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A COMPARATIVE STUDY INTO THE BONE HEALTH OF SOUTH AFRICAN PRE-PUBERTAL CHILDREN WHO PARTICIPATE IN PHYSICAL ACTIVITIES WITH VARIOUS AMOUNTS OF SKELETAL LOADING

By

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A Thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy.

Johannesburg, 2014

DECLARATION

I declare that this Thesis is my own, unaided work. It is being submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

Signed _____

This _____day of _____2014 in Johannesburg

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ACRONYMS AND ABBREVIATIONS

B^3Q	Bone specific physical activity questionnaire
BA	Bone area
BMC	Bone mineral content
BMD	Bone mineral density. Bone mineral density refers to areal bone density (g/cm^2) throughout the thesis
BSI	Bone strength index
CoA	Cortical area
CoD	Cortical density
CSA	Cross-sectional area
CT	Cortical thickness
DXA	Dual energy X-ray absorptiometry
EC	Endosteal circumference
Ethnicity	An ethnic group is referred to a population of humans whose members identify with each other, usually on the basis of a presumed common geneology or ancestry. Ethnic groups are also usually united by certain common cultural, behavioural, linguistic and ritualistic or religious traits (1,2).
IGF-1	Insulin-like growth factor 1
METS	Metabolic equivalents
NTx	Cross-linked N-telopeptides of Type I collagen
PA	Physical activity: Any bodily movement resulting in energy expenditure above the basal level. PA can be seen as an umbrella term for human behaviour with multiple dimensions and sub categories such as exercise, sport, leisure activities, dance and transportation (3–5).
PAQ	Physical activity questionnaire
PBSS	Peak bone strain score
PC	Periosteal circumference
pQCT	Peripheral quantitative computed tomography

ROI	Region of interest
SES	Socioeconomic status. SES is typically a composite of measures such as occupation, education, income, type of residence and access to certain amenities in the home (e.g., telephone, TV, motor car). For the purpose of this thesis, children's SES is regarded as that of their parents/caregivers.
SSI	Strength-strain index
ToA	Total area
ToD	Total density
TrbD	Trabecular density

“ The true explorer does his work not for any hopes of reward or honour, but because the thing he has set for himself to do is a part of his being, and must be accomplished for the sake of accomplishment. And he counts lightly hardships, risks, obstacles, if only they do not bar him from his goal.”

Admiral Robert E. Peary, Polar Explorer.

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PREFACE

The work presented in this thesis is a continuation from previous work done in South African children, which has shown that there are differences in measures of bone mass, size, strength and structure between black and white children. Although unclear, these differences have been largely attributed to a genetic advantage afforded to black children (6). The interplay between nature and nurture presents an ever interesting topic of study and thus for me the participation in physical activity (known to be beneficial to bone health) and the relationship to bone mass within different ethnic groups, was of considerable interest. In South Africa, the epidemiological transition is shifting the burden of disease from communicable to non communicable conditions and it is plausible that we will observe increasing incidences of physical inactivity. In order to observe the possible long term associations of physical inactivity and bone health, a thorough understanding of the influences of physical activity on bone within different ethnic groups is needed. Whilst living in a country such as ours, we are urged to find sustainable and affordable solutions to the ever growing challenges of health equity. Perhaps, because of the genetic advantage to bone health in black children, the influence of physical activity effects on bone health in these children has been overlooked. At the start of my studies, the ethnic-specific bone response to weight-bearing physical activity had not been well researched and certain questions remained unanswered in the field of physical activity, ethnicity and bone health in South African children. A one-size-fits-all approach to physical activity in children may not counteract the effects of the sedentary lifestyles that are threatening our children's health and healthcare system. Thus there was a need to understand the ethnic-specific physical activity response in order to

address unique needs of children in South Africa; research which may ultimately contribute to the maintenance of good bone health in adulthood.

This thesis has been written up and submitted in the following manner: Chapter 1 is the review of literature presented as an overview and an attempt at identifying unresolved issues in the field of physical activity and bone health in black and white children. Chapter 2 outlines the methodological approaches that were used throughout the thesis and details the methods used in each specific study of the thesis. Chapters 3, 4, 5 and 6 present the results of each of the studies that were conducted in the thesis. Finally, chapter 7 summarises the main conclusions of the thesis as a whole.

The work presented in this thesis is based on the following publications and presentations which have emanated from data collected for the thesis.

Peer Reviewed Publications:

Meiring, Rebecca M., Avidon, Ingrid, Norris, Shane A., McVeigh, Joanne A. A two-year history of high bone loading physical activity attenuates ethnic differences in bone strength and geometry in pre/-early pubertal children from a low-middle income country. *Bone,* 57 (2013) 522-530.

Meiring RM and McVeigh JA. Associations of Physical Activity Load with Volumetric Bone Properties in Pre-pubertal Children. Submitted to *South African Journal of Sports Medicine.*

Meiring RM, Micklesfield LK, Avidon I, McVeigh JA. Osteogenic Effects of a Physical Activity Intervention in South African Black Children. Submitted to *Journal of Musculoskeletal and Neuronal Interactions*.

Meiring RM, Micklesfield LK and McVeigh JA. Loading and Ethnic Effects on Annual Bone Accrual in Pre-Pubertal Black and White Children. Submitted to *Acta Paediatrica*.

Conference Presentations:

1. ACSM 58th Annual Meeting and 2nd World Congress on Exercise is Medicine, Denver, Colorado, June 2011: Poster presentation.

2. SASMA Congress, Johannesburg, October 2011: Oral presentation.

3. 8th International Workshop for Musculoskeletal and Neuronal Interactions, Ipswich, UK, June 2012: Oral presentation.

4. 2nd Joint Meeting of the International Bone and Mineral Society and the Japanese Society for Bone and Mineral Research, Kobe, Japan, May 2013: Oral presentation.

5. 18th Annual Congress of the European College of Sport Science, Barcelona, Spain, June 2013: Oral presentation.

6. 41st Congress of the Physiological Society of Southern Africa, Johannesburg, September 2013: Oral presentation.

Published Abstracts:

Meiring, RM, Avidon, I and McVeigh J. A study examining the effect of two-year physical activity history on current bone health status and body composition using pQCT in pre-/early pubertal South African children. *Journal of Musculoskeletal and Neuronal Interactions*, 12:2, p102-115, 2012.

Honours and Awards:

1. Awarded 2nd place for oral presentation at 8th International Workshop for Musculoskeletal and Neuronal Interactions, 2012, Ipswich, UK.

2. Awarded best student oral presentation at Faculty of Health Sciences Research Day, August 2012, University of the Witwatersrand, South Africa.

3. Awarded International Bone and Mineral Society Travel Grant 2013, for travel to the 2nd Joint Meeting of the International Bone and Mineral Society and the Japanese Society for Bone and Mineral Research, Kobe, Japan.

ABSTRACT

Osteoporosis is a disease that may be pre-determined from the condition of bone health during youth. In South Africa, the situation is quite unique in that the population of black people has a reduced fracture rate compared to white people. As lifestyle and dietary patterns change with urbanisation and there is a shift towards westernised diets and sedentary behaviour in youth, fractures in elderly South African blacks may become more prevalent. With these rapid lifestyle changes, it will become increasingly important to prioritise osteoporosis and its related conditions as a major public health concern in South Africa. Very few of the factors influencing osteoporosis have been well studied in children of different ethnic groups. Physical activity in childhood, especially in the pre-pubertal years, confers residual benefits to the adult skeleton. In this thesis, the associations between ethnicity, history of participation in physical activity and skeletal health were explored in a sample of pre-/early pubertal children from South Africa who participated in four different studies. Furthermore, a novel aspect of the thesis was the use of peripheral quantitative computed tomography (pQCT) to investigate the mechanistic role that physical activity plays on bone health in this unique population.

First the use of an existing physical activity questionnaire for the assessment of bone loading had to be validated in a sample of black and white boys and girls (n=38). A bone loading algorithm was used to calculate a peak bone strain score (PBSS) from the physical activity questionnaire. Therefore a bone specific physical activity questionnaire (B^3Q) was used in subsequent studies. The PBSS was shown to be reliable and reproducible with

significant ($p<0.001$) intraclass correlation coefficients. There were significant correlations between PBSS and moderate ($r=0.38$; $p=0.02$), vigorous ($r=0.36$; $p=0.03$) and combined moderate to vigorous intensity activity counts ($r=0.38$; $p=0.02$) as measured by accelerometry. The ability of the PBSS algorithm to classify children into high or low weight bearing groups was in moderate agreement with accelerometer derived combined moderate and vigorous activity counts ($\kappa=0.42$; $p=0.008$). PBSS was significantly correlated to body size adjusted bone mineral content at all sites scanned by DXA ($r=0.43$-0.57; $p<0.05$). Positive correlations were observed between PBSS and area, density and strength at the radius and tibia ($r=0.40$-0.64; $p<0.05$). At the radial metaphysis, significant correlations between moderate activity ($r=0.46$; $p=0.005$) and combined moderate and vigorous activity counts ($r=0.42$; $p=0.01$) were seen for bone strength. No associations were seen between accelerometer measured physical activity and bone outcomes at the tibial diaphysis. Multiple regression analysis showed that the PBSS was a better predictor of bone mass and structure than was accelerometry.

The next study sought to determine whether children who were classified as being high bone loaders for the past two years would present with greater bone mass and strength regardless of their ethnicity. Sixty six children [black boys, 10.4(1.4) yrs, n=15; black girls, 10.1(1.2) yrs, n=27; white boys, 10.1(1.1) yrs, n=7; white girls, 9.6(1.3) yrs, n=17] reported on all their physical activities over the past two years in the interviewer administered bone specific physical activity questionnaire (B^3Q). Children were classified as being either high or low bone loaders based on the cohort's median peak bone strain score estimated from the B^3Q. In the low bone loading group, black children had greater

femoral neck bone mineral content (BMC) (2.9 (0.08)g) than white children (2.4 (0.11)g; p=0.05). There were no ethnic differences in the high bone loaders for femoral neck BMC. At the cortical sites, the black low bone loaders had a greater radius area (97.3 (1.3) vs 88.8 (2.6) mm^2; p=0.05) and a greater tibia total area (475.5 (8.7) vs. 397.3 (14.0) mm^2; p=0.001) and strength (1633.7 (60.1) vs. 1271.8 (98.6) mm^3; p=0.04) compared to the white low bone loaders. These measures were not different between the black low and high bone loaders or between the black and white high bone loaders. Ethnic differences in bone area and strength apparent between children classified as having a lower bone loading physical activity history appear to have been attenuated when children partaking in high bone loading physical activities were compared. Greater levels of mechanical loading seemed to have no apparent benefits in black children.

Cross-sectional studies in black and white pre-pubertal children have observed significant ethnic differences in structural bone outcomes as measured by pQCT but there are a limited number of intervention studies that have been conducted in black children. The cortical bone of black and white children may respond differently to mechanical forces, yet no physical activity interventions and their effects on bone structure in black children have been done. The aim of the third study was to determine whether a weight-bearing physical activity intervention improves measures of bone mass, structure and strength in pre-pubertal black children. Children (9.7 ± 1.1 years) were randomised into an exercise (EX; n=12) and control (CON; n=11) group. The EX children performed a 20-week weight-bearing exercise program performed twice a week for 45 minutes per session, while CON children continued their regular activities. Changes in tibial trabecular volumetric bone

density, area and strength were greater in the EX than the CON group (all $p<0.01$). At the cortical site of the tibia, the change in bone density was greater in the EX group than the CON group (all $p<0.05$). The greater change in tibial periosteal circumference in the EX groups also resulted in a greater change in cortical thickness of the tibia compared to the CON group ($p<0.05$).

The final study assessed whether rates of bone accrual differed over one year between high and low bone loaders and also between black and white South African children. Forty seven children (18 boys, 29 girls) were followed up after one year. High bone loaders tended to have greater baseline BMC at all sites measured by DXA but the difference was only significant at the femoral neck ($p=0.03$). At the follow up visit, femoral neck BMC remained significantly higher in the high compared to the low bone loaders ($p=0.003$). Bone strength index (BSI) at the follow up visit was significantly greater in the high bone loaders compared to the low bone loaders ($p=0.05$). Although there was a trend for the high bone loaders to have greater indices of density and area at the 65% tibia compared to the low bone loaders, this was not significantly different at baseline or at follow up. High bone loaders had greater relative changes in whole body BMC ($p=0.002$), tibial cortical area ($p=0.03$), cortical density ($p=0.04$) and cortical thickness ($p=0.03$) compared to low bone loaders. There were no significant differences in DXA bone outcomes between black and white children at baseline and follow up. At baseline, total density at the 4% radius was greater in black than in white children ($p<0.001$) but total density at the follow up visit was not significantly different between black and white children ($p=0.06$). Trabecular density was greater in the black than in the white children

at baseline (p=0.01) as well as at follow up (p=0.04). BSI at baseline was greater in the black than in the white children (p=0.05) but this significance disappeared at follow up. Similar to the 4% radius, cortical density at baseline was significantly greater in the black compared to the white children at the 65% radius (p=0.01) and at the 65% tibia (p=0.04).

In conclusion, the PBSS algorithm from the B^3Q can be used to reliably and accurately collect data on previous participation in weight bearing exercise and is able to classify children as being either high or low bone loaders. It appears that in order for White children to reach the same bone mass/health levels as Black children, they may need to participate in higher levels of weight-bearing physical activity. Ethnic differences in bone area and strength apparent between children classified as having a lower bone loading physical activity history appear to have been attenuated when children partaking in high bone loading physical activities were compared. The associations may indicate that a strong environmental influence (i.e. high participation in physical activity) may offer similar or even superior benefits to bone over genetic (ethnic) influences. The use of pQCT appears to be sufficiently sensitive in detecting bone structural changes in response to mechanical loading interventions. As such, pQCT measures were able to determine the efficacy of a weight bearing physical activity intervention on trabecular and cortical sites in black children, and, similar to what has previously been observed in white and Asian children, our knowledge on the attainment of bone in response to an exercise intervention in black children is deepened. Moreover, the bone accrual that occurs in a population of black and white children from a low-middle income country may also differ between ethnicities and may reflect an environmental influence that modifies existing

paradigms on physical activity and bone health in children. The promotion of weight-bearing physical activity should occur in all youth, to oppose the possible lifestyle induced risks for developing osteoporosis in adulthood.

CHAPTER 1 – LITERATURE REVIEW

1.1 Introduction

Osteoporosis is defined clinically as having either a low bone mineral density (T-score < -2.5 SD) and/or a history of fragility fracture (7). Osteoporosis is a disease that generally occurs in the elderly, therefore as the average lifespan of the global population increases, it is likely that the incidence of osteoporosis will increase as well. In the United States, the incidence of osteoporosis is expected to increase by 50% between 2005 and 2025 (8). The estimated number of hip fractures worldwide was 1.7 million in 1990 and is likely to rise to 6.3 million in 2050 if age-adjusted incidence rates for hip fractures do not change (9). Of greater concern is that the incidence of osteoporosis in developing countries is predicted to overtake that of developed countries in the next 40 years (8,10) and along with this rise in disease incidence is the increase in the burden of annual costs which are estimated to be between 17 and 20 billion dollars in the US alone (11). Hip fracture data are limited in a country such as South Africa and as such extrapolated data from international statistics are used. The extrapolated prevalence of osteoporosis in South Africa according to the International Osteoporosis Foundation was approximately 2 million (±4% of the population) in 2011. Even though the cost to a hip fracture patient is ±USD 24 000 (±R240 000), osteoporosis is not yet recognized as a major health problem in South Africa due to the burden of the infectious diseases such as HIV/AIDS and tuberculosis. In addition the average number of days that one will spend in hospital after a hip fracture is 8 days, a length of hospital stay that already overcrowded hospitals cannot accommodate. The increased risks associated with bone fracture in adults and children alike have been well documented and studies suggest that lifestyle influences play a large role in increasing fracture risk in these populations (12–14). If the results

from a study by Clark and colleagues in 2006 (15), on over 6000 children from the United Kingdom, are reflective of children in South Africa, then the bone health of future generations is concerning. In this study, children, an average of 9.9 years old, were followed over 24 months. Bone mass was measured in the children at baseline, 12 and 24 months. After adjusting for height, weight and bone area, for every standard deviation (SD) decrease from the mean of total body bone mineral content, there was an 89% increase in fracture risk within this population. The latter study provided definitive evidence of what others have inferred - fracture incidence is associated with low bone density, poor bone structure, poor nutrition and high body mass index (BMI) (16–18). One theoretical analysis has also speculated that a 10% higher than average (computer simulated) peak bone mass, delayed the onset of osteoporosis by 13 years (19). Although this study was based on a computer simulation, other factors studied i.e. the variation in age at menopause and the variation in non-menopausal bone loss (decreased physical activity and changes in nutritional intake and requirements) delayed the onset of osteoporosis by only two years. A way of reducing the risk of osteoporosis then, would be to ensure that a maximum bone mass is attained before the onset of age-related bone loss. The maximum amount of bone one can gain can be exacerbated via participation in high levels of weight-bearing physical activity. The mechanical loading stimulus allows bone to adapt to loads placed on it by changing the mineral content and structure of the bone. The optimal period for adaptations to mechanical loading occurs in childhood during the years preceding puberty. The review that follows aims to highlight the inadequacies in the current literature pertaining to lifestyle and bone health in children, with a particular focus on children's participation in weight-bearing physical activity.

1.2 Bone physiology, genetics and the environment

Bone is a dynamic material, the structure of which changes many times in a lifetime. Connective tissue within a collagen framework laden with hydroxyapatite salts, (namely Ca^{2+} and PO_4^{3-}) forms the basis of bone. Bone, in both adults and children, consists of an outer layer of compact or cortical bone and an inner filling of trabecular or spongy bone (20). The design of the human skeleton is such that it is resistant to great impact forces but light enough to allow for easy movement. During one's lifetime bone undergoes periods of modeling and remodeling (21). Modeling is the attainment of new bone during growth and allows for increases in width of the bone (22). In comparison, remodeling is a continuous process that involves the modification of existing bone where old bone is resorbed and new bone mineral is laid down on the same surface (22). The bone turnover rate for cortical bone is 4% per year while that for trabecular bone is 20% per year (20). After growth is complete the remodeling that occurs in the adult skeleton is largely attributable to the stresses and strains placed on the skeleton by everyday movement.

The mechanostat theory (a short history of bone)

In the late 19th century, Julius Wolff, a German surgeon, wrote Wolff's Law which introduced the theory, although flawed, that bone can adapt to the loads that it is exposed to (23). In 1960, Harold Frost refined this idea and developed the Utah paradigm of skeletal physiology also known as the mechanostat theory (23). The mechanostat theory states that bone adapts in response to loads placed on it but the reverse is also true; that bone is resorbed if the amount of load placed on it is less than a threshold value of voluntary mechanical loads (23,24). Thus bone is only as strong or as weak as the

loads placed on it and will never do more or less than is required to maintain current skeletal integrity. The updated version of this theory, published in 2003, continues to strive to relate cellular mechanisms to bone remodeling as seen in loaded and unloaded bone (25). Although the cellular mechanisms are not altogether known, osteocytes, osteoblasts and osteoclasts are the cells involved in the adaptation to loading and unloading of bone.

The cell that regulates the remodeling process is the osteocyte. Osteocytes are the most abundant cell in bone (26) and they communicate with one another and other bone cells via their cell processes that extend out from the cell into caniliculi that run throughout the bone matrix (27). Osteoprogenitor cells differentiate into the bone forming cells called osteoblasts. Once osteoblasts have performed their function they 1) differentiate into osteocytes and are embedded in the bone matrix; 2) become inactive bone-lining cells present on the surface of bone or 3) undergo apoptosis (28). The osteocyte is now recognized as the “master cell” that acts as the conductor in the orchestra that is bone remodeling (29). The osteocyte acts as the mechanical sensor and initiates signals to other cells in the vicinity to act accordingly to those strains or stresses (28). Therefore, it follows that remodeling of bone occurs in areas most subjected to mechanical loading (30). The signalling pathways resulting from shear stress stimulation of osteocytes are outlined in Figure 1.1.

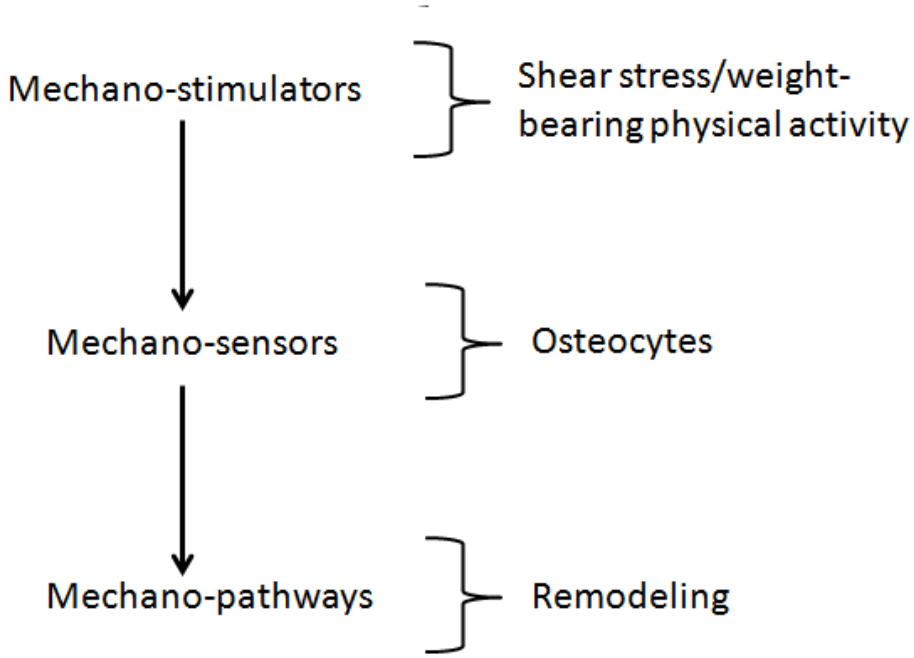

Figure 1.1 Mechanical signalling pathways in bone remodeling. Modified from Rochefort et al (20).

Osteocytes are sensitive to shear stresses generated by fluid flow within the caniliculi (31) and osteocytes translate these mechanical signals into biomechanical signals that promote bone gain or loss (32) (Figure 1.2). Researchers postulate that this is ultimately how loading promotes bone gain after birth but it is not known which signal from the osteocyte stimulates bone resorption by the osteoclasts (33). For example sclerostin, a protein highly expressed in osteocytes, is one of many molecular signals that can act on osteoblasts to inhibit bone formation (34).

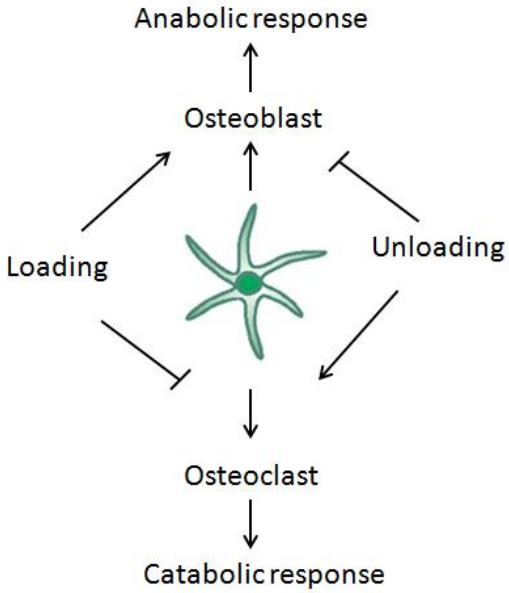

Figure 1.2 Diagram showing osteocyte activity under conditions of loading and unloading. Modified from Atkins and Findley (13).

Once the osteocyte has been stimulated to begin the process of remodelling at a specific site, osteoclasts adhere to the bone surface and the plasma membrane in contact with the bone surface develops a ruffled border. The ruffled border increases the surface area from which the osteoclast can secrete acids and proteases to dissolve the bone matrix (35). The collagen breakdown products (C- and N-terminal collagen cross-linked telopeptides) from bone resorption can be assayed in urine as a measure of the rate of bone resorption (35). The trail of bone dissolving osteoclasts is followed by bone laying osteoblasts that fill the areas of resorbed bone with new bone matrix (35). The entire process takes approximately three months (30). The concerted efforts of the osteocytes, osteoclasts and osteoblasts - together comprising the basic multicellular unit (BMU) - function to remodel bone in small packets along the surface of the bone (Figure 1.3) (36).

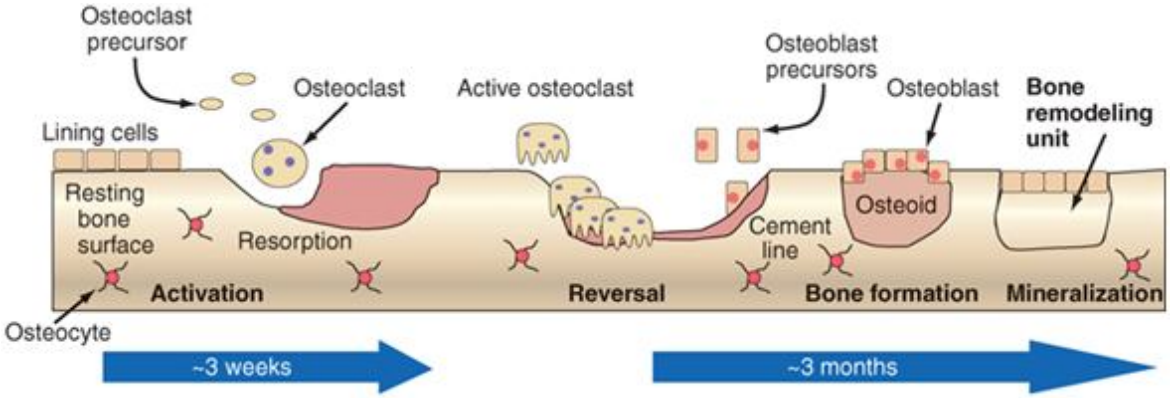

Figure 1.3 Schematic of the process of bone remodelling shown by resorption by osteoclasts and bone formation by osteoblasts. Source: Bringhurst et al (30).

1.3 Biological activity of bone turnover

As mentioned above, bone turnover is maintained by the ebb and flow of bone formation and bone resorption. Many serum and urinary markers of bone metabolic activity serve as indicators of whether bone turnover favours formation or resorption (37) and offer a more dynamic view of bone remodeling as opposed to scans which tend to give a more static analysis.

1.3.1 Bone formation markers

Bone Alkaline Phosphatase

Alkaline phosphatase (ALP) is an enzyme involved in the mineralisation of bone (38). Although there are many isoforms of ALP, the most common during skeletal growth is bone specific alkaline phosphatase (Bone-ALP) (39). Bone-ALP is solely associated with osteoblast activity (40,41).

Osteocalcin

Osteocalcin (OC) is also exclusively produced by osteoblasts. Although one would think that this makes its function in bone mineralisation clear, research has shown that mice without OC have an abnormal extracellular bone matrix however overexpression of OC does not influence mineralisation (37). Nonetheless OC is used as a marker of bone formation in humans and as such many studies have detected changes in OC after a single bout of some type of physical activity involving an element of mechanical loading such as volleyball (42) and running (endurance and short-distance) (43,44).

Insulin-like Growth Factor 1 (IGF-1)

Insulin-like growth factor 1 is positively correlated with markers of bone formation (45). Overall, the activation of IGF-1 signalling results in the proliferation and differentiation of osteoprogenitor cells into osteoblasts so that endochondral ossification can occur (46,47). Yakar and co-workers (48,49), showed that mice deficient in the IGF-1 gene exhibited impaired growth of cortical bone at the pubertal stage, however trabecular bone was not affected by the missing gene. The deletion of IGF-1 in osteocytes specifically affects bone longitudinal growth while the role of IGF-1 in osteoclasts is unclear (46) possibly due to the fact that osteoclasts express IGF-1 receptors, produce IGF-1 themselves and stimulate osteoclastogenesis in bone marrow stromal cells (50–52). The authors of one study hypothesised that there is a negative feedback effect on the osteocyte to stimulate production of osteoclasts (47). Sex steroids stimulate bone mass growth in conjunction with increases in serum IGF-1 levels and are positively correlated

with bone strength in both boys and girls in early puberty (53). Thus, when assessing environmental or hormonal impacts on bone, the role of IGF-1 should be considered.

1.3.2 Bone resorption markers

Amino-terminal cross-linking telopeptide (NTx)

Amino-terminal cross-linked telopeptides result from the degradation of the telopeptide in Type I collagen of bone (37). As NTx is completely cleared by the kidney it can be assayed in urine and is therefore a convenient marker of bone resorption especially in children. Maggio et al (54) showed that serum levels of Type I collagen cross-linking were decreased in response to a nine month weight-bearing exercise intervention, however this decrease was not associated with changes in total body areal bone mineral density.

Sclerostin

The Wnt signalling pathway (Figure 1.4) is gaining interest as a pathway that can be manipulated to influence the dynamics of bone remodelling. The pathway is initiated by the binding of Wnt ligands to a receptor complex composed of frizzled (FZD) and LDL related lipoprotein (LRP) 5. In the absence of ligand binding, β-catenin is degraded within the cell (36). The binding of Wnt ligands to the receptor complex results in β-catenin not being degraded and the transcription of genes for osteoblast differentiation and function is initiated (36,55) (Figure 1.4). The LRP5 receptor also occurs on osteoblasts and the binding of agonists to this receptor leads to stimulation of the Wnt signalling pathway and consequently bone formation. Sclerostin is an osteocyte selective marker (32) and is produced exclusively by mature osteocytes. Sclerostin, which is an intracellular inhibitory

signal, is involved in reducing bone formation. The binding of sclerostin to LRP5 inhibits this signalling pathway and thus sclerostin has been implicated as a signaller of bone loss (56).

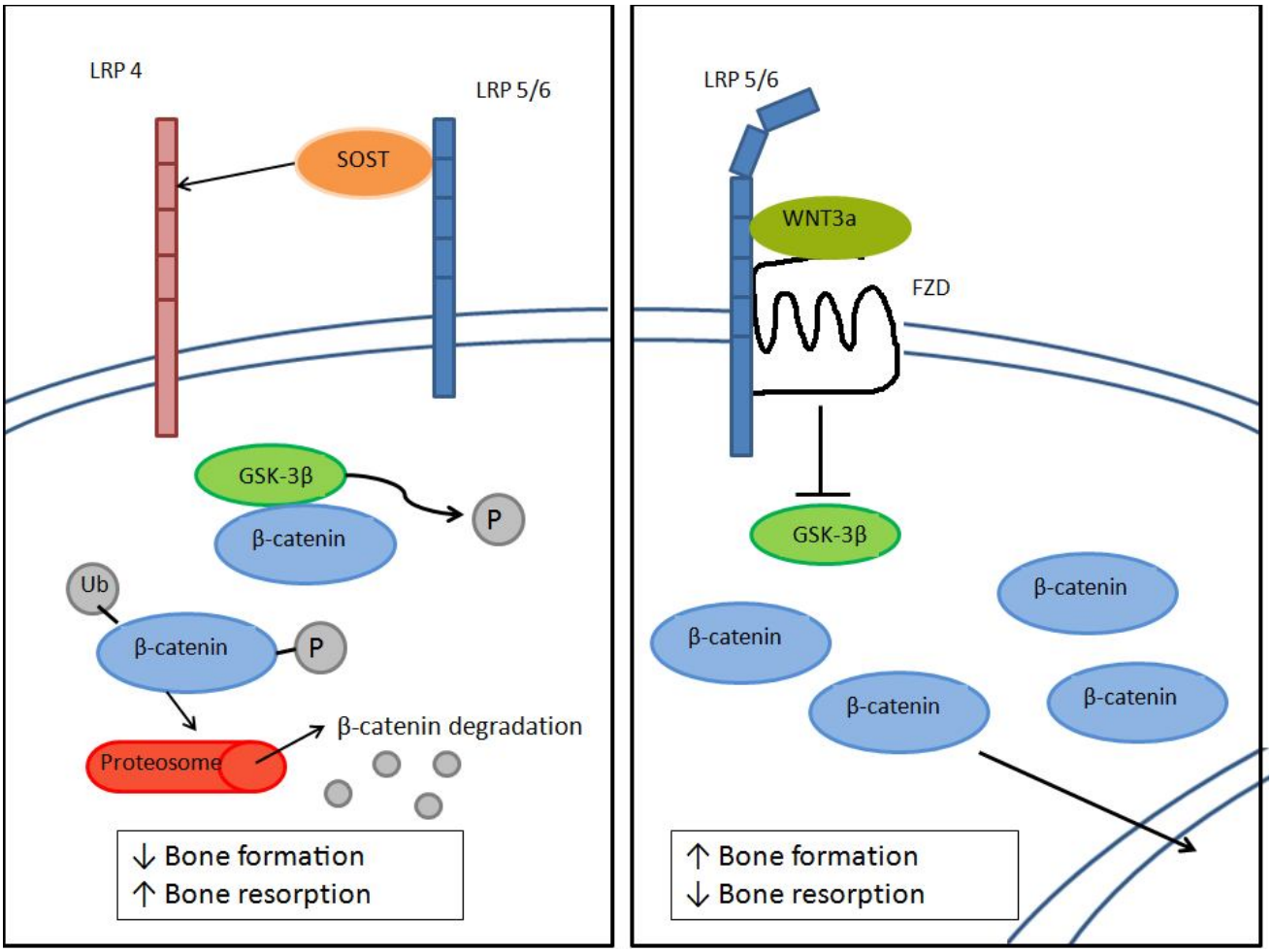

Figure 1.4 Simplified schematic showing Wnt signalling. Modified from Baron and Kniessel (55).

Studies of sclerostin in children are limited. One cross-sectional study has observed changing sclerostin levels in children and adolescents in response to growth (57). Sclerostin levels increased during early puberty up to the onset of puberty, and subsequently started to decline at the bone age point equivalent to the onset of puberty, while levels appeared in higher amounts in boys compared to girls during puberty (57).

The authors mentioned that the decline is due to the influence of sex hormones at the onset of puberty (57) and from a bone perspective, the increase in bone mass accrual rate that occurs during puberty may also contribute to the decline in sclerostin levels. The effect of physical activity on sclerostin has been investigated in a population of healthy adults and its expression is suppressed in a more physically active cohort (58). In addition, rats who have their hind limbs unweighted exhibit high levels of sclerostin compared to controls (59,60). The effect of physical activity on levels of sclerostin has not been investigated in children of pre-pubertal maturation status nor has any ethnic difference in sclerostin been documented in children or adults.

Although discrepancies in biochemical markers of bone metabolism have been reported in studies of mechanical loading, Banfi et al (37) concluded that the duration of exercise and length of time after exercise is performed is important in detecting changes in biochemical markers but due to the variability in the data, our understanding of the role of biochemical markers in mechanical loading is still limited.

1.4 Bone growth in children and the importance of the pre-pubertal years

Both modeling and remodeling occur during childhood growth and are influenced by factors such as genetics, nutrition and physical activity (22). During youth, the process of remodeling occurs at a rapid rate compared to that in adulthood. However, the coupling of linear growth and modeling overshadow the remodeling effects (35), making the remodeling process during youth a challenge to study accurately. Adult bone loss, due to

increased bone remodeling, may be offset by a greater peak bone mass attained during youth. Bones grow in length because of the presence of a cartilaginous growth plate that is found at the ends of long bones (61) (Figure 1.5). This growth plate contains a number of horizontal areas at various stages of chondrocyte differentiation. When resting chondrocytes at the epiphyses of long bones enter the next area of differentiation, they proliferate and subsequently start to hypertrophy (61). The hypertrophied chondrocytes then secrete matricellular proteins and calcium and the surrounding area becomes mineralized (62). At the junction between the growth plate and the diaphysis or shaft of long bones, i.e. the metaphysis, unmineralized areas are resorbed by chondroclasts (62,63). The spicules of bone left behind create spaces called trabeculae into which blood vessels occupy and onto which osteoblasts lay bone (61). Further away from the metaphysis, i.e. towards the centre of the bone, there is less trabecular bone since the bone has mostly been resorbed during the longitudinal growth of the bone (61). Increases in trabecular thickness result in the growth of trabecular bone but without an increase in trabecular number during the growing period (26). These changes are small in children and result in a slow increase in trabecular density with age (64).

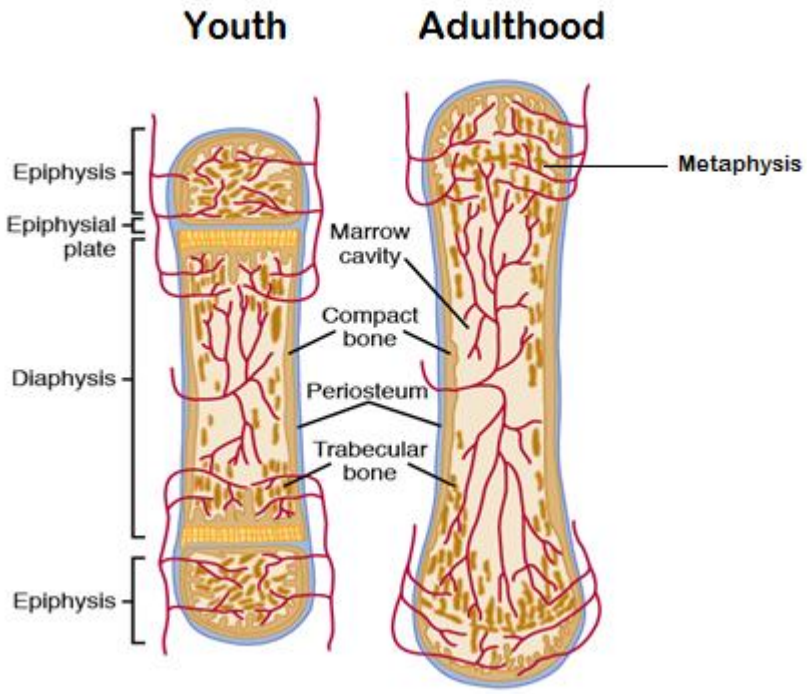

Figure 1.5 Diagram of a long bone during growth. Source: Barrett et al (12).

Bone growth in width is also important for bone stability during growth. The periosteum is the outer layer of bone (61,65). As bones grow in length, the laying down of bone by osteoblasts at the periosteum (periosteal apposition), results in a widening of the bone. (66). Osteoblasts are the cells that lay mineralised tissue at the periosteal surface (64) thus increasing the bone mineral content and size of the diameter of the bone at the diaphysis. The thickening of the cortex of the bone gives it structural stability to withstand mechanical forces because the forces are distributed over a greater area (66). Periosteal apposition rates peak during puberty and slow considerably after puberty when growth has ceased (67). It is important to understand the factors that influence periosteal apposition during childhood, since the increase in periosteal circumference increases bone strength (66). As mentioned above, osteocytes can detect shear forces

from mechanical loading of bone (28). It is most likely that continuous stimulation of the osteocytes increases the activity of osteoblasts on the bone surface, modifying the size and shape of the bone (68) and allowing the bone to adapt to forces placed on it. These adaptations have been shown to occur in children who take part in regular weight-bearing activity. Ultimately the goal of increasing bone mass in childhood is to create structurally stronger bones that will cope better with the inevitable gradual bone loss that occurs throughout adulthood. The responsiveness of paediatric bone to factors that influence bone growth (which will be discussed in section 1.4) may determine the condition of the skeleton throughout adulthood.

The maximum rate of bone accrual in a lifetime occurs at a similar time in both boys and girls; approximately eight months after peak height velocity is reached (69,70). Therefore the rate at which one gains bone mineral content during this rapid growth spurt plays an important role in determining the peak bone mass that is attained and available for the rest of one's lifetime (71) (Figure 1.6). Peak bone mass is the maximum amount of bone mass an individual will gain during their lifetime and is achieved around the end of the second decade of life (72). Nearly 40% of peak bone mass is accrued in the two years before and the two years after peak height velocity is reached (72). The amount of bone that an adult has at any point is merely the difference between the maximal amount of bone gained at skeletal maturity (peak bone mass) and the amount of bone lost up to that age as an adult. Yet, the relationship between bone mineral gain during childhood and adolescence, and bone mineral loss and skeletal fragility as an adult, are not fully understood (73).

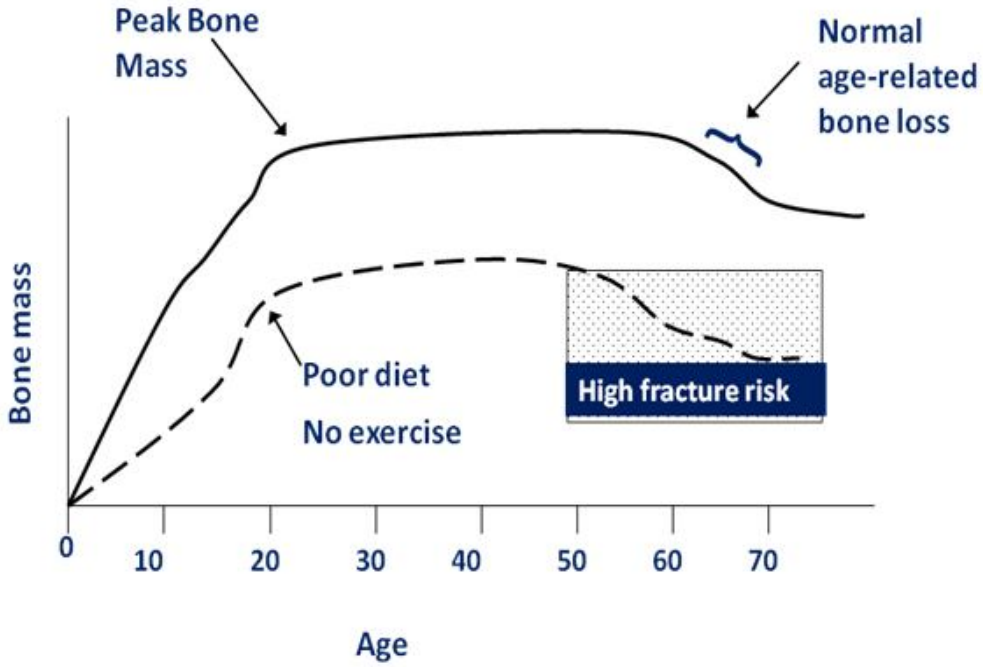

Figure 1.6 Schematic showing the environmental influences on the attainment of peak bone mass in childhood and the age-related bone loss that occurs after approximately the second decade of life. Modified from Heaney et al (63).

It has been suggested that peak bone mass is an important determinant of the risk of osteoporosis as well as development of fractures later in life (74,75). Studies have suggested that a low peak bone mass may be more important in contributing to osteoporosis than rapid loss of bone during ageing (76). These studies imply that the best chance of conserving bone health in later life lies with attaining a high peak bone mass during childhood, especially during the pre-pubertal years when the rate of peak bone mass accrual is at its highest (77–79). In children, rate of bone mineral accrual is at its highest between the ages of 11-14 years of age with boys still accruing bone mineral slightly later than girls (74,80). Bone mineral accrual then slows down after approximately

16 years of age (74) or two years after the age at which the maximum growth rate (peak height velocity) is achieved (80). Based on these findings, any intervention put in place to achieve maximum peak bone mass should occur at a pre-pubertal and early adolescent stage rather than at a post-pubertal age. Harnessing the power of the natural rapid rate of bone growth in order to maximise peak bone mass will undoubtedly prove an effective prevention for fracture risk later in life.

1.5 Measurement of paediatric bone

In order to gain insight into the mechanisms of bone growth and changes that occur as a result of intrinsic and environmental influences, accurate techniques for the measurement of bone content and structure are needed.

1.5.1 Dual Energy X-ray Absorptiometry (DXA)

DXA is a simple method of measuring bone mineral content and bone area over the whole body (81). Bone mineral density (BMD) is then calculated from bone area and bone mineral content and is reported in g/cm^2. BMD measured by DXA is a two-dimensional measure and is therefore referred to as areal BMD (aBMD) – because it is not a true volumetric measure (22). DXA not only measures peripheral skeletal sites such as the forearm and lower limbs but can also be used to measure sites of the axial skeleton including the hip and lumbar spine – two sites that are most often subject to osteoporotic fracture. DXA emits two proton energies which allows for the differentiation between hard (bone) and soft (lean and fat) tissue due to the difference in composition between

these two tissues (82). There are several limitations to using DXA for bone measurements. Because the body is composed of fat, lean and bone tissue, the differentiation between fat and lean tissue is estimated according to the known composition of these tissues; but, if the composition of the soft tissue surrounding the bone region of interest is unknown then BMD measures tend to be inaccurate in patients who are overweight or obese (82). DXA is also unable to distinguish between cortical and trabecular bone (83), an important factor in the determination of bone turnover. Another disadvantage of DXA is that BMD estimates are influenced by body size i.e. DXA underestimates BMD in very small patients (children), and overestimates BMD in very tall patients (83). Nonetheless DXA remains the gold standard method of clinical bone density measurement because hip BMD is the most reliable measure of predicting hip fracture risk (83,84). The reproducibility of DXA scans, like any assessment tool, is subject to precision errors but typically the coefficients of variation (CVs) are approximately 1-1.5% for lumbar spine and total hip BMD and 2-2.5% for femoral neck BMD (85). DXA emits relatively low levels of radiation. As an example, depending on location, natural background radiation that one is exposed to on an annual basis is estimated to be 240 – 2000 microSieverts (μSV) (86). In comparison, for a six to eight minute DXA scan of the proximal femur and lumbar spine, the radiation dose is between 0.1 and 0.5 μSV (87).

1.5.2 Peripheral quantitative computed tomography (pQCT)

Peripheral QCT is a method of determining detailed bone measures at peripheral sites, usually the forearm and lower leg. X-rays are taken in approximately 10mm sections at various sites along the length of the bone, rotating around the peripheral site and

allowing for determination of volumetric bone mineral density (vBMD) (86). Peripheral QCT provides three-dimensional analysis of the strength and geometry of a bone (88) and also has the ability to separate trabecular bone from cortical bone allowing for interpretation of bone geometry (86) (Figure 1.7). Trabecular bone is measured at the metaphysis (4% of the length) of the bone while cortical bone is measured at the diaphysis (38 and/or 65% of the length) of the bone.

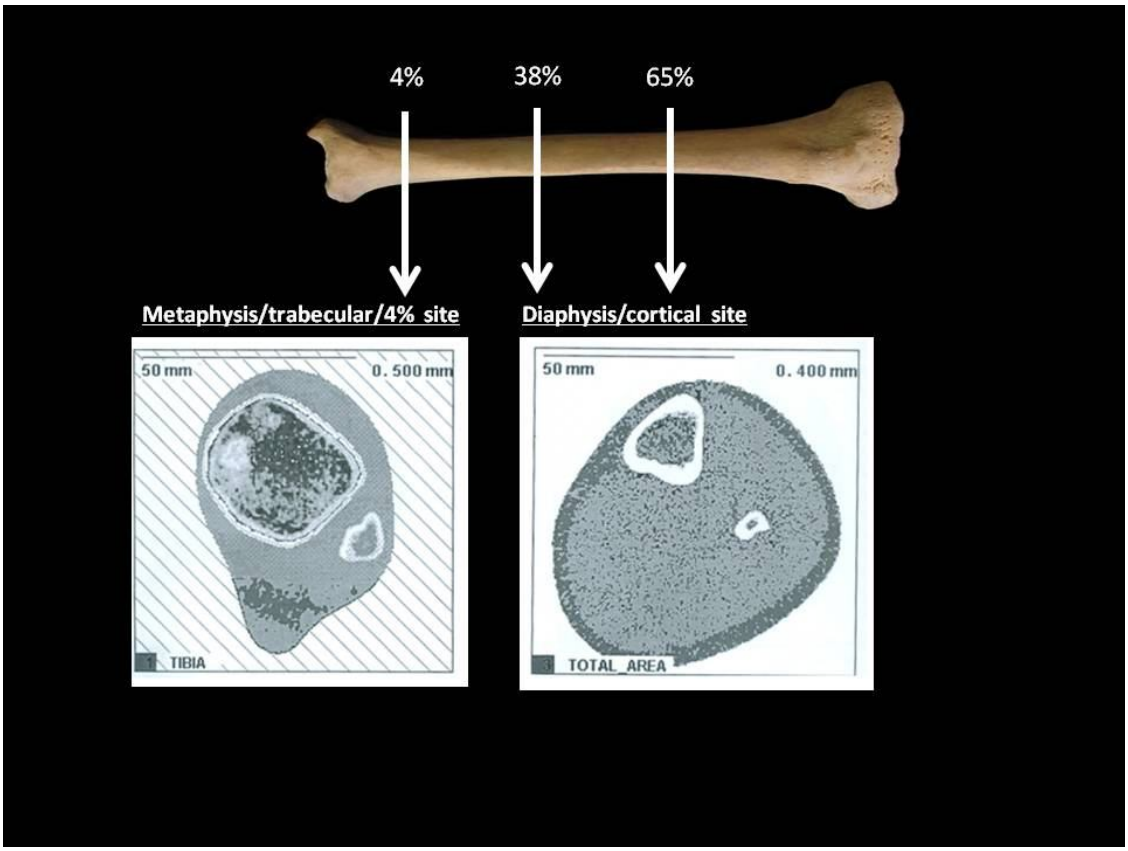

Figure 1.7 Metaphyseal (4%) and diaphyseal (65%) pQCT scans of the tibia.

Table 1.1 summarises the most commonly reported measurements that can be obtained at the trabecular and cortical sites of the radius and the tibia using pQCT. For abbreviations used throughout the thesis, the list of abbreviations appears on page xii.

Table 1.1 Peripheral QCT parameters and their definitions

Variable name (units)	**Definition**
Bone mineral density (BMD) parameters	
Total BMD (mg/cm^3)	Total volumetric bone mineral density
Trabecular BMD (mg/cm^3)	Trabecular volumetric bone mineral density at metaphyseal site only (4%)
Cortical BMD (mg/cm^3)	Cortical volumetric bone mineral density (65%)
Structural and geometrical parameters	
Total area (mm^2)	Total bone cross-sectional area (CSA)
Cortical area (mm^2)	Cortical bone cross-sectional area
Cortical thickness (mm)	Cortical shell thickness
Periosteal circumference (mm)	Outer circumference of bone
Endosteal circumference (mm)	Inner circumference of bone
Polar strength-strain index (mm^3)	Indication of cortical bone strength. This measure takes into account the material properties of bone and is derived from the cross-sectional moment of inertia which represents the distribution of bone surface in a given area.
Bone strength index (mg^2/mm^4)	Indication of trabecular bone strength. Total area x Total BMD^2

The parameters determined by pQCT also translate into biomechanical data such as measures of bone bending strength, bone strength in torsion as well as stress strain index (22) and in exercise studies it becomes important to measure these indices of bone strength using pQCT in order to understand the effect of exercise on bone in children and adults. The importance of reporting these indices is that they help explain how bone

surfaces adapt to a prolonged period of external stress such as weight-bearing exercise. Further advancements in pQCT technology have allowed for new interpretation of bone data as a tissue that is influenced by the muscle that surrounds it (89). This interpretation supports Frost's mechanostat theory (23) as studies have shown positive relationships between the geometrical measures of bone and muscle cross sectional area as determined by pQCT (90–92). Thus in addition to measuring material and geometric/structural properties of a bone, one can also measure the cross-sectional area of muscle (MCSA) surrounding that bone and the influence thereof. Peripheral QCT is also not without limitations. One such limitation in children is the variability that can occur in metaphyseal morphology due to the high rate of bone turnover at this site (93). Thus accurate positioning of the limb is required and it is favourable to use the same experienced and dedicated technician for the positioning and interpretation of the scans. Reference data for the radius and tibia of children have been published but are not yet available with the equipment's software (94). An additional limitation to consider is that of partial volume effects. The pQCT image is made up of many pixels with definite edges. If the bone is smaller e.g. in children, there may be a greater proportion of partial pixels because closer to the edge of the bone there is a higher chance that bone and soft tissue overlap. Thus the attenuation of the x-ray beam is lower than in reality and density measures may be underestimated (94,95). Newer software procedures can overcome or minimise this limitation by analysing individual pixel layers separately (95). Although higher than DXA, the radiation dose emitted from a forearm QCT scan is approximately 0.30 μSv (22).

1.6 Factors influencing bone

1.6.1 Sex and puberty

Sexual maturity is an important determinant of bone mass in boys and girls. Longitudinal studies have shown that before puberty, bone growth in length and width is similar in girls and boys (96,97) whereas sex differences in bone are mainly seen during and after puberty has begun (98–100). It is acknowledged however, that variation in sexual maturity between children may occur within the same category of sexual maturity classification. The Tanner stage criteria (101) involve pictorial representations of breast development (girls), gonadal (boys) and pubic hair stages; children, or their care-givers, are asked to self-select the stage that best describes themselves or their children (Appendix A). Children can be classified as being in one of five stages of sexual maturation (stages I – V). Stage I is when the child is pre-pubertal and stage V is when the child has reached pubertal maturity (post-pubertal). Gender differences in bone turnover markers in children also become evident from Tanner stage IV until complete sexual maturation is reached (98,102). Peak height velocity is reached at an approximate age of 14 years for boys and 12 years for girls after which, a greater gain in BMC is seen in boys than in girls (80). Because of these sex differences in bone after the onset of puberty, the assessment of sexual maturation is imperative in studies of paediatric bone. Ashby et al (103) found that sex differences in BMC at the whole body, leg and lumbar spine mostly occurred in the later stages of puberty i.e. stage IV and V. Differences in radial cortical density have also been observed between peri- and late pubertal boys and girls (104). Gender differences in total and cortical areas of the tibia occur as a result of greater periosteal

apposition in boys compared to girls (105), confirming that structural differences also become evident at the onset of and during puberty. Volumetric BMD increases at a greater rate in boys than in girls after puberty (90). In addition, measures of bone geometry and strength increase at a greater rate in boys than in girls after the onset of puberty (100). It has been speculated that this greater rate in boys occurs because androgens stimulate periosteal apposition causing bigger and therefore stronger bone, while oestrogens stimulate endosteal apposition (100,106). In summary, the abovementioned studies indicate that gender differences in measures of bone mass, bone density and bone structure become evident after the onset of puberty and that differences most likely occur because of the presence of different hormones in boys and girls after the onset of puberty.

1.6.2 Nutrition and socioeconomic status

Daily calcium requirements during growth in children are recommended at an intake of 1300 mg/day for children between the ages of 9-18 years (107,108). Calcium intake in children globally remains inadequate (107). All the same, little evidence is available to show associations between calcium intake and bone outcomes measured by DXA and calcium supplementation has been found to have no residual benefits to fracture risk in children (109). Moreover, despite lower levels of calcium intake reported in black versus white South African children, black children still display greater BMC compared to white children of the same age (110,111). This ethnic difference in BMC has been attributed to greater calcium absorption and retention in black children compared to white children (112,113). Vitamin D is also a vitamin required for bone metabolism, a deficiency of

which can lead to growth problems in children and osteoporosis as an adult (114). Sun exposure is the main source of vitamin D and although South Africa is generally a sunny country, one of the risk factors for low vitamin D status is non-white ethnicity (114). A review on the efficacy of vitamin D on bone health identified that there is a lack of studies in children as well as in different ethnic groups and therefore the efficacy of vitamin D supplementation in children is not well known (115). Although Ryan and co-workers, in a case-control study, found an association between lower bone mineral density and increased odds of forearm fracture in African American children (116), the proportion of children in each group (fracture and control) with a vitamin D deficiency was not significantly different and in addition it should be noted that the children with fractures had a greater BMI than those who did not have a fracture. Thus other factors may influence bone to a greater extent than calcium and vitamin D status (117).

South Africa is classified as a developing or low- middle income country. It is a country where there are disproportionate inconsistencies between the upper and lower income-earning citizens and basic healthcare is often not easily accessible to the majority of poorer people. Closely linked to and what may influence the nutritional status of an individual, is that of an individual's socioeconomic status (SES). Childhood socioeconomic status has thus been shown to influence health in later life (118,119) including musculoskeletal health (120). In a study of more than 300 nine to 10 year old children, current SES was found to be a strong predictor of whole body bone area and BMC but no associations were found between SES and hip or spine bone mass (120). In contrast, a study done in Spanish adolescents (aged 12-17 years old) reported no associations

between SES and whole body or hip BMC (121) even after adjusting for similar covariates as in the former study. An explanation for the differences found in whole body BMC between these two studies is that the former study took place in a population that was historically more disadvantaged in terms of SES, while the latter took place in a European population. On the other hand SES may in fact not appear to have any relationship with BMC and other factors have more of an influence on bone mass in childhood. Interestingly in a retrospective study of American middle aged adults, an association between childhood SES and adult femoral neck bone strength was seen in white men only but this relationship was attenuated when adjustment for factors such as age, current smoking status and current physical activity were accounted for (122). It is likely that the relationship between SES and bone health is a complicated one, influenced by many other genetic and environmental factors, making the relationship difficult to define clearly.

1.6.3 Ethnicity

The age-adjusted hip fracture incidence is 50% lower in African American women than in Caucasian women (123–125). What limited information there is, suggests that black South African adults also have markedly lower hip fracture rates than white South African adults (126,127). The situation in South Africa is quite unique, in that the black adult population has one of the lowest rates of hip fracture in the world. There is evidence however, that fragility fractures in African American women are underreported and very often go unrecognised (128). In addition, despite the lower incidence of osteoporosis in post-menopausal black women, those that do sustain a hip fracture have an increased

morbidity and mortality associated with the fracture compared to white women who suffer from hip fractures (125). Very few of the factors influencing osteoporosis have been well studied between race groups. Aloia and co-workers (129) found that a significantly greater number of black women over the age of 80 were classified as having osteoporosis compared to the number of women who were osteoporotic at 65 years of age. As lifestyle and dietary patterns change with urbanisation and there is a shift towards westernised diets and sedentary behaviour, fractures in an ageing population of South African blacks may become more prevalent (130,131). Results from the first South African Youth and Risk Behaviour Survey (SAYRBS) showed that 45% of 13-19 year olds participated in sufficient vigorous physical activity (131). Of concern, is the decline in this statistic reported six years later (in the 2008 SAYRBS results), which indicated that only 43% of adolescents in the same group would be considered to be sufficiently physically active to be beneficial to health (130). With these changes in lifestyle, it will become increasingly important to prioritise osteoporosis and its related conditions as a major public health concern in South Africa. A number of studies have confirmed that African American adults and children have denser and therefore stronger bones than their white counterparts (96,110,132,133). In South Africa distinct ethnic differences have also been observed for certain measures of bone health i.e. black children and adults have bigger, stronger and denser bones than those of their white peers (134–139). South African black children have greater hip and spine BMC compared to white children of the same age and sexual maturity (135). Using pQCT, Wetzsteon et al (133) have shown that African American children also have significantly stronger bones than their white peers. In a comprehensive study by Leonard and colleagues (96) in 665 black and white American

subjects between 5-35 yrs, section modulus (index of bone strength) had a positive non-linear relationship with age. Furthermore the relationship between section modulus and age did not differ between Tanner stages I-III but was greater in stages IV and V. Black race had a positive association with section modulus and section modulus in black children was greater than in white children in Tanner stage I. Ethnic differences were evident throughout Tanner stages I-IV with cortical BMD and BMC higher in blacks than in whites, but these differences were attenuated in Tanner stage V. Black children had greater endosteal circumference than whites over all pubertal stages (96). Longitudinal studies which track the ethnic differences in bone between black and white children are required in order to confirm whether or not the ethnic differences that exist in childhood are responsible for the lower fracture rate observed in black adults. Hui and co-workers (140) followed a group of American black and white boys who started the study when they were between 5-15 years of age. The boys were followed up between one and four years. Bone growth velocity for total body BMC and total body bone area were determined from follow up scans using DXA. The black children had greater velocities of change in BMC and bone area in the pre-pubertal stages only and this may be the reason for the greater peak bone mass observed in black versus white children (140). One limitation of this important study is that the children should have been matched for Tanner stage and not age although the authors argued that black children may be more sexually mature than a white child of the same age within a given Tanner stage. Future studies that match black and white children for Tanner stage or at least focus on the various stages of puberty should be done in order to answer the important question of whether rates of bone gain velocity are different between black and white children.

Only one study has examined ethnic differences in structural and geometric bone measures derived from pQCT scans in black and white South African children (141). In this study of adolescent (13 year old) children, black children had greater cortical area and strength at the tibia compared to white children. It has been postulated that bone differences between black and white children are attributed to a greater lean mass and lower rate of bone turnover in black children (78) but similar and more recent studies have either found no difference in lean mass between races (142) or have actually found that white children have greater lean mass than black children (136,143,144). Longitudinal studies on velocities of change in the strength and structure of bone between different ethnic groups are lacking. The availability of pQCT allows us to explore this research question. Following on from the Hui et al (140) study we may hypothesise that ethnic differences in exist in the way bone structure changes through growth and it is likely that this could be a possible mechanism as to how fracture risk is lessened in black adults. The availability of pQCT technology allows for the better interpretation of how and where bone is laid down in response to physical activity and growth and it gives an indication of how it may differ between ethnic groups.

1.7 Physical activity

Bone structure and content remains stable from the end of the second decade of life but with age, bone loss exceeds bone gain (Figure 1.6). Thus the shaping and moulding of bone into the optimum structure to compensate before bone loss begins is important. Bone remodeling from exercise is evident in both adults and children. Physical activity (PA) has the ability to alter the shape of bone such that it becomes more effective in

resisting external forces (145). The pre- and early pubertal epoch represents an opportune time for responding to external factors affecting bone's natural growth. Since the inception of the mechanostat theory, there have been a plethora of studies investigating the relationship between mechanical loading (via participation in weight-bearing exercise) and bone mass and density. Weight-bearing exercises which induce mechanical loads on bone include sports such as basketball, tennis, gymnastics or any activity that includes repetitive jumping. Bone adaptation to physical activity can occur either internally (via changes in the material properties of bone), or externally (via changes on the outer surface of bone) and these adaptations can be triggered via mechanical stimuli on the bone (146), which can be generated by participation in certain types of exercise. It is possible to mathematically model bone's response to mechanical forces (i.e. forces due to weight-bearing physical activity) (146–148). However the measurement of physical activity forms an integral part of this thesis in determining the relationships between physical activity and bone response and will therefore be measured objectively and subjectively.

1.7.1 Measurement of physical activity

In order to determine the effects of PA on bone health in children, it is important to have accurate and reliable methods of PA assessment available as tools in research studies. These tools however must also be practical and research-friendly especially when measuring PA in children. The two most common methods used to assess PA in paediatric studies are accelerometers and questionnaires.

Accelerometry

The best method of objectively assessing both habitual and daily PA is through the use of accelerometers (149). Accelerometers are small devices typically worn on the hip underneath clothing or on the wrist and so allow unhindered assessment of PA in daily free living. Accelerometers have been used successfully in correlating PA patterns to health risks in adults (150) and children (151,152). Accelerometers are piezoelectric and so detect speed and intensity of the body's movement in various planes depending on the type of accelerometer used (153,154). Movement is detected and stored in raw format until the data can be downloaded and analysed using software for that specific device (155). Algorithms within the software separate the PA data into time spent in light, moderate and vigorous activity as well as estimates of energy expenditure or metabolic equivalents (METS). Figure 1.8 is a graphical representation of outputs (in METS) as determined by an Actical accelerometer over eight days. The IOWA bone development studies, among others, have shown good associations between accelerometer measured time spent in moderate to vigorous intensity physical activity (MVPA) and changes in BMC, aBMD and bone area as measured by DXA in children (156–159). Accelerometers have also been shown to be useful in studies on the associations between detecting ground reaction force exerted by different types of activity and energy expenditure, although there is inconsistency between types/brands of accelerometers (160). One study has shown that accelerometer measured vigorous intensity activity, more so than moderate, is a significant predictor of BMC of the total body as well as at the femoral neck of nine year old children (161), while another has indicated that there is better agreement between increased BMC and moderate intensity exercise (162).

Although useful in objectively measuring PA, accelerometers do have limitations. Understandably, accelerometers are not able to detect the weight-bearing nature of the activity. As such, accelerometry may not necessarily accurately reflect activity that is classified as weight-bearing exercise; for example, hopping on one leg is high weight-bearing but is measured by an accelerometer as low intensity exercise. Similarly, cycling is high intensity but low-weight bearing. Second, there are a number of commercially available accelerometers, each with its own algorithms for the calculation of the intensity of physical activity and energy expenditure (163). Thus a limitation of using accelerometry is that in order for comparisons to be made, the same device should be used within studies. Thirdly, using accelerometers in large epidemiological studies of physical activity is expensive. Added to this, in paediatric studies, a researcher may be more reluctant to use accelerometry in case such a costly piece of equipment and therefore the data is misplaced. Therefore, although accelerometers provide an objective measure of PA, these limitations mean that there remains a need for other techniques for the estimation of PA.

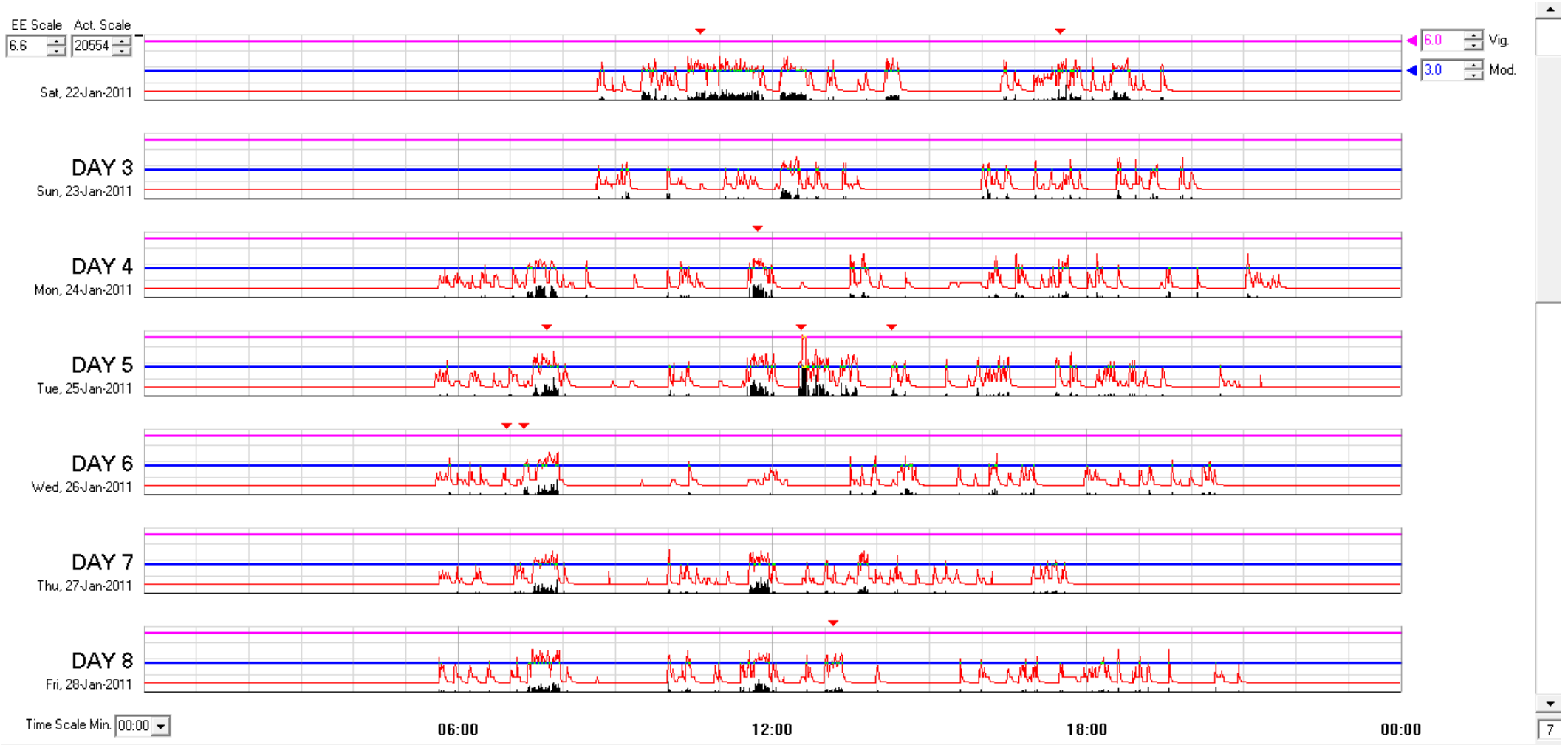

Figure 1.8 Graphical representation of daily outputs (in metabolic equivalents) as determined by the Actical accelerometer. The blue line represents the threshold for moderate activity while the pink line represents the threshold for vigorous activity. The black bars represent activity counts while the red graph represents energy expenditure.

Questionnaires

Self-report or interviewer administered questionnaires are frequently used to assess participation in PA. Their dominant use in paediatric exercise studies is due to the fact that they can be distributed *en masse*. Interviewer administrated questionnaires are likely to be more reliable than self-report questionnaires because the investigator is able to "interrogate" exact details on various domains of physical activity/inactivity such as structured and leisure activity, sedentary activity as well as habitual or work related activity (155). Several global physical activity questionnaires (PAQs) have been validated in various populations (154) and most questionnaires show agreement with accelerometer derived measures of PA in both adult and child populations (164–167). When choosing a questionnaire several factors need to be considered; including the population to be sampled, the time period of the recall and the method of administration (self-report or interviewer based). There are major limitations with the use of questionnaires and these include overestimating time spent in certain intensities of activity, difficulty with the recall period especially if longer than one week as well as demographic biases especially when used in children (154,155).

Similar to accelerometer derived measures of PA, self-report questionnaires that delineate the frequency and intensity of exercise, have been shown to be positively associated with BMD in young adult women (168). Weeks and Beck (169) developed a bone specific PAQ (BPAQ) for use in young adults which explained between 40% to 60% of the variance in aBMD at the femoral neck and lumbar spine in men and in calcaneal

bone ultrasound attenuation in women. Few studies have validated bone specific physical activity questionnaires in children (164,165) and those that have, have been limited by the use of estimates of weight-bearing factors to predict the bone loading that occurs in children either without measuring bone parameters themselves (160,165) or without measuring intensity of activity (170). Most studies on PA (measured by either accelerometry or questionnaires) and bone health in children have used DXA derived BMC as the primary outcome. Only recently has one study reported positive associations between a specific weight-bearing physical activity questionnaire and bone area, density and strength, as measured by high-resolution pQCT (HR-pQCT) in adolescent boys and girls (171). Because the effects of weight-bearing physical activity are more pronounced in pre-pubertal children, studies on the associations between PA as measured by questionnaires and accelerometry are needed using pQCT derived measures of bone size and strength in order to describe sport-specific changes induced by weight-bearing physical activity.

1.7.2 Physical activity and bone

Large longitudinal cohort studies such as the Saskatchewan Paediatric Bone Mineral Accrual Study (172) and the IOWA bone development study (158,173) that started in the late 90's have aimed to study the bone growth that occurs in children and adolescents in response to mechanical loading. Hind and Burrows (76) comprehensively reviewed the earlier literature (2005 and prior) on weight-bearing exercise and bone mineral accrual in pre-pubertal children. Most of the studies used DXA as the method of bone health

assessment and overall the authors found improvements in femoral neck and lumbar spine BMC as well as aBMD with an increased exposure to mechanical loading in children.

DXA studies

Many cross-sectional studies have shown strong associations between PA and DXA derived BMC, aBMD and strength in pre-/early pubertal children (161,174–178). Early DXA studies on pre-pubertal tennis players were key in showing the effects of weight-bearing physical activity on bone health. Most of these studies found increased BMC, bone area, aBMD as well as a greater lean mass in the dominant (playing) arm of the tennis players compared to their non-dominant arm (177,179), while lumbar spine BMC and aBMD were similar to controls (177). Findings from targeted weight-bearing exercise interventions in a school-based setting show substantial increases in bone mass for the whole body, femoral neck and lumbar spine over and above normal growth in as little as seven months (180,181). Gains in BMD and BMC have been seen after varying durations of exercise interventions. The ideal duration and intensity that a weight-bearing exercise intervention should be to have a significant effect on bone health has not yet been fully established (76). After an 11 month jumping intervention, MacDonald et al (182) reported greater changes in femoral neck BMC in pre-pubertal girls but not in boys, whereas McKay et al (183), reported greater total body, intertrochanteric and proximal femur BMC in pre-pubertal children after only eight months of a jumping intervention. Another issue to consider are the logistics of implementing plyometric exercise interventions into everyday practice. Ultimately the type of exercise must be practical and sustainable if it is to be carried out over a child's lifetime. In a similar nine-month

study in pre-pubertal and pubertal children, the combination of 10 minutes of jumping within a 45 minute exercise session resulted in greater increases in BMC at the total body, femoral neck and lumbar spine compared to children who did not perform the intervention (184). What has been established is that the bones of pre-pubertal children are more responsive to a bone loading exercise intervention compared to pubertal children, confirming the efficacy and importance of implementing weight-bearing exercise interventions before the onset of puberty. Historically, previous studies used DXA to elucidate structural responses of bone to mechanical loading. However, with the availability of pQCT technology, the effect of weight-bearing exercise interventions on bone health, as measured by pQCT, can now be fully investigated.

pQCT studies

DXA is advantageous in assessing physical activity effects on clinically relevant bone sites such as the femoral neck and lumbar spine (181). Although pQCT is only able to scan the trabecular and cortical bone at peripheral sites, the images allow researchers to assess where bone is laid down in response to mechanical loading as well as growth. Cross-sectional studies that report bone outcomes measured by pQCT indicate that weight-bearing exercise during growth in children results in the apposition of bone on the periosteal surface (Figure 1.9) (66,181) resulting in greater total and cortical bone areas.

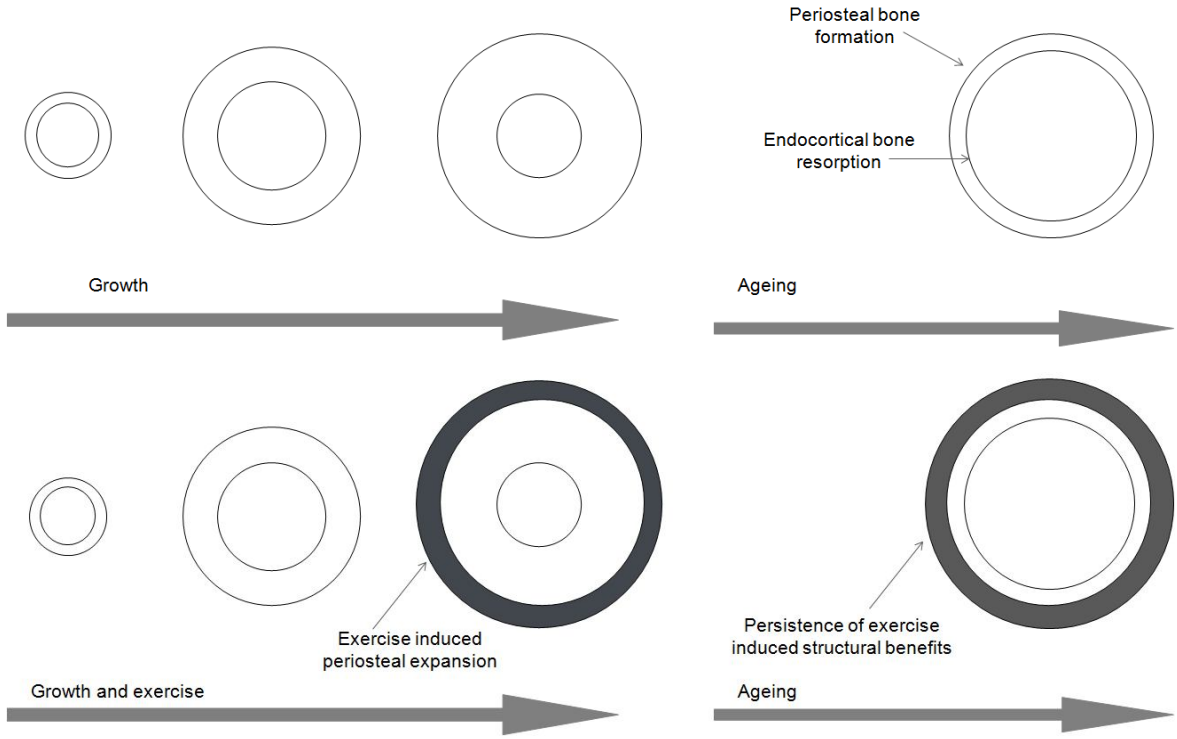

Figure 1.9 Physical activity results in the apposition of bone on the periosteal surface of bone. Picture modified from Warden and Fuchs (7).

Trabecular and cortical bone response to mechanical loading in children.

A meta-analysis that was published in 2013 determined the skeletal adaptation in pre-pubertal gymnasts (185). The authors identified only two out of 17 studies that used pQCT (one randomised controlled trial (186) and one cross-sectional study (187)) and both studies showed greater trabecular and cortical densities of the forearm in the gymnasts compared to controls. Another cross-sectional study in pre-menarcheal gymnasts between the ages of 9 and 13 years, showed that bone area at the trabecular site was not different between gymnasts and controls, but cortical area as well as periosteal circumference were significantly greater in the gymnasts than in the controls (188). In pre-pubertal children, increases in bone strength due to mechanical loading also

seem to be attributed to the bone apposition that occurs on the periosteal circumference of the cortical sites. When comparing various bone measures in gymnasts and controls, Ward et al (186) found that gymnasts had greater mid-radial strength as well as a larger cortical area at this site. Trabecular BMD was also increased in gymnasts compared to controls (186). Janz et al (176) found a positive association between moderate-vigorous physical activity (as measured using accelerometry) and bone area and strength of the hip. Using a physical activity questionnaire, Farr and co-workers (189) observed correlations between the load, frequency and duration of PA (as calculated from questionnaires) and periosteal circumference, bone strength index as well as strength-strain index at the tibia of girls aged 8-13 years. Similar to the DXA studies that have shown bone mineral content benefits, Ducher and Bass (190) have conclusively shown that the changes in bone structure and strength due to physical activity are greatest in the pre-pubertal years. In this 12-month follow up study on pre- and postmenarcheal female tennis players, magnetic resonance imaging was used to determine total bone area, cortical area and muscle cross-sectional area of the humeri of the playing and non-playing arms. The annual increase in total area and cortical area was greater in premenarcheal compared to postmenarcheal girls. In addition, studies using pQCT in gymnasts have documented that the sport-specific bone gain is greatest at sites that are the most loaded (191,192). Burt et al (191) found greater bone density and strength in the distal radius in female pre-pubertal gymnasts compared to non-gymnasts. In addition, the total bone area of the proximal radius was greater in gymnasts than in non-gymnasts and therefore strength of the proximal radius was greater in gymnasts (185). Falk and co-workers (192) reported higher radial densities in hockey players and higher tibial

densities soccer players, in pre-pubertal males. All these data suggest that the osteogenic nature of exercise in pre-pubertal children is sport-specific with the most loaded bones having the most significant changes in bone content, density and structure. In gymnasts, where the trabecular bone of the forearm is exposed to compressive forces, adaptations are noted at this site, whereas in soccer players, whose tibiae are exposed to torsional forces due to sprinting and turning actions, bone adaptations occur at cortical sites. The mechanisms for these adaptations remain poorly understood and biochemical markers of bone turnover are likely to assist in explaining the adaptations of bone to exercise.

The abovementioned cross-sectional data are invaluable as sport and site-specific bone gain detected using pQCT, may help to elucidate the dose-response elicited by weight-bearing physical activity interventions that DXA studies have been unable to confirm. However, the use of pQCT in exercise intervention studies is limited to a handful of studies in pre-pubertal children. Table 1.2 below is a summary of the various weight-bearing activity interventions that have been done in healthy pre-pubertal children and the effects thereof on bone size, structure and strength as measured using pQCT. In this thesis, literature that pertains to pre- and early pubertal children and more recent data from 2003 to the present date will be discussed. Databases searched were Pubmed, ScienceDirect and SPORTDiscus. Key search terms used were “weight-bearing exercise” AND/OR “physical activity” AND “children” AND “bone mass” OR “bone density” OR “bone structure”.

Table 1.2 Overview of exercise intervention studies (published from 2003-2013) in pre-pubertal children using pQCT variables as the outcome measures.

Reference	Participants	Intervention	pQCT ROI	Results
Specker and Binkley 2003 (193)	Children (3-5 years old) Fine motor group n=57 Gross motor group n=62	Fine motor group = activities done while sitting quietly Gross motor group = warm up, jumping/hopping and cool down 60 minutes per day 5 x per week One year intervention	20% distal tibia	Periosteal and endosteal circumferences significantly greater in gross motor compared to fine motor group
Johannsen et al 2003 (194)	Pre-pubertal boys and girls Jumpers n=13; age: 10.3±5.3 Non-jumpers n=13; age: 10.0±5.1	25 jumps per day off a 45 cm high box. 12 week intervention	4 and 20% tibia	No effect of intervention on pQCT measures at 4 and 20% tibia.
MacDonald et al 2007 (195)	INT: n=145 pre-pubertal boys, age: 10.2±0.6; n=136 pre-pubertal girls, age: 10.2±0.6 and CON: n=64 pre-pubertal boys, age: 10.3±0.6, n=65 pre-pubertal girls, age: 10.3±0.5.	Additional PA 15 min 5 days per week AND side to side jumping 3 min/day 4 days/week. 11 month intervention.	BSI, ToA, ToD at distal tibia (8%) SSI, ToA, CoA, CoD at tibial mid-shaft (50%)	Change in BSI greater in INT than CON for boys. Midshaft SSI no difference in boys or girls. Change in 8% ToD greater in INT than CON for boys. No differences in girls.
MacDonald et al 2009 (196)	INT: n=139 boys, age: 10.2±0.6 CON: n=63 boys, age: 10.3±0.6	Additional PA 15 min 5 days per week AND side to side jumps 3 x per day 4 days per week. 11 month intervention	Tibial midshaft I_{max}, I_{min}, CoA, CT	No difference in percentage change in CoA or CT between groups. Change in strength of bone was greater in INT compared to CON (I_{max}).
Anliker et al 2012 (197)	INT: n=12 boys and n=10 girls, age: 10.5±1.2 CON: n=16 boys and n=14 girls, age:10.8±1.1	10 minutes jumping activity 2 x per week. 9 month intervention	4, 14, 38, 66% tibia	No difference in the increase in BMC, BMD SSIpol, bone measures between INT and CON. EC smaller in CON than INT.

ROI: region of interest, INT: intervention, CON: control, PA: physical activity, BSI: bone strength index, ToA: total area, CoA: cortical area, ToD: total density, CoD: cortical density, CT: cortical thickness, I_{max}: maximum area moment of inertia (axis along which it is hardest to bend the bone), I_{min}: minimum area moment of inertia (axis along which it is easiest to bend the bone), EC: endosteal circumference.

From the table above, it is clear that intervention studies using pQCT measures of bone are lacking. The data show inconsistencies where the changes in bone structure and geometry occur even after 11 months of participation in weight-bearing activities. Furthermore, there are currently no data that compare the effects of physical activity on bone structure between black and white children as all of the studies outlined in Table 1.2 took place predominantly in white children. Indeed, if we consider the clear ethnic difference that exists in bone outcomes, as described in studies that have used DXA and from the few studies that have used pQCT, it is plausible that responses to mechanical loading may vary between black and white children. Only two cross-sectional studies have considered the influence of PA on bone health in an ethnically diverse population of pre-pubertal children (135,198) however, both these studies used DXA to measure of bone health outcomes. In the first study, which reported on the associations between PA and bone mass in nine year old South African children, white children with higher levels of PA had greater gains in BMC and BA over a year compared to their less active white peers (135). This relationship was not evident in black children although black children had significantly lower levels of PA compared to white children. Barbeau et al (198) implemented an exercise intervention in black girls to determine body composition responses; the girls that exercised compared to the controls had significant increases in aBMD and BMC. More studies are needed to determine the influence of habitual and daily physical activity on the bone health of black and white children.

Another important factor to consider is whether the benefits of weight-bearing exercise are sustained following the decrease or complete cessation of participation in PA. There are some longitudinal data to show that indeed this may be the case. Sustained activity throughout childhood has been shown to provide between 8-15% greater total body, hip, femoral neck and lumbar spine BMC in historically active versus inactive boys and girls (172). Sustained benefits have been observed one or more years after the cessation of weight-bearing exercise (69,172,199–202). Although the study was cross-sectional in nature, Bass et al (203) matched young gymnasts with those who had retired and found that compared to controls, aBMD was higher in the retired gymnasts implying that there was some residual effect of participation in gymnastics in youth. Another follow up study in gymnasts demonstrates that the skeletal benefits from weight-bearing exercise in childhood can be sustained at least four years after the sport was stopped and remained apparent in early adulthood (202). The side-to-side differences in tennis players also exists in adults who have a longer history of participation in the sport implying that continued participation also contributes to the sustained side-to-side differences in BMC in the playing arms of adults (204).

Structural changes induced by long-term mechanical loading appear to be the reason for the sustained differences in loaded versus unloaded bones. In one study in pre-pubertal tennis players the difference in BMC favouring the playing arm (versus the non-playing arm) was due to a change in the size of the bone (205). In addition, the differences were evident up to three years after the tennis players had retired. Structural changes also show the possibility of being sustained for some years post-cessation of a weight-bearing

sport. More recently, in a study of gymnasts who had been retired for six-years, pQCT scans of the radius and humerus showed greater cortical and total CSA at the cortical and distal sites in the gymnasts compared to their age-matched controls (199). Specker and Binkley (193) followed up their cohort of 3-5 year olds for an additional 12 months after a year-long intervention of jumping and hopping (see results in table 1.2). Similar to other studies that have shown sustained structural changes in exercising groups, the greater periosteal circumference of the gross motor group was maintained 12 months after the intervention while endosteal circumference was not quite significantly bigger after 12 months post-intervention ($p=0.08$) compared to the fine motor group.

The myriad of DXA studies on pre-pubertal boys and girls indicate that high levels of weight-bearing PA benefits children in terms of having greater bone mass compared to children with lower levels of PA. Peripheral QCT studies show that children with higher levels of PA also have greater cortical bone areas as well as greater cortical bone densities, periosteal circumferences and therefore greater bone strength. The majority of these differences occur at the bones most loaded (radius and/or tibia) depending on the sport they have previously taken part in. The differences in bone mass and structure can be sustained into young adulthood. Many studies on physical activity and bone health in children have therefore suggested that physical activity in childhood has a major role to play in the prevention of osteoporosis in adulthood (12,173,176,181,206). This becomes important as time spent in sedentary behaviour is increasing especially in children (207). Further research is required on whether there is an ethnic specific osteogenic response to PA and specifically differences in bone outcomes measured by pQCT need to be

determined. Longitudinal data which track influences of growth and physical activity between black and white children are also needed.

1.8 The muscle-bone unit

Although many studies (as described above) have shown that participation in weight-bearing exercise is an effective means of stimulating bone gain, the mechanostat theory has evolved to include the influence of muscle forces acting on bone. Researchers have coined the phrase "the functional muscle-bone unit" rather than sole reliance on the principles of the mechanostat theory (208). The theory proposes that muscle force is the largest force that bones have to overcome in order for the skeletal system to facilitate movement and therefore the most strain placed on bone comes from skeletal muscles, an amount of strain that is significantly greater than any ground reaction force generated by weight-bearing physical activity (209). In pre-pubertal children, the effects of the muscle-bone unit are amplified because, as children continue to grow in length, greater lever arms and bending moments are created which results in greater bone deformation (210). Consequently, muscle size increases and bones adapt to the increasing muscle forces placed on them. The importance of the muscle bone unit has been demonstrated in human and animal models of limb suspension. In humans, after 21 days of unilateral lower limb suspension (which mimics bone losses seen in bed rest) significant reductions in BMC on the periosteal surface of the distal tibia were seen. Decreases in BMD at the tibial metaphysis have been observed in a rat disuse model of tail suspension (211) because of muscle inactivation (212). Non weight-bearing resistance exercise (213) and

resistance exercise in combination with vibration therapy (214) prevent declines in BMC due to prolonged bed rest. Some researchers believe that muscle forces are a sufficient stimulus to drive exercise-induced bone gain (215) and the abovementioned data provide evidence to show that muscle forces alone can have an important benefit to bone. This concept becomes important in age-related sarcopenia (however this topic is beyond the scope of the current thesis). The correlations between muscle and bone cannot reflect causality and some researchers state that gravitational force and muscle force are inherently linked such that the associations between the two cannot explain whether one or the other has the greatest influence over bone (216). The bone gain that results from participation in weight-bearing physical activity, in combination with the bone response to muscle forces during participation in physical activity, are most likely to be significant contributers to the attainment of peak bone mass. Therefore, participation in all forms of physical activity and not just in weight-bearing sporting codes, is essential for optimal bone health (97,208,217).

Numerous studies have shown the close relationship between muscle and bone in pre-pubertal children. In pre-pubertal males and females, muscle CSA (as measured using pQCT), fat free/lean mass (as measured using DXA) as well as the power and force generated from a two-footed counter-movement jump, are all strongly and positively associated with bone strength at the tibia mid-shaft (20%) (89). Associations between thigh muscle strength and areal BMD have been observed in female soccer players aged 16 years (218). Dowthwaite et al (219) have shown similar correlations between muscle and bone measures (using pQCT) in gymnasts and in non-gymnasts. Forearm muscle CSA

was only moderately correlated to strength of trabecular bone against compression in the gymnasts, while in the non-gymnasts, forearm muscle CSA was associated with strength-strain index at the midshaft of the radius. Fat free mass (FFM) was also correlated to bone variables in both gymnasts and non-gymnasts. In this case, correlations in the gymnasts were observed between FFM and total arm BMC, total bone area at the 4% site, as well as cortical area and SSI at the midshaft of the radius. In the non-gymnasts, correlations were observed between FFM and total arm BMC, cortical CSA and SSI at the mid-shaft. The authors concluded that the bone gain due to exercise is not only because of muscle effects, but the actual loading of the bone is important in determining the ultimate size and content of the bone. It is important to highlight that these studies were all of a cross-sectional nature and therefore there is difficulty in defining causality of the bone gain. In addition, no studies of unloading have been done in healthy children since they are invasive and uncomfortable thus the cause and effect between muscle and bone in growing children remains difficult to study. One example where unloading may be studied is in children who suffer from cerebral palsy.

Although the muscle-bone relationship has been well characterized in children (220), it appears that the muscle-bone unit may not explain the ethnic differences in bone mass, size and structure observed between black and white children. Wetzsteon et al (133) have shown that the higher bone strength, area and density in African American compared to Caucasian children, were not attributable to differences in muscle CSA because bone outcomes were adjusted for any differences observed in muscle CSA. In addition, it has been consistently shown by Micklesfield and colleagues that muscle CSA is

greater in white compared to black South African children (136,143,144) but the same is not true in black and white North American children (144). As alluded to above, muscle CSA is one of many covariates influencing bone and a bigger muscle size is not necessarily related to the maximum force that a muscle can produce (221). Ethnic differences in muscle fibre size, type and recruitment during contraction may also confound our current perceptions of the muscle-bone unit in children. Therefore the reasons for ethnic variability in bone with regard to muscle force remain poorly understood mostly because this relationship has not been thoroughly studied in children of different ethnic groups. In studies observing the effects of weight-bearing physical activity on bone health between different ethnic groups of pre-pubertal children, the assessment of gravitational load as well as muscle force effects remain to be delineated.

1.9 Summary and objectives

In pre-pubertal children it is unclear why differences in measures of bone mass, size, strength and structure exist between black and white children. These differences have been attributed to a genetic advantage afforded to black children (6) and perhaps, because of this genetic advantage, studies on physical activity effects on bone health in black children have been overlooked. Few studies have compared black and white children with respect to physical activity participation, no studies have conducted physical activity interventions in black children and furthermore the use of pQCT as a measurement tool is limited in South African studies. Therefore the largest unresolved issue is: What are the effects of physical activity on the bone health of black and white South African pre-pubertal children? The remaining specific questions summarised below gave rise to the progression of this thesis. The rationale and aims of the four studies are outlined below.

Study 1 – Associations of objectively and subjectively measured physical activity with trabecular and cortical bone properties in pre-pubertal children.

- There is still a need to indirectly and non-invasively quantify the amount of bone loading that occurs in different types of physical activities. In South Africa, a specific questionnaire for the assessment of bone loading from participation in weight-bearing physical activity is lacking.
- From the bone-specific physical activity questionnaires that are available for assessment of bone loading in children, there are few studies in children that

correlate and validate the questionnaire data with actual bone outcome measures (rather than estimates of ground reaction forces).

- The questionnaire needs to be able to effectively classify children into a high or low bone loading group as effectively as accelerometry.
- The relationship between pQCT measured bone outcomes and physical activity assessment (using accelerometry and questionnaires) in children remains largely unknown.

The main objective of the first study was to therefore assess the relationship between the bone loading score obtained from a previously validated physical activity questionnaire, accelerometry and bone health in a group of South African pre-pubertal children. The test-retest reliability and criterion related validity of the bone specific questionnaire (B^3Q) with an accelerometer was assessed. The primary aim of this study was to assess the ability of the B^3Q to accurately categorize children into high and low bone loading groups based on time spent in moderate and vigorous intensity activities as measured using accelerometry. I also wished to determine whether the amount of weight-bearing physical activity as determined by the B^3Q was correlated to DXA and pQCT bone measures. Finally it was assessed whether the correlations between bone outcomes and B^3Q were similar to the correlations between bone outcomes and accelerometry measures. It was hypothesized that bone outcomes as measured using DXA and pQCT would be more closely associated with the B^3Q than with accelerometry.

Study 2 - A two-year history of high bone loading physical activity attenuates ethnic differences in bone strength and geometry in pre/-early pubertal children from a low-middle income country.

- There is a lack of research examining the effect of weight-bearing physical activity on bone mass in black and white children.
- Few reports have examined physical activity, ethnicity, muscle and bone strength in children.
- There is a lack of pQCT data on pre-pubertal black and white children.
- The role of the muscle-bone unit is not well understood with respect to ethnicity.

Once it had been established that the bone score obtained from the physical activity questionnaire was a sufficient surrogate measure of bone loading (study 1 results), the questionnaire (now called the B cubed questionnaire (B^3Q - "Building Better Bones questionnaire) was used in subsequent studies for the assessment of bone loading. The aim of study 2 was to use both DXA and pQCT technology to explore the interplay between bone health and ethnicity and analyze the differences in bone content, area and strength as well as geometric properties of bone due to history of bone loading in pre- to early-pubertal black and white South African children. The B^3Q served as a proxy measure of bone loading classification. This study had three hypotheses. First it was hypothesised that children who were classified as being high bone loaders for the past two years would present with greater bone mass and strength regardless of their ethnicity. The second hypothesis tested whether high levels of weight-bearing physical activity would negate

any ethnic bone differences that may exist in the study participants. Finally, it was tested whether muscle CSA could account for any differences observed in bone variables between high and low bone loading children.

Study 3 - Osteogenic effects of a physical activity intervention in South African black children.

- Data from cross-sectional/observational studies in white pre-pubertal children infer that a weight-bearing exercise intervention will increase bone mass in black children.
- This has not been confirmed because the lack of intervention studies done in black pre-pubertal children.
- There is a lack of research examining the role of an exercise intervention in bone health of pre-pubertal children using pQCT measures as an outcome (only five studies in the last 10 years with varying results; Table 1.2).
- The relationship between muscle and bone in a cohort of pre-pubertal black children is unknown.

The aim of this study was to determine whether a weight-bearing physical activity intervention improves measures of bone mass, structure and strength in a pre-pubertal black cohort. It was hypothesised that changes in bone content and geometry would be greater in children participating in an exercise intervention program compared with children not taking part in the exercise program.

Study 4 - Ethnic and loading effects on annual bone accrual in pre-pubertal black and white children.

- Longitudinal physical activity effects in a population of SA pre-pubertal children have not been studied.
- There is a lack of longitudinal pQCT data on black and white pre-pubertal children.
- It is unknown when/if ethnic divergence in bone mineral accrual begins.

The aims of this study were to 1) compare the effects of a history of bone loading on bone growth in content and structure over a one-year in pre-pubertal children and 2) to determine whether ethnicity has any effect on bone growth over a year of follow up in black and white children.

CHAPTER 2 – METHODOLOGY

2.1 Introduction

The following chapter will describe the methods that were used for data collection in this thesis. The thesis was divided into four main studies and this chapter will describe the design of each study in detail. Study one and two were cross-sectional, observational studies. Study three was an exercise intervention study and study four was a longitudinal study over a year. In all four studies, similar measurements were made, the details of which are outlined in the sections to follow. Any differences in methodology will be outlined in each study's specific design.

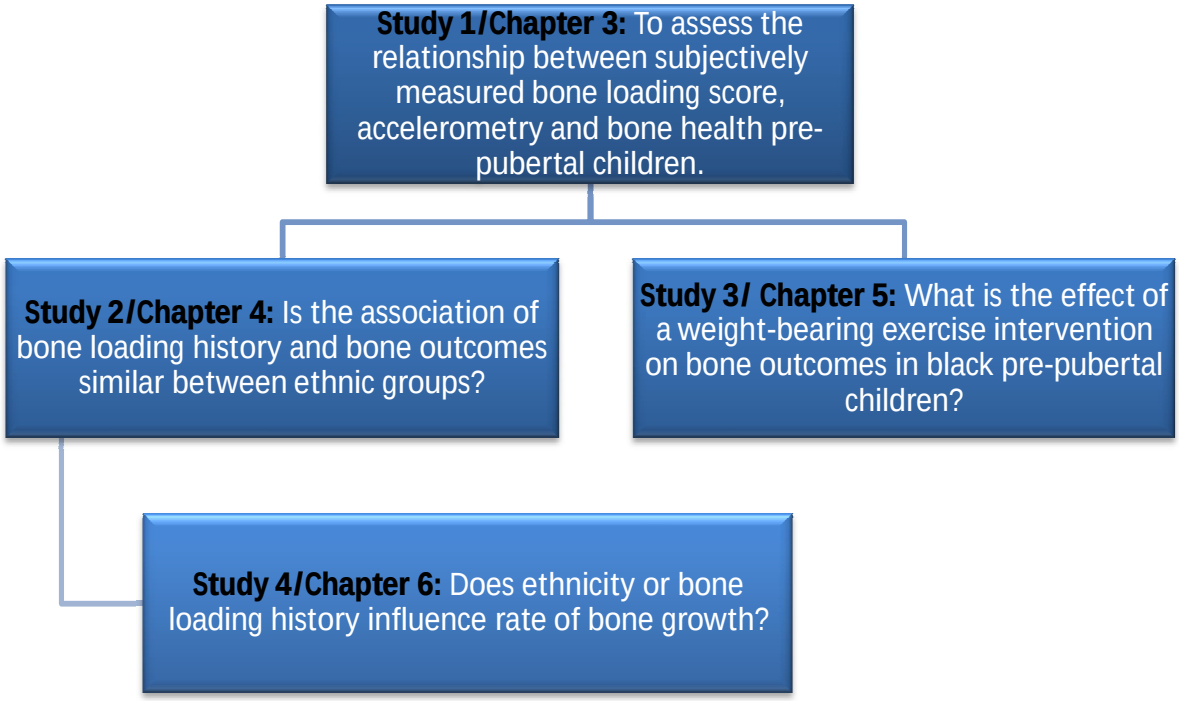

Figure 2.1 Schematic representation of the progression of the thesis.

2.2 Common methods used throughout the thesis

2.2.1 Participants

All participants who were recruited for participation in the project were children between the ages of 8 and 11 years. Children were recruited from schools, sports clubs and community centres around the greater Johannesburg area between August 2010 and July 2011. Information sheets detailing each study were sent home with all children from the appropriate grades, inviting their parents or primary caregivers to allow them to participate in the study (Appendix A). Details pertaining to the specific recruitment of participants for each study are outlined in the methods sections that follow. A total of 96 children were recruited and included in the screening procedures for the entire project. Prior to participation in any of the four studies in the project, all children were screened to determine their eligibility for the study. Parents/primary caregivers of children completed a general health questionnaire which pertained to the general health of their child including any medication use, as well as history of fractures and family history of osteoporosis (Appendix B). The assessment of pubertal status was done using the Tanner five stage classification criteria (101). The Tanner stage criteria involve pictorial representations of breast development (girls), gonadal development (boys) and pubic hair stages; the children or their caregiver were asked to self-select the stage that best described themselves or the child (Appendix C). The age at onset of menarche was also requested in the Tanner questionnaire for girls. Children were included in the study if they were categorised as being pre- to early pubertal or Tanner stage I to III. Socioeconomic status was determined using a household amenity questionnaire (222).

The amenity questionnaire gave a list of general household items (washing machine, indoor toilet, computer, television etc) and the parent or primary care-giver was asked to indicate the items that they owned (Appendix B). A score was then assigned to each child (sum of items owned) to calculate an amenity score, which was used to categorize their socioeconomic status. Although the relationship between SES and pQCT measured bone variables, whether in childhood or in adulthood, has not been investigated, in the present thesis, it was ensured that all participants came from a similar socioeconomic background (as assessed from the amenity score) in order to eliminate this potential confounder. All children had similar access to voluntary physical activities or after-school sports. Children were excluded if they were in Tanner stage IV or V, had been on corticosteroid medication consecutively for more than seven days in the past year, if they had any milk or lactose food allergies, if they were on a vitamin D or calcium supplement or if they had been ill or admitted to hospital in the last 3 months prior to participation in the study. Girls were excluded if they had attained menarche. All eligible children were recruited into study 1 and 2 and then children were invited to continue participation in the longitudinal study 4. Black children were invited to participate in the intervention study 3. Thus there was overlap in the studies that children could have taken part in, however children who took part in the intervention study were not included in the longitudinal study. The number of children involved in each study is summarised in Figure 2.7 on page 83.

2.2.2 Ethics

All children who participated in the project had the study protocol verbally explained to them and, if they agreed to participate, signed an assent form (Appendix D). Parents/primary caregivers of the children were required to consent to their child's participation in the project (Appendix E). The project (including all four studies) was approved by the Human Research Ethics Committee of the University of the Witwatersrand (protocol no.: M10635) which adheres to the principles of the Declaration of Helsinki (Appendix F).

2.2.3 Anthropometry

In each study, participant height and weight were recorded to the nearest millimetre (mm) and 100g respectively (stadiometer and scale: Holtain, Crosswell, UK). Participants were measured without shoes and while wearing light clothing. BMI percentile-for-age was calculated using software available from the World Health Organization (WHO, http://www.who.int/childgrowth/software/en). Radial and tibial lengths (to the nearest mm) were measured using sliding callipers (Holtain, Crosswell, UK) for the determination of the position of the bone scans. Radial length was defined as the distance from the tip of the olecranon process to the most distal end of the ulna styloid process. Tibial length was defined as the distance from the distal end of the medial malleolus to the superior aspect of the medial tibial condyle.

2.2.4 Dual Energy X-ray Absorptiometry (DXA)

Bone mineral content (BMC, g) and bone area (BA, cm^2) were reported from measures obtained using a DXA machine (Hologic QDR, Discovery W, Bedford, MA, USA) at the

following sites: right forearm, whole body (less head), lumbar spine (L1-L4) and total hip (for measurement of femoral neck) of the right leg. Fat and lean mass as well as body fat percentage were obtained from the whole body DXA scan. Children were asked to lie as still as possible while being scanned (Figure 2.2) and if there were significant motion artefacts whereby an acceptable reading could not be obtained, the scan was repeated. The same trained technician performed and analyzed all DXA scans. Throughout the project for the years 2010-2012, a quality control DXA phantom spine artificially made of similar material to bone was scanned each morning. The coefficients of variation (CVs) for BMC and BA were 0.36% and 0.32% respectively.



Figure 2.2 Example of participant placement for a whole body DXA scan.

2.2.5 Peripheral Quantitative Computed Tomography (pQCT)

Measures of the right side forearm and tibia were performed using peripheral quantitative computed tomography (Stratec XCT 2000, Stratec Medical, Pforzheim,

Germany). A scout view was performed for each subject and a reference line placed at the midline of the distal epiphyseal plate of the radius and the tibia. The forearm was scanned at 4% and 65% of the length of the radius from the reference line (representative of trabecular and cortical bone respectively). The tibia was then scanned (scan thickness was 2.3 mm thick), at 4, 38 and 65% of the length of the tibia from the reference line. The following measures were obtained from the 4% metaphysis (trabecular bone) of the radius and tibia: BMC, total cross-sectional area (ToA), total volumetric bone density (ToD) and trabecular volumetric bone density (TrbD). Diaphyseal measures (cortical bone) obtained from the 38 and 65% sites were: BMC, polar strength-strain index (SSI), cortical density (CoD) as well as cortical area (CoA). Muscle CSA measures were also obtained from the scans at the 65% site of the limbs. Bone strength index (BSI) of the trabecular bone was calculated (Equation 1) and reflects the strength of the bone against compression (223). Using periosteal diameter (equation 2) and endosteal diameter (equation 3) at the cortical sites, periosteal circumference (PC) (equation 4), endosteal circumference (EC) (equation 5) and cortical thickness (CT) (equation 6) were calculated using formulas previously described (141).

$$BSI\ (mg^2/mm^4) = total\ CSA \times (total\ vBMD)^2 \qquad \text{equation 1}$$

$$Periosteal\ diameter\ (TDiam, mm) = 2*\sqrt{(Total\ CSA/\pi)} \qquad \text{equation 2}$$

$$Endosteal\ diameter\ (EDiam, mm) = 2*\sqrt{(Total\ CSA - Cortical\ CSA/\pi)} \qquad \text{equation 3}$$

$$PC\ (mm) = \pi * TDiam \qquad \text{equation 4}$$

$$EC\ (mm) = \pi * EDiam \qquad \text{equation 5}$$

$$CT\ (mm) = (TDiam - EDiam)/2 \qquad \text{equation 6}$$

The CALCBD analysis algorithm was used for the analysis of all pQCT scans. For the trabecular (4%) measure, bone threshold was set at 180 mg/cm^3 and contour mode 1/peel mode 1 was used. For the cortical and bone geometry measures at the 65% radial and 38 and 65% tibial sites, bone threshold was set at 711 mg/cm^3 (contour mode 1/peel mode 2). Threshold for SSI at these cortical sites for both the radius and tibia was set at 480 mg/cm^3. For the measures of 65% muscle CSA, threshold was set at 40 mg/cm^3 (contour mode 3/peel mode 1) while for the measures of fat, threshold was set at -53 mg/cm^3 (contour mode 3/peel mode 1). Participants were asked to lie as still as possible while being scanned (Figure 2.3) and if there were significant motion artefacts such that an acceptable reading could not be obtained, the scan was repeated. The same independent technician performed all pQCT scans. A quality control pQCT phantom spine artificially made of similar material to bone was scanned each morning. The CVs for scans performed on the phantom were < 1% for bone density as well as area.

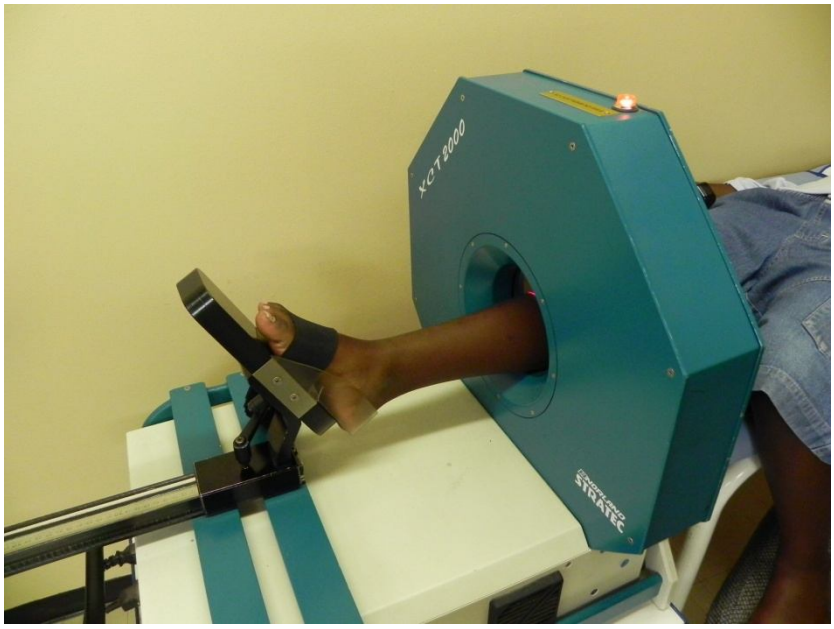

Figure 2.3 Example of participant placement for the 65% pQCT scan of the tibia.

It must be noted that a small number of scans could not be obtained from the 4% tibia for two reasons. The first is that it was only included halfway through the project. When the scan was included, it was found that most children had legs that were too short to reach the footrest (see Fig. 2.3). The pQCT machine scans in batches (i.e. it scans at 4, 14 and 38% in one go) therefore it was the thigh that ended up being scanned instead of 38% of the length of the tibia. Even if the child did not lay his/her foot on the foot rest it was impossible to obtain an acceptable scan as the foot was not secured in the footrest and motion artifacts occurred.

2.2.6 The bone specific physical activity questionnaire (B^3Q)

Children, with the assistance of their parent or caregiver, were required complete the B^3 questionnaire (B^3Q) pertaining to their participation in sport, exercise and leisure-time activities (Appendix G). This questionnaire was used to estimate the amount of bone loading a child had experienced for a prior period of time. The B^3Q was derived from a past year physical activity questionnaire that has been validated using accelerometry in South African children of a similar age (224). The physical activity questionnaire has been used extensively in other studies (135,225–227). The B^3Q was modified in order to take into account past week, past six month or past two year history of participation in weight-bearing sports. The questionnaire was interviewer administered each time the child and parent/caregiver visited the laboratory. Information was gathered from four activity question domains namely, physical activity participation during school, extra-mural/after-school physical activities, leisure time activity, as well as mode of transport to and from school. Time spent in sedentary activity during weekdays and on weekends was also

recorded but was not used in the current analyses. Children were asked to give details on the number of times per week they performed a particular activity as well as the amount of time they spent on each activity at any one time in order to determine the frequency and pattern of regularity of each activity. Regular participation in a specific activity was classified as the child performing an activity once a week for more than four months of the year (the usual length of a school semester in South Africa). After-school and leisure time activities were also reported on and assessed in the same way. Each regular activity was then assigned a bone strain score using a scoring system validated by Groothausen et al (228) where the ground reaction force produced by each activity was used to quantify the amount of bone loading of that activity. Table 2.1 is an example of the scores that were assigned to each sport recorded on the B^3Q. The sum of the scores for each activity made up the peak bone strain score (PBSS) that was assigned to each child. The median PBSS was calculated and based on the range of PBSS's attained for all the participants within each study, children scoring above the median score were classified as being high bone loaders while children who scored the median or below were classified as low bone loaders (Figure 2.4). Low bone loading did not necessarily mean children were physically inactive, for example, swimmers were classed as low bone loaders along with sedentary children.

Table 2.1 Peak bone strain scoring system based on associated ground reaction forces.

Peak score	Estimation criteria	Examples
3	Activities including jumping actions	Basketball, netball, gymnastics
2	Activities including sprinting and turning actions	Badminton, baseball, tennis
1	Weight-bearing activities	Dancing, jogging
0	All other activities	Bicycling, swimming

Adapted from Groothausen et al (228).

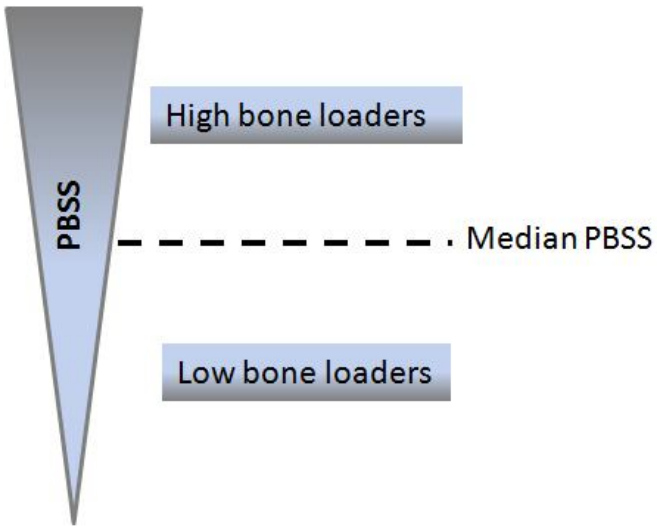

Figure 2.4 Schematic of how children were classified into the high and low bone loading groups. PBSS, peak bone strain score.

2.2.7 Accelerometry

Questionnaires provide a subjective measure of physical activity level and so the PBSS had to be compared to an objective measure of physical activity. Although energy expenditure of the child was not a primary outcome in this project, accelerometry was

used to objectively monitor participant activity. An accelerometer is a device that can be worn on the hip, thus directly measuring hip displacement and may therefore be a good proxy for weight bearing activity measurement. The Actical (Actical, Respironics, Phillips, USA) uses an omnidirectional sensor so that movement changes can be detected in various planes - but is more sensitive in one specific plane (229). The Actical detects human movement as an analogue signal and converts it to a digital signal where it is summed over a specified interval and stored (230). The Actical accelerometers are lightweight, small and have a data storage capacity of up to 32MB or 12 days of continuous recording. The Acticals are initialized and data can be downloaded via a pc interface and the results exported as an .xclx file. After measuring body height and weight, participants were fitted with the Actical on the left hip, along the iliac crest. Participants were asked to wear the Actical for seven consecutive days of assessment, and only to remove the Actical if they showered, bathed or swam. If participants removed the Actical to swim this was recorded on the seven day B^3Q. The Actical was then collected and the data downloaded and analysed independently of the B^3Q. Activity counts were collected in 15s epochs and data were reduced by removing only full days of non-wear time as assessed either by observation of the data where a full day of consistent zero activity counts was recorded, or as indicated by the participant if a day of wearing the Actical was missed. The remaining data are referred to as the “wear period” in a similar manner to that described by Hinkley et al (231). Participant data were included if out of the seven days, there was a minimum of four days of wear time where 10 hours of consecutive total activity and activity counts were recorded per day. Light, moderate and vigorous activity categories were defined according to activity count

thresholds based on guidelines recommended for children between the ages of 7 and 18 years (232). The moderate activity cut-point used was 1500 counts per minute and the vigorous activity cut-point was 6500 counts per minute (232). These cut-points are equivalent to an energy expenditure of 3 and 6 metabolic equivalents (METS) respectively. The Actical output variables were total activity counts per minute and counts per minute in each intensity category. Time spent in moderate and vigorous activity was also expressed as a percentage of the total wear time in a day.

The following sections will elaborate on some of the methods and describe the statistical analyses used in each study.

2.3 Study 1 - Associations of physical activity load with volumetric bone properties in pre- pubertal children.

As outlined in figure 2.1 above, the overarching theme presented in this thesis examines the role of physical activity on bone. In order to answer the specific study questions raised, I first needed to assess an accurate and reliable assessment of weight bearing physical activity. It was my intent to choose a method that could be used in the broader South African community for easy and reliable capture of weight bearing PA. To this end, the first study aimed to assess the relationship between accelerometry, a bone loading physical activity questionnaire (B^3Q) and bone health in a group of South African pre-pubertal children. I wished to assess the ability of both the B^3Q and the Actical accelerometer to accurately categorise children into similar high and low bone loading

groups. In order to do this, the PBSS algorithm that was derived from the B^3Q, and the time spent in moderate and vigorous physical activity thresholds, derived from the Actical, were used as determinants of high and low bone loading categories. Finally the relationship between PBSS, accelerometry measures and trabecular and cortical bone properties were assessed in pre- and early pubertal children.

2.3.1 Study design

Children came into our exercise laboratory to complete the interviewer administered B^3Q which pertained to their past two year history of participation in any type of physical activity and to have a DXA and pQCT scan and anthropometric measurements taken as described in section 2.1.3. At this visit, children were fitted with an Actical accelerometer (Actical, Respironics, Phillips, USA), which was placed on the hip of their right leg. The children were asked to wear the Actical for seven consecutive days in order to objectively measure physical activity. When the Actical was either collected or returned, children (with the aid of their parent/primary caregiver) were asked to complete the same interviewer administered activity questionnaire (but reworded accordingly) for the week that they wore the accelerometer. In other words, the same questionnaire was used to collect activity information for two year participation as well as for the past week's participation in sport and exercise. Six months after the initial visit, the B^3Q was administered again.

2.3.2 Participants

Cross-sectional data were obtained from a convenience sample of 45 participants recruited from local schools in the greater Johannesburg area. Participants that

responded to advertisements and flyer distribution were screened to determine their eligibility for the study. Based on previously published correlation coefficients between physical activity questionnaires and accelerometry data which range between 0.40 and 0.60 (164,165) I chose a value of 0.50 (moderate strength) and conducted a sample size calculation. It was estimated that a sample size of 38 children was needed at a power of 87% to ascertain a correlation coefficient of this size**.**

2.3.3 Dual energy X-ray absorptiometry (DXA)

The same bone mass measures described in section 2.2.4 were conducted on the participants for this part of the study.

2.3.4 Peripheral quantitative computed tomography (pQCT)

The forearm was scanned at 4% and 65% of the length of the radius from the reference line. The tibia was scanned at 65% of the length of the tibia from the reference line. The following measures were obtained from the metaphysis of the radius (4% slice): total cross-sectional area (ToA, mm^2), total volumetric density (ToD, mg/cm^3) and trabecular density (TrbD, mg/cm^3). Diaphyseal measures (cortical bone – 65% slice) obtained from the radius and the tibia were: polar strength-strain index (SSI, mm^3), cortical density (CoD, mg/cm^3) as well as total area (ToA, mm^2) and cortical area (CoA, mm^2). Muscle cross sectional area (CSA, mm^2) was also obtained from the scans at the 65% radius and tibia sites. Bone strength index (BSI, mg^2/mm^4) at the radial epiphysis, periosteal circumference (PC, mm), endosteal circumference (EC, mm) and cortical thickness (CT, mm) were also calculated using the formulas in section 2.2.5 above.

2.3.5 B^3Q

The B^3Q was interviewer administered for each child (with the assistance of their primary caregiver) on their participation in physical activity and leisure time activities for the previous two years as well as for the week during which the Actical was worn. PBSS was calculated as described in section 2.2.6. The two-year B^3Q (April 2010 – April 2012) was completed to account for seasonal and annual variation in physical activities and also to consider those children who had played a sport up to the first year but then changed to a different sport in the second year. For example, there were some children in the cohort that in the first year of PA assessment had participated in a weight-bearing sport but in the second year of assessment had participated in a non weight-bearing sport such as swimming. The rationale behind a two-year history as opposed to the conventional one-year history was to ensure inclusion of children who had been exposed to a season of bone loading. In addition, the sustained benefits of weight-bearing exercise are not well defined. A modified version of the questionnaire was also administered for the week in which the participants wore the Actical. This was done to ensure that the week during which the children wore the Actical was representative of a typical week of activity. The B^3Q (two-year and seven-day) was then re-administered six months later to compare monitor agreement between consecutive administrations of the questionnaire. Figure 2.5 is a timeline of administrations of the B^3Q.

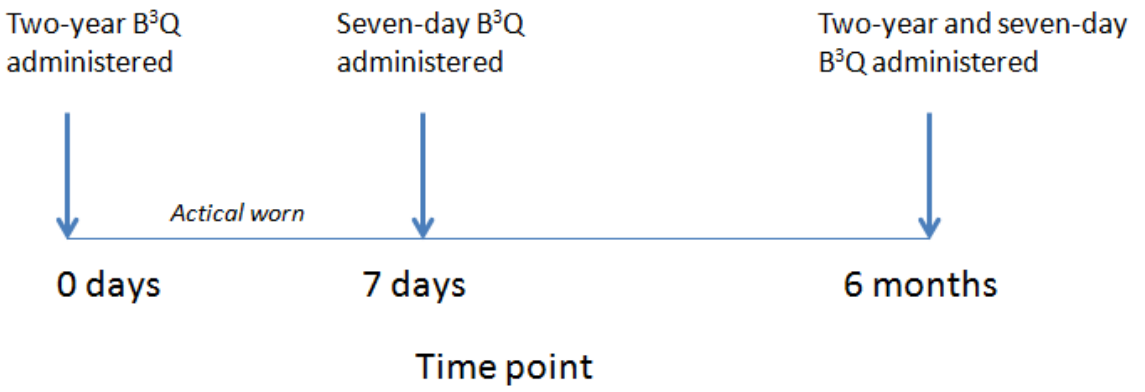

Figure 2.5 Timeline of B^3Q administrations.

2.3.6 Statistical Analysis

The difference in PBSS calculated from the two-year B^3Q and the PBSS from the seven day B^3Q was compared using a Wilcoxon signed rank test. Intra-class correlation coefficients (ICCs) (one-way random effects model) were used to evaluate the reproducibility of the estimates of the two administrations of the two-year and seven day PBSS. The two-year PBSS (indicative of bone loading history) was used for subsequent analyses. Weighted Kappa statistics were used to test the level of agreement between the high/low ranking of PBSS from the B^3Q and the Actical. The weighted Kappa statistic provides a measure of agreement between two scores which classify observations into one of several groups or categories. Kappa values were defined as poor (<0.20), fair (0.21 - 0.40), moderate (0.41 - 0.60) and good (0.61 - 1.00) agreement (233). PBSS and Actical activity (counts/min) were log transformed as data were not normally distributed. Pearson's correlations were then performed between PBSS and moderate, vigorous and moderate and vigorous combined intensity activity. Pearson's correlations were also performed between log-transformed PBSS, Actical activities and adjusted bone variables. DXA measurements were adjusted

for bone area, lean mass and sex, while pQCT values were adjusted for limb length, weight and sex. Even though the children were all pre-pubertal and sex differences in bone appear after puberty, sex was used as a covariate because the sexes were mixed within groups and sex does have an influence on bone status. Bone size was adjusted for in the form of bone area (for DXA) and limb length (for pQCT) to account for the variability in bone size in children. Unless otherwise specified, data are presented as mean and SE. Multiple regression analyses were used to determine the independant contribution of physical activity measures (PBSS and MVPA) on the variance of all DXA derived bone variables as well as on selected pQCT variables (cortical area, density and strength of the 65% radius and tibia) after adjustment for the abovementioned covariates. Data were analysed using SPSS 21.0 (IBM SPSS, NY, USA). Significance was set at $p \leq 0.05$.

2.4 Study 2 - A two-year history of high bone loading physical activity attenuates ethnic differences in bone strength and geometry in pre/-early pubertal children from a low-middle income country

The associations between physical activity, ethnicity and bone health using pQCT bone measures as an outcome have not been extensively researched. The aim of the second study was to explore the interplay between ethnicity and a two-year history of weight-bearing physical activity on the volumetric and geometric properties of bone in a pre- to early pubertal South African black and white population.

2.4.1 Study design

This study was of cross-sectional design. Participants were invited to come to the exercise laboratory at Wits Medical School at their convenience. At the exercise laboratory, participants were asked to fill out the B^3Q on their past two-year history of participation in bone loading sport. Children underwent a DXA and pQCT scan and provided a blood sample.

2.4.2 Participants

Participants were recruited from local schools and community centres around the greater Johannesburg area by distributing flyers containing study information during the summer months in South Africa (September 2010 to February 2011). All participants who volunteered for the study were from similar socioeconomic backgrounds with similar access to voluntary physical activities or after-school sports. Ninety six black and white, boys and girls between the ages of 8 and 11 years volunteered to participate in this study. As indicated by the parent or primary caregiver, children were considered black if both parents and grandparents were black, while children were considered white if both parents and grandparents were white.

2.4.3 B^3Q

In this study, the two-year recall bone specific questionnaire validated in study 1 was used. Similarly to study one, a two-year PBSS was calculated for each child using the scoring criteria described in section 2.2.6 above.

2.4.4 Dual Energy X-ray Absorptiometry (DXA)

The same bone mass measures described in section 2.2.4 were conducted on the participants for this part of the study.

2.4.5 Peripheral Quantitative Computed Tomography (pQCT)

The forearm was scanned at 4% (metaphyseal) and 65% (diaphyseal) of the length of the radius from the reference line. The tibia was scanned at 65% of the length of the tibia from the reference line. The following measures were obtained from the metaphysis (trabecular bone) of the radius and tibia: total cross-sectional area (ToA), total volumetric bone density (ToD) and trabecular volumetric bone density (TrbD). Diaphyseal measures (cortical bone) obtained from the 65% sites of the radius and tibia were: polar strength-strain index (SSI), cortical density (CoD) as well as cortical area (CoA). Muscle CSA measures were also obtained from the scans at the 65% site of the limbs. BSI, PC, EC and CT were calculated using the formulas described in section 2.2.5.

2.4.6 Statistical analysis

Data were analysed using Statistica Version 10 (Statsoft Inc, Tulsa, USA). An unpaired t-test (parametric data) or Mann-Whitney U (non-parametric data) test were used to determine any differences in anthropometric data between boys and girls as well as between black and white children. Differences in Tanner proportions between black and white children and between boys and girls were assessed using a chi-squared test. The relationship between PBSS and bone variables within each ethnicity was tested in a continuous manner and also in a categorical analysis. For the former analysis, the PBSS data was log-transformed because it was not normally distributed. Then Pearson's

correlations were performed to determine continuous associations between log-transformed PBSS and bone variables within each ethnic group. Covariates for bone analyses were determined by performing correlations between all variables. DXA bone measures were adjusted for sex, weight, sexual maturity and bone area. pQCT bone measures were adjusted for sex, weight, sexual maturity, limb length and muscle CSA. Sex was used as a covariate because the sexes were mixed within groups and sex has an influence bone status. Bone size was adjusted for in the form of bone area (for DXA) and limb length (for pQCT) to account for the variability in bone size in children. Weight was used as a covariate because there was a significant difference in percent body fat and fat mass between the groups. Therefore instead of adjusting for both variables and introducing possible colinearity, weight was chosen as a covariate in the analyses. In addition, sexual maturity was chosen as a covariate because there was a small number of participants who were classified as being in Tanner stage III. Ethnicity and sex interactions with adjusted bone variables were assessed using a two-way ANOVA with a Bonferroni post-hoc test to control for multiple comparisons. To categorically assess the effects of weight-bearing physical activity and ethnicity on bone health, children were then classified into two groups - low and high bone loading in their respective ethnic group (black and white). Again a two-way ANOVA was performed on adjusted bone outcomes between black and white, high and low bone loaders with a Bonferroni post-hoc test. Pearson's partial correlations were used to evaluate the relationship between muscle CSA and bone variables controlling for sex, weight, limb length and sexual maturity. Mean and standard deviation (SD) are reported for the unadjusted variables while mean and

standard error of the mean (SE) are reported for the adjusted variables. Significance was set at $p \leq 0.05$.

2.5 Study 3 - Osteogenic effects of a physical activity intervention in South African black children

The third study aimed to determine whether a weight-bearing physical activity intervention improves measures of bone volumetric density and geometry in a pre-pubertal cohort of black South African children.

2.5.1 Participants

Children between the ages of 8 and 11 years, from three urban primary schools in the greater Johannesburg area (Figure 2.6), were invited to participate in this cluster-randomised exercise intervention. Children from two of the schools attended the same after-school care community centre and participant recruitment and the intervention took place there. Study information sheets were sent home from school for parental consent. Only children whose mother and father were black were included in the study. Thirty seven children who met the inclusion criteria and were attending any of the three different primary schools volunteered to participate in the study. The children were cluster randomised into either an exercise (two schools, n=12; EX) and control (one school, n=10; CON) groups and 22 children completed the study i.e. complete data from before and after the intervention was available for 22 children. The remaining 15 children did not fulfil the inclusion criteria, moved away from the area or stopped attending the after-school care community centre. Using cortical density as a surrogate marker for bone

health in the children, a retrospective power analysis gave a power of 91% with the number of children that were included in the final analysis. All children came from similar low-middle income socioeconomic backgrounds as assessed using the household amenity questionnaire described in section 2.2.1 above.



Figure 2.6 Setting of the weight-bearing exercise intervention

2.5.2 Study design

Children were required to visit the laboratory for various measurements before and after a 20 week weight-bearing exercise intervention. These measurements included DXA and pQCT scans, filling out the B^3Q and giving a urine sample.

2.5.3 Measures of bone health

DXA and pQCT scans, as described in sections 2.2.4 and 2.2.5 above, were performed on each child in each group before and after the 20 week intervention period. The exercise intervention was a targeted lower body exercise intervention; therefore whole body and site specific scans were taken using DXA and only the tibia was scanned using pQCT. The 4% and 38% sites of the tibia were scanned while measures of muscle cross-sectional area (CSA) were taken at the 65% site. Measures of fat CSA (mm^2) were also taken at the 65% tibia site. The threshold for fat CSA was set at 40 mg/cm^3 (contour mode 3/peel mode 1).

2.5.4 Urinary cross-linked N-telopeptides of Type I collagen (NTX)

Children were asked to provide a urine sample before and after the intervention, for the analysis of urinary cross-linked N-telopeptides of Type I collagen (NTX). Urine samples were collected in the morning between 08:00 and 10:00 from all participants. NTX concentration before and after the intervention was analysed using a commercially available ELISA kit (Osteomark, Alere Scarborough, Inc., Scarborough, ME, USA). The NTX assay is a competitive-inhibition enzyme-linked immunoassay. NTX in the sample competes with the solid phase NTX for the binding sites of a monoclonal antibody labelled with horseradish peroxidase. The amount of antibody bound to the solid phase is inversely proportional to the amount of NTX in the sample. Assay values were corrected for urinary dilution by urinary creatinine analysis and expressed in nanomoles bone collagen equivalents per litre (nM BCE) per millimole creatinine per litre (mM creatinine).

2.5.5 Weight-bearing physical activity intervention

The 20 week exercise intervention took place between February and June 2012. The exercise sessions took place after school and were conducted by two facilitators who were employed by the school. Facilitators were instructed initially on how to conduct the series of weight-bearing exercises to be performed by the children for two 45 minute sessions per week. Because the intervention took place during the late summer to early autumn months, it was performed outside on the school's sports field. Each exercise session involved completing an exercise circuit consisting of five activities. A warm up of five minutes was performed before starting the circuit. The warm-up consisted of stretching the upper and lower body. Each activity was then performed for five minutes before moving on to the next activity in the circuit. Exercises included sprinting between cones set a certain distance apart, running and jumping to catch a 1kg medicine ball, ladder hopping (feet together jumping into each space in between the rungs of a ladder), hopping with one or both feet over 30 cm high hurdles and jumping rope for as long as they could. A competition within two or three of the activities was held for the next 10 minutes where children were divided into two groups and took part in a race to determine a winner of each activity. A cool-down was then performed for another five minutes which again involved upper and lower body stretches. The frequency and intensity of the exercises remained the same throughout the exercise intervention period. The facilitator was also required to keep a weekly log book of the children who performed the exercise intervention in order to monitor compliance. This log book was supplied by myself and the facilitator kept the log book for the entire duration of the 20 week intervention. On-site monitoring of the exercise intervention was conducted once a

month to maintain rigour and compliance with the intervention. The CON group continued with their regular activities throughout the intervention period.

2.5.6 B^3Q

The two-year B^3Q was administered to both groups of children at the start and at the end of the exercise intervention in order to monitor bone loading levels throughout the intervention period.

2.5.7 Statistical Analysis

Unless otherwise specified, data are presented as measures at baseline and follow up [mean (SD)] and adjusted change from baseline [mean (95% CI)] after 20 weeks of intervention. The median PBSS between and within the EX and CON groups were compared using a Kruskal-Wallis test. A two-way repeated measures analysis of variance (ANOVA) was used to determine time and group effects of the intervention on bone variables and to determine the within group changes over the intervention period. DXA derived BMC was adjusted for sex, follow-up sexual maturity and bone area while pQCT bone outcomes were adjusted for sex, follow-up sexual maturity, height and muscle CSA. Muscle CSA was adjusted for sex, follow-up sexual maturity and height. Sex was used as a covariate because the sexes were mixed within groups and sex has an influence on bone status. Follow-up maturity was also included as a covariate because some children changed Tanner pubertal status from pre- to post-intervention. Bone size was adjusted for in the form of bone area (for DXA). Height was used as a covariate in the adjustment for pQCT variables because the change in height between the CON and EX group was significantly different. Muscle CSA was also used in the adjustment of pQCT bone

variables at baseline and follow up to eliminate the possible confounding effects of the exercise intervention on muscle. Five-month absolute change of the bone variables was then compared between groups using an analysis of covariance (ANCOVA), adjusting for sex, Tanner stage at follow-up, and change in bone area for DXA , while pQCT outcomes were adjusted for sex, Tanner stage at follow-up, change in height and change in muscle CSA. A repeated measures ANOVA was used to determine differences in NTX concentrations between groups before and after the intervention. Adjustments for multiple comparisons were made using the Sidak correction for multiple comparisons. Data were analysed using SPSS 21.0 (IBM SPSS, NY, USA). Statistical significance was considered as $p \leq 0.05$.

2.6 Study 4 - Ethnic and loading effects on annual bone accrual in pre-pubertal black and white children

Children who participated in study 2 (A two-year history of high bone loading physical activity attenuates ethnic differences in bone strength and geometry in pre/-early pubertal children from a low-middle income country) were invited to continue participating in a longitudinal study over the following year. Forty seven children accepted the invitation to complete the follow up. The aim of the fourth study was to evaluate the influence of ethnicity and past history of physical activity on the acquisition of bone mass and bone structure over a period of one year in pre-pubertal children.

2.6.1 Study design

Children were asked to come into the exercise laboratory approximately one year after their baseline visit for a follow up visit. At this visit, children filled in a B^3Q, they had a whole body and site-specific DXA and pQCT scan, and they gave an optional blood sample.

2.6.2 B^3Q

Children who continued participation in this longitudinal study had already been classified into either a high or low bone loading group based on the PBSS score obtained at the first visit (study 1). At the follow up visit children were asked to fill out the B^3Q for their past year assessment of weight bearing physical activity in order to ensure that children remained in their respective bone loading group throughout the year. The average PBSS that was calculated and obtained (as described in section 2.2.6) from the baseline and follow up questionnaire was therefore an estimate of a three year history of bone loading.

2.6.3 Measures of bone health

Whole body and site specific DXA scans were taken for each child at the follow up visit (section 2.2.4 above). At this visit, pQCT scans were also taken for each child. The forearm was scanned at 4% and 65% of the length of the radius from the reference line. The tibia was scanned at 65% of the length of the tibia from the reference line. The following measures were obtained from the metaphysis of the radius (4% slice): total cross-

sectional area (ToA, mm^2), total volumetric density (ToD, mg/cm^3) and trabecular density (TrbD, mg/cm^3). Diaphyseal measures (cortical bone – 65% slice) obtained from the radius and the tibia were: polar strength-strain index (SSI, mm^3), cortical density (CoD, mg/cm^3) as well as total area (ToA, mm^2) and cortical area (CoA, mm^2). Muscle cross sectional area (CSA, mm^2) was also obtained from the scans at the 65% radius and tibia sites. Bone strength index (BSI, mg^2/mm^4) at the radial epiphysis, periosteal circumference (PC, mm), endosteal circumference (EC, mm) and cortical thickness (CT, mm) were also calculated using the formulas in section 2.2.5 above.

2.6.4 Blood

Children were asked to give an optional 10 ml blood sample. Samples were centrifuged and the serum was aliquoted and stored at -80˚C. Enzyme linked immunosorbent assays (ELISAs) were used for the determination of serum sclerostin (Adipo Bioscience, INC, Santa Clara, CA, USA) and serum IGF-1 (DRG Instruments GmbH, Germany). Sclerostin was chosen because of it's novelty and studies of exercise and sclerostin levels in children have not been done before. Serum IGF-1 was selected because of it's association between sexual maturity and bone growth in children.

Sclerostin

Briefly, the sclerostin assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for sclerostin is pre-coated onto microplates. Standards and samples are pipetted into the wells and any sclerostin present is bound by the immobilised antibody. After washing away any unbound substances, a biotinylated monoclonal antibody specific for sclerostin is added to the wells. Following a wash to

remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and colour develops in proportion to the amount of sclerostin bound in the initial step. The colour development is stopped and the intensity of the colour is measured through the use of spectrophotometry.

Insulin-like Growth Factor 1 (IGF-1)

The IGF-1 assay used was a solid phase enzyme-linked immunosorbent assay based on the principle of competitive binding. Participant samples, standards and controls were acidified and neutralised prior to the assay procedure. The microtitre plates were coated with a monoclonal antibody directed towards an antigenic site on the IGF-1 molecule. The pre-treated was incubated at room temperature with conjugate (biotinylated IGF-1). The wells were washed and then incubated with HRP linked Streptavidin. After the addition of the substrate solution, the intensity of the colour developed is inversely proportional to the concentration of IGF-1 in the participant sample.

2.6.5 Statistical analysis

Anthropometric characteristics at the baseline visit were analysed using a one-way ANOVA.

Effects of prior participation in weight-bearing sport on bone growth

The low and high bone loading groups were compared at baseline and at the follow up visit using repeated measures ANOVA. Bone variables at baseline were controlled for ethnicity, sex and bone size/limb length. Bone variables at follow up were controlled for ethnicity, sex, bone size/limb length and the baseline value of the corresponding variable. Ethnicity was used as a covariate in this analysis to eliminate the possible confounding effects of ethnicity on bone. Sex was again used as a covariate because the sexes were mixed in this study and sex has an effect on bone status. Bone area/limb length was used as the covariates to account for the bone size variability in children. At follow up, the baseline bone variable was added as a covariate to account for any influence of the baseline value on the rate of growth in the groups of children.

Effects of ethnicity on bone growth

Black and white children were also compared at baseline and follow up using a repeated measure ANOVA. The average PBSS between the baseline and the follow up visit was calculated and used as a covariate in the analyses. PBSS was used as a covariate to eliminate the possible confounding of a difference in loading on bone variables between the groups of black and white children. The same variables as above were used as covariates except baseline PBSS replaced ethnicity as a covariate for baseline variables and average PBSS over the follow up period replaced ethnicity as a covariate for follow up variables.

Because the duration of follow up ranged between 7-14 months (mean 12.1 months), relative annual changes were calculated according to formulas described by Ducher and colleagues (234) in order to normalise the change between baseline and follow up.

Absolute annual change =

(follow up measure – baseline measure)/follow up duration (months) x 12

Relative annual change, (%) =

Absolute annual change/baseline measure x 100

Only relative change is reported as a percentage change (95% CI) compared to baseline. Relative changes were then compared between the high and low bone loading groups and also between the black and white children using a one-way ANOVA. The relative changes in bone outcomes between high and low bone loaders were controlled for ethnicity, sex and the relative change in bone size/limb length. The relative changes in bone outcomes between black and white children were controlled for average PBSS over the year, sex and the relative change in bone size/limb length. Data from the blood biochemical markers were log-transformed and analysed using a repeated measures ANOVA between high and low bone loaders as well as between black and white children. A one-way ANOVA was used to compare the relative changes in IGF-1 and sclerostin concentrations over the year of follow up between low and high bone loaders and between black and white children. SPSS 21.0 (IBM SPSS, NY, USA) was used for all data analysis and data are presented as mean (SEM) unless otherwise specified. Significance was set at $p \leq 0.05$.

2.7 Summary of study designs

Figure 2.7 below summarises the number of children who participated in each study of the project as well as the basic procedures involved in the each study. In summary, the first study was required so that the use of a South African child-population-specific physical activity questionnaire (B^3Q) could be used to quantify the amount of bone loading that each child participates in. Thus following the results of study 1, the B^3Q was used throughout the rest of the project. In study 2, the questionnaire was used to assess a two-year history of bone loading and to determine then the effects of a history of bone loading as well as ethnicity on current bone health. In study 3, the B^3Q was used to monitor bone loading levels before and after a 20 week exercise intervention in black children. Finally, in study 4, the B^3Q was used at three time points over the course of a year to monitor bone loading and the effect thereof on bone health status in a longitudinal study over a year. In study 4, the effect of ethnicity on bone growth was also assessed.

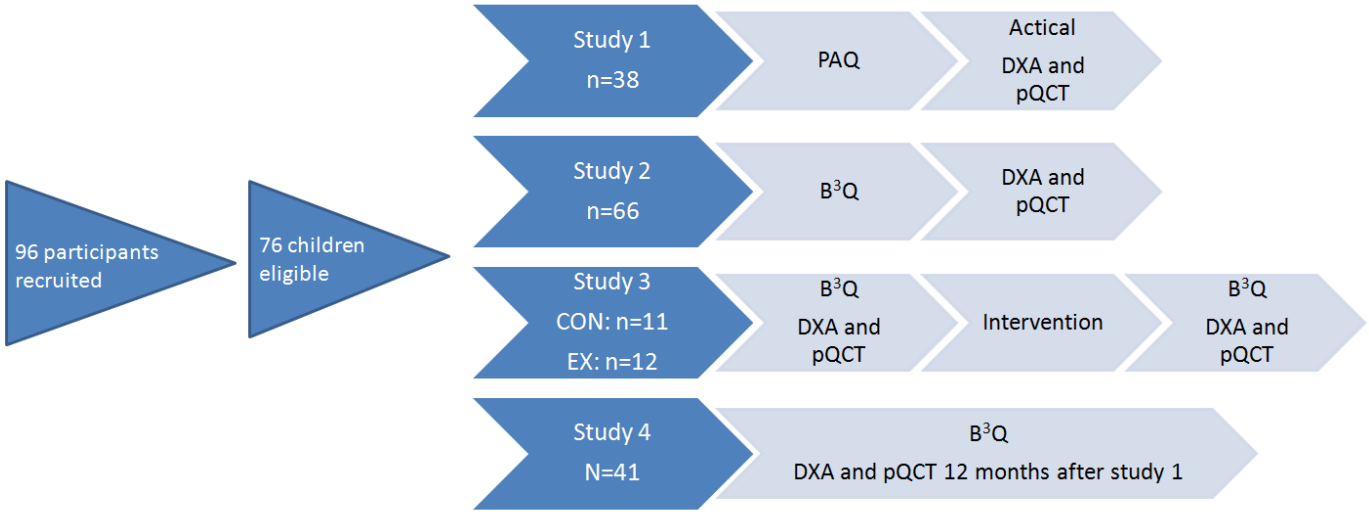

Figure 2.7 Schematic detailing the number of participants that were involved in each study of the thesis and the basic procedures involved in each study.

CHAPTER 3 – STUDY 1 - ASSOCIATIONS OF OBJECTIVELY AND SUBJECTIVELY MEASURED PHYSICAL ACTIVITY WITH VOLUMETRIC BONE PROPERTIES IN PRE-PUBERTAL CHILDREN[1]

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[1] Meiring RM and McVeigh JA. 2013. Submitted to South African Journal of Sports Medicine.

3.1 Introduction

The primary aim of the present study was to assess the ability of the B cubed questionnaire (B^3Q) to accurately categorize children into high and low bone loading groups based on time spent in moderate and vigorous intensity activities as measured using accelerometry. The test-retest reliability and criterion related validity of the bone specific questionnaire was assessed with actigraphy. A second aim was to determine whether the amount of weight-bearing physical activity as determined by the B^3Q was correlated to DXA and pQCT bone measures. Finally it was tested whether correlations between bone outcomes and the B^3Q were similar to the correlations between bone outcomes accelerometry measures.

3.2 Methods and materials

Please refer to section 2.3 for details on the methods for this study of the thesis. The schematic below (Figure 3.1) summarises the methods used for this study. Section 2.3.6 details the statistical analyses used for this study.

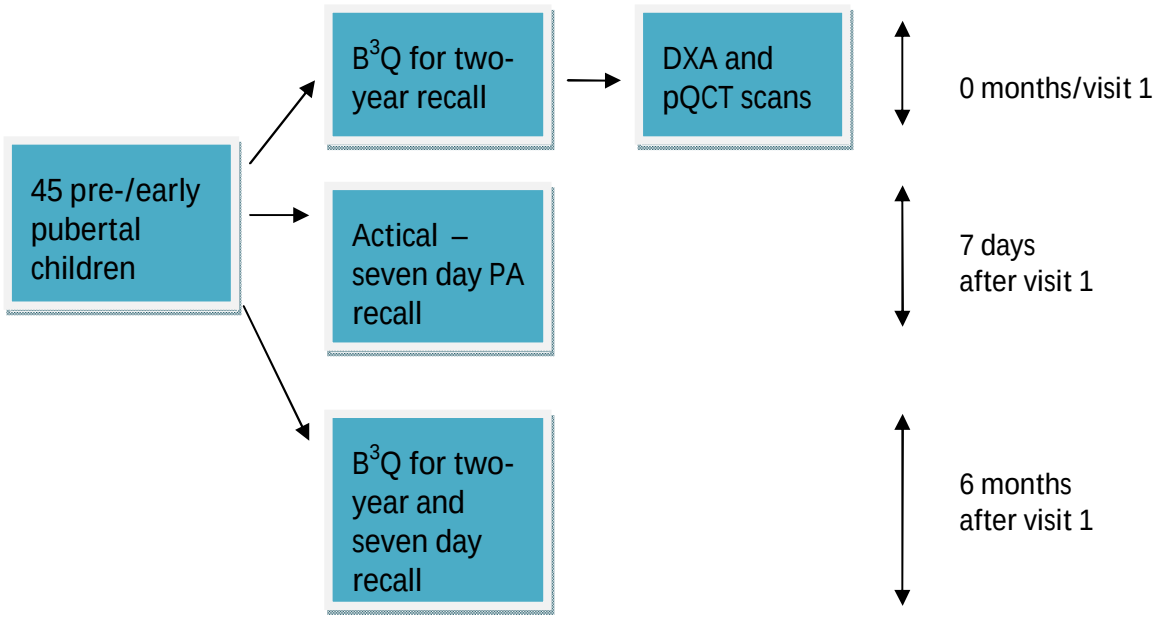

Figure 3.1 Schematic summarising the methods used for the B^3Q validation study

3.3 Results

Seven children were excluded from the analysis due to a lack of Actical data that met the criteria for a "wear" day. Therefore a total of 38 children's data were included in the final analysis. Participant characteristics for the whole group and for the low and high bone loaders (based on median PBSS score) are shown in Table 3.1. High bone loaders had greater forearm ($p=0.002$) and leg ($p=0.04$) muscle cross sectional area than low bone loaders. High bone loaders also spent more time engaged in moderate intensity activity ($p=0.005$) and had greater PBSS scores than low bone loaders ($p<0.001$). Body size adjusted BMC measured at all sites was significantly greater in the high bone loaders

compared to low bone loaders ($p<0.05$). Total bone area was significantly greater in the high bone loaders compared to low bone loaders at both the metaphysis ($p<0.05$) and diaphysis of the radius ($p=0.002$). High bone loaders also had greater cortical area ($p=0.01$), cortical density ($p=0.004$) and strength strain index (SSI) ($p=0.02$) at the radial diaphysis compared to low bone loaders and similarly, a greater cortical density ($p=0.01$), SSI ($p=0.04$), periosteal ($p=0.03$) and endosteal ($p=0.01$) circumference was evident at the tibia for the high bone loaders.

Table 3.1 Descriptive characteristics of participants.

Characteristic	Low Bone loaders (n=17)	High Bone Loaders (n=21)	Whole group (n=38)
Age (yrs)	9.8 (1.4)	9.9 (1.3)	9.9 (1.3)
Tanner (I/II)	12/5	10/11	22/16
Sex (male/female)	3/14	9/12	12/26
Ethnicity (White/Black)	15/2	17/4	32/6
Height (cm)	134.7 (8.8)	140.1 (10.3)	137.7 (9.9)
Body mass (kg)	30.5 (6.9)	34.9 (7.1)†	32.9 (7.2)
BMI-Percentile	46.5 (26.4)	57.3 (28.1)	52.5 (27.5)
Forearm muscle CSA (mm^2)	1595.3 (218.1)	1897.6 (319.8)*	1758.7 (313.7)
Leg muscle CSA (mm^2)	3318.1 (583.3)	3775.4 (683.7)*	3577.6 (673.9)
Physical Activity			
Moderate activity (min/day)	44.0 (13.0)	65.0 (26.1)*	55.6 (23.5)
Vigorous activity (min/day)	1.6 (1.6)	2.5 (3.7)	2.1 (3.0)
Wear time (hrs/day)	14.1 (0.8)	14.0 (0.9)	14.1 (0.9)
Days worn	7 (1)	6 (1)	6 (1)
PBSS score from PAQ (2 year)	3.5 (1.1)	8.6 (2.3)*	6.3 (3.1)
DXA			
Ulna BMC (g)	2.4 (0.4)	2.7 (0.4)*	2.5(0.4)
Radius BMC (g)	3.4 (0.6)	3.8 (0.7)*	3.6 (0.7)
Spine BMC (g)	21.4 (4.5)	24.7 (5.0)*	23.2 (5.0)
Hip BMC (g)	15.3 (3.2)	17.8 (3.8)*	16.7 (3.7)
Femoral neck BMC (g)	2.6 (0.5)	3.0 (0.5)*	2.8 (0.5)
Whole body BMC (g)	725.0 (139.2)	824.7 (136.6)*	781.6 (155.5)
pQCT			
Metaphysis-Radius			
ToA (mm^2)	210.4 (51.5)	241.8 (44.1)†	227.0 (49.6)
ToD (mg/cm^3)	289.1 (12.7)	289.0 (16.5)	289.1 (14.6)
TrbD (mg/cm^3)	210.2 (33.6)	205.5 (33.4)	211.1 (33.6)
BSI (mg^2/mm^4)	1748.9 (530.0)	1994.4 (487.5)	1878.9 (515.3)
Diaphysis- Radius			
ToA (mm^2)	93.5 (11.4)	108.0 (13.9)*	101.2 (14.6)
CoA (mm^2)	40.7 (9.4)	48.0 (7.0)*	44.6 (8.8)
CoD (mg/cm^3)	983.1 (29.4)	1013.0 (29.4)*	998.9 (32.7)
SSI (mm^3)	133.5 (34.8)	163.3 (35.4)*	149.2 (37.7)
PC (mm)	33.5 (3.2)	35.8 (2.7)	34.9 (3.1)
CT (mm)	1.4 (0.3)	1.5 (0.2)	1.5 (0.3)
EC (mm)	24.8 (2.9)	26.2 (3.3)	25.6 (3.2)
Diaphysis-Tibia			
ToA (mm^2)	415.4 (48.0)	452.8 (71.6)	436.6 (64.5)
CoA (mm^2)	207.8 (30.8)	217.7 (35.0)	213.4 (33.2)
CoD (mg/cm^3)	1025.9 (15.4)	1046.1 (14.3)*	1037.3 (17.8)
SSI (mm^3)	1400.6 (330.8)	1662.8 (406.0)*	1549.4 (393.1)
PC (mm)	71.9 (4.2)	75.6 (5.5)*	74.0 (5.3)
CT(mm)	3.4 (0.4)	3.4 (0.4)	3.39 (0.4)
EC (mm)	50.7 (3.3)	54.3 (4.3)*	52.7 (4.3)

Data are mean (SD) except for Tanner, sex and ethnicity which show proportions within each group and bone variables which are mean (SEM). BMC measures by DXA are adjusted for sex, lean mass and bone area while pQCT measures are adjusted for sex, body mass and limb length. ToA: total area, ToD: total density, TrbD: density of trabecular bone at 4% site, BSI: bone strength index, CoD: cortical density, SSI: strength-strain index, PC: periosteal circumference, CT: cortical thickness, EC: endosteal circumference.*p<0.05, † p<0.01, ‡p<0.001, high bone loaders significantly greater than low bone loaders.

Agreement between PBSS and Actical derived physical activity

The two year and seven day PBSS scores were comparable between administrations and demonstrated high intra class correlations (Table 3.2). There were significant positive correlations between PBSS and moderate (r=0.38; p=0.02), vigorous (r=0.36; p=0.03) and combined moderate to vigorous intensity activity (MVPA) (r=0.38; p=0.02). A weighted Kappa statistic (which assumes the categories to be ordered) was used to assess the strength of the PBSS algorithm at placing children into the same loading category as the Actical. The agreement between the Actical and PBSS algorithm with respect to categorizing participants into high or low loading categories, was κ=0.37 (p=0.02) for moderate intensity activity; κ=0.10 (p=0.54) for vigorous intensity activity and κ=0.42 (p=0.008) for MVPA.

Table 3.2 Intraclass correlation coefficients (r) comparing two administrations of the B^3Q.

	1st administration	2nd administration	ICC (CI)
Past two year PBSS	5.0 (4.0-8.0)	5.6 (3.0-8.8)	0.9 (0.8-0.9)‡
Past week PBSS	4.0 (2.0-5.0)	4.0 (4.0-6.0)	0.8 (0.2-0.9)*

Data are means (CI). *p<0.05, ‡p<0.001.

Correlations between activity and bone

PBSS was significantly correlated to body size adjusted BMC at all sites scanned by DXA (Table 3.3). In addition significant positive correlations between PBSS and bone variables were observed for total area (ToA; p=0.006), total volumetric density (ToD; p=0.003), density of trabecular bone (TrbD; p<0.001) and bone strength index (BSI; p=0.001) at the 4% radius site. At the 65% radius correlations were significant for ToA (p<0.001), cortical area (CoA; p=0.001), cortical density (CoD; p=0.01) and polar strength-strain index (SSI; p<0.001).

PBSS was also significantly correlated to periosteal circumference (p=0.002) and cortical thickness (p<0.05) of the radius at the 65% site. At the 65% site of the tibia, ToA (p=0.002), CoA (p=0.002), SSI (p=0.002), PC (p=0.004), EC (p=0.006) and CT (p=0.003) were all significantly and positively correlated to PBSS. PBSS was also significantly correlated to both arm (p<0.001) and leg (p=0.002) muscle CSA.

Total activity (counts/min) as measured by accelerometry was significantly correlated to body size adjusted BMC at the spine (p=0.04), hip (p<0.001) and femoral neck (p<0.001). Total activity counts were not correlated to any of the bone variables measured by pQCT. Similarly moderate but not vigorous intensity activity counts were significantly correlated to BMC at the femoral neck (p=0.04) whereas vigorous activity counts were correlated to BMC at the hip (p=0.03). When both moderate and vigorous activity counts were combined (MVPA), significant correlations were seen for BMC at the femoral neck only (p=0.05). At the 4% radius, significant correlations between moderate activity counts and MVPA were seen for ToD and BSI (p=0.005 and p=0.01 respectively). The association between vigorous activity counts and BSI at the 4% radius was not quite significant (p=0.06). Vigorous activity count was correlated to CoA (p=0.03) and forearm muscle CSA (p=0.01) at the 65% radius. There was no correlation between both moderate or vigorous activity count and other radial bone variables measured by pQCT.

At the 65% tibia site, no correlations were observed between activity counts and bone pQCT bone outcomes. However there was a trend for ToA (p=0.07) and CoA (p=0.08) of the tibia to be correlated to vigorous activity at this site.

Table 3.3 Correlation coefficients (r) between adjusted bone variables and log-transformed PBSS from the B^3Q as well as accelerometer derived activity.

		Counts/minute			
	PBSS	**Total activity**	**Moderate**	**Vigorous**	**Moderate - vigorous**
BMC (g)					
Ulna	0.46†	0.10	0.07	0.15	0.03
Radius	0.43†	0.27	-0.03	0.08	-0.05
Spine	0.47†	0.37*	0.17	0.30	0.15
Hip	0.50†	0.43*	0.26	0.36*	0.26
Femoral neck	0.57‡	0.50†	0.34*	0.30	0.32*
Metaphysis - Radius					
ToA (mm^2)	0.44†	0.07	0.23	0.26	0.19
ToD (mg/cm^3)	0.47†	0.09	0.41*	0.15	0.40*
TrbD (mg/cm^3)	0.61‡	-0.08	0.24	0.18	0.24
BSI (mg^2/mm^4)	0.56†	0.13	0.46†	0.33	0.42*
Diaphysis - Radius					
ToA (mm^2)	0.64‡	-0.18	0.17	0.20	0.16
CoA (mm^2)	0.52†	-0.07	0.03	0.37*	0.06
CoD (mg/cm^3)	0.40*	0.11	0.06	0.28	0.06
SSI (mm^3)	0.62‡	-0.16	-0.03	0.15	-0.03
PC (mm)	0.60†	0.003	0.34	0.22	0.35
CT (mm)	0.41*	-0.13	0.10	0.29	0.10
EC (mm)	0.35	-0.15	0.27	0.06	0.27
Forearm MCSA (mm^2)	0.64‡	0.27	0.31	0.41*	0.31
Diaphysis -Tibia					
ToA (mm^2)	0.49†	0.09	0.25	0.30	0.24
CoA (mm^2)	0.48†	0.10	0.15	0.30	0.14
CoD (mg/cm^3)	-0.16	-0.20	0.01	0.16	0.03
SSI (mm^3)	0.49†	0.06	0.21	0.32	0.19
PC (mm)	0.47†	0.04	0.23	0.28	0.21
CT(mm)	0.49†	0.03	0.02	0.19	0.003
EC (mm)	0.46†	0.02	0.25	0.22	0.24
Leg MCSA (mm^2)	0.52†	0.14	0.18	0.26	0.16

BMC measures by DXA are adjusted for sex, lean mass and bone area while pQCT measures are adjusted for sex, body mass and limb length. ToA; total area, ToD: total density, TrbD: density of trabecular bone at 4% site, BSI: bone strength index, CoD: cortical density, SSI: strength-strain index, PC: periosteal circumference, CT: cortical thickness, EC: endosteal circumference, MCSA: muscle cross-sectional area. * $p<0.05$, † $p<0.01$, ‡$p<0.001$.

Linear regression analysis

A summary of the results from the multiple linear regression analyses are presented in table 3.4. Although small, physical activity as assessed by the PBSS, explained significantly more variance in BMC of the femoral neck, spine, whole body and total hip compared to MVPA as measured by the Actical accelerometer. In addition, at the cortical sites of the radius and tibia, variance in area, density and strength were explained more by the PBSS than by MVPA.

Table 3.4 Results of multiple linear regression showing the proportion of bone variance explained by either the PBSS measured by questionnaire or MVPA as measured by accelerometry.

Variable	PA	β	t	p	SR^2, %
Femoral neck BMC (g)	PBSS	0.525	3.373	0.002	0.23
	MVPA	0.007	0.048	0.962	0.00
Spine BMC (g)	PBSS	0.514	3.209	0.003	0.22
	MVPA	-0.089	-0.557	0.581	0.01
Whole body BMC (g)	PBSS	0.479	2.932	0.006	0.20
	MVPA	-0.91	-0.556	0.582	0.01
Hip BMC (g)	PBSS	0.503	3.147	0.003	0.22
	MVPA	-0.044	-0.275	0.785	0.00
		65% radius			
Total area (mm^2)	PBSS	0.59	3.845	0.001	0.30
	MVPA	-0.078	-0.506	0.616	0.01
Cortical area (mm^2)	PBSS	0.599	3.885	<0.001	0.31
	MVPA	-0.219	-1.396	0.172	0.04
Cortical density (mg/cm^3)	PBSS	0.569	3.612	0.001	0.28
	MVPA	-0.151	-0.961	0.343	0.02
Strength-strain index (mm^3)	PBSS	0.594	3.843	0.001	0.31
	MVPA	-0.260	-1.678	0.103	0.06
		65% Tibia			
Total area (mm^2)	PBSS	0.541	3.424	0.002	0.25
	MVPA	-0.068	-0.430	0.67	0.00
Cortical area (mm^2)	PBSS	0.347	1.985	0.055	0.10
	MVPA	-0.090	-0.513	0.611	0.01
Cortical density (mg/cm^3)	PBSS	0.412	2.416	0.021	0.15
	MVPA	-0.131	-0.768	0.448	0.01
Strength-strain index (mm^3)	PBSS	0.546	3.456	0.001	0.26
	MVPA	-0.097	-0.614	0.544	0.01

MVPA is moderate-vigorous activity as measured using the Actical accelerometer. Results are expressed as partial regression coefficient (β), Student's t-test (t) and squared semi-partial

correlation (SR^2). Analysis was conducted using multiple linear regression adjusted for gender, body mass and bone area (for DXA variables) or limb length (for pQCT variables).

3.4 Discussion

The PBSS algorithm demonstrated a moderate ability to correctly classify children who participate in sports of a weight-bearing nature, into a group that would also be considered to be moderately or vigorously active as measured by an Actical accelerometer. All body size adjusted BMC measures and aspects of bone size and strength as measured by pQCT at both the radius and tibia were greater in children classified as being high bone loaders (according to the median PBSS) compared to low bone loaders. Whereas, moderate correlations between intensity of activity (assessed by the Actical) and strength of the radius and tibia were observed, the associations between accelerometry measures and bone area and content were weaker compared to those observed between the PBSS algorithm and size and content of bone. In the current study, moderate and vigorous intensity activity was moderately associated with PBSS scores. Nor Aini et al (in children of similar age to the present study), showed less strong associations between their physical activity questionnaire and Actical derived moderate activity, due in part to their participants over- or under-reporting vigorous activities (162). Stronger correlations may have been observed between objectively measured activity and the PBSS scores because activity was assessed over a two year period to account for variations in annual changes in sport.

Other studies have reported associations between physical activity questionnaires and BMC at the femoral neck, hip, spine (158) and whole body (157), whereas in this study PBSS was not only significantly associated with spine, hip and femoral neck BMC but also with ulna

and radius BMC. Children who take part in upper extremity sports such as gymnastics (185,191), hockey (192) and tennis (175), have greater bone mass, strength and area at the proximal and distal radius. On secondary analysis, it was found that participation in tennis (in both boys and girls) as well as netball (for girls), sports that use arm movement, was common in children who participated in this study with 24 out of the 38 children taking part in at least one of the abovementioned sports on a regular basis. In this study, PBSS was also associated with forearm and leg muscle cross-sectional area which indicates the ability of the score to reflect the close relationship that exists between muscle and bone. The present study also indicates the usefulness of the PBSS algorithm in predicting bone size and geometry as measured by pQCT. Farr and colleagues (189,235) investigated associations between a physical activity questionnaire and pQCT bone measures and reported that associations between a past year physical activity recall and bone strength indices were stronger than between pedometry and bone outcomes. Similarly, in the present study, stronger associations with PBSS and area of trabecular and cortical bone, strength indices as well as periosteal circumference were observed, than between accelerometry and bone outcomes. This study has shown that the bone specific nature of the B^3Q makes it an effective tool for obtaining information on bone loading participation in sport in a child population.

Total activity counts measured by accelerometry were associated with BMC at the spine, hip, and femoral neck - sites most affected by bone loss in later life. These findings underpin the importance of physical activity for bone health. The intensity of physical activity has been shown to be associated with bone health in children and specifically the combination

of moderate and vigorous physical activity has been most associated with greater BMC, BMD and bone area (156). In our study, vigorous intensity activity was not more closely related to BMC than was moderate intensity activity, as has been shown previously (161,236). Rather, moderate and combined moderate and vigorous activity was significantly associated with BMC at the femoral neck whereas vigorous activity was associated with BMC at the hip only. The positioning of the accelerometer on the hip as well as its limited ability to accurately detect movement in three-dimensional planes may have been a reason for associations in activity only being evident at the hip and femoral neck but not at any of the other sites assessed by DXA.

At the metaphysis of the radius, a site that is susceptible to wrist fracture during growth (61), moderate and combined moderate-vigorous activity were associated with bone strength, while at the cortical tibia, vigorous activity was not quite associated with bone strength ($p=0.05$). Previous studies, which have examined associations between bone strength indices at the radius and tibia and activity measured accelerometry (237,238), report similar findings to this study. Pedometers are not as accurate a method of assessing activity as accelerometry (239) and the strength of the relationships reported in the Farr et al study (235) (using pedometers) were not as strong as those seen in the present study. Farr and co-workers also showed that girls with the highest levels of duration and frequency of weight-bearing activity had greater strength at the tibia compared to girls with lower levels of weight-bearing activity without any changes in cortical density (189). In this study, vigorous activity was associated with cortical area at the radius but no significant association was seen between vigorous activity and radial strength. A study in pre-pubertal tennis

players however, has shown that resistance to torsion and bending (i.e. greater SSI) is due to increases in cortical area as a result of periosteal apposition, and not necessarily bone density (175).

The study has limitations which must be considered. Participants were a convenience sample and although there were strict inclusion and exclusion criteria, it is possible that a more physically active group of children may have inadvertently been selected. The two dimensional nature of DXA measurements is consistently problematic in interpreting bone data in children, which was controlled for by limiting participation in this study to children who were classified as being pre- or early pubertal as well as using appropriate body size covariates in the statistical analysis. The Actical has limited ability to accurately assess the intensity of specific types of activity such as weight-bearing activities, cycling, and swimming and the positioning of the Actical may also have contributed to the difference in associations seen between the PBSS and the Actical. The load values assigned to activities reported on in the B^3Q are based on peak strain scores reported in the literature (228) and we acknowledge that ground reaction forces were not measured in our study. In addition, vigorous activity was not quite significantly correlated to tibial total and cortical area ($p=0.07$ and $p=0.08$ respectively) but this may be due to the small sample size as well as the relatively low levels of participation in vigorous activity in this cohort of children.

The bone specific component of the B^3Q (PBSS algorithm) is a useful tool for the assessment of the relationship between participation in weight-bearing sport and bone health in pre-pubertal children. While the PBSS algorithm was associated with bone health measured by

DXA and pQCT, accelerometer measured activity did not show correlations with bone health to the same extent as the PBSS. In conclusion, the PBSS score generated from the B^3Q can be used to reliably and accurately collect data on participation in weight bearing exercise and is able to classify children as being either high or low bone loaders.

CHAPTER 4 – STUDY 2 - A TWO-YEAR HISTORY OF HIGH BONE LOADING PHYSICAL ACTIVITY ATTENUATES ETHNIC DIFFERENCES IN BONE STRENGTH AND GEOMETRY IN PRE/-EARLY PUBERTAL CHILDREN FROM A LOW-MIDDLE INCOME COUNTRY [1]

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[1] Meiring, Rebecca M., Avidon, Ingrid, Norris, Shane A., McVeigh, Joanne A. A two-year history of high bone loading physical activity attenuates ethnic differences in bone strength and geometry in pre/-early pubertal children from a low-middle income country. *Bone,* 57 (522-530), 2013.

4.1 Introduction

No studies have considered the relationship between ethnicity and physical activity on structural and geometric properties of bone in a healthy cohort of South African black and white children. This cross-sectional study used both DXA and pQCT technology to explore the interplay between bone health and ethnicity and analyze the differences in bone content, area and strength as well as geometric properties of bone due to history of loading in pre- to early-pubertal South African black and white children. A two-year history of physical activity was obtained in order to take into account seasonal variation in sport and also to consider those children who had played a sport up to the first year but then changed to a different sport in the second year. For example, there were some children in the cohort that in the first year of PA assessment had participated in a weight-bearing sport but in the second year of assessment had participated in a non weight-bearing sport such as swimming. The rationale behind a two-year history as opposed to the conventional one-year history was to ensure we included children who had been exposed to a season of bone loading. In addition, the sustained benefits of weight-bearing exercise are not well defined. The physical activity questionnaire served as a proxy measure of bone loading classification. It was hypothesised that children who were classified as being high bone loaders for the past two years would present with greater bone mass and strength regardless of their ethnicity and that high levels of weight-bearing physical activity would negate any ethnic bone differences that may have existed in the participants. It was also assessed whether muscle CSA could account for any differences observed in bone variables between high and low bone loading children.

4.2 Methods and Materials

Please refer to section 2.4 on page 66 for details on the methodology of this study. The statistical analyses used in this study are detailed in section 2.4.6.

4.3 Results

Of the 96 children who were recruited to participate, 14 children did not fulfil the Tanner pubertal classification of being pre-/early pubertal, two children were taking chronic medication for asthma and 4 children withdrew from the study before any procedures were performed. A further ten children (6 white boys, 2 white girls and 2 black boys) were excluded from the final analysis because their PBSS were either greater than or less than 2 SD from the mean PBSS. We excluded these children in order to match PBSS between groups and so that our ethnic groups were comparable for bone loading history. Thus 66 black and white boys and girls were included in the final analysis. Table 4.1 shows the descriptive characteristics of the children grouped according to ethnic group (black and white) and sex. All children were of similar age, height, weight, lean mass as well as BMI-for-age percentile and tibia length. Even though groups were matched for PBSS, white boys tended to have a higher PBSS although this was only significantly different to the black girls ($p=0.005$). There were significant interactions between sex and ethnicity for weight, fat mass, lean mass and forearm muscle CSA but post-hoc tests revealed no significant differences between groups for weight and lean mass. White boys had significantly greater fat mass compared to black boys ($p=0.01$) while black girls had greater fat mass and body fat percentage compared to black boys ($p=0.009$) and radius length compared to white girls

(p=0.02). White boys had greater arm muscle CSA compared to black boys (p=0.02), black girls (p=0.007) and white girls (p=0.002).

Table 4.2 shows the ethnic and sex effects on bone variables as measured by pQCT. There were significant interactions between sex and ethnicity for TrbD at the 4% radius and CT and EC at the 65% radius. There were no significant interactions between sex and ethnicity for bone outcomes at the 65% tibia. There were significant main effects of sex for TrbD and ToD as well as BSI at the 4% radius but no main effects of ethnicity were seen at the 4% radius. There was a significant main effect of sex as well as a main effect of ethnicity for CoA at the 65% radius site. There were no significant interactions between sex and ethnicity for the bone outcomes at the 65% tibia but there were significant main effects of ethnicity for ToA, CoA, PC and EC at the 65% tibia. No main effects of sex were seen at the 65% tibia. Post-hoc analyses are outlined in table 4.2.

Table 4.1 Descriptive characteristics of children grouped by sex and ethnicity.

	Black		White	
	Boys (n=15)	**Girls (n=27)**	**Boys (n=7)**	**Girls (n=17)**
Age (yrs)	10.4 (1.4)	10.1 (1.2)	10.1 (1.1)	9.6 (1.3)
Height (cm)	136.0 (6.7)	137.8 (8.3)	139.6 (11.8)	135.4 (8.8)
Weight (kg)	30.2 (3.8)	33.3 (7.3)	38.1 (11.3)	31.4 (6.2)
Tanner stage (n) I/II/III	7/5/3	14/9/4	6/1/0	14/2/1
***PBSS**	4.5 (3.2-5.7)	4.0 (3.0-6.0)[e]	8.0 (4.7-9.5)[e]	4.5 (4-10)
Fat mass (kg)	5.6 (0.8)[ab]	8.7 (3.1)[a]	9.8 (5.1)[b]	8.6 (2.6)
Lean mass (kg)	23.2 (3.7)	23.2 (4.7)	27.1 (6.6)	21.7 (4.0)
***% body fat**	19.3 (15.9-22.9)[a]	25.8 (22.3-28.7)[a]	21.9 (19.4-32.8)	28.6 (22.0-30.3)
***BMI percentile**	45.0 (17.4-53.8)	60.6 (28.2-75.0)	65.0 (23.5-98.0)	59.0 (28.1-78.7)
Radius length (mm)	220.5 (13.6)	228.6 (17.1)[c]	217.2 (21.0)	210.9 (18.4)[c]
Tibia length (mm)	314.0 (23.0)	325.0 (25.8)	320.33 (37.8)	315.8 (24.8)
Arm muscle CSA (mm^2)	1797.1 (391.5)[b]	1773.2 (316.4)	2364.5 (712.4)[bd]	1651.8 (256.8)[d]
Leg muscle CSA (mm^2)	3376.9 (581.0)	3402.3 (594.7)	3990.3 (1223.1)	3375.3 (627.3)

Data are mean (SD) except arm and leg muscle CSA which are mean (SE) and Tanner which show proportions within each group. * indicates data expressed as median (IQR). Matching superscript letters indicate significant differences between groups, e.g. if white low bone loaders and black low bone loaders have the same letter [a], then those are the two groups that are significantly different from each other; $p<0.05$. Forearm and leg muscle CSA were adjusted for weight, sexual maturity and limb length.

Table 4.2 Sex and ethnic differences in radius and tibia bone density, structure and strength.

	Black		White	
	Boys (n=15)	**Girls (n=27)**	**Boys (n=7)**	**Girls (n=17)**
4% Radius	(n=13)	(n=22)	(n=6)	(n=16)
ToA (mm^2)	232.6 (24.4)	225.0 (33.8)	253.2 (42.5)	215.4 (32.9)
ToD (mg/cm^3)	301.9 (6.3)	288.2 (10.7)	293.2 (12.5)	272.3 (6.7)
TrbD (mg/cm^3)	212.6 (11.2)[b]	214.3 (13.8)[d]	235.5 (17.3)[bc]	190.3 (9.6)[cd]
BSI (mg^2/mm^4)	2087.6 (210.4)	1846.6 (262.4)	2137.3 (320.7)	1610.8 (286.6)
65% Radius	(n=13)	(n=22)	(n=6)	(n=16)
ToA (mm^2)	103.8 (10.5)	94.0 (12.5)	108.2 (14.9)	98.3 (14.5)
CoA (mm^2)	43.2 (4.4)	43.6 (5.1)	52.0 (5.5)	41.6 (4.9)
CoD (mg/cm^3)	1012.8 (6.2)	1017.4 (6.9)	1019.3 (9.8)	988.9 (4.6)
SSI (mm^3)	162.5 (24.3)	139.2 (26.3)	169.9 (31.1)	137.5 (29.1)
PC (mm)	35.9 (1.8)	34.3 (2.2)	36.8 (2.6)	34.9 (2.6)
CT (mm)	1.4 (0.1)[ab]	1.5 (0.1)[ad]	1.6 (0.1)[bc]	1.4 (0.1)[cd]
EC (mm)	27.2 (1.7)[a]	24.9 (2.0)[a]	26.5 (2.3)	26.2 (2.4)
65% Tibia	(n=14)	(n=26)	(n=6)	(n=16)
ToA (mm^2)	472.5 (49.7)	461.4 (62.5)	485.2 (112.0)	418.5 (58.7)
CoA (mm^2)	226.2 (31.6)	224.8 (32.5)	219.9 (53.8)	208.5 (30.0)
CoD (mg/cm^3)	1053.1 (7.0)	1048.2 (6.7)	1025.5 (9.8)	1035.3 (3.9)
SSI (mm^3)	1624.1 (322.3)	1621.4 (373.9)	1813.5 (666.0)	1435.7 (361.5)
PC (mm)	76.9 (4.2)	76.0 (5.2)	76.7 (9.5)	72.3 (4.9)
CT (mm)	3.4 (0.3)	3.4 (0.3)	3.3 (0.5)	3.4 (0.3)
EC (mm)	55.4 (2.6)	54.3 (3.9)	56.3 (7.3)	51.1 (3.6)

Data are mean (SEM) and adjusted for weight, limb length, sexual maturity and muscle CSA. Similar superscript letters indicate significant differences; $p<0.05$. ToD = total bone mineral density, TrbD = trabecular bone mineral density, BSI = bone strength index, CoD = cortical bone mineral density, ToA = total area, CoA = cortical area, SSI = polar strength-strain index, PC = periosteal circumference, EC = endosteal circumference, CT = cortical thickness, CSA = cross-sectional area. Note: Scan numbers differ at each site as there were children in each group whose limbs were too short to fit comfortably in the pQCT machine.

4.3.1 Ethnicity, weight-bearing physical activity and bone

Table 4.3 shows the descriptive characteristics of the children according to ethnicity and bone loading group. White high bone loaders had a greater body mass compared to the white low bone loaders (p=0.03). There were significant interactions between ethnicity and bone loading for both forearm (p=0.03) and leg (p=0.01) muscle CSA. Arm muscle CSA was greater in the white high bone loaders compared to the black low bone loaders (p=0.003), Black high bone loaders (p=0.008) and the white low bone loaders (p=0.001) after adjusting for sex, weight and sexual maturity. After adjusting for sex, weight and sexual maturity, leg muscle CSA was greater in the white high bone loaders compared to both the black high bone loaders (p=0.03) and the white low bone loaders (p=0.006).

Table 4.3 Descriptive characteristics of children grouped by ethnicity and bone loading group

	Low		High	
	Black (n=30)	**White (n=11)**	**Black (n=12)**	**White (n=13)**
Age (yrs)	10.2 (1.1)	9.5 (1.4)	10.3 (1.6)	9.9 (1.2)
Height (cm)	137.5 (7.9)	133.1 (8.2)	136.1 (7.6)	139.6 (10.1)
Weight (kg)	32.5 (6.8)	29.0 (5.4)[d]	31.2 (5.3)	37.0 (8.8)[d]
Girls (n)	19	8	10	7
Tanner stage (n) I/II/III	14/11/5	8/2/1	7/3/2	12/1/0
***PBSS**	3.5 (3.0-4.0)[cf]	4.0 (3.3-4.0)[de]	6.0 (6-8.0)[ce]	8.0 (7.0-9.3)[df]
Fat mass (kg)	7.6 (3.2)	7.6 (2.5)	7.4 (2.0)	10.1 (3.7)
Lean mass (kg)	23.5 (4.3)	20.3 (3.4)[d]	22.3 (4.5)	25.8 (5.5)[d]
***% body fat**	22.6 (19.0-27.6)	23.0 (21.3-30.9)	23.7 (20.5-28.9)	27.5 (21.0-31.1)
***BMI percentile**	53.0 (25.5-70.7)	42.9 (14.1-76.1)	39.0 (27.8-60.5)	74.3 (42.2-91.5)
Forearm length (mm)	227.4 (13.9)[a]	204.0 (17.2)[a]	220.2 (21.4)	219.9 (17.8)
Tibia length (mm)	322.1 (25.7)	313.0 (24.5)	318.5 (24.3)	320.4 (31.3)
Arm muscle CSA (mm^2)	1792.6 (191.3)[af]	1563.3 (240.2)[ad]	1751.6 (259.7)[b]	2112.9 (317.8)[bdf]
Leg muscle CSA (mm^2)	3453.6 (499.5)	3153.1 (460.5)[d]	3253.1 (472.7)[b]	3868.0 (709.7)[bd]

Data are mean (SD) except arm and leg muscle CSA which are mean (SE). * indicates data expressed as median (IQR). Similar superscript letters indicate significant differences; p<0.05. Forearm and leg muscle CSA were adjusted for sex, weight, sexual maturity and limb length.

Figure 4.1 is a box-and-whisker plot of the PBSS between ethnic and bone loading groups. PBSS was significantly and positively correlated to most DXA and pQCT bone variables in the white children only (Table 4.4).

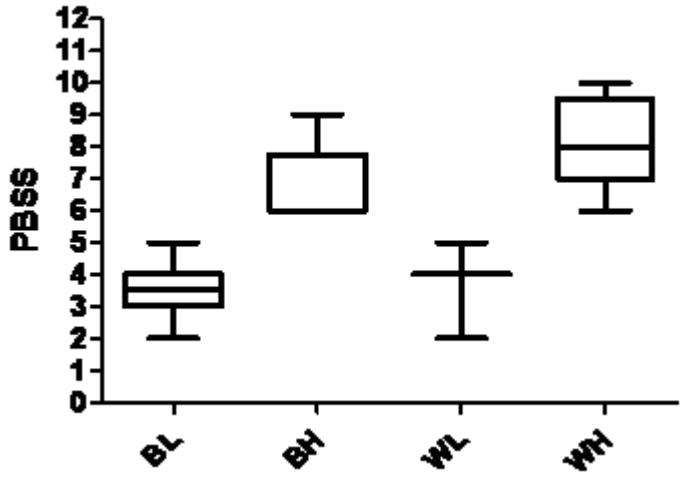

Figure 4.1 Box-and-whisker plot of PBSS between ethnic and bone loading groups. BL=Black low bone loaders (n=30); BH = Black high bone loaders (n=12); WL = White high bone loaders (n=11); WH = White high bone loaders (n=13).

Table 4.4 Pearson's correlations showing the r values of log-transformed PBSS with adjusted bone variables

DXA	Black (n=42)	White (n=24)
Ulna BMC (g)	0.01	0.56†
Radius BMC (g)	0.07	0.60†
Spine BMC (g)	-0.11	0.60†
Whole body BMC (g)	0.04	0.66†
Hip BMC (g)	0.03	0.55†
Femoral neck BMC (g)	0.07	0.55†
Radius (4%)		
ToD (mg/cm^3)	0.12	0.20
TrbD (mg/cm^3)	-0.19	-0.05
ToA (mm^2)	-0.18	0.54*
BSI (mg^2/mm^4)	-0.01	0.58†
Radius (65%)		
CoD (g/cm^3)	0.25	0.30
ToA (mm^2)	0.01	0.56*
CoA (mm^2)	0.14	0.54*
SSI (mm^3)	0.12	0.43*
PC (mm)	-0.01	0.58†
EC (mm)	-0.07	0.46*
CT (mm)	0.13	0.27
Arm MCSA (mm^2)	-0.13	0.54
Tibia (65%)		
CoD (mg/cm^3)	0.03	0.22
ToA (mm^2)	-0.07	0.54*
CoA (mm^2)	0.05	0.41
SSI (mm^3)	-0.07	0.65†
PC (mm)	-0.08	0.50*
EC (mm)	-0.07	0.41*
CT (mm)	-0.02	0.09
Leg MCSA (mm^2)	-0.20	0.51*

Data are r values. BMC measures by DXA are adjusted for sex, body mass, sexual maturity and bone area while pQCT measures are adjusted for sex, weight, sexual maturity, limb length and muscle CSA. ToA; total area, ToD: total density, TrbD: density of trabecular bone at 4% site, BSI: bone strength index, CoD: cortical density, SSI: strength-strain index, PC: periosteal circumference, CT: cortical thickness, EC: endosteal circumference, MCSA: muscle cross-sectional area. * $p<0.05$, †$p<0.01$.

4.3.2 DXA

Table 4.5 shows the DXA data obtained for all the participants grouped according to ethnicity and bone loading group. There were significant interactions between ethnicity and bone loading for BMC at the radius (p=0.005), spine (p=0.04), whole body (p=0.04) and femoral neck (p=0.05) but not for total hip BMC. There were significant main effects of bone loading for radius (p=0.001), whole body (p=0.004) and femoral neck (p=0.01). Post-hoc analysis revealed that there was an ethnic difference between black and white children in the low bone loading group for femoral neck BMC only (p=0.02). There was no difference in BMC between the black and white children in the high bone loading group for any site. White high bone loaders had greater BMC at the radius (p=0.01), whole body (p=0.04) and femoral neck (p=0.04) than the white low bone loaders. There were no within ethnic differences at any site between the high and low black bone loaders.

4.3.3 pQCT

There were significant interactions between ethnicity and bone loading for ToA at the 4% radius (p=0.01) (Table 4.5). There were no significant interactions between ethnicity and bone loading for any of the bone outcomes at the 65% radius. There were significant main effects of bone loading on ToA (p=0.04) and PC (p=0.04) at the 65% radius. Significant interactions between ethnicity and bone loading were present for ToA (p=0.02), SSI (p=0.02), PC (p=0.03) and EC (p=0.03) at the 65% tibia.

Table 4.5 Adjusted whole body and site-specific DXA and pQCT measures of the radial epiphysis and diaphysis between black and white children within each bone loading group

	Low		High	
	Black (n=28)	**White (n=9)**	**Black (n=9)**	**White (n=11)**
DXA				
Radius BMC (g)	3.7 (0.1)	3.0 (0.2)[c]	3.7 (0.2)	4.0 (0.2)[c]
Spine BMC (g)	23.7 (0.7)	20.6 (1.1)	22.9 (1.2)	24.6 (1.2)
Whole body BMC (g)	785.9 (24.2)	671.8 (35.5)[c]	800.8 (38.2)	827.8 (52.8)[c]
Total hip BMC (g)	17.5 (0.6)	14.9 (1.0)	17.8 (1.3)	17.6 (1.1)
Femoral neck BMC (g)	2.9 (0.1)[a]	2.4 (0.1)[ac]	2.9 (0.1)	2.9 (0.1)[c]
Radius (4%)				
ToD (g/cm^3)	290.4 (2.3)	272.7 (1.7)	297.3 (2.7)	286.6 (2.9)
TrbD (g/cm^3)	208.4 (2.0)	202.8 (2.7)	217.6 (4.0)	203.9 (3.1)
ToA (mm^2)	229.4 (4.6)[a]	198.8 (10.3)[ac]	215.0 (11.8)	243.3 (10.2)[c]
BSI (mg^2/mm^4)	1906.8 (38.7)	1450.0 (91.4)	1913.4 (116.8)	1999.5 (85.6)
Radius (65%)				
CoD (g/cm^3)	1013.6 (1.5)	989.4 (3.5)	1009.2 (4.3)	1008.0 (3.7)
ToA (mm^2)	97.3 (1.3)[a]	88.8 (2.6)[ac]	98.9 (3.7)[b]	109.9 (3.0)[bc]
CoA (mm^2)	43.0 (0.8)	41.9 (1.7)	43.6 (2.2)	46.7 (1.7)
SSI (mm^3)	145.4 (3.8)	130.5 (7.3)	151.6 (10.8)	159.4 (8.8)
PC (mm)	34.9 (0.2)[a]	33.2 (0.5)[ac]	35.0 (0.6)[b]	37.0 (0.5)[bc]
EC (mm)	25.9 (0.1)	24.1 (0.2)	25.9 (0.3)	27.8 (0.4)
CT (mm)	1.4 (0.02)	1.5 (0.04)	1.6 (0.05)	1.5 (0.04)

Data are means (SEM). DXA BMC data are adjusted for sex, weight, sexual maturity and bone area. Peripheral QCT data are adjusted for sex, weight, sexual maturity, limb length and arm muscle CSA. Similar superscript letters indicate between group differences; $p<0.05$. Note: Scan numbers differ at each site as there were children in each group whose limbs were too short for pQCT scanning.

Radial metaphysis (4%)

Table 4.5 shows the within and between ethnic differences between high and low bone loaders at the radius as measured by pQCT. The black low bone loaders had a greater ToA (p=0.04) at the epiphysis of the radius compared to the white low bone loaders. There was no difference in ToA between black and white high bone loaders. Within the white children, high bone loaders had greater ToA (p=0.007) compared to the low bone loaders.

Radial diaphysis (65%)

In the low bone loading group, the black children had greater total area (p=0.05) and periosteal (p=0.03) circumference than the white children (Table 4.5). In the high bone loading group, the white children had greater ToA (p=0.02) and periosteal (p=0.02) circumference compared to the black high bone loaders. There were only within-ethnic group differences in the White children, where the White high bone loaders had greater ToA (p<0.001), as well as periosteal (p<0.001) circumference compared to the White low bone loaders.

Tibial diaphysis (65%)

The data from the post-hoc analyses for the tibial diaphysis are shown in Figure 4.2. The black low bone loaders had greater ToA (Figure 4.2B) and SSI (Figure 4.2D) compared to the white low bone loaders (p=0.001 and p<0.001 respectively) but there was no difference in CoA or CoD between the two groups of low bone loaders (Figure 4.2A and C respectively). There were no significant ethnic differences in the high bone loading group for ToA, CoA, CoD or SSI. The white high bone loaders had significantly greater ToA (p=0.03), and SSI (p=0.02) but not CoA or CoD compared to the white low bone loaders. There were no

significant differences between the low and high bone loading groups of black children for all bone outcomes.

Tibial PC was smallest in the white low bone loaders compared to the black low ($p=0.001$) and white high ($p=0.04$) bone loading groups (Figure 4.2E). PC was not quite significantly smaller in the white low bone loading group compared to the black high group ($p = 0.06$). There was no difference in PC between the black and white children in the high bone loading group and there was no within-ethnic difference between low and high black bone loaders. Tibial EC was lowest in the White low bone loaders compared to the black low ($p<0.001$), black high ($p=0.001$) and white high ($p<0.001$) bone loaders (Figure 4.2G). The white high bone loaders were not significantly different to the black high bone loaders ($p=0.76$) in EC. Again there was no difference in EC between low and high black bone loaders. There were no statistically significant ethnic differences in CT in either the low or high bone loading groups (Figure 4.2F). Figure 4.3 is a schematic illustration of the differences in bone structure between low and high bone loading groups as well as between black and white children.

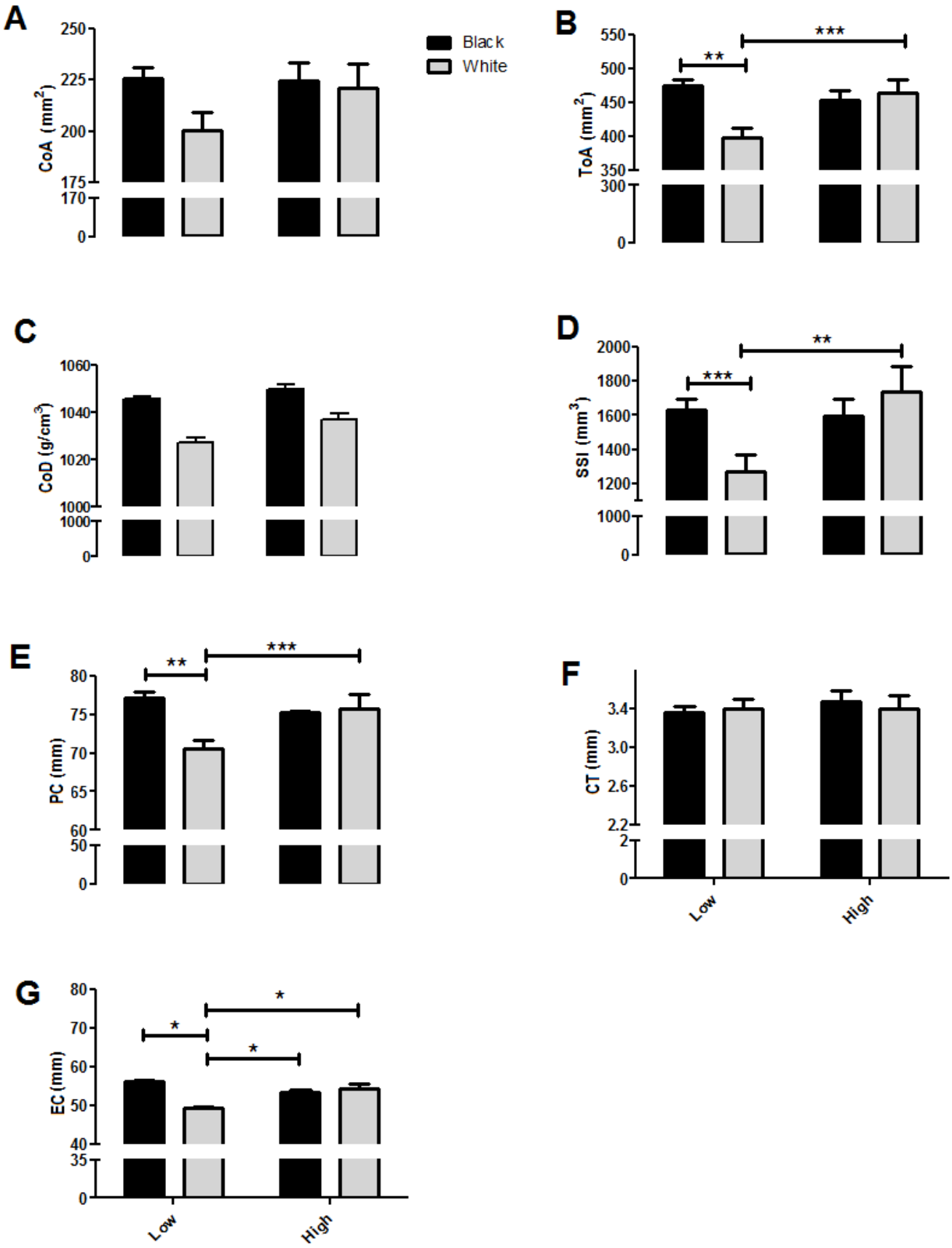

Figure 4.2 Bone and muscle geometry of the diaphysis of the tibia between black and white children within each bone loading group (Figures A-G). Bone variables were adjusted for sex, weight, sexual maturity, limb length and leg muscle cross-sectional area. Black low bone loaders: n=28; White low bone loaders: n=10; Black high bone loaders: n=12; White high bone loaders: n=12. *p<0.05, **p<0.01, ***p<0.001. Data are means (SE).

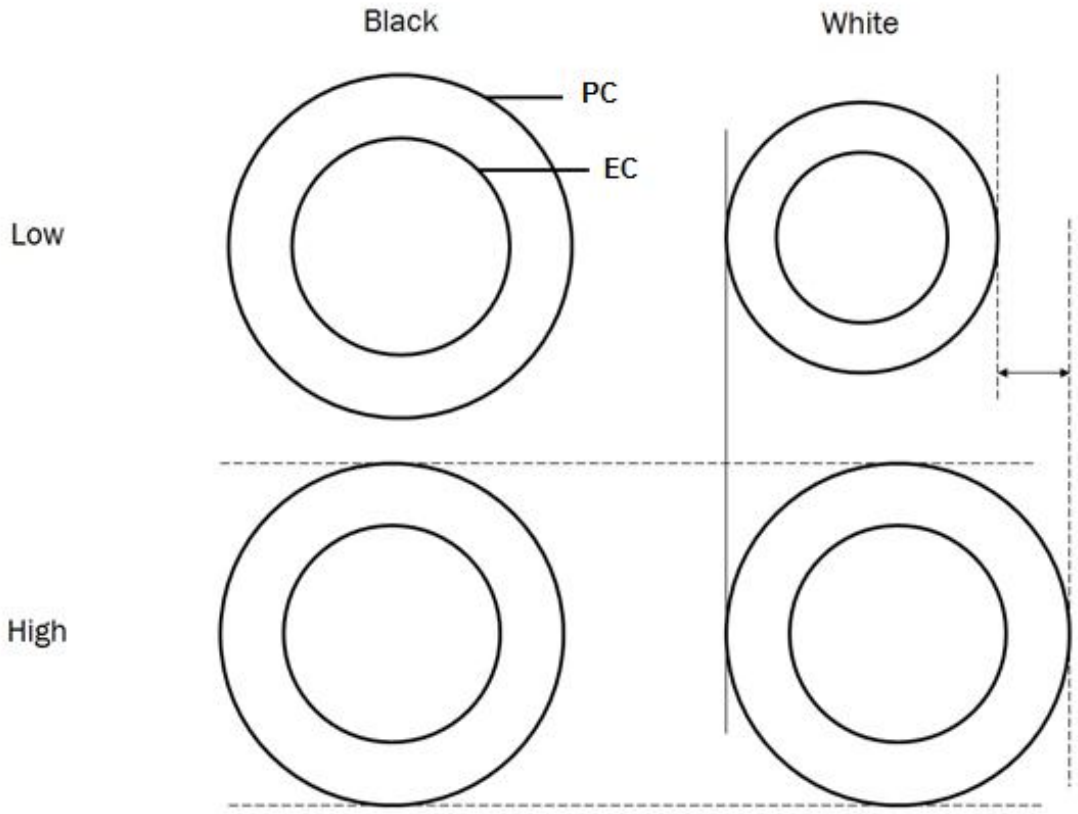

Figure 4.3 Schematic illustration of the differences in tibia bone structure between low and high bone loading groups as well as between black and white children. Diagram is not drawn to scale. PC = periosteal circumference; EC = endosteal circumference.

4.3.4 Muscle CSA

Forearm muscle CSA was not significantly correlated to any of the forearm pQCT bone outcomes in white children. There was an association between forearm muscle CSA and CoA (r=0.44, p=0.01), SSI (r=0.52, p=0.003) and PC (r=0.42, p=0.02) in the black children. Leg muscle CSA was significantly correlated to ToA (r=0.58, p=0.01), CoA (r=0.44, p=0.001), SSI (r=0.65, p=0.004), as well as PC (r=0.63, p=0.005) and CT (r=0.48, p=0.04) in the white children while leg muscle CSA was correlated to SSI (r=0.41, p=0.01) in the black children.

4.4 Discussion

In this study of pre- and early pubertal South African children, we have shown that in children who had a two-year history of low weight bearing physical activity, black children have higher bone mass and strength than white children. However, in the presence of a two-year history of high bone loading, white children had equal femoral neck bone mass compared to their black peers. Peripheral QCT measurements were used to show for the first time in South African children, that bone loading history may have a differential effect between ethnicities on femoral neck BMC, 4% radial and 65% tibial area as well as tibial structure and strength in black and white children. Structurally, black children and white high bone loaders exhibited larger periosteal circumferences compared to white low bone loaders, resulting in bigger cross-sectional areas of the bones of interest. The association of high bone loading was apparent at the diaphyseal (cortical) bone for the leg, where white children with the highest bone loading scores had a greater total area and periosteal circumference than their white peers with lower levels of bone loading. A greater tibial strength-strain index was also noted in the high bone loading group of white children compared to the low bone loaders, affording these children a more resistant appendicular skeleton. Interestingly, although PBSS score was significantly different between the black high and low bone loading children, the bone loading effect seen in the groups of white children was not observed in the black children. Out of the 13 children in the white high bone loading group 10 of them had a history of participation in tennis and netball and this may be a possible reason why there was a difference in muscle mass and bone area of the forearm between the white children. Studies have found that children who take part in upper extremity sports such as gymnastics (191,240), hockey (192) and tennis (175,177), have greater bone mass, strength and area at the proximal and distal radius. Netball is a

sport in which a ball is caught and thrown towards a goal and participation in this sport may well have also contributed to the differences seen in the White children.

Ethnic differences in measures of bone health are well documented in children. Vidulich and co-workers observed a greater BMC at the femoral neck, proximal femur and lumbar spine in black compared to white 10 year old South Africans, as measured by DXA (241). In the current study, ethnic differences in BMC as measured by DXA were observed at the femoral neck in the low bone loading group but not in the high bone loading group. Studies examining effects of ethnicity on measures of bone health using pQCT are scarce but those that have used pQCT, have been conducted in North American or European populations. In one North American cohort, ethnic differences in bone strength were shown to exist between black and white children and the effect of muscle on bone strength was found to be an important predictor of these differences, regardless of PA levels (133). In a study by Micklesfield et al (143) conducted in black and white South African 13 year olds, total bone area at the diaphysis of the tibia was significantly higher in black than in white children resulting in greater strength in black children even after adjusting for maturity and bone size. Ethnic differences in bone geometry and structure (as measured by pQCT) were also observed in this study. Black low bone loaders had greater area and strength measures at the tibia compared to the white low bone loaders.

McKay and co-workers (171) have used a similar questionnaire based method to assess the amount of participation in impact or non-impact PA and they found a positive association between impact PA and bone architecture and geometry as measured by high-resolution

pQCT (HR-pQCT). The authors did not include African Americans or black children in their study. The present study builds on previous knowledge of physical activity and bone health studies by considering the associations between ethnicity, participation in weight-bearing physical activity and bone health in a population of pre- to early pubertal children. When considering the children's history of participation in weight-bearing physical activity, the ethnic differences observed in the low bone loading groups were either reversed or attenuated in the high bone loading group. To the best of my knowledge this attenuation of bone difference between ethnic groups has not yet been observed in any populations when physical activity is used as a differentiating factor. South African black children and adults are generally considered to be afforded genetic protection in terms of bone health (126,242). McVeigh et al (135), using DXA, found that due to white children having higher levels of physical activity levels and therefore higher mechanical loading, increases in BMC in white South African children but not in black South African children occurred. Although black children did not report physical activity levels as high as those in white children, they still had greater bone mass compared to white children. The present study has further shown using pQCT, that the higher levels of weight-bearing physical activity that occurred in the high bone loading white children, was associated with greater bone density, area and strength at the trabecular and cortical site of the radius and the cortical site of the tibia compared to white low bone loaders. In addition, greater mechanical loading in the white children encouraged periosteal apposition which may have contributed to the greater bone area and strength, such that there appeared to be no ethnic difference in bone geometry and structure between the white and black high bone loaders. Therefore when physical activity levels are matched between ethnicities it may be possible for white high bone loaders to attain similar indices of bone health compared to black children.

Significant associations between physical activity and measures of bone geometry, including cortical area and thickness, as well as periosteal circumference have been observed in children and adolescents (171,189,196). Periosteal apposition increases bone width in pre-pubertal boys and girls (68). Weight-bearing physical activity leads to periosteal apposition and ultimately results in the formation of a much stronger bone (66). Even if one has a lower than normal bone mineral density, bone strength is maintained by the bigger bone area (243). Although studies are limited in whether ethnic differences in periosteal circumference exist due to the limited use of pQCT, the present study shows that there were no differences in periosteal circumference between white high bone loaders and the black group of children while white low bone loaders had smaller periosteal circumferences compared to the white high and black low bone loaders. High levels of weight-bearing physical activity in the white high bone loaders may have contributed to bone deposition on the periosteal surface such that bone area in the white high bone loaders was not different to that of the black group of children. Bass and colleagues (175) showed that mechanical loading through exercise resulted in greater periosteal apposition in pre-pubertal children compared to during late puberty. There certainly is a critical period where periosteal apposition is most evident in children during longitudinal growth after which periosteal apposition slows down greatly (66). Whether weight-bearing physical activity can influence the rate of periosteal apposition later in life, warrants further investigation.

Interestingly weight-bearing physical activity appeared to have no association with the bone health of black children implying that weight-bearing physical activity may either be more effective in those with a lower bone mass to start with or once a certain threshold of bone

mass is attained, physical activity levels offer little additional benefit over and above the genetic protection already afforded to black children. Furthermore, the fact that within-ethnic associations to weight-bearing exercise in BMC, bone area, strength and structure were seen in white children only, suggests that there may be ethnic-specific responses to mechanical loading in pre-early pubertal children. Nonetheless, this is yet to be determined as no studies have investigated the effects of weight-bearing physical activity on bone health in only black children and the cross-sectional nature of the present study does not allow for causal relationships to be made. Although it is possible that the tendency for the black children to be more mature than the white participants (but not significantly so) may have influenced the fact that no association was seen between PBSS and bone variables in the black children, both ethnicities were assessed with the same questionnaire. The lack of association may rather reflect a lower level of participation in exercise in general by black compared to the white children.

White South African children have greater leg muscle cross-sectional area compared to black South African children (137,143,144), a finding contrary to what has been reported in North American cohorts (133). In the present study muscle cross-sectional area of the forearm and lower leg was significantly higher in the white high bone loaders compared to the black high bone loaders even after adjustment for sex, , maturation and limb length. Although in this study a multivariate regression could not be done using muscle CSA as the dependant variable due to the small sample size, muscle CSA was significantly associated with bone variables in the white children only. Admittedly, these findings may have been due to the fact that there was no difference in muscle CSA between black high and low bone

loaders and indeed Warden et al (142) have suggested that muscle CSA did not contribute to the observed ethnic differences in bone variables seen in their cohort of black and white children. Micklesfield et al (143) found positive correlations between bone and muscle CSA in their black children, even though their black children had lower muscle CSA compared to white children. Neither of these studies took into account physical activity participation but in the latter study it has been suggested that ethnic-specific responses to mechanical loading and different types of physical activity may exist in black and white children (143).

There are limitations to this study. The use of physical activity questionnaires (PAQs) is problematic due to their subjective nature and investigator variability. There are also limitations associated with the retrospective recall of two year participation in physical activity but this was not a serious impediment to the measure of bone loading (i.e. PBSS) as all participants filled out the B^3Q assisted by their parent/caregiver and due to the seasonal nature of school sports, do not believe that it was difficult for children to recall their activities. In the case of the present study, the B^3Q used was one that has been validated for a South African population and I was responsible for the capture and analysis of all the B^3Q s. The B^3Q served as a means of obtaining a surrogate measure of high or low levels of physical activity hence the absolute value of physical activity was not imperative to this study. McKay and others (171) have used a similar questionnaire based tool to assess amount of participation in impact or non-impact PA and found a positive association between impact PA and bone architecture and geometry as measured by HR-pQCT. Participants were recruited into this study using a convenience sampling technique. Although there were strict inclusion and exclusion criteria, it is possible that a more

physically active group of white children may have been inadvertently selected. For this reason it was made sure that the PBSS was similar within high and low groups and thus selection bias is unlikely since the participants in this study had a similar range of physical activity levels. Ethnic differences in physical activity have been well described (135,207,244) and although it was attempted to match weight-bearing history as closely as possible between ethnic groups, the possibility exists that the positive (white) and negative (black) skewing of the scores in the high bone loading groups may have had an impact on the results. Larger sample sizes may be subjected to more robust multiple regressions, and since there were a limited number of participants, this analysis was not performed. The limited sample size and the cross-sectional design prevent causal conclusions from being established. As mentioned above, the absolute value of physical activity was not of the essence of this study and was merely a crude measure of estimating bone loading history, but further prospective studies are required to confirm the findings. The inherent limitations associated with DXA measurement in childhood must also be considered.

In conclusion, the present study has shown that in pre-/early pubertal children from a low-middle income country, white children appear to have a more sensitive bone response to weight-bearing exercise while black children may be afforded genetic protection of bone regardless of weight-bearing physical activity levels. It appears that in order for white children to reach the same bone mass/health levels as black children, they may need to participate in higher levels of weight-bearing physical activity. Moreover, ethnic differences in bone area and strength apparent between children classified as having a lower bone loading physical activity history appear to have been attenuated when children partaking in

high bone loading physical activities were compared. Greater levels of mechanical loading seemed to have no apparent benefits in black children.

CHAPTER 5 – STUDY 3 - OSTEOGENIC EFFECTS OF A PHYSICAL ACTIVITY INTERVENTION IN SOUTH AFRICAN BLACK CHILDREN[1]

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[1] Meiring RM, Micklesfield LK, Avidon I, McVeigh JA. Osteogenic Effects of a Physical Activity Intervention in South African Black Children. Submitted to *Journal of Musculoskeletal and Neuronal Interactions*.

5.1 Introduction

Although in study 2, higher levels of physical activity within the black children were not associated with bone outcomes, I wished to determine the bone response of black children to an increased threshold of physical activity (i.e. a targeted weight-bearing physical activity intervention). The aim of the next study was to determine whether a weight-bearing physical activity intervention improves measures of bone mass and structure in pre-pubertal black children. I hypothesised that changes in bone structure, geometry and content, would be greater in the children participating in the exercise intervention program compared with the children who did not partake in the program.

5.2 Methods and materials

Please refer to section 2.5 for details on the methods and materials used in this study. Statistical analyses used in this study are outlined in section 2.5.7.

5.3 Results

Thirty seven children were recruited into this study. One child from the control group and one child from the exercising group did not meet the inclusion criteria. Twelve children recruited into the exercising group moved away from the area or stopped attending the after-school care community centre and could not be followed up. Thus full (baseline and follow up) data are available for twenty two children (CON: n=10; EX: n=12). Seventy eight percent of children in the EX group completed all (100%) of the exercise sessions. The median peak bone strain score (PBSS) was similar between the two groups at the

start of the intervention (median, 95%CI; EX: 4.5, 3-8; CON: 4, 1-11; p=0.53). As expected, the median change in PBSS was significantly greater for the EX group compared to CON group (median, 95% CI; EX: 3, 2-5 vs. CON: 0, -1-1; p<0.001) and the PBSS for the EX group after the intervention was significantly greater than that of the CON group (median, 95%CI; EX: 7, 5-11 vs. CON: 4, 1-10; p=0.004) (Figure 5.1).

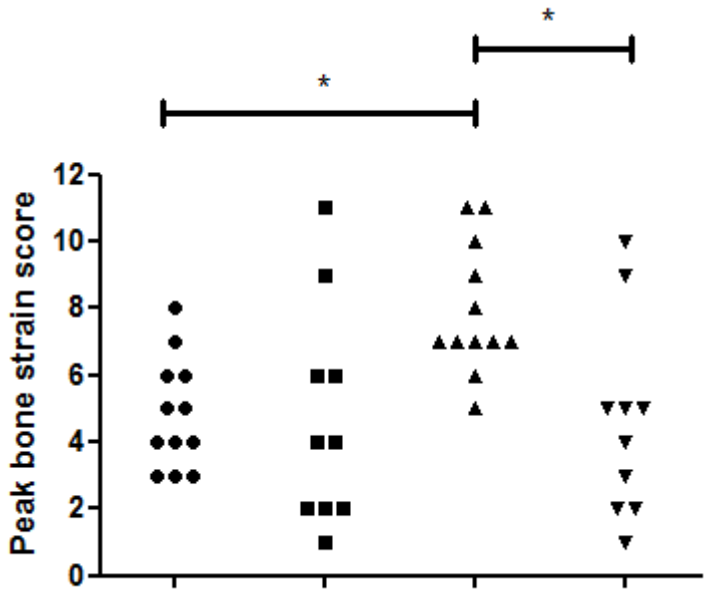

Figure 5.1 Peak bone strain score (PBSS) for the exercise (EX) and control (CON) groups before and after the 20 week intervention. PBSS was similar before the intervention between groups (p=0.53) but was significantly higher in the exercising group (* p<0.001) after the 20 week intervention. ●= EX baseline, ■ = CON baseline, ▲= EX post-intervention, ▼= CON post-intervention

Baseline and 20 week change in the anthropometric characteristics of each group are shown in Table 5.1. The change in height over the 20 week intervention period was significantly greater in the EX group (p=0.03). There was a difference in the proportion of children who were classified as being either Tanner stage I or II between the groups at baseline (p=0.03).Neither group were more pubertally advanced at follow up either

(p=0.68). There was no between or within group difference in fat CSA before or after the intervention and the change in these parameters were also not significantly different between groups.

Table 5.1 Baseline and change (where relevant) descriptive characteristics for control and exercising groups.

	Control (n=10)		Exercise (n=12)	
	Baseline (SD)	**Δ (95% CI)**	**Baseline (SD)**	**Δ (95% CI)**
Age	9.3 (0.9)	-	9.7 (1.2)	-
Girls (n)	7	-	8	
Tanner stage I/II (n)	9/1	-	5/7	-
Height (cm)	135.1 (8.2)	1.8 (1.2-2.4)	135.9 (8.7)	3.1 (2.1 - 4.2)[a]
Weight (kg)	30.6 (4.7)	1.0 (0.2-1.7)	30.0 (5.1)	1.6 (0.7-2.4)
BMI percentile	57.4 (22.4)	-5.1 (-15.0 - 4.9)	39.7 (20.1)	-3.1 (-8.6 - 2.4)
Fat mass (kg)	7.5 (1.9)	0.4 (-0.1 - 1.0)	6.7 (1.8)	0.3 (0.04 - 0.7)
Lean mass (kg)	21.4 (3.9)	1.2 (0.8-1.7)	21.9 (3.8)	1.6 (0.9 - 2.3)
% body fat	25.2 (5.4)	-0.02 (-1.3 – 1.3)	22.5 (3.8)	-0.3 (-1.3 - 0.7)
Fat CSA (mm^2)	1684.4 (129.1)	-5.6 (-13.3 – 2.0)	1538.0 (222.0)	-3.6 (-13.1 – 5.9)

Data are mean (SD). [a] Change is significantly greater in the intervention group, p<0.05. Cross sectional area (CSA). Post-intervention Tanner stage (I/II/III): CON, n=5/3/2; EX, n=5/6/1. The proportion of children in each Tanner stage at each visit is given as pre/post indicates the number of children at baseline and at the follow up visit.

5.3.1 DXA

Table 5.2 summarises the DXA data from each group at baseline and change after the 20 week intervention. There were significant time effects on the BMC of the hip, spine, radius, ulna and whole body (all p<0.001). There was also a significant overall interaction of time and group on the BMC of the total hip (p=0.04) but post-hoc analyses did not reveal any differences between groups. There was no difference in the change in bone mass for any of the sites measured by DXA over the 20 weeks intervention.

Table 5.2 Site-specific baseline and 20 week change in bone mineral content (BMC) measures by DXA.

	Control			Exercise			Adjusted p-values		
	Baseline	Post-intervention	Δ (95% CI)	Baseline	Post-intervention	Δ (95% CI)	Time	Group	Time*group
Femoral neck BMC (g)	2.7 (0.4)	2.7 (0.3)	-0.01 (-0.1 - 0.1)	2.9 (0.5)	3.0 (0.5)	0.1 (0.01 - 0.1)	0.04	0.19	0.25
Hip BMC (g)	16.3 (2.9)	16.5 (3.1)	0.2 (-0.5 - 1.0)	17.6 (4.9)	18.7 (5.5)	1.0 (-0.01 – 1.9)	<0.001	0.45	0.04
Spine BMC (g)	23.4 (4.6)	24.4 (4.7)	1.0 (-0.03 – 2.0)	23.1 (5.5)	24.3 (6.2)	1.3 (0.5 - 2.1)	<0.001	0.77	0.44
Radius BMC (g)	3.4 (0.5)	3.6 (0.6)	0.2 (0.1 - 0.2)	3.6 (0.8)	3.8 (0.8)	0.2 (0.1 - 0.3)	<0.001	0.35	0.69
Ulna BMC (g)	2.3 (0.4)	2.5 (0.4)	0.2 (0.1 - 0.2)	2.5 (0.6)	2.7 (0.6)	0.2 (0.1 – 0.2)	<0.001	0.11	0.57
Whole body BMC (g)	753.7 (103.6)	792.9 (116.7)	39.3 (23.2 - 55.3)	778.4 (164.0)	822.6 (195.5)	35.3 (17.3 – 53.3)	<0.001	0.62	0.55

Baseline and post-intervention data are unadjusted mean (SD). DXA change data are represented as mean (95% CI) and are adjusted for sex, Tanner at follow-up and change in bone area.

5.3.2 pQCT

Data obtained from the pQCT scans were compared between groups and the group and time effects are summarised in Table 5.3. There was a significant interaction of time and group on ToD (p=0.004) and TrbD (p=0.003) of the 4% site of the tibia. There was also a significant time and group effect on the BSI of the 4% tibia (p=0.006). When post-hoc tests were performed on the variables, ToA at the 4% site after the intervention was significantly greater in EX than in CON (p=0.03). No other differences in the absolute measures were seen between the groups at the 4% site of the tibia. The absolute changes in ToA (p<0.001), ToD (p=0.003), TrbD (p<0.001) as well as BSI (p<0.001) were all significantly greater for the EX group compared to CON (Table 3). There were significant overall interactions between group and time for cortical area (p=0.05) and cortical density (p=0.003) at the 38% site of the tibia. There were no between group differences for baseline and follow up values, when post-hoc tests were performed, although ToA at the 38% was not quite significantly different between EX and CON after the intervention (p=0.08). However the absolute change in CoA (p=0.04), CoD (p<0.001), PC (p=0.03) and CT (p=0.01) at the 38% tibia were all greater in the EX group than in CON. The change in muscle CSA was also greater in the EX group compared to the CON (p=0.01).

Table 5.3 Trabecular (4%) and cortical (38%) baseline and 20 week change in tibial bone measures by pQCT.

	Control			Exercise			Adjusted p-values		
	Baseline	Post-intervention	Δ (95% CI)	Baseline	Post-intervention	Δ (95% CI)	Time	Group	Time*group
4% Tibia									
ToA	738.5 (86.8)	741.3 (99.7)	2.8 (-7.1 – 12.7)	802.0 (136.9)	847.8 (146.3)*	48.8 (37.0 – 60.5)‡	0.13	0.22	0.34
ToD	319.6 (46.7)	306.2 (41.2)	-13.4 (-19.5 - -7.3)	304.6 (22.1)	306.2 (19.7)	2.3 (-5.5 – 10.2)‡	0.10	0.23	<0.01
TrbD	291.4 (59.1)	264.1 (54.1)	-27.3 (-36.9 – -17.6)	270.2 (29.2)	277.2 (24.6)	8.9 (-2.8 – 20.6)‡	0.13	0.23	<0.01
BSI	7685.4 (2470.6)	7095.6 (2270.7)	-589.8 (-828.0 - -351.6)	7503.6 (1753.1)	7978.2 (1688.1)	545.6 (209.2 – 882.1)‡	0.82	0.61	<0.01
38% Tibia									
CoA	160.9 (17.5)	170.1 (17.2)	9.1 (6.6 – 11.6)**	165.7 (26.1)	170.2 (25.0)	5.2 (2.2 – 8.2)	<0.01	0.44	0.05
CoD	1071.1 (27.0)	1071.1 (23.6)	0.004 (-3.8 – 3.8)	1059.8 (51.4)	1073.1 (44.4)	11.1 (6.9 – 15.3)‡	0.02	0.74	<0.01
SSI	761.6 (77.3)	808.5 (99.0)	46.9 (34.6 - 59.2)	840.9 (180.7)	888.7 (187.1)	45.0 (31.9 – 58.2)	<0.001	0.21	0.46
ToA	269.2 (19.0)	279.4 (21.6)	10.2 (7.7 – 12.7)	294.9 (48.8)	304.1 (48.2)	9.8 (6.9 – 12.8)	<0.001	0.19	0.23
PC	59.6 (1.8)	60.2 (1.6)	0.6 (0.3 - 1.0)	59.4 (3.3)	60.7 (3.8)	1.2 (0.8 – 1.6)†	<0.001	0.90	0.99
EC	38.8 (1.1)	39.2 (1.0)	0.4 (0.1 - 0.6)	38.3 (2.7)	39.1 (3.0)	0.7 (0.4 – 0.9)	<0.001	0.79	0.93
CT	3.3 (0.1)	3.4 (0.1)	0.04 (0.03-0.06)	3.4 (0.2)	3.4 (0.3)	0.08 (0.06-0.10)†	<0.001	0.27	0.28
MCSA	3281.0 (432.2)	3339.1 (425.5)	58.1 (-6.4 – 122.5)	2948.5 (414.9)	3142.4 (494.2)	193.9 (112.8 – 275.1)†	<0.001	0.49	0.93

Baseline and follow-up data are unadjusted mean (SD). Change data are represented as mean (95% CI) and are adjusted for sex, Tanner stage at follow-up, change in height and change in muscle CSA. ToD, total density; TrbD, trabecular density; ToA, total area; BSI, trabecular bone strength index, CoD, cortical density; CoA, cortical area; SSI, polar strength strain index, PC, periosteal circumference, EC, endosteal circumference, CT, cortical thickness. * significantly different between groups, $p<0.05$. ** change is significantly greater in control group, $p<0.05$. † change is significantly greater in exercise group, $p<0.05$. ‡ change is significantly greater in exercise group, $p<0.01$.

5.3.3 NTX

There were no differences in NTX concentration at baseline between the EX and CON group, or within each group at follow-up (EX: p=0.77; CON: p=0.06). After the 20 week intervention, however, the CON group had a significantly higher NTX concentration compared to the EX group (p=0.04) (Figure 5.2).

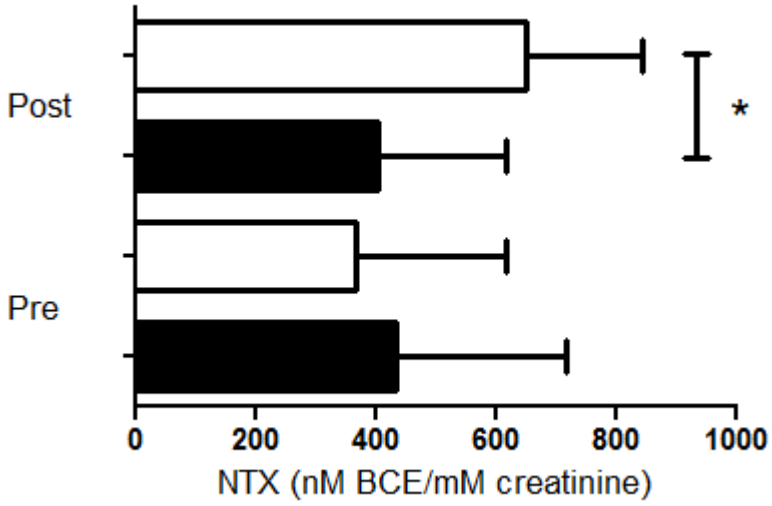

Figure 5.2 Urinary concentrations (mean and SD) of cross-linked N-telopeptides of Type I collagen (NTX) before and after the 20 week intervention. White bars are CON, black bars are EX. * p=0.04. Pre = before intervention, post = after intervention.

5.4 Discussion

The enhanced response of bone remodeling to an exercise intervention has been repeatedly shown in white children (175,182,183,196,197,245,246). Although black children have greater bone mass than their white peers at the femoral neck, proximal femur and lumbar spine (241), there are currently no studies that have considered the effects of an exercise intervention on the bone mass of black children. In this study, black

South African children who participated in a 20 week weight bearing exercise intervention showed significantly greater gains in hip BMC, as well as in density, area and strength at trabecular sites and density, area and structure at cortical sites of the tibia when compared to a non-exercising control group. As populations become increasingly sedentary and adopt poor dietary habits, genetic protection may not offer adequate protection against bone loss (6,71). This study has shown the possibility for black children to improve their bone health by taking part in a weight-bearing exercise program that may be easily incorporated into a school curriculum.

The inability of the DXA scans to detect changes in BMC in the present study (apart from at the hip) supports others who have suggested that the two dimensional DXA scan is limited in detecting a bone response to mechanical loading (83). The use of pQCT in this study enabled the elucidation of small structural changes as a result of the weight-bearing exercise intervention. No studies have used pQCT to assess the efficacy of an exercise intervention in black children, although the intervention studies that are available from other ethnic groups show similar results to those observed in the present study. Increases in tibial bone strength at the trabecular site but not the cortical site were seen after an 11-month-long weight bearing intervention in exercising pre-pubertal boys (195,196). Similarly in the present study there was no significant difference in the change in strength of the cortical site of the tibia between the EX and CON groups. In the studies described above, no other structural changes were observed, while in the present study, area and density changes were greater in the EX compared to CON group and were likely contributors to the greater change in bone strength seen at the trabecular site of the

tibia. Whereas responses to a longer intervention than was conducted in this study have been observed (195,196), five months appears to be have been long enough to elicit significant bone gains in this cohort of black children.

Interestingly, the children in the CON group tended to have a decrease in total and trabecular bone density with an overall (although not significant) decrease in BSI. Similarly, a study done comparing the effects of aerobic and resistance exercise on trabecular and cortical bone in young adult women (approximately 19 years of age), found that the control group had a slight decrease in trabecular density of the tibia after the intervention period (247). This decrease in density may be due to a number of reasons. The first is that the decrease in density may be due to a lag in the time it takes to deposit bone on the trabecular surface after the bone has grown in area. In addition the bone turnover rate is faster than that of cortical bone (20) and may explain the overall decrease seen after the intervention period because at any one point trabecular bone may appear less dense if it is in a period of bone resorption compared to at the baseline point. Another reason may be that participation in only high intensity weight-bearing physical activity may be associated with an increase in density as has recently been suggested by Michalopoulou and co-workers (248). The greater concentration of urinary cross-linked N-telopeptides of Type I collagen (NTX) in the CON group following the intervention may also in part explain the mechanism for the decrease in trabecular density. Only one other study, that has documented NTX concentrations in relation to exercise in children, showed that NTX accounts for only a small, but significant percentage of variation in change in BMD in peripubertal girls with exercise (200) while

maturity status is a large determinant of NTX levels (249). The difference in NTX levels between groups in the present study also suggests that the inhibition of bone resorption in the presence of a weight-bearing intervention may contribute to accelerated bone gain. The anomaly of a decrease in bone density warrants further investigation and mechanisms of bone deposition due to growth and participation in weight-bearing exercise interventions need to be considered.

The bone response to mechanical loading is site-specific (175). The intervention targeted bones of the lower body and resulted in increases in tibial periosteal circumference, and a significant increase in cortical thickness, in the EX group compared to the CON group. The increase in endosteal circumference was not quite significant in the EX compared to CON group ($p=0.06$) and may have been a reason why significant changes in strength at the cortical tibia were not observed. After completing nine months of a jumping intervention, 8-12 year old white boys and girls showed no difference in the increase in periosteal circumference between exercising and control groups at the mid-shaft of the tibia however the change in endosteal circumference was less in the intervention group compared to their control group (197). This suggests that the exercising children may have resorbed bone on the inner surface at a slower rate compared to the control group and therefore they may have had thicker cortices even though cortical thickness was not reported in that study. Similar to the results of the present study, Wang and co-workers (in an observational study) showed that Finnish girls who had the highest levels of leisure time physical activity, had greater cortical thickness at the tibia compared to girls in the lowest tertile of leisure time physical activity (250).

It is also possible that ethnic differences may exist in the way that bone is laid down in response to exercise between black and white children. Currently, no studies have looked at an ethnic comparison of bone apposition due to an exercise intervention in black and white children and further comparative studies need to be done to delineate the changes in bone geometry after an exercise intervention between race groups. In an observational study, Leonard and co-workers reported greater tibial periosteal and endosteal circumferences and strength in black compared to white children but they did not report differences in cortical thickness (96). Black children have been shown to have thinner cortices than white children (141), but it appears from the present study that an exercise intervention in black children has the ability to promote bone apposition at the periosteum, which may have contributed to a greater change in cortical thickness in the EX group. That said there was no difference in absolute cortical thickness between the groups after the intervention. Absolute differences in bone geometry may have been observed if the intervention period was longer but the significantly greater changes in all bone outcomes in the EX group show promise for future research on bone gain as a consequence of exercise in black children.

The change in muscle CSA over the 20 weeks, was greater in the EX group than the CON group. The weight-bearing intervention was effective in increasing muscle CSA and this is a likely explanatory mechanism as to how bone area and strength increased at a greater rate in the EX group compared to the CON group. It is widely accepted that muscle CSA (an increase in which is highly attributable to the amount of physical activity that one

partakes in) plays a role in determining the amount of bone density gain as well as the beneficial changes in bone geometry and structure (92,251).

Although there was a difference in the proportion of children classified as being either Tanner stage I or II at baseline, the children were still considered as being pre-pubertal. Pubertal status however is closely associated with bone gain, and it must be acknowledged that the change in pubertal status may have resulted in a greater rate of change in some of the bone variables in the EX group. However I believe this aspect was controlled for to the best of my ability in the present study by adjusting for follow up sexual maturity in the statistical analyses. The fact that the change in height was greater in the EX group compared to the CON also suggests that this group may have been more mature than the CON even though these children were considered pre-pubertal. This may have influenced the rate of change in bone variables in the group but also importantly highlights the fact that children within a certain Tanner stage classification may be more pubertally advanced than others in the same Tanner stage. Other ethnic groups were not included in this study therefore the results may only be applicable to black pre- early pubertal children. A number of children in the EX group could not return for their post intervention scans due to moving away from the area or school and thus the sample size was reduced. In addition, the small sample size resulted in the inability to compare gender differences in the response to mechanical loading. The biochemical interpretation of bone remodelling is also limited due to the fact that blood data was obtained only for bone resorption and not formation markers.

To my knowledge, pQCT has not been used in a cohort of black children who have undergone an exercise intervention. The results show greater absolute gains in volumetric bone density for the EX group compared to the CON group, at the trabecular and cortical sites, in addition to the greater changes in area, size and strength compared to those seen in the CON group. The results demonstrate the efficacy of weight bearing physical activity on the trabecular and cortical sites in black children, and, similar to what has previously been observed in white and Asian children, this study deepens our knowledge on the attainment of bone in response to an exercise intervention. In a low-middle income country such as South Africa, physical activity models and interventions need to be feasible, affordable, sustainable and relevant to the population that they are being conducted in. Primary schools are an important environment where large numbers of children can be encouraged to participate in exercise interventions. Further exploration into the ethnic specific effects of weight-bearing physical activity interventions in children is needed. The present study is an ideal example of how a simple low-cost, exercise intervention improved bone health in pre- and early pubertal black children.

In conclusion, pre- and early pubertal black South African children who undertook a 20 week weight-bearing exercise intervention had greater gains of bone density, area and strength at the tibia, compared to children who did not take part in the exercise intervention.

CHAPTER 6 – STUDY 4 - LOADING AND ETHNIC EFFECTS ON ANNUAL BONE ACCRUAL IN PRE-PUBERTAL BLACK AND WHITE CHILDREN [1]

[1] Meiring RM, Micklesfield LK and McVeigh JA. Part of this work has been submitted to Acta Paediatrica.

6.1 Introduction

The aims of this study were to 1) compare the effects of a history of bone loading on bone growth in content and structure over a one-year in pre-pubertal children and 2) to determine whether ethnicity has any effect on bone growth over a year of follow up in black and white children.

6.2 Methods and Materials

The methods have been described in section 2.6 above. Statistical analyses have been outlined in section 2.6.5.

6.3 Results

From the sample of 66 children that were included in the analysis for study 2, 47 children (18 boys, 29 girls) continued participation to follow up. Two white girls, one black girl and two white boys changed from Tanner stage II to III over the year and were excluded from data analysis. One white boy was classified as obese and was also excluded from the study. Thus 41 children were included in the final data analysis. Table 6.1 shows the baseline characteristics of the sample of children that completed the follow up study. All children were of similar age and were in either Tanner stage I or II at baseline. The proportion of children within Tanner stage I or II was not significantly different between the black and white children at baseline ($p=0.55$) or at follow up ($p=0.14$). Similarly the proportion of children in Tanner stage I or II was not significantly different between the

high and low bone loading groups at baseline (p=0.32) or at follow up (p=0.61). The proportion of children who changed from Tanner stage I at baseline to Tanner stage II at follow up was not significantly different between black and white children (p=0.22) as well as between the low and high bone loading group (p=0.54). The median PBSS was different between the high and low groups with both the black and white high bone loaders having significantly higher scores compared to the black and white low bone loaders. There were no within group differences in median PBSS between black and white low or black and white high bone loaders.

Table 6.1 Baseline anthropometric characteristics of children grouped by ethnicity and bone loading.

	Black (n=16)	**White (n=25)**	**Low (n=20)**	**High (n=21)**
Age (years)	9.4 (1.0)	9.5 (1.1)	9.3 (1.0)	9.6 (1.1)
Height (m)	1.33 (0.06)	1.36 (0.07)	1.34 (0.08)	1.35 (0.06)
Weight (kg)	29.4 (3.2)	31.9 (6.1)	30.5 (6.1)	31.3 (4.5)
Girls (n)	10	16	15	11
Tanner (I/II) (n)	15/1	22/3	19/1	18/3
BMI percentile	51.8 (20.8)	55.8 (28.6)	53.0 (25.7)	55.4 (26.1)
Fat mass (kg)	7.3 (1.9)	8.2 (3.1)	8.0 (2.9)	7.6 (2.6)
Fat free mass (kg)	20.7 (2.7)	22.6 (3.8)	21.2 (3.9)	22.5 (3.0)
Body fat percentage (%)	25.1 (5.5)	25.1 (6.0)	26.1 (5.6)	24.1 (5.8)
Radial length (mm)	216.3 (15.7)	209.2 (15.6)	211.8 (19.3)	211.4 (12.6)
Tibial length (mm)	308.8 (19.0)	311.3 (21.8)	312.6 (22.6)	308.3 (18.7)
PBSS baseline	4.5 (2.9)	7.4 (4.1)*	3.8 (2.2)	8.6 (3.7)†
Average PBSS	4.5 (2.7)	6.3 (3.3)	3.3 (1.4)	7.8 (3.7)†

Data are mean (SD) except for Tanner which show proportions within each group. PBSS = peak bone strain score. *p<0.05 black versus white; †p<0.001 high versus low.

6.3.1 Effect of mechanical loading on bone growth

Table 6.2 shows the baseline, follow up and annual absolute change in DXA and pQCT outcomes for the groups of low and high bone loading children. High bone loaders tended to have greater baseline BMC at all sites measured by DXA but the difference was only significant at the femoral neck (p=0.03). At the follow up visit, femoral neck BMC remained significantly higher in the high bone loaders compared to the low bone loaders (p=0.003).

There were no differences in baseline bone outcomes between high and low bone loaders at the 4% radius. There were no differences in baseline or follow up bone outcomes between the high and low bone loading groups of children at the 65% radius.

Although there was a trend for the high bone loaders to have greater indices of density and area at the 65% tibia compared to the low bone loaders, this was not significantly different at baseline or at follow up.

Table 6.2 Baseline and follow up data for bone outcomes measured by DXA and pQCT at the 4 and 65% radius and the 65% tibia for children grouped by bone loading.

	Baseline		Follow up				
	Low (n=20)	**High (n=21)**	**Low (n=20)**	**High (n=21)**	**Time**	**Load**	**Interaction**
Spine BMC (g)	21.4 (0.8)	22.8 (0.8)	23.6 (1.1)c	24.7 (0.9)[b]	<0.001	0.38	0.62
Ulna BMC (g)	2.2 (0.1)	2.6 0.1)	2.4 (0.1)[c]	2.8 (0.1)	0.001	0.05	0.46
Radius BMC (g)	3.3 (0.1)	3.6 (0.1)	3.6 (0.2)c	4.0 (0.1)[c]	<0.001	0.19	0.81
Whole body BMC (g)	696.9 (24.1)	763.2 (23.0)	783.5 (30.0)[c]	852.9 (28.4)[c]	<0.001	0.10	0.80
Hip BMC (g)	15.1 (0.5)	16.6 (0.5)	16.3 (0.6)[c]	18.3 (0.7)[c]	<0.001	0.07	0.15
Femoral neck BMC (g)	2.4 (0.1)[d]	2.8 (0.1)	2.6 (0.1)[ae]	3.1 (0.1)[b]	<0.001	0.001	0.07
4% Radius							
ToA (mm^2)	203.5 (3.4)	209.6 (4.1)	211.0 (7.2)	226.4 (8.5)[a]	0.008	0.25	0.28
ToD (mg/cm^3)	289.1 (3.9)	292.4 (2.7)	282.2 (6.4)	292.9 (6.5)	0.41	0.14	0.28
TrbD (mg/cm^3)	209.3 (4.4)	213.9 (2.9)	193.2 (5.6)[a]	198.6 (4.9)[b]	<0.001	0.29	0.88
BSI (mg^2/mm^4)	1699.3 (63.4)	1787.7 (42.0)	1629.7 (83.6)[d]	1980.5 (106.9)	0.34	0.02	0.01
65% Radius							
ToA (mm^2)	96.5 (2.3)	101.1 (2.8)	96.9 (4.1)	103.7 (4.2)	0.57	0.21	0.59
CoA (mm^2)	41.7 (0.8)	42.8 (0.9)	45.3 (1.4)[a]	48.0 (1.2)[c]	<0.001	0.25	0.09
CoD (mg/cm^3)	1001.1 (3.1)	1003.6 (2.3)	1011.5 (7.5)	1031.8 (6.4)[b]	0.005	0.05	0.03
SSI (mm^3)	139.9 (4.3)	147.8 (4.5)	147.2 (7.6)	167.4 (9.4)	0.03	0.12	0.23
PC (mm)	34.6 (0.4)	34.5 (0.5)	34.8 (0.7)	35.9 (0.7)	0.56	0.21	0.64
CT (mm)	1.4 (0.02)	1.4 (0.02)	1.5 (0.04)[a]	1.6 (0.04)[c]	<0.001	0.55	0.22
EC (mm)	25.9 (0.4)	26.7 (0.5)	25.4 (0.8)	26.1 (0.8)	0.30	0.31	0.95
Muscle CSA (mm^2)	1670.2 (40.8)	1751.6 (51.1)	1715.5 (74.5)	1878.9 (71.2)	0.03	0.13	0.37
65% Tibia							
ToA (mm^2)	425.4 (5.6)	430.6 (5.0)	446.9 (16.1)	460.2 (10.1)[a]	<0.001	0.53	0.19
CoA (mm^2)	203.1 (4.5)	204.4 (3.9)	221.5 (7.8)[a]	231.1 (7.2)[b]	0.006	0.61	0.46
CoD (mg/cm^3)	1036.9 (3.5)	1037.0 (2.5)	1043.7 (6.6)	1050.6 (5.9)	0.02	0.34	0.44
SSI (mm^3)	1405.4 (52.6)	1423.6 (45.3)	1592.7 (95.4)	1693.2 (80.8)[b]	<0.001	0.57	0.35
PC (mm)	73.0 (0.5)	73.4 (0.4)	74.7 (1.3)	75.9 (0.8)[a]	0.01	0.43	0.54
CT (mm)	3.2 (0.1)	3.2 (0.1)	3.5 (0.1)[b]	3.6 (0.1)[a]	0.60	0.51	0.92
EC (mm)	52.6 (0.2)	53.0 (0.2)	52.9 (1.1)	53.5 (0.9)	<0.001	0.69	0.40
Muscle CSA (mm^2)	3314.2 (79.1)	3416.1 (97.8)	3429.3 (138.0)	3639.8 (133.8)	0.01	0.47	0.61

Data are mean (SEM). Baseline measures are adjusted for sex, ethnicity and bone area/limb length, follow up measures are adjusted for sex, ethnicity, bone area/limb length and baseline value. ToD, total density; TrbD, trabecular density; ToA, total area; BSI, trabecular bone strength index; CoD, cortical density; CoA, cortical area; SSI, strength strain index; PC, periosteal circumference; EC, endosteal circumference; CT, cortical thickness; CSA, cross-sectional area. [a] p<0.05, [b] p<0.01, [c] p<0.001 versus baseline. [d] p<0.05, [e] p<0.01, [f] p<0.001 versus high.

There was a significant difference in the relative change in whole body BMC between low and high bone loaders (p=0.002) (Figure 6.1 and Table 6.4). The relative change in femoral neck BMC was not significantly different between high and low bone loaders (p=0.07). Figure 6.2 shows the annual relative changes in bone outcomes at the 65% tibia. High bone loaders had greater relative changes in CoA (p=0.03), CoD (p=0.04) and CT (p=0.03) compared to low bone loaders. There was no difference in the relative change in SSI between high and low bone loaders (p=0.11). Low bone loaders tended to have a greater relative change in EC but this was not significant (p=0.06).

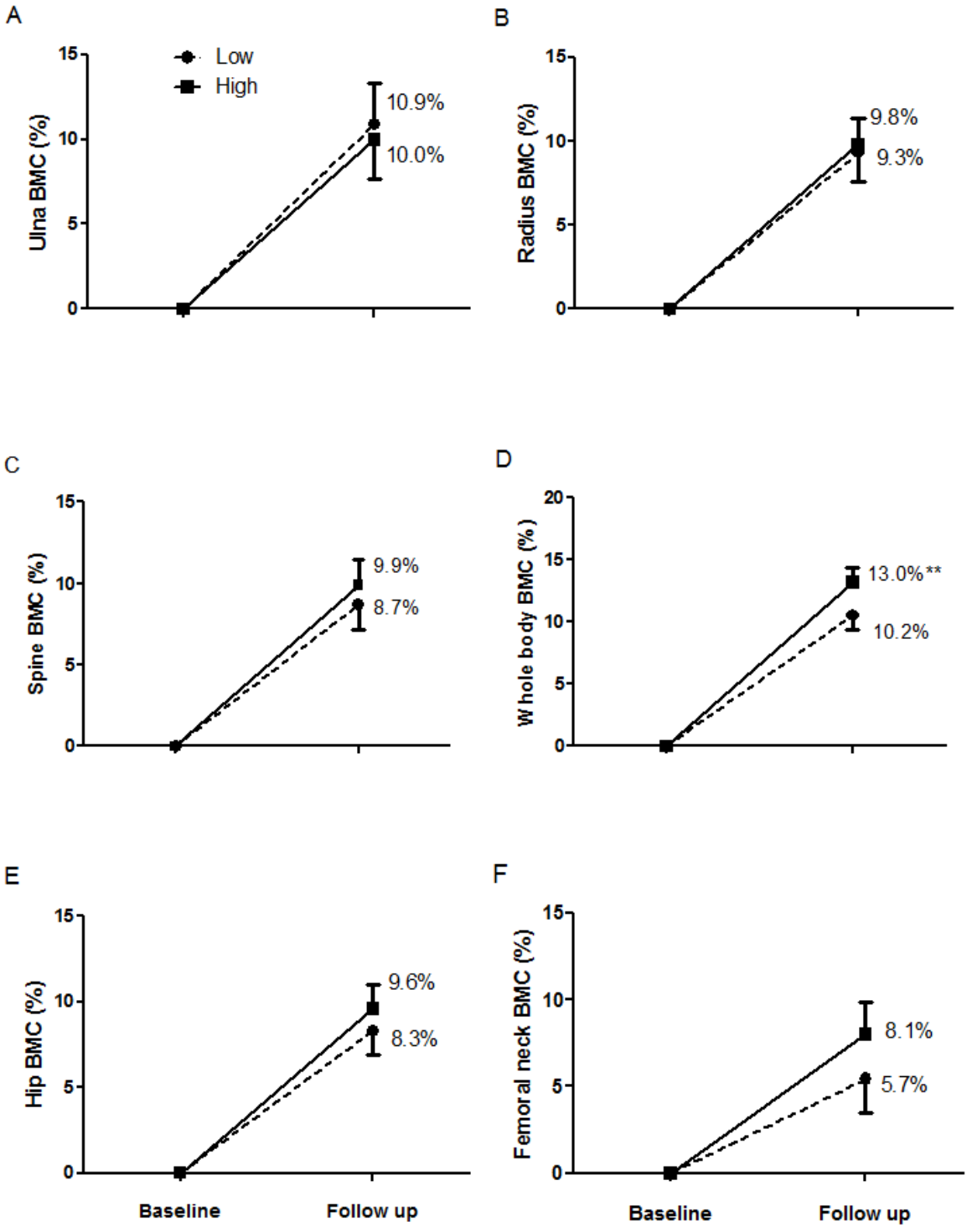

Figure 6.1 Annual relative change (%) in A) ulna, B) radius, C) spine and D) whole, E) hip and F) femoral neck bone mineral content (BMC) for the high and low bone loading groups. •and the solid line represents the relative change for high bone loaders; ▪ and the dashed line represents the relative change for low bone loaders. Error bars represent 95% confidence intervals. Significant difference between low and high bone loading groups, **p<0.001.

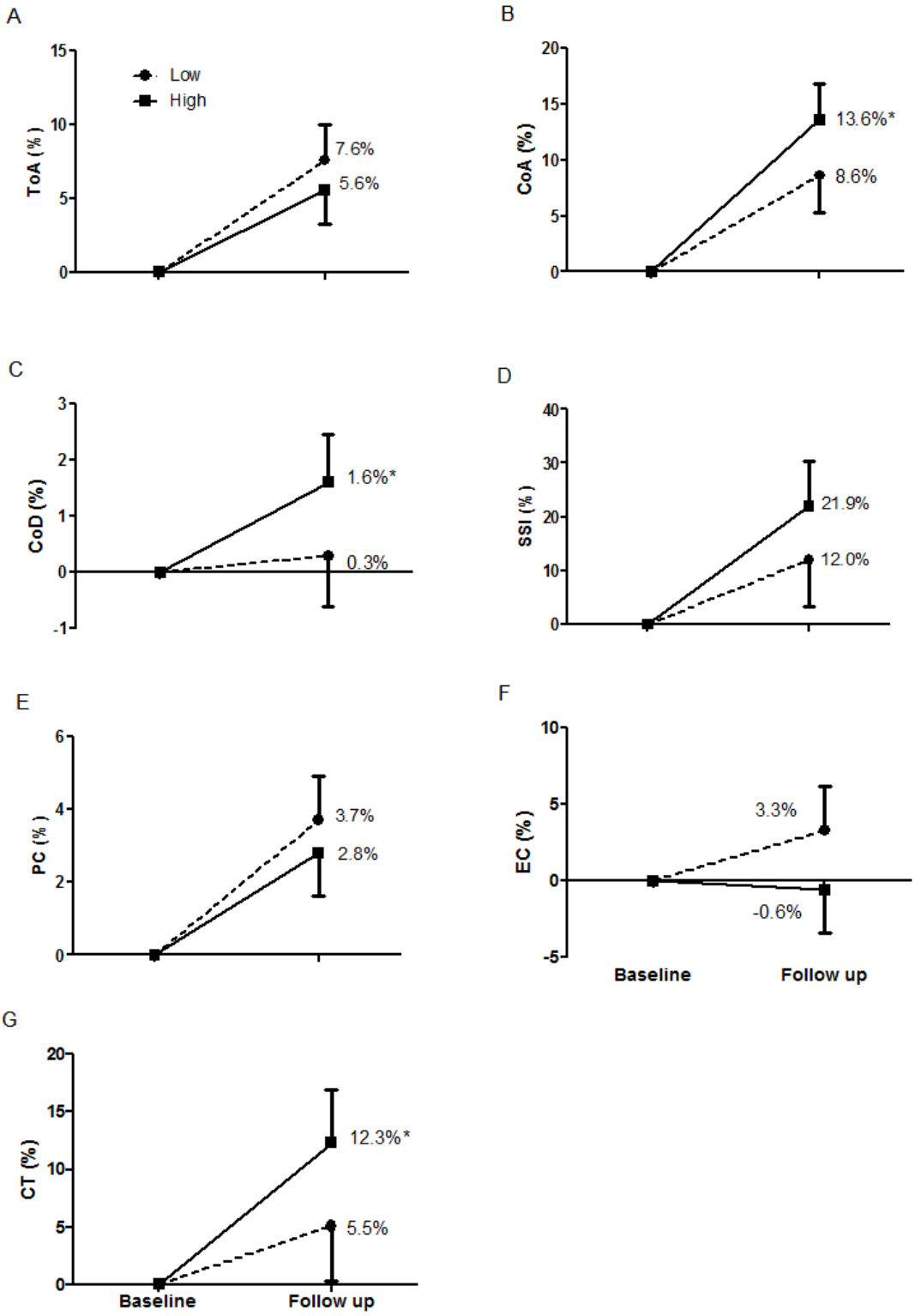

Figure 6.2 Annual relative change (%) in A) total area (ToA), B) cortical area (CoA), C) cortical density (CoD), D) strength-strain index (SSI), E) periosteal circumference (PC), F) endosteal circumference (EC) and G) cortical thickness (CT) at the 65% tibia for the high and low bone loading groups. •and the solid line represents the relative change for high bone loaders; ▪ and the dashed line represents the relative change for low bone loaders. Error bars represent 95% confidence intervals. Significant differences between high and low bone loaders, *p<0.05.

6.3.2 Effect of ethnicity on bone accrual

Similar comparisons were made between black and white children (Table 6.3). There were no significant differences in DXA bone outcomes between black and white children at baseline and follow up. At baseline, ToD at the 4% radius was greater in black than in white children ($p<0.001$) but ToD at the follow up visit was not significantly different between black and white children ($p=0.06$). TrbD was greater in the black than in the white children at baseline ($p=0.01$) as well as at follow up ($p=0.04$). BSI at baseline was greater in the black than in the white children ($p=0.05$) but this significance disappeared at follow up. Similar to the 4% radius, CoD at baseline was significantly greater in the black compared to the white children at the 65% radius ($p=0.01$) and at the 65% tibia ($p=0.04$). There were no ethnic differences in bone variables at the 65% radius or tibia at the follow up visit.

Table 6.3 Baseline and follow up data for bone outcomes measured by DXA and pQCT for children grouped by ethnicity.

		Baseline		Follow up				
		Black (n=16)	White (n=25)	Black (n=16)	White (n=25)	Time	Ethnicity	Interaction
	Spine BMC (g)	21.7 (0.8)	21.2 (1.0)	23.9 (1.1)[b]	22.7 (1.1)[a]	<0.001	0.59	0.32
	Ulna BMC (g)	2.3 (0.1)	2.2 (0.1)	2.6 (0.1)[c]	2.4 (0.1)[a]	<0.001	0.36	0.05
	Radius BMC (g)	3.4 (0.1)	3.3 (0.2)	3.7 (0.1)[c]	3.6 (0.2)[c]	<0.001	0.56	0.90
	Whole body BMC (g)	724.6 (21.2)	698.3 (30.2)	799.0 (27.9)[c]	791.1 (35.5)[c]	<0.001	0.70	0.24
	Hip BMC (g)	15.9 (0.5)	15.3 (0.7)	16.6 (0.6)	17.1 (0.9)[c]	<0.001	0.96	0.03
	Femoral neck BMC (g)	2.6 (0.1)	2.5 (0.1)	2.8 (0.1)[b]	2.7 (0.1)	<0.001	0.53	0.84
4% radius	ToA (mm^2)	201.9 (3.5)	201.9 (4.7)	201.3 (8.7)	221.3 (7.6)[a]	0.09	0.28	0.04
	ToD (mg/cm^3)	304.0 (1.8)[f]	279.2 (2.7)	306.9 (5.1)[d]	278.8 (7.6)	0.74	0.001	0.71
	TrbD (mg/cm^3)	224.8 (2.4)[e]	199.9 (3.4)	212.1 (4.4)[d]	186.0 (5.5)	0.001	0.001	0.88
	BSI (mg^2/mm^4)	1852.4 (46.7)[d]	1567.3 (70.4)	1901.6 (114.1)	1699.0 (112.2)	0.10	0.10	0.57
65% radius	ToA (mm^2)	95.9 (2.5)	98.4 (3.1)	100.2 (3.1)	98.8 (5.9)	0.42	0.91	0.26
	CoA (mm^2)	41.2 (0.8)	41.4 (0.9)	44.9 (1.2)[a]	45.5 (1.7)[a]	0.001	0.80	0.83
	CoD (mg/cm^3)	1011.1 (11.9)[d]	996.7 (9.7)	1013.8 (8.4)	1024.9 (6.5)[a]	0.01	0.15	0.08
	SSI (mm^3)	141.8 (4.1)	138.4 (5.8)	157.4 (6.0)	148.6 (10.1)	0.02	0.52	0.49
	PC (mm)	34.6 (0.4)	34.9 (0.6)	35.4 (0.6)	35.0 (1.0)	0.42	0.97	0.27
	CT (mm)	1.4 (0.03)	1.4 (0.02)	1.5 (0.1)	1.5 (0.1)[a]	0.002	0.88	0.38
	EC (mm)	25.9 (0.5)	26.4 (0.5)	26.1 (0.8)	25.7 (1.1)	0.64	0.98	0.23
	Muscle CSA (mm^2)	1646.2 (43.1)	1708.2 (56.5)	1719.5 (61.3)	1782.3 (94.5)	0.17	0.46	0.99
65% tibia	ToA (mm^2)	428.4 (4.5)	420.6 (7.2)	451.4 (11.0)	442.6 (19.1)	0.07	0.52	0.95
	CoA (mm^2)	202.0 (3.6)	200.0 (5.4)	219.5 (6.1)[a]	219.4 (9.1)[a]	0.01	0.91	0.79
	CoD (mg/cm^3)	1043.5 (2.5)[d]	1029.2 (3.5)	1058.2 (5.4)[a]	1035.9 (7.3)	0.005	0.02	0.41
	SSI (mm^3)	1399.0 (42.4)	1368.8 (63.7)	1594.5 (82.7)[a]	1536.3 (109.8)	0.009	0.69	0.73
	PC (mm)	73.3 (0.4)	72.4 (0.6)	75.2 (0.9)	74.3 (1.6)	0.06	0.42	0.96
	EC (mm)	53.2 (0.2)	52.3 (0.3)	53.8 (0.9)	52.6 (1.4)	0.63	0.15	0.84
	CT (mm)	3.2 (0.1)	3.2 (0.1)	3.4 (0.1)	3.5 (0.1)[b]	<0.001	0.75	0.58
	Muscle CSA (mm^2)	3148.3 (69.9)	3430.4 (105.3)	3288.7 (94.9)	3563.6 (182.1)	0.08	0.12	0.96

Data are mean (SEM). Baseline measures are adjusted for sex, baseline PBSS and bone area/limb length, follow up measures are adjusted for sex, average PBSS, bone area/limb length and baseline value. ToD, total density; TrbD, trabecular density; ToA, total area; BSI, trabecular bone strength index; CoD, cortical density; CoA, cortical area; SSI, strength strain index; PC, periosteal circumference; EC, endosteal circumference; CT, cortical thickness; CSA, cross-sectional area. [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$ versus baseline. [d] $p<0.05$, [e] $p<0.01$, [f] $p<0.001$ versus white.

There were no differences in relative changes between black and white children for any of the bone outcomes measured (Table 6.4). However, the total area and total density at the 4% radius and the cortical density at the 65% radius are shown in Figure 6.3 as an indication of the changes that occurred between black and white children.

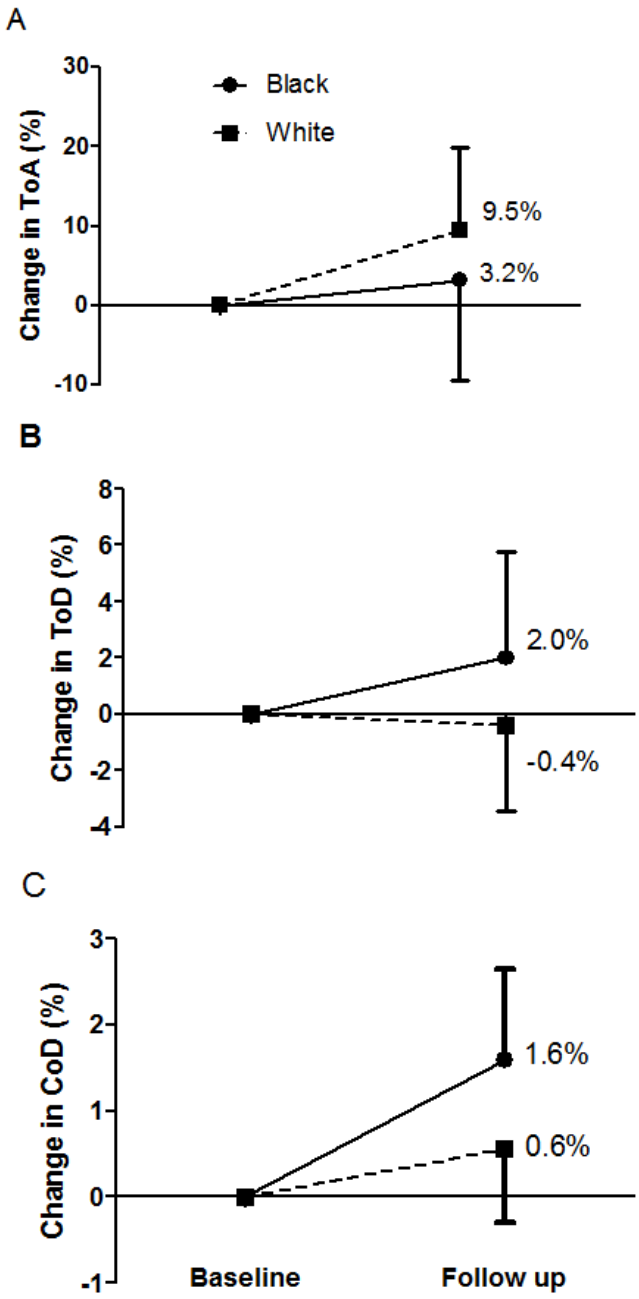

Figure 6.3 Annual relative change (%) in A) 4% radius total area (ToA), B) 4% radius total density (ToD) and C) cortical density (CoD) of the 65% tibia for the black and white bone loading groups. •and the solid line represents the relative change for black children; ▪ and the dashed line represents the relative change for white children. Error bars represent 95% confidence intervals. Significant differences between high and low bone loaders.

Table 6.4 Relative annual change (%) from baseline in bone outcomes for black and white children and high and low bone loaders.

	Black (n=16)	White (n=25)	Low (n=20)	High (n=21)
Spine BMC (g)	9.8 (8.2 - 11.6)	9.0 (7.6 - 10.4)	8.7 (7.2 - 10.3)	9.9 (8.4 - 11.5)
Ulna BMC (g)	11.8 (9.3 - 14.4)	9.5 (7.4 - 11.6)	10.9 (8.6 - 13.3)	10.0 (7.7 - 12.2)
Radius BMC (g)	9.0 (7.2 - 10.8)	10.0 (8.5 - 11.5)	9.3 (7.6 - 10.9)	9.8 (8.2 - 11.4)
Whole body BMC (g)	12.4 (11.1 - 13.7)	11.6 (10.6 - 12.6)	10.5 (9.4 - 11.6)	13.2 (12.2 - 14.3)†
Hip BMC (g)	8.5 (6.8 - 10.1)	9.3 (8.0 - 10.6)	8.3 (6.9 - 9.7)	9.6 (8.3 - 10.9)
Femoral neck BMC (g)	6.5 (4.4 - 8.6)	6.8 (4.4 - 8.6)	5.4 (3.5 - 7.3)	8.0 (6.1 - 9.9)
4% radius				
ToA (mm^2)	2.6 (-6.6 - 11.7)	5.8 (-1.9 - 13.6)	13.2 (2.5 - 23.9)	1.0 (-9.5 - 11.4)
ToD (mg/cm^3)	2.2 (-1.4 - 5.8)	-0.5 (-3.5 - 2.6)	-0.9 (-4.2 - 2.3)	2.1 (-1.1 - 5.2)
TrbD (mg/cm^3)	6.9 (-13.4 - -0.4)	-5.4 (-10.9 - 0.1)	-5.5 (-12.4 - 1.5)	-8.7 (-15.4 - -1.9)
BSI (mg^2/mm^4)	4.2 (-2.3 - 10.7)	4.2 (-1.3 - 9.7)	7.3 (1.3 - 13.3)	2.2 (-3.6 - 8.1)
65% radius				
ToA (mm^2)	4.3 (1.4 - 7.1)	4.6 (2.1 - 7.0)	3.3 (0.5 - 6.0)	4.6 (1.9 - 7.2)
CoA (mm^2)	12.9 (5.1 - 20.7)	9.4 (2.8 - 15.9)	10.0 (1.9 - 18.1)	9.8 (1.9 - 17.7)
CoD (mg/cm^3)	1.4 (0.01 - 2.8)	1.6 (0.4 - 2.8)	2.1 (0.7 - 3.4)	0.9 (-0.4 - 2.2)
SSI (mm^3)	12.4 (6.1 - 18.7)	9.8 (4.5 - 15.1)	12.0 (5.6 - 18.4)	7.8 (1.5 - 14.0)
PC (mm)	2.0 (0.9 - 3.4)	2.1 (0.9 - 3.3)	1.5 (0.1 - 2.8)	2.1 (0.8 - 3.4)
CT (mm)	12.9 (3.8 - 22.0)	9.1 (1.4 - 16.8)	10.1 (0.8 - 19.4)	9.6 (0.6 - 18.6)
EC (mm)	-0.3 (-3.1 - 2.5)	1.3 (-1.1 - 3.7)	0.1 (-2.5 - 2.8)	0.9 (-1.5 (3.5)
Muscle CSA (mm^2)	7.0 (4.3 - 9.7)	6.8 (4.4 - 9.0)	5.2 (2.8 - 7.6)	8.1 (5.8 - 10.5)
65% tibia				
ToA (mm^2)	5.6 (2.9 - 8.2)	7.3 (5.1 - 9.4)	7.6 (5.2 - 9.9)	5.6 (3.3 - 7.9)
CoA (mm^2)	8.8 (5.1 - 9.4)	12.6 (9.6 - 15.5)	8.6 (5.3 - 11.9)	13.6 (10.3 - 16.8)*
CoD (mg/cm^3)	1.5 (0.5 - 2.5)	0.6 (-0.2 - 1.4)	0.3 (-0.6 - 1.2)	1.6 (0.7 - 2.5)*
SSI (mm^3)	13.8 (4.0 - 23.5)	19.2 (11.4 - 26.9)	12.0 (3.3 - 20.7)	21.9 (13.4 - 30.4)
PC (mm)	2.7 (1.4 - 4.0)	3.5 (2.5 - 4.6)	3.7 (2.6 - 4.9)	2.8 (1.6 - 3.9)
EC (mm)	1.3 (-1.9 - 4.5)	1.3 (-1.2 - 3.9)	3.3 (0.4 - 6.2)	-0.6 (-3.4 - 2.2)
CT (mm)	6.6 (1.4 - 11.9)	10.1 (5.9 - 14.3)	5.1 (0.3 - 9.8)	12.3 (7.6 - 16.9)*
Muscle CSA (mm^2)	4.5 (1.7 - 7.3)	5.6 (3.4 - 7.8)	3.7 (1.2 - 6.2)	6.6 (4.1 - 9.0)

Data are mean (95% CI) and are adjusted for loading group, sex, and relative change in BA or limb length of the respective bone variable. ToD, total density; TrbD, trabecular density; ToA, total area; BSI, trabecular bone strength index; CoD, cortical density; CoA, cortical area; SSI, strength strain index; PC, periosteal circumference; EC, endosteal circumference; CT, cortical thickness; CSA, cross-sectional area. *p<0.05, †p<0.01.

6.3.3 Blood biochemical markers

There were no differences in serum IGF-1 or sclerostin concentrations between any of the groups at any of the visits (Table 6.5). In addition, there was no difference in the relative change in IGF-1 concentrations between the low and high ($p=0.99$) or between the black and white children ($p=0.99$). Similarly there was no difference in the relative change in sclerostin concentrations between low and high bone loaders ($p=0.32$) as well as between black and white children ($p=0.12$). There was however a main effect of time for sclerostin in the high and low bone loaders and also in the groups of black and white children.

Table 6.5 IGF-1 and sclerostin concentrations for low and high bone loaders as well as for black and white children.

	Baseline (median (min-max))		Relative change (mean (95% CI))		Follow up (median (min-max))				
	Low (n=11)	**High (n=15)**	**Low (n=11)**	**High (n=15)**	**Low (n=11)**	**High (n=15)**	**Time**	**Load**	**Interaction**
IGF-1 (ng/ml)	153.8 (65.3-341.7)	131.3 (71.1-255.3)	41.9 (-24.1 - 107.9)	48.7 (-0.1 - 97.5)	187.1 (67.3-258.8)	141.1 (52.7-394.5)	0.51	0.27	0.68
Sclerostin (pg/ml)	520.8 (290.7-820.0)	504.1 (337.7-1419.2)	48.4 (2.7-94.1)	37.8 (-5.6-81.2)	629.7 (319.5-1017.4)	574.2 (482.7-1474.6)	0.009	0.51	0.79
	Black (n=10)	**White (n=16)**	**Black (n=10)**	**White (n=16)**	**Black (n=10)**	**White (n=16)**	**Time**	**Ethni-city**	**Interaction**
IGF-1 (ng/ml)	124.6 (65.3-208.9)	155.5 (73.1-341.7)	62.5 (-17.9 - 143.1)	35.4 (-5.2 - 75.9)	143.4 (52.6-394.5)	175.5 (87.5-271.8)	0.37	0.43	0.19
Sclerostin (pg/ml)	485.6 (290.7-832.1)	591.6 (342.9-1419.2)	29.4 (-9.1 - 67.9)	52.8 (9.2-96.5)	520.5 (319.5 - 989.1)	869.4 (434.8 - 1474.6)	0.01	0.17	0.21

Data are median (min - max).

6.4 Discussion

This study showed the sustained benefits of participation in weight-bearing exercise in a group of pre- and early pubertal children using DXA and pQCT to assess bone structure, density and strength changes over approximately a year of follow up. The sustained femoral neck BMC one year after the start of the study, support the inferences from other studies that participation in weight-bearing activity provides long-term benefits to bone health in adolescence and early adulthood (172,206,245). In addition, the greater gain in whole BMC that occurred in the high bone loading children suggest that bone growth may have been accentuated by participation in high levels of weight-bearing physical activity. These results justify the need for lifelong participation in physical activity for the maintenance of good bone health. Certainly these data are important when considering that non-traumatic fractures due to osteoporosis often lead to increased morbidity and a decreased quality of life (252).

This study is one of a limited number of studies that have shown longitudinally the effect of high bone loading on bone health using pQCT (234,245). Participation in high bone loading exercise favoured greater measures of bone strength at the 4% radius, whereas there were no differences observed between high and low bone loaders for the absolute bone values of the 65% radius and tibia. The relative changes in tibial cortical area, density and cortical thickness were however greater in the high bone loading group, while the low bone loaders showed a greater relative change in endosteal circumference. Although the size of the tibia was not significantly different between groups at follow up,

the relative change in the structure of the bone suggest that the cortex of the tibia of the high bone loaders became thicker and these structural changes may have contributed to the greater gains in total area, cortical density and strength of this bone in the high bone loaders; that which was not observed in the low bone loaders. In a well-controlled longitudinal study that compared the exercise-induced growth effects between the playing and non-playing arm of pre-/peri-pubertal tennis players, it was found that repetitive loading induced greater annual changes in total and cortical area of the humerus of the playing arm were greater compared to those in the non-playing arm (234). Specker and Binkley (245) followed up a group of children 12 months after they had completed a 12 month weight-bearing exercise intervention (i.e. 24 months after the start of the intervention) and found that periosteal and endosteal circumferences remained increased in the exercising group compared to the non-exercising group. Although density was not reported in both the aforementioned studies, it has been suggested that in young adult male and female athletes, the changes in bone due to long-term mechanical loading results in bone structural changes (area, cortical thickness and endosteal circumference increases) and not changes in volumetric bone mineral density (205,240). In a cross-sectional study of tennis players, Daly et al (2004) found that post-pubertal children had greater BMC and cortical areas in the non-playing arm indicating that natural growth in children results from a decrease in bone resorption on the endocortical surface rather than periosteal expansion.

There was no effect of ethnicity on BMC as measured by DXA for the whole body as well as the femoral neck. This is an interesting finding as previous studies in South African

children using DXA derived bone outcomes have shown that black children have greater BMC at whole body as well as at specific sites including femoral neck (139,241); a result that was also seen in study 2 of this thesis. Although in this study black children tended to have greater DXA derived BMC measures, the greater absolute gain in white children may be due to the fact that they participated in higher levels of physical activity. In addition, the lack of significance between ethnic groups at baseline and follow up may be due to the small sample size.

The ethnic differences in density and strength at the trabecular bone of the radius may provide evidence as to how bone growth of black and white children differs. In the present study after one year, we found that after adjusting for physical activity, white children gained a significantly greater total bone area while this did not occur in black children. Instead at follow up black children had a greater total and trabecular bone density compared to white children. Cortical density was also greater in the black compared to white children at the 65% radius and tibia. Although only the gain in total area was significant for white children, the trend for black children to accrue bone density at a faster rate than their opposite ethnic group was present at trabecular bone. The annual relative changes in area and density may be a reflection of differential growth in these children. White children had a 9.5% (versus 3.2%) increase in total area at the wrist, while black children gained 2.0% and 1.6% in total density at the 4% radius and 65% tibia respectively (versus -0.4% and 0.6% in white children). Hui and co-workers (2010) used DXA, to follow up a group of black and white children (5-15 years old) for between one to

four years. These authors showed that the growth rate of bone area in white boys and girls appeared to increase beyond that of the black children at approximately 11 years of age for boys and 9 years of age for girls. A similar increase in total body BMC in white children (which would have contributed to an increase in BMD) was seen but was less prominent than the increase for bone area (140). To the best of our knowledge the present study is the only one to date that has examined a history of bone loading physical activity history in black and white children and observed their influence on growth over a year. The findings in this study are in agreement with some other studies that have observed the ethnic effects on bone in pre-pubertal children. In a recent large scale cross-sectional study, Warden and co-workers (142) found that in early pubertal children (aged 10–12 years) black children had greater tibial cortical density compared to white children however it was also reported that bone size was also greater in black than in white children. Similarly, black children in Tanner stages I – IV also have greater cortical bone densities and size at the tibia (96). Only one other study in South African children has found a similar result to the present study where white children tended to have greater bone areas at the trabecular radius and tibia as well as at the cortical tibia compared to black children (143). The results of this study and those of the latter studies suggest a possible mechanism behind strength gain at trabecular bone in black and white children.

Sclerostin concentrations increased significantly from baseline to follow up in both the low and high bone loading groups as well as in the black and white children. This finding is interesting because sclerostin is an inhibitor of the Wnt bone formation pathway (56)

and higher levels of sclerostin are present in animal models of hindlimb unloading (253). The present study is the only study to date that has investigated sclerostin concentrations in pre-pubertal children. Another study done in adolescent athletes (age 15-21 years) found that sclerostin levels were higher in female athletes compared to controls (254) and the authors suggest possible reasons for this may be that different types of physical activity have different effects on sclerostin levels. Indeed resistance exercise in rats with their hind limbs unloaded, resulted in a suppression of sclerostin levels and a restoration of cortical bone growth compared to controls (255). Other reasons for the unexpected finding in sclerostin concentrations in the study of female athletes are that nutritional deficiencies due to the lower energy availability from excessive exercise may play a role or that sclerostin actually acts as a regulatory signal to prevent excessive bone formation from participation in exercise (254). In pre-pubertal children, however the latter reason may not be plausible as it is during this period of growth where bone mass gain is at its highest and one would expect a lower concentration of sclerostin during growth. That said, IGF-1 concentrations did not change significantly and as IGF-1 is closely associated with bone growth (45), it is suggested that in the current study the children may not have been in the correct phase of growth for the detection of IGF-1 changes.

The main limitation of this study is the small number of children that were followed up after a year. The difference in bone turnover rate during growth - 4% per year for cortical bone, and 20% for trabecular bone (20) - must be considered as a potential confounder and may account for the differences observed at the trabecular and cortical sites. Because only two scans were performed (as opposed to scans every 3 months), this

difference in bone turnover rate may introduce variation in results simply due to each child not necessarily being at the same stage of bone turnover, even within the same Tanner stage. The suggestion that the way in which black and white children accrue bone may be different, must be therefore made with caution. Ethnic differences in bone outcomes were not observed in any other variables except density and this may have been because white children tended to have higher PBSS although adjustment for physical activity was made when comparing black and white children.

As has been shown previously, continued participation in high levels of weight-bearing physical activity can benefit the bone health of pre-pubertal children for up to one year at a clinically relevant site such as the femoral neck. The way that mechanical loading contributes to the laying down of bone in order to confer structural changes was not conclusive although participation in high levels appeared to contribute to a greater strength of the wrist. The effect of weight-bearing physical activity on bone may also not be exclusively due to periosteal apposition but may in fact contribute to the inhibition of endosteal resorption, resulting in a thicker and denser cortex. In addition, the specific regions and structural changes in bone accrual that occur due to growth in a population of black and white children from a low-middle income country may also differ to that which has been previously shown in populations in higher income countries. These differences may reflect an environmental influence that modifies existing paradigms on physical activity and bone health in children.

CHAPTER 7 – CONCLUSIONS

Four studies were conducted in series and parallel to establish a better understanding of physical activity effects on bone mass and structure in children. While all studies conducted focused on the global research framework, the first study was primarily methodological in design. The main purposes of the Actical and B^3Q study was to attempt to compare an objective measure of PA and set up a B^3Q which could be used as a reliable estimate for weight bearing PA in SA children. Studies two to four were more in-depth studies directed at describing the associations of historical exposure to weight bearing PA, effects of an exercise intervention and providing longitudinal follow up data on physical activity with bone mass acquisition in Black and White South African children.

The main hypotheses tested in this thesis are outlined in Table 7.1.

Table 7.1 Hypotheses tested in the thesis.

Hypothesis	Chapter	Main finding
A bone specific physical activity questionnaire is more closely associated to bone outcomes than is PA measured by accelerometry.	3	The bone specific physical activity questionnaire was positively correlated to bone outcomes as measured via DXA and pQCT in black and white children more so than accelerometer-derived measures of physical activity.
Black and white children who take part in high levels of weight-bearing physical activity have denser, larger and stronger bones than their low bone loading peers.	4 and 6	In order for white children to reach the same bone mass as black children, they may need to participate in higher levels of weight-bearing physical activity. Ethnic differences in bone outcomes that were apparent between children classified as being low bone loaders, appear to have been attenuated when greater mechanical loading was undertaken. There were no differences in bone outcomes between black high and low bone loaders.
Black children will exhibit an osteogenic response to a weight-bearing physical activity intervention.	5	Pre- and early pubertal black South African children who undertook a 20 week weight-bearing exercise intervention had greater gains of bone density, area and strength at the tibia, compared to children who did not take part in the exercise intervention.
Children with a 2 year history of high bone loading physical activity will have greater bone gain during a year of growth compared to their low bone loading peers.	6	Continued participation in high levels of weight-bearing physical activity can benefit the bone health of pre-pubertal children at a clinically relevant site such as the femoral neck. The way that mechanical loading contributes to the laying down of bone in order to confer structural changes was not conclusive although participation in high levels appeared to contribute to a greater strength of the wrist. The effect of weight-bearing physical activity on bone may also not be exclusively due to periosteal apposition but may in fact contribute to the inhibition of endosteal resorption, resulting in a thicker and denser cortex.
Black children will have a greater bone gain during one year of growth compared to white children	6	Black children did not have greater gains in bone mass compared to white children. Instead, there may also be ethnic-specific responses to bone growth in black and white children

The results from this thesis fit into some of the conceptual frameworks that have previously encompassed bone health in pre-pubertal children. Overall, children who took part in high levels of weight-bearing physical activity had greater bone mass, area and strength compared to their low bone loading peers. This thesis, in agreement with previous studies, has also documented ethnic differences in bone health. Where this thesis has added to existing paradigms on bone health in children, is in the fact that the studies presented, focussed on the combined assessment of weight-bearing physical activity for the purposes of understanding bone mass acquisition patterns and the factors influencing them in a racially diverse group of children. The data obtained from each of the studies also adds to the existing body of literature of children living in a developing country – a place where there is certainly a role for incorporation of daily physical activity. Additionally, this thesis made use of newer technology in pQCT, a technique that is limited in South African paediatric studies of bone health.

Two main themes emerged from this thesis: that of physical activity, ethnicity and bone health as well as the theme of the use of pQCT in the assessment of bone health in pre-pubertal children. The results presented in this thesis currently provide the only longitudinal physical activity and pQCT measured bone mass data available on normal pre- and early-pubertal children living in South Africa. Consistent with previous exercise and bone health analyses in predominantly white children of European descent, the findings of this thesis indicate that higher levels of participation in weight-bearing activity confer density, area and structural benefits to bones of the pre-/early pubertal skeleton. The studies described suggest that the bones of white children respond to mechanical

loading whereas any loading (low or high) shows no associations with bone health on the already strong bones of black children. Thus there may appear to be ethnic differences in the threshold required to attain optimum bone health between black and white pre-pubertal children. This finding was detailed in study 2 (chapter 4) where a history of high bone loading offered distinct benefits to bone in white children. This conclusion must be made with caution however, because although it was attempted to ensure match loading between black and white children, there may have been some negative skewing of PBSS.

Prior to the study presented in Chapter 4, it was not known whether ethnic differences were associated with mechanical loading as the majority of studies described in the literature thus far have focussed on white children. Moreover, the results presented in Chapter 5 detail the osteogenic response of black children to a weight bearing exercise intervention. Intervention studies in the literature have also been conducted predominantly in the developed world (i.e. the US and Europe), however this thesis presents results from a study that has for the first time been conducted in a) a black only population and b) in a low income context. The importance of establishing this principle in the context as described above, lies in the fact that despite black Africans as well as black African-Americans having the lowest incidence of osteoporosis, the increasing amount of time spent in sedentary activity in low-middle income countries (South Africa in particular) is becoming increasingly concerning. In addition, as discussed in the literature review, black adults with fractures related to osteoporosis have increased risks of morbidity and mortality compared to white adults. Thus it becomes a public health priority to ensure that the prevention of osteoporosis occurs from an early age.

Even though in study 2 mechanical loading appeared to have no benefit to the bone health of black children, it seems that a threshold must be exceeded in order for those benefits to occur. In study 3 (chapter 5), it was shown that a 5-month long, weight-bearing physical activity intervention that consisted of two 45 minute sessions a week, afforded greater changes in bone outcomes to black children who took part in the intervention, i.e. the skeleton of black children **can** respond to positively to a weight-bearing physical activity intervention. The ethnic differences in bone mass cannot be explained by differences in muscle mass between black and white children (as was seen in chapter 4) but from the intervention study described in chapter 5, it was shown that the change in muscle mass of children who took part in the intervention, was greater compared to those children who did not take part in the intervention. The bones of black children therefore may respond to changes in muscle mass, suggesting that any type of physical activity that increases muscle mass (as most do), should confer additional bone benefits in children.

From the studies in chapter 4 and 5, there is a suggestion that the way black and white children lay down bone in response to exercise may be different. Although a white group of children was not included within the exercise intervention study (Chapter 5), others have shown that weight-bearing activity exerts its effects on changing the strength of the bone via periosteal apposition in white children (197,250). In the intervention study described in this thesis, black children had a greater change in bone density but a lower change in bone area in response to the exercise intervention when compared to the control group. Even though one may have less bone content, a bigger bone area

contributes to a greater strength in that bone. However in black children, studies have consistently shown greater bone densities compared to white children and therefore a bone strength gain related to physical activity in black children may be associated with the additional deposition of bone within the cortex, which may not necessarily be concomitant with an increase in area.

The data from the final study (chapter 6), observed bone mass changes over a one year period and three year historical exposure to weight-bearing physical activity in a population of black and white pre-and early-pubertal children living in South Africa. Over the course of 12 months, past history of weight-bearing physical activity (as measured using the PBSS) seemed to benefit those children who were classified as high bone loaders compared to children who were low bone loaders. This was especially evident for the weight-bearing bones of the femoral neck but more than that the change in the structure of the wrist and the cortex of the tibia appeared to favour the formation of a physiologically stronger bone. The sustenance of these benefits into adulthood has been shown in studies in white children and one would assume from the volumetric density responses of black children to the weight-bearing intervention, the same may hold true for black children, although this remains to be determined.

Throughout this thesis, the use of pQCT allowed for the discovery of possible mechanisms of bone change in black and white children in response to mechanical loading and growth. Changes in bone strength that cannot be detected by DXA, can be inferred from

data collected through the use of pQCT, i.e. changes in bone area, structure and volumetric density may result in beneficial strength changes. This newer technique has certainly allowed the field of physical activity and bone gain to grow extensively and what has arisen from this thesis is a broader understanding of the ethnic differences that may occur in bone's response to physical activity.

The studies presented in this thesis provide important information for gaining understanding about the complex relationship between race and bone during skeletal development, particularly in young black and white South African children. Within the scope of the present thesis, important achievements towards a more accurate understanding of ethnic differences in bone mass with respect to physical activity and growth have been made. Lack of physical activity is a major public health issue not only globally but also in South Africa, and intervention is necessary at the population level. As lifestyle and dietary patterns change with urbanisation and there is a shift towards westernised diets and sedentary behaviour, fractures in an ageing population of South African blacks may become more prevalent. Participation in weight-bearing physical activity in South African children will therefore become increasingly important for decreasing the risk of osteoporosis, mainly because of the burden of the decreased quality of life that is associated with fractures as well as the substantial costs that are incurred to a country. This thesis contributes to public health policy by suggesting that in rolling out physical activity interventions, consideration must be given to ethnic-specific responses in order to optimise bone health benefits for all children.

Study limitations

Overall participants who volunteered for the studies presented in this thesis were a convenience sample and although there were strict inclusion and exclusion criteria, it is possible that a more physically active group of children may have inadvertently been selected. Ethnic differences in physical activity have been well described (135,207,244) and although it was attempted to match weight-bearing history as closely as possible between ethnic groups, the possibility exists that the positive (white) and negative (black) skewing of the scores in the high bone loading groups may have had an impact on the results. In study 2, the limited ability of the Actical to accurately assess the intensity of specific types of activity such as weight-bearing activities, cycling, and swimming must be considered. Furthermore the positioning of the Actical may also have contributed to the difference in associations seen between the PBSS and the Actical. The load values assigned to activities reported on in the B^3Q are based on peak strain scores reported in the literature (228) and thus acknowledgement that ground reaction forces were not measured in our sample is made. As each of the studies relied largely on the categorisation into bone loading groups based on the B^3Q, the limitations associated with the retrospective recall of two year participation in physical activity must be acknowledged. This was not a serious impediment to the measure of bone loading (i.e. PBSS) as all participants filled out the B^3Q assisted by their parent/caregiver and due to the seasonal nature of school sports, I do not believe that it was difficult for children to recall their activities. In the case of the present study, the B^3Q used was one that has been validated for a South African population and I was responsible for the capture and analysis of all the questionnaires. The B^3Q served as a means of obtaining a surrogate

measure of high or low levels of physical activity hence the absolute value of physical activity was not imperative to this study. The limited sample size and the cross-sectional design of study 2 prevent causal conclusions from being established. In addition, other ethnic groups were not included in the intervention study therefore the results may only be applicable to black pre- early pubertal children. The biochemical interpretation of bone remodelling is also limited due to the fact that only blood data for bone resorption and not formation markers were obtained. In addition, the data from sclerostin and IGF-1 limit any inferences to be made about the process of bone remodeling during a year of growth.

Other limitations associated with the studies include the self assessment of pubertal status. Pubertal status is closely associated with bone gain. Even though every attempt was made to ensure only pre-/early pubertal children were included in this study, there may be sexual maturity differences between children even within a certain Tanner stage. For example, the white low bone loaders in study 2, tended to be (although this was not significant) younger, lighter, shorter and less mature than the black low bone loading group, which may have accounted for the differences in bone variables between the two ethnic groups in the low bone loading group. This variation in sexual maturity may also influence the results especially when attempting to longitudinally observe bone growth between groups of children. The two dimensional nature of DXA measurements is consistently problematic in interpreting bone data in children, which again was controlled for by limiting participation in this study to children who were classified as being pre- or early pubertal as well as using appropriate body size covariates in the statistical analysis. The small sample size is a limitation for the interpretation of the data. This is a limitation

because in paediatric studies of bone growth, ethnicity and pubertal status play an important role in the bone status of children. Bigger sample sizes would have allowed for more comprehensive interpretation of results as one is able to then stratify the sample by sexual maturity and ethnicity in order to eliminate these confounders on bone growth in children. The results of the thesis indicate the questions that need to be answered in future large scale studies. Although they were beyond the scope of the present thesis and not dealt with, other environmental factors such as diet and socioeconomic factors play a role in determining the bone health status of children. While the relationship between nutrition and bone health may be well described, that of socioeconomic status and bone health is less clear as outlined in the introduction. Again future research will need to encompass the effects of socioeconomic status on bone health especially in a low to middle income country such as South Africa.

Future research

The findings presented in this thesis raise a number of questions which require further investigation. In particular, future studies should aim to focus on delineating the specific type of exercises that confer changes in strength due to structural and density changes in order to determine the type, intensity and duration of mechanical loading required to allow for the greatest benefits to peak bone mass in childhood in pre-/ early pubertal black and white children. Dose-response exercise intervention studies within each ethnic group would be useful in answering this question. Also, whether or not ethnic specific responses to mechanical loading are sustained needs to be investigated in a longitudinal

manner. The use of pQCT in this thesis has also given rise to the question of what constitutes a stronger bone; greater density or greater bone area. This directs future research to study similar structural variables (i.e. density or area) within ethnic populations while between different ethnicities; comparisons between different structural measures as well as strength should be made. The clarification of exercises that then target either increases in bone density or increases in bone area (in order to confer increases in overall bone strength) should be studied in older black and white populations that may be at risk of or already have osteoporosis. Indeed just as the association with a history of weight-bearing activity is ethnic specific, bone accrual due to physical activity may also differentially affect the bone of black and white children.

The work presented in this thesis has built on previous work in the field of physical activity and bone mass in South African children. In particular the studies presented have shown that ethnic differences exist in the geometry and structure of bone. In addition the bones of black children have the capacity to increase not only via gains in content but also by changes in structure. Since continued physical activity contributes to the natural growth gains in bone and these benefits can be sustained in children, the next step will be to build on the work described here in order to develop and implement physical activity intervention programs specific to populations within the frameworks described.

In conclusion, participation in weight-bearing physical activity is beneficial for bone health in both black and white children but possibly not to the same extent i.e. ethnic-specific physical activity thresholds may exist in order for bone gain to occur. In addition, the use of pQCT assists in the elucidation of the ethnic-specific response to weight-

bearing physical activity in order to confer the greatest benefits to bone gain during the pre-pubertal years.

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“I have walked that long road to freedom. I have tried not to falter; I have made missteps along the way. But I have discovered the secret that after climbing a great hill, one only finds that there are many more hills to climb. I have taken a moment here to rest, to steal a view of the glorious vista that surrounds me, to look back on the distance I have come. But I can only rest for a moment, for with freedom come responsibilities, and I dare not linger, for my long walk is not ended.”

Nelson Rolihlahla Mandela

APPENDICES

APPENDIX A – Information sheet

UNIVERSITY OF THE WITWATERSRAND

SCHOOL OF PHYSIOLOGY

INFORMATION SHEET

Influence of Weight-Bearing Activity on Bone Density

Hello, my name is Rebecca Meiring. My co-investigators, Dr Ingrid Avidon and Dr Joanne McVeigh and I work in the exercise laboratory at Wits Medical School. We are conducting research in the field of exercise and bone density (bone health). We are interested in the effects of exercise on the bone health of South African children.

Children gain most of their bone mass before they reach puberty. High impact sports such as football, running and netball have beneficial strengthening and building effects on the bones of the body. We would like to find out what effect previous and current participation in high and low impact sports has on the development of the bone mass in children just before they reach puberty (ages of 8 to 11 years).

We would appreciate it if you would assist us with our research by allowing your child to participate as a subject in our research project. If you agree, and your child is willing to participate, you will be asked to visit the Exercise Laboratory, at your convenience, which is located at the Wits Medical School Campus in Parktown, Johannesburg on **three** separate occasions over the course of a year. For all of the visits, we will pay for your and your child's travel expenses to and from the Laboratory.

Visit 1 to Exercise Laboratory

When you bring your child into the Exercise Laboratory, you will be asked to help them fill out some questionnaires. You are welcome to look at these questionnaires in advance.

The principal investigator (Rebecca) will help you complete these questionnaires if needed. The first questionnaire is a general health questionnaire which informs us of your child's general health. The second questionnaire is a seven day dietary recall questionnaire which asks questions about your child's diet and calcium intake for seven days. The third questionnaire is a physical activity questionnaire which asks questions about your child's current level of physical activity and their level of physical activity in the previous two years. In order to ensure the visit is as quick as possible, the 2nd and 3rd questionnaires can be taken home with you and filled out at your leisure and can be collected at an alternative time. The fourth questionnaire will need to be filled out at the lab by your child with your help. This pictorial questionnaire is a self-assessment of their sexual maturity and is needed to determine their stage of puberty. It will take approximately 30 minutes to complete all the questionnaires. During the visit to the laboratory we would also like to perform a bone scan on your child. This scan takes about 30 minutes to complete and your child will need to lie still while he/she is being scanned. The scan emits an extremely low level of radiation (less than that of a dentists x-ray) making it safe for children and even safe for pregnant women. This scan will give us a lot of information about your child's bone and body composition. If your child is willing, we would also like to take a small blood sample from a vein in their arm. A nurse will use a needle to draw 10 millilitres (about 2 tablespoons) of blood from their arm. To take away the pain your child might experience from the needle, an anaesthetic cream can be applied to the arm area before the blood is collected. I would like to stress that giving blood is completely optional. At some stage during the visit, when your child needs the toilet, we would like your child to provide a urine sample (about half a cup). The blood and urine will be used to test for markers that give us information about the health of your child's bones. The blood will be tested for substances that give us an indication of bone being formed. Specifically the substances are sclerostin an insulin-like growth factor 1. The urine sample will allow us to look for a substance that shows bone being broken down. The first visit to the lab should last about two hours. You and your child are free to end participation in the study at this point, however a follow up option is available and is described below.

If you agree to your child's continued participation, and your child is willing, we would like to invite you to continue in our research project for a further 12 months. During these 12 months your child will be asked to continue their normal physical activities and activities of daily living. Please note that your child will not need to change their routine or diet in any way during this year but continue in their normal activities as before. They are allowed to begin new sports and activities if they wish. During this time you would be required to visit the Exercise Laboratory for two more visits that are spaced six months apart.

Visit 2 (after six months)

After six months your child will need to visit the Laboratory for a blood test, urine test and bone scan, as described in the first visit. In addition, we will require you to complete a physical activity questionnaire and dietary recall questionnaire as described in visit 1. This visit will take approximately one hour. Seven days before this visit, we would like your child to wear a small activity data logger on their hip. This logger is a square plastic cube about the size of a bottle top and is light and unobtrusive. The logger is fitted on a belt and worn under the clothing. The logger monitors the level of activity of your child during the day by measuring movement. The logger needs to be taken off when your child baths, showers or swims. It can also be removed when your child goes to sleep at night and then put on again in the morning. We would require your child to wear the logger for one full week (seven days). When the week is over the logger can be dropped off at the lab when your child comes in for their six month visit.

Visit 3 (after 12 months)

After 12 months your child will need to visit the Laboratory for a blood test, urine test and bone scan, as described in the first visit. In addition, we will require you to complete a physical activity questionnaire and dietary recall questionnaire as described in visit 1. This visit will take approximately one hour. Seven days before this visit, we would like your child to wear a small activity data logger on their hip as described above. When the week is over the logger can be dropped off at the lab when your child comes in for their 12 month visit.

In summary, the flow chart below describes the order of measurements and visits:

Visit 1 (approximately 2 hours)

- Four questionnaires
- Bone scan
- Blood and urine test

Visit 2 – 6 months after visit 1 (approximately one hour)

7 days before visit wear logger for 7 days

- Physical activity and dietary questionnaire
- Bone scan
- Blood and urine test

Visit 3 - 12 months after visit 1 (approximately one hour)

7 days before visit wear logger for 7 days

- Physical activity and dietary questionnaire
- Bone scan
- Blood and urine test

Please note that transport to the University from your child's school and back, can be arranged for your child. Your child's participation in the study is voluntary and you will be able to withdraw your child's participation at any stage during the study without any prejudice to yourself or your child. All the results will be kept confidential and will be made available only to the researchers involved in the study and yourself. If you wish, we will discuss your child's results with you so that you can gain information on their bone health and how to improve or maintain their bone strength. All records of your child's results will be identified by a reference number, so that he/she remains anonymous. All the data from the study will be put together, analysed and the results published in research papers written for the scientific community. We have obtained approval for the study from the Committee for Research on Human Subjects of the University of the Witwatersrand (ethics clearance number: M10635). If you have further questions please do not hesitate to ask me or one of my co-investigators.

Thank you for considering your child's participation in this study. Please read the information sheet carefully before signing the consent form. If you have any queries or are interested in allowing your child to participate in the study, please contact myself, Rebecca.

Tel: 076 311 2725 or 011 717 2363 Email: Rebecca.Meiring@students.wits.ac.za.

If you have any doubts to your rights as a subject please feel free to contact Anisa Keshav, Wits Research Office, 10th Floor Senate House, East Campus.

Tel: 011-717-1234 Fax: 011-717-1265 Email **anisa.keshav@wits.ac.za**

Sincerely,

Rebecca Meiring

APPENDIX B - General Health Questionnaire

SCREENING GENERAL HEALTH QUESTIONNAIRE

We would like to know about your child's health. Please answer the questions below as accurately as possible. To tick a box, double click the box and under default value select the checked option. Click ok. If you need to type, click in the box provided and write as much as is needed.

Thank you very much for your co-operation.

Your child's date of birth: ☐

1) Compared to other children of your child's age, how would you rate his/her health in the last TWO years? Please tick one box.

Better than other children	☐	Worse than other children	☐
Same as other children	☐	Much worse than other children	☐

*2) **In the last TWO years:***

a) Has your child ever gone to hospital? Yes ☐ No ☐

*b) If **yes**, what has your child gone to hospital for?*

c) How long did your child stay in the hospital for?

d) Has your child had any surgical procedures in the last year? Yes ☐ No ☐

3) Please tick any illnesses that your child has had in the last SIX months.

Illness		**Fully recovered?**	
Asthma	☐	Yes ☐	No ☐
Leukaemia	☐	Yes ☐	No ☐
Influenza ('Flu)	☐	Yes ☐	No ☐
Cold	☐	Yes ☐	No ☐
Headaches	☐	Yes ☐	No ☐
Rickets	☐	Yes ☐	No ☐
Chicken pox	☐	Yes ☐	No ☐
Measles	☐	Yes ☐	No ☐
Mumps	☐	Yes ☐	No ☐
Diabetes	☐	Yes ☐	No ☐
None	☐		

4) Please tick the medication/treatment on the list that your child may have taken in the last SIX months.

Asthma medication	☐
Blood transfusions	☐
Vitamin D supplements	☐
Cortisone	☐
Antibiotics	☐
None	☐

5) Has your child ever fractured (broken) a bone? Yes ☐ No ☐

a) If yes, how old was the child when the bone was ☐ *fractured?* YEARS

b) Please tick which of the following bones has your child broken?

Forearm ☐
Upper arm ☐
Lower leg ☐
Upper leg ☐
Finger ☐
Toe ☐
None ☐

6) As far as you know, have you (as the biological parent of the child or any other biological member of the child's family) been told that they have osteopoenia, osteoporosis or low bone density?

7) Please tick whether you think your child is pre-pubertal or whether you think they have already entered puberty.

Pre-pubertal ☐ Already entered ☐

THANK YOU FOR TAKING THE TIME TO COMPLETE THIS QUESTIONNAIRE

Appendix C – Tanner self-questionnaire

Code:

The assessment of physical development in adolescence

Female

Section A

We would like to ask you a few questions about your physical development.

Question 1

Have you grown taller in the last 6 months?

(Please mark the appropriate block)

☐ No ☐ Yes, a little ☐ Yes, some ☐ Yes, a lot ☐ Don't know

Question 2

Have you started puberty (i.e. do you have pubic hair or have your breasts enlarged)? (Please mark the appropriate block)

☐ No ☐ Yes

Question 3

Have your breasts started to develop (grow) yet?

(Please mark the appropriate block)

☐ No ☐ Yes, a little ☐ Yes, some ☐ Yes, a lot ☐ Don't know

Question 4

Do you have any hair underneath your arms or in the pubic region?

(Please mark the appropriate block)

☐ No ☐ Yes, a little ☐ Yes, some ☐ Yes, a lot ☐ Don't know

Question 5

Has your skin started to change (pimples)?

(Please mark the appropriate block)

☐ No ☐ Yes, a little ☐ Yes, some ☐ Yes, a lot ☐ Don't know

Question 6

Have you begun to menstruate (have your period)?

(Please mark the appropriate block)

☐ No ☐ Yes

Question 7

If **Yes** to *Question 6*, at what age did you begin to menstruate? ☐

Question 8

If **Yes** to *Question 6*, do you have regular menstrual cycles (periods)?

(Please mark the appropriate block)

☐ No ☐ Yes

Question 9

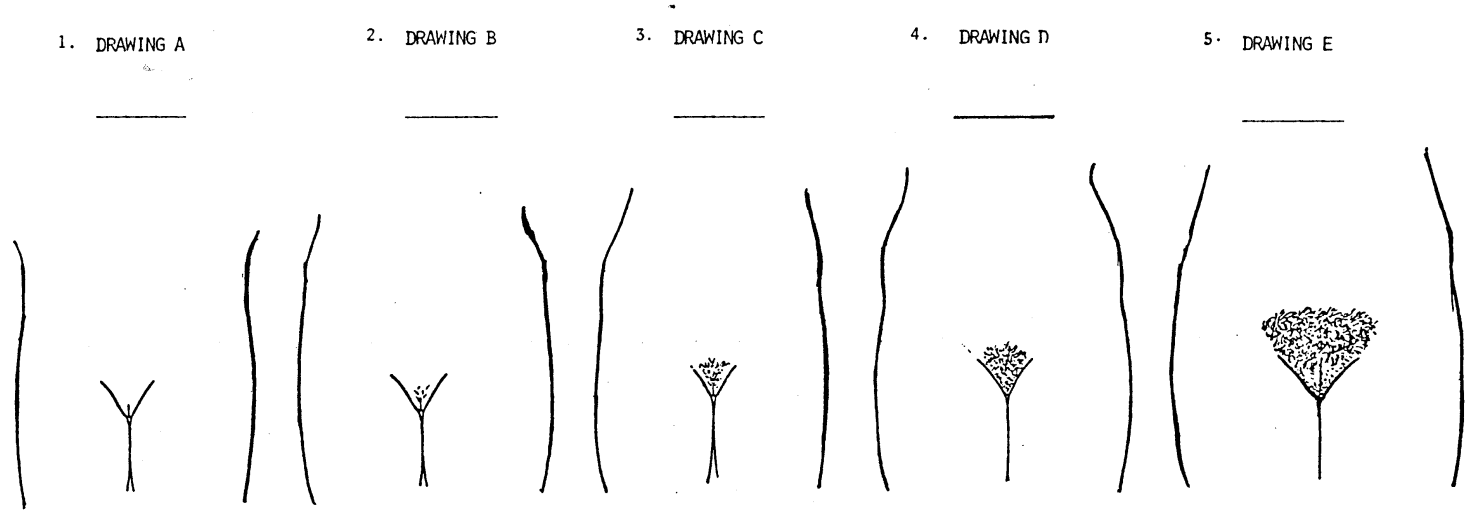
If **Yes** to *Question 6*, have you been having periods for more than two years?

(Please mark the appropriate block)

No ☐ Yes ☐

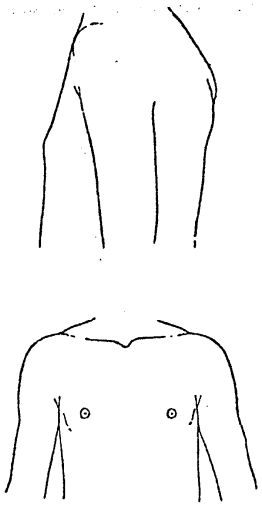
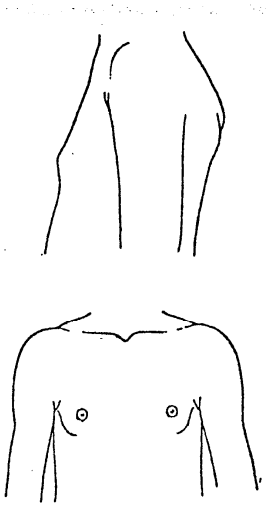
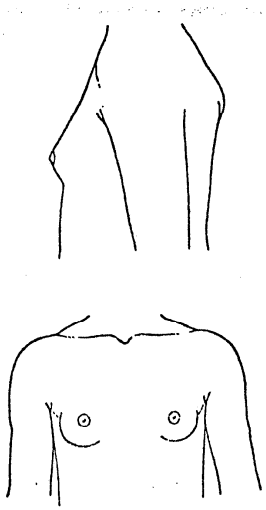
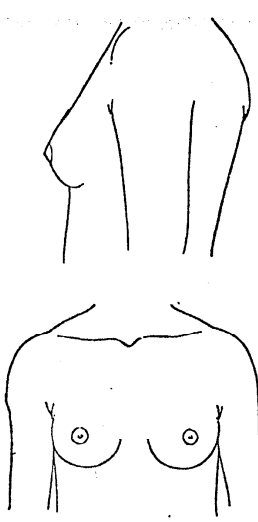
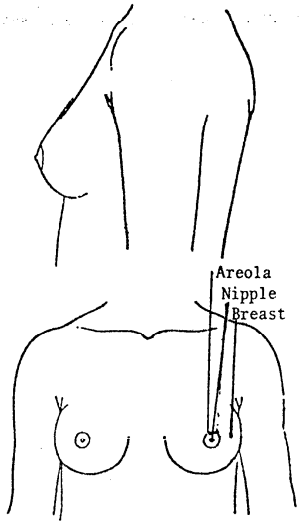
Section B

The drawings below show different amounts of pubic hair. A teenager passes through each of the five stages shown by these drawings. Please look at each drawing and read the sentences under the drawings. Then choose the drawing closest to your stage of hair development and mark it.



1. DRAWING A	2. DRAWING B	3. DRAWING C	4. DRAWING D	5. DRAWING E
There is no pubic hair	**Texture** of the hair is soft. **Density** of the hair is little. **Distribution** of the hair is along the mid-line. Pubic hair may be straight or curly	**Texture** of the hair is coarser. **Density** of the hair is a little more. **Distribution** of the hair has spread out thinly, up the mid-line, and covers a somewhat larger area.	**Texture** of the hair is coarse. **Density** of the hair is much more. **Distribution** of the hair covers a large area in a triangular shape, but has not spread to the thighs. The hair is a darker colour.	**Texture** of the hair is coarse. **Density** of the hair is that of an adult. **Distribution** of the hair has spread to the thighs and moved up the mid-line.
☐	☐	☐	☐	☐

The drawings below show the different stages of development of breasts. A teenager passes through each of the five stages shown by these sets of drawings. Please look at each set of drawings and read the sentences under the drawing. Then choose the set of drawings closest to your stage of breast development and mark it.

1. DRAWING A	2. DRAWING B	3. DRAWING C	4. DRAWING D	5. DRAWING E
				
The breast is flat.	The breast is a small mound. The **areola** (circle around the nipple) is larger. The nipple is slightly raised.	The **areola** circle has gotten bigger, and the **breast** has increased in size and more cone-shaped	The **areola** and the **nipple** make up a mound that sticks up above the shape of the breast. Small bumps may be developing on the areola. The breast may have gotten bigger and rounder.	The **breasts** are fully developed and extend to the arm pit, there are small bumps on the areola around the nipple, and only the **nipple** sticks out in this stage.
☐	☐	☐	☐	☐

Code:

The assessment of physical development in adolescence

Male

Section A

We would like to ask you a few questions about your physical development.

Question 1

Have you grown taller in the last 6 months?

(Please mark the appropriate block)

No ☐ Yes, a little ☐ Yes, some ☐ Yes, a lot ☐ Don't know ☐

Question 2

Do you have to shave your face because you have facial hair?

(Please mark the appropriate block)

No ☐ Yes, a little ☐ Yes, some ☐ Yes, everyday ☐ Don't know ☐

Question 3

Have you started puberty (i.e. do you have pubic hair or have your genitalia enlarged)?

(Please mark the appropriate block)

No ☐ Yes ☐

Question 4

Has your skin started to change (pimples)?

(Please mark the appropriate block)

No ☐ Yes, a little ☐ Yes, some ☐ Yes, a lot ☐ Don't know ☐

Question 5

Has your voice started breaking (do you speak in a deeper voice)?
(Please mark the appropriate block)

No ☐ Yes ☐

If YES, have you been speaking in a deeper voice for *more than* two years?

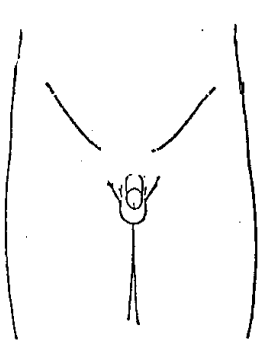
No ☐ Yes ☐

If YES, how old were you when your voice broke? ☐

Section B

The drawings below show different amounts of **pubic hair**. A teenager passes through each of the five stages shown by these drawings. Please look at each drawing and read the sentences under the drawings. Then choose the drawing closest to your stage of hair development and mark it.

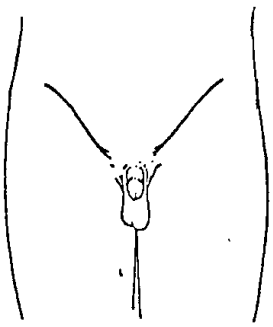
1. DRAWING A



There is no pubic hair

☐

2. DRAWING B



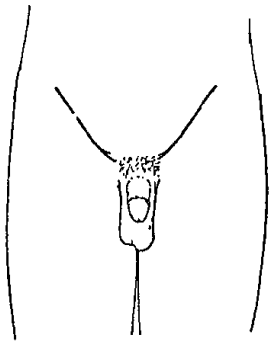
Texture of the hair is soft.

Density of the hair is little.

Distribution of the hair is at the base of the penis, especially on the sides. Pubic hair may be straight or curly

☐

3. DRAWING C



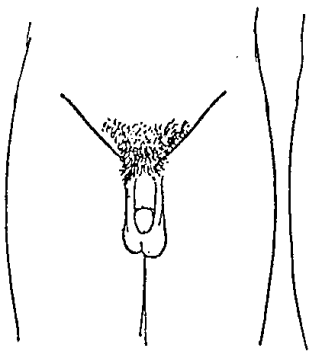
Texture of the hair is coarser.

Density of the hair is a little more.

Distribution of the hair has spread out thinly and covers a somewhat larger area around the base of the penis.

☐

4. DRAWING D



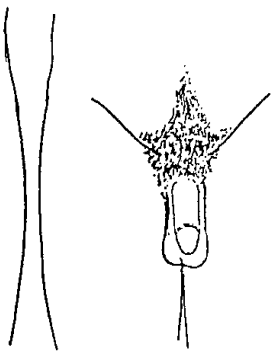
Texture of the hair is coarse.

Density of the hair is much more.

Distribution of the hair covers a large area but has not spread to the thighs. The hair is a darker colour.

☐

5. DRAWING E



Texture of the hair is coarse.

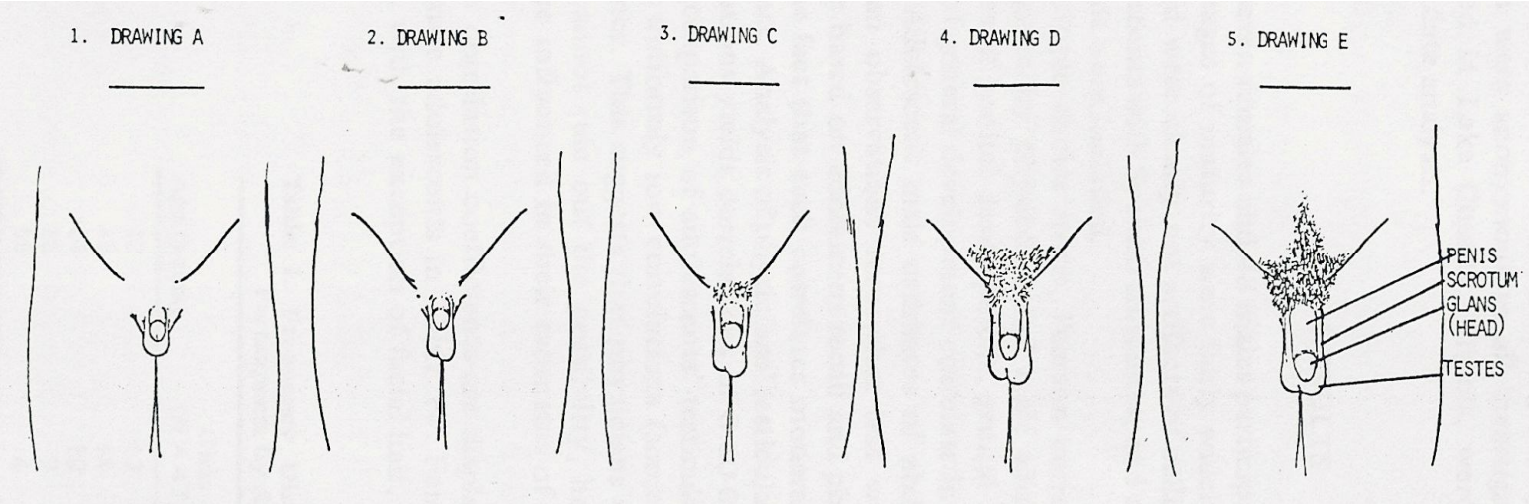
Density of the hair is that of an adult.

Distribution of the hair has spread to the thighs and moved up the mid-line.

☐

Section C

The drawings below show the different stages of development of genitals. A teenager passes through each of the five stages shown by these sets of drawings. Please look at each set of drawings and read the sentences under the drawing. Then choose the set of drawings closest to your stage of genital development and mark it.



There has been no change in the **testes** (balls), **scrotum** (sack holding the balls), and **penis**.	The **scrotum** has lowered a bit, and the skin of the scrotum has changed (smoother). The **testes**, **scrotum** and **penis** have gotten a little larger.	The **scrotum** has dropped lower than in stage 2. The **testes**, **scrotum** and **penis** have grown more.	The **scrotum** is bigger because the **testes** have gotten bigger. The **penis** has grown even larger and wider. The head of the **penis** is bigger	There have been no further size changes in the **penis, scrotum,** and **testes.**
☐	☐	☐	☐	☐

Appendix D – Child Assent form

EXERCISE AND BONE STUDY

Hi there! My name is Rebecca Meiring. I work at Wits University.

I am asking you to take part in a research study because I want to learn whether playing a sport can make your bones stronger than the bones of other kids who don't play a sport.

I will need you and your parents/primary caregiver to come and visit me 3 times over the course of a year at my University. Each visit will be the same.

If you agree to be a part of my study, you will be asked some questions. You will be asked how you are feeling on that day and if you have been feeling sick for the past few weeks. You will also be asked about the kind of food you eat and how much you eat in a day. We will also ask you some questions about what your body looks like. We will also measure how tall you are and how much you weigh. Answering these questions will take about 30 minutes. You do not have to put your name on the questionnaire. Next we will go and visit someone who is going to take a picture of your bones. All you have to do for this is lie on a bed and try and keep still for about 30 minutes.

If you want we would also like to take a little bit of blood from your arm. This might be a little bit sore but we can put some cream on your arm at the beginning so you won't feel any pain. You don't have to do this if you think it will be too sore and that's ok – you can still be part of my project. I also would like you to wee in a cup.

You do not have to be in this study. No one will be mad at you if you decide not to do this study. Even if you start, you can stop at any time if you want. You can ask me any questions you have about the study. If you decide to be in the study I will not tell anyone else what you say or do in the study. Even if your caregiver or teachers ask, I will not tell them about what you say or do in the study.

Thanks!

Rebecca!

PARTICIPANT ASSENT FORM

I, _____ (full name and surname of child) agree to take part in the study called "**Exercise and Bone** Study".

Rebecca has explained the study to me and I understand everything.

I understand that I do not have to carry on with the study if I do not feel like it and I can leave the study whenever I want.

Signature of child (if applicable): _____

Signature of investigator: _____

Date_____

.....

To be detached and kept separately

Participant name: _____

Participant code: _____

Appendix E – Parent/primary caregiver consent form

CONSENT FOR CHILD TO ACT AS PARTICIPANT IN RESEARCH

I,_____(full name and surname of parent/primary caregiver) agree to allow my child, _____ (full name and surname of child) to participate in the study titled: *Influence of Weight-Bearing Activity on Bone Density.*

The procedures/questionnaires have been explained to me and I understand and appreciate their purpose, any risks involved, and the extent of my child's involvement. I have read and understand the attached information sheet.

I understand that the procedures form part of a research project and may not provide any direct benefit to me or my child. I understand that any blood that may be drawn by my child will be used exclusively for detection of substances that give an indication of bone being formed and the urine sample will be used for detection of a substance that shows bone being broken down.

I understand that all experimental procedures have been sanctioned by the Committee for Research on Human Subjects, University of the Witwatersrand, Johannesburg.

I understand that my child's participation is voluntary and that I am free to withdraw my child from the project at any time without prejudice.

_____ _____

Primary caregiver name and signature Date

_____ _____

Investigator name and signature Date

Please provide a contact telephone number in order for a researcher to contact you about a visit to the lab: _____

Appendix F –Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Mrs Rebecca Meiring

CLEARANCE CERTIFICATE	**M10635**
PROJECT	A Comparative Study into the Bone Health of South African Pre-Pubertal Children who Participate in Physical Activities with Different amounts of Skeletal Loading
INVESTIGATORS	Mrs Rebecca Meiring.
DEPARTMENT	School of Physiology
DATE CONSIDERED	25/06/2010
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 23/07/2010 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr I Avidon

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

Appendix G - Physical Activity Questionnaire

Participant code:

PHYSICAL ACTIVITY QUESTIONNAIRE

1. ACTIVITIES AT SCHOOL

1.1 Do you attend physical education/P.E./games lessons at school (i.e. during school hours)?

What do you do in these classes? How many years have you had these classes for? How often are classes held & how long are classes?

Activities	Times / week	Hours / time	How many years?

2. INFORMAL ACTIVITIES

Do you engage in any physical activity outside school but NOT in a sports club e.g. riding a bike, playing in the street or yard, etc? What activities do you engage in, how often, how many years have you been doing these activities for & how long?

Activities	Times / week	Hours / time	How many years?

3. SEDENTARY ACTIVITIES

3.1) Do you engage in any of the following activities before or after school (MON - FRI)? If so, for how many hours per day?

a) Watching TV and videos

Yes☐ No☐

Hours per day ☐

b) Reading, drawing, music lessons, homework

Yes☐ No☐

Hours per day ☐

c) Playing video games during the week

Yes☐ No☐

Hours per day☐

Have you been doing these activities regularly for the past 2 years? Yes☐No☐

If no, for how long? _____

3.2. Do you engage in any of the following activities on the weekend (SAT & SUN)? If so, for how many hours per day?

a) Watching TV and videos

Yes☐ No☐

Hours per day on Saturday☐

Hours per day on Sunday☐

b) Reading, drawing, music lessons, homework

Yes☐ No☐

Hours per day on Saturday☐

Hours per day on Sunday☐

c) Playing video games during the weekend

Yes☐ No☐

Hours per day on Saturday☐

Hours per day on Sunday☐

Have you been doing these activities regularly for the past 2 years? Yes☐No☐

If no, for how long? _____

During the WEEK, on average, what time do you go to bed? _____

Wake up? _____

On the WEEKEND, on average what time do you go to bed? _____

Wake up? _____

4. TRANSPORT

How do you get to school and how long does it take to get there and back? Please choose one of the 5 options:

4.1 BY CAR, BUS, TAXI, TRAIN ETC? ☐ Yes ☐ No

How long to get there? _____ minutes.

How long to get back? _____minutes

4.2 WALKING? ☐ Yes ☐ No

How long to get there? _____ minutes.

How long to get back? _____minutes

When you walk, at what pace (how fast) do you usually walk? Please circle:

At a vigorous pace, that makes me breathe much harder than normal	At a medium pace that makes me breathe somewhat harder than normal	At a slow pace when there is no change in my breathing

4.3 BICYCLE? ☐ Yes ☐ No

How long to get there? _____ minutes.

How long to get back? _____minutes

When you cycle, at what pace (how fast) do you usually cycle? Please circle:

At a vigorous pace, that makes me breathe much harder than normal	At a medium pace that makes me breathe somewhat harder than normal	At a slow pace when there is no change in my breathing

4.4 COMBINATION: (WALKING AND TAXI, BUS AND WALKING) ☐ Yes ☐ No

How long to get there walking/cycle? _____ minutes

How long to get there vehicle? _____ minutes

How long to get back walking/cycle? _____ minutes

How long to get back vehicle? _____ minutes

When you walk/cycle, at what pace (how fast) do you usually walk/cycle? Please circle:

At a vigorous pace, that makes me breathe much harder than normal	At a medium pace that makes me breathe somewhat harder than normal	At a slow pace when there is no change in my breathing

4.5 OTHER? ☐ Yes ☐ No

Please give details:_____

5. ANY EXTRA MURAL ACTIVITIES OR PRIVATE LESSONS AFTERSCHOOL (ORGANISED SPORT)?

Please think of your activity over the past month	Number of Practises/Week	Hrs/ Practise	Competitions/Wk	Intensity? (1 – 5)
Athletics (track events)				
Athletics (field events)				
Archery				
Badminton				
Ballet				
Basketball				
Cricket				
Cycling				
Dancing				
Fencing				
Golf				
Gymnastics				
Hockey				
Horse riding				
Judo/Karate				
Netball				
Rowing				
Rugby (Touch)				
Rugby (Contact)				
Soccer				

Squash				
Swimming				
Tennis				
Volleyball				
Waterpolo				
Other				

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Original Full Length Article

A two-year history of high bone loading physical activity attenuates ethnic differences in bone strength and geometry in pre-/early pubertal children from a low-middle income country

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ABSTRACT

We examined the interplay between ethnicity and weight-bearing physical activity on the content and volumetric properties of bone in a pre- to early pubertal South African Black and White population. Sixty six children [Black boys, 10.4 (1.4) yrs, n = 15; Black girls, 10.1 (1.2) yrs, n = 27; White boys, 10.1 (1.1) yrs, n = 7; White girls, 9.6 (1.3) yrs, n = 17] reported on all their physical activities over the past two years in an interviewer administered physical activity questionnaire (PAQ). All participants underwent a whole body and site-specific DXA scan and we also assessed bone structure and estimated bone strength with pQCT. Children were classified as being either high or low bone loaders based on the cohort's median peak bone strain score estimated from the PAQ. In the low bone loading group, Black children had greater femoral neck bone mineral content (BMC) (2.9 (0.08) g) than White children (2.4 (0.11) g; p = 0.05). There were no ethnic differences in the high bone loaders for femoral neck BMC. At the cortical site, the Black low bone loaders had a greater radius area (97.3 (1.3) vs 88.8 (2.6) mm^2; p = 0.05) and a greater tibia total area (475.5 (8.7) vs. 397.3 (14.0) mm^2; p = 0.001) and strength (1633.7 (60.1) vs. 1271.8 (98.6) mm^3; p = 0.04) compared to the White low bone loaders. These measures were not different between the Black low and high bone loaders or between the Black and White high bone loaders. In conclusion, the present study shows that there may be ethnic and physical activity associations in the bone health of Black and White pre-pubertal children and further prospective studies are required to determine the possible ethnic specific response to mechanical loading.

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Introduction

Participation in weight-bearing physical activity is an effective way of increasing bone mass [1]. The best time to partake in this type of physical activity is during the years preceding puberty when bone mass accretion is greatest [2]. Pre- and early pubertal children who perform weight-bearing exercise on a regular basis have geometrically bigger and stronger bones [3,4] with significantly higher areal bone mineral density (aBMD) [5], volumetric bone mineral density (BMD) [6], bone mineral content (BMC) and bone area than children who are less active [7,8]. Longitudinal studies have shown that weight bearing activities in early childhood provide sustained benefits to bone health in adolescence [9–11] and lifelong participation in physical activity is now recommended as a means of preventing osteoporotic fracture in later life [12,13].

Physical activity and bone health studies that have taken place in South African children have been limited in their interpretation due to the sole reliance on dual energy X-ray absorptiometry (DXA). One of the main disadvantages of DXA is the two dimensional nature of the scan resulting in inaccurate aBMD reporting in participants who are very large or very small [14]. A number of studies have confirmed that African American adults and children have denser and therefore stronger bones than their White counterparts [15–18] and these differences remain after adjustment for bone size. In South Africa distinct ethnic differences have also been observed for certain measures of bone health i.e. Black children and adults have bigger, stronger and denser bones than those of their White peers [19–24]. It is suggested that this advantage in bone may be lessened [25] as people living in a low-middle income country such as South Africa adopt increasingly sedentary and Westernized lifestyles [20,26,27]. What remains to be elucidated is whether in the future, the genetic advantage of stronger bones afforded to Black South African children will be attenuated with increasingly sedentary lifestyles.

Using pQCT technology, African American children have been shown to have significantly stronger bones as a result of having greater cortical volumetric BMD and cortical area compared to their White peers [18].

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More recently greater bone area and strength in Black compared to White children has been observed in South African children [25]. Although the muscle–bone relationship has been well characterized [28], Wetzsteon et al. [18] have shown that race differences in bone geometry are not attributable to differences in muscle cross sectional area (CSA). Also, it has been consistently shown by Micklesfield and colleagues that muscle CSA is greater in White compared to Black South African children [21,29,30] but the same is not true in North American children [29]. Thus the reasons for ethnic variability in bone remain poorly understood with regard to physical activity.

Higher levels of physical activity have been shown to be associated with higher DXA derived BMC at the hip and spine in South African children [20,31]. No studies have considered the relationship between ethnicity and physical activity on structural and geometric properties of bone in a healthy cohort of South African Black and White children. This cross-sectional study used both DXA and pQCT technology to explore the interplay between bone health and ethnicity and analyze the differences in bone content, area and strength as well as geometric properties of bone due to history of loading in pre- to early pubertal South African Black and White children. The physical activity questionnaire we used served as a proxy measure of bone loading classification. Our study had three hypotheses. First we hypothesized that children who were classified as being high bone loaders for the past two years would present with greater bone mass and strength regardless of their ethnicity. Our second hypothesis tested was that high levels of weight-bearing physical activity would negate any ethnic bone differences that may have existed in our participants. Finally we assessed whether muscle CSA could account for any differences observed in bone variables between high and low bone loading children.

Materials and methods

Participants

Participants were recruited from local schools and community centers around the greater Johannesburg area by distributing flyers containing study information during the summer months in South Africa (September 2010 to February 2011). Participants volunteered by contacting the primary investigator. All participants who volunteered for the study were from similar socioeconomic backgrounds with similar access to voluntary physical activities or after-school sports. Ninety six Black and White, boys and girls between the ages of 8 and 11 years volunteered to participate in the study. As indicated by the parent or primary caregiver, children were considered Black if both parents and grandparents were Black while children were considered White if both parents and grandparents were White. Maturational status was self-assessed using breast (girls), gonadal (boys) and pubic hair stages as the Tanner five stage classification criteria [32]. The age at onset of menarche was also requested in the Tanner questionnaire for girls. Children were included in the study if they were pre- to early pubertal or Tanner stage I to III. A general health questionnaire was administered to the parents or caregivers of each child to collect information on socioeconomic status, state of health of the child, including any medication use, as well as history of fractures and family history of osteoporosis. Children were excluded if they had been on corticosteroid medication consecutively for more than seven days in the past year, if they had any milk or lactose food allergies, if they were on a vitamin D or calcium supplement or if they had been ill or admitted to hospital in the last 3 months prior to participation in the study. Girls were excluded if they had attained menarche. All children who participated in the study had the protocol verbally explained to them and if they agreed to participate, signed an assent form. Parents/primary caregivers of the children were required to consent to their child's participation in the study. The study was approved by the Human Research Ethics Committee of the University of the Witwatersrand (protocol no.: M10635) which adheres to the principles of the Declaration of Helsinki.

Physical activity questionnaire

Children, with the assistance of their parent or caregiver, were required to fill out a physical activity questionnaire, previously validated for South African children [33]. Briefly, the PAQ was validated by assessing agreement with activity recorded using accelerometry [33]. In the present study, the questionnaire was modified to include a two-year as opposed to one year, history of the child's participation in all types of physical activity with similar access to voluntary physical activities or after-school sports. A two-year history of physical activity was obtained in order to take into account seasonal variation in sport and also to consider those children who had played a sport up to the first year but then changed to a different sport in the second year. For example, there were some children in the cohort that in the first year of PA assessment had participated in a weight-bearing sport but in the second year of assessment had participated in a non weight-bearing sport such as swimming. The rationale behind a two-year history as opposed to the conventional one-year history was to ensure that we included children who had been exposed to a season of bone loading. In addition, the sustained benefits of weight-bearing exercise are not well defined. Children and their parents listed all the physical activities that the child had participated in for the past two years. Information was gathered from four activity question domains namely, physical activity participation during school, extra-mural/after-school physical activities, leisure time activity as well as mode of transport to and from school. Children were asked to give details on the number of times per week they performed that activity as well as the amount of time they spent on each activity at any one time in order to determine the frequency and pattern of regularity of each activity. Regular participation in a specific activity was classified as the child performing an activity once a week for more than four months of the year (the usual length of a school semester in South Africa). After-school and leisure time activities were also reported on and assessed in the same way. Each regular activity was assigned an estimated bone strain score using a scoring system developed by Groothausen et al. [34], which was validated by using the ground reaction force produced by each activity to quantify the amount of bone loading of that activity. The sum of the scores for each activity made up the estimated peak bone strain score (PBSS) that was assigned to each child and allowed us to classify them as high or low bone loading. Based on the range of PBSS attained, children were divided into two groups: a high and a low bone loading group. This classification was based on the median PBSS attained for the whole sample, such that we had four groups i.e. Black high bone loading, White high bone loading, Black low bone loading and White low bone loading. Low bone loading did not necessarily mean children were physically inactive, for example, swimmers were classed as low bone loaders along with sedentary children.

Anthropometry

Participant height and weight were recorded to the nearest millimeter (mm) and 100 g using a stadiometer (Holtain, Crosswell, UK) and a digital scale (Dismed, Halfway House, South Africa) respectively. Participants were measured without shoes and while wearing light clothing. BMI percentile-for-age was calculated using software available from the World Health Organization (WHO, http://www.who.int/childgrowth/software/en). Radial and tibial lengths (to the nearest mm) were measured using sliding calipers (Holtain, Crosswell, UK) for the determination of the position of the bone scans. Radial length was defined as the distance from the tip of the olecranon process to the most distal end of the ulna styloid process. The side that was chosen for scanning was determined by asking the child which hand they wrote with and that side was scanned. Tibial length was defined as the distance from the distal end of the medial malleolus to the superior aspect of the medial tibial condyle.

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Dual energy X-ray absorptiometry (DXA)

Bone mineral content (BMC, g) and bone area (BA, mm^2) were reported from measures obtained using a DXA machine (Hologic QDR, Discovery W, Bedford, MA, USA) at the following sites: the dominant forearm, whole body (less head), lumbar spine and total hip (for measurement of the femoral neck) of the dominant leg. Fat and lean mass as well as body fat percentage were obtained from the whole body DXA scan. The same trained technician performed and analyzed all DXA scans. The CVs for BMC and BA were 0.36% and 0.32% respectively.

Peripheral quantitative computed tomography (pQCT)

Measures of the forearm and tibia were also performed using peripheral quantitative computed tomography (Stratec XCT 2000, Stratec Medical, Pforzheim, Germany). A scout view was performed for each subject and a reference line placed at the midline of the epiphyseal plate of the radius and the tibia. The forearm was scanned at 4% and 65% of the length of the radius from the reference line. The tibia was scanned at 65% of the length of the tibia from the reference line. The following measures were obtained from the metaphysis of the radius (4% slice): total cross-sectional area (Tt.Ar, mm^2), total volumetric density (Tt.BMD, mg/cm^3) and trabecular density (Tb.BMD, mg/cm^3). Diaphyseal measures (cortical bone — 65% slice) obtained from the radius and the tibia were: polar strength–strain index (SSI.Po, mm^3), cortical density (Ct.BMD, mg/cm^3) as well as total area (Tt.Ar, mm^2) and cortical area (Ct.Ar, mm^2). Muscle cross sectional area (CSA, mm^2) was also obtained from the scans at the 65% radius and tibia sites. Bone strength index (BSI, mg^2/mm^4) at the radial metaphysis, periosteal circumference (Ps.C, mm), endosteal circumference (Es.C, mm) and cortical thickness (Ct.Th, mm) were calculated using formulas previously described [35]. Participants were asked to lie as still as possible while being scanned and if there were significant motion artifacts whereby an acceptable reading could not be obtained, the scan was repeated. For the 4% radial measures, the bone threshold was set at 180 mg/cm^3 and contour mode 1/peel mode 1 was used. For the cortical and bone geometry measures at the 65% radial and tibial sites, bone threshold was set at 711 mg/cm^3 (contour mode 1/peel mode 2). Threshold for SSI.Po at these sites for both the radius and tibia was set at 480 mg/cm^3. For the measures of muscle CSA, threshold was set at 40 mg/cm^3 (contour mode 3/peel mode 1). The same independent technician performed and analyzed all pQCT scans. A phantom of similar and known composition to human bone was scanned each morning.

Statistical analysis

Data were analyzed using Statistica Version 10 (Statsoft Inc., Tulsa USA). We performed an unpaired t-test (parametric data) or Mann–Whitney U (non-parametric data) test to determine any differences in anthropometric data between boys and girls as well as between Black and White children. Differences in Tanner proportions between Black and White children and between boys and girls were assessed using a chi-squared test. We tested the relationship between PBSS and bone variables within each ethnicity in a continuous manner and also in a categorical analysis. For the former analysis, we log-transformed the PBSS data because it was not normally distributed. Then Pearson's correlations were performed to determine continuous associations between log-transformed PBSS and bone variables within each ethnic group. Covariates for bone analyses were determined by performing correlations between all variables. DXA bone measures were adjusted for sex, weight, maturity and bone area. pQCT bone measures were adjusted for sex, weight, sexual maturity, limb length and muscle CSA. Ethnicity and sex interactions with adjusted bone variables were assessed using a two-way ANOVA with a Bonferroni post-hoc test to control for multiple comparisons. To categorically assess the effects of weight-bearing physical activity and ethnicity on bone health, children were then classified into two groups — low and high bone loadings in their respective ethnic group (Black and White). Again a two-way ANOVA was performed on adjusted bone outcomes between Black and White, high and low bone loaders with a Bonferroni post-hoc test. Pearson's partial correlations were used to evaluate the relationship between muscle CSA and bone variables controlling for sex, weight, limb length and sexual maturity. Mean and standard deviation (SD) are reported for the unadjusted variables while mean and standard error of the mean (SE) are reported for the adjusted variables. Significance was set at $p \leq 0.05$.

Results

Of the 96 children who were recruited to participate, 14 children did not fulfill the Tanner pubertal classification of being pre-/early pubertal, two children were taking chronic medication for asthma and 4 children withdrew from the study before any procedures were performed. A further ten children (6 White boys, 2 White girls and 2 Black boys) were excluded from the final analysis because their PBSS were either greater than or less than 2 SD from the mean PBSS. We excluded these children in order to match PBSS between groups and so that our ethnic groups were comparable. Thus sixty six Black and White boys and girls were included in the final analysis. Table 1 shows the descriptive characteristics of the children grouped according to ethnic group (Black and White) and sex. All children were of similar age, height, weight, lean mass as well as BMI-for-age percentile and tibia length. Even though groups were matched for PBSS, White boys tended to have a higher PBSS although this was only significantly different to the Black girls ($p = 0.005$). There were significant interactions between sex and ethnicity for weight, fat mass, lean mass and forearm muscle CSA but post-hoc tests revealed no significant differences between groups for weight and lean mass. White boys had significantly greater fat mass compared to Black boys ($p = 0.01$) while Black girls had greater fat mass and body fat percentage compared to Black boys ($p = 0.009$) and radius length compared to White girls ($p = 0.02$). White boys had greater arm muscle CSA compared to Black boys ($p = 0.02$), Black girls ($p = 0.007$) and White girls ($p = 0.002$). Table 2 shows the ethnic and sex effects on bone variables as measured by pQCT. There were significant interactions between sex and ethnicity for Tb.BMD at the 4% radius and Ct.Th and En.C at the 65% radius. There were no significant interactions between sex and ethnicity for bone

Table 1
Descriptive characteristics of children grouped by sex and ethnicity.

	Black		White	
	Boys (n = 15)	Girls (n = 27)	Boys (n = 7)	Girls (n = 17)
Age (yrs)	10.4 (1.4)	10.1 (1.2)	10.1 (1.1)	9.6 (1.3)
Height (cm)	136.0 (6.7)	137.8 (8.3)	139.6 (11.8)	135.4 (8.8)
Weight (kg)	30.2 (3.8)	33.3 (7.3)	38.1 (11.3)	31.4 (6.2)
Tanner stage (n) I/II/III	7/5/3	14/9/4	6/1/0	14/2/1
PBSS*	4.5 (3.2–5.7)	4.0 (3.0–6.0)[e]	8.0 (4.7–9.5)[e]	4.5 (4–10)
Fat mass (kg)	5.6 (0.8)[ab]	8.7 (3.1)[a]	9.8 (5.1)[b]	8.6 (2.6)
Lean mass (kg)	23.2 (3.7)	23.2 (4.7)	27.1 (6.6)	21.7 (4.0)
% body fat*	19.3 (15.9–22.9)[a]	25.8 (22.3–28.7)[a]	21.9 (19.4–32.8)	28.6 (22.0–30.3)
BMI percentile*	45.0 (17.4–53.8)	60.6 (28.2–75.0)	65.0 (23.5–98.0)	59.0 (28.1–78.7)
Radius length (mm)	220.5 (13.6)	228.6 (17.1)[c]	217.2 (21.0)	210.9 (18.4)[c]
Tibia length (mm)	314.0 (23.0)	325.0 (25.8)	320.33 (37.8)	315.8 (24.8)
Forearm muscle CSA (mm^2)	1797.1 (391.5)[b]	1773.2 (316.4)	2364.5 (712.4)[bd]	1651.8 (256.8)[d]
Leg muscle CSA (mm^2)	3376.9 (581.0)	3402.3 (594.7)	3990.3 (1223.1)	3375.3 (627.3)

Data are mean (SD). Matching superscript letters indicate significant differences between groups, e.g. if White low bone loaders and Black low bone loaders have the same letter [a], then those are the two groups that are significantly different from each other; $p < 0.05$. Forearm and leg muscle CSA were adjusted for weight, sexual maturity and limb length.
* Indicates data expressed as median (IQR).

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Table 2
Sex and ethnic differences in radius and tibia bone density, structure and strength.

	Black		White	
	Boys (n = 15)	Girls (n = 27)	Boys (n = 7)	Girls (n = 17)
4% Radius	(n = 13)	(n = 22)	(n = 6)	(n = 16)
Tt.Ar (mm^2)	232.56 (24.4)	225.0 (33.8)	253.2 (42.5)	215.4 (32.9)
Tt.BMD (g/cm^3)	301.9 (6.3)	288.2 (10.7)	293.2 (12.5)	272.3 (6.7)
Tb.BMD (g/cm^3)	212.6 (11.2)[b]	214.3 (13.8)[d]	235.5 (17.3)[b,c]	190.3 (9.6)[c,d]
BSI (mg^2/mm^4)	2087.6 (210.4)	1846.6 (262.4)	2137.3 (320.7)	1610.8 (286.6)
65% Radius	(n = 13)	(n = 22)	(n = 6)	(n = 16)
Tt.Ar (mm^2)	103.8 (10.5)	94.0 (12.5)	108.2 (14.9)	98.3 (14.5)
Ct.Ar (mm^2)	43.2 (4.4)	43.6 (5.1)	52.0 (5.5)	41.6 (4.9)
Ct.BMD (g/cm^3)	1012.8 (6.2)	1017.4 (6.9)	1019.3 (9.8)	988.9 (4.6)
SSI.Po (mm^3)	162.5 (24.3)	139.2 (26.3)	169.9 (31.1)	137.5 (29.1)
Ps.C (mm)	35.9 (1.8)	34.3 (2.2)	36.8 (2.6)	34.9 (2.6)
Ct.Th (mm)	1.4 (0.1)[a,b]	1.5 (0.1)[a,d]	1.6 (0.1)[b,c]	1.4 (0.1)[c,d]
En.C (mm)	27.2 (1.7)[a]	24.9 (2.0)[a]	26.5 (2.3)	26.2 (2.4)
65% Tibia	(n = 14)	(n = 26)	(n = 6)	(n = 16)
Tt.Ar (mm^2)	472.5 (49.7)	461.4 (62.5)	485.2 (112.0)	418.5 (58.7)
Ct.Ar (mm^2)	226.2 (31.6)	224.8 (32.5)	219.9 (53.8)	208.5 (30.0)
Ct.BMD (mg/cm^3)	1053.1 (7.0)	1048.2 (6.7)	1025.5 (9.8)	1035.3 (3.9)
SSI.Po (mm^3)	1624.1 (322.3)	1621.4 (373.9)	1813.5 (666.0)	1435.7 (361.5)
Ps.C (mm)	76.9 (4.2)	76.0 (5.2)	76.7 (9.5)	72.3 (4.9)
Ct.Th (mm)	3.4 (0.3)	3.4 (0.3)	3.3 (0.5)	3.4 (0.3)
En.C (mm)	55.4 (2.6)	54.3 (3.9)	56.3 (7.3)	51.1 (3.6)

Data are mean (SD) and adjusted for weight, limb length, sexual maturity and muscle CSA. Similar superscript letters indicate significant differences; $p < 0.05$. Note: Scan numbers differ at each site as there were children in each group whose limbs were too short for pQCT scanning.

Table 3
Descriptive characteristics of children grouped by ethnicity and bone loading group.

	Low		High	
	Black (n = 30)	White (n = 11)	Black (n = 12)	White (n = 13)
Age (yrs)	10.2 (1.1)	9.5 (1.4)	10.3 (1.6)	9.9 (1.2)
Height (cm)	137.5 (7.9)	133.1 (8.2)	136.1 (7.6)	139.6 (10.1)
Weight (kg)	32.5 (6.8)	29.0 (5.4)[d]	31.2 (5.3)	37.0 (8.8)[d]
Girls (n)	19	8	10	7
Tanner stage (n) I/II/III	14/11/5	8/2/1	7/3/2	12/1/0
PBSS*	3.5 (3.0–4.0)[c,f]	4.0 (3.3–4.0)[d,e]	6.0 (6–8.0)[ce]	8.0 (7.0–9.3)[d,f]
Fat mass (kg)	7.6 (3.2)	7.6 (2.5)	7.4 (2.0)	10.1 (3.7)
Lean mass (kg)	23.5 (4.3)	20.3 (3.4)[d]	22.3 (4.5)	25.8 (5.5)[d]
% body fat*	22.6 (19.0–27.6)	23.0 (21.3–30.9)	23.7 (20.5–28.9)	27.5 (21.0–31.1)
BMI percentile*	53.0 (25.5–70.7)	42.9 (14.1–76.1)	39.0 (27.8–60.5)	74.3 (42.2–91.5)
Forearm length (mm)	227.4 (13.9)[a]	204.0 (17.2)[a]	220.2 (21.4)	219.9 (17.8)
Tibia length (mm)	322.1 (25.7)	313.0 (24.5)	318.5 (24.3)	320.4 (31.3)
Forearm muscle CSA (mm^2)	1792.6 (191.3)[a,f]	1563.3 (240.2)[a,d]	1751.6 (259.7)[b]	2112.9 (317.8)[b,d,f]
Leg muscle CSA (mm^2)	3453.6 (499.5)	3153.1 (460.5)[d]	3253.1 (472.7)[b]	3868.0 (709.7)[b,d]

Data are mean (SD). Similar superscript letters indicate significant differences; $p < 0.05$. Forearm and leg muscle CSA were adjusted for sex, weight, sexual maturity and limb length.
* Indicates data expressed as median (IQR).

outcomes at the 65% tibia. There were significant main effects of sex for Tb.BMD and Tt.BMD as well as BSI at the 4% radius but no main effects of ethnicity were seen at the 4% radius. There was a significant main effect of sex as well as a main effect of ethnicity for Ct.Ar at the 65% radius site. There were no significant interactions between sex and ethnicity for the bone outcomes at the 65% tibia but there were significant main effects of ethnicity for Tt.Ar, Ct.Ar, Ps.C and Es.C at the 65% tibia. No main effects of sex were seen at the 65% tibia. Post-hoc analyses are found in Table 2.

Ethnicity, weight-bearing physical activity and bone

Table 3 shows the descriptive characteristics of the children according to ethnicity and bone loading group. White high bone loaders had a greater body mass compared to the White low bone loaders ($p = 0.03$). Fig. 1 is a box-and-whisker plot of the PBSS between ethnic and bone loading groups. There were significant interactions between ethnicity and bone loading for both forearm ($p = 0.03$) and leg ($p = 0.01$) muscle CSA. Arm muscle CSA was greater in the White high bone loaders compared to the Black low bone loaders ($p = 0.003$), Black high bone loaders ($p = 0.008$) and the White low bone loaders ($p = 0.001$) after adjusting for sex, weight and sexual maturity. After adjusting for sex, weight and maturity, leg muscle CSA was greater in the White high bone loaders compared to both the Black high bone loaders ($p = 0.03$) and the White low bone loaders ($p = 0.006$). PBSS was significantly and positively correlated to DXA and pQCT bone variables in the White children only (Table 4), with the exception of radius and ulna BMC (as measured by DXA) as well as Ct.Th at the 65% tibia.

DXA

Table 5 shows the DXA data obtained for all the participants grouped according to ethnicity and bone loading group. There were significant interactions between ethnicity and bone loading for BMC at the radius ($p = 0.005$), spine ($p = 0.04$), whole body ($p = 0.04$) and femoral neck ($p = 0.05$) but not for total hip BMC. There were significant main effects of bone loading for the radius ($p = 0.001$), whole body ($p = 0.004$) and femoral neck ($p = 0.01$). Post-hoc analysis revealed that there was an ethnic difference between Black and White children in the low bone loading group for femoral neck BMC only ($p = 0.02$). There was no difference in BMC between the Black and White children in the high bone loading group for any site. White high bone loaders had greater BMC at the radius ($p = 0.01$), whole body ($p = 0.04$) and femoral neck ($p = 0.04$) than the White low bone loaders. There were no within-ethnic differences at any site between the high and low Black bone loaders.

pQCT

There were significant interactions between ethnicity and bone loading for Tt.Ar at the 4% radius ($p = 0.01$). There were no significant interactions between ethnicity and bone loading for any of the bone outcomes at the 65% radius. There were significant main effects of bone loading on Tt.Ar ($p = 0.04$) and Ps.C ($p = 0.04$) at the 65% radius. Significant interactions between ethnicity and bone loading were present for Tt.Ar ($p = 0.02$), SSI.Po ($p = 0.02$), Ps.C ($p = 0.03$) and Es.C ($p = 0.03$) at the 65% tibia.

Radial metaphysis (4%)

Table 5 shows the within and between ethnic differences between high and low bone loaders at the radius as measured by pQCT. The

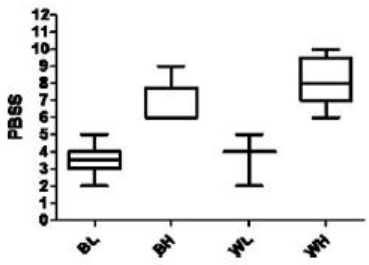

Fig. 1. Box-and-whisker plot of PBSS between ethnic and bone loading groups. BL = Black low bone loaders (n = 30); BH = Black high bone loaders (n = 12); WL = White high bone loaders (n = 11); WH = White high bone loaders (n = 13).

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Table 4
Pearson's correlations showing the r values of log-transformed PBSS with bone variables.

	Black	White
DXA		
Ulna BMC (g)	0.01	0.56[b]
Radius BMC (g)	0.07	0.60[b]
Spine BMC (g)	−0.11	0.60[b]
Whole body BMC (g)	0.04	0.66[b]
Hip BMC (g)	0.03	0.55[b]
Femoral neck BMC (g)	0.07	0.55[b]
Radius (4%)		
Tt.BMD (mg/cm^3)	0.12	0.20
Tb.BMD (mg/cm^3)	−0.19	−0.05
Tt.Ar (mm^2)	−0.18	0.54[a]
BSI (mg^2/mm^4)	−0.01	0.58[b]
Radius (65%)		
Ct.BMD (g/cm^3)	0.25	0.30
Tt.Ar (mm^2)	0.01	0.56[a]
Ct.Ar (mm^2)	0.14	0.54[a]
SSI.Po (mm^3)	0.12	0.43[a]
Ps.C (mm)	−0.01	0.58[b]
Es.C (mm)	−0.07	0.46[a]
Ct.Th (mm)	0.13	0.27
Forearm muscle CSA (mm^2)	−0.13	0.54
Tibia (65%)		
Ct.BMD (mg/cm^3)	0.03	0.22
Tt.Ar (mm^2)	−0.07	0.54[a]
Ct.Ar (mm^2)	0.05	0.41[a]
SSI.Po (mm^3)	−0.07	0.65[b]
Ps.C (mm)	−0.08	0.50[a]
Es.C (mm)	−0.07	0.410[a]
Ct.Th (mm)	−0.02	0.09
Leg muscle CSA (mm^2)	−0.20	0.51[a]

Data are r^2 values. Tt.BMD = total bone mineral density, Tb.BMD = trabecular bone mineral density, BSI = bone strength index, Ct.BMD = cortical bone mineral density, Tt.Ar = total area, Ct.Ar = cortical area, SSI.Po = polar strength–strain index, Ps.C = periosteal circumference, Es.C = endosteal circumference, Ct.Th = cortical thickness, CSA = cross-sectional area.
[a] $p < 0.05$.
[b] $p < 0.01$.

Table 5
Adjusted whole body and site-specific DXA and pQCT measures of the radial epiphysis and diaphysis between Black and White children within each bone loading group.

	Low		High	
	Black (n = 28)	White (n = 9)	Black (n = 9)	White (n = 11)
DXA				
Radius BMC (g)	3.7 (0.1)	3.0 (0.2)[c]	3.7 (0.2)	4.0 (0.2)[c]
Spine BMC (g)	23.7 (0.7)	20.6 (1.1)	22.9 (1.2)	24.6 (1.2)
Whole body BMC (g)	785.9 (24.2)	671.8 (35.5)[c]	800.8 (38.2)	827.8 (52.8)[c]
Total hip BMC (g)	17.5 (0.6)	14.9 (1.0)	17.8 (1.3)	17.6 (1.1)
Femoral neck BMC (g)	2.9 (0.1)[a]	2.4 (0.1)[a,c]	2.9 (0.1)	2.9 (0.1)[c]
Radius (4%)				
Tt.BMD (g/cm^3)	290.4 (2.3)	272.7 (1.7)	297.3 (2.7)	286.6 (2.9)
Tb.BMD (g/cm^3)	208.4 (2.0)	202.8 (2.7)	217.6 (4.0)	203.9 (3.1)
Tt.Ar (mm^2)	229.4 (4.6)[a]	198.8 (10.3)[a,c]	215.0 (11.8)	243.3 (10.2)[c]
BSI (mg^2/mm^4)	1906.8 (38.7)	1450.0 (91.4)	1913.4 (116.8)	1999.5 (85.6)
Radius (65%)				
Ct.BMD (g/cm^3)	1013.6 (1.5)	989.4 (3.5)	1009.2 (4.3)	1008.0 (3.7)
Tt.Ar (mm^2)	97.3 (1.3)[a]	88.8 (2.6)[a,c]	98.9 (3.7)[b]	109.9 (3.0)[b,c]
Ct.Ar (mm^2)	43.0 (0.8)	41.9 (1.7)	43.6 (2.2)	46.7 (1.7)
SSI.Po (mm^3)	145.4 (3.8)	130.5 (7.3)	151.6 (10.8)	159.4 (8.8)
Ps.C (mm)	34.9 (0.2)[a]	33.2 (0.5)[a,c]	35.0 (0.6)[b]	37.0 (0.5)[b,c]
Es.C (mm)	25.9 (0.1)	24.1 (0.2)	25.9 (0.3)	27.8 (0.4)
Ct.Th (mm)	1.4 (0.02)	1.5 (0.04)	1.6 (0.05)	1.5 (0.04)

Data are means (SE). DXA BMC data are adjusted for sex, weight, sexual maturity and bone area. Peripheral QCT data are adjusted for sex, weight, sexual maturity, limb length and arm muscle CSA. Similar superscript letters indicate between group differences; $p < 0.05$. Tt.BMD = total bone mineral density, Tb.BMD = trabecular bone mineral density, BSI = bone strength index, Ct.BMD = cortical bone mineral density, Tt.Ar = total area, Ct.Ar = cortical area, SSI.Po = polar strength–strain index, CSA = cross-sectional area, Ps.C = periosteal circumference, Es.C = endosteal circumference, Ct.Th = cortical thickness.

Black low bone loaders had a greater Tt.Ar ($p = 0.04$) at the epiphysis of the radius compared to the White low bone loaders. There was no difference in Tt.Ar between Black and White high bone loaders. Within the White children, high bone loaders had greater Tt.Ar ($p = 0.007$) compared to the low bone loaders.

Radial diaphysis (65%)

In the low bone loading group, the Black children had greater total area ($p = 0.05$) and periosteal ($p = 0.03$) circumference than the White children (Table 5). In the high bone loading group, the White children had greater Tt.Ar ($p = 0.02$) and periosteal ($p = 0.02$) circumference compared to the Black high bone loaders. There were only within-ethnic group differences in the White children, where the White high bone loaders had greater Tt.Ar ($p < 0.001$), as well as periosteal ($p < 0.001$) circumference compared to the White low bone loaders.

Tibial diaphysis (65%)

The data from the post-hoc analyses for the tibial diaphysis are shown in Fig. 2. The Black low bone loaders had greater Tt.Ar (Fig. 2B) and SSI.Po (Fig. 2D) compared to the White low bone loaders ($p = 0.001$ and $p = 0.04$ respectively) but there was no difference in Ct.Ar or Ct.BMD between the two groups of low bone loaders (Figs. 2A and B respectively). There were no significant ethnic differences in the high bone loading group for Tt.Ar, Ct.Ar, Ct.BMD or SSI.Po. The White high bone loaders had significantly greater Tt.Ar ($p = 0.03$), and SSI.Po ($p = 0.02$) but not Ct.Ar or Ct.BMD compared to the White low bone loaders. There were no significant differences between the low and high bone loading groups of Black children for all bone outcomes.

Tibial Ps.C was smallest in the White low bone loaders compared to the Black low ($p = 0.001$) and White high ($p = 0.04$) bone loading groups (Fig. 2E). Ps.C was not quite significantly smaller in the White low bone loading group compared to the Black high group ($p = 0.06$). There was no difference in Ps.C between the Black and White children in the high bone loading group and there was no within-ethnic difference between low and high Black bone loaders. Tibial Es.C was lowest in the White low bone loaders compared to the Black low ($p < 0.001$), Black high ($p = 0.001$) and White high ($p < 0.001$) bone loaders (Fig. 2G). The White high bone loaders were not significantly different to the Black high bone loaders ($p = 0.76$) in Es.C. Again there was no difference in Es.C between low and high Black bone loaders. There were no statistically significant ethnic differences in Ct.Th in either the low or high bone loading groups (Fig. 2F).

Muscle CSA

Forearm muscle CSA was not significantly correlated to any of the forearm pQCT bone outcomes in White children. There was an association between forearm muscle CSA and Ct.Ar ($r = 0.44$, $p = 0.01$), SSI.Po ($r = 0.52$, $p = 0.003$) and Ps.C ($r = 0.42$, $p = 0.02$) in the Black children. Leg muscle CSA was significantly correlated to Tt.Ar ($r = 0.58$, $p = 0.01$), Ct.Ar ($r = 0.44$, $p = 0.001$), SSI.Po ($r = 0.65$, $p = 0.004$), as well as Ps.C ($r = 0.63$, $p = 0.005$) and Ct.Th ($r = 0.48$, $p = 0.04$) in the White children while leg muscle CSA was correlated to SSI.Po ($r = 0.41$, $p = 0.01$) in the Black children.

Discussion

In this study of pre- and early pubertal South African children, we have shown that in children who had a two-year history of low weight bearing physical activity, Black children have higher bone mass and strength than White children. However, in the presence of a two-year history of high bone loading, White children had equal femoral neck bone mass compared to their Black peers. We used pQCT measurements to show for the first time in South African children, that bone loading history may have a differential effect between ethnicities on femoral neck BMC, 4% radial and 65% tibial area as well as tibial structure and

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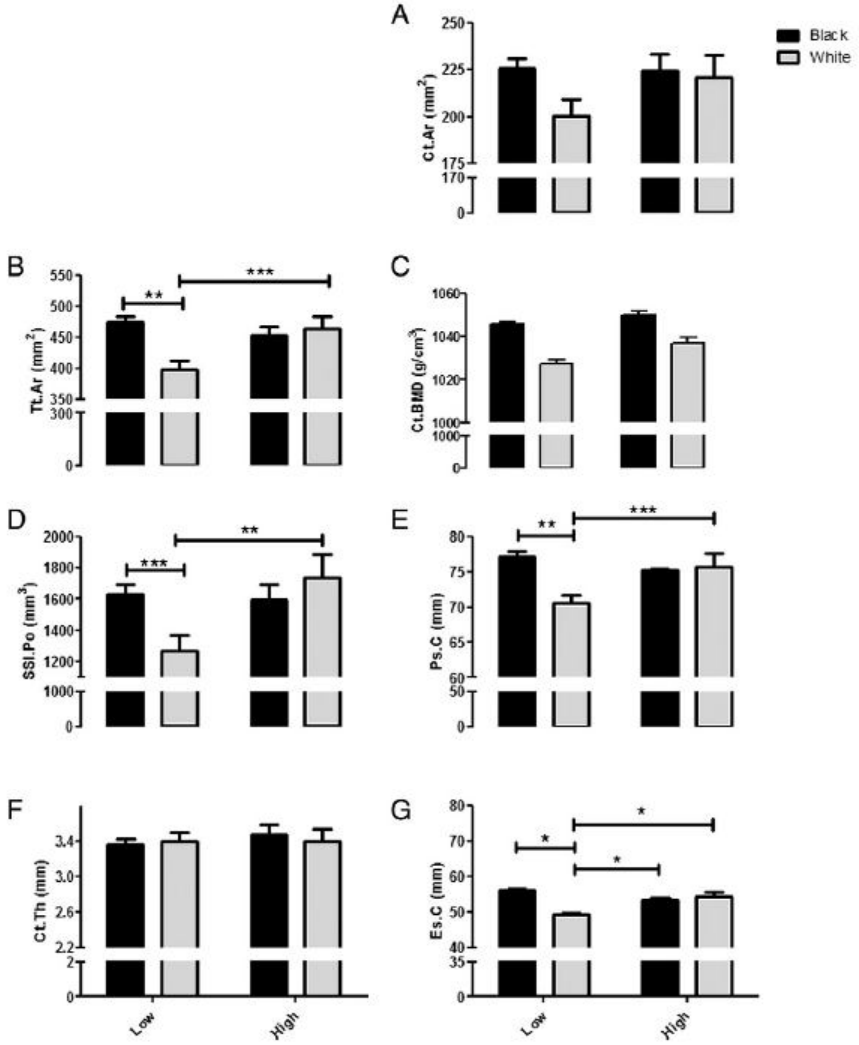

Fig. 2. Bone and muscle geometry of the diaphysis of the tibia between Black and White children within each bone loading group (panels A–G). CtAr = cortical area, TtAr = total area, Ct.BMD = cortical bone mineral density, SSI.Po = polar strength–strain index, Ps.C = periosteal circumference, Ct.Th = cortical thickness, Es.C = endosteal circumference. Bone variables were adjusted for sex, weight, sexual maturity, limb length and leg muscle cross-sectional area. Black low bone loaders: n = 28; White low bone loaders: n = 10; Black high bone loaders: n = 12; White high bone loaders: n = 12. *p < 0.05. Data are means (SE).

strength in Black and White children. Structurally, Black children and White high bone loaders exhibited larger periosteal circumferences compared to White low bone loaders, resulting in bigger cross-sectional areas of the bones of interest. The association of high bone loading was apparent at the diaphyseal (cortical) bone for the leg, where White children with the highest bone loading scores had a greater total area and periosteal circumference than their White peers with lower levels of bone loading. A greater tibial polar strength–strain index was also noted in the high bone loading group of White children compared to the low bone loaders, affording these children a more resistant appendicular skeleton. Interestingly, although PBSS score was significantly different between the Black high and low bone loading children, the bone loading effect seen in the groups of White children was not observed in the Black children. Out of the 13 children in the White high bone loading group 10 of them had a history of participation in tennis and netball and we suspect that this is the possible reason why there was a difference in muscle mass and bone area of the forearm between the White children. Studies have found that children who take part in upper extremity sports such as gymnastics [6,36], hockey [1] and tennis [37,38], have greater bone mass, strength and area at the proximal and distal radius. Netball is a sport in which a ball is caught and thrown towards a goal and participation in this sport may well have also contributed to the differences seen in the White children. The fact that the arm chosen for scanning was based on the hand that the child wrote with may have influenced the results.

Ethnic differences in measures of bone health are well documented in children. Vidulich and co-workers observed a greater BMC at the femoral neck, proximal femur and lumbar spine in Black compared to White 10 year old South Africans, as measured by DXA [39]. In the current study, ethnic differences in BMC as measured by DXA were observed at the femoral neck in the low bone loading group but not in the high bone loading group. Studies examining effects of ethnicity on measures

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of bone health using pQCT are scarce but those that have used pQCT, have been conducted in North American or European populations. In one North American cohort, ethnic differences in bone strength were shown to exist between Black and White children and the effect of muscle on bone strength was found to be an important predictor of these differences, regardless of PA levels [18]. In a study by Micklesfield et al. [30] conducted in Black and White South African 13 year olds, total bone area at the diaphysis of the tibia was significantly higher in Black than in White children resulting in greater strength in Black children even after adjusting for maturity and bone size. Ethnic differences in bone geometry and structure (as measured by pQCT) were also observed in the current study. Black low bone loaders had greater area and strength measures at the tibia compared to the White low bone loaders.

McKay and co-workers [40] have used a similar questionnaire based method to assess the amount of participation in impact or non-impact PA and they found a positive association between impact PA and bone architecture and geometry as measured by high-resolution pQCT (HR-pQCT). The authors did not include African Americans or Black children in their study. The present study builds on previous knowledge of physical activity and bone health studies by considering the associations between ethnicity, participation in weight-bearing physical activity and bone health in a population of pre- to early pubertal children. When considering the children's history of participation in weight-bearing physical activity, the ethnic differences observed in the low bone loading groups were either reversed or attenuated in the high bone loading group. To the best of our knowledge this attenuation of bone difference between ethnic groups has not yet been observed in any populations when physical activity is used as a differentiating factor. South African Black children and adults are generally considered to be afforded genetic protection in terms of bone health [41,42]. McVeigh et al. [20], using DXA, found that due to White children having higher levels of physical activity levels and therefore higher mechanical loading, increases in BMC in White South African children but not in Black South African children occurred. Although Black children did not report physical activity levels as high as those in White children, they still had greater bone mass compared to White children. The present study has further shown using pQCT, that the higher levels of weight-bearing physical activity that occurred in the high bone loading White children, also resulted in greater bone density, area and strength at the trabecular and cortical site of the radius and the cortical site of the tibia compared to White low bone loaders. In addition, greater mechanical loading in the White children encouraged periosteal apposition which may have contributed to the greater bone area and strength, such that there appeared to be no ethnic difference in bone geometry and structure between the White and Black high bone loaders. Therefore when physical activity levels are matched between ethnicities it may be possible for White high bone loaders to attain similar indices of bone health compared to Black children.

Significant associations between physical activity and measures of bone geometry, including cortical area and thickness, trabecular bone geometry as well as periosteal circumference have been observed in children and adolescents [3,7,40]. Periosteal apposition increases bone width in pre-pubertal boys and girls [43]. Weight-bearing physical activity leads to periosteal apposition and ultimately results in the formation of a much stronger bone [44]. Even if one has a lower than normal bone mineral density, bone strength is maintained by the bigger bone area [45]. Although studies are limited in whether ethnic differences in periosteal circumference exist due to the limited use of pQCT, the present study shows that there were no differences in periosteal circumference between White high bone loaders and the Black group of children while White low bone loaders had smaller periosteal circumferences compared to the White high and Black low bone loaders. High levels of weight-bearing physical activity in the White high bone loaders may have contributed to bone deposition on the periosteal surface such that bone area in the White high bone loaders was not different to that of the Black group of children. Bass and colleagues [37] showed that mechanical loading through exercise resulted in greater periosteal apposition in pre-pubertal children compared to during late puberty. There certainly is a critical period where periosteal apposition is most evident in children during longitudinal growth after which periosteal apposition slows down greatly [44]. Whether weight-bearing physical activity can influence the rate of periosteal apposition later in life, still warrants further investigation.

Interestingly weight-bearing physical activity appeared to have no association with the bone health of Black children implying that weight bearing physical activity may either be more effective in those with a lower bone mass to start with or once a certain threshold of bone mass is attained, physical activity levels offer little additional benefit over and above the genetic protection already afforded to Black children. Furthermore, the fact that within-ethnic responses to weight-bearing exercise in BMC, bone area, strength and structure were seen in White children only, suggests that there may be ethnic-specific responses to mechanical loading in pre–early pubertal children. Nonetheless, this is yet to be determined as no studies have investigated the effects of weight-bearing physical activity on bone health in only Black children and the cross-sectional nature of the present study does not allow us to draw causal relationships.

White South African children have greater leg muscle cross-sectional area compared to Black South African children [22,29,30], a finding contrary to what has been reported in North American cohorts [18]. In the present study muscle cross-sectional area of the forearm and lower leg was significantly higher in the White high bone loaders compared to the Black high bone loaders even after adjustment for sex, gender, maturation and limb length. Although in our study we could not perform a multivariate regression with muscle CSA due to the small sample size, we did find that muscle CSA was significantly associated with bone variables in the White children only. Admittedly, these findings may have been due to the fact that there was no difference in muscle CSA between Black high and low bone loaders and indeed Warden et al. [46] have suggested that muscle CSA did not contribute to the observed ethnic differences in bone variables seen in their cohort of Black and White children. Micklesfield et al. [30] found positive correlations between bone and muscle CSA in their Black children, even though their Black children had lower muscle CSA compared to White children. Neither of these studies took into account physical activity participation but in the latter study it has been suggested that ethnic-specific responses to mechanical loading and different types of physical activity may exist in Black and White children [30].

There are limitations to our study. The use of physical activity questionnaires (PAQs) is problematic due to their subjective nature and investigator variability. We acknowledge the limitations associated with the retrospective recall of two year participation in physical activity but do not regard this as a serious impediment to our measure of bone loading (i.e. PBSS) as all participants filled out the PAQ assisted by their parent/caregiver and due to the seasonal nature of school sports we do not believe that it was difficult for children to recall their activities. In the case of the present study, the PAQ used was one that has been validated for a South African population and one investigator was responsible for the capture and analysis of all the PAQs. In addition we only wished to obtain a surrogate measure of high or low levels of physical activity hence the absolute value of physical activity was not imperative to this study. McKay and others [40] have used a similar questionnaire based tool to assess amount of participation in impact or non-impact PA and found a positive association between impact PA and bone architecture and geometry as measured by HR-pQCT. We recruited participants into this study using a convenience sampling technique. Although we had strict inclusion and exclusion criteria, it is possible that we may have inadvertently selected a more physically active group of White children. For this reason we made sure that the PBSS was similar between high and low groups and thus selection bias is unlikely since our participants in this study had a similar range of physical

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activity levels. Ethnic differences in physical activity have been well described [20,47,48] and although we attempted to match weight-bearing history as closely as possible between ethnic groups, the possibility exists that the positive (White) and negative (Black) skewing of the scores in the high bone loading groups may have had an impact on our results. Larger sample sizes may be subjected to more robust multiple regressions, and since we had a limited number of participants, we chose not to perform this analysis. Our limited sample size and the cross-sectional design prevent causal conclusions from being established. As mentioned above, the absolute value of physical activity was not of the essence of this study and was merely a crude measure of estimating bone loading history, but further prospective studies are required to confirm our findings. The inherent limitations associated with DXA measurement in childhood must also be considered.

Conclusion

In conclusion, the present study has shown that in pre-/early pubertal children from a low-middle income country, White children appear to have a more sensitive bone response to weight-bearing exercise while Black children may be afforded genetic protection of bone regardless of weight-bearing physical activity levels. It appears that in order for White children to reach the same bone mass/health levels as Black children, they may need to participate in higher levels of weight-bearing physical activity. Moreover, ethnic differences in bone area and strength apparent between children classified as having a lower bone loading physical activity history appear to have been attenuated when children partaking in high bone loading physical activities were compared. Greater levels of mechanical loading seemed to have no apparent benefits in Black children.

Disclosures

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