise. Establishing a causal relationship between OSO and Vitamin D could yield a

better understanding in the underlying biological mechanism of the interrelationship of bone, fat and muscle mass. To increase the knowledge and understanding in this area, a consensus on the definition of OSO is needed, which will allow for accurate diagnosis and comparisons between studies. The first step that is critical in this process is to standardize the operational definition of OSO.

CHAPTER 6

CONCLUSION

6.1 Conclusion

In this study involving multiethnic Malaysian women, we have described the current status of musculoskeltal health of young and older cohort. Majority of postmenopausal women in this study sample were obese, albeit having good musculoskeltal health according to standard definitions. Regardless, their low physical performance begged re-analysis of cut-off values suitable for the obese. We presented alternative measures and cut-off values for the physical diagnosis of OSO using portable equipment, allowing the syndrome to be screened in the general population. With regards to Vitamin D, although both total and bioavailable 25(OH)D were positively correlated to bone density (BUA) and muscle mass (appSMMI), and the bioavailable fraction of 25(OH)D was marginally stronger than total 25(OH)D in its correlation with bone and muscle, it is still too early to draw any conclusion on the relationship. The small differences in the R values and the small effect size does not warrant the conclusion that bioavailable 25(OH)D is superior to total 25(OH)D in its association to the BUA. More research is needed, with a larger sample size in order to validate these findings. Nevertheless, the significant correlation found between bioavailable 25(OH)D levels and the BUA shows that it may be helpful in clinical practices when assessing bone health in Malaysian women.

We also found that hypovitaminosis D was associated with a higher likelihood of concurrent condition of obesity and osteoporosis (Osteopenic

obesity) in postmenopausal women. The findings suggest that maintaining high levels of Vitamin D may potentially protect against combined abnormalities in body composition, especially Osteopenic obesity. Longitudinal and cause-effect studies are needed to increase our understanding of the impact of vitamin D levels on body composition disorders in the elderly. In addition, ongoing controversies in the Vitamin D area requires resolutions in order to effectively guide future research and clinical practices. For example, there should be a refinement of the gold-standard methods for the quantification of vitamin D metabolites, ideally with a use of similar methods and assay standardization so that serum values are directly comparable between studies and clinical centers. A consensus on optimal cut-offs and on what constitutes as adequate vitamin D levels is also needed. However, due to various evidence, perhaps optimal levels should be tailored to the specific background, conditions or disease states.

OSO is a progressive syndrome that requires early interventions. The screening of osteosarcopenia in asymptomatic obese women may potentially reduce their risk of adverse health outcomes at a later age. Acknowledging OSO as an emerging problem in public health will increase scientific and public awareness for the proper diagnostic criteria, prognosis, public health costs, and ultimately the development of behavioral, nutritional, and even pharmacological interventions to prevent or reverse this condition. To the best of our knowledge, the current study is the first study in estimating the prevalence of OSO, SO and OO in a cohort of overweight/obese Malaysian postmenopausal women. Further, this study is also among the first in proposing preliminary cut-off values for the diagnosis of OSO in Malaysian

population. The study of OSO as a single entity is still in infancy and its diagnostic criteria needs further refinement and validation studies.

6.2 Limitations

This study has some limitations. First, the participants in this study were mostly healthy and relatively young (~60 years), resulting in a low prevalence of OSO, thus limiting the generalizability of the results, albeit having better homogeneity. Skin color was not directly measured, limiting the ability to validate the differences in 25(OH)D levels between ethnicities. In addition, for the correlation test between BUA and both 25(OH)D indices (total and bioavailable), the sample size was underpowered (total 25(OH)D; effect size=0.2, Power=0.602, bioavailable 25(OH)D; effect size=0.2, Power=0.703). A post-hoc power analysis (calculated using G*Power 3.0.10) also revealed that on the basis of the mean, between-groups comparison and the effect size of d=0.291, Sigma=16.7 and alpha=0.05, the sample size was underpowered (Power=0.657) to detect differences of total 25(OH)D between the musculoskeletal disorder groups (ANOVA). Nevertheless, the power was much higher for other variable of interest such as the HGS (the effect size=0.475, and Power=0.991, Sigma=4, alpha=0.05). Similarly, the study was also adequately powered to perform analysis comparing the differences of 25(OH)D between ethnicities (the effect size=0.495 and Power=0.999, alpha=0.05, Sigma=16). For HGS, the effect size=0.312 and Power=0.917, alpha=0.05, Sigma=5.

Genotype-specific affinity constants was also not used in the calculation of free and bioavailable 25(OH)D due to unavailable data on VDBP

polymorphisms in the Malaysian population. This may be a confounding factor. Nevertheless, Li et al. (2017) suggests that the VDBP variants account for only a small proportion of the bioavailable 25(OH)D variation as they found no associations between the VDBP level and the presence of VDBP variants rs4588 and rs7041 with bone density. Finally, the cross-sectional design of the study prevents it from making any causal inferences.

6.3 Strengths

Notwithstanding the above limitations, the current study had several strengths. One of the strengths of this study lies in the representativeness of the study sample which include the 3 major ethnicity in Malaysia, crediting the external validity of the results. Further, the participants were non-institutionalized, allowing direct extrapolation to the population at large. Moreover, potential confounders were adjusted in the analysis, demonstrating the robustness of the findings. In addition, the current study determined the associations of total and bioavailable 25(OH)D levels with bone, fat and muscle indices, which could lead to elucidation of the underlying mechanisms for the effects of 25(OH)D on OSO.

The current study also described the prevalence of OSO, SO and OO in a cohort of Malaysian postmenopausal women, and proposed screening test criteria for osteosarcopenia in obese women. In addition, the current study described musculoskeletal health of both younger and older Malaysian women and the relationship between OSO and Vitamin D. To the best of our knowledge, this is the first study to investigate age disparities in musculoskeletal health status between younger and older Malaysian women, thereby bridging the research gap in the pool of currently available data.

6.4 Recommendation for Future Works

Further research is needed validate the proposed cut-offs values using a more robust diagnostic tools such as DXA. For example, in the case of the ROC Curve method, DXA-derived measures such as the T-score of the femoral neck, the appendicular muscle mass and/or body fat percent should be used as external criteria. Further research is also needed to investigate whether the screening tools (QUS, the BIA and the SPPB) do measure the risk of OSO and have similar clinical performances between populations and gender. Since the present study is limited by its small sample size, further research with more sample size and higher predictive power is needed to strengthen the study. The accuracy of the cut-off values needs to be validated using a higher number of samples. Future works should aim at developing the cut-off values for OSO using a larger sample size, possibly also exploring the definition of OSO for different population groups as defined by gender and ethnicity. While our proposed criteria and cut-off values were always meant to be improved upon, there are still more work to be done to achieve a complete and most comprehensive diagnosis of OSO syndrome. Due to inadequate technology for some aspects of body composition assessment, it is presently difficult to measure the degree of fat infiltration in the subjects. Currently, there is still no convenient tool to assess infiltrated fat into bone and muscle other than magnetic resonance imaging (MRI) which is still not suitable for routine general screening. However, there is an opportunity to add the diagnosis of OSO to scheduled abdominal CT/MRI scans (Murray, Williams, & Lee, 2017), which ultimately may help with treatment plans and improve outcomes. Additionally, it was suggested that newer technologies,

such as quantitative computed tomography (CT), EchoMRI (Quantitative Magnetic Resonance), OsteoProbe and ultrasonography be utilized for the diagnosis and management of OSO (Ilich, 2017). Although these are reliable tools that could be utilized and further developed for the future diagnosis of OSO and its components, they are unfeasible to be used in mass screenings. There needs to be a concerted effort to develop devices that can be conveniently used in the field/clinic as well as in a research setting to accurately assess body composition, so more studies can be done to refine the diagnostic criteria for OSO. Using these sophisticated technologies, physicians will be able to select therapy that targets body composition and skeletal characteristics at risk.

In the case of Vitamin D, further studies should be done to gather data on VDBP polymorphisms in a larger sample of multiethnic Malaysian women. There should also be a study to determine the influence of VDBP polymorphism on vitamin D status among Malaysian women. The outcomes of these study will help us to understand more on the role of VDBP and consequently, the bioavailable fraction of vitamin D on musculoskeletal health.

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RESPONDENT INFORMATION SHEET AND CONSENT FORM

Title of study: Osteosarcopenic Obesity: The Development Of Screening Test Criteria And The Association With Bioavailable 25(OH)D

Name of investigator and institution: Dr. Soma Mitra and Nurdiana Zainol Abidin/University of Nottingham Malaysia

Introduction:

You are invited to participate in a research study because you are a Malaysian woman and have reached menopause. The details of the research process are described in this document. It is important that you understand why the research is being done and what it will involve. Please take your time to read through and consider this information carefully before you decide if you are willing to participate. Ask the project staff if anything is unclear or if you like more information. After you are properly satisfied that you understand this study, and that you wish to participate, you must sign this informed consent form. To participate in this study, you will be required to provide a clinician/investigator with information of your medical history.

Your participation in this study is voluntary. You do not have to be in this study if you do not want to. You may also refuse to answer any question you do not want to answer. If you volunteer to be in this study, you may withdraw from it at any time. If you withdraw, all your data will be removed from this study. Your refusal to participate or withdraw will not affect any medical or health benefits to which you are otherwise entitled.

This study has been approved by the Science and Engineering Research Ethics Committee, (SEREC), University of Nottingham Malaysia Campus.

What is the purpose of the study?

The purpose of this study is to identify osteosarcopenic obesity, osteopenic obesity, and sarcopenic obesity in a population of postmenopausal Malaysian women and find associations, if any, with vitamin D indices. Eligibility criteria for study participants are as follows: 1) woman of Malaysian nationality, 2) no menstrual bleeding, or spotting during the 12 months prior to enrollment.

You are NOT eligible to participate in this study if you: 1) Have inability to stand for height, weight and gait speed assessments, 2) Have artificial limbs and/or metal implants, 3) Have severe cardiac, pulmonary, or musculoskeletal disorders, 4) Have severe cognitive impairment or any disability that makes communication impossible, 5) Have presence of terminal illness, 6) Have co-morbidities associated with high risk of falls (e.g. Parkinson's disease) or that may directly affect gait speed.

What will happen if I decide to take part and what kind of study procedures will I receive?

At the onset, information will be collected from study participants by an investigator on medical history. If you are eligible, the investigators will collect information from you on basic characteristics, e.g educational level and physical activity using a validated questionnaire. Then, your physical measurements will be taken and your physical capability will be assessed. You will be required to give 8ml of blood which will be collected by a trained phlebotomist. Your participation will be for about 30-40 minutes for all of the above assessments.

Study Procedure

Each participant will be evaluated according to the procedures bellow. Procedures may take 30-40 minutes to be completed:

a) Questionnaire

This section will be administered by the interviewer. The questionnaire will include:

i. Socio-demographic questions such as age, sex, educational level, physical activity, and health status.
ii. FRAX® - a fracture risk assessment tool formulated by the World Health Organisation.

b) Anthropometric measurements of :

i. Height and weight
ii. Body composition
iii. Waist and hip circumference

c) Blood will be collected (8ml) to assess the levels of, vitamin D indices, calcium, parathyroid hormone and albumin.
d) Assessment of physical performance:

i. 6 meter walk test: Gait speed will be measured by timing the 6-meter normal walk. The 6-meter course is marked by two cones or pieces of tape. You will start at one end of the course, walking at a normal pace and walk past the other end of the course.

ii. Sit-and-stand test (as many as you can in 30 seconds): You will be seated in an armless chair, arms crossed over chest, back straight, and feet flat on the floor. You will then be asked to rise from the chair and sit down again as many times as possible in a 30-second period.

iii. One-Leg stance (right and left leg, max. 30 seconds): For one-leg stance, you will be asked to stand on one leg while lifting the other leg, for up to 30 seconds, performed on both the right and left legs.

iv. Handgrip strength: Muscle strength will be determined by handgrip strength and will be measured in each hand using a hand dynamometer.

What are the benefits of being in this study?

You will have firsthand information about your current body composition in terms of fat mass and muscle mass. From ultrasound bone measurements you will have information of the status of your bone health. If you happen to be at risk of osteoporosis, the research team will intimate you on the same and you will be referred to an orthopedic at a health centre of your choice.

For the benefit of the population at large, such data from you and many other participants of this study will be analyzed to establish suitable cut-offs for easy identification of osteosarcopenic obesity, osteopenic obesity, and sarcopenic obesity in postmenopausal women and to identify a better biochemical indicator for the assessment of vitamin D status.

Sharing your health information with others

Research information and test results will not be placed in your medical record or used in your care.

Payment

There will be no payment required for participation nor any compensation will be given for participation.

Storage of your sample

The investigators will keep your data for a period of 5 years from the time of sample collection. On completion of the investigation, we will destroy all your records. Blood samples will be stored in repository at UNMC. However, before any researcher uses your specimen with your information, the study will be reviewed and approved again by SEREC, University of Nottingham in Malaysia in order to review research and protect the rights and welfare of research subjects.

What are my responsibilities when taking part in this study?

It is important that you answer all of the questions asked by the project staff honestly and completely.

What are the potential risks and side effects of being in this study?

The physical risks of testing is usually minimal, typically no risk. We will protect your confidentiality by using codes. Your specimen will have a coded number that only the study team will know. We will protect all data and they will not be used to identify you.

There is no direct benefit to you from allowing us to store your samples. The use of your samples in this research could help us learn more about the musculoskeletal health of postmenopausal Malaysian women and how to improve it. This knowledge could help other investigators in the future.

Will my medical information be kept private?

All your information obtained in this study will be kept and handled in a confidential manner, in accordance with applicable laws and/or regulations. When publishing or presenting the study results, your identity will not be revealed without your expressed consent.

Who should I call if I have questions?

If you have any questions about the study and you want information about results, please contact the investigators: Dr. Soma Mitra at +6 (03) 8725 3433 or Ms. Nurdiana Abidin at + 6019-2706303.

In case you have a complaint in your treatment by a member of staff or anything to do with the study, you can initially approach the lead investigators. If this achieves no satisfactory outcome, you can then contact the secretary of SEREC, UNMC at ethics@nottingham.edu.my.

What does your signature on this consent form mean?

Your signature on this consent form means:

- You have been informed about the purpose, procedures, possible benefits and risks of this study.
- You have been given the chance to ask questions before you sign.
- You have voluntarily agreed to be in this study.

APPENDIX A1

Respondent's Information and Consent Form (Signature Page)

Research Title: Osteosarcopenic Obesity: The Development Of Screening Test Criteria And The Association With Bioavailable 25(OH)D

Investigator's name: Dr. Soma Mitra and Nurdiana Zainol Abidin

To become a part this study, you or your legal representative must sign this page. By signing this page, I am confirming the following:

- I have read all of the information in this Respondent's Information and Consent Form including **any information regarding the risk in this study** and I have had time to think about it.
- I understand the purpose of the research project and my involvement in it.
- I understand that while information gained during the study may be published, I will not be identified and my personal results will remain confidential.
- I voluntarily agree to be part of this research study, to follow the study procedures, and to provide necessary information to the investigator, or other staff members, as requested.
- I may freely choose to stop being a part of this study at any time.
- I understand that data will be stored in the investigator's own personal computer and will be accessible only by the research team.
- I have received a copy of this Respondent's Information and Consent Form to keep for myself.
- I understand that I may contact the researcher or supervisor if I require further information about the research, and that I may contact the SEREC, University of Nottingham, if I wish to make a complaint relating to my involvement in the research.

Respondent's name	**Respondent's I.C number**
Signature of Respondent or Legal Representative	**Date (ddMMyy)**

BORANG MAKLUMAT DAN KEIZINAN RESPONDEN

Tajuk Kajian: Osteosarkopenik obesiti: Penentuan kriteria saringan dan perkaitan dengan *bioavailable* 25(OH)D

Nama penyelidik dan institusi: Dr. Soma Mitra/Nurdiana Zainol Abidin University of Nottingham Malaysia

Pengenalan:

Anda dijemput untuk mengambil bahagian dalam penyelidikan ini kerana anda adalah seorang wanita warganegara Malaysia dan telah mencapai menopaus. Butir-butir proses penyelidikan adalah seperti yang dinyatakan dalam dokumen ini. Ia adalah penting untuk anda memahami mengapa penyelidikan ini dilakukan dan apakah prosedur-prosedur yang dilibati. Sila luangkan masa untuk membaca dan mempertimbangkan maklumat-maklumat ini dengan teliti sebelum anda membuat keputusan dan jika anda bersedia untuk mengambil bahagian. Sila tanya salah seorang kakitangan projek ini jika ada apa-apa yang tidak jelas atau jika anda memerlukan maklumat lanjut. Selepas anda betul-betul berpuas hati dan memahami kajian ini, dan anda ingin menyertainya, anda perlu menandatangani borang persetujuan ini. Untuk mengambil bahagian dalam penyelidikan ini, anda mungkin dikehendaki memberitahu doktor atau penyelidik maklumat sejarah perubatan anda.

Penyertaan anda dalam kajian ini adalah secara sukarela. Anda tidak perlu menyertai kajian ini jika anda tidak mahu. Anda juga boleh enggan menjawab apa-apa soalan yang anda tidak mahu menjawab. Jika anda menawarkan diri untuk menyertai kajian ini, anda boleh menarik diri pada bila-bila. Jika anda menarik diri, mana-mana data yang telah dikumpul tidak akan digunakan untuk kajian. Keengganan anda untuk mengambil bahagian atau menarik diri tidak akan menjejaskan apa-apa faedah perubatan atau kesihatan yang sepatutnya anda dapat.

Kajian ini telah diluluskan oleh *Science and Engineering Research Ethics Committee, (SEREC)*, Universiti Nottingham Kampus di Malaysia.

Apakah tujuan kajian ini?

Tujuan kajian ini adalah untuk mengenal pasti individu yang mempunyai osteosarkopenik obesiti, osteopenik obesiti, dan sarcopenik obesiti dikalangan populasi wanita warganegara Malaysia yang telah mencapai menopaus dan mencari perkaitan, jika ada, dengan indeks vitamin D. Kriteria kelayakan untuk peserta kajian adalah seperti berikut: 1) wanita warganegara Malaysia, 2) telah putus haid selama 12 bulan berturut-turut sebelum pendaftaran dalam kajian.

Anda TIDAK layak untuk mengambil bahagian dalam kajian ini jika anda: 1) Tidak boleh berdiri dengan sendiri tanpa bantuan untuk mengukur berat, tinggi dan kelajuan gaya jalan, 2) Mempunyai tangan/kaki palsu atau implant besi, 3) Menghidapi penyakit jantung, pernafasan atau penyakit otot dan tulang yang serius, 4) Mempunyai penyakit kognisi yang teruk atau kurang upaya yang

mengganggu komunikasi, 5) Mempunyai penyakit terminal, 6) Mempunyai penyakit yang memberi risiko tinggi untuk jatuh (contoh: penyakit Parkinson's) atau penyakit yang menggangu kelajuan gaya jalan.

Apa yang akan berlaku jika saya membuat keputusan untuk mengambil bahagian dan apakah jenis-jenis prosedur kajian yang akan saya terima?

Pada permulaan, maklumat daripada peserta kajian akan dikumpul oleh seorang pegawai penyelidik mengenai sejarah perubatan. Jika anda layak menyertai kajian ini, pegawai penyelidik akan mengumpul maklumat mengenai kriteria-kriteria asas, seperti tahap pendidikan dan aktiviti fizikal menggunakan borang soal selidik yang telah disahkan. Kemudian, ukuran fizikal anda akan diambil dan keupayaan fizikal anda akan dinilai. Anda akan dikehendaki untuk memberi 8ml darah yang akan dikumpulkan oleh *phlebotomist* yang terlatih. Penyertaan anda akan mengambil kira-kira 30-40 minit untuk semua penilaian atas.

Prosedur-prosedur kajian

Setiap peserta kajian akan ditinjau berdasarkan prosedur berikut:

a) Soal selidik

Bahagian ini akan dijalankan dengan pengisian borang soal selidik termasuk:

i) Socio-demografik seperti jantina, umur, taraf pendidikan, status kesihatan, dan aktiviti fizikal.
ii) Pengisian borang soal-selidik FRAX® (sejarah penyakit tulang) yang telah diformulasi oleh World Health Organisation (WHO).

b) Penilaian anthropometrik:

i. Berat dan tinggi
ii. Jisim otot, jisim lemak badan dan jisim tulang
iii. Ukur lilit pinggang dan pinggul

c) Pengambilan darah sebanyak 8ml untuk peninjauan kandungan indeks vitamin D, kalsium, hormon paratiroid, dan albumin dalam darah.

d) Penilaian fizikal:

i) Masa yang diambil untuk berjalan sepanjang 6 meter: kelajuan gaya berjalan (*gait*) akan diukur dengan mengambil masa yang anda ambil untuk berjalan dalam kelajuan normal sepanjang 6 meter. Jarak 6 meter akan ditandai dengan dua kon atau kepingan pita. Anda akan bermula pada satu hujung jarak ini, berjalan pada kadar yang normal dan berjalan sekali lalu dari satu hujung jarak ke hujung jarak yang lain.
ii) Bilangan duduk diatas kerusi dan berdiri sebanyak yang mungkin dalam masa 30 saat: Anda akan diminta duduk di kerusi, dengan tangan melintasi atas dada, belakang lurus, dan kaki rata di atas

lantai. Anda kemudian akan diminta untuk bangkit dari kerusi dan duduk semula seberapa banyak kali yang mungkin dalam tempoh 30 saat.

iii) Kebolehan untuk berdiri dengan satu kaki selama 30 saat (kaki kiri dan kanan): Untuk menguji keseimbangan badan, anda akan diminta untuk berdiri dengan sebelah kaki sambil mengangkat kaki yang lain, sehingga 30 saat, dilakukan ke atas kedua-dua kaki, kiri dan kanan.

iv) Kekuatan genggaman tangan: kekuatan otot akan dinilai menggunakan kekuatan genggaman dan akan diukur dalam setiap tangan menggunakan *hand dynamometer*.

Apakah faedah-faedah yang akan saya dapati dengan menyertai kajian ini?

Anda akan mempunyai maklumat secara langsung mengenai status pemakanan anda daripada keputusan penilaian jisim lemak dan jisim otot. Daripada keputusan penilaian tulang (menggunakan mesin *ultrasound*), anda akan mempunyai maklumat mengenai status kesihatan tulang anda. Jika anda berada pada risiko tinggi untuk osteoporosis, pasukan penyelidik akan memberitahu anda dan anda akan dirujuk kepada pakar ortopedik di pusat kesihatan pilihan anda.

Demi manfaat populasi secara umum, data daripada anda dan peserta-peserta lain akan dianalisa untuk mewujudkan "cut-off" yang sesuai untuk mengenal pasti osteosarkopenik obesiti, osteopenik obesiti, dan sarcopenik obesiti di kalangan wanita yang telah mencapai menopaus dan juga untuk mengenal pasti penunjuk biokimia yang lebih baik bagi penilaian status vitamin D.

Perkongsian maklumat kesihatan dengan pihak lain

Maklumat penyelidikan dan keputusan ujian tidak akan dimasukkan di dalam rekod perubatan anda atau digunakan dalam penjagaan anda.

Bayaran

Tiada sebarang bayaran dikenakan atau diberi untuk penyertaan.

Penyimpanan sampel anda

Pegawai-pegawai penyelidik akan menyimpan data anda selama tempoh 5 tahun dari masa pengumpulan sampel. Setelah selesai kajian, kami akan memusnahkan semua rekod anda. Sampel darah akan disimpan di dalam tabung di UNMC sebelum dianalisa. Walau bagaimanapun, sebelum penyelidik kajian menggunakan spesimen dan maklumat anda, kajian tersebut akan dilihat semula dan diluluskan oleh SEREC, Universiti Nottingham di Malaysia untuk melindungi hak-hak dan kebajikan subjek kajian.

Apakah tanggungjawab saya semasa mengambil bahagian dalam kajian ini?

Adalah penting untuk anda menjawab semua soalan-soalan yang ditanya oleh kakitangan projek ini secara jujur dan menyeluruh.

Apakah potensi risiko-risiko dan kesan sampingan daripada kajian ini?

Risiko ujian fizikal biasanya minimum, dan selalunya tiada risiko. Kami akan melindungi kerahsiaan anda dengan menggunakan kod. Spesimen anda akan diberikan kod dan hanya pegawai penyelidik sahaja yang akan tahu. Kami akan melindungi semua data dan data tersebut tidak akan digunakan untuk mengenal pasti anda.

Tidak ada manfaat secara terus daripada kebenaran untuk kami menyimpan sampel-sampel anda. Penggunaan sampel dalam kajian ini boleh membantu kami mengetahui lebih lanjut mengenai kesihatan otot wanita yang telah mencapai menopaus dan bagaimana untuk memperbaikinya. Informasi ini boleh membantu masyarakat pada masa akan datang.

Adakah maklumat perubatan saya akan disimpan secara rahsia?

Semua maklumat anda yang diperolehi daripada kajian ini akan disimpan dan dikendalikan secara sulit, mengikut undang-undang dan/atau peraturan yang berkenaan. Apabila kami menerbitkan atau membentangkan keputusan kajian, identiti anda tidak akan didedahkan tanpa kebenaran anda.

Siapakah yang patut saya hubungi jika saya mempunyai soalan?

Jika anda mempunyai sebarang soalan mengenai kajian ini dan anda ingin maklumat tentang hasilnya, sila hubungi pegawai penyelidik: Dr. Soma Mitra di +6 (03) 8725 3433 atau Cik. Nurdiana Abidin di + 6019-2706303.

Sekiranya anda mempunyai aduan mengenai anggota kakitangan atau apa-apa kaitan dengan kajian ini, anda pada mulanya boleh menghubungi pegawai penyelidik. Jika ini tidak mempunyai keputusan yang memuaskan, anda boleh menghubungi SEREC, UNMC di ethics@nottingham.edu.my.

Apakah makna tanda tangan anda pada borang persetujuan ini?

Tandatangan anda di borang persetujuan ini bermakna:

• Anda telah dimaklumkan mengenai tujuan, prosedur, manfaat dan risiko yang mungkin daripada kajian ini.

• Anda telah diberi peluang untuk bertanya soalan sebelum anda menandatanganinya.

• Anda telah secara sukarela bersetuju untuk menyertai kajian ini.

APPENDIX A2

Borang Maklumat Dan Keizinan Responden (halaman tanda tangan)

Tajuk Kajian: Osteosarkopenik obesiti: Penentuan kriteria saringan dan perkaitan dengan *bioavailable* 25(OH)D

Nama penyelidik dan institusi: Dr. Soma Mitra dan Nurdiana Zainol Abidin/ University of Nottingham Malaysia

Untuk menyertai kajian ini, anda atau wakil sah anda mesti menandatangani muka surat ini.

Dengan menandatangani muka surat ini, saya mengesahkan yang berikut:

- Saya telah membaca semua maklumat dalam Borang Maklumat dan Keizinan Responden ini **termasuk apa-apa maklumat berkaitan risiko yang ada dalam kajian** dan saya telah pun diberi masa yang mencukupi untuk mempertimbangkan maklumat tersebut.
- Saya faham tujuan projek penyelidikan dan penglibatan saya di dalamnya.
- Saya faham, walaupun maklumat yang diperolehi semasa kajian boleh diulang terbit, saya tidak akan dikenal pasti dan keputusan peribadi saya akan kekal sulit.
- Saya, secara sukarela, bersetuju menyertai kajian penyelidikan ini, mematuhi segala prosedur kajian dan memberi maklumat yang diperlukan kepada penyelidik utama dan juga kakitangan lain yang berkaitan apabila diminta.
- Saya bebas memilih untuk berhenti menjadi sebahagian daripada kajian ini pada bila-bila masa.
- Saya faham bahawa data akan disimpan dalam komputer peribadi penyelidik dan boleh diakses hanya oleh pasukan penyelidikan.
- Saya telah menerima satu salinan Borang Maklumat dan Keizinan Responden untuk simpanan peribadi saya.
- Saya faham bahawa saya boleh menghubungi penyelidik atau penyelia jika saya memerlukan maklumat lanjut mengenai penyelidikan, dan saya juga boleh menghubungi SEREC, Universiti Nottingham, jika saya ingin membuat aduan berkaitan dengan penglibatan saya dalam penyelidikan.

APPENDIX B

Demographic Data

1. What is your gender O male O female
Apakah jantina anda? *lelaki* *perempuan*

2. What is your date of birth? ☐ / /
Apakah tarikh lahir anda?

3. What is best described your ethnicity?
Apakah kumpulan etnik anda?
O Malay O Chinese O Indian O Other: _____
Melayu *Cina* *India* *Lain-lain:* _____

4. What is the highest qualification you have completed?
Apakah kelayakan tertinggi anda?
- O No formal education/*Tiada pendidikan rasmi*
- O Primary school/*Sekolah rendah*
- O Secondary school/*Sekolah menengah*
- O Certificate/Diploma/*Sijil/Diploma*
- O University degree/*Ijazah*
- O Higher University/Postgraduate degree/*Ijazah Sarjana/PhD*

5. Are you currently taking: (leave blank if none)
Adakah sekarang anda mengambil: (tinggalkan kosong jika tiada)

O Calcium tablet. How many IU/mg per day _____
Tablet kalsium. Berapa IU/mg _____
O Vitamin D. How many IU/mg per day _____
Vitamin D. Berapa IU/mg _____
O Anticonvulsant medication (i.e antiepileptic or antiseizure medication) _____
Ubat untuk penyakit sawan atau epilepsi _____

6. How would you describe your current menstrual status?
Apakah status haid anda sekarang?

O Pre-menopause (before menopause; having regular periods)
Belum menopaus; haid datang secara normal
O Peri-menopause/menopause transition (changes in periods but have not gone 12 months in a row without a period)
Transisi untuk ke menopaus (haid datang tidak normal tetapi BELUM sampai ke tahap 12 bulan berturut-turut tanpa haid)

O Post-menopause (after menopause. Have gone 12 months in a row without period)
Telah mencapai menopaus (12 bulan berturut-turut tanpa haid)

a) Was your menopause…….(please tick √)
Adakah menopaus anda……. (sila tandakan √)

O Spontanoeus ("natural")
Semulajadi ("natural")

O Surgical (removal of both ovaries)
Disebabkan pembedahan (kedua-dua ovari telah dikeluarkan)

O Due to chemotheraphy or radiation theraphy.
Reason for therapy: _____
Disebabkan oleh kemoterapi atau terapi radiasi. Sebab terapi: _____

O Other. Explain: _____
Lain-lain. Terangkan: _____

b) What is your age at first menstrual period? :_____
Apakah umur anda semasa haid yang pertama?: _____

c) If not still having periods, what was your age when you had your last period?
Jika anda telah putus haid, apakah umur anda semasa haid terakhir? _____

d) Are you currently using hormone therapy (HRT) for menopause? _____
Adakah anda sekarang menggunakan terapi hormone disebabkan menopaus? _____

7. The following questions are about your level of activity
Soalan berikut adalah mengenai tahap aktiviti fizikal anda

Please choose **ONE** activity category that best describes your usual pattern of daily physical activities, including activities related to house and family care, transportation, occupation, exercise, wellness and leisure or recreational purposes.
*Sila pilih **SATU** kategori aktiviti yang menerangkan tabiat seharian aktiviti fizikal anda, termasuklah aktiviti yang berkaitan dengan rumah dan penjagaan keluarga, pengangkutan, pekerjaan, senaman, kesejahteraan, aktiviti masa lapang atau rekreasi.*

O Inactive or little activity other than usual daily activities
Tidak aktif atau melakukan sedikit aktiviti selain daripada aktiviti harian biasa.

O Regularly (5 days per week or more) participate in physical activities requiring low levels of exertion that result in slight increases in breathing and heart rate for at least **10 minutes** at a time
Kerap (5 hari seminggu atau lebih) melakukan aktiviti-aktiviti fizikal yang memerlukan tahap kekuatan yang rendah yang mengakibatkan sedikit peningkatan dalam kadar penafasan dan denyut jantung untuk sekurang-kurangnya 10 minit pada suatu masa.

O Participate in aerobic exercises such as brisk walking, jogging or running, cycling, swimming, or vigorous sports at a comfortable pace or other activities requiring similar levels of exertion for **20 to 60 minutes** per week
Melakukan senaman aerobik seperti berjalan dengan pantas, berjoging atau berjalan pada kadar yang selesa atau aktiviti-aktiviti lain yang memerlukan tahap kekuatan yang sama selama 20 hingga 60 minit seminggu.

O Participate in aerobic exercises such as brisk walking, jogging, or running at a comfortable pace, or other activities requiring similar levels of exertion for **1 to 3 hours** per week
Melakukan senaman aerobik seperti berjalan dengan pantas, berjoging atau berjalan pada kadar yang selesa atau aktiviti-aktiviti lain yang memerlukan tahap kekuatan yang sama selama 1 hingga 3 jam seminggu.

O Participate in aerobic exercises such as brisk walking, jogging, or running at a comfortable pace, or other activities requiring similar levels of exertion for **over 3 hours** per week
Melakukan senaman aerobik seperti berjalan dengan pantas, berjoging atau berjalan pada kadar yang selesa atau aktiviti-aktiviti lain yang memerlukan tahap kekuatan yang sama lebih daripada 3 jam seminggu.

8. How much bodily pain have you had during the past 4 weeks? Tick ONE answer
Sejauh manakah kesakitan badan yang anda alami sejak 4 minggu yang lepas?
[Tandakan satu jawapan]

O None/*Tiada*
O Very mild/*Sangat ringan*
O Mild/*Ringan*
O Moderate/*Sederhana*
O Severe/*Teruk*
O Very severe/*Sangat teruk*

9. **Do you use sunscreen?** O Yes O No
   Adakah anda menggunakan pelindung mata hari (sunscreen)?
   O Ya O Tidak

10. **Do you wear headscarves?** O Yes O No
    Adakah anda memakai tudung kepala? O Ya O Tidak

11. **On average, how much sun exposure have you had in the past week?**
    Secara purata, berapa lamakah anda terdedah kepada matahari pada minggu lepas?

    O less than 5 minutes per day
    kurang dari 5 minit setiap hari

    O 5 to 15 minutes per day
    5 ke 15 minit setiap hari

    O 15 to 30 minutes per day
    15 ke 30 minit setiap hari

    O More than 30 minutes per day
    lebih dari 30 minit setiap hari

12. **How many servings of milk do you get daily? (1 serving is 250ml)** _____ (leave blank if none)
    Berapa banyak hidangan susu yang anda ambil setiap hari?
    (1 hidangan 250ml) _____
    (tinggalkan kosong jika tiada)

13. Have you ever been DIAGNOSED with or TREATED for:
Pernahkah anda MENGIDAP atau DIRAWAT untuk:

	Yes/*Ya*	No/*Tidak*	Don't know/*Tidak tahu*
High blood pressure (hypertension) *Tekanan darah tinggi (hypertensi)*	O	O	O
Diabetes Type 2 (high blood sugar) *Diabetes Jenis 2 (tahap gula yang tinggi dalam darah)*	O	O	O
Angina / Heart attack / other heart problems *Angina / serangan jantung / masalah jantung yang lain*	O	O	O
Osteoarthritis	O	O	O
Rheumatoid arthritis	O	O	O
Osteoporosis	O	O	O
Parkinson's Disease/ *Penyakit Parkinson*	O	O	O
Stroke/*Angin ahmar*	O	O	O
Kidney disease/ *Penyakit buah pinggang*	O	O	O
Depression/Anxiety *Kemurungan / Kebimbangan*	O	O	O
Cancer/*kanser* _____	O	O	O

14. FRAX ® Fracture Risk Assessment Tool

a) Any previous fracture?
(spontaneously, or a fracture arising from trauma)
Adakah anda pernah mengalami fraktur tulang sebelum ini?(tulang retak atau patah secara semulajadi, atau disebabkan oleh trauma)

O Yes/Ya O No/Tidak O Don't know/Tidak tahu

b) Parent fractured hip?
(history of hip fracture in your mother or father)
Adakah ibu atau bapa anda pernah mengalami fraktur di tulang pinggul? (tulang retak atau patah di pinggul)

O Yes/Ya O No/Tidak O Don't know/Tidak tahu

c) Current smoking?
Adalah anda merokok?

O Yes/Ya O No/Tidak O Don't know/Tidak tah

d) Are you taking Glucocorticoids? (steroid hormone)
Adakah anda mengambil Glucocorticoids?

(currently exposed to oral glucocorticoids or has been exposed to oral glucocorticoids for more than 3 months at a dose of prednisolone of 5mg daily or more (or equivalent doses of other glucocorticoids)

(sedang mengambil oral glucocorticoids atau pernah mengambil oral glucocorticoids selama lebih dari 3 bulan pada dos untuk prednisolone sebanyak 5mg setiap hari atau lebih) [glucocorticoids adalah sejenis hormon steroid]

O Yes/Ya O No/Tidak O Don't know/Tidak tahu

e) Do you have secondary osteoporosis?
Adakah anda mempunyai penyakit yang berkait rapat dengan osteoporosis? (secondary osteoporosis)

(Example: type 1 diabetes (insulin dependent), osteogenesis imperfecta in adults, untreated long-standing hyperthyroidism, hypogonadism or premature menopause (<45 years), chronic malnutrition, or malabsorption and chronic liver disease)

(contoh: diabetes jenis 1 (insulin dependent), osteogenesis imperfecta in adults, hyperthyroidism yang tidak pernah dirawat, hypogonadism atau premature menopause (<45 years), malnutrisi kronik, atau malabsorption dan penyakit hati yang kronik)

O Yes/Ya O No/Tidak O Don't know/Tidak tahu

f) Are you taking alcohol 3 or more units/day?
Mengambil alcohol 3 unit atau lebih setiap hari?

O Yes/Ya O No/Tidak O Don't know/Tidak tahu

-END OF QUESTIONNAIRE-

THANK YOU FOR ANSWERING ALL QUESTIONS

Physical Capability Tests

A. Hand grip strength

Left: ☐ **1st trial** **Right:** ☐ **1st trial**

☐ **2nd trial** ☐ **2nd trial**

B. Next, I would like to ask you do three tests to assess your physical capabilities.

1. Sit-to-Stand Chair test

Instruction: "Please sit down on a regular kitchen chair with your arms folded in front of your chest. Stand up and sit down as many times as you can without using your hands, but you are allowed to rest if needed. You are required to do this for 30 seconds. I am going to record the time you need to complete this test. You may start when I say when."

- Able to perform the test: O Yes O No
- How many sit to stands were completed: ☐ times
- Time needed to complete the test: ☐ seconds (max 30 sec)
- Particularities:

_____

iv. Walk test

This is our walking course. If you use a cane or other walking aid when walking outside your home, please use it for this test. I want you to walk at your usual pace to the other end of this course (a distance of 6 meter). Walk all the way past the other end of the mark before you stop. Are you ready?

Grading: Press the start button to start the stopwatch as the participant begins walking. Measure the time take to walk 6 meters. Then complete ordinal scoring.

- Able to perform the test: O Yes O No
- Time needed to complete the test: ☐ seconds

- Particularities (i.e use walking aid?):

_____

v. Balance Test

This test helps us to assess your standing balance. I want you to stand on one leg for as long as you can, or until I say stop. Watch while I demonstrate. (Demonstrate using chair/table/counter for initial support.) You are required to test both **left and right** legs.

You may choose either foot to stand on first. Place your arm by your sides and try not to move your feet or grab a support unless you need to gain your balance. Hold this position until I say stop. When you are ready, pick up one of your feet from the floor and hold it as long as you can.

Start timing when hand leaves the chair/table (if you are not using a support, start when the foot is lifted). Stop timing when their free foot touches the ground, their hand contacts the chair/table, their foot moved, or 30 seconds has passed.

Make sure you are close enough to guard the participant and they understand that they should put their foot down before they fall.

- Able to perform the test: Left: O Yes O No
  Right: O Yes O No

- Time position was held (max 30 sec): Left: [sec]

  Right: [sec]

- Particularities:

_____

Calculations based on Vermeulen's equations:

Free 25(OH) vitamin D = Free [D]:

Free [D] = $\frac{-b+\sqrt{b^2-4ac}}{2a}$

Bioavailable (non-DBP bound vitamin):

Bio [D] = Free [D] + [DAlb] = (Kalb · [Alb] + 1) · Free [D]

EXAMPLE CALCULATION

Total 25(OH)-vitamin D = [Total] = 40 ng/mL = 1.0 x 10^{-7} mol/L

Total serum DBP = [Total DBP] = 250 ug/mL = 4.3 x 10^{-6} mol/L

Total serum albumin = [Alb] = 4.3 g/dL = 6.4 x 10^{-4} mol/L

Kalb = 6 x 10^{5} M^{-1}

KDBP = 7.0 x 10^{8} M^{-1}

a = 2.7 x 10^{11}

b = 3325

c = - 1 x 10^{-7}

Calculated concentration of free 25(OH)D = 3.01 x 10^{-11} mol/L = 12.1 pg/mL

Calculated concentration of bioavailable 25(OH)D = 1.09 x 10^{-8} mol/L = 4.6 ng/mL

Then, values were subtituted based on the conversion units from Powe's paper:

Example:

Total 25(OH) vitamin D = [Total 25OHD] = 66.00 nmol/L = convert to mol/L = [Total] nmol/L x 10^{-9} = 66.00 x 10^{-9} = **6.6×10^{-8} mol/L**

Total serum DBP = [Total DBP] = 272.5 ug/mL = convert to mol/L = [Total DBP] ug/mL x 1.72 x 10^{-8} = **4.6×10^{-6} mol/L**

Total serum albumin = [Alb] = 50.00 g/L = convert to mol/L = [Alb] g/L x 1.4884 x 10^{-5} = **7.4×10^{-4} mol/L**

Kalb = 6 x 10^{5} M^{-1}

KDBP = 7.0 x 10^{8} M^{-1}

a = 3.133 x 10^{11}

b = 3682.22

c = - 6.6 x 10^{-8}

a = KDBP · Kalb · [Alb] + KDBP

= 7.0 x 10^{8} x 6 x 10^{5} x 7.4 x 10^{-4} mol/L + 7.0 x 10^{8}

= 3.133 x 10^{11}

b = KDBP · [Total DBP] – KDBP · [Total 25OHD] + Kalb · [Alb] +1

= [7.0 x 10^{8} (4.6 x 10^{-6} mol/L)] – [7.0 x 10^{8} x 6.6 x 10^{-8} mol/L] + [(6 x 10^{5} x 7.4 x 10^{-4} mol/L) +1]

= 3280.9 – 46.2 + 447.5

= 3682.22

Free [D] = $\frac{-b+\sqrt{b^2-4ac}}{2a}$

$$= - \frac{3682.2 + \sqrt{(-3682.2)^2 - (4 \times 3.133 \times 10^{11} \times [(- 6.6 \times 10^{-8})]}}{(2 \times 3.133 \times 10^{11})}$$

$$= - \frac{3682.2 + \sqrt{13,558,596.8 + 82,701.7}}{6.266 \times 10^{11}}$$

$$= - \frac{3682.2 + 3693.4}{6.266 \times 10^{11}}$$

$$= \frac{11.2}{6.266 \times 10^{11}} = 1.78742 \times 10^{-11} \text{ mol/L}$$

= convert to pmol/L = Free [D] x 10^{12} = 1.78742 x 10^{-11} mol/L x 10^{12} = **17.897pmol/L**

Free 25(OH) D from Table 1 (pmol/L) = xx.xx ± xx.xx

Bio [D] = [D] + [DAlb] = (Kalb ·□[Alb] + 1) ·□[D]

= [(6 x 10^5 x 7.4 x 10^{-4} mol/L) + 1] x 1. 78742 x 10^{-11}mol/L

= 447.5 x 1. 78742 x 10^{-11}

= 8.0 x 10^{-9} mol/L

= convert to pmol/L = Bio [D] x 10^9 = 8.0 X 10^{-9} mol/L x 10^9 = **8.0 nmol/L**

Bioavailable 25(OH) D from Table 1 (nmol/L) = x.xx ± x.xx

Example with real values:

Calculations based on Vermeulen equations

Free 25(OH)-vitamin D = [D]:

$$[D] = \frac{-b+\sqrt{b^2-4ac}}{2a}$$

Bioavailable (non-DBP bound vitamin):

$$[Bio] = [D] + [DAlb] = (Kalb \cdot [Alb] + 1) \cdot [D]$$

So I tried out the equation first using their example – managed to get their answer:

EXAMPLE CALCULATION

Total 25(OH)-vitamin D = [Total] = 40 ng/mL = 1.0×10^{-7} mol/L

Total serum DBP = [Total DBP] = 250 ug/mL = 4.3×10^{-6} mol/L

Total serum albumin = [Alb] = 4.3 g/dL = 6.4×10^{-4} mol/L

Kalb = 6×10^{5} M^{-1}

KDBP = 7.0×10^{8} M^{-1}

a = 2.7×10^{11}

b = 3325

c = $- 1 \times 10^{-7}$

Calculated concentration of free 25(OH)D = 3.01×10^{-11} mol/L = 12.1 pg/mL

Calculated concentration of bioavailable 25(OH)D = 1.09×10^{-8} mol/L = 4.6 ng/mL

Then substituted values from the Powe's paper (Table 1):

Total 25(OH)-vitamin D = [Total] = 64.23 nmol/L = 6.423×10^{-8} mol/L

Total serum DBP = [Total DBP] = 4.19 μmol/L = 4.19×10^{-6} mol/L

Total serum albumin = [Alb] = 42.47g/L = 4.247 g/dL = 6.32×10^{-4} mol/L

Kalb = 6×10^{5} M^{-1}

KDBP = 7.0×10^{8} M^{-1}

$a = 2.6614 \text{ X } 10^{11}$

$b = 3268.239$

$c = -6.423 \text{ x } 10^{-8}$

a = KDBP · Kalb · [Alb] + KDBP

$= 7.0 \text{ X } 10^{8} \text{ X } 6 \text{ x } 10^{5} \text{ X } 6.32 \text{ x } 10^{-4} + 7.0 \text{ x } 10^{8}$

$= 2.6614 \text{ X } 10^{11}$

b = KDBP · [Total DBP] – KDBP · [Total] + Kalb · [Alb] +1

$= [7.0 \text{ x } 10^{8} (4.19 \text{ x } 10^{-6})] – [7.0 \text{ x } 10^{8} \text{ X } 6.423 \text{ x } 10^{-8}] + [(6 \text{ x } 10^{5} \text{ X } 6.32 \text{ x } 10^{-4}) +1]$

$= 2933 – 44.961 + 380.2$

$= 3268.239$

$$[D] = \frac{-b+\sqrt{b^2-4ac}}{2a}$$

$$= -\frac{3268.239 + \sqrt{(-3268.239)^2 – (4 \text{ X } 2.6614 \text{ X } 10^{11} \text{ X } [(- 6.423 \text{ x } 10^{-8})]}}{(2 \text{ X } 2.6614 \text{ X } 10^{11})}$$

$$= \frac{- 3268.239 + \sqrt{10,681,386.2 + 68,376.688}}{5.3229 \text{ X } 10^{11}}$$

$$= \frac{- 3268.239 + 3278.683}{5.3229 \text{ X } 10^{11}}$$

$$= \frac{10.44441027}{5.3229 \text{ X } 10^{11}} = 1.96217 \text{ X } 10^{-11} \text{ mol/L} = \textbf{19.622 pmol/L}$$

Free 25(OH) D from Table 1 (pmol/L) = 25.37 ± 18.52

$[Bio] = [D] + [DAlb] = (Kalb \cdot [Alb] + 1) \cdot [D]$

$= [(6 \times 10^{5} \times 6.32 \times 10^{-4}) + 1] \times 1.96217 \times 10^{-11}$

$= 380.2 \times 1.96217 \times 10^{-11}$

$= 7.46017 \times 10^{-9}$ mol/L

= **7.46017 nmol/L**

Bioavailable 25(OH) D from Table 1 (nmol/L) = 9.58 ± 6.74

APPENDIX D

FREE & BIOAVAILABLE VITAMIN D CALCULATIONS

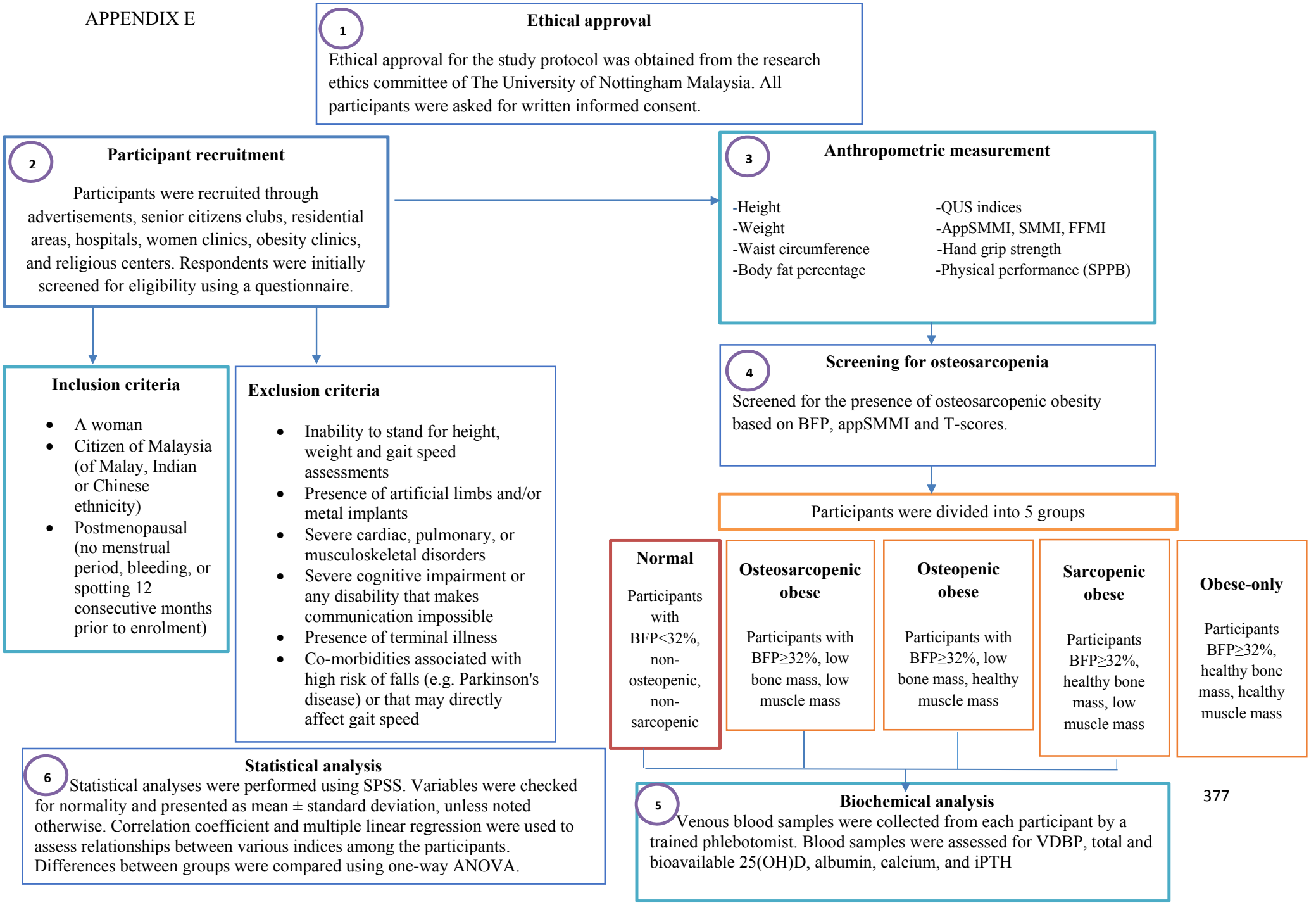
Table 1

Column	Variable	Unit conversion	Calculation	Vermeulen supplementary material
E	Albumin	g/L → mol/L	$\frac{\text{value (B) X } 6.4 \text{ X} 10^{-4}}{43}$ value (B) X $1.4884 \text{ X } 10^{-5}$	4.3g/dL = $6.4 \text{ X } 10^{-4}$mol/L 43g/L = $6.4 \text{ X } 10^{-4}$mol/L
F	25OHD	nmol/L → mol/L	value (C) X 10^{-9}	
G	VDBP	µg/mL → mol/L	$\frac{\text{value (D) X } 4.3\text{X}10^{-6}}{250}$ value (D) X $1.72 \text{ X } 10^{-8}$	250µg/mL = $4.3\text{X}10^{-6}$ mol/L
H	KDBP	-	-	$7.0 \text{ x } 10^{8}$ M^{-1}
I	Kalb	-	-	$6 \text{ x } 10^{5}$ M^{-1}
J	a	-	H * I * E + H	KDBP · Kalb · [Alb] + KDBP
K	b	-	H * G – H * F + I * E + 1	KDBP·[Total DBP]–KDBP ·[Total] +Kalb·[Alb] +1
L	c	-	- (value F)	-[Total Vit D]

APPENDIX D

Column	Variable	Unit conversion	Calculation	Vermeulen supplementary material
M	Free Vit D	-	(-K + SQRT (K * K – 4 * J * L)) / (2*J)	$\frac{-b+\sqrt{b^2-4ac}}{2a}$
N	Bio Vit D	-	(I * E + 1) * M	(Kalb ·[Alb] + 1) ·[D]
O	Free Vit D	mol/L → pmol/L	value (M) X 10^{12}	-
P	Bio Vit D	mol/L → nmol/L	value (N) X 10^{9}	-

APPENDIX E


1
Ethical approval
Ethical approval for the study protocol was obtained from the research ethics committee of The University of Nottingham Malaysia. All participants were asked for written informed consent.
2
Participant recruitment
Participants were recruited through advertisements, senior citizens clubs, residential areas, hospitals, women clinics, obesity clinics, and religious centers. Respondents were initially screened for eligibility using a questionnaire.
3
Anthropometric measurement
-Height
-Weight
-Waist circumference
-Body fat percentage
-QUS indices
-AppSMMI, SMMI, FFMI
-Hand grip strength
-Physical performance (SPPB)
4
Screening for osteosarcopenia
Screened for the presence of osteosarcopenic obesity based on BFP, appSMMI and T-scores.
Inclusion criteria
A woman
Citizen of Malaysia (of Malay, Indian or Chinese ethnicity)
Postmenopausal (no menstrual period, bleeding, or spotting 12 consecutive months prior to enrolment)
Exclusion criteria
Inability to stand for height, weight and gait speed assessments
Presence of artificial limbs and/or metal implants
Severe cardiac, pulmonary, or musculoskeletal disorders
Severe cognitive impairment or any disability that makes communication impossible
Presence of terminal illness
Co-morbidities associated with high risk of falls (e.g. Parkinson's disease) or that may directly affect gait speed
Participants were divided into 5 groups
Normal
Participants with BFP<32%, non-osteopenic, non-sarcopenic
Osteosarcopenic obese
Participants with BFP≥32%, low bone mass, low muscle mass
Osteopenic obese
Participants with BFP≥32%, low bone mass, healthy muscle mass
Sarcopenic obese
Participants BFP≥32%, healthy bone mass, low muscle mass
Obese-only
Participants BFP≥32%, healthy bone mass, healthy muscle mass
6
Statistical analysis
Statistical analyses were performed using SPSS. Variables were checked for normality and presented as mean ± standard deviation, unless noted otherwise. Correlation coefficient and multiple linear regression were used to assess relationships between various indices among the participants. Differences between groups were compared using one-way ANOVA.
5
Biochemical analysis
Venous blood samples were collected from each participant by a trained phlebotomist. Blood samples were assessed for VDBP, total and bioavailable 25(OH)D, albumin, calcium, and iPTH

APPENDIX F

GANNT CHART

Activities	Aug-Sep 2016	Oct 2016	Nov 2016-Jan 2017	Feb-Mar 2017	April 2017-Mar 2019	April 2019	May-Dec 2019	Dec 2019	April 2020
Ethical approval applied									
Ethical approval obtained									
Materials for data collection purchased									
Pilot study completed									
Data collection completed									
Biochemical analysis completed									
Data analysis completed									
First draft of thesis submitted									
Final thesis submitted									

APPENDIX G

MILESTONES AND DATES

Description	Date	Cumulative project Completion Percentage
Ethical approval applied	30/09/2016	5
Ethical approval obtained	19/10/2016	10
Disposable materials for data collection and lab kits purchased	31/1/2017	15
Data collection started	29/4/2017	20
Data collection completed	30/3/2019	60
Biochemical analysis completed	30/4/2019	60
Data analysed (statistical analysis completed)	25/12/2019	70
Draft of thesis submitted	31/12/2019	80
Final thesis submitted	15/4/2020	100