A cross-sectional study of radial and tibial ultrasonography between different ethnic and physically active groups

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Thesis Abstract

Research has attempted to quantify the effect physical activity has on bone health measured by ultrasonography as well as clarify the differences in bone health between ethnic groups. An original thesis was produced as part of a greater research project investigating ultrasonography and muscle strength in different ethnic groups. Quantitative ultrasound (Sunlight MiniOmni®) of the distal radius and mid-shaft tibia was measured in 132 male students aged 18-25 (69.22±11.04kg, 1.74±0.08m) as well as quadricep strength and anthropometrics. An aim of the study was to determine if frequency and type of physical activity affect radial and tibial ultrasound (SOS). Using the Stanford Patient Research Questionnaire, 66 multi-ethnic British males (21.04±1.57 yrs; 73.97±7.6 kg; 1.80±0.06m) were stratified for frequency of total physical activity and strength activity. Radial and tibial ultrasound were not significantly different between any of activity groups ($p > .05$), attributed to lack of difference in fat free mass. A second aim was to determine a main effect or interaction between exercise and ethnicity. Significant ethnic differences were found between Caucasian British (n=48; 21.45±1.32yrs; 72.56±6.7kg; 1.79±0.07m) and Malay Malaysian (n=66; 20.17±0.59yrs; 64.47±12.01kg; 1.68±0.06m) men for adjusted radius SOS (3984.745 and 4077.982, respectively) ($p < .005$) and tibia SOS (3885.47 and 3956.27, respectively) ($p < .01$). Ethnic group determined radial (6.3%) and tibia (5.7%) ultrasound. Body mass is strongest determinant of radial ultrasound (7.3%). No other variables impact tibia SOS ($p < .05$).

An interaction effect existed between ethnicity and exercise for radius SOS ($p = .005$). Greater competition and training as a district athlete largely reduced radial SOS in Malaysians but not British, suggesting a negative association between volume of training and bone health for Malaysians only. Factors associated with bone mass changeable under

exercise were different between Malaysians but not British groups, suggesting activity levels between controls and athletes were not consistent, misinterpreting an interaction effect.

Ethnic differences in radial and tibial ultrasound varied. No group had consistently higher SOS for both sites, the size of the difference was not consistent and different external factors affected the difference. Better quantification of physical activity with a focus on physiological adaptation of factors associated with osteogenesis, along with more control of groups is required.

<u>Acknowledgements</u>

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CHAPTER

<center>Thesis Introduction</center>

Overview

A collaborative research team was used to collect the data for this study. These included Shanks, J, Taylor, M., Ooi, F.K., Chen C.K. & Li, X. Data was collected on two separate sites including University of Essex main campus and University Sains Malaysia main campus. Ooi, F.K and Chen C.K were responsible for the inception of the study. Their aim was to assess the difference in quadricep strength and quantitative ultrasound of bone between controls and athletes of British and Malaysian ethnicities. Their research proposal was a 'Comparison of bone health and isokinetic strength between young male athletes and non-athletes'. Other anthropometric measures were gathered to observe physiological differences. Shanks, J and Li, X were responsible for data collection for the British and Malaysian sample, respectively. Both researchers followed an identical design but had different research objectives.

Before completion of the current thesis, Shanks et al (2015) submitted a presentation for the International Conference of Physical Education (ICPE). The sample included 66 British male students aged 18-25. It explored differences in quantitative ultrasound and anthropometrics between those competing at different sporting and physical activity levels.

Ethnicity, exercise and bone health

Interventions that elevate bone mass span many approaches (Aloia et al, 1994; Baltzer et al, 2001; Marques et al, 2011) due to bone tissue being influenced by many factors. Like many tissues, bone tissue responds to exercise. Studies cite physical activity at a young age as important to delay onset of age related osteoporosis (Liberato et al, 2007). In a young male population specifically, quantification of physical activity and identification of groups that respond more strongly to physical activity may identify those at greater risk. Ethnicity describes the product of genetics and environment. Ethnic groups exist that have higher bone

mineral density than their age matched counterparts (Nam et al, 2010) with different rates of growth (Gilsanz et al, 1991) and decay (Tracey et al, 2005). As well as age, ethnic groups may have a different osteogenic response to exercise.

The current study reduced the variation within the sample by controlling for anthropometric measures and quadricep strength in chapter 3 and 4. Chapter 3 investigated the effect of frequency and type of physical activity on QUS. A physical activity questionnaire was completed by the multi-ethnic British group but not the Malaysian group; therefore chapter 3 focused on the British sample. Chapter 4 investigated an interaction between athletic standard and ethnicity. Athletic standard was used to describe controls and athletes, collectively. Ethnicity described White British and Malay Malaysian participants.

The current study chose to observe QUS between these groups to compliment the literature. Total stimulus from physical activity sees the greatest improvements in bone health (Gomez-Cabello et al, 2013). However, strength training provides the strongest stimulus. Adaptations in strength and muscle volume have been shown to encourage site specific bone growth in all ethnicities, ages and genders (Taafe et al, 2001). The current literature has not taken into account type and frequency of training and the effect this has on QUS accounting for key variables that change under different exercise conditions. An investigation into this was carried out.

It is understood that risk factors for bone health interact such as age and gender. This has flagged specific high risk groups for BMD assessments and interventions when a certain age is reached. What is missing from the literature is how different ethnic groups interact with risk factors for low bone mass. If an interaction between exercise and ethnicity is established, interventions could become more specific to ethnic group.

Structure

The thesis will begin with a literature review (Chapter 1). Quantitative ultrasound will be described and compared with other bone health assessment tool with particular attention to accuracy and precision. The role of physical activity will be reviewed followed by factors such as muscle strength and lean muscle mass that significantly affect bone properties. The extent to which they do and whether this is different between physically active or ethnic groups is important to discuss. Research designs span numerous approaches in bone health studies (Mackelvie et al, 2002; Bielemann et al, 2014; Gouieva et al, 2014). Exercise interventions are long and costly as adaptations in response to exercise are chronic, whereas cross-sectional design are cost and time effective but maybe lacking validity due to multiple factors affecting bone properties. The review will assess the advantages and disadvantages of these designs. Finally, the role genetics and ethnicity will be reviewed.

Due to similarities in methodology between studies within the present thesis, a single methods Chapter will address both with clear distinction made where they were different (Chapter 2).The main body of the thesis is split into two sections both of which investigate the impact of different factors on peripheral bone properties determined by quantitative ultrasound. Participants were grouped according to physical activity, athletic standard and ethnicity. The first scientific paper format (SPF) observed the effect of physical activity, stratified into low moderate and high groups (Chapter 3). The second SPF assessed the individual main effect and interaction between ethnicity and athletic standard (Chapter 4). A conclusion chapter will evaluate the thesis explaining the implications for the literature and what future research should include (Chapter 5).

Research Question, objectives and hypothesis

The first research question is 'A comparison of quantitative ultrasound measurement of bone in young males with different physical activity levels'. The objective of this research question is to determine if frequency of total (TA) or strength (SA) physical activity changed QUS-SOS significantly, controlling for body mass (kg), height (m), fat free mass (kg), fat mass (kg) and quadricep strength (Nm). It was hypothesised there would be a significant difference in SOS measurements between the physical activity groups for both total and strength categories. This difference would be partially determined by one or more covariates.

The second research question is 'Analysis of quantitative ultrasound measurement of bone in British and Malaysian groups'. The objective of this research question was to determine if a significant interaction or main effect exists between ethnic group and athletic standard in determining quantitative ultrasound measurements of bone, controlling for body mass (kg), height (m), fat mass (kg) and quadricep strength (Nm). It was hypothesised there would be a significant interaction and main effect between ethnic group and exercise. This would be will be partially determined by one or more covariates.

<u>Literature Review</u>

Osteoporosis is a skeletal disease characterised by low bone mineral density ($g.cm^{-2}$) by the World Health Organisation (WHO). The value is obtained using Duel-energy X-ray absorptiometry (DEXA). The disease affects the activity of osteoclast and osteoblast activity, inhibiting the natural remodelling of bone tissue. In terms of burden of disease measured by disability adjusted life years (DALYs), osteoporotic fractures are ranked higher than hypertension, prostate cancer, breast cancer and rheumatoid arthritis (National Osteoporosis Foundation: Clinicians Guide revised, 2013). Due to reduced bone mass, susceptibility to fracture under trauma or loads is increased. Osteoporotic fractures relating to low bone mass are debilitating often presenting no symptoms, coining the term 'the silent killer' (Parsons 2005). In elderly men specifically, 20-25% of total reported hip fractures occur. The mortality rate is roughly 20% within the first 12 months, a rate higher than women in people over 60 (Seeman et al, 1995; Center et al, 1999). WHO favour DEXA to identify low bone mass.

Other tools such quantitative ultrasound (QUS) has had equal success in predicting and identifying low bone mass. Risk factors and interventions for low bone mass branch many approaches. This is mainly due the interaction populations have with different risk factors. As a result developing a research design can be complex as bone tissue is so multifaceted. This thesis is focusing on the role of ethnicity and physical activity, both of which are prevalent risk factors for low bone mass in men.

Research tools in bone health studies

The following table provides the aim, method, results and conclusion of studies investigating the structures and mechanical properties observed by QUS.

Table 1: Research tools in bone health studies.

Researcher	Aims	Method	Results	Conclusion
Bouxsein and Radloff (1997)	Determined whether densitometric variables and QUS are associated with mechanical properties of cadaver feet.	31 intact cadaveric feet of men and women (Avg yrs 77) Measured broadband ultrasound attenuation (BUA), ultrasound transit velocity (SOS), Duel energy absorptiometry (DEXA), Elastic modulus and ultimate strength of bone.	QUS moderately to strongly associated with mechanical properties (r^2=0.48-0.68) Strongest associations with calcaneal BMD (r^2= 0.66-0.88)	QUS associated with mechanical properties of bone.
Muller et al (2008)	Prediction of bone mechanical properties by QUS and peripheral quantitative computed tomography (pQCT)	pQCT measurements of BMD and cortical thickness (CT) and bi-directional axial transmission of QUS. Compared failure to load and elastic properties.	Best predictor of failure load was the pQCT. Best predictor of elastic modulus was bi-directional QUS.	Combination of assessment tools can provide a good prediction of bone mechanical properties.
Cavani et al (2008)	Evaluate the potentiality of quantitative ultrasound (QUS).	15 cylinders of spongy bone demineralized and assessed at different mineralized levels using QUS, QCT and DEXA. Compression test to calculate the Elastic modulus.	Correlation analysis showed speed of sound (SOS) correlated with BMD, young's modulus and majority of QCT parameters.	SOS influenced by BMD and elastic modulus.
Moayyeri et al (2012)	Assessed the predictive power of heel QUS for fractures using a meta-analysis.	Inverse variance random effects meta-analysis. Measurements of baseline and fracture outcome using heel QUS.	Heel QUS can predict risk of different fracture outcomes in an elderly population.	Studies with QUS measures adjusted for hip BMD significantly and independently predict fracture risk.

Many tools exist to assess the health of bone tissue. DEXA is the preferred tool in the majority of cases reporting bone mineral density (BMD) (g.cm^{-2}). This is because alternative methods do not match the precision (Maulalen et al, 1995; Turner et al 1995) of DEXA, are less accurate when measuring high risk sites such as the femoral neck (Clowes et al, 2005), demonstrates greater sensitivity to change (Lehtonen-Veromaa et al 2000) and contain low dose radiation (Clowes et al, 2005). Area bone density (g/cm^2) and bone mineral content

(BMC) (g) are the two measures taken from the projection image of bone produced by X-rays. The World Health Organisation (WHO) uses this method to accumulate normative data. This data is used to establish t-scores, helping establish an individual's relative risk of fracture. T-scores refer to the standard deviations away from mean, an individual is. -1 and -2.5, WHO qualifies as low bone mass and osteoporosis, respectively. This is the clinical standard tool and method used for assessing those at risk of osteoporosis. As such other methods of assessment bone health will adjust their units such as estimated bone mineral density (eBMD) adjusted from calcaneal quantitative ultrasonography (Kolbe-Alexander et al, 2004) or volumetric BMD (g.cm^{-3}) derived from quantitative computed tomography (QCT). The current section will review the methods involved in bone health assessments.

QUS uses the time taken for sound waves to transmit through bone as they pass through peripheral skeletal sites to measure bone characteristics. Broadband Ultrasound Attenuation (Hz/dB) (BUA) measures the reduction in strength of a signal. Alternatively, Speed of Sound (m.s^{-1}) (SOS) measures the time taken for a sound wave to pass through a material. Both methods indirectly infer density by observing the change in signal. With respect to QUS-SOS the higher the value the higher the density of the material.

In recent years QUS has been adapted to comply with WHO recognised units for determining osteoporosis (Kolbe-Alexander et al, 2004). Earlier cadaver studies identified that QUS measurements are associated with the material properties of trabecular bone as well as failure to load at the proximal femur (Bouxsein et al 1995; Grimm and Williams 1993; Langrana et al 1996). In osteoporosis, trabecular bone is more severely affected than cortical bone. Furthermore, trabecular bone accounts for more of the elastic properties of bone tissue. This suggests QUS is better at determining the ability of bone tissue to deform under loads.

Muller et al (2008) agree with this. A low elastic modulus (Young's modulus) characterises a linear material that can deform under stronger loads and return to its original shape. Peripheral QUS (pQUS) was significantly better at identifying this property in bone tissue than other preferred radiation based assessments of bone properties such at quantitative computed tomography (QCT) or DEXA. Table 1 highlights a combination of QUS and QCT may better identify bone mechanical properties.

Compressive modulus and ultimate strength correlated significantly with BUA and SOS of an intact heel in a cadaver study by Bouxsein and Randoff (1997). Again, concurring that QUS can identify fracture resistant bone properties. However BMD had a stronger relationship with fracture resistant properties. BUA and SOS together explained 7-12% of the variance in trabecular bone mechanical properties (Hans et al, 1999). This value appears low. Hans et al (1999) report the variance in SOS can be largely explained by BMD, with a small contribution from elasticity. This suggests that SOS and BMD observe similar properties of bone. In vivo Böttcher et al (2006) reports a strong relationship between QUS-SOS and DEXA-BMD (r=.71) in patients with bone pathology. Later cadaver studies by Cavani et al (2008) suggest as much as 93.34% of variation in SOS can be attributed to BMD and Young's modulus, providing even more support for the comparability between SOS and BMD (Table 1).

Research has investigated how well a combination of QUS and BMD measurements can predict fracture. Chan et al (2012) reported a combination of femoral neck BMD and BUA was better at predicting fragility fractures then BMD alone. In relation to Cavani et al (2008) this suggests that QUS identifies bone properties relating to fracture resistance that BMD does not, namely elastic properties as suggested by Muller et al (2008). The ability of bone tissue to absorb impact loads associated with physical activity may be better assessed using

QUS methods. The similarities between QUS and BMD, regarding their accuracy have been scrutinised (El Maghraoui et al, 2009). Marín et al (2006) concluded from a meta-analysis that the similarities between QUS and BMD extend to non-spinal and femoral neck fractures. This does withdraw from the fact that QUS methods including SOS and BUA have significantly predicted fractures in a meta-analysis reviewing 21 heterogeneous studies by Moayyeri et al (2012) (Table 1).

The following table provides examples of studies that critique QUS comparatively with other bone health assessment tools.

Table 2: Research tools in bone health studies.

Researcher	Aims	Method	Results	Conclusion
Frost et al (2000)	Establish a T-score threshold appropriate to identify women at risk of osteoporosis using QUS.	420 healthy women aged 20-79 years and 97 postmenopausal women with vertebral fractures. Established healthy mean with a subgroup of 102 women aged 20-40. DEXA measurements of hip and spine. QUS measurements of heel.	Average T-score for a woman aged 65 years was -1.2 for QUS and -1.75 for the BMD.	T-score threshold of -1.80 using QUS would classify the same percentage of women as osteoporotic as would T-score threshold of -2.5 using DEXA.
Cowes et al (2005)	Evaluate the ability of different peripheral and central bone techniques to discriminate fractures.	Women with proximal femoral, vertebral, distal forearm or proximal humeral fractures (n=281), and 500 population-based women (age 55–80 years). Multi-site measurement using DEXA, QCT and QUS.	Heel BUA and SOS was comparable with hip and spine DXA in discriminating osteoporotic fractures.	Discriminating between fracture cases and controls is device- and site-specific.
Baroncelli et al (2008)	Review methodological principles of ultrasounds and the QUS variables	Simplicity, lack of radiation, low cost and portability provide clear advantages over DEXA and QCT. There is a lack of normative data for application in a clinical setting. There are modest correlations between peripheral sites for QUS within and between measurement tools.		

QUS presents itself as a positive alternative. However there are difficulties in drawing direct comparisons with DEXA. First of all, T-scores are used to in DEXA-BMD assessments to determine relative risk (-1 SD low bone mass, -2.5 osteoporosis) may under or overestimate a person risk using QUS. Frost et al (2000) concludes the t-score ranges for BMD may be

inappropriate at skeletal sites such as the spine and forearm when QUS is used. In order to classify osteoporosis properly, using BUA, SOS and eBMD, Frost et al (2000) recommends- 1.61, -1.94 and -1.90, respectively using a Hologic Sahara ultrasonometer. Numbers also differ between brands of devices. These factors question the reproducibility of QUS and studies have reported that QUS does not yet match the precision of bone densitometry techniques (Mautalen et al, 1995; Turner et al 1995).

QUS can accurately discriminate fracture risk, but may lack accuracy and precision when compared with DEXA. It is a cost effective, radiation free alternative to DEXA and QCT. It is cost effective as it is cheaper than both DEXA and QCT, cheaper to run and cheaper to transport. It could be used in early prognosis or in accordance with densitometry tools to more accurately determine relative risk of fracture (Baroncelli 2008) (Table 2). More specifically it may be applied to younger healthy population to determine the effect of risk factors and interactions between them. QUS was chosen as a bone health assessment tool in the present study.

QCT, using a low kilovolt technique, takes an 8-10mm slice (in vitro) along the mid plane of the relevant vertebrae in spinal cases. This technique is particularly sensitive to trabecular bone. It can determine true volumetric density (mg/cm^3) of trabecular and cortical bone at any skeletal site. A greater sensitivity to change in trabecular bone and a distinction between cortical and trabecular bone (integral bone density) makes QCT arguably a better assessment tool. Peripheral QCT (pQCT) refers to the appendicular skeleton such as legs or arm therefore is less general than QCT (Engelke et al, 2008). Clowes et al (2005) compared pQCT, four QUS techniques, peripheral and central DEXA to determine the association with fracture risk. QCT and DEXA more accurately predicted hip and vertebral fractures, but equally predicted fractures at peripheral sites with QUS (BUA and SOS). QCT and DEXA

better comply with the WHO criteria for osteoporosis as they are able to imply true density in high risk sites for osteoporosis. As a general tool for assessing peripheral osteology not necessarily in high risk groups, but to determine the effects of changing risk factors, QUS may prove a promising alternative.

Lenox et al (2015) propose an ultrasound based tomographic transmission (QTUS) capable of producing a 3D image of a bone structure using only sound waves. This technology has not been specifically testing on bone structures yet, but success has been reported with breast imaging (Wiskin et al, 2013). QTUS may prove to be a radiation free comprehensive alternative to DEXA in bone health assessments in the future.

QUS

Table 3 shows the results of studies using QUS tools on various populations.

Table 3: QUS reference data

Researcher	Tool	Participants	Measurement technique	Measurement site	SOS (m/s) (SD)	CV	t-score
Kendler et al (1999)	Sunlight Omnisense	573 women 25-35years	Peak Speed of sound (m.s⁻¹)	TIB MET	3945 (151) 3799 (202)	3.8% 5.3%	0.23 (1.11) -1.12 (0.97)
Njeh et al (2001)	Sunlight Omnisense	334 adult women 48.8 (+/- 17.4)	Speed of sound (m.s⁻¹)	RAD TIB	4087 (147) 3893 (150)	3.5% 3.8%	0.41 (1.22) -0.11 (0.98)
Zhu et al (2008)	Achilles Sonometer	2927 Chinese men (35-45 years)	Speed of sound (m.s⁻¹)	RAD TIB	4075 (124) 3990 (115)	3.0% 2.8%	0.19 (0.87) 0.09 (1.21)
Nguyen et al (2004)	Omnisense Sonometer	472 non-fracture women aged 49-88	Speed of Sound (m.s⁻¹)	RAD TIB PHA	4017 (151) 3880 (141) 3806 (207)	3.8% 3.6% 5.4%	-1.41 (1.55) -0.47 (1.23) -1.39 (1.44)
Kendler et al (1999)	Sunlight Omnisense	573 women 35-45 years	Peak Speed of Sound (m.s⁻¹)	RAD PHA	4167 (102) 4092 (161)	2.4% 3.9%	
Hans et al (2001)	14 Omnisense devices	6000 women 20-29	Peak Speed of sound (m.s⁻¹)	RAD	4108 (119)	2.8%	
Weiss et al (2000)	Omnisense Sonometer	1521 healthy Israeli women (20-90 yrs)	Maximum Speed of Sound	RAD (35-45yrs) MET (35-45yrs) PHA (35-45yrs) TIB (25-35 yrs)	4169 3663 4047 3939		
Drake et al (2001)	Omnisense Sonometer	545 health Caucasian Women (20-90yrs)	Peak Speed of Sound	RAD (40 yrs) TIB (40 yrs) MET (40 yrs) PHA (40 yrs)	4161 3929 3786 4092		

CV = Coefficient of variance. RAD = radius; TIB= tibia; PHA = Phalanx; MET= Metatarsal.

Chinese men reached peak radius and tibia SOS at 35-45 years (4075 and 3990m.s^{-1}, respectively) (Zhu et al, 2008). Both t-scores were <0 indicating values are higher than young adult male average. Beamed Sunlight Omnisense and Hologic Sahara Sonometer do not publish male reference data, therefore values are difficult to obtain. Peak radius was 4169 for age matched women (Weiss et al, 2000). Peak tibia SOS was 10 years earlier (3939m.s^{-1}). Drake et al (2001) concur, female SOS peaks higher than male, yet declines quicker. Nguyen et al (2004) report SOS values of 4017 and 3880 for radius and tibia respectively, in women aged 49-88, without incidents of fracture. T-scores were -1.41 and -0.47, respectively.

Barkmann et al (2000) carried out a precision test for the Omnisense Sonometer 7000®. Testing each of the 29 healthy subjects 3 times, they report a coefficient of variance (CV) of between 0.2 and 0.3% for the same experimenter (Intra-observer) and 0.3-0.7% for different experimenters (Inter-observer). Weiss et al (2001) suggest the short term coefficient of variance is ≤1% for women in all sites observed using an Omnisense Sonometer®. Nearly all studies in QUS reference data have CVs below 5% showing of the populations they studied, there was small variability within the samples. Patel et al (2000) carried out precision testing over 7 years for in post-menopausal women. CV was around 1−1.5% for spine and total hip BMD and 2−2.5% for femoral neck BMD. Precision using both tools was high. Vignolo et al (2006) stated increments in growth measured by SOS and bone transmission time were similar to most bone growth velocity curves, strongly associated with age. This highlights the accuracy of the tool. Njeh et al (2001) recognises the accuracy of the QUS compared to DEXA, but highlights that ultrasound attenuation observes structural components opposed to density of the tissue.

Physical activity

Table 4 shows three studies, two of which are systematic reviews and one a cross-sectional study. They identify high PA specifically, strength exercise improves bone health.

Table 4: Physical Activity – Main areas of research

Researcher	Aim	Method	Results	Conclusion
Pettersson et al (1999)	investigated any differences in bone mass at different sites between young adults subjected to a different physical activity levels	Areal bone mineral density (BMD) was measured in total body a various anatomical sites.	BMD was significantly higher in the total body (8.1%), spine (12.7%), femoral neck (10.3%) and proximal tibia (9.8%) in the high activity group. High activity group also had a significantly higher lean body mass (5.4%) and isokinetic strength.	Elevated quad strength has a notable relationship with BMD whereas the high PA has none. High PA provides a platform for physiological adaptation.
Gomez-Cabello et al (2012)	Systematic review of exercise programmes effect on bone-related variables in elderly people.	Systematic review - 59 controlled trials, 7 meta-analyses and 8 reviews. Strength exercise seems to be a powerful stimulus to improve and maintain bone mass during the ageing process. Multi-component exercise may help to increase or at least prevent decline in bone mass with ageing. Future research is recommended including longest-term exercise training programmes.	
Bolam et al (2013)	Systematically review trials examining the effect of weight-bearing and resistance-based exercise modalities on the BMD of hip and lumbar spine	Systematic Review – RCT that investigated resistance-based exercise interventions on BMD measured by dual-energy x-ray absorptiometry in middle aged men Resistance training alone or in combination with impact-loading activities are most osteogenic for this population.	

Liberato et al (2013) suggests that the most important primary prevention of osteoporosis is to promote physical activity (PA) in young populations as at this age, bone is seen to respond more sharply to PA. Furrer et al (2014) reported similar exercise related improvements in an elderly population. Increased physical performance was correlated with reduced fracture risk. These studies suggest that if it's within the individual's capacity to do so, participation in PA can elevate bone integrity, in all ages but especially younger groups. The consequences of physical inactivity (PI) and the risk factors of developing fragility fractures are highlighted (Nguyen et al, 1998). PI women lost 0.9% more BMD per year (p<.001) than physically active women.

Bielemann et al (2014) and Falk et al (2007) help to quantify the cumulative effects of PA on bone structure in youths. Both suggest a positive dose response relationship between PA and BMD. Furthermore, PA at both 18 and 23 years of age was associated with greater BMD at 30 years in males, suggesting it has long term effects on BMD. These studies infer that PA provides the platform for adaptation in BMD. This means PA encourages greater bone loads, increased strength and muscle mass that in turn stimulate osteogenesis. Pettersson et al (1999) and McCroy et al (2013) help distinguish whether types of PA can influence bone structure. McCroy et al (2013) concluded that high levels of athletic activity do not have a significant effect on BMD over controls, whereas those from a high impact sport background, such as Ice Hockey, have significantly greater BMD over controls (Pettersson et al, 1999). Ireland et al (2014) suggest that primarily, types of forces subjected to bone (bending, shear and torsion) are important in promoting adaptations in bone tissue as well as exercise induced adaptations of surrounding tissues. They also found a strong relationship between muscle size and bone size in both arms of tennis players ($p<.001$). This may be why high contact sports such as ice-hockey see changes in BMD opposed to athletics. Ireland et al (2014) infer that applying stress on bone tissue through planes where it is not typically strong is the best way to facilitate osteogenesis. This advocates multi-directional loading sports as a method of increasing bone mass (Platena et al, 2001).

With lean tissue mass being a prominent risk factor reviews have taken place to assess methods of elevating muscle mass which increases bone health. Bolam et al (2013) through a systematic review reported resistance training alone or in combination with impact-loading activities is the most osteogenic in males. Marques et al (2011) supports the theory that variety in types on bone loading elevates BMD. Resistance training for 8 months instigated a 2.8% increase in BMD in elderly women; often deemed an unresponsive group to PA. However, Armamento-Villareal et al (2014) highlight again the importance of maintaining

overall mass when attempting to encourage muscle hypertrophy, in order to see improvements in bone health. Villareal et al (2006) lessen the importance of fat mass when using exercise as an intervention to elevate bone health. Caloric restricted induced weight loss, but not exercise induced was associated with a reduction in BMD. Overall these studies suggest resistance based training combined with loading activities alongside a calorie appropriate diet to maintain body mass is the most advantageous to improve bone health and stay healthy.

Other forms of training to improve bone health have been met with differing success. Whitfield et al (2015) aimed to assess the minimum requirement of aerobic endurance exercise to elevate BMD in lumber spine (LS) and femoral neck (FN).The findings were complex. In order to see changes in LS and FN BMD women had to exceed recommended aerobic activity by 2-4 times to see improvements in FN. Whereas males should exceed by 4+ times to see improvements in LS and FN. 150 minutes of moderate and 75 minutes of vigorous aerobic activity a week is the physical activity guideline for Americans. This equates to a between 450-900 minutes (18.75-37.5 hours) of activity per week to see changes in BMD. This could reference the inadequacies of one directional, slow loading force as a method of improving BMD as it requires an almost impractical amount of work for the average person to see changes. Gomez-Cabello et al (2012) suggest that strength training is a strong osteogenic stimulus but a combination of strength, aerobic, high impact and/ or weight-bearing training could be equally as advantageous, suggesting total stimulation is what is important. If this is the case, qualifying PA into relative categories and in turn, quantifying this is important to test this theory.

Factors affecting bone tissue

Table 5 lists studies that identify lean mass as a prominent risk factor for bone health.

Table 5: Factors affecting bone health – Main areas of research.

Researcher	Aims	Method	Results	Conclusion
Nelson et al (1994)	Determine how multiple risk factors for osteoporosis change under high intensity strength training (HIST)	30 50-70yr old women. High-intensity strength training exercises 2 days per week for 1 year. Dual energy x-ray absorptiometry for bone status, one repetition maximum for muscle strength.	Muscle mass and muscle strength increased in the strength-trained women ($p<.01$). Femoral neck and lumber spine BMD significantly increased in strength trained women.	HIST is effective and feasible in improving BMD.
Taafe et al (2001)	Examined the independent effects of lean mass (LM), fat mass (FM), and muscle strength on regional and whole body bone mineral density (BMD).	Cohort of 2619 well-functioning older adults (70-79 yrs) multi-ethnic. BMD of the femoral neck, whole body, upper and lower limb and whole body.	LM was a significant ($p < 0.001$) determinant of BMD, except in women.	Increase LM and strength in the elderly to elevate BMD.
Ginty et al (2005)	Evaluate the relationships between BMD and self-reported participation time in physical activities and fitness measurements.	16- to 18-year-old boys. Absorptiometry (DXA), VO2 max, grip strength, and back strength. EPIC (European Prospective Investigation of Cancer) physical activity questionnaire.	At most skeletal sites BMD and bone area correlate with fitness, strength measurements and high physical activity. Size adjusted bone mineral content at the distal radius correlated with grip strength. Whole body BMD correlated with time spent at high physical activity.	High intensity impact activities are positively associated with greater bone size and mineral content. Those in high activity group had significantly higher lean mass and back strength.

The literature has reported that muscle strength is a predictor of bone density independent of body weight in men (Nguyen et al, 1994; Snow-Harter et al, 1992; Glynn et al 1995). Many variables affect the outcome of bone mineral density (BMD). Madsen et al (1995) reported quadriceps strength was better at predicting tibia BMD than body height, mass or age. Seebra et al (2012) reports this is true for all body sites. Knee extensor strength was significantly associated with BMD and bone mineral content (BMC) at the femoral neck, lumber spine, distal radius and calcaneus. Previous research had contradicted this, however. Madsen et al (1993) and Hughes et al (1995) state that adaptations in BMD as a result of muscle strength are site specific. In addition, the relative contribution of muscle strength to BMD can differ according to anatomical site, age and gender (Bevier et al, 1989; Taaffe et al 2001). In middle

aged men only, back strength proved to be the most robust predictor of spinal BMD, predicting 19% of the variation (Bevier et al, 1989). The extent to which muscle strength effects bone tissue, the relative effect is has in comparison to other variables and the differences between anatomical sites and measurement tools will be explored in this section.

In detail the literature has reported the effect of local muscle strength on adjacent bone tissue (Blain et al, 2001). In men, higher hand grip strength was associated with reduced fracture risk of the radius (Furrer et al, 2014). Similarly, Ginty et al (2005) reported that at forearm site grip strength was significantly positively associated with BMC. Both studies sampled adolescent males and adjusted the outcomes for body weight and age. Menkes et al (1993) using a resistance exercise intervention measured before and after femoral neck BMD and Isokinetic knee strength, in men. A significant knee extensor strength increase of 32Nm $\pm$ 4% was met with a femoral neck BMD increase of 3.8 g/cm^2 $\pm$ 1%. This study did not control for training outside of testing, nor control for common factors such as body mass or age. However, it becomes clearer that training which targets muscle strength has a positive effect on local BMD, whatever the anatomical site in young men.

Not exclusive to young males, postmenopausal women have experienced gains in response to resistance exercise (RE). Marques et al (2011), report that 8 months of RE was sufficient to see significant changes in both BMD and muscle strength. Over a year, Nelson et al (1994) using bi-weekly high intensity strength training saw similar significant increases in BMD at the femoral neck as well as muscle mass and muscle strength. Conroy et al (1993) applied this to junior weight lifters (JWL). When compared to controls and adult male reference data, JWLs had significantly higher lumber spine and femoral neck BMD. Multiple regression analysis reported as much as 30-65% of BMD variance could be explained by muscle strength in JWLs. This is very different from the value explained by Bevier et al (1989) (19%). Jawed et al (2001) using a similar protocol observed power lifters, but used

broadband ultrasound attenuation (BUA) to determine a difference with a control group. The powerlifters had 6.1% greater (dB/MHz) than controls adjusted for age. These studies have highlighted strength training can facilitate increases in BMD. Training gains are also unaffected by age (Bauer et al, 1993) or gender (Nelson et al, 1994). Studies also suggest that constant strength training will continue to see rises in BMD. It may also have a larger relative impact on BMD compared to other variables. Moreover, exercise specificity appears to be important. Pettersson et al (1999) reported that high overall physical activity weakened the relationship between strength and BMD. A study which observes the relative and overall impact of strength straining on osteology may prove complimentary to the literature.

Studies have supported the role of muscle strength on BMD accrual, but attributed the gains to lean muscle mass. Taafe et al (2001) concluded that both lean mass and muscle strength contribute to limb BMD. However, when lean mass and muscle strength are introduced to a regression model together, lean mass diminished the effect of muscle strength on bone. In a twin study investigating the role of muscle strength on BMD, BMD was associated with muscle strength before, but not after adjusting for lean mass (Seeman et al, 1996). This is understandable as strength is proportional to muscle cross-sectional area (Taafe et al, 2001). This would indicate that muscle hypertrophy is what is important to elevate BMD.

Table 6 lists studies that identify fat mass as a risk factor for bone mass.

Table 6: Factors affecting bone health – Main areas of research

Researcher	Aims	Method	Results	Conclusion
Baumgartner et al (1996)	Associations of fat and muscle masses with bone mineral status.	301 men and women aged > or = 65 y.	In men, muscle was closely associated with adjusted BMC. In women, fat mass was associated significantly with BMC.	Body fat important in women for maintaining BMC
Armamento-Villareal et al (2014)	Relationships among strength, muscle mass, and bone mineral density (BMD) with lifestyle change.	107 obese older adults. Control, diet, exercise, and diet-exercise groups for 1 year. Diet was caloric restrictions. Thigh muscle volume – MRI BMD – DEXA Knee strength – Dynamometry Bone markers - immunoassay	Thigh muscle volume correlated with changes in hip BMD (r = 0.55, P = <0.001) No correlations between BMD changes and knee strength.	Elevate muscle volume and maintain body mass to elevate BMD.
Ho-pham et al (2014)	Comparison of the magnitude of association between LM, FM, and BMD.	20,000+ men and women. Between 18-92 years. Meta-analytic study - 44 studies that had examined the correlation between LM, FM, and BMD.	The effect of LM on FNBMD in men (r=0.43) was greater than that in women (r=0.38).	LM exerts a greater effect on BMD than FM in men and women.

The "muscle-bone unit" is a term coined by Frost et al (2000). It refers to the adaptations in bone tissue being largely responsible by adjacent muscle tissue. They state that the two form an *"operational unit"* meaning other factors, mechanical or chemical affect the unit jointly such as physical activity or human growth hormone, respectively.

This is reinforced by Armamento-Villareal et al (2014) and Blain et al (2001). The later reported that lean mass to a large extent supported the association between BMD and body weight. In reference to the "muscle-bone unit" this implies the added body weight required more muscular support therefore more stress upon the bone leading to elevated BMD. Armamento-Villareal et al (2014) reported a significant relationship between thigh muscle volume and femoral neck BMD close to 0.5 (r = 0.55, P = <0.001). A unit increase in thigh muscle volume equals the same rate of increase in BMD. The same research project used an exercise intervention. Thigh muscle volume increased in this group by 2.7-3.1%. It is unclear whether this was enough to facilitate a significant BMD increase. Taafe et al (2001) stated that lean mass was a significant independent contributor to BMD regardless of race, sex or age. BMD increased 4.7-5.9% with a 7.5kg increase in lean mass. In a monozygotic

twin study Seeman et al (1996) suggest BMD can increases by as much 10% per 6kg increase of lean tissue mass. The findings from all three studies conform to the "muscle-bone unit" theory.

Physical activity is commonly used to elevate muscle volume and strength to, which then elevates BMD. Pettersson et al (1999) reported at those in the high physical activity group had significantly greater BMD (7.4%-12.7% at seven different sites) and lean body mass (5.4%), than the low physical activity group. The high physical activity group contained many engaged in contact sport. In a study similarly stratifying physical activity Ginty et al (2005) reported back strength, lean mass and lumber BMD were all significantly greater in the high physical activity group.

Baumgartner et al (1996) in a large cohort longitudinal study suggest that 21% of the variance in BMD of people above 65 can be explained by the appendicular skeletal muscles, when age, and total body fat were controlled for. This value seems low in comparison to the "muscle-bone unit" theory. A meta-analysis by Ho-pham et al (2014) which examined 44 studies found the correlation between lean mass and femoral neck BMD was $r=.43$ in men. This was a comprehensive study including a large (n=20,000) multi-ethnic and multi-aged (20-92) sample. One can confidently assume the independent association between BMD and lean mass and can postulate that all factors (hormone balance, exercise or body mass) impact the "muscle-bone unit" and not just the bone.

Table 7 provides studies that investigate the role of body mass and BMI affects bone health.

Table 7: Factors affecting bone health – Main areas of research.

Researcher	Aims	Method	Results	Conclusion
De Laet et al (2005)	Association of BMI with fracture risk in relation to age, gender and BMD.	Meta-analytic study - 60,000 men and women from 12 prospective population-based cohorts.	Any type of fracture increased significantly with lower BMI. BMI of 20 had 2 fold more risk of fracture than 25, irrespective of gender. 17% less risk of fracture in BMI 30 over 25.	Low BMI a considerable risk factor.
Lloyd et al (2014)	Examine the association between body mass index (BMI) and bone mineral density (BMD)	US adults ages 50 and older (n = 3,296)	Unit increase in BMI = increase of 0.0082 g/cm(2) in BMD (p < 0.001). Race, age and sex do not significantly affect this relationship.	Positive association between BMI and BMD
Greco et al (2010)	Characterise lumbar bone mineral density (BMD) in overweight (BMI > 25 < 29.9) and obese (BMI > 30) patients.	398 Italian participants (291 women, 107 men, age 44.1 + 14.2 years, BMI 35.8 + 5.9 kg/m(2)) underwent body composition assessments.	Overweightness (BMI > 25 < 29.9) was neutral or protective of BMD. Obesity (BMI>30) associated with low bone mass.	High BMI associated with low bone mass.
Yao et al (2011)	Examine the role of physical activity in determining SOS in overweight girls.	Speed of sound (SOS) and physical activity levels were examined in overweight (OW) girls and adolescents. Controls (NW) were normal weight (n=75).	Tibial SOS was lower in OW compared with NW in both age groups. Moderate-to-vigorous physical activity higher in NW females.	Differences partially attributed by physical activity perhaps.
Rocher et al (2008)	Determine the influence of obesity on bone status in prepubertal children.	20 obese prepubertal children and 23 maturation-matched controls. Bone mineral parameters and body composition assessed using DEXA. Broadband ultrasound attenuation (BUA) and speed of sound (SOS) at the calcaneus	After adjustment for body weight and lean mass, Overweight had lower whole body BMD After adjusting for fat mass, overweight showed no difference in BMD or ultrasound with controls.	In reference to anthropometric changes, BMD in those in overweight group does not adapt sufficiently to deal with heavier load.

Bone health studies will often control for body mass (George et al 2014; Gerosso-Neto et al, 2014; Ginty et al 2005). This is because it can create variance in a sample when trying to observe the effect of another variable such as exercise. Huang et al (2014) using a healthy cohort of 19,980 Chinese men and women reported that body mass significantly correlated with radius SOS (r=.42). Lloyd et al (2013) calculate that for every unit increase of BMI the individual will see 0.0082 g.cm^{-2} increase in BMD (p < 0.001). Studies suggest that body mass has a linear relationship with bone density. Greco et al (2010) studied the BMD

difference between overweight (BMI > 25 < 29.9) and obese (BMI > 30) patients. 45% of men showed t-scores (standard deviations away from average normative data) of -1.84 ± 0.71. Findings by Lloyd et al (2013) would suggest they should be a value higher than 0. Greco et al (2010) concluded obesity (BMI > 30) was associated with a low bone mass and overweightness had a protective quality. Greco et al (2010) suggest an inverted 'J' curve whereby BMI has a linear relationship with BMD until a certain point where it decreases. De laet et al (2005) suggests the relationship between hip fracture and BMI is not linear in meta-analysis. A BMI of 20 (kg/m^2) was 2 times more at risk of hip fracture, than that of 25 whereas a BMI of 30 was only 17% less at risk of hip fracture than a BMI of 25. De Laet et al (2005) infer the protective nature of BMI increases sharply then plateaus rather than declines.

Immobility associated with being obese (30 kg/m^2) may cause muscle atrophy, resulting in adjacent loss in bone mass as reported by Greco et al (2010). Adjustment for lean mass in a study by Rocher et al (2008) significantly lowered values of whole body BMD in obese children. A common finding from Yao et al (2011) and Falk et al (2008) is that overweight participants were less physically active than their normal weight counterparts. In addition in the DEXA study by Hoy et al (2013) the higher tibial BMD reported in overweight participants was attenuated by lean tissue mass. Lean mass may be largely responsible for the relationship between body mass and BMD (Blain et al, 2001). The difference between obese BMD reported by De Laet et al (2005) and Greco et al (2010) may be explained by activity levels and lean mass.

Research designs in bone health studies

Table 8 provides examples of research designs used to investigate bone health.

Table 8: Research designs in bone health studies – Main areas of research.

Design	Researcher	Aims	Findings	Advantages	Disadvantages
Randomized Control Trial (RCT) Intervention based study that randomly allocates participants into experimental and control groups.	Marques et al (2011)	Effects of a resistance training protocol and a moderate-impact aerobic training protocol on bone mineral density (BMD).	Increased BMD at the trochanter (2.9%) and total hip (1.5%) for resistance trained group.	Unbiased allocation of participants. Intervention design closely controls independent variable. Easily measurable.	Adherence strict to exercise plan over time.
Cross-sectional Defines a population at one point in time.	Lehtonen-Veromaa et al (2000)	Investigate whether two types of physical exercise affect the growing skeleton differently.	65 gymnasts, 63 runners, and 56 nonathletic controls. Physical activity correlated weakly with all measured BMD and ultrasonographic values in the pubertal group (r = 0.19–0.35).	Can observe populations in their environment without manipulation. Can produce large data sets with a single assessment. Cost effective.	Causality is not certain. Cannot control for many extraneous variables. Independent variables not controlled.
Longitudinal Can be observational or intervention, it looks at how a population changes over time.	Biellemann et al (2014)	Evaluate a prospective association between physical activity (PA) and bone mineral density (BMD) in young adults.	A positive dose-response effect was found for the association between PA at 18 y and BMD. Males in the two highest quartiles of PA at 23 y had significantly greater BMD at all anatomical sites than males in the lowest quartile.	Overcomes issues of causality faced by cross-sectional designs. Can monitor extraneous variables. Monitor changes over time of the group and an individual.	Requires a lot of funds, resources and time.

Research designs will vary in approach based on the aim and resources available to the researcher. This section of the review will discuss different research designs used in osteology studies with greater attention given to the role of exercise and ultrasonography.

Randomised control trials (RCTs) are common in experimental research design. It allows unbiased allocation to intervention groups thus providing a true representation of the sample one is observing. Inclusion criteria in meta-analyses will often require an RCT design. Due to their greater reliability, they will be reviewed in this section. Marques et al (2011) used this method. They randomly allocated 71 older women to resistance exercise (RE), aerobic

exercise (AE) or a control group (CON). The aim was to determine if resistance training affected BMD more so in an 8 month period than moderate impact aerobic training. RE group exhibited increases in BMD at the trochanter (2.9%) and total hip (1.5%), and improved body composition. Even in cases where improvements are discrete an experimental design was able to determine differences created by an exercise intervention. Mackelvie et al (2002) used a 7 month jumping intervention (10 minutes, 3 times per week) to determine if it affected bone mineral gains in prepubertal Asian and White boys (10.3 ± 0.6 years, 36.0 ± 9.2 kg). Children were randomly split into control (n=60) or experimental (n=61) groups. Bone changes were similar for experimental and control groups. The study was measureable, unbiased and can establish causality. Cross sectional designs sometimes lack these criteria. This would be the most favourable design to use as the researcher is directly influencing the sample. With direct control if the independent variable there is more validity. Cross-sectional studies offer no control of the independent variable meaning the same population may have contrasting results dependent on the time frame. Furthermore, there would not be any biased allocation of participants from the experimenter and the study would be reproducible. Due to expense and changes in bone tissue occurring over long periods, it is difficult to implement an intervention based design.

Cross-sectional designs allow a snap-shot of a sample population. It aims to describe a population and or subgroups with respect to an outcome and a set of risk factors. The prevalence of an outcome is determined but no definitive outcome. This is because the sequence of events prior to testing is not definitively known. This makes it impossible to infer causality. However, this method is used to a great extent in osteoporosis studies. As such, it is important to review the use of this research model, relative to bone health.

Lehtonen-Veromaa et al (2000) aimed to determine if two types of physical exercise affected the growing skeleton differently using a cross sectional design. Calcaneus BUA was

13.7% higher in prepubertal gymnasts (n=65) than non-athletic controls (n=56). Mean BMD of the femoral neck in the pubertal gymnasts was 20% higher than non-athletic controls. Lehtonen-Veromaa et al (2000) have established that a cross sectional design can be used to observe changes in QUS and BMD in relatively small samples. Stepwise regression analysis reported that physical activity accounted for much more of the variance in BMD than QUS. Using a cross sectional study to investigate the effects of exercise on QUS, was lacking. Similar positive associations between physical performance (PP) and all bone health parameters (BUA, SOS and BMD) were reported by Furrer et al (2014) in a cross-sectional study. The positive association seen between PP and bone health parameters were similar to Lehtonen-Veromaa et al (2000). Furrer et al (2014) controlled for confounding factors and used a much larger cohort. Controlling for confounding factors and having a larger cohort increase the power of the study. The validity of the study improves thus the probability that the independent variable affects the outcome measures. This appears important to witness changes in QUS as a result of exercise with a cross-sectional design.

Longitudinal designs use many data collection points over time. This helps overcome issues of causality faced by cross sectional studies. Some include an intervention, yet some are observational. This lends itself well to bone health studies, as changes in osteology in relation to environment are chronic. Bailey et al (1999) conducted a longitudinal study with multiple testing points, investigating the long term effects of physical activity on multisite bone mineral content (BMC) in growing adolescents. Covariate analysis was used, similar to Furrer et al (2014) accounting for key confounding variables related to the sample. Two factors were established, physical activity and gender. Significant main effects for physical activity and gender were found at peak BMC accrual at the femoral neck. Controlling for maturation and size between groups a 9% greater total body BMC in active boys over their inactive peers was reported. By following the development of the sample and measuring all

factors and outcomes, the sequence of events prior to testing is known. Therefore, without manipulating the sample, Bailey et al (1999) can more confidently assume causality.

Biellemann et al (2014) reported that physically active patterns are important in the first three decades of life. This follows a 15 year longitudinal study of 3454 young men. A positive dose-response effect was reported for the association between PA at 18 years and BMD. This reiterates the strength of a longitudinal design, in the absence of an experimental design to establish the effect of physical activity on bone.

Using a similar design Bachrach et al (1999) tested ethnic and gender differences in bone mineral acquisition on an annual basis over 4 years. Bachrach et al (1999) did not use a covariate model but did report significant main effects for ethnicity but no interaction with gender. Between subjects analysis, report consistent differences in areal and volumetric bone density between Black and Non-Black subjects. There may be potential for a cross-sectional or longitudinal design to incorporate physical activity and ethnicity into a two-way analysis of covariance model to determine if different ethnic groups respond differently to exercise.

Genetics & Ethnicity

Arden and Spector (1996) explain the genetic influences of muscle strength, lean body mass and bone mineral density in a twin study. They stipulated that no studies existed that quantified the size of the genetic component. They proposed two aims. Determine the heritability of lean muscle mass and muscle strength and estimate how much of the genetic variance in BMD could be explained by muscle. Arden and Spector (1996) suggest BMD and lean mass is predisposed to a certain extent. The effect lean mass has on BMD is therefore also inherent. Genetic factors that affect BMD are inclusive of lean mass and muscle strength.

The overall effect on BMD from muscle variables relating to genetics was small (6.8-18.6%). The heritability of lean mass in this study was .56. Over 50% of lean muscle in 45–70 year old women was inherited, yet of that percentage 6.8-18.6% affects BMD, inherently. This also shows that as much as 50% of muscle bulk was to down to environmental factors. This proves optimistic for interventions such as exercise that aim to elevate muscle variables, in order to improve BMD. In addition there are large genetic factors independent of lean mass and muscle strength that determine BMD. This highlights the importance of investigating bone-specific genes (Arden and Spector, 1996).

Polymorphisms in receptor genes FGFR2, ERalpha, ERbeta and vitamin D receptor can cause significant adaptations in BMD or QUS (Thijssen, 2006; Dong et al, 2015; Correa-Rodríguez et al, 2015). Changes in these genes affect the chemical signalling in bone remodelling. Correlations between these gene polymorphisms and populations with low bone mass have yet to be reported. These genetics studies suggest large amounts of variation in bone density attributed to genetics remains unknown. Furthermore, what is known is related to phenotype, rather than what is happening in the DNA.

Pollitzer and Anderson (1989) explain that the term ethnicity makes no firm commitment in attributing differences to genetics or environment, rather it establishes a product of the two. The term race is purely genetic. It is important to understand that non-visible traits are as much a part of an ethnic group as visible traits. An early study by Garn et al (1965) reported that South American natives of Tiera del Fuego had naturally elevated metabolisms at night to deal with the cold at altitude, providing an example of environmental adaptation not uniform to greater native American populations.

Ethnicity can affect bone properties (Mazes and Mathers, 1974; Melton et al, 1987; Tracey et al, 2005). This can be observed by looking at trends relating to fracture incidents. Melton et al (1987) conducted a survey into the incidents of proximal femur fracture of two

ethnic groups of New Zealand. Per 100,000 years White women had 178.3 incidents of fracture, whereas Maori women had 88.3. The margin was much closer for men, 80.9 and 70.9, respectively. In a survey of orthopaedic admissions of a 5 year period Moldawer et al (1965) report that hip fractures are 5.6 times more likely in White males than Black males of the same age. In fact, cross-sectional studies stipulate that peak accrual and rate of decline in BMD among US Hispanic and US Caucasian men are lower and faster, respectively than African Americans (Araujo et al 2007; Travison et al, 2009).

Table 9 provides examples of studies that observe ethnic differences in bone health.

Table 9: Ethnicity: Main areas of research.

Researcher	Aims	Method	Results	Conclusion
Nam et al (2010)	Investigate men's bone mineral density (BMD) levels across race/ethnic groups and geographic locations.	Cross-sectional design. 208 African-American, 422 Afro-Caribbean, 4,074 US Caucasian, 157 US Asian, 116 US Hispanic, 1,747 Hong Kong Chinese and 1,079 Korean men.	Afro-Caribbean and Afro-American males had a difference of 0.091 $g.cm^{-2}$ whereas US Asian and Hong Kong Asian had a difference of .001. The difference in BMD between the four US groups was 0.074.	Substantial race/ethnic differences in BMD even within African or Asian origin. Body size important between Asians men and others
Travison et al (2011)	Determine the contributions of risk factors to racial/ethnic differences in bone mineral content (BMC) and density (BMD).	Cross-sectional design. Afro-Americans (n=335), US Hispanics (n=394) and US Caucasian (n=441). BMD, Socioeconomic status, health history and dietary intake Multivariate analyses and multiple regression analysis.	Afro-Americans had a BMD of 1.07 ($g.cm^{-2}$), US Hispanics 1.09 and US Caucasian 0.98. Lean mass, fat mass and Socioeconomic status influence ethnic differences.	Variation in body composition, diet and socioeconomic status account for differences between ethnic groups.
Liang et al (2007)	What extent do diet, lifestyle factors and anthropometrics determine BMD	115 young 20 - 35 year-old women of Asian (n=40), Hispanic (n=39) and Caucasian (n=36). BMD, lean and fat mass, CV fitness, leg strength, diet and lifestyle questionnaire.	BMD values are significantly lower in Asians than Caucasians and Hispanics (p<.001). Adjusted BMD at the wrist was 7.3% higher in Asian than Caucasian.	Significant factors underlying BMD in ethnically diverse young women vary as a function of ethnicity and include leg strength and dietary calcium as well as anthropometric characteristics.

Many studies have approached this area of the literature using large epidemiological studies with multiple ethnic groups. Nam et al (2010) used a final data set of 208 African-American,

422 Afro-Caribbean, 4,074 US Caucasian, 157 US Asian, 116 US Hispanic, 1,747 Hong Kong Chinese and 1,079 Korean men. Adjusting for many confounding variables including age, weight and dietary intake of calcium they report the following. Afro-Caribbean men (1.008 g.cm^{-2}) and African American men (0.917 g.cm^{-2}) had the highest bone mineral density of the femoral neck. Koreans had the next highest (0.906). US Hispanics, US Asians and Hong Kong Asians had values between 0.849-0.843. US Caucasians had the lowest (0.820). Afro-Caribbean and Afro-American males had a difference of 0.091 whereas US Asian and Hong Kong Asian had a difference of .001. The difference in BMD between the four US groups was 0.074. The role of genetics appears more uniform and the role of environment more changeable. It could also be argued that the genetics of ethnic groups interacts with environment differently.

A cross sectional study by Araujo et al (2007) reports similar genetic trends. African American males (n=367) had 13.3% and 5.6% higher BMD than US Caucasian (n=451) or Hispanic males (n=401), respectively. The difference between Afro-American males and Caucasian males was 10.6% and the difference between Afro-American males and Hispanic males was 8.1%, according to Nam et al (2010). Both studies suggest similar differences between ethnic groups. Lifestyle, socio-economic status and diet may vary between these ethnic groups however, despite all being from the US. Travison et al (2008) again at the femoral neck report that Afro-Americans (n=335) had a BMD of 1.07 (g.cm^{-2}), US Hispanics (n=394) 1.09 and US Caucasian (n=441) 0.98. The general rank from highest to lowest BMD is the same as studies by Nam et al (2010) and Araujo et al (2007) but they are markedly higher in Travison et al (2008). Afro-American BMD of the Travison et al (2008) study (1.07) is more similar to Afro-Caribbean BMD (1.008) than the Afro-American BMD (0.917) of the Nam et al (2010) study.

Studies that involve similar ethnicities are reporting similar ranked differences. Between studies, there are differences between those of the same ethnicity. This highlights again, the effect of the environment. Pollitzer and Anderson (1989) describe that genetics provides the platform for environment to operate. Despite this point, studies 7 years apart of the same population (Hong Kong Asian) report almost identical femoral neck BMD of men. Lau et al (2003) report 0.85 g.cm^{-2} and Nam et al (2010) 0.849 g.cm^{-2}. Perhaps there is a difference to the way bone tissue responds to the environment, based upon ethnic group. Differences between the BMD of ethnic groups can also be seen in the magnitude of growth and decay. Gilsanz et al (1991) addressed this. In prepubertal girls, they report no differences in vertebral bone density between US Black (n=75) and US White girls (n=75). The magnitude of change and peak BMD accrual was much higher in Black than White girls. At Tanner stage 3, the middle stage of sexual development, White girls had a BMD (mg.cm^{-2}) of 157±14 and Black girls 161±19. At Tanner stage 4 White girls had 166±19 and Black girls 202±21. Ethnicity significantly interacted with stage of sexual development past tanner stage 3 (p<.001). This constitutes a large genetic component for the formation of bone tissue between ethnic groups.

A greater volume of studies observe the degradation of bone tissue due to age between ethnic groups. Wang et al (2005) report that although cortical thickness was 0.35 standard deviations lower in Chinese than Caucasian men, with age the thickness diminished less. Sheu et al (2001) the annual rate of decline of BMD is greatest in African American (-.42%) then Caucasians (-.32%) and Asians (-0.9%) in those aged 65 and over. Tracey et al (2005) disagree with these figures. The rate of annual BMD decline of the femoral neck was 2.1±3.7% for Caucasian men and 1.1±3.3% for Afro-American men. Contrasting figures suggest factors other than genetics that contribute to bone loss. With respect to Hispanic

Americans Travison et al (2008) suggest a large genetic component in bone loss, supported by Araujo et al (2007) in comparison with Caucasian and Afro-American males.

Nam et al (2010) suggest the difference between Asian and Caucasian men is inconclusive, meaning the research is limited and the reasons for differences not established. Liang et al (2007) conclude that ethnicity affects the response to risk factors such as physical activity, hormone balance and nutrition rather than having a genetic predisposition, despite being unable to explain 16% of the variance in BMD after controlling for common risk factors.

The understanding of ethnic differences in bone may come from biochemical analysis. Three main hormones regulate the metabolism of bone tissue. These are Parathyroid Hormone (PTH), Calcitonin and the hormone form of vitamin D – Calcifediol. Osteoblasts create bone tissue and osteoclasts remove bone tissue. PTH promotes the uptake of calcium ions from the kidneys and gut as well as osteoclast activity. Calcitonin does the opposite. It inhibits uptake of calcium and promotes osteoblast activity. Calcifediol helps with the absorption of calcium stimulated by PTH. Osteocalcin and alkaline phosphatase are products of osteocyte metabolism often used as markers for bone remodelling.

Modlin (1967) reported that osteoporosis was uncommon among Bantu people; an ethnic group spanning Central-Southern Africa. Their life long calcium intake was low (250-400mg/day) and they excreted significantly less calcium than their Caucasian counterparts. This lack of dietary calcium resulted in an inherent biochemical adaptation to naturally retain more of the mineral. It is suggested that this lead to greater resistance to osteoporosis. Bell et al (1988) observed differences in calcium regulating hormone between Black and White participants. On same diet of calcium, Black participants had significantly higher PTH and lower osteocalcin. This promotes serum calcium and perhaps explains higher BMD in Black subjects.

Dibba et al (1999) carried out a cross sectional study of Black Gambian and White British males, living in the UK. Gambian males had higher size adjusted bone mineral content than British males. However there were no significant differences in bone turnover markers. Urinary calcium was also similar between the two groups. A biochemical explanation for ethnic differences in BMD is refuted by this study. Again, Henry et al (2000) reports multi-site significant differences in BMD between Black and White participants (p≤.0005) yet similar concentrations of bone turnover markers. Leder et al (2006) yield contrasting results. In the study of 1029 men (aged 30–79 years) mean Osteocalcin was 17.6 and 20.5% higher in Hispanic (P = 0.02) and White men (P < 0.01), respectively compared with Black males. Osteocalcin is produced by osteoblasts and is often as a marker for bone formation.

Hormones associated with bone formation seem to be connected to White and Hispanic populations whereas hormones associated with retention seem to be connected to Black populations. This is inconclusive however. For the individual the use of biochemical markers as a mean to diagnosing or combating osteoporosis may prove advantageous, but at distinguishing between ethnic groups it is lacking.

Summary

QUS proves to be a promising alternative to DEXA. Precision, compliance to World Health Organisation (WHO) figures and identification of bone qualities separate the two tools. Together they have improved the prediction of fragility fractures (Chan et al, 2012). Cavani et al (2008) suggest QUS can be explained by BMD (93%) and elastic modulus (7%). It closely identifies with BMD derived from DEXA and accounts for elastic properties. It may prove a reliable cost effective alternative to investigate young adult male bone properties in response to the mechanical stress of increasing physical activity.

Muscle strength and lean mass are all factors that impact bone properties. It is argued that muscle strength alone does not improve local bone mineral density (BMD). Conversely intervention based studies state that gains in muscle strength as a result of resistance training improves local and global BMD regardless of age and gender. Gains in strength are strongly linked with muscle hypertrophy. Taafe et al (2001) suggest when muscle strength and lean body mass are introduced into regression models together, lean body mass diminishes the effect of muscle strength on bone.

Frost et al (2000) propose the "muscle-bone unit". Factors chemical or mechanical affect the unit jointly such as resistance training or growth hormones. With relative consistency studies have quantified the linear increase in lean mass and bone mineral density, irrespective of age, race and gender. In a meta-analysis of 44 studies Ho-Pham et al (2014) reports a correlation co-efficient of .48 in men between lean tissue mass and total BMD. Controlling for variables so inexplicably linked with bone properties helps remove variance caused by them. Removing muscle strength and lean mass variability enables the researcher to be more confident they are observing differences created by the independent variables.

Factors affecting bone properties are numerous and the response to these varies between groups. Secondly, adaptation in bone happens slowly overtime. Developing a research design has challenges and various approaches have been used. Observational cross-sectional designs with a large sample are the most common. These are cost effective and generally involve a single assessment. As the sequence of events prior to testing is unknown, establishing causality is difficult. Furthermore many different conditions could be affecting the sample on a single day, creating more variability. As such a large number of variables are controlled for based upon existing literature. Longitudinal designs increase the confidence in causality. They help overcome the issue of chronic bone adaptation and the researcher becomes aware of the sequence of events. This design has been successful in determining the

effect of physical activity on bone mineral density (Bielemann et al, 2014). Muscle and bone at adulthood forms as a result of genetic and environmental factors. Arden and Spector (1996) stipulate this genetic component for muscle will have a hereditary effect on bone regardless of adaptation or environmental factors. The genetic component for BMD includes the genetic component for muscle. They calculated this using an MZ-DZ twin study. 6.8-18.6% of muscle mass derived purely from genetics inherently contributes to overall BMD. Different ethnic groups may be on alternate ends of this range. In women aged 45-70 56% of muscle mass was inherited, meaning environment is 44%. This proves optimistic for interventions that target lean mass to combat low bone mass.

Ethnicity can affect bone properties. Afro-Caribbean and Afro-American male BMD was significantly different (0.091 $g.cm^{-2}$) ($p<.005$) (Nam et al, 2010). This suggests a large environmental component to ethnic differences in BMD. Studies have disagreed on BMD of specific ethnicities. However, they consistently rank Black, Hispanic, Asian and White in that order for BMD. Genetics provides the platform for environment to work (Arden and Spector, 1989). Discrepancies in in the literature regarding the magnitude of degradation of BMD with age, suggest a large environmental component for bone loss. Using biochemical analysis to determine a difference between ethnicities proves inconclusive.

The literature cites physical activity at a young age, as a best deterrent for osteoporosis later in life. Studies have cited strong multi-directional loading activities as the most osteogenic form of physical activity. Research highlights changes in BMD happen in accordance with improvements in muscle strength and lean mass. The literature is lacking an interaction between exercise and ethnicity. An aim of the study was to determine if frequency and type of physical activity affect radial and tibial ultrasound (SOS). It was hypothesised there would be difference in QUS-SOS between physical activity groups, significantly affected by covariates. A second aim was to determine if there was a difference in radial or

tibial SOS between different ethnic groups in addition to an interaction effect between ethnic group and level of exercise. It was hypothesised there would be a significant interaction and main effect between ethnic group and exercise. This would be will be partially determined by one or more covariates.

Methods

Overview 2.1

The research proposal by Chen C.K and Ooi F.K was a 'comparison of bone health and isokinetic strength between young male athletes and non-athletes'. Their research objectives were to determine any significant differences in in bone health (QUS), determine any significant differences in isokinetic muscular strength and determine any associations between anthropometrics in British and Malaysian athletes and non-athletes. Using the same sample, the current thesis investigated to what extent physical activity determines QUS (Chapter 3) and whether an interaction and/or main effect exists for ethnicity and athletic standard (Chapter 4). Athletic standard defines controls (non-athletes) and athletes. The study follows a cross-sectional design, commonly used in bone health studies (Goueveia et al, 2014; Erlandson et al 2012). This was a single assessment of dominant radial and tibial ultrasonography (m.s^{-1}), dominant quadricep strength (Nm) and anthropometrics. Both researchers (Shanks, J and Li, X) followed identical lab protocols, but on different sites. Shanks, J collected data for the British population in the UK and Li, X for Malaysian in Malaysia.

Sampling

A power calculation was carried out by Chen C.K and Ooi F.K to determine the correct number of participants for their research project using PS Power and Sample Size Calculation version 3.0.43. The power of the study was set at 80% with 95% confidence interval. The calculated sample size for each group was 33. They had 4 experimental groups therefore a total 132 were needed, half of which were British and half Malaysian. Therefore, 132 male students aged 18-25 were recruited through quota and opportunity sampling. This sampling method was resourceful, economical, convenient and commonly used in for this research

design. However, it can create bias on behalf of subjects and experimenters as physically active people are more likely to step forward.

66 multi-ethnic British students were recruited from the University of Essex campus, UK as per the power calculation. These participants were used in chapter 3, investigating the effect frequency and type of physical activity on SOS measurements. 66 Malay Malaysian were recruited from University Sains campus, Malaysia. These were used in chapter 4 along with 48 White British males from the British sample. This study investigated the effect of ethnic group and athletic standard on SOS measurements.

Grouping

Chapter 3 sampled the British group only (n=66; 21.04 ± 1.57 y; 73.97 ± 7.6 kg; 1.80 ± 0.06m) and grouped them according to levels of physical activity. The Stanford patient research questionnaire has been commonly used in the community for collecting data on lifestyle factors that affect chronic illness and their recovery (Osborne et al, 2007; Ritter et al, 2014). Elements of the Stanford Patient Education Research Centre questionnaire were used to assess level of total and strength activity in the current study. This questionnaire was used as it allowed the researcher to concisely measure total physical activity account for a range of exercise modalities including strength training.

The questionnaire (Appendix) contained 6 questions graded on a scale of 0-4 asking how much of a certain activity is carried out in a typical week (0 = none; 1=0-30 mins; 2=30-60 mins; 3=60-180 mins; 4=>180 mins). 0, 1, 2, 3 and 4 were averaged at 0, 15, 45, 120 and 180 minutes, respectively. Question 2 asked 'to what extent do you walk for exercise'. This was removed as 180 minutes of walking by this method would carry the same weight 180 minutes of swimming or aquatic exercises, understandably appropriate for people with low mobility but it may misrepresent the difference between young adult males engaged in high

and low physical activity. Total minutes were then added for questions Q1, Q3, Q4, Q5 and Q6. This established a continuous variable that was easily stratified into low, moderate and high groups similar to a physical activity study by Pettersson et al (1999). Question one only, was used for strength grouping.

The research title comprised by Chen C.K and Ooi F.K was a 'comparison of bone health and isokinetic strength between young male athletes and non-athletes'. They compared QUS and strength measurements between athletes and non-athletes of British and Malaysian groups. The method for grouping athletes is the same method used in this thesis. Chapter 4 investigated an interaction between athletic standard and ethnicity, controlling for confounding factors such as strength. Chapter 4 sampled the White British (n=48; 21.45±1.32y; 72.56±6.7kg; 1.79±0.07m) participants from the University of Essex campus and all the Malay Malaysian (n=66; 20.17±0.59y; 64.47 ± 12.01kg; 1.68 ± 0.06m) participants from the Universiti Sains campus. The grouping variables established a British control, British athlete, Malaysian control and a Malaysian athlete group.

Participants were not grouped for physical activity because data regarding physical activity levels for Malaysians was not collected. Athletic standard was used as a measure for level of exercise as it retroactively accounts for 1 year of regular competition and training at district level or higher, maintained to date. This is important as bone adaptations are chronic in response to exercise. Under this design, the researcher cannot collect data prior to testing, therefore grouping in this way helps establish a group characterised by long term training and competition.

Protocol 2.2

Table 12: Order of lab protocol

Order	Test	Duration
1	PARQ; Informed Consent; Physical activity questionnaire; Athlete questionnaire; Participant interview.	20 minutes
2	Body Composition Analyser; height and body mass	15 minutes
3	Sunlight Miniomni bone Sonometer	25 minutes
4	Isokinetic dynamometry	30 minutes.

The lab protocol will be described in order in which it was undertaken (Table 12). A PAR-Q, informed consent form, Physical activity questionnaire, Athlete questionnaire and Participant interview were done prior to testing. A PAR-Q informs the experimenter of physical readiness. The informed consent form provides a brief overview of the study and notification that they can withdraw at any time. This interview was necessary for the following reasons. Those with fracture injuries of the measurement sites were excluded from the study as the lab protocol may exacerbate the injury further. Furthermore, QUS-SOS measurements of the site may not be representative of healthy bone tissue for the participant. For similar reasons muscular or osteological issues were discussed with the experimenter prior to testing. Unless recommended not to by a physician or causing pain, those reporting chronic injuries as a result of exercise such as medial tibial stress syndrome (MTSS) or apophysitis were included in the study. If excluded from the study the population may not be representative of the effect of physical activity on QUS-SOS.

Body composition analyser, height and body mass

Body height (cm) and mass (Kg) were measured with a wall mounted stadiometer (SECA 213, UK) and digital weighing scales (SECA 813, UK) respectively. The accuracy of the wall mounted stadiometer was ±0.5mm and scales were accurate to 0.1 Kg.

A body composition Analyser (TANITA, model TBF-140, Japan) was used to measure percentage body fat (%) and fat free mass (Kg) to the nearest 0.1% and 0.1kg,

respectively. Participants were instructed to arrive at the experiment hydrated as arriving dehydrated can affect body composition measured by Tanita body scanners. The device required the height (m) and body mass (kg) of the individual. Participant stood barefoot on the elevated platform. After 5-10 seconds, the bio-impedance device provided body fat (%) and fat free mas (kg).

Bio electrical impedance analysis works well in healthy subjects with stable water and electrolytes balance with respect to age, sex and race (Kridger 2006). Body fat and fat free mass are calculated knowing the resistance and conductivity of fat mass and water, respectively. Studies have reported it can make accurate estimations of fat free mass (kg) (Kotler et al, 1996) but it underestimates results compared to DEXA for fat mass ($p<.05$) (Lazzer et al, 2003) and fat free mass (Kotler et al, 1996). However, it is a cost effective alternative to the current researcher, enabling the observation of key confounding variables.

Quantitative ultrasound

A Sunlight Miniomni[tm] bone sonometer (BeamMed Ltd) with ultrasound probe type CMC was used to collect quantitative ultrasound- speed of sound (QUS-SOS) data at the dominant mid-shaft tibia and distal radius. Systems quality verification (SQV) was required before each test to ensure the probe was functioning correctly. This involved the CMC probe, ultrasound gel, a phantom and BeamMed Ltd software. The phantom allowed expected SOS to be compared with actual SOS, thus calibrating the unit. The Phantom is displayed in figure 1.

Figure 1: Phantom used for calibration

 A layer of ultrasound gel was applied to the upward facing probe and the phantom mounted on top. Three measurement cycles of the SQV using the Beamed Ltd software was enough to

establish actual SOS. The temperature gauge aside the phantom and corresponding SOS values were also manually entered into the software to establish expected SOS. The room temperature of testing was between 19-21°C. This completed SQV.

A gauge was used to determine the measurement location of the distal radius. The elbow of the subjects arm was placed on the gauge platform elbow at 90 degrees with fingers fully extended and palm facing the subject. The distance between the gauge platform and tip of the third finger was measured. At the half way point a mark was drawn. This mark was then extended covering the radius and half the diameter of the arm and the lateral surface of the wrist was then placed on the hand rest. A uniform layer of ultrasound gel was applied to the ultrasound probe and measurement site. During data collection, the probe was moved 140° around the longitudinal axis of the bone, back and forth with scans lasting 25 seconds, ensuring good contact with the skin. The current study consistently recorded 4 scan cycles. The Beamed system required 4 scans for precision purposes. Dividing standard deviation by the mean, a coefficient of variation (CV) was established. If this value was over .06 or 6%, the test required a repeat as it did not satisfy the precision quality of the unit.

The second measurement site was the mid-shaft tibia. With the patient sitting and the knee at a 90 degree angle, the gauge platform was placed under the heel and measured up to the tip of the patella. The half-way point was marked on the anterior surface of tibia. The leg was then elevated at the same height of the chair they were sitting on. The same protocol as the radial site follows except each scan lasts 15 seconds. Speed of sound (m.s^{-1}) (SOS) was noted. Reliability was ensured by measuring each anatomical site twice with the gauge platform. The same amount of ultrasound gel was applied to the probe surface and measurement site prior to scanning. Furthermore, the order of the procedure was kept the same throughout testing. The researcher was taught how to use the testing equipment by a lab technician yet not pilot or re-test reliability tests were carried out for the equipment.

Quantitative ultrasound- speed of sound (QUS-SOS) (m.s^{-1}) is a valuable prognostic tool used in bone health assessments (Barkmann et al, 2000; Böttcher et al, 2006; Muller et al, 2008). Böttcher et al (2006) report it correlates significantly (p<.01, r=0.71) with DEXA-BMD, the gold standard measurement tool in patients with bone pathology. Its primary application is testing for osteoporosis in high risk patients (Knapp et al, 2004) but it has been widely applied to large epidemiological studies (Huang et al, 2015), longitudinal aging studies (Furrer et al, 2014) and exercise studies (Falk et al, 2007). What these studies suggest that QUS is a valid tool in discriminating between healthy and unhealthy bone tissue and successful in seeing adaptations in radial and tibial bone tissue under different exercise conditions. It is the comparative success of QUS compared to DEXA and its ability to identify discrete changes in radial and tibial SOS that make it a plausible tool to use in this study.

Radial and tibial sites were chosen as much of the literature using QUS has analysed these sites (Wang et al, 2008; Williams et al, 2012; Nguyen et al 2004). Radial SOS is of particular importance as it is highlighted by the WHO to be a high risk site, mainly due to its relative fragility to the rest of the skeleton and its susceptibility to trauma from falls or impacts etc. As a high risk site, collecting data concerning it will have value and relevance to current literature. Knapp et al (2004) argues that the anatomical areas targeted by WHO (Femoral neck, lumber spine and distal radius) are not widely applicable to all bone sites. As such, in order to be eclectic and utilize the BeamMed: Sunlight Mini Omni[tm] to a more comprehensive extent, the tibia is used also.

Isokinetic Dynamometry

An Isokinetic dynamometer (CHATTECX – KINCOM 125E – PLUS; Software version 5.30 Chattanooga Group) was used to measure peak torque (Nm) as an indicator of quadricep strength. Calibration of unit included adjusting for gravity, weighing of the limb, lever length adjustment, range of
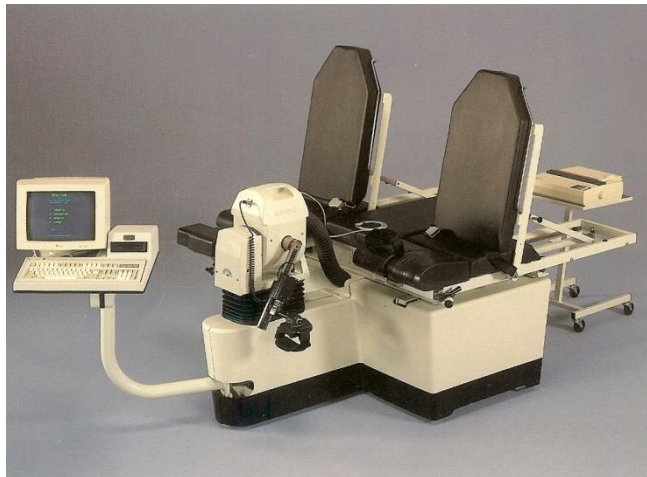

Figure 2: KINCOM Isokinetic Dynamometer

active motion and attachment points to the dynamometer. A warm up program was carried to get participants accustomed to the equipment. By self-report participants were free of any musculoskeletal injury that would have inhibited them from testing. The warm up involved a low effort continuous concentric and eccentric quadricep contraction of the dominant leg only at $30^{o}.s^{-1}$. Verbal instructions were given to produce 50% effort. Leg extension using this apparatus can feel unnatural. This familiarisation allows the individual to gain confidence in the movement. This means when it comes to maximal concentric contraction they are more likely to produce their maximal force, without any fatigue brought on by the warm up. Concentric strength was then measured at $60^{o}.s^{-1}$ on the dominant side. Maximal extension was measured 3 times and the maximum taken of the three. Motivational aid was kept to a minimum.

Muscle strength as well as muscle volume has been shown to be a firm predictor of bone health (Gouveia et al, 2014). Functionally, muscle power and torque is measured by dynamometers capable of measuring dynamic force whilst controlling the velocity. Barnes (1980) states that muscular torque decreases with increased angular velocity (AV), satisfying the force-velocity equation (Fenn and Marsh, 1935; Hill, 1938). Isokinetic dynamometry has

been used to assess muscle strength in bone health studies (Blain et al, 2001). Research by Moffroid et al (1969); Perrine and Edgerton (1978) and Lesmes et al (1978) maintain that force plateaus between 0-144 degrees per second, citing limitations from neural mechanisms at higher speeds. The current study measured concentric strength of the dominant quadricep (Nm) to ensure a voluntary contraction. Secondly, a $60^{o}.s^{-1}$ velocity was used to ensure maximum recruitment of motor units, for maximum force production.

Statistics 2.3

Table 10: Independent variable, groups, factors and dependent variables.

Chapter 3	Model 1	Model 2
Independent Variables	Total Physical Activity (TPA)	Strength Activity (SA)
Groups	Low, Moderate, High	Low, Moderate, High
Factors	Body Mass (kg), Height (m), Body Fat %, Fat Free Mass (kg), Quadricep Strength (Nm)	
Dependent Variables	Dominant Radius SOS, Dominant Tibia SOS	
Chapter 4	Model 1	
Independent Variables	Athletic Standard (AS), Ethnicity, AS*Ethnicity	
Groups	British Control, British Athlete, Malaysian Control, Malaysian Athlete	
Factors	Body Mass (kg), Height (m), Body Fat %, Fat Free Mass (kg), Quadricep Strength (Nm)	
Dependent Variables	Dominant Radius SOS, Dominant Tibia SOS	

AS*Ethnicity = interaction between athletic standard and ethnicity.

Table 10 shows the structure of the study. Chapter 3 has two related independent variables needing two separate statistical models for each. Groups were stratified into low moderate and high. Chapter 4 sought an interaction between two unrelated independent variables. Groups were separated into four groups based upon athletic standard and ethnicity. Both chapter 3 and 4 studies had the same factors. These factors may be used as covariates dependening on the relationship they have with the dependent variables.

Table 11: Statistical testing in Chronological order for both chapters 3 and 4.

1	Descriptive Statistics
	Mean
	Standard Deviation
2	Analysis of variance
	Homogeneity of variance
	Between Subject Effects
	Post Hoc
3	Homogeneity of Regression
	Correlation
	Univariate test
4	Analysis of Covariance
	Homogeneity of Error Variance
	Between Subject Effects
	Estimated Marginal Means Post Hoc
5	Multiple Regression Analysis

Statistical analyses were performed using SPSS ver19.0 (SPSS Chicago, IL, USA)

Step 1: Descriptive statistics provide the mean and standard deviation for all groups regarding all measureable variables (Table 11).

Step 2: Secondly, an analysis of variance (ANOVA) test was carried out to determine if differences existed between the independent groups given in table 10. Homogeneity of variance test was required to ensure all groups have similar distribution of results (Table 12). Between subject effects shows the result of the ANOVA. A post hoc was undertaken where a significant overall difference was found in the ANOVA ($p<.05$). This establishes, if there are more than two groups, which groups were specifically different. The adjusted p score for this is .016 for chapter 3 and .0125 for chapter 4. This is because chapter 3 has three groups (.05/3) and chapter 4 has four groups (.05/4).

Step 3: A homogeneity of regression (HOR) is test required before an analysis of covariate test. It ensures the covariates relate to the dependant variables, radius and tibia SOS and the covariates do not strongly correlate. Two-tailed Pearson's correlation coefficients were used to calculate correlation. Lastly, HOR ensured all groups respond similarly to covariates. It was carried out by a univariate interaction test (table X). If different factors affected radius and tibia SOS then two univariate rather than one multivariate test were necessary.

Step 4: An analysis of covariance (ANCOVA) test takes away variance created by other factors and more confidently observes the effect of the independent variable. Homogeneity of Error Variance ensures the variability is equal in SOS measurements and covariates between groups (table X). If this is true (p>.05), the test can continue. Between subject effects shows the result of the ANCOVA. A p value of ≤.05 for an independent variable denotes it significantly affects SOS measurements. A p value of ≤.05 for an interaction denotes two independent variables have a joint effect on SOS measurements.

If covariates significantly affect the outcome of radius and/or tibia SOS, then Estimated Marginal Means can be observed. This looks at group specific means in radius and tibia SOS taking away variance created by covariates. Because the two independent variables in chapter 3 were related, there were two statistical models for each independent variable. Chapter 4 has one statistical model to determine an interaction between the independent variables.

Step 5: A multiple regression analysis was carried out specifically for chapter 3. This was to determine the relative effect of covariates on radius and tibia SOS.

Introduction 3.1

Four factors that play a major role in the attainment of peak bone mineral density (BMD) are genetics, hormonal status, physical activity (PA) and nutrition (Dalsky et al, 1990; Kelly et al, 1990 and Politzer et al 1989). PA is reported to elevate bone mineral density (BMD) in all ages, genders and races (Bailey et al, 1999; Biellemann et al 2014; Liberato et al (2013) but sensitivity to the osteogenic stimulus of PA is greatest in a young male population (Mcveigh et al 2014; Liberato et al 2014). PA can facilitate osteogenesis, based upon the type and magnitude of its application (Biellemann et al, 2013; Gouveia et al, 2014). As such, the frequency of strength and total activity could change this relationship.

Quantitative ultrasound- Speed of sound (QUS-SOS) is a valuable prognostic tool used in bone health assessments (Barkmann et al, 2000; Böttcher et al, 2006; Muller et al, 2008). Its primary application is testing for osteoporosis in high risk patients (Knapp et al, 2004) but it has been widely applied to large epidemiological studies (Huang et al, 2015), longitudinal aging studies (Furrer et al, 2014) and exercise studies (Falk et al, 2007). This tool will be utilized in the current study.

Bone density shares a relationship with lean mass (kg) (r=.42), fat mass (kg) (r=.28) and body mass (kg) (r=.41) in men (Ho-Pham et al, 2014; Huang et al, 2015). In boys aged 6-21, weight was an independent predictor of SOS (Van den burg et al, 2000). As well as local muscle strength (Nm) (Pettersson et al, 1999) these factors are often controlled for in intervention (Marques et al, 2011) longitudinal (Biellemann et al, 2013) and cross-sectional (Pettersson et al, 1999) exercise studies as they significantly and indiscriminately impact bone health. As such, controlling for these variables is essential, adjusting the values for bone

density accordingly to exclude the variance caused by them. This means the main effects for physical activity can be more accurately observed.

Groups typically unresponsive to PA (55+ women) have seen positive adaptations in BMD as a result of resistance training alone after eight months (Marques et al, 2011). Layne et al (1999) conclude the osteogenic response to exercise is greater felt after resistance based training, rather than aerobic. These adaptations have been largely attributed to improvements in strength and lean mass. Increasing bone loads in order to increase bone mass, is prevalent conclusion. Bolam et al (2013) through a systematic review reported resistance training alone or in combination with impact-loading activities is the most osteogenic in males. Monitoring the amount of strength training individuals do when observing the osteogenic response to PA in different groups, is important.

Aerobic training has a minimal osteogenic response and only in extremely aerobically active adults (Whitfield et al, 2015). Gomez-Cabello et al (2012) suggest that strength training is a strong osteogenic stimulus but a combination of strength, aerobic, high impact and/ or weight-bearing training could be equally as advantageous, suggesting total stimulation is what is important. Observing the response of independent groups to total and strength activity may provide information regarding the relationship bone density has with physical activity with respect to frequency and type.

High PA in a young male population has been demonstrated to increase bone mineral density (Biellemann et al, 2013). Studies have investigated adaptations in BMD in response to different frequencies and types of physical activity namely, resistance based (weight training), aerobic (road running) and weight bearing exercise (soccer) (Marques et al, 2011; Whitfield et al, 2015; Falk et al, 2007).

What in unclear, is what amount of PA will instigate change in QUS-SOS in a young male population and whether is determined by type of activity. The current study will categories (low, moderate and high) total and strength activity based upon average weekly exercise. The objective of the study is to determine if frequency of total and strength activity change QUS-SOS significantly, controlling for body mass (kg), height (m), fat free mass (kg), fat mass (kg) and quadricep strength (Nm). This study aimed to identify whether young adult males are more sensitive to the osteogenic stimulus of strength or total PA, measured by QUS-SOS. This study sought to identify key factors that influence bone density in young males using ultrasound which as an accessible cost effective tool. It is hypothesised that there will be a difference in QUS-SOS between physical activity groups. This difference will be significantly affected by covariates.

Methods 3.2

The method for this section is detailed in chapter 2. In summary, participants were put into low, moderate or high physical activity groups based upon the total amount of activity and more specifically strength activity. The questionnaire in question summated total activity in minutes. This allowed the researcher to separate groups based upon the amount of time spent undertaking physical activity. Body mass, height, fat free mass, body fat, quad strength, radius and tibia SOS were measured by cross sectional design.

Results 3.3

Table 13 provides an overview of the data. It shows how participants were grouped for low, moderate and high activity and the means for each group based upon this stratification. The descriptive data includes body mass (kg), height (m), fat free mass (kg), body fat %, quadricep strength (Nm), radius SOS (m.s^{-1}), radius t-score, tibia SOS (m.s^{-1}) and tibia t-score. Total physical activity was used as a grouping variable as well as Strength activity. They form separate models as they contain the same participants. The P denotes the results of an ANOVA determining the difference between groups for each variable. A post hoc test was carried out where appropriate to determine specific differences

Table 13: Descriptive data of measureable variables Mean(SD).

	LOW	MOD	HIGH	P
Total Physical Activity (N)	20	24	22	
Activity per week (mins)	0-120	120-240	>240	
Body mass (kg)	73.47(8.02)	73.65(7.20)	74.80(7.35)	.825
Height (m)	1.80(0.06)	1.79(0.06)	1.81(0.06)	.474
Fat Free Mass (kg)	57.59(4.86)	59.13(5.87)	61.19(5.77)	.126
Body Fat (%)	16.71(4.66)*	15.28(3.56)	13.24(3.00)	**.017**
Quadricep Strength (Nm)	151.55(43.00)*	182.83(52.28)	194.91(55.36)	**.026**
Radius SOS (m.s^{-1})	4014.65(129.32)	3993.00(118.10)	4045.91(122.61)	.367
Radius t-score	-0.69(1.08)	-0.96(0.97)	-0.48(0.99)	.298
Tibia SOS (m.s^{-1})	3961.95(94.10)	3945.58(96.47)	3962.77(106.63)	.812
Tibia t-score	-0.33(0.84)	-0.47(0.83)	-0.31(0.91)	.802
	LOW	MOD	HIGH	P
Strength Activity (N)	20	25	21	
Activity per week (mins)	0-30	30-60	>60	
Body mass (kg)	71.83(7.33)	75.84(8.10)	73.80(6.37)	.213
Height (m)	1.79(0.06)	1.80(0.05)	1.80(0.06)	.830
Fat Free Mass (kg)	58.65(6.29)	60.20(6.20)	58.99(4.24)	.636
Body Fat (%)	14.44(3.00)	16.02(3.94)	14.41(4.66)	.303
Quadricep Strength (Nm)	164.30(54.48)	182.32(48.34)	183.95(56.94)	.438
Radius SOS (m.s^{-1})	4045.25(102.02)	4022.00(146.55)	3984.76(108.85)	.303
Radius t-score	-0.5(0.09)	-0.71(1.21)	-0.94(0.91)	.404
Tibia SOS (m.s^{-1})	3989.95(100.98)	3935.24(105.19)	3949.24(81.51)	.179
Tibia t-score	-0.08(0.84)	-0.56(0.94)	-0.44(0.70)	.163

NOTE: MOD = Moderate; SOS = Speed of Sound; P values in bold are significant (p<.05); * significantly different from HIGH (p<.05).
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Total Physical Activity (Table 13)

Body fat % and quadricep strength were significantly different between groups ($F_{(2)}$ =4.318 p<.05 and $F_{(2)}$ = 3.857 p<.05, respectively) for total physical activity grouping. Post hoc reports the difference lies between low (16.71%, 151.55 Nm) and high (13.24% and 194.91 Nm) groups for both variables (body fat % and quadricep strength, respectively) (p<.05). The mean difference for body fat % was 3.47% and for quadricep strength was 43.36 (Nm). Those that engaged in high total physical activity had greater quadricep strength and lower body fat % than those in low. There is a clear progression in FFM between low (57.59kg), moderate (59.13kg) and high (61.19kg) but no significant difference (p>.05). T-scores state that in all groups participants were below the average for healthy young adult males (t-score< 0). With respect to total physical activity, the moderate group had the lowest t-score (radius -0.96, tibia -0.47). SOS scores for radius and tibia were not significantly different between groups for total physical activity ($F_{(2)}$ = 1.018 p>.05 and $F_{(2)}$ = .209 p>.05, respectively). For Radius SOS low, moderate and high groups had scores of 4014.65, 3993.00 and 4045.91 m.s^{-1}, respectively. For Tibia SOS low, moderate and high groups had scores of 3961.95, 3945.58 and 3962.77 m.s^{-1}, respectively. The moderate group has the lowest SOS for both radial (3993.00 m.s^{-1}) and tibial (3945.58 m.s^{-1}) sites. There is no obvious progression in SOS from low to high activity.

Strength Activity (Table 13)

There are no significant differences between strength groups (p>.05). There are no distinctive increases or decreases between groups for FFM and body fat %. Quadricep strength is marginally higher than low (164.30 Nm) in the moderate (182.32 Nm) and high (183.95 Nm) groups. For Radius SOS low, moderate and high groups had scores of 4045.25, 4022.00 and 3984 m.s^{-1}, respectively. There was no significant difference ($F_{(2)}$ = .918 p>.05). For Tibia

SOS low, moderate and high groups had scores of 3989.95, 3935.24 and 3945.24 m.s^{-1}, respectively. There was no significant difference (F(2) = 1.767 p>.05). Radius SOS gradually declines from low to high, suggesting strength exercise has a negative impact on bone health. The same can be seen for Tibia SOS. Again in all cases the t-scores are negative, suggesting the population observed are below average.

Figure 3 shows the rise and fall in Radius SOS between high, moderate and low groups. SOS appears to peak at high (>240 min PW) and low (<120 min PW) and trough at moderate (120-240 min PW). This implies total physical activity at 120-240 min PW is detrimental to bone health measured by quantitative ultrasound. In order to gain from total physical activity, one must engage in >240 min PW.
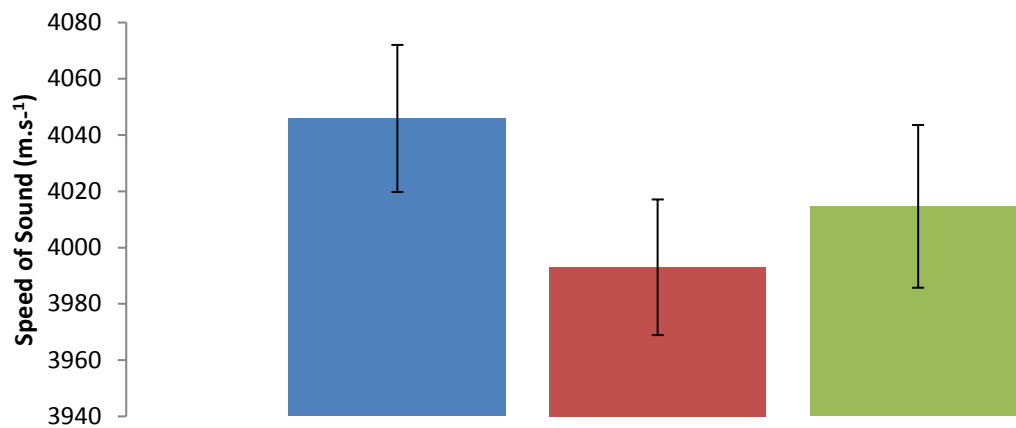
Figure 3: Radial ultrasound between Total Physical Activity Groups.

■ High ■ Moderate ■ Low

Figure 4 shows a similar pattern for Tibia SOS. SOS peaks at high and low and toughs at moderate. The effect of total physical activity is not exclusive to a particular site.

■ High ■ Moderate ■ Low
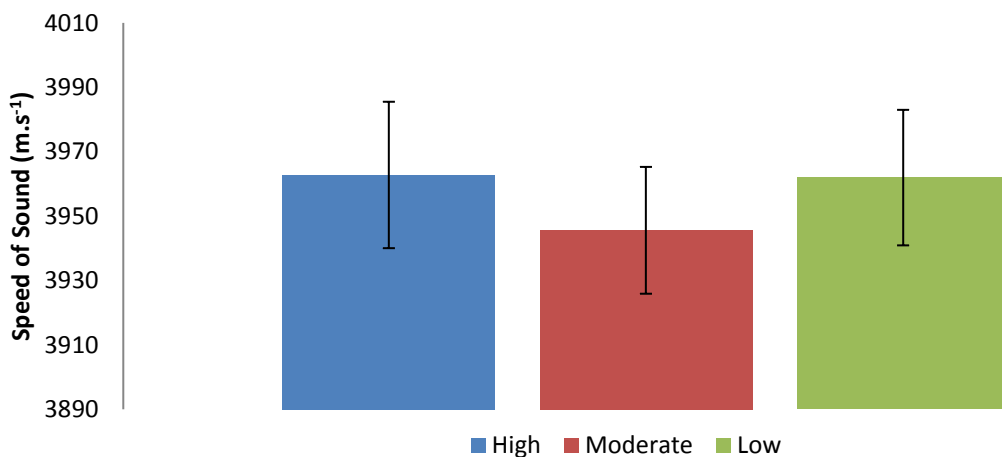
Figure 4: Tibial ultrasound for Total Physical Activity Grouping

<u>Correlation</u>

A correlation matrix (Table 14) was carried out for a number of purposes. The variables used are the dependent variables, Radius SOS, Radius t-score, Tibia SOS and Tibia t-score and potential covariates body mass, height, BF, FFM and QS. EMPA was used in later regression models.

Table 14 – Correlation between covariates and dependent variables

	1	2	3	4	5	6	7	8	9
5. Body Mass	.300*	.333**	-.087	-.082	-				
6. Height	.319**	.248*	.084	.069	.426**	-			
7. BF	-.159	-.065	-.055	-.043	.443**	-.184	-		
8. FFM	.383**	.379**	.006	.014	.797**	.497**	.015	-	
9. QS	-.019	-.027	-.205	-.207	.346**	.007	.039	.400**	-
10. EMPA	.054	.051	-.034	-.032	.106	.117	-.356**	.271*	.300*

Note.   Total sample n=66. * denotes Coefficients are significant ($p<.05$); ** denotes coefficients are significant ($p<.01$). FFM = Fat Free Mass; BF= Body Fat %; QS = Quadricep Strength; EMPA = Estimated Minutes of Physical Activity. Dependant: 1= Radius SOS (m.s^{-1}); 2= Radius t-score; 3= Tibia SOS (m.s^{-1}); 4= Tibia t-score.

The main purpose of table 14 was to determine a relationship between SOS measurements and covariates. T-score are given to provide context for the population but will not be observed in further statistical testing. If the covariates have a significant relationship with SOS measurements, they can be taken forward in statistical testing. Body mass, height and FFM all had a significant relationship with Radius SOS (.300 $p<.05$, .319 $p<.01$ and .383 $p<.01$, respectively). BF, QS and EMPA did not share a significant relationship with Radius SOS (-.159 $p>.05$, -.019 $p>.05$, .054 $p>.05$, respectively). None of the covariates had a significant relationship with Tibia SOS ($p>.05$).

Homogeneity of regression (HR) testing dictates that a relationship must exist between covariate and dependent variable, for them to be used in the same model. It further dictates that there cannot be a strong relationship between covariates (r=.5, $p<.05$). The

variables taken forward, body mass, height and FFM, are related. Both body mass and FFM correlate strongly with height (.426 p<.01 and .497 p<.01, respectively). Body mass and FFM correlate strongly (.797 p<.01). Heights' relationship with body mass and FFM is highly significant and close to .5. Subsequently, it cannot be used as a covariate. Although highly significant, the correlation coefficient between FFM and body mass is not close to .5. Both covariates were taken further in statistical testing. The final HR test is to ensure all groups (low, moderate and high) respond similarly to covariates. This was carried out by observing the interaction between the physical activity grouping variable and covariates for each dependent variable using a univariate test. This included physical activity, body mass and FFM. F(3) = 2.429 p>.05, meaning all groups responded similarly to covariates.

EMPA appears to have no relationship with SOS measurements (P>.05). It appears to have strong relationships with variables that can change with training. These include BF (-.356 p<.01), FFM (.271 p<.05) and QS (.300 p<.05). Of these variables FFM has a significant relationship with Radius SOS (.383 p<.01), only. FFM has a strong significant relationship with QS (.400, p<.01) confirming the link between muscle volume and strength.

Table 15: Results of ANCOVA to determine if a difference in radial ultrasound exists between groups categorised for total and strength activity, adjusted for body mass and fat free mass.

Independent Variable	Covariate	F	P
Total Physical Activity		.671	.515
	Body Mass	.009	.942
	Fat Free Mass	3.702	.059
Strength Activity		1.521	.227
	Body Mass	.034	.855
	Fat Free Mass	3.531	.065

Note: total sample n=66.

<u>Univariate Analysis</u>

The univariate test permits the research to determine if a difference exists between the 3 groups whilst accounting for covariates that may or may not influence the outcome. Specifically the test is called an analysis of covariance (ANCOVA).

As no covariates affect the outcome of Tibia SOS the ANOVA test can be taken (Table 13). Therefore it is not necessary to carry out an ANCOVA for this dependent variable. It is necessary to carry out an ANCOVA for Radius SOS as both body mass and FFM have a significant relationship with it. No significant main effect was found for total physical activity ($F(2) = .671$ $p>.05$). Body mass ($F(1) = .009$ $p>.05$) nor FFM ($F(1) = 3.702$ $p=.59$) significantly affected this difference. This means that total physical activity grouped in this manner holds no bearing to the outcome of Radius SOS, which in unaffected by body mass and FFM.

No significant main effect was found for strength activity ($F(2) = 1.521$ $p>.05$). Body mass ($F(1) = .034$ $p>.05$) nor FFM ($F(1) = 3.531$ $p=.065$) significantly affected this difference. This means that strength activity grouped in this manner holds no bearing to the outcome of Radius SOS, which in unaffected by body mass and FFM.

<u>Multiple Regression Analysis</u>

The current study sought to determine the relative contribution of variables found to have a relationship with SOS measurements. More specifically, establish how strongly the covariates used in univariate tests predict radius SOS. This may help explain differences between physical activity groups.

A multiple linear regression model was calculated to predict radius SOS based upon body mass (BM) and fat free mass (FFM). A significant regression equation was found ($F(2, 63) = 5.424$, $p<.01$) with an adjusted R^2 of .120. As the F value is significant the model has

explanatory power. This means that BM and FFM together can in part explain Radius SOS. Specifically, 12% (adjusted $R^2 = .120$) of the variability of Radius SOS can be explained by BM and FFM.

In this model BM did not significantly predict radius SOS (t = -.085, p>.05). FFM did significantly predict radius SOS (t=2.054, p<.05). Participants' predicted Radius SOS is equal to 3523.39 + 8.652 *(FFM) where FFM is coded as kilograms. Participants' Radius SOS increases 8.652 (m.s^{-1}) for each kilogram of FFM, holding all other independent variables constant. The unique contribution of FFM to radius SOS is 5.7% (.239^2).

It appears when FFM and BM are added to a regression model together, FFM makes BM redundant. FFM is largely responsible for the relationship between BM and Radius SOS (r=.300, p<.05). Findings also infer that many factors that determine SOS measurements are not observed.

<u>Summary</u>

Results suggest that there is no difference in Radius or Tibia SOS between groups engaging in difference frequencies of total physical activity or strength activity. There is evidence that an increase in total physical activity causes significant changes in quadricep strength and body fat % (P<.05). Although not significant, FFM increased progressively between the low, moderate and high total physical activity groups. T-scores in total and strength categorisation suggest the population observed have consistently lower than average bone health.

Figures 4 and 5 could infer moderate total physical activity (120-240 mins PW) is detrimental to bone health. To see gains relating to bone health measured by quantitative ultrasound one must exceed 240 mins per week. However in relation to Tibia SOS low and high groups are almost identical.

Table 14 reports body mass, height and FFM had a significant relationship with Radius SOS (.300 p<.05, .319 p<.01 and .383 p<.01, respectively). BF and QS did share a significant relationship with Radius SOS (p>.05). No variables had a significant relationship with Tibia SOS (p>.05). Homogeneity of regression testing determined that body mass and FFM are used as covariates.

EMPA had no relationship with SOS measurements (p>.05), yet had significant relationships with BF (-.356 p<.01), FFM (.271 p<.05) and QS (.300 p<.05), suggesting training gains. Physical activity appears to have no impact on SOS measurements directly but indirectly. Adjusting for BM and FFM in a univariate test, may help determine what influences the outcome between physical activity groups. There were no main effects (F(2) = .671 p>.05, F(2) = 1.521 p>.05) nor significant effects from covariates (p>.05). Frequency of physical activity appears to hold no bearing to the outcome of SOS measurements. Physical activity appears to be detrimental or even mask the impact of other variables on SOS measurements. Multiple regression analysis explains that 5.7% of the variance in in Radius SOS can be explained by FFM. Also, FFM is largely responsible for the relationship between body mass and radius SOS.

Discussion 3.4

Overview of research question, objective and hypothesis

The current study investigated to what extent physical activity affected quantitative ultrasound measurements of bone (QUS), in a young male population. A sample of 66 multi-ethnic males was split into three groups according to frequency in total and strength activity. These physical activity categories underwent individual statistical testing. The objective of the study was to determine if frequency of total or strength physical activity changed quantitative ultrasound measurements of bone (QUS) significantly, controlling for body mass (kg), height (m), fat free mass (kg), fat mass (kg) and quadricep strength (Nm). It was hypothesised that there would be a significant difference in QUS measurements (Speed of Sound $m.s^{-1}$) between the physical activity groups for both total and strength categories. This difference would be partially determined by one or more covariates.

Overview of method and main findings

The two outcomes measures were Radius and Tibia ultrasound (SOS $m.s^{-1}$). The grouping variables were total and strength physical activity. No covariates affected the outcome of Tibia SOS; therefore an ANOVA test was sufficient for total and strength activity grouping. Covariates affected the outcome of Radius SOS; therefore an ANCOVA was necessary for total and strength activity grouping. The covariates were body mass (BM) and fat free mass (FFM) after significance testing. ANCOVA tests (table 15) report the differences between groups with respect to radius SOS. There was no difference between groups for total physical activity (TPA) ($F(2) = .671$ $p>.05$). This difference was unaffected by body mass ($F(1) = .009$ $p>.05$) or fat free mass ($F(1) = 3.702$ $p>.05$). There was no significant difference between groups for strength activity ($F(2) = 1.521$ $p>.05$). This difference was unaffected by body mass ($F(1) = .034$ $p>.05$) or fat free mass ($F(1) = 3.531$ $p>.05$). Levels of total or strength

activity do not affect radius SOS. ANOVA tests (Table 13) report the differences between groups with respect to tibia SOS. For both total and strength grouping there were no significant differences between the groups ($F_{(2)} = .209$ $p>.05$ and $F_{(2)} = 1.867$ $p>.05$, respectively).

Discussion of descriptive data

Current literature states factors such as body mass, fat free mass, fat mass and muscle strength significantly impact bone health (Huang et al, 2014; Ho-Pham et al 2014; Blain et al, 2001). Therefore, in a cross-sectional design, it is important to observe these variables when investigating differences in bone health. In reference to TPA, body mass, height and fat free mass were not significantly different between groups ($P>.05$). Both body fat % and quadricep strength were significantly different between groups ($P<.05$). In both cases, the low TPA group were significant different from the high TPA group (Table 13). >240 minutes per week of TPA was enough to significantly reduce body fat % and increase quadricep strength above 0-120 minutes per week between groups. Blain et al (2001) found strong associations between femoral neck and lumbar spine BMD and quadricep strength, arguing the site specific effects of muscle strength on bone tissue. This may imply that the high TPA group should have higher tibia SOS due to significantly stronger site specific muscle strength than the low TPA group. Taafe et al (2001) agree strength elevates bone mass but in accordance with elevated lean tissue mass.

There is no evidence in the current study that fat free mass changed in response to physical activity, despite table 14 reporting quadricep strength and fat free mass correlate strongly ($r=.400$, $p<.01$). Physical activity grouping appears to hinder the relationship between strength, fat free mass and bone properties. Petterson et al (1999) concluded that high physical activity in fact weakens the relationship between BMD and muscle strength. In

a meta-analysis Ho-Pham et al (2014) conclude that body fat correlates with total BMD (r=.28). This may suggest the low TPA should have improved SOS measurements as they have significantly higher body fat % than the high TPA group, in the current study.

Figures 4 and 5 could demonstrate that gains seen in body fat % and quadricep strength (Nm) for the low and high TPA groups respectively, are similar giving both groups marginally higher radial and tibial SOS than the moderate group. Body mass does not significantly differ between groups (table 13). Maintaining body mass is important to see improvements in bone mass (Armamento-Villareal et al, 2014) but fat free mass does not significantly differ which is a factor significantly linked with improvements in bone mass in men ($p<.001$) (Taafe et al, 2001). This may ultimately be why without adjusting for confounding factors, radial and tibial SOS is not significantly different between TPA groups. TPA in the current study was perhaps not sufficient to elevate fat free mass to a necessary degree.

Participants grouped for strength activity (SA) had no significant differences for any of the variables ($p>.05$). Interestingly, for both radius and tibia SOS the low SA group had higher SOS values than the high SA group, perhaps implying that strength training reported by the current population is detrimental to bone health although not significantly ($p>.05$). Although not significant, table 14 reports a correlation coefficient of -.205 between quadricep strength and tibia SOS. Furthermore, quadricep strength was marginally higher in high SA group than Low SA group (+19.65 Nm), but not significantly ($p>.05$). Seabra et al (2012) report knee extensor strength gains are significantly associated with elevated BMD and 4 central and peripheral sites; contradictory to findings in the present study. More specifically, in response to a year's strength training, Nelson et al (1994) saw significant increases in BMD at the femoral neck as well as muscle mass and muscle strength. Again, in the current

study for SA no differences in strength or fat free mass were seen (p>.05), reiterating that physical activity has to change these variables to change bone properties.

For TPA the radius SOS measurements were 4014.65, 3993.00 and 4045.91 (m.s^{-1}) for low, moderate and high groups, respectively (Table 13). Zhu et al (2008) reports of 2927 Chinese adult males ages 18-55 the average radius SOS was 4075 m.s^{-1}. Using an Omnisense device similar to the present study Njeh et al (2001) report of 334 adult women (48.8±17.4) the average radius and tibia SOS was 4087 and 3893m.s^{-1}. Normative data for men was difficult for this researcher to obtain, but Kendler et al (1999) and Hans et al (2001) state peak radius SOS in young adult women (20-29) can reach 4167 and 4108 m.s^{-1}, respectively.

It appears in the present study the male population have a lower than average radial SOS in all groups. T-scores, derived from reference data supplied by Beamed®, concur for low, moderate and high groups radius SOS is below average (-0.69, -0.96, -0.48, respectively) (Table 13). Zhu et al (2008) report average tibia SOS to be 3990 m.s^{-1} in adult males. SOS values in the current study are closer to this value, justifying the higher t-scores for low, moderate and high groups (-0.33, -0.47, -0.31, respectively). Although all groups are below average the moderate group, based upon physical activity, are more at risk of low bone mass. This may infer where total physical activity is concerned, much more than 240 minutes per week (high TPA group) is necessary to see changes in SOS measurements. Whitfield et al (2014) state exceeding physical activity guidelines 4 fold (450-900 minutes) may beneficial for proximal femur BMD. Less emphasis on frequency and more on modality to elevate strength (Seabra et al, 2014), FFM and FM (Ho-Pham et al, 2014) and maintain body mass (Armamento-Villareal et al, 2014) may be a better approach to physical activity.

Discussion of correlations

Correlations were observed to determine what variables have a relationship with SOS measurements. If the relationships are significant, they could impact the difference in SOS between physical activity groups. As well as univariate tests, correlations were necessary to complete homogeneity of regression (HR) test, prior to an ANCOVA (see methods section). After HR testing, body mass (r=.300 p<.05) and fat free mass (r=.383 p<.01) were used as covariates.

Of the potential covariates for an ANCOVA, radius SOS had a significant relationship body mass, height (r=.319 p<.01) and fat free mass. Tibia SOS did not have a significant relationship with any of the covariates (p>.05) (Table 14). Huang et al (2015) report body mass correlates with radius SOS in a large cohort of males (n=28702) (r=.410 p<.001). Furthermore, combining body mass and height, body mass index (BMI) correlated with radius SOS in males (r = 0.403 p<.05) (Krieg et al, 1998). Falk et al (2007) reported that muscle volume correlated with radius SOS (r=.370 p<.01). Findings in the literature seem to agree with findings of the current study. Ho-pham et al (2014) suggest there should a significant relationship between body fat and bone health parameters (r=.28 p<.001). This was not the case for radius and tibia SOS in the current study (-.159 p>.05 and -.055 p>.05, respectively). There are discrepancies in bone health and body fat assessment tools between the studies, perhaps citing the differences. Overall, participants in the current study seem to be typical of the wider population. There is evidence therefore, with respect to radius SOS, body mass, height and fat free mass should be used as covariates.

The current study used total physical activity to group participants. Participants were stratified into low, moderate and high groups, equally. A continuous variable was established also, to look at the relationship physical activity had with SOS measurements. Table 14 reports that estimated minutes of physical activity (EMPA) has no significant relationship

with SOS measurements (r = -.034-.054 p>.05). It becomes clearer that more physical activity (frequency) does not equate improved bone properties, in young adult men. However, EMPA has a significant relationship with body fat %, fat free mass and quadricep strength (-.356 p<.01, .271 p<.05 and .300 p<.05, respectively). Total physical activity significantly reduces body fat and increases fat free mass and quadricep strength. The strongest correlation is with body fat %; a negative relationship perhaps reinforcing that physical activity is not the best intervention for low bone mass as, in a meta-analysis, body fat positively correlated with total BMD (r=.28) (Ho-Pham et al, 2014).

Conversely, EMPA correlated positively with fat free mass and quadricep strength. With no direct relationship between EMPA and SOS measurements and studies supporting links between quadricep strength, fat free mass and bone properties (Taafe et al, 2001), it could be inferred that elevating physical activity level is a method of indirectly improving bone properties. Pettersson et al (1999) concluded that a high amount of physical activity provides the 'platform' for physiological adaptation, but high PA weakens the relationship between quad strength and BMD. It seems PA needs to target specific physiological adaptations such as muscle hypertrophy rather than overall exercise.

Discussion of ANCOVA

The statistical test in this study allowed the researcher to investigate whether physical activity affects the outcome variables when controlling for covariates. The advantage of this is one can see independent effects of covariates and physical activity, without one influencing the other.

Current literature suggests there should be difference in bone density between high, moderate and low PA groups (Biellemann et al, 2013; Babaroutsi et al, 2005) in accordance with significant differences in FFM and BM. The current study reported no significant main

effects from TPA or SA ($F_{(2)} = .671$ p>.05 or $F_{(2)} = 1.521$ p>.05, respectively) but adjusted for body mass and fat free mass. Neither body mass nor fat free mass significantly adjusted the outcome between TPA or SA groups (p>.05).

Although not significantly affecting the outcome variables in the present study, improvements in lean mass as a result of elevated physical activity may be largely responsible for the link between elevated physical activity and elevated bone properties. As such, adjusting the value of the outcome variables may be counterproductive to determine the effect of physical activity on SOS. This may be why, Biellemann et al (2013) and Babaroutsi et al (2005) found statistically significant results. Table 13 shows the results of ANOVA testing between groups. Radius SOS was $F_{(2)} = 1.018$ (p=.367). Table 15 shows the results of ANCOVA testing between groups for Radius SOS, only. Difference between groups was not significant ($F_{(2)} = .671$, p=.515). Without adjusting for covariates, the p value is closer to rejecting the null hypothesis (Table 13). FFM is close to significantly affected the outcome in the ANCOVA ($F_{(2)} = 3.702$ p=.059). Taking away the effects of fat free mass reduced the significance suggesting it is partially responsible for links between higher physical activity and bone properties. Effect sizes would help explain to what extent fat free mass influences the outcome of radius SOS between physical activity groups. However, fat free mass did not significantly impact the difference between physical activity groups. Therefore, the effect size is unreliable. Conversely, a multiple regression analysis would help explain to what extent body mass and fat free mass, account for variation in radius SOS.

Multiple Regression Analysis

A multiple regression analysis was used to determine how much in terms of %, body mass and fat free mass affect radius SOS. This may help explain differences between physical activity groups. Radius SOS was used as the dependent and body mass and fat free mass as

the factors. A significant regression equation was found ($F_{(2, 63)} = 5.424$ p<.01) with an adjusted r^2 of .120. This says 12% of the variance in radius SOS can be, in part explained by body mass and fat free mass collectively, holding all other factors. However, with fat free mass (t=2.054 p<.05) in the regression model, body mass is made redundant (t= -.085, p>.05). Blain et al (2001) draw a similar conclusion, as cited in the literature review.

This has connotations of the Mechanostat theory (Frost et al, 2000). In this case, more weight requires more structural support from muscle and this in turn, exerts more force on respective bone tissue, encouraging osteogenesis. Although body mass has links bone density, as reported by the current study this relationship might be attributed to fat free mass. Radius SOS was equal to 3523.39 + 8.652*FFM. For every kilogram of fat free mass, radius SOS increased $8.652 m.s^{-1}$. The unique contribution of fat free mass to radius SOS was 5.7%. Hind et al (2012) conclude, using this method, lean and total mass explain the majority of the variance in BMD. This was not the case in the present study. 88% of the variation in in radius SOS is not accounted for in this multi-ethnic sample. Although SOS measurements seem largely unaffected by total physical activity, there appear to be many other factors that contribute to SOS measurements not observed in this study. Predisposed factors such as ethnicity may be one (Travison et al, 2008; Nam et al, 2010).

Limitations

The use of a cross sectional design, despite being convenient, cost effective and used in bone health studies (Gouveia et al, 2014), could be draw back in the current study. The design provides a snapshot of a population that may not be the same after testing. This means it is difficult to establish causality, due to the numerous extraneous variables. This has been successfully overcome by monitoring participants overtime, constituting a longitudinal study (Biellemann et al, 2014), but ensuring participants return for multiple days of testing is

difficult also. Due to adaptations in bone tissue being chronic, a longitudinal observational study that controlled for key variants would be the ideal design. However, under this method QUS-SOS may not be a suitable tool. Wang et al (2008) state that for monitoring changes in bone densitometry the axial transmission method-derived SOS is not comparable to DEXA and peripheral quantitative computed tomography (pQCT).

The Stanford patient research questionnaire has been commonly used in the community for collecting data on lifestyle factors that affect chronic illness and their recovery (Osborne et al, 2007; Ritter et al, 2014). As such, there are drawbacks for this questionnaire in this study. It is specific to frail individuals that have reduced mobility, therefore perhaps ill-equipped to measure the physical activity levels of active young adult males. It could also incur a ceiling effect. The highest amount of exercise an individual could report was >3 hours. This was established as 180 minutes, whereas in reality this value could have been much higher. This may have established a 'high' physical activity group with much more variation in physical activity than the moderate or low group. Stratification of physical activity in this way could misrepresent what constitutes low, moderate and high physical activity, thus reducing its comparability with existing studies. However, this method is used for time spent at high impact activities (Ginty et al, 2005; Petterson et al, 1999). In a cross-sectional design, physical activity levels will be retrospective, therefore could be subject to bias. However a well cited alternative physical activity measurement might be the International Physical Activity Questionnaire (IPAQ) (Booth et al, 2003).

Conclusion

A greater specification of physical activity focusing on muscle growth is needed and a multi-stage design where the monitoring of physical activity over time can take place and the osteological response observed. Less emphasis should be place upon frequency and more on

modality to encourage physiological adaptations. The objective of the current study was to determine if frequency of physical activity changes QUS measurements of bone. Frequency physical activity did not significantly change QUS measurements of bone. The null hypothesis is accepted that there is no significant difference in QUS measurements between physical activity groups for both total and strength categories. This difference was not determined by covariates.

Further Research

88% of the variation in in radius SOS is not accounted for in this multi-ethnic sample. There are factors that affect bone health determined by QUS not observed by this study. Ethnicity is an independent risk factor for bone density (Nam et al, 2010) that interacts with different risk factors such as exercise (Liang et al, 2007) similar to age and gender (Bachrach et al, 1999). This means ethnic groups have a varied osteological response to exercise. Identifying these groups and comparing them could prove advantageous when addressing issues with bone health. In turn, it may help to determine whether exercise interventions are suitable or not. Chapter 4 will investigate the impact both exercise and ethnicity have on QUS measurements of bone.

Introduction 4.1

Exercise in particular has been associated with BMD in all ages (Gouveia et al, 2014) and genders (Burger et al, 1994) as well as ethnicity (Ross et al, 1996). Liang et al (2007) state that 16% of the variance of BMD in an ethnically diverse population cannot be explained by common risk factors, suggesting ethnicity is an inherent predictor of bone density. This variance may exist in response to level of exercise. The current study defines two groups by ethnicity and athletic standard. The Malaysian group is exclusively ethnic Malay and the British group White.

Linear relationships exist between bone health parameters and independent factors such as lean mass (kg) (r=.42), fat mass (kg) (r=.28) and body mass (kg) (r=.41) (Ho-Pham et al, 2014; Huang et al, 2015) in Asian and European populations. As well as local muscle strength (Nm) (Pettersson et al, 1999) these factors are often controlled for in intervention (Marques et al, 2011) longitudinal (Biellemann et al, 2013) and cross-sectional (pettersson et al, 1999) exercise studies as they significantly and indiscriminately impact bone health. To adjust QUS-SOS outcomes accordingly, controlling for these confounding variables proves important to determine any main effects for ethnicity or athletic standard.

Athletic standard has been reported to significantly elevate regional and global BMD above untrained and sedentary counterparts (Lariviere et al 2003; Sutton et al 2009; Jackman et al 2013) and inhibit age related declines in footballers (Uzunca et al 2003). Adaptations in BMD as a result of professional level have been reported in footballers and water polo players independently of BMI and age (Wittich et al, 1998; Ebrahimi et al 2013) but not LM (Guadalupe-Grau et al, 2001). Research discriminating between those of varying ethnicity

and their response to athletic standard is limited. Comparable improvements have been reported as result of high athletic standard among those with similar ethnicities. Gerosa-Neto et al (2014) report Brazilian elite soccer players have 0.12g/cm3 greater volumetric BMD than controls (p<.001), agreeing with Guadalupe-Grau et al (2001) who reported 0.06g/cm3 greater BMD among professional Spanish footballer than untrained controls (p<.001).

Ross et al (1996) report significantly reduced arm (-2.20%; $P < 0.05$) and leg (-1.65%; $P < 0.05$) BMD in Asians than Caucasians, adjusted for quadricep strength, lean body mass and fat mass. Liang et al (2007) suggest these adjusted variables and their effect on BMD vary as a function of ethnicity, meaning underlying traits relating to ethnicity can dictate the rate of change in bone density. This may explain why a greater BMD in Caucasians males than Asian males was attenuated or even reversed when adjusting for body weight (Nam et al, 2010) and a difference still found after adjusting for common risk factors (Liang et al, 2007). These studies suggest ethnicity in part explains variance in bone density.

Studies that investigate differences in bone density through either quantitative ultrasound-speed of sound (m.s-1) (QUS-SOS) or duel energy x-ray absorptiometry- bone mineral density (g.cm^{-2}) (DEXA-BMD) between athletes and controls are numerous, homogeneous of ethnicity (Guadalupe-Grau et al, 2001; Gerosa-Neto et al, 2014; Jackman et al, 2013). What is unclear is to what extent different ethnicities, respond to exercise (defined in this case by athletic standard) in reference to QUS-SOS, comparatively. The objective of the current study primarily is to determine any main effects for athletic standard and ethnicity containing different ethnic groups. Secondly, the study aims to determine if an interaction exists between ethnic groups, and athletic standard. The study will identify ethnicities that are more sensitive to the osteogenic stimulus of exercise, between athletes and controls. This has the potential to identify those more at risk of stress related fractures and flag those more

responsive to exercise in cases where exercise prescription is a possibility to overcome low bone mass.

The literature in this area focuses on high risk osteoporosis populations such as the postmenopausal women. Using a healthy young male population the current study hopes to observe ethnic differences in QUS-SOS. Athletic standard has been shown to elevate BMD above untrained sedentary levels between sports (Wittich et al, 1998; Malandish et al, 2013) and within similar ethnicities (Gerosa-Neto et al, 2014; Guadalupe-Grau et al, 2001). To the knowledge of this researcher there is limited research that observes the response of different ethnic groups to Athletic standard. Using a cross-sectional design the current study will observe differences in QUS-SOS between white British and Malaysian (ethnic Malay) athletes and controls. It is hypothesised that there will be significant main effects from ethnicity and Athletic standard as well as an interaction between them. A significant difference between groups will be partially determined by covariates.

Results 4.2

Table 16: Descriptive data of dependent variables and covariates Mean (SD).

	BRIT (n=48)		MALAY (n=66)		
	CON	ATH	CON	ATH	
	(n=22)	(n=26)	(n=33)	(n=33)	P
Body mass (kg)	73.44	74.51	64.18	64.75	
	(7.98)	(7.01)	(13.79)^{AB}	(9.69)^{AB}	.000
Height (m)	1.79	1.81	1.68	1.68	
	(0.06)	(0.06)	(0.06)^{AB}	(0.05)^{AB}	.000
Fat Free Mass (kg)	57.51	61.18	48.56	57.68	
	(5.27)	(5.57)	(6.95)^{AB}	(6.98)^{C}	.000
Body Fat (%)	16.94	13.12	21.93	10.43	
	(4.20)	(2.70)	(7.49)^{AB}	(4.15)^{BC}	.000
QS (Nm)	160.73	194.03	157.56	176.05	
	(45.38)	(56.29)	(33.06)^{A}	(35.47)	.004
Radius SOS (m.s^{-1})	3993.21	4041.18	4071.84	4019.20	
	(125.26)	(120.17)	(180.71)	(134.54)	.152
Radius t-score	-0.91	-0.53	-0.37	-1.00	
	(1.06)	(0.98)	(1.68)	(1.12)	.135
Tibia SOS (m.s^{-1})	3948.21	3964.33	3896.97	3873.97	
	(102.31)	(96.16)	(125.72)	(117.21)^{A}	.004
Tibia t-score	-0.45	-0.30	-0.63	-0.99	
	(0.90)	(0.82)	(1.43)	(1.25)	.091

Total sample n= 114. Post hoc Bonferroni α =0.0125; Significant ANOVA in bold (p<.05); BRIT = British, MALAY = Malaysian, CON = Control, ATH = Athlete; QS=Quadricep Strength; A significantly different from BRIT-ATH (p<.0125); B significantly different from BRIT-CON (p<.0125); C significantly different from MALAYCON (p<.0125).

Descriptive data

Table 16 provides an overview of the data. White British and Malay Malaysian participants were split into control and athlete groups. The means and standard deviations for each covariate and dependent variable are reported. The descriptive data includes body mass (kg), height (m), fat free mass (kg), body fat %, quadricep strength (Nm), radius SOS (m.s-1),

radius t-score, tibia SOS (m.s-1) and tibia t-score. The P denotes the results of an ANOVA determining the difference between groups for each variable. Subsequent post hoc tests followed, to determine which groups were significantly different with an adjusted α at .0125. Table 16 shows that body mass (F(3) =9.803 p<.000), height (F(3)=46.812 p<.000), fat free mass (F(3) 24.268 p<.000), quadricep strength (F(3)=4.714 p<.004) and Tibia SOS (F(2)=4.684 p<.005) have a significant overall difference (p<.005). However, homogeneity of variance is only assured for height, fat free mass, quadricep strength and tibia SOS (p>.05). No significant differences between groups were found for radius SOS, radius t-score or tibia t-score (P>.05).

Body mass (kg), height (m), fat free mass (kg) and body fat (%) were significantly different between Malaysian Controls (MC) (64.18kg, 1.68m, 48.56kg and 21.93%, respectively) and British Controls (BC) (73.44kg, 1.79m, 57.51kg and 16.94%, respectively) and British Athletes (BA) (74.51kg, 1.81m, 61.18kg and 13.12%, respectively) (p<.0125). Quadricep strength was significantly different between MCs (157.56Nm) and BAs (194.03Nm) (p<.0125). Body mass and height were significantly different between MAs (64.75kg and 1.68m) and BCs (73.44kg and 1.79m) and BA (74.51kg and 1.81m). MAs (10.43%) were significantly different from BCs (16.94%) and MCs (21.93%) for body fat %. MAs (57.68kg) were also different from MCs (48.56kg) for fat free mass. Tibia SOS was significantly different between MAs and BAs (3873.97 and 3964.33, respectively). British controls and athletes appear to be heavier and taller than both their Malaysian counterparts as well as have different amounts of fat free mass and body fat than Malaysian controls. Malaysian groups appear to have a larger range of body fat and the British athlete group have the largest quadricep strength 194.03Nm, which is significantly greater than Malaysian controls. T-scores indicate that that all groups for radius and tibia SOS are below average (-.30 to -1.00). The lowest for both radius (-1.00) and tibia SOS (-0.99) were Malaysian

Athletes. The highest for radius (-0.37) and tibia SOS (-0.30) were Malaysian Controls and British Athletes, respectively. Observing the raw data, for both radius and tibia SOS, The British group see improvements in SOS measurements as athletes but the Malaysian group see a decline.

Table 17: Correlation between measureable variables

	1	2	3	4	5	6	7	8
5. Body Mass	.301*	.136	-.059	-.070	-			
6. Height	.046	.080	.212*	.086	.533**	-		
7. BF	.255*	.109	-.116	.004	.311*	-.083	-	
8. FFM	.114	.097	-.018	-.096	-.061	.514**	-.061	-
9. QS	.015	.032	-.217*	-.116	.226**	.112	-.053	.323**

Note.   Total sample n=114. * denotes Coefficients are significant ($p<.05$); ** denotes coefficients are significant ($p<.01$). FFM = Fat Free Mass; BF= Body Fat %; QS = Quadricep Strength; Dependant: 1= Radius SOS ($m.s^{-1}$); 2= Radius t-score; 3= Tibia SOS ($m.s^{-1}$); 4= Tibia t-score.

Correlations

Radius SOS, Radius t-score, Tibia SOS, Tibia t-score, body mass, height, BF, FFM and QS are the variables used for correlation testing. As stated in chapter 3 the main purpose of correlation testing is to determine if SOS measurements have a relationship covariates. This forms part of homogeneity of regression testing (methods section). If they did affect the outcome of SOS measurements, they were controlled for in univariate tests.

Body mass and body fat had a significant relationship with Radius SOS (.304 $p<.05$ and .255 $p<.05$, respectively). Height and quadricep strength had a significant relationship with Tibia SOS (.212 $p<.05$ and -.217 $p<.05$, respectively). None of the other variables affected the SOS measurements significantly ($p>.05$).

Homogeneity of regression (HR) testing says that a relationship must exist between covariate and dependent variable, for them to be used in the same model. This has been

shown in table 17. Table 17 also reports that different covariates affect different SOS measurements. This dictates that separate statistical tests are needed for radius and tibia SOS. HR testing further dictates that there cannot be a strong relationship between covariates (r=.5, p<.05). Body mass correlates with height and body fat (.533 p<.01 and .311 p<.05) and with quadricep strength (.226 p<.01). FFM correlates with height (.514 p<.01) and FFM correlates quadricep strength (.323 p<.01). Where radius SOS is concerned, body mass and body fat are important. The relationship is not strong (r=.311 p<.05) so both can be used in univariate testing. Regarding tibia SOS, quadricep strength and height are important. These variables do not correlate (r=.112 p>.05) so both can be used in univariate testing.

Table 18: Univariate testing - Test Between-Subject Effects for Radius and Tibia SOS

Radius SOS				
Independent variable	Covariate	Effect Size	F	P
Athletic		.026	3.365	.069
Ethnicity		.063	8.438	.004
Interaction		.062	8.313	.005
	Body Mass (kg)	.072	9.835	.002
	Body Fat %	.037	4.888	.029
Tibia SOS				
Independent variable	Covariate	Effect Size	F	P
Athletic		.001	.160	.690
Ethnicity		.058	7.801	.006
Interaction		.011	1.386	.241
	Height (m)	.000	.025	.875
	Quadricep Strength (Nm)	.030	3.834	.052

Note: sample = 114; Athletic = Grouping of controls and athletes.

Univariate analysis

The univariate test permits the research to determine if a difference in SOS measurements exists between the groups for ethnicity and athletic independent variables. It also allows the researcher to determine if an interaction exists between the independent variables. Lastly, variance created by other factors can be controlled for as covariates. The outcome variable can be adjusted accordingly. Specifically the test is called an analysis of covariance (ANCOVA).

For radius SOS there were significant main effects for ethnicity (F(1)=8.438 p=.004) but not Athletic (F(1)=3.365 p=.069) (table 18). This means that ethnicity, independently affects radius SOS but athletic standard does not. There was a significant interaction between athletic and ethnicity grouping (F(1)=8.313 p=.005). This means that ethnic groups respond differently to athletic standard where radius SOS is concerned. These outcomes were significantly adjusted by body mass and body fat (F(1)=9.835 p=.002 and F(1)=4.888 p=.029, respectively). This means that body mass and body fat are partially responsible for the significant differences between ethnic groups, and the interaction between the independent variables. The effect size for ethnicity was .063, indicating that 6.3% of the variance in radius SOS can be attributed to this factor. The interaction effect size is similar (.062). The effect size for body mass and body fat was 7.2 (.072) and 3.7 % (.037), respectively.

For tibia SOS there were significant main effects for ethnicity (F(1)=7.801 p=.006). No significant main effect for athletic (F(1)= .160 p=.690) or an interaction (F(1)=1.386 p=241) existed (table 18). The covariates did not significantly affect the difference between the group (p= .875 and p=.052, respectively). The effect size for ethnicity (.058) indicated that 5.8% of the variance in tibia SOS can be explained by ethnic group.

Table 19: Radius SOS and adjusted Radius SOS for each independent group including athletic, ethnicity and the interaction between them.

	N	Mean (STD)	Adjusted Mean (STE)*
Athletic			
CON	55	4032.53 (161.60)	4066.549 (22.675)
ATH	59	4030.20 (126.18)	3996.178 (22.675)
Ethnicity			
BRIT	48	4017.20 (126.02)	3984.745 (20.094)
MALAY	66	4045.53 (160.47)	4077.982 (20.094)[A]
Interaction			
CON-BRIT	22	3993.21 (127.21)	3979.661 (24.737)
CON-MALAY	33	4071.84 (183.51)	4153.437 (39.562)
ATH-BRIT	26	4041.16 (122.03)	3989.829 (30.097)
ATH-MALAY	33	4019.21 (131.15)	4002.527 (28.004)

Note: sample n-114. * denotes Covariates fixed (body mass = 69.2205 kg; body fat = 15.6038 %). STD = Standard deviation; STE = Standard Error. CON = Control; ATH = Athletic; BRIT = British; MALAY = Malaysian. A denotes significantly different from BRIT (p<.01).

The variables reported in table 19 are mean radius SOS and adjusted mean radius SOS. Body mass and body fat create variation in radius SOS (Table 18). Radius SOS is adjusted to account for this variation by fixing body mass (69.2205 kg) and body fat (15.6038 %) for all participants. This provides a hypothetical situation where all the participants have the same body mass and body fat, thus variation caused by it is removed.

As there were no significant main effects for Athletic grouping (p<.05) (Table 18) there is no significant difference between controls (4066.549) and athletes (3996.178) for the adjusted mean. T-tests show there is no significant difference between controls (4032.53) and athletes (4030.20) (F(130)= 2.661 p>.05) for the non-adjusted mean. There was a significant main effect for ethnicity grouping (p<.05). This means, as there are only two groups, there is a significant difference between British (3984.75) and Malaysian (4077.98) for adjusted mean. T-tests show there is no significant difference between British (4017.20) and Malaysian (4045.53) (F(130)=2.040 p>.05) for non-adjusted mean.

Participants split into their respective four groups showed no significant difference as the ANOVA showed in table 16. The interaction between athletic and ethnicity was significant (P<.05) (Table 18). Observing the adjusted values, this means that difference between British controls and British athletes (+10.17m.s^{-1}) is significantly different from the difference between Malaysian controls and Malaysian athletes (-150.91m.s-^{1}).

Table 20: Mean height, quadricep strength and tibia SOS of controls, athletes, British and Malaysian groups.

	N	Height (m) (STD)	QS (Nm) (STD)	Mean (STD)
Athletic				
CON	55	1.73 (0.08)	159.14 (39.73)	3922.59 (117.44)
ATH	59	1.74 (0.05)	185.04 (35.47)[B]	3919.15 (117.21)
Ethnicity				
BRIT	48	1.80 (0.06)	177.38 (53.77)	3956.27 (100.37)
MALAY	66	1.68 (0.05)[A]	166.80 (35.78)	3885.47 (123.02)[A]

Note: sample n-114. STD = Standard deviation; QS = Quadricep Strength (Nm). CON = Control; ATH = Athletic; BRIT = British; MALAY = Malaysian. A denotes significantly different from BRIT (p<.000); B denotes significantly different from CON (p<.05).

Table 20 shows the means for each independent group for height, quadricep strength and tibia SOS. They are included in table 20 to help explain the results, although the main focus is British and Malaysian mean tibia SOS. There were no significant differences in tibia SOS between the control (3922.59) and athletic group (3919.15) ($F_{(130)}$=.209 p>.05). There were also no significant differences height ($F_{(130)}$= .364 p>.05) but significant differences for quadricep strength ($F_{(130)}$=1.089 p<.001). Athletes had a mean quadricep strength of 185.04Nm and controls 159.14Nm.

There were significant differences in Tibia SOS between British (3956.27) and Malaysian (3885.47) groups ($F_{(1)}$=7.801 p=.006). Effect size indicates that ethnicity can explain 5.8% of the variance in tibia SOS (Table 18). There were significant differences in height ($F_{(130)}$=.966 p<.000) but no significant differences in quadricep strength ($F_{(130)}$=10.256 p>.05) (Table 20).

Summary

There was no difference between the four independent groups (BC, BA, MC and MA) for tibia or radius SOS (p>.05). BCs and BAs were significantly heavier and taller than both their Malaysian counterparts. Athletic standard appears to have a much larger impact on fat free mass and body fat % in Malaysians than British participants. All groups had negative t-scores, indicating they have below average SOS values for their age. Observing the raw data for radius and tibia SOS, the British group see improvements in SOS measurements as athletes but the Malaysian group see a decline. This indicates an interaction of some kind.

Different potential covariates had a relationship with radius and tibia SOS. Radius SOS shared a relationship with body mass and body fat and tibia SOS shared a relationship with height and quadricep strength. The relationship with quadricep strength was negative. Radius and tibia SOS respond differently to their environment.

For radius SOS, there was a significant main effect for ethnicity (p<.05) adjusted for covariates. There was also a significant interaction between athletic and ethnicity (p<.05) for radius SOS. This differences between groups was significantly influenced by body mass and body fat (p<.05). The interaction explains 6.2% of the variation in radius SOS. For radius SOS, there was a significant main effect for ethnicity (p<.05) but not adjusted by covariates. No main effect for athletic or a significant interaction, was found (p>.05).

Adjusting for covariates made groups significantly different (p<.05). Before this they were not (p>.05) for radius SOS. With body mass (69.2205 kg) and body fat (15.6038 %) fixed for all participants and adjusted for ethnicity and athletic standard, Malaysian controls (4153.437) have the highest SOS value and British controls (3979.661) the lowest. Athletic standard strongly, negatively and significantly impacts Malaysians. The difference between British controls and British athletes ($+10.17 \text{m.s}^{-1}$) is significantly different from the difference between Malaysian controls and Malaysian athletes (-150.91m.s^{-1}).

British had significantly greater non-adjusted tibia SOS than Malaysians. No covariates affected this outcome. This is the opposite of adjusted radius SOS values. As well as a difference in the way radius and tibia respond to environment, there is a difference in the way ethnic groups respond to environment.

Discussion 4.3

Overview of research question, objective and hypothesis

The current study analysed quantitative ultrasound measurement of bone in British and Malaysian groups. British (n=48) and Malaysian (n=66) participants were further grouped for athletic standard, creating two independent variables. Athletic standard was a method used to determine level of exercise. The objective of the study was to determine if a significant interaction exists between athletic standard and ethnicity, controlling for body mass (kg), height (m), fat free mass (kg), fat mass (kg) and quadricep strength (Nm). This will determine if ethnic groups respond differently to exercise. The second objective was to determine if ethnicity or athletic standard affects quantitative ultrasound measurements of bone independently, controlling for body mass (kg), height (m), fat free mass (kg), fat mass (kg) and quadricep strength (Nm).

It was hypothesised there would be an interaction and significant independent effects from ethnicity and athletic standard. The differences between groups would be significantly affected by covariates body mass (kg), height (m), fat free mass (kg), fat mass (kg) and quadricep strength (Nm).

Overview of main findings

The two outcomes measures were Radius and Tibia ultrasound (SOS m.s^{-1}). Covariates affected both radius and tibia SOS; therefore an ANCOVA was necessary for both. Different covariates had relationships with radius and tibia SOS, therefore separate models for each outcome variable was necessary. Table 18 reports the results of said ANCOVA. For radius SOS, there was a significant interaction between ethnicity and athletic standard ($F(1)=8.313$ $p=.005$). There was a significant main effect from ethnicity ($F(1)=8.438$ $p.004$). Body mass

and body fat significantly affected these differences (F(1)=9.835 p=.002 and F(1)=4.888 p=.029, respectively). When body mass (69.22kg) and body fat (15.06%) are fixed, Malaysians respond differently to athletic standard than British. There was a significant main effect for ethnicity for tibia SOS (F(1)=7.801 p=.006). There was no interaction and no significant impact from covariates (P>.05).

Athletic standard is marginally beneficial (+10.17m.s^{-1}), and greatly detrimental (-150.91m.s^{-1}) to radius SOS for British and Malaysian participants, respectively. The differences between non-adjusted and adjusted mean demonstrates how strongly body mass and body fat effects the differences between these two ethnic groups, where radius SOS is concerned. Effect sizes for ethnicity were 6.3 and 5.8% for radius and tibia SOS, respectively. It is an important factor to consider when addressing bone health.

Descriptive data and Correlations

The aim of the study is to determine the effect of ethnicity, athletic standard and an interaction on SOS measurements of bone, controlling for other factors that affect bone health. Observing group differences in these factors may indicate what to expect in univariate analysis and help understand the sample better.

Both British controls (BC) and British Athletes (BA) had significantly greater body mass and height than their Malaysian control (MC) and athlete (MA) counterparts (Table 16). Huang et al (2014) state radius SOS correlates with body mass (r=.304 p<.05) in a multi-ethnic sample similar to the relationship reported in the current chapter (r=.301 p<.05) (Table 17) and chapter 3 (r=300, p<.05). Samples for the three studies include Chinese males, British males and Malaysian males. Body mass affects radius SOS consistently across ethnic groups. This infers a difference in radius SOS attributed to body mass, is likely to exist between ethnic groups in the present study. Foldes et al (1995) report a correlation coefficient

of r=.29 (p<.001) between tibia SOS and height in young Caucasian women, similar to the present study (r=.212 p<.05) but not chapter 3 (r=.084 p>.05). The correlation coefficient in the present chapter, accounts for Malaysian and British males, but only British in chapter 3.This may imply that the strength of the relationship between tibia SOS and height is stronger in Malaysian males. Overall, risk factors affect radius and tibia ultrasound differently. Ethnic groups may also respond differently to risk factors.

As discussed in chapter 3, both body mass and height have a significant impact on bone mineral density and are often used as covariates in cross-sectional studies (Mcveigh et al, 2014; Ginty et al, 2005; Lloyd et al, 2014). The current study should therefore expect to see differences in bone properties, between ethnic groups, attributed to body mass and height. Both British (Control – 57.51; Athlete – 61.18kg) groups and MA (57.68kg) group had significantly greater fat free mass than the MC (48.56kg) group. Athletic standard facilitated gains in fat free mass for Malaysians but not British Participants. If the training undertaken by athletes was uniform, there are significant differences in the FFM response to training between ethnicities. A strong link between lean mass and bone health (Armamento-Villareal et al, 2014; Seabra et al, 2014; Bielleman et al 2014) suggest Malaysians would benefit greater from being athletes.

However training modalities were not measured for controls or athletes and may be different between British and Malaysian groups. The British groups may be as physically active as each other, indicated by a lack of significant difference in FFM (p>.05). In this case, an interaction is to be expected between athletic standard and ethnic group as the FFM difference between the BC and BA groups is contrasting to the difference between MC and MA groups. This may be a false representation of the response to exercise between ethnic groups. However, Table 17 indicates that without grouping for athletic standard or ethnicity there was no relationship between FFM and radius SOS (.114 p>.05) and tibia SOS (-.018

p>.05). This suggests FFM holds no bearing to the outcome of SOS measurements for British and Malaysian males.

Chapter 3 reported a significant relationship between FFM and radius SOS (r=.383 p<.01) but not tibia SOS (.006 p>.05). Similar to mass and height, there are differences in the response to FFM between ethnic groups and anatomical sites.

Table 16 reports that body fat is significantly greater in MC (21.93%) than all other groups (≤16.94%). MAs (10.43%) have significantly less body fat than BCs (16.94%). Athletes have lower body fat % than control groups, except between BAs and BCs. The difference between British groups is small (3.82%) (p>.05). The difference between Malaysian groups is large (11.50%) (p<.05). Findings may suggest the MCs are engaged in more aerobic fat burning activities. Furthermore, BCs are perhaps less sedentary than MCs highlighting again that BC are not necessarily less active than the BA group.

Ho-pham et al (2014) suggest there is a significant relationship between body fat and BMD (r=.28 p<.001). Böttcher et al (2006) reports that QUS-SOS correlates with DEXA-BMD (r=.71 p<.01). There is an argument that QUS will correlate with body fat %. In the current study, body fat % correlated with radius SOS (r =.255 p<.05) but not tibia SOS (r=-.116 p>.05). This would imply that the MC group should have higher radius SOS than the other groups markedly so with the MA group. For British only in chapter 3, There were no significant correlations between body fat and SOS measurements (p>.05). This implies an ethnic difference in response to this factor.

MAs have significantly lower tibia SOS (m.s^{-1}) than BAs (3873.97 and 3964.33, respectively). No other groups were significantly different for tibia SOS. The trend from highest to lowest for tibia SOS is BA followed by BC, MC and MA (3964.33 followed by 3948.21, 3896.97 and 3873.97, respectively). British groups have higher SOS values than Malaysian groups, implying a link between ethnicity and tibia SOS. Zhu et al (2008) reported and average tibia SOS of 3990m.s^{-1} in Chinese males 18-25 year olds, similar in age and gender to the current study. SOS values for all groups were below this in the current study. Beamed® software calculates t-scores based upon its reference database. This software was used in the current study along with the Beamed® Sonometer. Tibia t-scores were between -0.99 to -0.30 (table 16) for all groups. This reiterates that all groups were below average.

Both British groups were significantly heavier and taller than their Malaysian counterparts. This may support the association between ethnicity and tibia SOS (Ginty et al, 2005). Nam et al (2010) concluded that body size is important between Asians men and others for total BMD, making size adjustments important when observing differences between Malaysians and British bone properties. The BA group also had significantly higher quadricep strength (Nm) than the MC group (p<.05), a factor linked to elevated bone density (Seabra et al, 2014).

There were no significant differences in radius SOS between groups (p>.05). The highest SOS value was 4071.84 and the lowest 3993.21 for MC and BC, respectively. This difference is considerable, yet not significant suggesting large variation within the groups. The next largest SOS value radius SOS is 4041.18 followed by 4019.20 for BA and MA, respectively. There is no consistently higher ethnic or athletic group. Both points suggest other factors are involved. Zhu et al (2008) report average radius SOS to be 4075 m.s^{-1}, similar to Malaysian controls but dissimilar to other groups.

Significantly lower body mass and height than British groups (p<.05) and significantly lower FFM than all groups (p<.05) did not mean lower radius SOS for Malaysian controls. The literature and current study support relationships between body fat and total BMD and radius SOS. MCs had significantly greater body fat % than all other groups and have higher radius SOS. However, if body fat % was the sole determinant of radius SOS, BC should have the second highest radius SOS value. This is not the case meaning there are other factors or interactions that cause this difference. Travison et al (2011) conclude that lean mass, fat mass and socioeconomic status influence ethnic differences the most. This increases the requirement for a factorial analysis of covariance.

Univariate testing

Looking at the difference between the four independent groups is not enough to answer the research objectives. Table 16 has shown the differences in factors and SOS measurements between groups. What has become clearer is factors affect groups differently for each anatomical site. Removing the variation created by factors measured in this study, helps establish the effect of ethnicity and athletic standard more accurately. Homogeneity of regression testing determines that body mass and body fat % are used as covariates for radius SOS and height and quadricep strength are used as covariates for tibia SOS.

In the current study ethnicity affects radius SOS (p=.004). Malay Malaysians had significantly greater adjusted radius SOS than White British (4077.98 and 3984.75m.s^{-1}, respectively) (Table 19). Values were adjusted for radius SOS as body mass and body fat significantly affect the outcome (p=.002 and p=.029, respectively) (table 19). Adjusting fixes at body mass at 69.22kg and body fat at 15.60%, respectively for all participants. Non-adjusted radius SOS between groups was 4017.20 and 4045.53m.s^{-1} for British and Malaysian groups, respectively. These were not significantly different (p>.05).

Ethnicity is a strong independent predictor of radius SOS as indicated by adjusted values. Body mass and body fat % also strongly determine radius SOS, for these groups. A differences in average body mass (5.91kg) and body fat (-1.15%) meant the non-adjusted SOS values between British and Malaysian groups were not significantly different. This highlights the importance of size adjustments when comparing ethnic groups (Nam et al, 2010). Body mass accounted for more variance in radius SOS (7.3%) than ethnicity (6.3%). Despite body mass and body fat reducing the difference between ethnic groups, Malaysians still had higher average radius SOS, perhaps highlighting strong ethnic differences regardless of factors. Independently, ethnicity accounted for 6.3% (effect size = .063) of the variance in radius SOS (table 18).

40 Asian males had significantly lower radius BMD values than 36 Caucasian males aged 20-35 years in a study by Liang et al (2007). Adjusted for anthropometrics, dietary calcium and leg strength Asians had 7.3% significantly higher radius BMD than Caucasians. The Malaysian group in the current study had 2.3% significantly greater adjusted radius SOS. Both studies, although using different units report the Asian group have significantly greater adjusted radial bone health than the Caucasian group.

As suggested by Liang et al (2007) the osteogenic response to exercise may differ in relation to ethnicity. An interaction between two factors infers one has an impact on the other. There was a significant interaction between ethnicity and athletic standard for radius SOS (p=.005). SOS values were significantly adjusted by body mass and body fat (p<.01) (Table 18). This suggests athletic standard has either a positive or negative effect on radius SOS depending on the ethnic group. 4153.44, 4002.53, 3989.83 and 3979.66m.s^{-1} were the adjusted values for MC, MA, BA and BC, respectively. What is notable is both Malaysians groups still have higher radius SOS values than their British counterparts. The effect size was .063 (6.3%) for the interaction, very similar to ethnicity alone. Ethnic difference is still

prevalent. 10.17m.s^{-1} was the difference between BC and BA. -150.91m.s^{-1} was the difference between MC and MA.

Differences are not uniform in either direction or magnitude. This means the difference in radius SOS is neither consistently positive or negative nor equal in size between controls and athletes, of British and Malaysian groups. This suggests that for Malaysians, being an athlete is detrimental to radial bone health, whereas for British it is moderately beneficial.

Independently, athletic ability did not significantly affect radius SOS between groups (=.069). Athletic ability significantly changed certain factors for the Malaysian groups namely, body fat ($p<.01$) and fat free mass ($p<.01$) but no factors for British ($p>.05$) (table 16). This suggests either ethnic differences in response to exercise or a difference in exercise. Without information on physical activity levels for either controls or athletes, the latter is more likely. Rather an ethnic difference in the response to exercise as the interaction portraits, there is more likely a lack of adaptation in factors associated with bone health between British controls and athletes. Similar to the conclusion drawn in chapter 3, athletic ability may significantly change factors that in turn, affect radius SOS without having a direct impact on it independently.

This does not explain why Malaysians have a negative association with athletic standard. This difference in radius SOS prior to adjustments was -52.64m.s^{-1} between MA and MC (Table 16). A significantly larger body fat % for the MC group (+11.5%) may explain the higher SOS value. If this is the case, adjustments for body fat would reduce this difference. Accounting for body fat and body mass the difference in SOS in between MA and MC is -150.91m.s^{-1}. The larger difference suggests athletic standard is more likely to have a negative impact on radius SOS in Malaysians directly. Monitoring of training habits for both Athletes and controls is needed for understand this difference in greater detail.

There was no interaction for tibia SOS (p>.05). Ethnicity significantly affected the outcome of tibia SOS (p=.006) which was unaffected by covariates (p>.05) (Table 18). Interestingly, no factors cited as affecting bone health including local muscle strength (Marques et al, 2011), body mass (Ginty et al, 2005), lean mass (Armamento-Villareal et al, 2014) or body fat (Ho-Pham et al, 2014) significantly affect the outcome between ethnic groups. The effect size was .058 suggesting ethnicity can explain 5.8% of the variance in tibia SOS. No covariate affecting the outcome makes a stronger case for the independent effects of ethnicity on this anatomical site. No main effect for athletic standard (p=.690) suggests a change in activity level holds no bearing to the outcome either.

Limitations

As suggested in chapter 3, there are issues relating to causality in the present study. Without knowing what factors affect the population before testing, one cannot definitively assume that ethnicity is an independent predictor of radius SOS.

There are limitations to this study when considering the definition of athletic standard and its purpose as an independent variable. This study was part of a greater research group investigating the role of quadricep strength on tibial SOS in athletes. The current study opted to use this grouping variable to establish a group, in theory, subjected to different physical demands. The criterion for an athlete was 1 year minimum, maintained to this date of district level competition and training within their chosen discipline. It retroactively accounted for activity levels which was advantageous because changes in bone health in response to exercise are chronic (Biellemann et al, 2014). Any participant that did not match this criterion became part of the control group. This was the same for British and Malaysian groups.

The primary issue with this grouping method is the researcher is unaware of quantity, intensity, time or type of training undertaken by any of the athletes. The literature review

states that training type and intensity matters greatly in changing bone properties (Nelson et al, 1994). As such, members of the athlete group may be getting different stimuli from exercise. This is same for within groups and between ethnicities. This creates issues when measuring independent effects of athletic standard and interactions with ethnicity.

Not only athletes, the control groups were not measured for physical activity levels. Just because they do not compete does not necessarily mean the group are completely sedentary. This is most likely highlighted by a lack of difference in physiological parameters between British controls and athletes in the current study.

Summary

An objective of this study was to determine if an interaction between ethnicity and athletic standard existed when observing radius and tibia SOS. The current study confirms an interaction for radius SOS but suggests a lack of difference in physiological factors between British groups means the training undertaken in the athlete group was not different from controls. This implies the exercise involved in athletic standard is lacking uniformity between ethnic groups.

Another objective of this study was to determine if a significant main effect existed for either ethnicity or athletic standard. There is evidence to suggest that for both radius and tibia SOS, ethnicity affects the outcome between British and Malaysian groups. Neither ethnic group had the greatest SOS value for both anatomical sites. In addition, factors affected each site differently. Using quantitative ultrasound, it is clear bone health changes a lot in young adult males in response to genetics and environment.

Chapter 5

Aims and objectives

These studies set out to determine how exercise and ethnicity affected quantitative ultrasound measurements of bone. Chapter 3 "A comparison of quantitative ultrasound measurement of bone in young males with different physical activity levels" aimed to determine a difference in peripheral SOS between levels of total and strength physical activity. Chapter 4 "Analysis of quantitative ultrasound measurement of bone in British and Malaysian groups" had two objectives. First was to determine if ethnicity or exercise (measured by Athletic Standard) had individual effects on the outcome of peripheral SOS. Secondly the study sough to determine to what extent ethnicity and exercise interact.

Rationale

Literature surrounding the bone health of young adult males is lacking. With physical activity being an increasingly used intervention for osteoporosis, an ability to quantify the level required to instigate change was considered important. Using physical activity as an intervention for low bone mass may become more specific. To a large extent, the literature has identified the role of ethnicity in bone health. Liang et al (2007) conclude ethnic groups react differently to their environment where osteological adaptations are concerned. The current study aimed to determine if an interaction existed between exercise and ethnicity, identifying groups perhaps better suited to an exercise intervention for low bone mass.

Findings

Within a multi-ethnic British sample, frequency of total physical activity or strength activity did not affect radius or tibia SOS values. Between a White British and Malay Malaysian sample, ethnicity significantly affected radius and tibia SOS, whereas athletic standard did

not. An interaction was found between athletic standard and ethnicity for radius SOS, suggesting the difference between controls and athletes of the British group is different to the Malaysian group. Body mass and body fat % significantly change radius SOS values between ethnic groups. No other factors significantly changed tibia SOS between groups.

Implications

This study suggests that volume of physical activity is very unlikely to improve bone health determined by quantitative ultrasound (Chapter 3). Body mass and fat free mass correlate with radial ultrasound, yet hold no bearing to the outcome between physical activity groups. There is evidence that the groups respond typically to risk factors for bone mass but there is no evidence that frequency of physical activity causes any significant physiological adaptations measured in this study. The study can therefore not specify the amount of physical activity needed to change SOS significantly.

This study suggests that White British males are more at risk of radial low bone mass than Malay Malaysian males (Chapter 4). The opposite is true for the tibia. Body mass and body fat are very important when comparing between ethnic groups for radial but not tibial ultrasound. This study implies differences are site specific between these ethnic groups and risk factors affect these sites differently. Effect sizes suggest body mass and ethnic group (7.3 and 6.2%, respectively) should be valued as prominent risk factors for radial bone mass, determined by QUS.

The interaction effect implies a Malay male will see a large decline in radial SOS as a result of being an athlete, whereas a White male will see a marginal increase. This suggests the competition and training involved with athlete status defined by this study, is largely detrimental to Malays but not Whites. The study draws attention to lack of difference in physiology among the White compared to the Malay, citing that training induced adaptations

are clearer in the Malaysian group than the White group. This was attributed to an active British control group, but no steps were taken to measure this.

Malaysian athletes with significantly higher fat free mass had much lower radius SOS than Malaysian controls who had higher body fat %. Body mass was the same and fat free mass did not correlate with radius SOS, therefore this implies for this ethnic group body fat is a much prominent factor than fat free mass, contrary to the current literature. Fat free mass was prominent factor for a multi-ethnic British sample in chapter 3, not including Malays.

Limitations

No re-test reliability or pilot studies were undertaken prior to data collection. A re-test reliability study involves testing the individual under the same conditions at different times to establish how consistent the experimenter is. Without this the consistency of testing was not known, therefore how accurate the experimenter was. No pilot study suggests any errors encountered in the design could not be rectified once data collection had begun. However, the design carried out by the experimenter is Britain was identical to the one in Malaysia which was already underway at the time of testing. Any adaptations would compromise the comparability of the groups. Acknowledging this, it may have been more reliable for the same researcher to collect data on behalf of the British and Malaysian groups.

Discussions in chapter 3 address the limitations regarding the physical activity questionnaire and the stratification of the groups. The discussion in chapter 4 highlights the reasons for using athletic standard to define level of exercise, relating to the design of the study and research objectives from others on the research team. Both chapters address the issue of causality when using a cross-sectional design. Without directly influencing the sample a monitoring change over time it is difficult to confidently conclude the independent variable is causing the change.

Future Research

The aim of the study in chapter 3 sought to quantify physical activity to establish what was needed to change SOS. The questionnaire used may have not addressed strength training adequately and created a ceiling effect therefore misrepresenting high physical activity. A second aim in chapter 4 was to determine an interaction between physical activity and ethnicity. The grouping variable used to define physical activity did not clearly and consistently establish groups undertaking higher amounts of exercise. It was lacking specificity and qualified exercise rather than quantified it.

Future research needs to address the issue of defining physical activity. Physical activity needs to target adaptations in lean tissue mass while maintaining body weight, fat mass and caloric intake. It also needs to be measureable and not qualitative as it becomes subjective and exposed to bias. A randomized control trial, blind to the participant could be beneficial. A 7-8 month intervention based design (Marques et al, 2011) of strength or heavy loading exercises (Nelson et al, 1994) whilst comprehensively monitoring adaptations in anthropometrics (Bielemann et al, 2014) may highlight the quantity and type of exercise capable of elevating bone health significantly above sedentary levels, thus answering the research objective in chapter 3. Furthermore, it would provide a reliable measure of physical activity to compare the difference between ethnic groups answering the research objective in chapter 4.

Alternatively, a longitudinal study with a large cohort of ethnic groups and their environment may better answer the research question. Along with a better model for quantifying exercise (Bailey et al, 1999; Biellemann et al, 2013) and a comprehensive control of confounding variables (Nam et al, 2010) a better understanding of the effects of exercise on bone properties between ethnic groups could be understood.

<center>Appendices</center>

Table 21: Homogeneity of Variance tests for both chapters 3 and 4

Variable	Chapter 3 Total Activity	Chapter 3 Strength Activity	Chapter 4 All Groups
Body mass	.678	.443	.005
Height	.942	.262	.000
Radius SOS	.771	.434	.105
Radius t-score	.697	.480	.009
Tibia SOS	.624	.799	.409
Tibia t-score	.698	.597	.008
Body Fat	.229	.479	.000
Fat Free Mass	.855	.194	.151
Quadricep Strength	.595	.986	.051

P value >.05 means equal variance assured for ANOVA.

Table 22: Univariate Interaction tests for both chapters 3 and 4

		Radius F	Radius df	Radius p	Tibia F	Tibia df	Tibia p
Chapter 3	Total Activity	.905	3	.445	.687	3	.564
Chapter 3	Strength Activity	.314	3	.815	.177	3	.911
Chapter 4	Al Groups	.830	4	.508	1.602	4	.178

P value >.05 means all the groups respond similarly to covariates.

Table 23: Levene's Test of Equality of Error Variance for both chapters 3 and 4

		Radius F	Radius df	Radius P	Tibia F	Tibia df	Tibia P
Chapter 3	Total Activity	.235	2, 63	.791	-	-	-
Chapter 3	Strength Activity	.416	2, 63	.661	-	-	-
Chapter 4	Al Groups	3.904	3,128	.010	1.170	3, 128	.324

P value >.05 means equal variance assured for ANCOVA. No values for Chapter 3 as no covariates affect SOS measurements

List of Abbreviations

QUS – Quantitative ultrasound

DEXA – Duel Energy X-ray Absorptiometry.

BMD – Bone mineral Density

FFM – Fat Free Mass

FM – Fat Mass

QS – Quadricep Strength

BM – Body Mass

pQCT – Peripheral quantitative computed tomography

eBMD – Estimated bone mineral density

SOS – Speed of sound

HR – Homogeneity of variance

EMPA – Estimated minutes of physical activity

IPAQ – International physical activity questionnaire

PW – Per week

ICPE -

SPF – Scientific paper format

TA – Total activity

SA – Strength activity

DALY – Disability adjusted life years

WHO – World health organisation

BUA – Broadband ultrasound attenuation

CT – Computed tomography

TPA – Total physical activity

BMC – Bone mineral content

BMI – Body mass index

RE – Resistance exercise

AE – Aerobic exercise

ANOVA – Analysis of variance

ANCOVA – Analysis of covariance

QTUS - Ultrasound based tomographic transmission

RAD - Radius

TIB - Tibia

MET - Metatarsal

PHA – Phalanx

CV – Coefficient of variance

RCT – Randomized control trial

FN – Femoral neck

LS – lumbar spine

LM – Lean mass

MZ - Monozygotic

DZ - dizygotic

MTSS - medial tibial stress syndrome

PARQ – Physical activity readiness questionnaire

AS – Athletic standard

BC – British control

BA – British athlete

MC – Malaysian control

MA – Malaysian athlete.

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