BONE HEALTH AND PHYSICAL ACTIVITY THROUGH THE VARIOUS LIFE STAGES

by

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DECLARATION

I, **Lisa Kim Micklesfield**, do hereby declare that the experiments presented in this thesis were conceived and executed by myself except where otherwise indicated.

Neither the substance nor any part of this thesis has been submitted in the past, or is being, or is to be submitted for a degree in the University or any other University.

The thesis is presented in fulfillment of the requirements for the degree of PhD.

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LIST OF PUBLICATIONS

Peer-reviewed publications leading to the development of this dissertation

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ABSTRACT

The aims of this thesis were to investigate the interaction between physical activity and bone health through various life stages, in order to better understand the determinants of adult bone mass and consequently, osteoporosis. All the studies have been carried out on the various ethnic groups that make up the population of South Africa, with widely divergent economic and socio-cultural experiences, and therefore provide us with insight into determinants of bone health within the South African context. In this dissertation, we will explore the relationship and interactions between physical activity, and other lifestyle, physiological and demographic factors, on BMD and bone QUS parameters in the South African population. What sets this thesis apart is that we consider groups that are largely unstudied, groups undergoing demographic and epidemiological transitions, and even groups for whom physical activity may no longer provide protection for bone health.

To a large extent, the populations selected for study in this dissertation, and thereby the study designs, have been opportunistically linked to existing study populations, or been logistically limited to the use of calcaneal QUS for field work. However, our data strongly support the important role of physical activity, along with other factors, as a determinant and an ongoing modulator of bone health throughout life. Moreover, our data suggest certain interaction effects exist between traditional or commonly associated determinants of bone health, ethnicity and early life influences. Finally, there may be a sub-population of individuals for whom physical activity does not provide protection for bone.

In the first part of this thesis we investigated the relationship between lifetime physical activity and bone mineral density (BMD) in South African women using data collected in a case-control study of breast cancer in relation to BMD. Subjects (N=144) were of black African or mixed ancestral origin, and < 60 yrs of age (mean age: 42.6 ± 8.9 years). Cases had newly diagnosed breast

cancer (N=62) and controls were referred for conditions unrelated to BMD or breast cancer (N=82). Physical activity data consisting of household, occupational and leisure-time activity, and activity for transport, were collected via questionnaire at 4 life stages (epochs), viz.14-21, 22-34, 35-50, and 50+ years of age. Total energy (MET hrs) and peak bone strain scores (TPBSS) were calculated for each epoch. Lumbar spine (LS) and total proximal femur (PF) BMD were measured using dual-energy x-ray absorptiometry (DXA). BMD measures were similar between cases and controls and therefore, data were combined. The major determinants of PF BMD in these groups included age, transport activity including walking and bicycling between the ages of 14 and 21 years, and current weight (adjusted R^2 = 0.33, $p<0.0001$). Further, the major determinants of LS BMD included age, household energy expenditure between the ages of 14 and 21 years, and current weight (adjusted R^2 = 0.23, $p<0.0001$). TPBSS for activities between 14-21 years of age was also significantly correlated with LS BMD ($r=0.18$, $p<0.05$). Intraclass correlation coefficients to assess tracking of activity through epochs 1, 2 and 3 were high for total physical activity (0.96; 95%CI: 0.94-0.97), household (0.98; 95%CI: 0.97-0.99) and occupational activity (0.78; 95%CI: 0.71-0.84), and activity for transport (0.92; 95%CI: 0.89-0.94). These data suggest that walking or activities resulting in impact loading at a young age are associated with higher BMD in later years. In addition our findings suggest that in some groups physical activity patterns track over time. Having highlighted the importance of the possible cumulative effect of lifetime physical activity on BMD, and the potential modulating effect of lifetime physical activity on the interpretation of BMD and current physical activity, the second study attempted to determine whether there is a link between physical activity and bone early in life.

Therefore, in the second study we specifically targeted pre-adolescent girls in an attempt to characterize the potential effect of physical activity patterns on bone accretion in children prior to the influence of puberty. In particular, we wished to examine and compare the effect of physical activity and bone loading on bone development in contrast to other known effects such as body composition and diet. We obtained calcaneal QUS measurements in 198 girls between 7.5-11.7

years of age, representing ethnic groups [black (n=80), white (n=41), mixed ancestral origin (n=77)] in South Africa. Anthropometry was also assessed. Demographics, physical activity, habitual dietary calcium intake and pubertal development were quantified by questionnaires. Broadband ultrasound attenuation (BUA) and speed of sound (SOS) of the left calcaneus were measured. Girls in Tanner breast stage 5 and/or those menstruating were excluded from analysis. Black girls were lighter than white girls (31.4 ± 7.8 vs. 34.8 ± 7.5 kg; $p<0.05$), and shorter than girls of mixed ancestral origin (1.29 ± 0.08 vs. 1.34 ± 0.07m; $p<0.001$) and white girls (1.35 ± 0.07m; $p<0.001$), after adjusting for age. Reported calcium intake scores were higher in black than white girls (21.6 ± 11.1 vs. 16.1 ± 8.4; $p<0.01$). TPBSS was higher in white compared to black girls (6.8 ± 4.8 vs. 5.0 ± 4.7; $p<0.05$), while walking energy expenditure (MET hrs.wk^{-1}) was higher in black girls compared to the other groups ($p<0.001$). BUA and SOS were higher in the black girls (59.6 ± 13.7 dB/MHz; 1575.1 ± 22.6 m/s; $p<0.001$) and girls of mixed ancestral origin (59.0 ± 12.5 dB/MHz; 1567.8±26.1 m/s; $p<0.01$) than in the white girls (50.4 ± 8.7 dB/MHz; 1552.1 ± 19.5 m/s). Co-varying for age, weight and height did not affect these results. Walking energy expenditure ($r=0.20$) and calcium score ($r=0.17$) were correlated ($p<0.05$) with SOS for the whole group. In summary, QUS parameters were lower in the white compared to the black girls, who consumed more calcium on average, but who were lighter, shorter and performed less impact activity. These findings suggest that interactions between ethnicity and lifestyle factors determine bone quality even in premenarcheal girls. In addition, this study suggests that high impact physical activity at a young age is beneficial for the optimization of bone health.

There is increasing evidence that factors even earlier in life, in fact as early as in-utero, may also impact on bone health later in life. These effects need to be considered within the context of demographic and lifestyle influences, as well as maternal factors and genetic potential. Therefore, in the third study of this dissertation, we examined the effects of the intrauterine environment and early life influences on calcaneal QUS measures, by characterizing various maternal, intrauterine and lifestyle factors in children of a similar age to that of our previous study.

Maternal stress and an adverse intrauterine environment due to lifestyle and socio-demographic factors, may affect multiple developmental systems, including various neuro-hormonal axes, of the unborn child, either dependently or independently of birth weight. These changes, often referred to as fetal or early life "programming" have been implicated in the later development of certain adult diseases, such as hypertension, diabetes, and more recently, osteoporosis.

We collected demographic and maternal data from 122 7-9 year old children of mixed ancestral origin from a working class community, whose mothers had participated in a nutrition and pregnancy study at the time of their birth. Anthropometry was also assessed, as well as data concerning developmental milestones, childhood illness, physical activity (school and organized after-school sport), television viewing time, and certain dietary factors (not presented here). BUA and SOS were measured in the children and a sub-sample of the mothers (n=94) using QUS. Hand radiographs were used to measure metacarpal morphometry. We found no associations between the QUS parameters, birth weight, current weight or height. However, ponderal index was correlated with BUA ($r=0.25$; $p<0.05$). Furthermore, BUA was lower in children whose mothers smoked during pregnancy compared to children whose mothers did not smoke ($p<0.05$). Children whose mothers consumed alcohol during pregnancy had a lower Barnett-Nordin metacarpal index compared to children whose mothers did not consume alcohol ($p<0.05$), after co-varying for height and weight. Child's BUA was negatively correlated with housing density ($r=-0.22$; $p<0.05$) and positively correlated with maternal SOS ($r=0.22$; $p<0.05$). There were no associations between QUS measures and physical activity or inactivity levels in this sample. There were no gender differences in the QUS parameters, metacarpal measures, age, height or weight, however, the boys had a significantly higher ponderal index, and lower current sitting height, height-for-age Z-score and weight-for-age Z-score than the girls. In this study we found an interaction between maternal and early life influences on bone QUS parameters and metacarpal morphometry, in prepubertal children, which were also influenced by socio-demographic and environmental factors.

We have established an association between lifetime physical activity and BMD, and have demonstrated that physical activity in early life may interact with various demographic and lifestyle factors to affect bone as measured by QUS. Further, we have established that even maternal factors and the intrauterine environment may contribute to bone accretion early in life. However, there is surprisingly little data on the relative contributions of these various factors in adults who have already achieved peak bone mass. Therefore the fourth study attempted to examine the relationship between various lifestyle factors, including current physical activity, and adult bone properties as determined by QUS. This is of particular relevance as the prevalence of osteoporosis in the South African population, a population that is undergoing epidemiological transition, is unknown. However by introducing early screening programmes, the morbidity and mortality rates associated with osteoporosis may be reduced. In addition, the properties of bone, as measured by QUS, may be different to those assessed by DXA.

We aimed to develop a tool to assess risk of low bone mass, over a broad age range, with a view to targeted early intervention. The sample consisted of 167 Caucasian women, recruited as part of a free community health risk factor survey, between the ages of 19 and 79 years, with a mean BMI of 24 ± 4.7 $kg.m^{-2}$. A questionnaire was designed to collect data on variables such as history of osteoporosis, current leisure and occupational physical activity, calcium, alcohol and caffeine intakes, smoking, and various measures of reproductive history including past menstrual irregularities and pregnancies. Historical physical activity data was collected via questionnaire at 4 life stages (epochs), viz.14-21, 22-34, 35-50, and 50+ years of age. A lifetime energy expenditure score (MET) and a lifetime bone impact score (IMP) were calculated for each subject. Estimated BMD values were obtained from calcaneal BUA and SOS measurements using the Hologic Sahara Clinical Bone Sonometer. Odds ratios were generated in order to determine the odds of having a low T-score (<-1 SD) compared to a high T-score (>-1 SD), in relation to exposure to the aforementioned risk factors. Significant odds ratios were obtained for age (OR:

1.03; 95%CI: 1.005-1.059), current physical activity (≥ 3 times/wk) (OR:0.290; 95%CI: 0.120-0.702) and lifetime MET score (OR:0.967; 95% CI: 0.938-0.997). Age and weekly physical activity were included in a model to predict osteopenia, and a score of ≥ 50 correctly identified 60% of women with or without osteopenia in this sample. These findings corroborate previous research, and our own data, to show that physical inactivity is a key risk factor for compromised bone health. We have also demonstrated in Caucasian and non-Caucasian adults that lifetime, and current, physical activity will influence current bone properties, after the achievement of peak bone mass, as measured by DXA and QUS. In addition, our findings suggest that several forms of physical activity may be beneficial to the bone, including leisure-time, transport and household physical activity.

In our final study we introduce a "cautionary tale", or caveat. We suggest that despite the almost incontrovertible evidence that physical activity and bone loading are beneficial for bone health in most populations, even those undergoing transition, and that the effects present in a dose-dependant manner, there are some groups in whom physical activity may not be beneficial for bone health. In the fifth study we followed a group of previously competitive ultra-marathon runners, over a period of approximately 7 years, to investigate the change in BMD associated with changes in training and menstrual status. LS and PF BMD were measured by DXA on 3 different occasions, over a seven-year period, in a sample of 14 women, and on two occasions, over the same period, in two women. Anthropometry, reproductive and medical histories, as well as training history, were collected on all subjects during the various testing sessions. Some of these data were used to calculate menstrual history index (MHI), as an indicator of prior or current menstrual irregularity. Reported lifetime milk intake was determined for four life stages (epochs), viz. under the age of 14 years, 14-21, 22-34, and 35-50 years. The mean age of the sample was 42.6 ± 4.9 years, current body weight was 59.7 ± 7.2 kg and mean height of the sample was 166.8 ± 5.6 cm. Of the sixteen subjects, 9 women had gained weight, 6 women had lost weight and one woman's weight had remained the same over the seven-year period. There were no

significant differences in any of the BMD measurements between tests 1, 2 and 3. At test 3, five of the subjects presented with osteopenia (T-score <1) of the LS and two women presented with osteopenia of the PF. In the whole sample, using a repeated-measures ANOVA, the current weekly running time was significantly less than that reported during the previous 5 years (178.6 ± 118 vs. 276.9 ± 139 mins.wk^{-1}; $p<0.05$). A statistical model which best predicted current LS BMD was determined by multiple stepwise regression, and included age, age at menarche, current MHI and weekly running time over the previous 5 years (adjusted $r^2=0.611$; $p<0.01$). These data suggest that history of menstrual irregularity is an important determinant of current BMD, and that high levels of physical activity may further increase the risk of reduced BMD in these individuals, rather than protect against it.

In summary, this thesis has demonstrated that physical activity, and in particular lifetime physical activity, and other lifestyle factors, have a significant role to play in determining adult bone mass. Physical activity, especially loaded physical activity, during childhood and adolescence will influence future bone health, however this effect will be modulated by certain socio-cultural factors. Lifestyle habits that are adopted during adulthood will continue to explain some of the variance in adult bone mass, however these factors may also alter the metabolic programming of ones offspring which may in turn alter their potential to achieve an adequate bone mass.

ABBREVIATIONS

BMD/aBMD – bone mineral density/areal bone mineral density

BMC – bone mineral content

DXA – dual energy x-ray absorptiometry

DPA – dual-photon absorptiometry

SPA – single-photon absorptiometry

QCT – quantitative computed tomography

QUS – quantitative ultrasound

MHI – menstrual history index

MI – metacarpal indices

BUA – broadband ultrasound attenuation

SOS – speed of sound

FFM – fat free mass

BMI – body mass index

PF – proximal femur

LS – lumbar spine

FN – femoral neck

TB – total body

MHI – Menstrual History Index

BMAD – Bone mineral apparent density

D – Metacarpal diameter

d – medullary cavity diameter

CT – combined cortical thickness

BN – Barnett-Nordin Index

CHAPTER ONE

LITERATURE REVIEW

1.1. INTRODUCTION

Research in the field of bone health is ultimately carried out with the aim of preventing osteoporosis and reducing the future risk of fracture. Osteoporosis, often described as the "silent disease", is a public health threat for approximately 44 million Americans, 10 million of whom have the disease, and 34 million who are at an increased risk of becoming osteoporotic (Statistics from the National Institutes of Health Osteoporosis and Related Bone Diseases – National Resource Centre, revised 2/2003). Unfortunately no statistics are available on the prevalence of osteoporosis in South Africa, however as the population undergoes epidemiological transition, with the accompanying lifestyle changes, the prevalence may be expected to rise. The morbidity and mortality associated with osteoporosis indicate that approximately 24% of people over the age of 50 years, who sustain a hip fracture, will die within one year [177]. One in three women, and one in eight men over the age of 50 years are predicted to sustain an osteoporosis-related fracture in their lifetime [228].

There is a growing body of evidence that although the risk of osteoporosis increases with age, it is the childhood years that form the basis of an individual's risk of developing the disease. During childhood and growth the size and shape of the skeletal tissue is determined, during early adulthood the skeleton is maintained, and after peak bone mass is reached, the skeleton deteriorates and BMD is reduced. Bone is a dynamic tissue that is constantly being broken down and re-formed. Bone density is determined by bone mass acquisition during growth up until the time peak bone mass has been attained, and then the subsequent rate of bone loss thereafter. There is still no consensus as to the critical period during which peak bone mass is reached. Literature exploring this issue is reviewed in Section 1.4.1.

Bone mass is a major determinant of the incidence of fractures in later life [267]. To date, the most common way of assessing future fracture risk is through the measurement of BMD [154].

The World Health Organisation [1] criteria for classification/diagnosis of osteoporosis are as follows:

- with osteopenia (a BMD of > 1SD below the young adult mean, the T-score, but <2.5 SD below this value); and
- with osteoporosis (a BMD of 2.5 standard deviations or more below the young adult mean, the T score) with or without pre-existing fragility fractures.

Various risk factors are responsible for influencing the process of growth leading to peak bone mass, and subsequently, the rate of bone loss. Most of the risk factors have a role to play in both processes, however the contribution of each may be different during varying stages of bone development and may depend on whether or not peak bone mass has been reached. These factors include genetics, body composition, diet, and physical inactivity, amongst others. In addition, other factors such as ethnicity may interact with demographic and lifestyle risk factors in determining BMD.

1.2. PHYSIOLOGY OF BONE

1.2.1. General

Skeletal maturation is determined primarily by the processes of growth, modeling and remodeling [21]. These three processes alter bone structure by way of a mediator called the basic multicellular unit (BMU). This consists of a group of cells that undergo a sequence of events beginning with activation of the unit, which may occur in response to a number of factors including mechanical strain. Once activated, osteoclastic activity is increased. Osteoclasts are bone cells that are responsible for bone resorption. The resorption phase is followed by the bone formation phase, which is characterized by increased osteoblastic activity. This sequence of BMU activation, resorption and formation is known as the ARF sequence. This is followed by a period of mineralization, which is responsible for bone stiffness, and then a maintenance stage [21].

BMU's do not resorb and form equal amounts of bone. At different parts of the bone, bone balance may be positive (BMU formation greater than resorption resulting in net bone gain) or negative (BMU formation less than resorption resulting in net bone loss). At the cortico-endosteal surface, activation of BMUs results in bone loss, and at the periosteal surface, bone balance is positive resulting in bone gain at this surface. During skeletal growth, the function of which is to increase tissue volume, bone balance is positive and will result in increased bone mass. In the long bones such as the femur, growth in bone length occurs by endochondral bone formation at the growth plates, whereas increases in bone width occur by apposition of subperiosteal bone [21]. In the vertebrae, growth occurs by endochondral ossification, which commences in the central area of the cartilage and expands towards the periphery in all directions.

The process of modeling is responsible for the increases in size and changes in the shape of the bone that occur during childhood and adolescence, as well as the adaptation of the bone cells to the mechanical strain environment, a process known as mechanotransduction [104]. During the modeling phase, periosteal BMUs are activated resulting in a positive bone balance, and therefore there is an increase in the width and cross-sectional area of the bone, as well as the width and cross-sectional area of the cortical region. There are also increases in the thickness of trabeculae, but there is no increase in the number of trabeculae. Strains on the bone greater in magnitude or distribution than those needed for steady state remodeling will cause a modeling response that increases bone mass to meet the increasing load requirement [274].

Once the skeleton is established, remodeling of the bone occurs. During remodeling, BMUs on the cortico-endosteal surface are activated resulting in a negative bone balance and net bone loss [21]. In addition, after peak bone mass has been achieved, continuous bone remodeling will result in a net bone loss.

Factors that affect BMU function will affect growth, modeling and remodeling. These regulating factors include, amongst others, physical factors such as mechanical load (mentioned above), calcium-regulating hormones [192] [146] [78], and circulating growth factors such as insulin like growth factor-1 [128].

Calcium-regulating hormones are responsible for maintaining calcium homeostasis, and include parathyroid hormone (PTH) [192], 1,25-dihydroxyvitamin D (vitamin D) [146] and calcitonin [78]. The maintenance of calcium homeostasis is crucial for bone health as 98% of total body calcium is stored within the bone and is therefore used as a pool to maintain calcium stores in the body. Decreased levels of serum calcium result in an increased secretion of PTH from the parathyroid gland. These elevated levels of PTH are then responsible for increasing 1.25-dihydroxyvitamin

D synthesis resulting in increased intestinal calcium absorption. In addition, PTH secretion increases calcium reabsorption from the renal tubules of the kidney, as well as stimulates bone resorption. Vitamin D, also known as cholecalciferol, is a hormone that is produced endogenously by the liver and the kidney, after the activation of 7-dehydrocholesterol in the skin in response to ultraviolet exposure. Another source of Vitamin D is animal products. It's functions include increasing intestinal calcium absorption, calcium transport, stimulation of osteoclasts in order to mobilize calcium from the bone, and renal calcium reabsorption.

An observational study by Slemenda et al., [286] has proposed that the accrual of FN BMD, which mainly consists of cortical bone, is not as strongly influenced by maturational status as BMD accrual at the LS, which is largely made up of trabecular bone. Their study concluded that trabecular bone may be more sensitive to changing hormone concentrations than cortical bone. BMD values for cortical bone may be 8 times higher than trabecular BMD values [112]. Puberty, once begun, is complete within 3 years. Growth spurts and accelerated natural bone accumulation correspond to the onset of Tanner stage 2, reach a peak at stages 3 to 4, and end at stage 5. Menarche usually occurs during stage 4, and the longitudinal growth and natural bone accumulation rates markedly decrease soon after menarche, so that increases are only minimal in Tanner stage 5. Menarche is the first sign of cessation of bone development.

1.2.2. Endocrine axes associated with bone metabolism

1.2.2.1 Hypothalamic-pituitary-adrenal axis (HPAA)

Activation of the HPA axis is responsible for the secretion of cortisol by the adrenal cortex [128]. Increased circulating levels of endogenous cortisol concentrations influence bone mass by regulating bone formation through a modulating effect on IGF 1 and II, the IGF-binding proteins, as well as certain markers of bone turnover, namely, osteocalcin [148].

1.2.2.2 Growth hormone/Insulin like growth factor-1 axis (GH/IGF-1)

One of the important sites of action for growth hormone (GH) is on the epiphyseal growth plate [128]. It is responsible for stimulating the various processes of epiphyseal cartilage growth, which is then converted into new bone to elongate the shaft and to push the epiphyses further apart. This ensures growth of the long bones that occur during childhood, however once the epiphyses have united with the shafts, no further lengthening of the long bone can occur, and GH no longer has a role to play in lengthening the bones. Throughout life, however, GH is important for bone remodeling which it does by stimulating the osteoblasts, which are responsible for depositing new bone on older bone.

The release of GH is pulsatile and circadian, with the highest pulse amplitudes observed at night with the onset of sleep [128]. The pattern of GH release is also a function of age and sex, with the frequency and magnitude of the pulses increasing as children pass through puberty. Exercise, stress and sleep increase the production of GH [128]. Exercise may modulate levels of GH and influence bone mineral through that mechanism. After the age of 35 years, there is a decline in the secretion of GH, which until then has largely been responsible for bone accretion rather than the maintenance of bone with increasing age.

Growth hormone is responsible for stimulating chondrocytes (cartilage cells) in the growth plate to secrete IGF-1 from the liver [128]. IGF-1 stimulates proliferation of chondrocytes (cartilage cells), resulting in cartilage formation and bone growth. The IGF system includes IGF-1 and IGF-II and at least six IGF-binding protein (IGFBPs). IGF-1 is also responsible for stimulating the activity of osteoblasts.

Although IGF-1 levels decrease with increasing age, due to their influence on the osteoblasts they have an important role in maintaining bone mass in adulthood.

1.3 ASSESSMENT OF BMD

There are various methods of assessing bone status for research and clinical purposes.

1.3.1 Dual-energy x-ray absorptiometry

Bone densitometry, which includes dual-energy x-ray absorptiometry (DXA) and dual-photon absorptiometry (DPA) measures BMC, unadjusted for bone size, or areal BMD, which is adjusted for the area of the region, but not the depth. Therefore, the measurement of mass per area determines bone mineral density (BMD) and thus, will be influenced by the size of the area of interest.

DXA is considered to be the "gold standard" in assessing BMD, and diagnosing osteoporosis, which in most cases, is done using the WHO classification [1]. The most common areas that are assessed by DXA include the postero-anterior LS and the total PF, which consists of the FN, the trochanteric region and Ward's area. Some systems are also able to evaluate the intertrochanteric region and the radius, as well as soft tissue, which includes fat mass and lean mass.

Quality control is maintained by repeated measurements of the same area over a short (precision), or long (stability), period of time. This is usually expressed as a coefficient of variation and should be reported in all research studies. The coefficients of variation of some of the studies included in this Literature Review are presented in Table 1.1.

Table 1.1.: The coefficient of variation (%) of repeated measures on dual x-ray absorptiometry (DXA) of studies included in this Literature Review:

Reference	Sample	LS	PF or FN	TB	N/S
Snow-Harter et al., [287]	20	0.5%	0.9%		
Ramsdale et al., [260]	21-43	1.4%	1.9-4%		
Welten et al., [310]		1.3%			
Bassey & Ramsdale, [24]	43	6 (lat. sp.)	1.5%		
Cooper et al., [63]		1.0%	1.3%		
Fehling et al., [96]	20	1.8%	1.8%	1.7%	
Friedlander et al., [103]		1%	1-2%		
Houtkooper et al., [153]	104	1.3%	2.2%	0.7%	
Kirchner et al., [184]	10	0.63%	0.89%	0.58%	
Pruitt et al., [259]		1.5%	1.5%		
Prince et al., [257]		1%	1.5%		
Taaffe et al., [297]		0.5%	0.5%	1%	
Heinonen et al., [141;142]					0.5-0.8%
Kerr et al., [179]			1.5%		
Bennell et al., [31]	15	1.3%	0.7%	0.6%	
Bonjour et al., [37]					1-1.6%
Boot et al., [39]		1.04%		0.64%	
Cooper et al., [65]		1.1%	1.8%		
Dook et al., [83]	5		2%	0.96%	
Ettinger et al., [91]	20	1.4%	2.2%	0.9%	
Suleiman et al., [294]	1 (x5)	1.7%	0.65%		
Aloia et al., [9]					1-1.5%
Bassey et al., [25]	21-43	1.4%	2%		
Salamone et al., [277]		1.5%	1.3%		

Table 1.1 cont.

Reference	Sample	LS	PF or FN	TB	N/S
Teegarden et al., [299]		1%	1.4%		
Coupland et al., [67]	428	1.5%		0.9%	
Lehtonen-Veromaa et al., [197]	10 (x2)	1.3%	0.8%		
Gale et al., [106]		1.0%	3.0%		
Finkelstein et al., [100;101]	5 (x3)	1.4%	2.2%		
Antoniades et al., [12]	40				0.6-1.6%
Hawkins et al., [139]					0.8-1.5%

LS=lumbar spine; PF=proximal femur; FN=femoral neck; TB=total body; NS=site not specified

Some studies [221] have shown that there is a difference in the measurements of BMD between different manufacturers of DXA instruments. These authors stress the importance of developing manufacturer-specific data in order to adjust data for instrument specificity to enable comparison of the results of different studies.

There are several limitations of DXA. Although BMD, as measured by DXA, is used in most studies, it divides the amount of bone (BMC) by the size of the bone (bone area) and therefore does not take into account the volume of the bone. This can be particularly problematic if there is calcification or mineralization of the bone, which may result from aging or repair of an injury, as these will be interpreted incorrectly as an increased BMD. An increase in volumetric BMD may be achieved by increasing cortical thickness, trabecular number or thickness, or the true BMD of these structures. In addition, the values may increase during growth as a result of increases in size in the absence of a true change in BMD. Thus, DXA may overestimate true bone mass in larger individuals and underestimate bone mass in smaller individuals. Therefore when comparing the results of studies assessing changes in BMD with age (Sections 1.4.1 and 1.5.1) or

as a result of a lifestyle influence (Section 1.4.2 and 1.5.2), bone data should be adjusted for size in order to ensure that the same absolute measure is being compared.

Several investigators have provided some solutions to these limitations. Carter et al., [54] have developed a model to estimate volumetric BMD. They describe a parameter, bone mineral apparent density (BMAD), which is defined as the mineralized tissue mass per total tissue volume, and it is calculated by dividing BMC by a reference volume which depends on identifying some reference linear measurement from the patient's body. This approach provides several advantages over conventional DXA testing as it reduces the confounding effect of bone size and is height- and weight- independent. It is particularly useful for longitudinal studies, as well as cross-sectional studies, which involve subjects of varying sizes. Horlick et al., [152] have also suggested using total body bone area (TBBA) as a covariate, rather than as a component of calculated areal BMD when looking at the differences in total body BMC (TBBMC). Parsons et al., [244] adjust for size differences by correcting BMC for bone area (measured as bone width), as well as body weight and height.

Another limitation of DXA is that it does not distinguish between trabecular and cortical bone, which may have implications for bone health as trabecular bone is known to be more metabolically active and therefore may respond more readily to metabolic or hormonal intervention. In addition, DXA does not provide information concerning bone architecture or the material properties of the bone, which are an important part of evaluating the quality of the bone and predicting future fracture risk.

Kalla et al., [172] developed a local reference database of South African women, scanned using the Hologic QDR-1000. They performed DXA on 311 white women and women of mixed ancestral origin between the ages of 18 and 75 years, over a 2-year period. These results were compared to the reference database on the Hologic software, which uses American normative

data based on healthy American women aged between 20 and 80 years. Their results showed significantly higher LS BMD of South African women compared to the American reference over the age of 45 years. At the Ward's triangle, South African values were also significantly higher than American values within various age groups, including 25 to 30 years, 35 to 40 years, 60-70 years and 75 to 80 years. These results suggest a need for more population-specific normative data.

For research purposes, DXA is currently being used as the method of choice for assessment of bone parameters in children, particularly due to the low level of radiation exposure. However with specific reference to children it is crucial to take into account the influence of continuous changes in body size that occur with growth, and how these may alter bone measurements. McKay et al., [223] suggest a need for standardizing the analysis of DXA scans, particularly of the proximal femur, in children. This is of additional importance in prospective studies when serial scan analysis is necessary.

Reference data necessary for interpreting pediatric bone densitometry results have been developed by Bachrach et al., [15], from their longitudinal study on 423 subjects between the ages of 9 and 25 years. Normative curves that provide a standard deviation, or z-score, for BMD and volumetric BMAD for the LS, PF and the TB are available for various ethnic groups. However, this reference data can only be used when interpreting bone scans obtained on the Hologic 1000W in the pencil beam mode.

1.3.2 Quantitative computed tomography (QCT)

Quantitative computed tomography (QCT) provides various advantages over DXA as it determines the volumetric density of trabecular and cortical bone, which has important implications for prevention and treatment of osteoporosis. It measures a mass per volume and

therefore allows for accurate measurements of the size and the density of bone in the axial and appendicular skeletons [163].

This method is seldom used in children for research purposes due to high radiation exposure.

1.3.3 Quantitative ultrasound (QUS)

DXA and QCT are two techniques for assessing BMD, which is a determinant of bone strength and which in turn has been shown to be a strong predictor of osteoporotic fracture risk. However, there are other determinants of bone strength including bone structure, and QUS has been identified as a technique to provide important structural information on the bone [115;116]. Other advantages of QUS include no radiation exposure, reduced cost and greater accessibility.

The outcome measures of QUS include broadband ultrasound attenuation (BUA; dB/MHz) and speed of sound (SOS, m/s). BUA reflects the frequency dependence of ultrasound attenuation in the range 0.2-0.6 MHz. This has been based on the fact that bone attenuates high frequency sound waves more than low frequency sound waves. SOS is a measure of the speed of sound and is determined by measuring the heel width and dividing it by the time the sound waves take in moving from one transducer to the other. In the study by Gluer et al., [116], BUA was one of three ultrasound parameters that were found to be significantly associated with the bone structure of 20 cubes of trabecular bone. More specifically, Gluer et al., [115], have shown that BUA signals depend on trabecular orientation. In their in-vitro study on ten specimens of trabecular bone, the BUA signals changed significantly between specimens, as well as within specimens, when the direction of transmission was changed. The BUA measurement was 50% higher along the axis of compressive trabeculae, ie. parallel to the principal orientation of the trabeculae, and the site at which weight-bearing is at its highest. SOS has also been shown to be dependent on direction and is associated with bone density and elasticity [137]. In the study by Hans et al.,

[137], trabecular bone density was measured by QCT, magnetic resonance imagery was used to measure trabecular structure, the elasticity and strength were determined in a non-destructive compression test, and QUS was used to measure SOS. The highest univariate association was found between SOS and density, which also explained approximately 88-93% of the variance in SOS in the multivariate analysis. An additional 3-5% of the variance in SOS was explained by elasticity. The dependence of BUA and SOS on the direction of the trabeculae, suggests that they are more sensitive to trabecular structure, and possible structural changes. The mechanical properties of bone measured by QUS, are additional indicators of bone strength, and are useful when combined with a measurement of BMD, in assessing fracture risk (see below).

1.3.3.1. Quantitative ultrasound in adults

Although it is suggested that QUS and DXA are measuring different properties of bone, previous studies have demonstrated a good correlation between QUS measurements and BMD, as measured by DXA, in adults [9;119;292;296] (See Table 1.2.). There are no standardized norms for QUS and it is therefore limited with regard to predicting future fracture using T-scores. However, several studies have investigated the role of QUS in determining fracture risk in adults, through in-vitro methods by testing failure loads of cadaveric femurs [40;41] and through in-vivo methods by designing cross-sectional [27] and prospective studies [26;136]. Bauer et al., [27] measured BUA and SOS using QUS, and BMD using DXA, on 484 postmenopausal women between the age of 55 and 80 years. Average BUA was 10% lower in the women with vertebral fracture, confirmed by lateral thoracic and LS radiographs, than in women with normal radiographs. In order to determine the relationship between BUA and fracture, independent of BMD, multiple logistic models were examined. After adjusting for BMD of the whole body, spine or hip, as well as other potential confounders, the relative risk of a vertebral fracture per 1 SD decrease of BUA was 1.6 (95% CI: 1.1-2.1). Therefore a lower BUA was associated with an increased likelihood of vertebral fracture, independent of BMD. This relative risk was similar to

that obtained by Hans et al., [136] in a prospective study of 5662 women over the age of 75 years (RR: 1.7; 95% CI: 1.4-2.2) and Bauer et al., [26] in a prospective study in 6189 women over the age of 65 years (RR: 1.5; 95% CI: 1.0-2.1), after adjusting for FN BMD and the same potential confounders. In the study by Bauer et al., [27] the relative risk per 1 SD decrease of FN BMD, adjusted for BUA and other potential confounding factors, was 1.5 (95% CI: 1.2-1.9), while the study by Hans et al., [136] reported a relative risk of 1.8 (95% CI: 1.4-2.2) and Bauer et al., [26] reported a relative risk of 2.2 (95% CI: 1.5-3.3) after adjusting for the same factors. The results of the studies above indicate that QUS and DXA are measuring independent predictors of hip fracture, and a combination of both may be useful in predicting hip fracture risk.

Table 1.2.: Correlation coefficients (r) between QUS parameters (BUA, SOS) and BMD of the total body (TB), femoral neck (FN) and lumbar spine (LS) as measured by DXA, in adult studies

Reference	QUS Densitometer	Subjects	BUA vs TB	SOS vs TB	BUA vs FN	SOS vs FN	BUA vs LS	SOS vs LS
Brahm et al., [44]	Lunar ACHILLES	19-54 yrs Runners (n=30)	NS					
		Controls (n=30)	0.83					
Taaffe et al., [296]	Walker Sonix UBA 575+	19 yrs Gymnasts & controls (n=42)	0.55	0.74	0.58	0.74	0.54	0.67
Aloia et al., [9]	Lunar ACHILLES	20-70 yrs Black & white women (n=285)	0.71	0.75	0.65	0.68	0.61	0.63
Blanchet et al., [36]	Lunar ACHILLES	33-84 yrs Women (n=1162)			0.45	0.46	0.44	0.48

If not specified, subject sample consists of males and females; Correlations significant, unless specified

1.3.3.2. Quantitative ultrasound in children

Previous studies have demonstrated a good correlation between QUS measurements and BMD, as measured by DXA, in children (see Table 1.3.). Several studies have published reference data for the ultrasonographic parameters, BUA and SOS, for children of various ages, however reference data will depend on the QUS densitometer used. Jaworski et al., [162], using the Lunar ACHILLES ultrasonographer, list normative data for children between the ages of 6 and 13 years, while Mughal et al., [236] collected data on 367 children between the ages of 6 and 15 years using the CUBA (McCue) machine. Reference data using the SAHARA ultrasonographer are also available in children [315].

BMD, as measured by DXA, has been correlated with age, height and weight in children [17;275;276]. Similar relationships have been found between BUA, as measured by QUS, and age [162;235;236;295], height [236;295;315] and weight [235;236;295;315]. Although Sundberg et al., [295] showed a similar magnitude of association between these parameters, and BUA and BMD, there was a weaker association between SOS and age, height and weight. This is further evidence that QUS is measuring an important aspect of bone growth, and that BUA reflects some aspect of BMD.

To date no studies have investigated the role of QUS in determining fracture risk in children. Reasons for this may be that the interest in measuring bone in children is more with regard to determining the timing of peak bone mass attainment, as well as the factors which influence peak bone mass. Therefore validation studies of QUS in children are necessary.

Table 1.3.: Correlation coefficients (r) between QUS parameters (BUA, SOS) and BMD of the total body (TB), femoral neck (FN) and lumbar spine (LS) as measured by DXA, in paediatric studies

Reference	QUS densitometer	Subjects	BUA vs TB	SOS vs TB	BUA vs FN	SOS vs FN	BUA vs LS	SOS vs LS
Jaworski et al., [162]	Lunar ACHILLES	6-13 yrs (n=89)	0.80	0.67	-	-	0.83	0.67
Mughal et al., [235]	CUBA	7-17 yrs (n=58)	0.74					
Mughal et al., [236]	CUBA	6-15 yrs (n=367)						
Sundberg et al., [295]	Lunar ACHILLES	11-16 yrs Boys (n=148)	0.73	0.61	0.56	0.54	0.68	0.56
		11-16 yrs Girls (n=132)	0.58	0.64	0.51	0.55	0.57	0.55
Lehtonen-Veromaa et al., [197]	Hologic SAHARA	Pre-pubertal girls (n=52)	-	-	0.29	0.31	0.29	0.40
		Pubertal girls (n=132)	-	-	0.44	0.46	0.51	0.46

If not specified, subject sample consists of boys and girls.

Table 1.4.: The coefficient of variation (%) of repeated measures using calcaneal ultrasound (QUS), of the studies that have reported it, and which are included in this Literature Review:

Reference	Sample	QUS densitometer	Site	BUA	SOS
Bauer et al., [27]	In vivo	Walker-Sonix UBA 575	Calcaneus	4.0-5.8%	
Bouxsein et al., [40]	In vitro	Walker-Sonix UBA 575+	Calcaneus	6.1%	0.3%
Jaworski et al., [162]	In vivo	Achilles (Lunar, Madison, WI)	Calcaneus	1.39%	0.15%
Hans et al., [136]	In vivo	Achilles (Lunar, Madison, WI)	Calcaneus	1.8%	0.2%
Bauer et al., [26]	In vivo	Walker-Sonix UBA 575	Calcaneus	5%	
Brahm et al., [44]	In vitro	Achilles (Lunar, Madison, WI)	Phantom	2.6%	0.2%
Daly et al., [72]	In vivo	CUBA (McCue)	Calcaneus	2.8%	0.13%
Aloia et al., [9]		Achilles (Lunar, Madison, WI)	Calcaneus	2.0%	0.4%
Taaffe et al., [296]	In vivo	Walker-Sonix UBA 575^{+} (Hologic)	Calcaneus	2.9%	0.1%
Gregg et al., [120]	In vivo	Walker-Sonix UBA 575^{+} (Hologic)	Calcaneus	4.6%	0.08%
Hans et al., [137]	In vitro	DBM Sonic 1200	Trabecular cube	0.44%	

Table 1.4 cont.

Reference	Sample	QUS densitometer	Site	BUA	SOS
Lehtonen-Veromaa et al., [197]	In vitro	Sahara (Hologic)	Phantom	2.04%	0.28%
	In vivo		Calcaneus	1.46%	1.42%
			Phantom	1.0%	0.25%
Stewart et al., [292]	In vivo	McCue CUBA	Calcaneus	2.87%	2.99%
Wunsche et al., [315]	In vitro	Sahara (Hologic)	Phantom	5.4%	0.3%
			Radius		0.34%
			Phalanx		0.39%
	In vitro		Phantom	2.8%	0.6%
Blanchet et al., [36]	In vivo	Achilles (Lunar, Madison, WI)	Calcaneus	0.8-1.4%	0.2%
Kung et al., [193]	In vitro	Sahara (Hologic)	Phantom	4.6%	0.39%
	In vivo		Calcaneus	3.2%	0.3%

1.3.4 Metacarpal morphometry

Metacarpal morphometry is a technique which measures appendicular cortical bone mass, and was originally proposed as a means of diagnosing osteoporosis. [20]. Radiographs of the left hand and wrist are obtained, and the measurements are normally performed on the second metacarpal. These measurements include the metacarpal diameter (D) and medullary cavity (d), which are measured at exactly halfway up the metacarpal using a digital caliper. These measurements are then used to calculate the combined cortical thickness which is the medullary cavity subtracted from the metacarpal diameter (C=D-d) and the Barnett-Nordin index (BN=C/D x 100), which is a ratio of the combined cortical thickness to the total thickness, or diameter, of the

bone.

This technique has been used as a means of assessing bone characteristics in a community-based sample [305;306]. Wagener and Hough [305] have provided normative data for the Caucasian and mixed-ancestral origin populations, between the ages of 10 and 80 years, living in the Western Cape of South Africa. These results showed combined cortical thickness values of females of mixed ancestral origin to be significantly lower than Caucasian females between the ages of 20 and 50 years, and for men of all ages of mixed ancestral origin compared to Caucasian males. Peak values for metacarpal cortical thickness were achieved between the ages of 25 and 35 years, and values started to decrease from 40 years of age in all subjects. The rate of decline was also more pronounced in females compared to males.

Ma et al., [210] investigated the association between bone mass, as assessed by DXA and metacarpal morphometry, and upper limb fractures in boys and girls between the ages of 9 and 16 years. The cortical width and metacarpal index (described as the Barnett-Nordin index above) were significantly lower in the children who had previously suffered an upper limb fracture compared to those who had not. The odds ratios for DXA and metacarpal morphometry were similar for wrist and forearm fractures for the boys and girls, and a combination of LS BMAD and the metacarpal index were significant predictors of wrist and forearm fractures according to multivariate analysis.

1.4 BONE HEALTH DURING CHILDHOOD AND ADOLESCENCE

1.4.1 Evolution of bone through childhood and adolescence

The most critical period for bone health during childhood and adolescence appears to be the time around puberty which, once begun, is complete within 3 years. Tanner staging of the breast and pubic hair region in girls, and the genital and pubic hair region in boys is used to estimate the pubertal stage of children/adolescents [298]. Growth spurts and accelerated natural bone accumulation begin at the onset of Tanner stage 2, reach a peak at stages 3 to 4, and end at stage 5. Menarche in girls usually occurs during stage 4, and the longitudinal growth and natural bone accumulation rates markedly decrease soon after menarche, so that increases are only minimal in Tanner stage 5. Menarche is the first sign of cessation of bone development.

Studies that have monitored the evolution of bone throughout childhood and adolescence have described changes in terms of bone mineral content and/or bone mineral density. Bone mineral content (BMC) can be defined as the total grams of bone mineral tissue as hydroxyapatite within a certain bone area. Bone mineral density (BMD) is the grams of bone mineral tissue per unit of bone scanned and should therefore more correctly be referred to as areal bone mineral density (aBMD). Therefore, studies that define bone changes in terms of BMC changes are only taking into account the content of bone tissue itself, whereas BMD changes are changes in bone mineral content corrected for the size of the structure.

An early study by Gilsanz et al., [110] highlights the importance of bone changes that occur with puberty. In a cross-sectional study of 101 boys and girls between the ages of 2 and 18 years, they found a significant difference in trabecular vertebral density between prepubertal and pubertal children. A cross-sectional study by Sabatier et al., [276] measured the BMD and BMC of 574 girls between the ages of 10 and 24 years. To control for differences in maturity at the

same chronological age, all subjects were grouped according to skeletal age. Their results showed the most significant increases (53% in L3 BMD and 35% increase in LAT-BMD) in lumbar BMD between the skeletal ages of 10 and 14 years. A 2-year follow-up study by the same author [275] showed that the greatest annual change in height occurred approximately one year before peak bone accrual, both of which were prior to menarche. In the same study, an adult reference population of 206 women was followed up after a period of 4 years, and the results showed that during the 4 years around menarche, the young sample acquired 46% of the total bone mass of the adults, and three years after menarche almost 95% of the reference adult bone mass was acquired. No significant increases in height, BMD or BMC occurred after the seventh or eighth year following menarche.

Results from the University of Saskatchewan Pediatric Bone Mineral Accrual Study [17] show a similar order of events to the study by Sabatier et al., [275], with the highest rate of bone mineral accrual being reached within approximately one year after peak height velocity, which is the highest rate of linear growth and a common maturational landmark which can be used to compare children at the same developmental age. Their study investigated the bone mineral accretion of approximately 200 boys and girls between the age of 8 and 18 years, over a period of 6 years. Their results show that the age of peak bone mineral accrual is slightly earlier in the girls at 12.5 +/- 0.9 years of age compared to 14.1 +/- 1.0 years in the boys. Using adult reference data, at the time at which peak height velocity is attained, boys and girls had attained approximately 90% of adult height, 70% of adult FN BMC, and 60% of adult BMC of the LS and the TB. Quantifying bone mineral accrual in the 4 years surrounding peak height velocity (13.5 years in boys and 11.6 years in girls), commonly referred to as the adolescent growth spurt, the researchers showed an increase of 36% in TB and LS BMC. These findings are similar to those of Slemenda et al., [286] who showed an increase of 29% in LS BMD in a group of peripubertal girls over a 3 year period, compared to an 11% increase in the prepubertal girls over the same period. As similar changes were not seen at the radius during the pubertal period, Slemenda et al., [286] stresses the

importance of the skeletal site, and the proportions of trabecular and cortical bone, in response to the onset of puberty. In addition, a longitudinal study by Bass et al., [22] showed that the various regions of the skeleton grow at different rates during the various stages of puberty, and this may predispose to future fracture risk of particular sites. They showed that increases in length and size occur earlier than bone mineral accrual and that the skeleton is not a single functioning entity. They concluded that the effect of growth, aging and other influences vary according to whether the region is axial or appendicular, cortical or trabecular, and whether the surface is periosteal or endosteal.

Peak bone mass can be defined as the highest BMC that can be achieved. Sabatier et al., [276] suggests that peak bone mass is reached before the end of the second decade and that the term "plateau" should be used, rather than peak. There is still controversy as to when "peak" bone mass is achieved, however from the literature outlined above it is evident that the childhood and adolescent years are an important time for bone mass accretion.

The role of childhood growth in determining future bone health has been investigated by epidemiological studies. Cooper et al., [64] obtained birth and growth records for a cohort of 7086 Finnish men and women between the ages of 38 and 71 years. The total number of hip fractures during this time was a total of 112 and this was significantly influenced by the rate of childhood height and weight gain. Poor gain in height and weight when the women were between 7 and 11 years of age significantly increased fracture risk as an adult.

1.4.2 Factors influencing bone development in children and adolescents

Maternal and paternal history of osteoporosis is associated with a 1.5-2 fold greater risk of osteopenia in women [289;289]. In addition, the significant correlation in BMD between monozygotic twins (r=0.92) compared to dizygotic twins (r=0.36) [254] and the results of mother-

daughter studies [97;97;208;218;218;282;282], are evidence that genetics makes a significant contribution to peak bone mass. In the study by Ferrari et al., [97], the proportion of the variance in BMD that was attributable to genetic factors, in a sample of 8-year-old pre-pubertal girls and their mothers, was 33 and 36% at the LS and the PF, respectively. These results do suggest, however, that differences in bone accretion may also be attributed to other environmental factors, such as physical activity and nutrition.

1.4.2.1 Body composition

It still needs to be more clearly established as to whether body composition influences BMD in children as much as it does in adults, as well as which components of body composition have the most effect. In addition, it is unclear as to the exact timing of when the relationship between skeletal mass and body weight and height are established. A 3-year longitudinal study by Slemenda et al., [286] in ninety children aged between 6 and 14 years, showed that changes in height paralleled changes in radial BMD, whereas changes in weight were similar to changes in lumbar spine BMD.

In 580 children (6-20 years of age) from South Africa, Patel et al., [247] found that weight was most consistently and most significantly correlated with the bone mass parameters in boys and girls. However, in this study there was a negative correlation between the bone parameters, bone width and BMC, and body fat for the whole sample. Similar results in a later study by Patel et al., [246] of children of a similar age, also showed a negative correlation between bone width and BMC, and the sum of four skinfold sites. Conflicting results of a study by Slemenda et al., [286] found increases in skinfold measurements to be weakly associated with increases in BMC and BMD in a group of prepubertal children, however these associations were negative in the peripubertal children.

These results suggest that it may be more important to consider lean body mass, in terms of the influence of body composition and weight on bone mass parameters. A ten-month intervention study by Morris et al., [234], in a sample of premenarcheal girls, showed significant increases in lean body mass in the exercising group, which mirrored changes in BMD. Using multiple regression, change in lean body mass in the exercise group was found to be an independent predictor of changes in TB, LS, PF and FN BMD, and accounted for between 10-58% of the variance in bone accrual. Other studies in children [212;239] have clearly shown an association between lean body mass and BMD.

There may be an interaction effect between body composition, including lean mass, and lifestyle, environmental and demographic influences, on bone mass in children. A study by MacKelvie et al., [214] found significant increases in BMC of an exercise intervention group compared to the control group in boys of average BMI, while no significant differences were found in the boys with BMI > 75th percentile.

1.4.2.2 Calcium intake

The rapid increase in bone that occurs during puberty, as discussed above, is associated with an increase in both calcium absorption and the deposition of calcium in the bone. Just prior to menarche in girls calcium deposition in the bone is at its maximum, reaching approximately five times that of adulthood. There is a reduction in this deposition rate after menarche. Therefore, during childhood and adolescence, calcium intake may be an important component of bone health.

Dietary calcium studies in children vary in the magnitude of the reported bone response. The difference in these results may be due to differences in methodology such as the use of dietary recall in observational studies compared to the findings of randomized, double-blind, placebo-

controlled trials. Potential errors with self-reported calcium intake have been suggested by Carter et al., [55]. In addition, the length of the intervention, whether dairy products or calcium supplements were used and the nature of the calcium supplement, and whether the age of the children is reported chronologically or according to their maturity status, must all be considered when reviewing this literature.

A 12-month trial by Chan et al., [57] randomly assigned 48 girls between the ages of 9 and 13 years into a dairy group, in which their diet was supplemented with 1200mg of dairy products, or a control group where the girls were required to maintain their normal diet. At the beginning of the study all of the girls were classified as Tanner stage 2, however the paper does not give any details of the changes in maturity of any of the subjects during the one-year study. There was no significant difference between the groups diets at baseline and although calcium intake, phosphorus and vitamin D were increased significantly in the girls being supplemented with dairy products, there were no differences in total energy intake or fat intake at follow-up. There were no differences in any of the bone parameters at baseline, however at follow-up the girls being supplemented with dairy products had a 12% and 18% increase in their TB BMC and LS BMD respectively, compared to a 6.7% and 11% increase in the control group. The gain in weight and height was similar in both groups, and therefore was not associated with calcium intake. Although the authors have mentioned obtaining information on routine physical activity, this data was not included in the paper, however girls who participated in school team sports were excluded from the study.

The findings of a study by Johnston et al., [164] emphasise the importance of pubertal stage when investigating the influence of calcium intake on bone in children and adolescents. Their three-year, double-blind, placebo-controlled study in 45 pairs of identical twins between the ages of 6 and 14 years, showed significant changes (0.9-4.9%) in BMD with calcium supplementation in prepubertal subjects, however no benefit (-1.6-2.2%) was found in the pairs who underwent

puberty during the study or who were post-pubertal. In this study, one twin of each pair served as a control and the calcium supplementation consisted of an additional 1000mg of calcium citrate malate per day. In the whole group and the prepubertal twins, the greatest increases in the calcium-supplemented group compared to the placebo group were noted at the various radial sites (2.5-5.1% over the 3 years), with smaller differences (0.4-3.5% over the 3 years) at the LS and the PF sites. The increase in weight and height were similar in both groups over the study period.

To minimize the influence of maturity and to further investigate the influence of calcium supplementation at various skeletal sites, Bonjour et al., [37] conducted a randomized, double-blind, placebo-controlled trial in a cohort of pre-pubertal girls between the ages of 6.6 and 9.4 years. In this study, calcium intake was increased in the calcium-supplemented group by adding two calcium enriched products per day to the subjects diet (~ 850mg). The authors reported no change in pubertal status in any of the subjects during the one-year study. In contrast to the findings of Chan et al., [57], Bonjour et al., [37] did not show a significant difference in LS BMD measurements between the calcium supplemented and placebo groups at follow-up, however there were significantly greater increases in the various radial and femoral sites, excluding the FN, in the calcium supplemented group compared to the placebo group (3.5-5%/yr). Similar to the findings of Johnston et al., [164] who also showed greater increases at all the radial sites, this suggests that the appendicular skeleton may be more responsive to calcium supplementation than the axial skeleton.

In addition, in the study by Bonjour et al., [37], the girls who presented with spontaneously low calcium intakes and who were in the calcium supplemented group, showed the most significant increases in BMD, BMC, bone area and height over the study period, and one year after treatment discontinued. These findings suggest that there is a calcium intake threshold below which calcium supplementation, or an increased dietary calcium intake, may be particularly

beneficial for bone health in prepubertal children.

Studies within communities accustomed to a low calcium diet provide further evidence for the influence of an increased calcium intake on bone mineral accretion. An 18-month randomized, double-blind, controlled trial by Lee et al., [196] was conducted on 162 7-year-old Chinese children with habitually low calcium intakes (<300mg/day). The experimental group in this study received 300mg elemental calcium in the form of a calcium citrate tablet. This study showed significantly greater increases in the midshaft radial areal density (BMC/BW) in the calcium supplemented group compared to the placebo group after 6 months (3.07% vs 2.02%), 12 months (7.67% vs 5.27%) and 18 months (9.45% vs 6.31%). Unfortunately no other bone measurements were included in this study to determine site differences, and these results were not compared to a sample with a higher habitual calcium intake in order to investigate the possibility of a calcium threshold. There were no differences in weight or height between the 2 groups at the 18-month follow up. A similar study by Dibba et al., [82] investigated bone mineral accretion in rural Gambian children in response to 1000mg calcium supplementation in the form of two calcium carbonate tablets. They showed increases in midshaft radial BMD of the calcium-supplemented group when compared to the placebo group (8.0% vs 4.2%), similar in magnitude to the 12-month results of the study by Lee et al., [196].

Calcium supplementation in the form of dairy products has been investigated by Cadogan et al., [52]. In this randomized controlled intervention trial in 80 adolescent girls, the 'milk' group was provided with 568 ml (~660mg) of milk over a one-year period while the control subjects were asked to continue with their habitual diet. There were no differences between the groups in any anthropometric or bone measurements at baseline, however on completion of the 18-month study period, the girls in the milk group presented with a 9.6% increase in TB BMD compared to the control group who showed an 8.5% increase. This difference was significant (p=0.017). The absolute change in BMD in the milk group and the difference between the milk group and the

control group were noticeably less than the change and differences shown by Chan et al., [57] in their 12-month study. In their study, dairy products were also used to increase calcium intake, however it seems that tighter control of the intake of these dairy products was implemented in the Chan et al., [57] study and the average calcium intake at the end of the study was 1437 ± 366 mg compared to 728 ± 321 mg of the control group. In the study by Cadogan et al., [52] calcium intake of the milk group at the end of the study was 1125 ± 44 compared to 703 ± 205 mg in the control group. A cross-sectional study by Black et al., [35] compared the BMD of children between the ages of 3 and 10 years who avoided milk consumption, and who consequently consumed a lower calcium intake, to sex-specific and age-adjusted z-scores provided by a group of controls who drank milk. Femoral neck, trochanteric, LS, ultradistal radius and radial BMD were significantly lower than the z-scores in the milk avoiders.

It has been suggested that dairy products not only increase calcium intake, but also provide other beneficial nutrients such as protein, phosphorus, magnesium, and zinc, which may also optimize bone health. The association between other dietary nutrients such as potassium and sodium, and BMD, have been investigated by Jones et al., [169]. In their study, there was a positive significant correlation between urinary potassium and TB, PF and LS BMD. Urinary potassium was associated with potassium intake in the diet, and has been shown to be beneficial for bone health by reducing urinary calcium excretion. In addition, higher levels of urinary potassium may be an indication of a more nutritious diet.

The literature above suggests that calcium, and in particular dairy products, are beneficial for bone health of children. However, this positive association is only beneficial if bone changes are maintained. Bonjour et al., [38] have investigated whether the positive effects of an increased calcium intake during childhood are maintained after discontinuation of calcium supplementation. This study followed up a group of 149 pre-pubertal girls who had participated 3.5 years previously, in a 12-month randomized, double-blind, placebo-controlled trial. At follow-up there were no

differences in body weight, height, BMI or pubertal maturation between the girls who had been in the calcium-supplemented group or the placebo group. There was also no difference in the spontaneous calcium intake of their diets. However, 3.5 years after the completion of the 1-year intervention trial, BMD of the LS, radial diaphysis, FN, femoral trochanter and femoral diaphysis were still significantly greater in the calcium-supplemented group compared to the placebo group. There was a significant difference in the gain in lumbar vertebrae height between the 2 groups at the 3.5-year follow-up ($p<0.01$) suggesting a modification of the trajectory of bone growth. These data suggest that the benefits of an increased calcium intake during childhood are maintained for several years after calcium intake returns to normal. Conflicting results from Lee et al., [195] who followed up 84 prepubertal children after a period of 18 months, showed that the benefits gained by calcium supplementation during childhood are lost, when supplementation ceases.

Various studies have investigated whether calcium intake during childhood and adolescence has a positive influence on adult bone health. These studies are reviewed in Section 1.5.2.2. (ii), Lifetime calcium intake.

1.4.2.3 Physical activity

It is thought that physical activity has the greatest influence on the skeleton during the period of linear growth [17;174;286]. But there are still questions as to what type of physical activity is most beneficial and whether or not habitual physical activity is sufficient to result in a bone mineral response. Observational and longitudinal studies have been completed [17;286] to try to determine whether more active children have a higher BMD than less active children, and if so, at what age these differences are evident. The limitation of cross-sectional studies comparing active populations to inactive populations is the potential for selection bias. The higher BMD values seen in athletes may be due to them choosing to participate in higher levels of activity due to larger musculoskeletal mass, rather than as a result of the activity itself. The advantage of

studies looking at general physical activity in normally active, non-athletic children is that selection bias is minimized. The studies by Slemenda et al., [286] and Bailey et al., [17] have determined a general activity score in normally active children. The observational study by Slemenda et al., [286] found physical activity to be a significant predictor of BMD at all sites in prepubertal children, and they reported a 4-7% greater increase in BMD over the 3-year period in the active children compared to the inactive children. Similarly, Bailey et al., [17] found physical activity to have the main effect on TB and FN peak BMC accrual, as well as with BMC accrual in the 2 years surrounding this peak. Therefore, both studies have demonstrated that bone mineral is accrued at higher rates in children who participate in a wide range of everyday physical activities compared to inactive children.

Active loading of the skeleton, rather than just general physical activity, has been shown to have additional benefits on the bone in several cross-sectional studies comparing athletes participating in high impact activities to controls. A study by Grimston et al., [124] found a significant difference in FN BMD between competitive athletes participating in activities with high impact loads compared to other athletes in whom active load was applied to the bone via a non weight-bearing activity. As there was no significant difference in LS BMD between the groups, the local mechanical pull of the muscle on the bone may be implicated, however this study had low statistical power due to a sample size of only 17 children participating in the various sports. In a study by Dyson et al., [89], pre-adolescent gymnasts were compared to a group of controls who were not participating in any particular sport. This study determined an estimate of volumetric bone density called bone mineral apparent density (BMAD; see Section 1.3.1), and also used peripheral quantitative computerized tomography to differentiate total, cortical and trabecular bone densities and areas. BMAD of the TB, as well as the FN and LS, was 7-20% higher in gymnasts compared to the controls. Similarly, trochanteric BMD was 8-16% higher in the gymnasts, even after adjusting for height and weight. Differences in radial volumetric bone density between the gymnasts and the controls (16-27%), may again suggest a response to a local mechanical load as

gymnastics does involve large mechanical strains on the arms and wrists. The effect of loading on the trabecular and cortical bone compartments of the gymnasts was similar. Further, a study by Bass et al., [23] compared prepubertal gymnasts to bone age-matched controls. Their results showed an 8-20% higher areal BMD at all sites in the gymnasts compared to the controls. Follow-up after 12 months showed 30-85% greater increases in areal BMD in the gymnasts compared to the controls. From these studies it seems that certain kinds of activities are more beneficial for increasing bone mass than others due to the site-specific response of the bone to the activity.

There is also evidence that different types of bone respond in a specific manner to different types of activity loading. In an intervention study by Heinonen et al., [144] in which pre- and post-menarcheal girls underwent 9 months of moderate intensity exercise including jump training, there was a clear training effect at the LS and FN, sites that are largely trabecular, with no training effect in the cortical bone of the tibial midshaft, of the premenarcheal girls. There were no differences between the training and control groups of postmenarcheal girls. Petit et al., [251] has also demonstrated, using a hip structural analysis technique, that compression and impact activities may have a greater influence on areas which are primarily cancellous or trabecular bone, while bending or torsional activities will increase the diameter of the bone in the areas that are primarily cortical bone. These conclusions are based on the results of a 7-month intervention study, which showed an increase in BMD, cross sectional area, cortical thickness and sectional modulus of the neck and intertrochanter (areas that are primarily trabecular bone) of the exercising group who participated in a jumping programme, compared to the control group. However, the changes in BMD in the shaft of the femur, a site that is primarily cortical bone, were not different between intervention and control groups. Similarly, studies of racquet sport athletes in whom loading is more as a result of bending than compression, have shown greater differences in BMC in the proximal humerus, humeral shaft, radial shaft and distal humerus, all of which are predominantly cortical bone, between the dominant arm and the non-dominant arm in the athletes

compared to the controls [129;174].

Intervention studies in children have consisted of modifying existing exercise and physical education programmes [212;214;225;251;312], or designing separate and specific exercise programmes in addition to current activity [42;234]. All these interventions have incorporated jumping and higher impact activities, allowing for progression over time. In addition, a variety of high-impact aerobic workouts together with 10 weeks of strength training were included in the study by Morris et al., [234], while a progressive jumping programme consisting of 5 jumping activities, has been included within the studies of Petit et al., [251], Mackelvie et al., [212], Mackelvie et al., [214] and McKay et al., [225]. The duration of the exercise sessions range from as little as 10 minutes, 3 times a week [212;214;225;251] to 30 minutes, 3 times a week in the studies by Morris et al., [234] and Bradney et al., [42]. Thus, there is a lack of consistency in the "exposure" that is being compared in intervention studies. These differences in study design do make the results of intervention studies more difficult to interpret.

In one of the few studies that have examined the relationship between bone and physical activity during early childhood (4-6 years of age), Janz et al., [161] found physical activity to be associated with various bone measures. Their results showed an 11.9% greater mean BMC of the hip for children in the highest quartile of vigorous physical activity compared to the lowest quartile. However, the influence of exercise on the bone in children appears to be maturity specific, and bone growth and development during childhood may be more dependant on level of sexual maturity than chronological age. Most intervention studies in children have been done before or during puberty and have shown significant increases in BMD and BMC in the intervention group compared to controls [105;212;214;234;251]. However, a 9-month intervention study by Witzke et al., [312] did not show a significant change in BMC in the exercisers compared to controls and this may be due to the fact that their subjects were post-pubertal and the bone may not be as responsive to impact loading as it is prior to puberty. This was confirmed by

Kannus et al., [174] who reported a greater difference in BMC between the dominant and non-dominant arms of tennis and squash players who had started their playing careers before or at menarche, compared to those who started after menarche. They concluded that the exercise-induced benefit on bone gain is approximately two times greater if women start their playing careers at or before menarche (during puberty) than after menarche.

Similarly, Haapasalo et al., [131], when comparing humeral BMD, BMC as well as several measures of bone geometry, found greater side-to-side differences in a group of young tennis players (0.5-45.2%) who starting playing competitively at an early age, compared to a group of older tennis players (0.1-12.4%) who only became competitive in adulthood. Although all playing groups showed greater side-to-side differences than age, weight and height-matched controls, these differences were lower in the women who started their playing career later. In addition, results of an intervention study by Heinonen et al., [144] showed a significant training effect on BMC of the LS and FN in premenarcheal girls (Tanner stages I-III). No training effect was seen in the postmenarcheal girls (Tanner stage III-IV).

Contrary to these findings, Haapasalo et al., [129] showed that although there were significant side-to-side differences in BMD at the proximal humerus and the humeral shaft for the tennis players within all the Tanner stages, these were only significantly different to the side-to-side differences in areal BMD in the controls in Tanner stages III, IV and V. Lumbar spine BMD was only significantly greater in the players compared to the controls in Tanner stages IV and V. Also the correlation between training parameters and BMD was only significant in Tanner stages III, IV and V, suggesting that the effect of physical activity on BMD is only evident in the latter stages of puberty. Similarly, McKay et al., [225] reported no differences in LS or FN BMD between the intervention and control groups at the end of an 8-month moderate intensity intervention programme. 89% of the subjects in this study were still in Tanner stage 1. The only significant change in the intervention group compared to the control group was at the trochanter, which may

be more responsive to exercise due to the pull of the large muscle groups. Mackelvie et al., [212] found significantly greater changes in BMC and BMD of the LS and FN after a 7-month jumping programme, in the intervention group compared to the control group only in those girls who were in early puberty (Tanner stages 2 and 3). There were not significant differences between the intervention and control groups in the pre-pubertal girls (Tanner stage 1).

Possible reasons for the greater responsiveness of bone during early puberty compared to pre-puberty is that BMC accrual velocities are accelerating [17], bone is laid down on the inner (endosteal) and outer (periosteal) surfaces of the bone cortex [22] and the amplitude of bone-enhancing systemic hormones such as growth hormone, IGF-1, estrogen and andogrens, are increased. In girls, growth hormone peaks during Tanner stage 2/3 [88] which is approximately between the ages of 10.6 and 12.6 years, and then decreases immediately after menarche. In boys, growth hormone peaks during Tanner stage 4/5. IGF-1, which rises during puberty, increases trabecular bone formation [220]. In girls, serum oestrogen starts to increase gradually at approximately 10 years of age, and more dramatically in Tanner stages 3 to 4, while androstenedione increases at the age of 10 years. It is therefore between the ages of 10-12 years that all of these hormones are simultaneously increased which will provide the most opportune time for bone's response to other signals such as exercise.

When looking at the influence of exercise on bone in children, it is important to dissociate what is due to normal growth and what is due to the exercise itself. In addition, as there are different forms of exercise, perhaps the bending and compression forces that occur in racquet sports are different to the loading that occurs during weight bearing exercise. During normal growth there is no increase in volumetric BMD of long bones as the increase in mass and size is proportional. For volumetric BMD to increase during growth, the increment in mass must be greater than the increment in size. With normal growth, the increase in size is due to periosteal expansion together with an increase in endocortical (medullary) diameter so this will not result in a change in cortical

thickness. However with exercise, cortical thickness is increased as there is the normal periosteal expansion that happens with growth but there is increased endocortical/periosteal apposition of bone which may be due to the surface being exposed to greater mechanical stresses [130]. This will result in greater mineral being accrued within the periosteal envelope of a bone. The increase in cortical area with exercise may be due to greater periosteal apposition of bone and/or reduced endocortical (medullary) diameter, which may be a result of reduced endocortical resorption or increased endocortical apposition. The components of exercise such as intensity and frequency in terms of bone loading, may be important, as well as the maturational stage of the bone. It has been suggested that exercise may affect the periosteal surface in early adolescence, when the periosteal surface is growing rapidly, while exercise may have greater effects on the endocortical surface later in puberty, when endocortical apposition is increasing cortical thickness and reducing the medullary diameter. This is not supported by the results of the intervention study by Bradney et al., [42] in prepubertal males in whom there was no difference in periosteal width between the control and the intervention groups after 8 months of exercise. However, there was decreased endocortical diameter in the exercise group compared to the controls, with a result increased cortical thickness. Similarly, Bass et al., [23] found no difference in periosteal diameter between a group of prepubertal gymnasts and a group of bone age-matched controls. However, the endocortical diameter was lower in the gymnasts resulting in greater cortical thickness in the athletes. These findings suggest that the endocortical diameter is reduced in exercising prepubertal males and females, however the factors determining whether exercise results in changes on periosteal, endocortical, or both surfaces are not clear.

There is increasing evidence to suggest that it is also the mechanical pull of the muscle on the bone that may be important in bone accretion. Schonau et al., [262;279] have proposed a functional model of bone development, which suggests that bone is increased at the site at which mechanical requirements are increased, which are a result of the action of the muscles attached to the site. The correlation between muscle strength, as determined by grip strength, and an

index of bone strength [280], provides further evidence of this relationship and illustrates the importance of evaluating the muscle-bone relationship in bone development.

Several studies have illustrated the sensitivity of QUS to physical activity in children [72;197]. A recent study by Lehtonen-Veromaa et al., [197] on peripubertal girls showed the mean SOS values of the gymnasts and runners to be significantly higher than the controls. This study also reported a significant correlation between the calcaneal QUS parameters, SOS and BUA, and a measure of physical activity, the MET index in the pre-pubertal and pubertal girls ($r=0.19$-0.31; $p<0.05$). Daly et al., [72] compared calcaneal, radial and proximal phalanx ultrasound data in 33 male gymnasts and 40 controls of 9.4 ± 1.1 years of age. Although there were no significant differences in BUA between the groups, the SOS at all the sites was 1.2-3.2% higher in the gymnasts compared to the age-, height- and weight-matched controls. These data suggest that QUS is also able to discriminate between active and inactive children.

Although the evidence is overwhelming with regard to the influence of exercise on BMD during childhood and the adolescent growth spurt, these gains in BMD may only have a significant influence on bone health and fracture risk in later life if they are maintained. Kontulainen et al., [189] completed a 20-month follow-up of a 9-month intervention programme to determine whether the changes that occur with exercise are maintained on completion of the intervention. The results of their study showed a 5% greater LS accrual at the end of the follow-up period in the girls who had previously participated in the intervention programme at the age of 12.5 ± 1.5 years, compared to the controls. Although the trend was similar for the hip, this relationship was not significant. For a more thorough review of the literature see section 1.5.2.3 (Lifetime physical activity).

Calcium intake, physical activity and body composition are the most important childhood factors to ensure the attainment of an optimal peak bone mass. Several studies have compared the relative

importance of each during childhood in determining BMD. A 15-year longitudinal study by Welten et al., [310] assessed calcium intake and weight-bearing activity in 182 males and females between the ages of 13 and 27, and entered both, together with current body weight, in a multiple regression to determine the most successful factor in predicting BMD at the age of 27 years. In the males, weight-bearing activity was the most significant predictor of LS BMD between the ages of 13 and 27 years, however it could only account for 15.8-17% of the variance in BMD. Neither weight-bearing activity nor calcium intake significantly predicted LS BMD in the females. In the females, current body weight was the only significant predictor and accounted for 9-14% of BMD. A cross-sectional study by Boot et al., [39] assessed the association between height, weight, pubertal stage, calcium intake and physical activity in determining BMD in 500 males and females between the ages of 4 and 20 years. Calcium intake and physical activity were not significant predictors of TB or LS BMD in the girls, as Tanner stage and weight accounted for 80 and 85% of the variance in LS and TB BMD, respectively. Weight and height predicted 85% of the variance in LS BMD of the boys, however it was weight and calcium intake that predicted 88% of the variance in TB BMD. Age was not included in the multiple regression.

Although physical activity was associated with LS and TB BMD in the boys after adjusting for age in the study by Boot et al., [39], it was not divided into weight-bearing and non-weight bearing components which may explain it's lack of significance in the multiple regression model. In addition, subjects of various ethnic groups were included in the study and the possible differences in habitual physical activity may have affected these results. Ilich et al., [159] assessed body composition, calcium intake, physical activity and BMD in 456 pre-adolescent Caucasian girls in order to determine the factors which best predicted TB BMD. The model which predicted 47.8% of TB BMD in this sample included total bone area, body fat, lean body mass, stature, skeletal age and dietary calcium. Total physical activity was assessed by analyzing two 24-hour activity records, however it was not divided into weight-bearing and non weight-bearing activity. In addition, the age range of the sample included in this study may have been too narrow to pick up

the influence of physical activity on BMD. A one-year longitudinal study by Molgaard et al., [232] recorded calcium intake and physical activity using a food frequency questionnaire and a 24-hour activity recall, respectively. The subject sample included 201 girls and 142 boys between the ages of 5 and 19 years and this data was collected at baseline, 6 months and on completion of the study at 1 year. Unfortunately this study did not include calcium intake and physical activity in a multiple regression in order to determine their relative contribution to BMD, however the authors did report that TB BMC, adjusted for bone area, height and weight, was associated with average calcium intake in the girls. In the boys, the accretion of size-adjusted whole body BMC over the one-year was also significantly associated with change in calcium intake. No association was found with physical activity.

Similarly, calcium supplementation significantly increased size-adjusted BMC in older adolescent girls (17.3 ± 0.3 yrs) who were recruited for a 15.5-month intervention study. There was no relationship between the exercise intervention and BMC, however this may be due to only 27% of the subjects participating in >50% of the exercise classes. When the relationship was assessed only in the girls who attended >50% of the exercise sessions, there was a significant relationship with total hip and trochanter BMC. There was no interaction between the supplement effect and the exercise intervention at any site.

Due to the range of ages of the samples included in the various studies above, as well as the various tools used to measure physical activity and calcium intake, it is not possible to make an overall conclusion regarding the relative contributions of physical activity, calcium intake and body composition to bone accretion in children. However, there is sufficient evidence to suggest that they all have a role to play and should be optimized to ensure adequate bone health in children.

1.4.3 Ethnic influences on bone health in children and adolescents

Inter-ethnic differences in the incidence of osteoporosis and hip fracture in later life have been found [94;122]. Some data has suggested that anatomical, genetic and/or biochemical influences may explain these differences [69;240;302]. Other research has suggested that modifiable lifestyle factors around the age of puberty may play a role in determining adult bone mass and risk of fracture [171;174;180], however results of this research has not been considered with specific reference to ethnic differences. It may be a combination of these genetic and environmental factors that explain the apparent differences in BMD. Relevant literature with regard to Ethnic influences on bone health in adults will be discussed further in Section 1.5.3.

When reviewing the literature on the differences in BMD between the children and adolescents of various ethnic groups, it is important to consider maturation and pubertal development as maturation may occur at different chronological ages in different ethnic groups. Puberty results in significant changes in BMD in children of various ethnic groups [15]. A longitudinal study by Bachrach et al., [15] of males and females between the age of 9 and 25 years from various ethnic backgrounds, found an increase in BMD of 50% at the LS, 25% at the FN, and 30% at the PF in girls as they progressed through adolescence. A study by Gilsanz et al., [111] attempted to determine whether the changes in BMD that occur with sexual development varied between ethnic groups. They found no ethnic differences in vertebral bone density prior to puberty, as measured by QCT, however after puberty vertebral bone density was substantially higher in the black girls compared to the white girls. These results were corroborated in a later study by Gilsanz et al., [112] on 80 black and white children, matched for age, gender, height, weight and stage of sexual development. In this study the ethnic difference in vertebral bone density in the later stages of sexual development was found to exist in boys as well.

Interestingly the study by Gilsanz et al., [112] and others [15] did not find a similar developmental pattern in femoral bone density. Slemenda et al., [286] have shown that trabecular bone may be more sensitive to changing hormone concentrations than cortical bone. Therefore, this data

suggests that the hormonal and metabolic changes that are occurring with sexual development may differ between ethnic groups, resulting in different rates of trabecular bone growth. Plasma IGF-1 levels, which are increased during puberty and which are associated with bone formation, have been found to be higher in African-American girls of various ages compared to Caucasian girls [113;314]. However, research in prepubertal girls by Girgis et al., [113] also reported significantly higher IGF-1 levels in black girls compared to white girls. Other findings from this study, showed approximately twice the level of dehydroepiandrosterone sulfate (DHEA-S), a marker of adrenal androgen production, in the black girls compared to the white prepubertal girls. Body composition changes, such as increases in height and weight, around the time of puberty may also play a role in the different BMD changes in black and white subjects. A study by Nelson et al., [239] reported greater increases in TB BMC and BMD, and LS BMC, that mirrored the changes in height and weight, with increasing age around the time of puberty, in black compared to white girls.

Other studies have shown significant differences in PF BMD between ethnic groups [213], which may be due, in part, to the response of cortical bone to mechanical loading. Unfortunately, physical activity was not quantified in the study by Gilsanz et al., [112]. Mackelvie et al., [213] found increased differences in TB, FN and PF BMD between Asian and Caucasian children in Tanner stage II compared to Tanner stage I. To ensure that the differences between ethnic groups were not a result of differences in body size or composition, all comparisons controlled for lean mass, fat mass and segment lengths. The physical activity profiles between the two ethnic groups were significantly different, and the findings suggest that the ethnic difference in PF BMD may be due to differences in loading rather than endocrine differences between the ethnic groups. No differences were found in LS BMD in either group. This may be due to the fact that all of the subjects in this study were either in Tanner stage I or II, which can still be considered early puberty, and findings from Gilsanz et al., [111;112] only found ethnic differences in vertebral bone density from Tanner stages III-IV.

The difference in physical activity profiles between the ethnic groups in the study by Mackelvie et al., [213] suggest that there must be an interaction between cultural and other ethnic determinants of BMD. The various ethnic groups that make up the population of South Africa are unique in that they represent various dietary and lifestyle habits [271;293]. There are limited data published on ethnic differences in BMD in South African children [246;247]. Patel et al., [247] found no difference in radial bone mass, as measured by SPA, between age-matched black and white South African children between the ages of 6 and 20 years. After adjusting for height, bone mass parameters tended to be greater in black than white children, but these results were not significant. The Birth-to-Twenty study in South Africa has also failed to demonstrate inter-ethnic differences in BMD at the LS and radius for both 9-year-old children and their mothers, however, black children and mothers did have greater hip BMD, and were significantly shorter than the white groups (personal communicaton, Norris, S). Patel et al., [246] found height- and weight-adjusted radial bone mass to be greater in girls of mixed ancestral origin compared to white girls between the ages of 6 and 18 years. There is a need for more studies to compare the bone status of children of the various ethnic groups in South Africa.

Several studies have compared the BMDs of African-American and Caucasian children [29;200]. One of the first studies to investigate the ethnic differences in BMC in early childhood was by Li et al., [200]. They compared 78 black children between the ages of 1 and 6 years to 53 white children of the same age. After adjusting for weight, which was higher in the black children compared to the white children, BMC of the distal radius was significantly higher in the black children. A study by Bell et al. [29] found a difference in BMD of the midradius (as measured by single photon absorptiometry), spine and hip (both measured by dual photon absorptiometry), as well as BMC, lean body mass and body fat between African-Americans and Caucasian-Americans prior to puberty (7-12 years of age).

Similarly, ethnic differences in BMD between Asian and Caucasian children have been investigated [152;213;224]. McKay et al., [224] showed that prepubertal Canadian-Caucasian boys had significantly higher FN BMD when compared to Asian boys. In a study by Mackelvie et al., [213], in a slightly older group of girls, differences were found in TB, PF and FN BMD between Asian and Caucasian groups. A study by Horlick et al. [152], comparing 336 Asian, black, and white prepubertal children, confirmed that there is an ethnic difference in TB BMC, adjusted for TB bone area, age, height, and weight between the black and the nonblack children.

All of these data suggest that the changes that occur during puberty, that are responsible for influencing bone status, may differ in magnitude between various ethnic groups. There may also be an interaction between these factors and environmental factors such as physical activity, which may also influence bone health. In addition, anatomical, biochemical and genetic differences may explain the variation in bone status found between ethnic groups.

The results of Gilsanz et al., [112] corroborate the findings of others who have shown that there are significant racial differences in the lengths of the axial and appendicular skeletons. Their study found that black children had shorter sitting heights (upper body segment lengths) and greater leg length ratios (leg length: sitting height) than white children. In addition, Gilsanz et al., [112] suggests that there may be ethnic differences in bone growth between the axial and appendicular skeletons. In the axial skeleton, these differences are based on greater bone density, while in the appendicular skeleton, they are due to greater bone size. African American girls have been shown to be taller, heavier, and to mature earlier than Caucasian girls [239;314].

Other studies have suggested that ethnic differences in VDR genotype frequencies, and the association between these genotypes and bone mass, may also explain ethnic differences in bone mass [240]. The mechanism of these differences in the frequencies of the vitamin D receptor is through its influence on skeletal retention of calcium in children. There is a difference

in the distribution of VDR genotypes between ethnic groups [240], and the polymorphism of the vitamin D receptor gene may have an effect on bone mass during puberty as peak bone mass is accumulated.

Pratt et al., [256] found overnight urinary calcium excretion to be approximately 30% lower in black children compared to white children, however there was no difference in the excretion rates of the markers of bone resorption: hydroxyproline and the pyridinium cross-links. This may suggest that there are differences in bone formation in children of various ethnic groups. The results of a study by Bell et al., [30] showed no difference in calcium absorption between black and white boys and girls however urinary calcium loss was between 56-81 mg less in the black children compared to the white children. There were also racial differences in Vitamin D metabolites in this study as the black children had lower levels of serum 25-hydoxyvitamin and significantly higher levels of serum 1.25 dihydroxyvitamin D compared to the white children. Greater calcium absorption and lower urinary calcium excretion have been shown in African-American compared to Caucasian girls [5]. In another study by Abrams et al., [4] they looked at differences in calcium metabolism between prepubertal girls from different ethnic groups, viz. Mexican-American girls and Caucasian girls. Although there was increased serum total alkaline phosphatase activity and bone specific alkaline phosphatase activity in the Mexican-American girls compared to the Caucasian girls, there was no difference in fractional calcium absorption or urinary calcium excretion between the groups. Ethnic differences were also found in serum 25-hydroxyvitamin D and PTH concentrations, however groups were similar in terms of TB BMC and BMD. Therefore the bulk of evidence suggests that ethnic differences exist with regard to Vitamin D and calcium metabolism, even in childhood. However, the extent to which ethnic differences in lifestyle account for these is not clear.

The influence of positive lifestyle habits have been shown to be particularly relevant during growth when increased calcium intake [171] and increased physical activity [180] during childhood and

adolescence have been associated with increased BMD in adulthood. The idea of a "window of opportunity" when bone is most responsive to physical activity has been reviewed and investigated by MacKelvie et al., [211;212]. However, the impact of these modifiable lifestyle factors on the BMD of children from different ethnic backgrounds has only been investigated comparing Asian and white children [213;214;224]. McKay et al., [224] found calcium intake and daily weight bearing activity to be significantly lower in 58 Asian children compared to 110 Caucasian children, with the Asian boys also having significantly lower FN and PF BMD compared to the Caucasian boys. Studies by McKay et al., [224] and Mackelvie et al., [214] in which an exercise intervention programme was instituted for 8 months and 7 months respectively, found no difference between response to exercise in the Asian and Caucasian children. There is a need for more research comparing the physical activity profiles in children of various ethnic groups from different demographic backgrounds, in order to investigate the interactions of these environmental factors in determining bone quality and quantity during this development period.

1.5 BONE HEALTH DURING ADULTHOOD

1.5.1 Evolution of bone through adulthood

Although the literature is inconsistent with regard to the age at which peak bone mass is attained, it is generally accepted that childhood and adolescence are an important time for bone growth and accretion (Section 1.4.1.). After peak bone mass has been attained, bone maintenance during adulthood, and the slowing down of bone loss in later life, are necessary to reduce osteoporosis and fracture risk.

Several studies have investigated at what age peak bone mass is attained, for how long is it maintained, and at what age it starts to decline. A longitudinal study by Mazess and Barden [222] of 250 women between the ages of 20 and 39 years showed no significant changes with age over the 2-year period. An early study by Krolner and Pors Nielsen [191] showed no relationship between age and BMC in premenopausal women, however a significant negative linear relationship was shown in the postmenopausal women. They concluded from this cross-sectional study that maximum lumbar BMC is obtained by the age of 34 years in most individuals. Some longitudinal studies [244;263;275] still show bone gains during adulthood. In the study by Recker et al., [263] the median gain in TB bone content was 12.5% during the third decade of life. In the study by Parsons et al., [244], the sample was also young, 18-21 years of age, and increases of 0.6-2% of BMC for various sites in the body, were recorded for the study period of just less than 2 years. A two-year longitudinal study by Sabatier et al., [275] showed a mean annual gain of 0.7% for LS BMC and 0.4% for LS BMD in their sample of 206 slightly older women between the ages of 27 and 40 years. All of the studies did record lifestyle factors such as physical activity and calcium intake, which were shown in some studies to be related to bone gain [263].

Results by Lindquist et al., [203] showed bone loss with increasing age in middle-aged women of

the same menopausal status, as well as a difference in BMD between pre-menopausal women and early menopausal women of the same age. It seems that age, as well as menopausal status, are important in determining bone loss in older adults. An early study by Riggs et al., [267], on 187 men and women, assessed the patterns of bone loss in order to more clearly understand osteoporosis in older adults. In women between the ages of 20 and 89, these authors showed a loss of 0.0092 g/cm^2 per year, predicting that by the age of 90 years vertebral BMD is 47% less than at the age of 20 years (0.67%/yr). However, the pattern of bone loss was different in the radius, a site that is predominantly cortical bone. At the mid-radius and distal radius bone loss only started occurring at the age of 50 years, and then seemed to slow down after age 65 years. These results and others [48] show different patterns of bone loss in the axial and appendicular skeletons, and suggest that trabecular and cortical bone may respond to hormonal and mechanical stimuli in different ways.

1.5.2 Factors influencing bone development in adults

This review of the literature covers the lifestyle influences on BMD independently, rather than their combined effects, however it is important to consider that many of the factors can, and do, interact with each other and may therefore, modulate their effects. For example, it has been suggested that the positive influence of physical activity on the LS in younger [263] and older women [257;294], is maximized when combined with a high calcium intake. In addition, it is evident from the literature that various lifestyle factors such as physical activity and calcium may influence the bone in different ways [153;294]. Where relevant, this literature will be reviewed.

In addition, the presence, or absence, of some of these lifestyle influences may increase the risk of future fracture and osteoporosis. Several studies have also compared the usefulness of QUS measurements, in the context of various well-known risk factors, as a method of screening for risk of future fracture and osteoporosis [34;49;51;76;118;138;188]. Stewart et al., [292] compared the

predictive "power" of various factors such as menopausal status, body mass index, maternal fracture history, exercise, previous use of HRT and fracture history, to QUS, in identifying patients at risk for osteoporosis. Osteoporosis was then confirmed by DXA according to the WHO guidelines [1]. Their study showed a significant agreement (kappa statistic: 0.19-0.31) between the QUS measures, BUA and SOS, and LS and FN BMD. There was, however, very low agreement (kappa statistic: 0.06-0.09) between risk, as determined by the number of risk factors present, and the QUS parameters. Area under the curve (AUC) was calculated using Receiver operator characteristic (ROC) analysis of the sensitivity and specificity of QUS compared to the risk factor classification, in diagnosing osteoporosis of the hip and lumbar spine, as assessed by DXA. The QUS parameters, and in particular speed of sound, were better at predicting women with osteoporosis than the risk factor categorization of osteoporosis (AUC: 0.799 vs 0.515). A similar study by Kung et al., [193] showed a stronger AUC value (0.80) for their clinical assessment tool, the Osteoporosis Self-assessment Tool for Asians, however the AUC value for QUS was similar to the previous study (0.78). As both studies used the WHO criteria for the diagnosis of osteoporosis, other possible reasons for the difference in the performance of the assessment tools in the two studies may be due to the more homogenous sample included in the study by Kung et al., [193] as all of the subjects were Chinese women in comparison to the study by Stewart et al., [292] in which 250 women referred by their physician were included. These studies do illustrate however, that QUS is a useful means of identifying women at risk for osteoporosis in order that appropriate measures can be recommended with regard to treatment. In addition, clinical risk assessment tools need to be culturally sensitive in order to be a useful tool for assessing osteoporosis risk.

1.5.2.1 Body composition

The influence of body composition and it's various components, namely weight, height, lean tissue mass and fat tissue mass, on bone has been extensively researched. Several studies have

found a strong, positive correlation between current body weight and BMD in adults [63;65;260], as well as body mass index and BMD [77;260]. Teegarden et al., [301] assessed TB BMC and BMD in a sample of 247 women, between the ages of 11 and 32 years. Although age explained approximately 30% of the variance in both measures, this was increased to 52% and 70% respectively, with the inclusion of weight in the model. The addition of height to the model did not have a significant effect on TB BMD, however together with age and weight, the model was able to explain 74% of the variance in TB BMC. Similarly, weight loss may negatively influence BMD [277]. Salamone et al., [277], randomly assigned 236 healthy, premenopausal women to an experimental group, placed on a low-fat dietary plan and a physical activity programme, or a control group. The loss of PF BMD was twice as high in the experimental group, who showed a weight loss of 3.2 ± 4.7 kg compared to the control group who experienced a weight gain of 0.42 ± 3.6kg. Although exercise was included as part of the intervention study there was no additional benefit of exercise on the BMD changes.

The contribution of lean and fat body mass to body weight, and its influence on bone, has also been investigated. Parsons et al., [244] showed a significant positive relationship between BMC measured at various sites in a population of 40 women between the ages of 18 and 21 years, and estimates of lean fraction (lean body mass/body weight). However, in this population, fat fraction (fat mass /body weight) showed a negative effect on BMC. An early study by Heinrich et al., [145] on a group of collegiate athletes found fat-free weight to be significantly correlated with BMC at each of the 6 sites measured. Aloia et al., [10] assessed fat free mass and fat mass in sample of 164 nonobese women using various techniques in order to increase accuracy. Their results averaged the values of these techniques and found that fat free mass accounted for 56% of the variance in bone mass in the premenopausal women, and 50% of the variance in the postmenopausal women. Results for the contribution of fat mass to bone mass were not significant in the premenopausal women. Similarly, other data from studies of older women support the relationship between lean body mass and BMD [59;83;139].

The influence of body composition on bone may differ between various groups, such as premenopausal and postmenopausal women [10], as well as exercising and non-exercising women [265]. In the study by Aloia et al., [10], fat mass explained a greater % of variability in bone mass in the postmenopausal women (12-21%) compared to the premenopausal women (1-9%). The possible reason for this is the conversion of androgens to estrogens in the adipose tissue provides valuable estrogens necessary for adequate bone metabolism to continue in postmenopausal women in whom estrogen deficiency increases the rate of bone loss. However, in an 18-month study by Houtkooper et al., [153] in a group of women between the ages of 28 and 39 years, fat mass and change in fat mass were also positively associated with TB BMD, but not with any of the regional sites measured.

Weight, height and the components of body composition, including fat mass and fat-free mass, contribute significantly to the variance in BMC and BMD. This may be due to the response of the bone to increased mechanical (gravitational) forces, however Parsons et al., [244] cautions this interpretation. They suggest that body size is not adequately adjusted for when studies report on the relationship between BMD and weight. So although weight is related to BMC it may be due more to a bigger person having bigger bones, rather than a causal effect.

1.5.2.2 Calcium intake – current and lifetime

(i) Current calcium intake

Various methods have been used to assess calcium intake in adults, and this may account for the equivocal results when investigating the influence of calcium intake on bone. These include food frequency questionnaires [83;95;133;157;260;294;299], 24 hour dietary recall [171;222], food records of different lengths [153;257;278] and some researchers have combined various methods [173]. There is considerable debate as to what is the most valid and reliable method of assessing

calcium intake, however to evaluate all of this literature is not within the scope of this Literature Review.

Some studies have found no direct relationship between current calcium intake and BMD [83;103;222;278]. Current calcium intake, in the study by Sandler et al., [278], was estimated using a three-day food record and the average intake of calcium for the 255 postmenopausal women included in the study was 720 mg/day. Although their study showed that women who reported drinking more milk during childhood and adolescence had higher bone densities than women who reported drinking milk less frequently, there was no correlation between current calcium intake and bone density. A longitudinal study by Mazess and Barden [222] was one of the first to look at calcium intake in premenopausal women, as most studies had been done on postmenopausal women. The 200-300 subjects included in this study were between the ages of 20 and 39 years and calcium intake was assessed using a 24-hour food record taken three times over a period of 2 years. BMC and BMD of the LS and FN were assessed by DPA and various radial measurements were taken using SPA. There was no correlation between calcium intake and any of the bone measurements and calcium intake was not associated with change in BMD over the two-year period.

An intervention study by Friedlander et al., [103] also did not show any effect of calcium intake on BMD in a sample of 127 women of a similar age, who were randomized into exercise and calcium-supplemented groups. BMD, which was assessed using QCT and DXA, was not significantly different between the women taking calcium supplements and those on placebo, and none of the groups showed a significant BMD change over the duration of the study.

Despite the fact that these studies relied on three-day food records, which are considered to be one of the most accurate ways to assess and measure the dose-response effect of dietary calcium intake on the bone, they failed to find an association between calcium intake and BMD in

subjects of various ages. A possible explanation could be the limited records that were obtained on other lifestyle factors such as smoking and alcohol intake, as well as hormone replacement therapy in the study by Sandler et al., [278]. These factors have been shown to influence BMD (see Section 1.5.2.5.) and may have modulated the effects of dietary calcium intake.

Other research has found a positive relationship between current calcium intake and BMD [95;171;260;294;299]. There does not seem to be a pattern that distinguishes these studies from those that have shown a negative association between calcium intake and bone as subjects in these studies have included relatively young women (20-23 years, [95]; 18-31 years, [299]), women of a wide range of ages (21-47 years, [260]; 20-49 years, [171]) and older women (52-62 years, [294]). The methods used to assess calcium intake in these studies have also not been consistent and range from food frequency questionnaires in the studies by Fehily et al., [95], Ramsdale et al., [260], Suleiman et al., [294] and Teegarden et al., [299], and a 24 hour dietary recall by Kalkwarf et al., [171].

However, the results of intervention studies seem to be more conclusive than observational or cross-sectional studies [140]. Prince et al., [257] showed that calcium supplementation, in the form of tablets or milk powder, was effective in attenuating bone loss between 0.07-0.17% per year, in post-menopausal women. It is generally accepted that calcium supplementation or an increase in dietary calcium intake in postmenopausal women may be more effective the greater the number of years that have lapsed since menopause due to the over-riding hormonal effect on the bone in the first three years after menopause [257;309].

However, the results of Kanders [173] and others [133] suggest that there is a threshold above which additional calcium no longer results in increased BMD. In the study by Kanders et al., [173] when the group of premenopausal women (n=60) was divided into high (> 800mg calcium per day) and low calcium (< 800mg calcium/day) there was a significant difference in BMD between

the groups. The group with the high calcium intake had a significantly higher BMD. The relationship between calcium intake and BMD was significant up to 1000 mg/day, however for calcium intakes above this value, there was no additional increment in BMD. Unfortunately, these results were not adjusted for differences in weight or body size, and subjects were selected on the basis of weight-for-height so none of the women were overweight for their height.

Some studies have found a relationship between total energy intake and BMD [153]. In one such study, Houtkooper et al., [153] assessed BMD and various lifestyle factors in 66 Caucasian women between the ages of 28 and 39 years. These women were part of an exercise intervention study and their diets were analysed for a total of 12 days using four-day food records at various intervals throughout the trial. Although calcium intake was not associated with rate of change of BMD in this 18-month study, total energy intake was a significant predictor of LS BMD and explained 17% of it's variance, together with exercise group status. Nutrients such as vitamin A, carotene, dietary fibre, magnesium, phosphorus and sodium all contributed significantly to the variance in the TB BMD slope when included in separate models, together with initial TB BMD and fat mass. Therefore, energy intake may be an indication of higher intakes of other nutrients that may indirectly influence bone mass.

When discussing the influences of various lifestyle factors on bone health in adults, it is important to consider body composition, either as a direct cause-effect relationship on bone metabolism and BMD, or via an indirect influence on bone as a consequence of lifestyle factors associated with a specific body composition. To highlight this point, in a study by Parsons et al., [244], a direct relationship was found between current calcium intake and whole body BMC, as well as between various activity patterns and BMC. However when BMC was adjusted for bone size (bone width) and body size (height and weight), these relationships were no longer significant. Thus, these investigators concluded that physical activity and diet may directly influence BMC, or that larger people with higher BMDs are consuming more food and participating in more physical activity.

However, in their study when comparing people of similar size, but with a diet of differing calcium content, there was no difference in BMC. Therefore, they could not provide evidence for a cause-effect relationship between calcium and BMC.

(ii) Lifetime calcium intake

Several researchers have attempted to quantify lifetime calcium intake in order to relate calcium nutrition during childhood to adult bone mass [95;133;171;278;299]. There are conflicting data, in part, due to the differences in study design. Fehily et al., [95] found no long-term effects of milk supplementation during a 2-year period in childhood, on adult BMC. Conversely, in studies in which individuals self-selected calcium content of the diet [133], and were classified as having a low, intermediate or high calcium intake, a low calcium intake was significantly associated with low radial BMD and BMC. Studies by Sandler et al., [278], Teegarden et al., [299] and Kalkwarf et al., [171] only assessed milk consumption as a proxy for calcium intake, assuming that the primary source of dietary calcium was milk. Results of the study by Sandler et al., [278] and Teegarden et al., [299] suggest that there is tracking of milk consumption throughout life. In the study by Sandler et al., [278] women who reported drinking milk with every meal during childhood, adolescence and adulthood had a significantly higher current calcium intake as assessed by a three-day diet record, compared to the women who reported consuming milk less frequently. Similarly, in the study by Teegarden et al., [299], childhood and adolescent milk intakes were significantly correlated with current calcium intake, as assessed by a food frequency interview. Sandler et al., [278] also showed BMD of the radius was significantly higher in the women who reported consuming milk with every meal during childhood and adolescence than the women who consumed milk less frequently. This difference was not found for milk consumption reported during adulthood. In a sample of more than 3250 women aged 20-80 years [171], hip BMD or BMC of women who reported consuming <1 milk serving per week during childhood or adolescence was 2-3% lower than women who reported consuming >1 milk serving a day during the same age period. These results were adjusted for potential confounders including current

calcium intake, age, weight, height and physical activity status. In addition, low milk intake during childhood (<1 milk serving/week) was associated with a two-fold increased risk of osteoporotic fracture, defined as a fracture after the age of 50 years, in the women over the age of 50 years. Teegarden et al., [299] showed that reported milk intake during adolescence (13-19 years of age), and not childhood, predicted TB and radial BMC and BMD in young women (< 31 years of age), but not that of the LS or PF BMC or BMD.

Many factors have been shown to alter the effectiveness of calcium intake or supplementation on bone. These factors include magnesium and phosphorus as well as dietary fibre, and high and low protein diets. Reviewing the literature on each of these factors, their interaction with calcium, and its influence on BMD, is not within the scope of this thesis, however this literature has been extensively reviewed by Ilich et al., [158].

1.5.2.3. Physical activity – current and lifetime

There is a growing body of evidence implicating the importance of physical activity on BMD during the adult years. Physical activity exposure may be quantified according to intensity (METs), impact loading [125], frequency and duration of the exposure, as well as the age at which subjects were exposed to the physical activity ("epochs"). Some of the research has been focused on quantifying the influence of current physical activity, in various forms such as weight-bearing activity and strength training. Other research has attempted to determine the effects of lifetime physical activity on adult bone mass. The following sections will only discuss the role of physical activity in the maintenance of bone mass and the prevention of bone loss in adulthood, and will not cover the very extensive literature that is available on exercise in the treatment of osteoporosis and the prevention of falls. This literature has been reviewed by Khan et al., [182], Ernst et al., [90], Marcus et al., [217], and Forwood and Burr, [102].

(i) Current physical activity

Physical activity levels decline with age and may be a cause of bone loss that is seen with aging. However the question remains as to whether physical activity serves only to maintain bone mass, or whether it is still possible to increase bone mass in adulthood? Many researchers who have investigated the influence of physical activity on the bone have compared athletic populations to each other, and to non-athletic controls [31;96;145;268;297]. In these circumstances it is important to consider that the athletic population may be a biased sample as they may have a genotype that favours success in a particular sport, and thereby may also favour a greater BMD. However, they do provide an accessible sample on which to study the influence of wide-ranging exposure to physical activity on the bone, and the results of these studies are meaningful.

The sensitivity and specificity of calcaneal QUS to changes in bone which may be associated with physical activity, and in particular impact loading, have been illustrated by several studies [36;44;120;296]. In a study by Taaffe et al., [296] BMD and QUS parameters were compared in young collegiate gymnasts and healthy controls. The difference between the athletes and controls for the ultrasound parameters (21%) was similar to the difference reported in BMD of the various sites (7.3-28%) as measured by DXA. Although Brahm et al., [44] also showed significant differences in BUA and SOS, and BMD values, between runners and age- and sex-matched controls (QUS: 3.1-9.2%; BMD: 3.6-11.8%), these differences were not as large as those shown by Taaffe et al., [296], but were still similar between the methods. The smaller difference between the exercise and control groups in the study by Brahm et al., [44] may be due to their age as they were significantly older at 32 years (range 19-54 years) than the subjects in the study by Taaffe et al., [296] who had an average age of 19 years. The relationship between QUS and physical activity also exists for leisure-time activity in older adults. Blanchet et al., [36] collected QUS, bone densitometry and physical activity data on 1162 postmenopausal women with an average age of 58 years. There was a significant difference in QUS parameters between the sedentary,

moderately-active and active women, and activity was an independent predictor of SOS and BUA in these women. More extensive physical activity data was collected by Gregg et al., [120] in their study investigating the association between QUS and various lifestyle and genetic variables in 393 women enrolled in the Women's Health Lifestyle Project. Physical activity data included past year physical activity as well as historical physical activity during various epochs from the age of 14 years. Although historical physical activity between the ages of 14 and 35 years was not associated with the QUS parameters, BUA and SOS, recent physical activity and physical activity from age 35 years to present were associated with SOS and BUA. In addition, the variables which were significant predictors of SOS, including past-year physical activity, were also significant predictors of FN BMD, measured by DXA. High impact exercise in particular has been shown to increase BMD as measured by DXA [96;184;297], and BUA, as measured by QUS [160]. Jakes et al., [160] have reported a strong association between time spent in high impact recreational activity and BUA of the calcaneus, after adjusting for confounding factors such as age and weight. Although BUA and SOS measure different properties of bone, as discussed in Section 1.3.3.1., there does not appear to be any consistency in the literature with regard to which parameter is most responsive to physical activity. Some studies [44;296] comparing athletes to controls, have shown a greater difference in BUA (9.2-21%) compared to SOS (0.9-3.1%). In addition, leisure-time physical activity in a study by Blanchet et al., {374) is more strongly correlated with BUA ($r=0.11$; $p<0.001$) than SOS ($r=0.07$; $p<0.05$). In a study by Gregg et al., {1999) SOS was more closely associated with physical activity than BUA. These studies suggest that QUS is sensitive enough to measure changes in the bone in response to physical activity and differences in bone loading, and is a useful technique in discriminating active individuals from inactive individuals in the same way as DXA does. However, it is not yet clear, which QUS parameter, and therefore which property of bone, is most responsive to mechanical loading.

In non-athletic populations, cross-sectional studies have shown a significant correlation between BMD and habitual physical activity as measured by activity monitors {18}[263], questionnaires

[67;173;294] and physical fitness [255]. However, longitudinal and intervention studies may be the most appropriate way to assess change in BMD with changing physical activity. A longitudinal, prospective study by Recker et al., [263] on 156 college students found a significant positive correlation between self-selected activity levels and change in spinal BMD over a 3-5 year period, in women between the ages of 18 and 26 years.

Various questionnaire studies to determine the contribution of certain factors to low bone mass or osteoporosis have collected data on physical activity. Cadarette et al., [51] classified no current physical activity as less than 20 minutes of physical activity once a week, and reported a signifcant odds ratio of 1.6 (95%CI: 1.0-2.4) for low BMD at the FN. However, physical activity was not included in their final 3-item Osteoporosis Risk Assessment Instrument. Hawker et al., [138] obtained extensive physical activity information, which included physical activity as an adolescent, level of physical activity at work, average distance walked each week and intensity of physical activity. 19.6% of the sample reported inactivity as an adolescent, and this was the only variable that predicted low bone mass at the various DXA sites, as well as according to QUS measures. The odds ratio for inactivity as an adolescent, for low BMD by either DXA or QUS, was 2.37 (1.60-3.52). Other questionnaire studies have collected physical activity data but have not shown it to be a significant predictor of low bone mass [34;76;118;188] compared to other risk factors.

Intervention studies comparing exercise groups to control groups have been useful in assessing the influence of various forms of physical activity on BMD, however the intensity and progression of the exercise programmes need to be considered when reviewing this literature. An early study by Gleeson et al., [114] compared LS BMD of premenopausal women assigned to a weight-training group or a control group, after the implementation of a one-year intervention programme. The results of this non-randomised study showed that although a 0.5% reduction in LS BMD was noted in the controls, there was an increase of 0.8% in the weight-training group, suggesting that

exercise may attenuate the decrease in BMD that occurs once peak bone mass is achieved. An 8-month intervention study of 31 young (mean age 19.9 years) [287] showed significant increases in LS BMD in a weight-training (1.2%) and running (1.3%) group, compared to a control group. Another resistance training study in previously sedentary premenopausal women who were randomly assigned to a resistance training group or who remained sedentary [205], showed a 2% increase in LS BMD after 5 months of training, which remained at a similar level at 12 (1.6% above baseline) and 18 (1.4% above baseline) months. Lumbar spine BMD decreased by 0.8% in the sedentary group when BMD was measured at 5 months. Significant increases in BMD of the femoral trochanter were also recorded at 12 and 18 months in the exercise group.

There are few studies that have shown a negative effect of weight-training on BMD, however interpretations of these may be limited due to small sample sizes and lack of adequate control. For example, Rockwell et al., [270] showed a decrease of approximately 4% of LS BMD in a slightly older group (average age of 36 years) of women, after 9 months of weight-training. Concerns with interpreting the results of this study include the small sample size (10 women in the exercising group and 7 controls), the possible self-selection of subjects into groups due to nonrandomized group assignment, and a relatively low training stimulus (2 sets of 12 repetitions of 8 exercises, twice a week, at 70% of 1RM).

A longer intervention study (2 years) by Friedlander et al., [103] randomly assigned women between the ages of 20 and 35 years to an exercise group (including aerobic exercise and strength training) or a stretching group. In contrast to the study by Lohman et al., [205] who showed the greatest changes in BMD after 5 months of a resistance training programme, Friedlander et al., [103] found no significant changes in either group after one year of intervention. However, after the two-year intervention, significant increases were noted in LS, FN and trochanteric BMD. Results were also significantly different to the stretching group who showed significant bone losses from baseline in spinal trabecular BMD (as measured by QCT), and when

compared to the exercising group in FN, trochanteric and calcaneal BMD.

Although all of these studies clearly support the role of exercise in slowing down age-related bone loss, and in some cases increasing BMD, all of the programmes implemented in these studies consist of very different regimens. The study by Gleeson et al., [114] used a weight-training programme that consisted of 2 sets of 20 repetitions of 8 exercises at 60% of 1RM within a 30 minute session, 3 times a week. However, the studies by Snow-Harter et al., [287] and Lohman et al., [205] assigned more intensive programmes that included one hour long exercise sessions consisting of 12-14 exercises for 3 sets of 8-12 repetitions at 70-80% 1RM .

The benefit of exercise for postmenopausal women may be greater in terms of maintaining bone mass, and in particular lowering the risk of falling and resultant hip fracture. Research from the Nurse's Health Study [98] showed the incidence of hip fracture to be significantly inversely proportional to physical activity. As walking was the primary activity in most of the postmenopausal women included in their study, they analysed walking data separately. Their findings included a significant reduction in the risk of hip fracture in those women who walked for more than 4 hours a week compared to women who walked for one hour or less a week. In addition, walking pace was also a significant predictor of hip fracture. Similar results with reference to walking pace were obtained from an observational study by Coupland et al., [67]. They categorised the physical activity habits of 580 postmenopausal women and found an association between two habitual forms of physical activity, namely stair-climbing and walking speed, and TB BMD and LS BMD.

One of the first exercise intervention studies on postmenopausal women [71] showed a 6.1% increase in LS BMD in a group of women who participated in a weight-bearing exercise programme, which consisted of mainly walking, jogging, and stair-climbing, over a period of approximately 22 months. The same study showed a 4.8% loss in BMD after 13 months of

detraining. An intervention study by McMurdo and colleagues [226] randomly allocated a group of 118 volunteers between the ages of 60 and 73 years, to an exercise group combined with calcium supplementation or calcium supplementation alone, for a two year period. Although there were no significant differences in LS BMD between the 2 groups at the end of the intervention period, there was a significant increase in forearm BMC (measured using SPA) in the exercising group, and they experienced significantly fewer falls than the group who did not exercise in the two-year period. Findings by Prince et al., [257] showed no difference in BMD at the end of a 2-year intervention period between various calcium supplementation groups and a calcium plus exercise group, however all of these groups did not show a loss in BMD as was seen in the placebo group.

Other researchers have also investigated whether resistance training, and/or the components of resistance training, produce sufficient intensity or stimulus to prevent bone loss with aging [259], or even increase BMD [179]. In the study by Pruitt et al., [259], 26 postmenopausal women were randomly assigned to a low or a high intensity strength training group, or a control group. The low intensity programme consisted of performing 2 sets of 10 exercises at 40% of one-repetition maximum for double the number of repetitions (14 repetitions) in order to ensure similar training volumes as the high intensity programme who performed 2 sets of 7 repetitions of the same exercises at 80% of one-repetition maximum. The results of this study showed no significant changes in BMD in any of the groups at the end of the 12-month intervention period. Significant improvements in muscular strength were noted in both groups. Significant improvements in muscle strength, as assessed by a one-repetition maximum test, were also shown in a study by Kerr et al., [179] in which postmenopausal women were randomly assigned to a one year progressive resistance training programme consisting of high-load low repetitions (3 x 8RM) or low-load high repetitions (3 x 20RM). Using each subject as their own control, significant increases in BMD of the exercising limb compared to the non-exercising limb were found in the strength group who performed high loads of exercises, while no significant changes were found in the endurance group who performed more repetitions. The discrepancy in the findings of Pruitt et

al., [259] and Kerr et al., [179] may be due to a difference in the training volume between the studies as only 2 sets were performed by the subjects in the study by Pruitt et al., [259] and 3 sets were included in the Kerr et al., [179] study. However, the findings by Kerr et al., [179] suggest that load may be more important than the number of loading cycles.

It is clear that there is a local influence of physical activity on the bone. This finding has been confirmed by many researchers who have related changes in BMD to several factors associated with weight-bearing and impact loading activities [11;31;96;173;179;215]. Improvements in strength that occur with physical activity have been associated with positive bone adaptations at the site of the muscle insertion [215]. In the study by Madsen et al., [215], BMD of the forearm was also correlated with isometric and isokinetic quadriceps strength, however in a multiple regression while isokinetic quadriceps strength and age explained approximately 40% of tibial BMD, it was age and weight that were the significant predictors of forearm BMD. In addition, muscle stabilization, as a consequence of weight-training, has been associated with improvements in BMD. This relationship has been shown to also exist in postmenopausal women [179]. In a one year intervention study [179] in which women were assigned to either a high-load low-repetition group or a low-load high-repetition group, the increases in trochanter BMD, intertrochanter BMD and Ward's triangle were significantly correlated with the associated strength increases. In a study by Davee et al., [77] the exercise patterns of 27 healthy, premenopausal women were compared against differences in BMD. Some of the subjects were sedentary, others participated regularly in various forms of aerobic activity, while others supplemented their aerobic activity with weight-training (but did not exercise significantly more than the aerobic group). The results of this study showed LS BMD of the weight-training group to be significantly higher than the other two groups, and "muscle-building exercise", together with body mass index, explained approximately 40% of the variance in LS BMD. The local skeletal adaptation to mechanical loading activities is illustrated by studies that compare athletes who compete in various forms of exercise. Bennell et al., [31] compared the BMD of power athletes, endurance athletes and non-

athletic controls and showed a significant difference in upper limb BMD between the power athletes and the controls. There was however, no difference in upper limb BMD between the endurance athletes and controls, suggesting local skeletal adaptation of the upper limb to the mechanical loading activities common in power sports and the increased participation in weight training necessary to excel in power sports.

From the literature above, it is generally accepted that weight-bearing exercise, due to the mechanical stress on the bone, and strength training exercise, due to the pull of the muscle on the bone, are beneficial in the conservation, and in some cases the attainment, of BMD in adulthood. However, is the muscle activity on the bone, referred to as active loading by Grimston et al., [124], in the absence of weight-bearing activity or impact loading, sufficient to result in a beneficial effect on BMD? One of the earliest studies by Risser et al., [268] in which they compared the BMD of the LS and the calcaneus of a group of swimmers to a "matched impact athlete group" and a group of non-athletic controls, found the swimmers to have significantly lower BMD at both sites when compared to both groups. Similar findings by Taaffe et al., [297] compared the bone mineral densities of swimmers, gymnasts and controls. After adjusting for differences in weight, swimmers had significantly lower LS, FN, trochanteric and TB BMD compared to the gymnasts, and significantly lower FN, trochanteric and TB BMD compared to the sedentary controls. Research by Fehling et al., [96] compared collegiate swimmers to volleyball players and gymnasts, and although the swimmers did not have significantly lower BMD in comparison to the control group as in the previous two studies, the volleyball players and gymnasts had significantly greater BMD at the LS, PF and TB, after adjusting for height and weight, compared to the swimmers and controls.

Higher BMDs seen in gymnasts [184;297], and other sports people, who participate in high impact activities [31;83;96;143;268] has led to more research investigating the type of weight-bearing activity that is most beneficial for the bone. It has been suggested that high peak forces

(magnitude) of loading, such as those in jumping and sprinting, may have a greater influence on bone mass than a large number of completed loading cycles that occur in for example, distance running or walking. A cross-sectional study by Heinonen et al., [143] compared the bone densities of young athletes competing in sports of varying impacts, including squash (high impact), aerobic dance (moderate impact), and speed skating, with a group of physically active students and controls who were not participating in any structured exercise programme. The results of their study showed squash players to have the highest weight-adjusted BMD values at all sites, however they only compared the BMD's of the various sports to the sedentary group, and not between sporting groups. However, in older athletes there was no difference in BMD between the athletes participating in netball and/or basketball (high impact sports) compared to those participating in running and/or field hockey (medium impact sports), although TB BMD, regional leg BMD and regional arm BMD in the "high impact" athletes was significantly higher than the swimmers and controls [83]. To determine whether the bone response to activities of various impacts differs over time Bennell et al., [31] compared the BMD of power athletes (sprinters, jumpers, hurdlers), endurance athletes (middle distance and distance runners), and non-athletic controls at baseline and after a 12-month period, during which time all of the subjects maintained their training status. At baseline the female power athletes had a significantly higher LS BMD than the endurance athletes and controls, and although there were significant increases in LS BMD in all of the groups at the end of the 12-month period, the increase in the power athletes (2.6%) was greater than the endurance athletes (1.7%) and the controls (0.4%).

Some research has shown differences in the response of bone to high impact activities in both premenopausal and postmenopausal women [25]. In one exercise programme subjects were asked to perform 50 jumps (approximately 3-4 times body weight) on 6 days of the week. This exercise protocol did not require any special equipment or clothing, and was easy for most women to participate in. Follow-up at 5 months showed significant increases in BMD of the LS, FN and trochanter in the 30 pre-menopausal women who were randomly assigned to the exercise

group, compared to their baseline measurements. When compared to the 25 controls, who also showed a significant increase in LS BMD at 5 months, there was a significantly greater increase in BMD of the trochanter in the exercising group. In contrast to these findings, there were no significant differences in the changes in BMD between the exercise and control groups of the postmenopausal women who had participated in the same exercise programme for a 12-month period. These findings suggest that the bone of post-menopausal women may not be as responsive to high impact forces as it is to activities that involve cyclic loading forces, such as walking, jogging or stair-climbing, and which has been positively associated with BMD in post-menopausal women [74;241].

The role of exercise in post-menopausal women may be more important in the prevention of bone loss that occurs in older adults. Humphries et al., [155] divided a group of peri- and post-menopausal women into a high intensity strength training group and a walking group, and further into those women on hormone replacement therapy and those women not on HRT. Although all of the groups showed a loss in LS BMD at the end of the six-month programme, the only significant change from baseline was in the walking group who were on HRT, who lost 1.3%. However, this study gives no details of the training intensity used in the walking group, which may be an important factor in analyzing bone changes. Bassey and Ramsdale, [24] investigated change in the BMD of a group of postmenopausal women, in response to additional weight-bearing, and in particular impact, activity in a one year intervention study. They randomly assigned 44 women to either a high-impact exercise group, which consisted of performing 50 'heel-drops' per day, or a control group. Their results showed no significant change in the BMD of either group at the end of the two-year intervention period. These results suggest that a certain amount of activity is required to provide a sufficient stimulus to prevent bone loss with increasing age, and it may be necessary to implement more high intensity programmes in ageing populations in order to slow down bone loss. Several studies have shown a slowing down of bone loss in postmenopausal women who participate in higher impact activities. Grove et al., [126] showed no

significant change in BMD after a year, in a group of postmenopausal women randomly assigned to a low impact or a high impact exercise group, or a control group. There was however a significant decrease of approximately 6% in LS BMD in the control group over the one-year period. Similar results were obtained by Hawkins et al., [139] who followed a group of masters runners over a five-year period. They found that the runners maintained their bone mass over this time, and this was not affected by menopausal status or hormone replacement therapy.

A cross-sectional study by Pocock et al., [255] used a measure of physical fitness (predicted VO_2 max) as a proxy for habitual physical activity, and examined the relationship between fitness and BMD at 3 different sites. Using multiple stepwise regression, VO_2 max was able to explain a significant amount of the variance in FN and LS BMD in the whole group. However, in the premenopausal women onlyk, its contribution to BMD was lower. This may be due, in part, to the fact that VO_2 max is considered to be a poor measure of fitness and therefore, may not reflect habitual physical activity [242]. However, these findings do illustrate the fact that the influence of physical activity may differ between premenopausal and postmenopausal women. An improvement in cardiovascular function, as well as other components of "fitness" such as strength, power, co-ordination and balance, with regular physical activity may also be important in reducing the risk of future hip fracture.

Although it is generally accepted in the literature that physical activity is beneficial for bone health, some research has found a negative association between endurance running distance and BMD [50]. For example, although Burrows et al., [50] found a negative association between distance run (km/week) and BMD of the LS and the FN, even after adjusting for body size and age, they did not consider controlling for menstrual status. In their sample, 23% of the women were oligo/amenorrhea and it is unclear from the study as to whether these athletes were the ones presenting with the higher training volumes. The implication of excessive exercise, in combination with a restricted energy intake, is explored in further detail in section 1.5.2.4. (Menstrual

irregularity and reproductive history).

The aim of regular physical activity during the postmenopausal years is to slow down the loss of bone mass that occurs with aging, as well as reduce the incidence of hip fracture by decreasing the risk of falling. The literature suggests that high intensity activities are particularly effective in maintaining BMD. In addition, research has also shown that the benefits of physical activity are only maintained for as long as the exercise programme is continued.

Whether any of the changes that occur as a result of exercise intervention, are maintained when the intervention ceases is important in the consideration of bone health. An intervention study on postmenopausal women [71] showed a 4.8% loss in BMD after 13 months of detraining after an original increase of approximately 6% in LS BMD after a 22 month period. In the Nurses Health Study [98] in the women who reported a decrease in activity between 1980 and 1986, there was a linear increase in the risk of hip fracture. Heinonen et al., [142] followed up 30 of their original 39 pre-menopausal subjects used in an 18-month intervention trial that showed a significant increase in LS and lower limb BMD, as well as some of their original control group. At the end of the programme, subjects were instructed to continue with unsupervised exercise in the form of aerobics and step classes, 3 times a week. Follow-up after 8 months showed a continued increase at all of the lower limb sites despite a reduction in the intensity of the programme. Longer term follow up studies are recommended.

(ii) Lifetime physical activity

Past physical activity has been shown to influence adult bone mass [23;95;133;178;185;300;304]. Teegarden et al., [300] found that high school energy expenditure was correlated with current BMD and BMC of the TB, LS and FN, in a sample of minimally active women between the ages of 18 and 31 years. Similar relationships were found with occupational and leisure time activity in

the preceding 5 years. A study by Kirchner et al., [185] compared BMD of former college gymnasts (average age of 36.3 years) with a group of age, weight and height matched controls. They found that even when controlling for the influence of current physical activity and physical activity over the last 10 years, the differences between the two groups for LS, FN, Ward's triangle and TB BMD were 16, 18, 22 and 9% respectively, higher in the retired gymnasts compared to the controls. Bass et al., [23] compared the BMD of retired elite gymnasts between the ages of 18 and 35 years with age, weight and height-matched controls. Training history, including age at the start of training, number of years of training, years since retirement and number of hours of training per week, were collected on each of the gymnasts. Total body and regional (PF, LS, arms and legs) BMD were 6-16% higher in the retired gymnasts compared to the controls. A similar study by Zanker et al., [318] on former gymnasts who had been retired, and consequently sedentary, for 3-12 years, and age-matched controls, reported a 6-11% higher BMD in the retired gymnasts compared to the controls. In the Amsterdam Growth and Health Longitudinal Study [178], both LS and PF BMD (at different sites) measured at 28 years of age was significantly associated with physical activity measured during adolescence. However the subjects used in all of these studies are relatively young (below the age of 45 years) and previous physical activity has been within the last 10-20 years, rather than in childhood and adolescence. As the evidence above shows, exercise during childhood and adolescence seems to have a large influence on young adult BMD. When looking at the reduction in the risk of osteoporosis and the incidence of fractures in later life, it may be more relevant to look at an older population and to consider whether bone mineral acquisition during childhood is maintained into adulthood, and eventually older age.

Some studies have also found past activity to be associated with current BMD in older women [73;180;190]. In a study of retired ballet dancers and matched controls (average age of 51 years), Khan et al., [180] obtained detailed information on childhood ballet training including the age of starting ballet and the number of hours per week of formal ballet classes throughout childhood

and adolescence. They found a significant relationship between within-pair differences in FN and PF z-scores, and weekly hours of ballet training between the age of 10 and 12 years. The study by Kriska et al., [190], obtained details on leisure-time physical activity, including walking, during four lifetime periods (14-21, 22-34, 35-50, and 50+ years), and converted the data into kilocalories of energy expenditure. Their study found a consistent dose-response relationship between increasing quartiles of historical physical activity, excluding walking, and bone area and density in their sample of 223 postmenopausal women. Unfortunately this study did not include other means of physical activity such as occupational and household physical activity, which may also contribute significantly to daily physical activity. In a more recent study by Brahm et al., [43], of Swedish men and women up to 85 years of age, data on lifetime occupational and sport activities, as well as bed-rest, were also collected during specific life periods, namely childhood, young adulthood, and recent past. A total lifetime activity score was calculated from scores obtained from various levels of occupational activity and sport activities. However, no association was found between lifetime physical activity and BMD in this study. It is not clear from this paper whether these scores were based on intensity of the activity and how they were combined with frequency and duration to obtain the total lifetime activity score. Few longitudinal cohort studies have assessed the relationship between lifetime physical activity and BMD, and to our knowledge none have extended through to older adulthood. These data support the research that has shown that the childhood years are of particular importance in the accretion of bone, and as physical activity has been shown to track throughout life [303], this period of time will continue to play a crucial role in bone health.

1.5.2.4 Menstrual irregularity and reproductive history (age at menarche, parity, lactation, oral contraceptive use)

Physical inactivity is one of a number of risk factors that have been shown to impact on bone health. A women's lifetime estrogen exposure, and the factors that contribute to it, is associated

with bone. Most studies will take into account one, or all, of these factors when reporting on the determinants of bone in adult women. Armamento-Villareal et al., [13] developed a scoring system to quantify a woman's lifelong exposure to estrogen based on age at menarche, frequency and length of menstrual cycles and use of birth control medications. These factors were weighted according to their relative importance and the results of their study showed a significant correlation between vertebral bone density and the estrogen score in 63 premenopausal women between the ages of 19 and 40 years. The important factors that contribute to a woman's estrogen exposure during her lifetime are summarized below.

(i) Age at menarche

As menarche is associated with a more favourable hormonal environment, including increased oestrogen levels, for the accretion of bone, a delay in the onset of menarche may have negative consequences for long-term bone health. Early age at initiation of training has been significantly related to a later onset of menarche [297]. Delayed menarche has been associated with reduced BMD and an increased frequency of stress fractures [266;308]). However, although Fehily et al., [95] found women who had an early menarche tended to have higher BMD than women with a later menarche, their results were not significant. Athletes have been shown to have a later age of menarche than controls [31], and amenorrheic athletes have been shown to have a later age at menarche than eumenorrheic athletes [238;266]. This may be due to a disruption of the HPA-axis and subsequent LH pulsatility resulting from restricted energy availability, which is the difference between dietary energy intake and exercise energy expenditure [149;320].

(ii) Oral contraceptive use

The findings of a study by Myburgh et al., [238] reported that athletes presenting with stress fractures were less likely to be using oral contraceptives. However, in this study of nineteen female athletes, they did not adjust for lifestyle factors. Other cross-sectional studies have found

no difference in BMD between oral contraceptive users and women who do not use oral contraceptives [95;222], while others have show a negative association between BMD and oral contraceptive use [258]. The study by Prior et al., [258] was in slightly older women (mean age 36.3 ± 5.9 years) who had used oral contraceptives for an average of 6.8 ± 4.8 years. The findings of a recent 36-month prospective study [264] found no significant changes in BMD of 245 women who had been using oral contraceptives for between 0.1 and 14.9 years, and those who were not currently using oral contraceptives. In contrast to these findings, Pasco et al., [245] showed that women who had been using oral contraceptives for between 11 and 48 years had a 3.3% greater LS BMD than women not using oral contraceptives. Their sample of 710 subjects consisted of premenopausal and postmenopausal women, however the relationship between oral contraceptive consumption and BMD did not exist in the postmenopausal subgroup of women. These contrary findings illustrate that the association between oral contraceptive use and bone is unclear. Women who present with amenorrhea have traditionally been prescribed oral contraceptives as a means of regulating oestrogen levels, however the findings of recent studies [149;320] suggests that therapy needs to concentrate on correcting the energy deficit that occurs when energy expenditure is greater than energy intake, and therefore the perceived benefits of oral contraceptives in menstrual dysfunction, are not warranted (see Section iv). A more comprehensive evidence-based analysis of the relationship between low-dose oral contraceptives and BMD, has been published by Kuohung et al., [194].

(iii) Lactation and parity

There is conflicting evidence on the relationship between lactation, parity and BMD due to the potential confounding associated with pregnancy and lactation. Several studies have reported that lactation history is not associated with BMD [95;187]. In a study by Karlsson et al., [175], LS BMD immediately postpartum was 7.6% lower, after controlling for differences in fat and lean mass, than a group of age-matched controls. In addition, LS and FN BMD was decreased with lactation of 1-6 months compared to baseline results, and BMD loss was greater in lactating

mothers compared to non-lactating mothers. However, lactation between 6 and 12 months did not show any additional bone loss, although there was incomplete restoration of FN BMD in the women who stopped breastfeeding at 6 months, but follow up only continued to 12 months. Lumbar spine BMD, which was decreased by 4.1 ± 0.8% during the first 6 months of lactation, increased by 5.1 ± 0.8% between 5 and 12 months postpartum in women who stopped breastfeeding at 6 months. A prospective study by More et al., [233] also followed women from conception to 12 months postpartum. The decrease in LS BMD during pregnancy was 2.1%, which was significantly lower than the 7.6% decrease shown by Karlsson et al., [175]. This may be due to a difference in subject number as the study by More et al., [233] had only 38 subjects compared to the 73 subjects in the Karlsson et al., [175] study. In addition, the women in the More et al., [233] study were younger (19-36 years) that the women in the Karlsson et al., [175] study (20-44 years). Proximal femur BMD was not measured in the study by More et al., [233]. Although LS BMD decreased in the 6 months of lactation by 4.9% in the women who stopped breastfeeding at 6 months, similar to the study by Karlsson et al., [175] and Sowers et al., [290], the increase in BMD at the 12 month visit was only 2.3% which is significantly lower than the 5.1% shown by Karlsson et al., [175]. This suggests incomplete restoration of LS BMD in women 12 months after delivery, who stopped breast-feeding at 6 months. In the women who breast-fed for between 6 and 12 months there was continued bone loss up until the 12-month visit.

Thus if it is possible that the bone loss during lactation is not completely restored, as suggested by More et al., [233], then subsequent pregnancies would result in a net loss of bone. However, Karlsson et al., [175] showed no difference in BMD between women who had four or more pregnancies compared to those who had one or two pregnancies. In contrast to these findings, Paton et al., [248] showed in 1354 individual females, that women with 1-3 pregnancies had significantly higher LS BMD (2.2-3.1%) than nulliparous women, after adjusting for age, height and fat mass. They also showed that women who had breast-fed had significantly higher BMD and BMC than parous women who had not breast-fed. These findings suggest that pregnancy

and lactation may play a beneficial role in increasing BMD, which may be due to the hormonal changes that take place. As part of a sub-study [248] they also compared the BMD of twin pairs, one of whom was parous and the other, nulliparous. BMD was similar despite differences in parity within the twin pairs. In another sub-study in which groups of twins were compared when one or both were parous, there was a difference in BMC between twins who had no difference in pregnancies between them (-0.4%) and twins who had a difference of more than 3 pregnancies between them (5.1%). In addition, there were no differences in BMD within the twin pairs, one of whom had breast-fed while the other twin was nulliparous. These findings suggest that there is no detrimental effect on the bone as a result of pregnancy or lactation and in fact pregnancy may have a positive effect on the skeleton. These contradictory findings suggest that the restoration of bone mass after pregnancy and lactation does not occur immediately, as the studies that have reported a net loss of bone [233] have followed up their subjects within a short space of time after pregnancy. This is in comparison to the studies that have shown no difference [175] or a net gain in bone mass [248], who have followed up subjects after a longer period of time.

(iii) Menstrual dysfunction

There is extensive research on the influence of current and past menstrual status on bone health. Much of this work has been conducted on athletes or people with anorexia and eating disorders, with limited research [308] investigating menstrual dysfunction in non-exercising women. In general, menstrual dysfunction has been associated with an energy imbalance between intake and expenditure that has also been linked to reduced bone turnover and decreased bone formation causing a net bone loss [319;319]. These relationships have often been described as the Female Athlete Triad. However, recent research has also started to question the definition of the Female Athlete Triad as defined by the American College of Sports Medicine [243]. Khan et al., [183] has suggested that the term "osteopenia" should replace "osteoporosis" in order to ensure that the definition is more widely applicable, and they have also suggested that perhaps

the definition should not apply only to athletes. What the evidence points to is a specific group of individuals for whom physical activity is not beneficial for bone health and does not protect against bone loss. This is discussed in more detail below.

The most obvious forms of menstrual dysfunction included amenorrhea, which is defined as fewer than 3 cycles per year or no cycles for the past six months, or oligomenorrhea which is 3-6 cycles per year [176]. Eumenorrhea is a term that refers to regular menses and can be defined as 10-13 cycles per year.

Cross-sectional studies: Most of the early studies investigating the relationship between menstrual dysfunction and bone mass have only considered current menstrual status [53;62;85;216]. One of the earliest studies by Drinkwater et al., [85] compared the BMD of 14 amenorrheic athletes to 14 matched eumenorrheic athletes. Their study found the vertebral BMD of the amenorrheic athletes to be 86% of the vertebral BMD of the eumenorrheic athletes. In a similar study by Marcus et al., [216], they compared amenorrheic runners to eumenorrheic runners and sedentary controls. Similar to the results of Drinkwater et al., [85], the amenorrheic runners had significantly lower LS BMD compared to the cyclically menstruating women, however, in this study BMD was also lower than a group of age-matched nonathletic controls. There was no difference in radial BMD between the groups in either of the studies.

A later study by Micklesfield et al., [230] also took into the account the importance of including oligomenorrhea when examining the relationship between BMD and menstrual status. The results of this study showed that ultramarathon runners who had either current or prior oligomenorrhea or amenorrhea, had a LS BMD of only 87% of those women who had had regular menstrual periods. These findings are similar to an earlier study by Lloyd et al., [204] who showed that women who had missed more than 50% of their expected menses (moderately oligomenorrheic) had a trabecular bone density of 88% that of the eumenorrheic group. There

was not a significant difference between the groups, however this could be due to the small sample size with only 6 women being classified as eumenorrheic and 10 women were moderately oligomenorrheic. However, the women who were classified as severely oligomenorrheic (missed more than 50% of their expected menses but had had at least three menses per year; n=4) had a trabecular bone density of 69% that of the eumenorrheic group ($p<0.05$).

The studies by Micklesfield et al., [230] and others [84;204;238] have illustrated the importance of menstrual history as well as current menstrual status.

In the study by Micklesfield et al., [230], periods of menstrual irregularity were included in a calculation of a menstrual history index which was shown to be significantly correlated with LS BMD ($r=0.67$; $p<0.001$). This menstrual history index was adapted from that of Grimston et al., [123] and was a measure of the average number of periods per year from the age of 13 years to the current age. In a study by Drinkwater et al., [84], female athletes were classified as always having regular menses, or having had episodes of oligomenorrhea or amenorrhea, and then a combination of their past and present menstrual patterns were then ranked. The results of their study showed that women who had always had regular menstrual cycles had a significantly higher LS BMD than women who had a history of oligo/amenorrhea combined with periods of regular menses, and both were significantly higher than the LS BMD of women who had never had regular menstrual cycles.

Longitudinal studies: Limited literature has provided follow-up on the current bone status of women who have previously experienced menstrual dysfunction. Drinkwater et al., [86] were the first to characterize changes in BMD when regular menses were resumed in athletes with menstrual dysfunction in a follow-up study after a period of 15.5 months. Seven of the 9 women who had been previously amenorrheic had regained regular menses. These were compared to seven matched eumenorrheic controls from the previous study. There was a significant increase

in vertebral BMD (6.3%) in the formerly amenorrheic athletes, while these measurements were maintained (-0.3%) in the eumenorrheic athletes. Similar results were shown at the end of a 15 month follow up study by Lindberg et al., [202] (6.6% increase in vertebral BMD) and a 2 year follow up study by Jonnavithula et al., [170] (13.3% increase in vertebral BMD). A more recent study by Warren et al., [308] showed a 17% increase in wrist and vertebral BMD in previously amenorrheic women, including dancers and controls, who had resumed regular menses.

However, in all of these studies, the BMD of the formerly amenorrheic subjects was still lower than the regularly menstruating subjects and it is unclear from studies of this length, how long the initial increase in BMD that occurs with the resumption of menses will continue. The results of a longer follow-up study by Keen et al., [176] showed that even when menses have been resumed for a significantly longer period of time (± 8 years) in previously amenorrheic athletes, their vertebral bone was still significantly lower (84.4%) than those athletes who had always had regular menses [172]. Similar results in a study by Micklesfield et al., [231] found LS BMD of women with a history of menstrual irregularity to be 89.7% of regularly menstruating women after a follow up period of 3-5 years, and 85.6% of sedentary controls.

Extensive literature has been reviewed in sections 1.3.3.3 and 1.4.3.3 to show that exercise training increases BMD. However the findings by Micklesfield et al., [231] and others, which show that the BMD of amenorrheic athletes is compromised even when compared to sedentary controls, raises the question as to whether the exercise stimulus is sufficient to be protective against bone loss in the presence of current or past menstrual irregularity. Results from Myburgh et al., [237] reported significantly lower LS BMD, as well as all areas of the proximal femur, in a group of 9 amenorrheic runners compared to 12 runners who were classified as eumenorrheic. There was, however, no difference in tibial midshaft BMD between the amenorrheic and eumenorrheic athletes. Similar findings by Rencken et al., [266] in a larger sample of 49 female athletes suggest that impact loading during exercise is not sufficient to protect against the bone

loss that results from menstrual irregularity. Both studies reported LS and PF BMD of the amenorrheic athletes to be between 81-88% of that of the eumenorrheic athletes. As in the study by Myburgh et al., [237], the deficit in BMD of the shafts of the tibia and the femur were less than the other sites (92-94%), however these results were still significant. Data from Pearce et al., [250] on adolescent ballet dancers showed decreased BMD of weight-bearing and non weight-bearing sites with a longer duration of oligomenorrhea, suggesting that increased BMD resulting from exercise may be lost with increased periods of menstrual dysfunction.

Conversely, Snyder et al., [288] showed no difference in radial or vertebral BMC between non-athletic controls and a group of elite oarswomen, some of whom had regular menses and others who were oligomenorrheic or amenorrheic. This suggests that their activity, which in this case is rowing, utilising extensive back musculature, may protect these athletes from bone loss associated with menstrual irregularity. However, the sample size in this study was small, only 4 of the 19 women could be classified as amenorrheic, and no details were obtained on menstrual history. Similarly, although Gremion et al., [121] found significantly lower LS BMD in oligo-amenorrheic long distance runners compared to eumenorrheic running controls, there was no difference in BMD of the proximal and midshaft femur, areas which are maybe being protected by the high impact forces of their activity.

In order to investigate the long term effects of menstrual dysfunction and other lifestyle factors, Khan et al,. [181] compared the BMD and various lifestyle factors of 101 retired elite female ballet dancers to 101 controls. Although the retired ballet dancers had several risk factors for reduced BMD such as a high alcohol intake, smoking and menstrual irregularity, their BMD at the various hip sites and the LS was not significantly different to the controls. In addition, there was no significant difference in the incidence of osteopenia (46% dancers vs 40% controls) and osteoporosis (24% dancers vs 39% controls) at any site between the two samples.

From the literature reviewed above in section 1.4.3.4 it is seems that LS BMD (predominantly trabecular bone) is more sensitive to the hormonal environment, with many studies showing no difference in femoral or radial BMD (predominantly cortical bone) between regularly menstruating women and women with menstrual dysfunction.

1.5.2.5 Other lifestyle factors

(i) Smoking

The evidence concerning the relationship between smoking and BMD is conflicting [11;32;95;108;222]. The lack of association in the study by Fehily et al., [95] may be due to no record of current smoking status or dose of smoking being obtained. In addition, the results were not adjusted for possible confounding factors such as weight. The sample included in the study by Fehily et al., [95] was also young (20-23 yrs), and included both men and women, compared to the sample in the study by Aloia et al., [11] which included women with a mean age of 39 ± 1.39 years. Aloia et al., [11] showed a significant negative relationship, using multiple stepwise regression, between smoking and BMD of the radius and the LS in a sample of 24 healthy, premenopausal women. Similar results from Mazess and Barden, [222] in their sample of 200-300 women between the age of 20 and 39 years, found LS BMD to be significantly lower in smokers compared to non-smokers. An epidemiological study by Cornuz et al., [66] showed a reduction in the risk of hip fracture in past smokers but only ten years after cessation of smoking. Thus, there is evidence that the negative effects of smoking on BMD may be, in some cases, partially reversible.

Various mechanisms have been proposed to explain lower BMD and incidence of hip fracture in smokers compared to non-smokers. These include: a reduction in calcium absorption [47;283], altered bone formation (as measured by osteocalcin) [32], and a decreased response of BMD to estradiol [32]. In the study by Bjarnason et al., [32], smokers and non-smokers were randomized

to 1 or 2 mg estradiol/day. The response of BMD to 1mg of estradiol in the smokers was only half that of the non-smokers, however at 2mg of estradiol there was a similar response in the smokers and the non-smokers. The smokers in this study had also experienced menopause one year earlier than the non-smokers. The literature is therefore conclusive, smoking is detrimental to bone health, however some of these detrimental consequences of smoking are partially reversible, with time, on the cessation of smoking.

(ii) Alcohol consumption

An early prospective study over a 6-year period [147], reported an increased risk of both hip and forearm fractures in women who reported moderate alcohol intake. The women in this study were between the 34 and 59 years of age, and the results of the study also showed a dose-response relationship between alcohol intake and risk of fracture. In the sub-sample of men included in a study by Fehily et al., [95], there was an inverse relationship between alcohol consumption and radial BMD. Similar results in a study by Clark et al., [60] on alcohol-dependant women between the ages of 18 and 70 years, showed that after controlling for age and menopausal status, the FN and LS BMD of women being treated for alcohol abuse was 7.7% and 6.3% lower, respectively, than the non-alcohol abusing women. There were significant differences in other lifestyle factors between these groups such as smoking, oral contraceptive use and amenorrhea, and when all of these factors were included in a multiple regression, current alcohol dependence still significantly predicted FN and LS BMD, however the difference between PF BMD between women in recovery and non-alcohol dependent women was lost. This suggests that BMD may be restored when alcohol consumption ceases.

In contrast to these findings, Ilich et al., [157] showed that small to moderate measures of alcohol were actually positively associated with LS BMD. They collected data on the long-term frequency of alcohol consumption, as well as the amount and source of alcohol consumed, from the 136 women with a mean age of 68.6 ± 7.1 years. The group was divided into alcohol consumers and

non-consumers and the bone variables were compared between the groups. These data suggest that the dose of alcohol consumed may have the greatest impact on BMD and must be considered when comparing the BMD between alcohol consumers and non-consumers.

Various mechanisms have been proposed to explain the influence of alcohol consumption on BMD. They include reduced bone formation due to the toxic effect of alcohol on the osteoblasts. Indirect influences include impairment of liver function and the disruption of vitamin D and calcium metabolism.

(iii) Caffeine consumption

In the study by Ilich et al., [157], caffeine and alcohol intake were positively associated. In addition, FN and trochanter BMD were significantly lower in the caffeine-consumers compared to the women who did not consume caffeine, in the group that were consuming low levels of calcium. Similarly, results from Hernandez-Avila et al., [147] also showed that women who consumed significant amounts of caffeine had nearly a 3-times higher risk of hip fracture than women who consumed small amounts of caffeine. Conversely, a study by Conlisk et al., [61] in much younger women between the ages of 19 and 26 years found caffeine intake, in the range consumed by young adult women, to be an unimportant risk factor for low BMD. The women in the study by Conlisk et al., [61] were significantly younger than the women in the other two studies [147;157] suggesting that years of caffeine consumption may influence bone.

This section has summarized the various lifestyle factors that are associated with bone in adults. Although there is conflicting evidence surrounding each of these factors, it is accepted that lifestyle does play a role in determining the risk of osteoporosis, and lifestyle interventions, in order to reduce the risk of osteoporosis and fracture, should be implemented if necessary.

1.5.3 Ethnic influences on bone health in adults

Ethnicity has been clearly associated with differences in BMD. Several studies have found that black persons have a higher BMD [9;75;91;100;186;201;207;227;302] and a lower fracture rate [122] compared to white persons of various ages in adulthood. Results of an early study by Liel et al., [201] showed radial, LS, trochanteric and FN BMD to be significantly lower in a group of white women of 35 ± 1 years of age compared to black women of the same age.

In order to determine whether ethnic differences in the incidence of osteoporosis and hip fracture is a result of increased peak BMD or a slower rate of bone loss after the attainment of peak bone mass, a cross-sectional study by Luckey et al., [207] was designed to investigate differences in BMD with age, in black and white women between the ages of 24 and 65 years. After adjusting for age and body mass index, BMC at the radial and vertebral sites was 6.5% ($p<0.0001$) higher in black women compared to white women. The cross-sectional age change of the whole sample of women showed that although BMC decreased with age in the black and the white women, the change in BMC at the radius in the black women was less than that found in their white counterparts (2.6% per decade vs 5.1% per decade). Vertebral BMD was also lower with increasing age in the black women (3.5% per decade), and the white women (4.2% per decade), however these differences were not statistically significant. In addition, when the women were divided into premenopausal and postmenopausal groups, bone loss occurred in the premenopausal white women (-3.2% per decade), however in the premenopausal black women, radial bone density showed an increase of approximately 3.8% per decade. These data require the confirmation of a longitudinal study.

In an older sample (55-75 years of age) of post-menopausal black and white women investigated by Kleerekoper et al., [186], the differences in BMD between black and white women still exist. Although the black women in this sample were significantly heavier and taller than the white

women, after co-varying for body mass index, the BMD of the forearm, LS and FN were 9.8%, 8.7% and 14.7% higher respectively, compared to the white women.

The role of various risk factors well-known for influencing BMD were compared between black and white subjects in a study by Ettinger et al., [91]. These included anthropometry, physical activity and dietary factors, as well as biochemical markers of bone and mineral metabolism. Although this study showed significant racial differences in most of the variables measured including weight and height, lean body mass and sum of skinfolds, total physical activity and calcium intake, as well as 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, parathyroid hormone and urinary calcium, the only factors that consistently explained the differences in BMD between the two ethnic groups were the anthropometric variables. Adjusting for all of the variables included in the assessment, excluding race, explained only 22-45% of the variance in BMD in men, and 10-34% in women. Therefore although many anthropometric, lifestyle and biochemical factors are significantly different between black and white subjects, only a few of these are able to explain the ethnic differences in BMD. A similar study by Finkelstein et al., [100] compared the BMD of women of various ethnic groups including Caucasian and African-American. They collected extensive lifestyle data on all 2131 women and after co-varying for the factors that were related to BMD, the BMD of the African-American women was 3-6% and 6-9% higher than the other groups, at the LS and FN respectively. Due to the over-riding influence of body weight on BMD, these authors compared the various ethnic groups only for the women below 70kg. Although the African-American women still showed significantly higher BMD at the FN, this difference was no longer significant at the LS.

Aloia et al., [9] have suggested that there may also be ethnic differences in bone quality. In their study they compared 285 black and white women between the ages of 20 and 70 years of age. Although they found significant ethnic differences in BMD as measured by DXA at all the sites, there were no differences in BUA or SOS, the two parameters of QUS, between the

premenopausal ethnic groups. BUA was significantly higher in the postmenopausal black women compared to the white women, however, after adjusting for age and weight. Although extensive research reports a significant relationship between BMD, as measured by DXA, and the QUS parameters (see Section 1.3.3), there is literature to suggest that these two techniques are measuring different bone properties. This literature is discussed in detail in Section 1.3.3.

Ethnic differences in hip geometry have been suggested by Cummings et al., [69] and others [302]. A shorter hip axis length, the distance from the trochanter to the inner pelvis, has been hypothesized as a reason for the lower incidence of hip fracture in Asian and black women compared to white women [69]. In one study [69], the age-adjusted odds ratio for hip fractures were 0.53 (95%CI: 0.37-0.76) and 0.68 (95%CI: 0.55-0.85) for Asian and black women respectively, compared to white women due to their shorter hip axis length. This effect was independent of femoral BMD. In a study by Daniels et al., [74], femoral neck axis length (FNAL) was significantly longer in the whites compared to the blacks, even when correcting for height. Unfortunately there is limited current data on the incidence of hip fractures in the white South African population compared to the black South African population. In the study by Theobald et al., [302], all the radiographic width and length measurements of the PF were consistently lower in the black samples, while the cortical thickness of the FN was significantly higher in the African-American and Nigerian women compared to the Caucasian women. These authors have used this data to predict risk of future hip fracture and show that the increased medial cortical thickness of the black subjects compared to the white women will translate into an odds ratio of 0.74-0.77 for the African-American and Nigerian women respectively.

Ethnic differences in BMD may be due, in part, to ethnic differences in the PTH-vitamin D-endocrine system. These differences, resulting in greater accumulation of bone in black individuals, may be due to the better renal calcium handling (shown by lower urinary calcium excretion) and skeletal resistance to bone absorption by PTH (shown by lower serum levels of

osteocalcin, bone-specific alkaline phosphatase, and urinary hydroxyproline). Meier et al., [227] compared vitamin D metabolites and PTH, as well as BMD, between 70 premenopausal black women and 67 premenopausal white women of similar socioeconomic, educational and dietary status. They found no differences in serum 25-hydoxyvitamin D, 1.25-dihydroxyvitamin D or PTH between the two ethnic groups, who also reported similar dietary calcium and vitamin D intakes. However, urinary calcium excretion was significantly lower in the black women compared to the white women, even after being adjusted for dietary calcium intake. In a study by Kleerekoper et al., [186], and others [74;91] serum 25-hydoxyvitamin D was significantly lower, and 1.25-dihydroxyvitamin D and PTH were significantly higher, in black women compared to white women. As PTH is responsible for inducing the conversion of 25-hydroxyvitamin D to 1,25-di-hydroxyvitamin D in the kidneys, relatively higher PTH levels may influence intestinal calcium absorption and increase renal tubular reabsorption of calcium thereby decreasing urinary calcium excretion. The higher level of PTH observed in blacks may be due to a lower dermal production of Vitamin D precursors, resulting in increased PTH secretion in order to increase 1.25-di-hydroxyvitamin D production.

In addition, markers of bone formation (osteocalcin and bone-specific alkaline phosphatase) and bone resorption (hydroxyproline/creatinine ratio) were lower in the black women compared to the white women [186]. Similarly, results of a study by Finkelstein et al., [101] also reported significantly higher serum osteocalcin and urinary N-teleopeptide (to assess osteoclast activity) in white subjects compared to subjects of various ethnic groups, included African-Americans, suggesting ethnic differences in bone turnover. These results were still significant after adjusting for numerous factors either shown, or known, to influence bone turnover such as cigarette smoking, daily calcium intake, menopausal status and age. However, all of the variables that could account for more than 1% of the variance, including race, were only able to account for 16% of the variance in osteocalcin and 9% of the variance in urinary N-teleopeptide. These results may suggest lower rates of bone remodeling or bone turnover in black subjects compared to white

subjects.

One of the first studies to investigate ethnic differences with specific reference to the South African population is by Kalla et al., [172]. The purpose of their study was to establish reference data for South African women, and it included white women and women of mixed ancestral origin. Their results showed no significant differences in BMD of the LS or the PF in the women of mixed ancestral origin compared to the white women. However, LS BMD was significantly lower in the South African women over the age of 45 years compared to an American database of 650 age-matched women. These data suggest that different cultures and geographical regions will also present various factors that may influence bone. A later South African study by Daniels et al., [75] assessed age-related changes in BMD in black and white female nurses between the ages of 20 and 64 years. The results of this cross-sectional study report 7%, 10% and 13% higher PF BMD in the black women compared to the white women in the premenopausal, perimenopausal and postmenopausal groups respectively. This was after adjusting for differences in height and weight. In addition, in the premenopausal group, age was significantly correlated with LS, PF and radial BMD in the black women, however not in the whites. However, in the postmenopausal women, BMD at all three sites was negatively correlated with number of years since menopause in the white group but not in the black group. These results are similar to those of Luckey et al., [207] discussed previously, and suggest that there may be different mechanisms influencing the attainment of peak bone mass and subsequent bone loss in black and white women. In addition, the study by Daniels et al., [75], reported no ethnic differences in BMD of the LS or the radius.

This is in contrast to the findings of various American studies also reviewed in this thesis [9;91;186;201;207;227] that have reported significant differences between blacks and whites at all sites. A subsequent study by Daniels et al., [74] using 294 of the original 364 subjects included in the previous study, reported similar findings. Although Daniels et al., [74] has adjusted for size differences between blacks and whites, they have also calculated BMAD, as an estimate of bone

volume, and have shown this to be significantly higher for the FN, as well as the LS, in the black nurses compared to the white nurses. A study investigating the risk factors for the development of osteoporosis in the South African population [33] identified a low body weight and height, smoking, high alcohol intake and fat distribution around the waist to be some of the common risk factors in 125 males and females included in their study. Although these factors have also been identified as increasing the risk of osteoporosis in other populations, it is critical to investigate those risk factors relevant to the population under investigation. An important example of this point is West Africa, illustrated in a study by Aspray et al., [14] on rural Gambian women. In this population of women between the ages of 45 and 85 years, there was a 29-60% decrease in BMC of the various sites with increasing age. This decrease was lowest in the femur and highest in the distal radius. When compared to a sample of women from the United Kingdom, BMD of the LS and the midshaft of the radius was significantly lower in the Gambian women between the ages of 45 and 64 years, after adjusting for age, height and weight. Lumbar spine BMD was also significantly lower in the Gambian women over the age of 65 years compared to the British women, however there was no significant difference in the BMD of the midshaft of the radius. Despite these findings, there is a low fracture incidence among Gambian women, suggesting that other factors unrelated to preserving bone mass, may be protecting this population.

In summary, the literature is conclusive that black women have a different BMD and a lower fracture rate than white women which may be due to a number of factors, including hip geometry, bone quality and/or the PTH-vitamin D-endocrine system. Different risk factors and incidence of fracture within the different African populations suggest that consideration of the population under investigation must be made when comparing populations.

1.6 COMING A FULL CIRCLE: THE INFLUENCE OF BIRTH WEIGHT ON BONE

Birth weight has been identified as a marker of the influence of the pre-natal environment on the intrauterine growth of the fetus. Barker et al., [19] introduced the concept known as "fetal programming" which is defined as changes in the structure and function of certain metabolic and endocrine systems in response to intrauterine stress. These intrauterine stresses may include maternal smoking, maternal under-nutrition and malnutrition and stress, as well as physical activity. Barker et al., [19] were the first to show that these "early life" events have an influence on the risk of various chronic diseases such as hypertension and type 2 diabetes later on in life. There is also recent evidence to suggest that early life events may influence adult BMD. It has been proposed that the interaction between these fetal events, and the individual's subsequent growth, determines their risk of adult disease.

Alteration or "programming" of various endocrine axes, namely, the hypothalamic-pituitary-adrenal (HPA) axis and the growth hormone/insulin-like growth factor 1 (GH/IGF 1) axis have been suggested as possible mechanisms to describe the relationship between birth weight and BMD (see Section 1.2.2). Increased cortisol levels, evidence of increased activity of the HPA axis, have been associated with low birth weight [252;253], and decreased BMD [79]. A recent study by Dennison et al., [79] on 34 healthy men between the ages of 61 and 72 years found a significant negative association between integrated cortisol concentrations and BMD of the LS and PF, however this relationship was lost after adjusting for body mass index. However, integrated and trough cortisol concentrations were positively correlated with bone loss rate over a period of 4 years, at the PF, even after adjusting for body mass index and several other possible confounding factors. These data suggest that elevated levels of cortisol, associated with low birth weight, may be detrimental for BMD later in life.

Alteration of the growth hormone/insulin like growth factor-1 axis has also been shown to have

negative implications for BMD. Birth weight and weight at one year data were collected from 37 elderly men in a study by Fall et al., [93]. Weight at one year of age was not related to peak GH concentration, however it was significantly associated with median GH concentration (the value below which 50% of the samples fall), which was also negatively correlated with BMD. Peak growth hormone and fasting IGF-I concentrations were positively correlated with FN BMD. Birth weight was not correlated with median or peak growth hormone concentrations, or IGF-I. These data suggest a dual effect of GH secretion on BMD, and that the pattern of GH secretion, resulting in median GH concentrations, may be programmed in early life due to its association with weight at one year of age. A study by the same authors [80] on women between the age of 60 and 75 years also measured BMD at baseline and four years later, as well as growth hormone and IGF-1 levels. Their results reported a positive association between growth hormone (trough, median, integrated and total) and LS BMC after adjusting for various confounders. In addition, all of the measures of the circulating GH profile were significantly higher in the highest tertile of birth weights compared to the women born in the lowest tertile of birthweights. This suggests that a lower GH concentration may be associated with low birth weight, which will compromise the bone health of these individuals later in life. Whether this is due to a failure to achieve peak bone mass or due to an increased rate of bone loss is unknown.

Several studies have suggested that socio-cultural and environmental factors may influence birth weight, resulting in these adaptations and physiological changes. Evidence for this influence is provided by the difference in mean birth weights reported by various developed and developing countries (Table 1.5).

Table 1.5.: Mean birth weights reported by various developed and developing countries

Author	Country	Sample (n)	Sample (age)	Mean BW: whole sample (g)	Mean BW: males (g)	Mean BW: females (g)
Cooper et al., [63]*	UK	153	21 yrs			3307 ± 514
Cooper et al., [65]**	UK	413	63-73 yrs		3598 ± 607	3460 ± 532
Yarbrough et al., [316]**	USA	305	70 yrs			3400 ± 800
Jones et al., [165]**	Australia (Tasmania)	330	8 yrs		3232 ± 678	2764 ± 671
Levitt et al., [215]*	SA	137	20 yrs	2674 ± 410		
Cooper et al., [64]**	Finland	7086	62-71 yrs		3440 (z score 0.52)	3320 (z score 0.49)
Gale et al., [106]**	UK	143	70-75 yrs		3390 ± 520	3230 ± 440
Godfrey et al., [117]*	UK	145	Neonates		3480 ± 422	3321 ± 420
Rao et al., [261]*	India	633	Neonates	2665 ± 358		
Norris et al., (personal communication)*	SA	2611	11 yrs		3215 ± 525	3095 ± 480

* All infants born at full term; ** Gestational age not specified

In order to understand early life influences on bone parameters, independent of current body weight in individuals of various body sizes, current body size must be adjusted for. A recent longitudinal study by Ichiba et al., [156] compared bone mineral densities of infants of various birth weights and gestational ages, from the age of 40 weeks postconception to 3 years of age. Although LS BMD was significantly lower in the low birth weight groups than the normal birth weight groups at an early age, this difference disappeared by the age of 1 year. The results also showed, however, that the low birth weight groups had a significantly lower body weight and length at 40 weeks postconception than the other groups, although this was not adjusted for when comparing their BMD. At the age of 2 years, there was no longer a difference in LS BMD, weight or length between the groups of various birth weights.

The availability of birth and school records have provided a number of researchers with the opportunity to investigate the relationship between adult bone parameters with birth data and changes in physiological parameters over time. Several studies have investigated the relationship between birth weight and adult bone parameters [63;65;106;134;316]. Data from Yarbrough et al., [316] showed a relationship between birth weight and BMC, however, when adjusting for adult body weight, the relationship for BMC of PF and the forearm was no longer significant. However, the relationship was maintained at the LS. Gale et al., [106] also show a significant increase in BMC with increasing tertiles of birth weight in their sample of 143 men and women between the age of 70 and 75 years, however their data were adjusted for age and not for current body weight. In the sub-sample of 41 women there were also weaker, but significant, increases in LS, FN and TB BMD with increasing tertiles of birth weight, however again these data were not adjusted for current weight. When the authors introduced current weight as a co-variate with birth weight, to predict BMC at the various sites, the relationship became non-significant. A cross-sectional study by Hamed et al., [134] comparing the BMD of 230 women between the ages of 20 and 23 years who were either of normal weight at birth (>2500g) or low birth weight (≤ 2500g) showed no difference in their adult BMD. This study did

not adjust for current differences in weight or height, and the low birth weight group included premature as well as small for gestational age subjects.

The trajectory of bone growth throughout childhood is a critical determinant of adult bone mass. Weight in infancy may be used as a marker of the intrauterine and early post-natal environment, as well as determine the trajectory of body weight changes throughout. Cooper et al., [63] investigated the relationship between weight at various stages during childhood and current bone parameters in adults. In their study of 153 young adult women they showed a tracking of body weight from 1 year of age up to 21 years of age. After adjusting for current weight there was a significant relationship between weight at 1 year and current FN and LS BMC in this young adult sample. However, when adult height was introduced into the multiple regression model, weight at age 1 was no longer significant. In addition, there was no relationship between weight at age 1 and BMD, which is BMC adjusted for the area of the region, or bone mineral apparent density which is BMC adjusted for the volume of the area using the equation by Carter et al., [54]. Their study also obtained extensive data on various lifestyle factors including physical activity, calcium intake and reproductive status. When all of these factors were entered into a multiple regression to explain current bone status, several lifestyle factors including physical activity and alcohol consumption, as well as current weight were found to be the most significant predictors of BMD. Weight at 1 year of age was not included into this multiple regression. Similar, but weaker relationships were shown in another study by the same authors [65] on an older population of men and women between the ages of 63-73 years.

It is unclear from these data whether the relationship between early growth parameters and adult bone parameters are as a result of bone size as determined by body size, or due to BMD which is partially adjusted for bone size. In addition, these studies suggest that growth in infancy is associated with skeletal status in adulthood, however lifestyle factors may also have an important role to play in optimizing bone health. It may be simplistic to only look at the

relationship between birth weight, childhood growth as determined by weight and height, and adult BMD, without considering the influence of various environmental factors throughout life, particularly during childhood which has been shown to be the most critical period for the optimization of bone health [276]. Unfortunately neither historical physical activity or past calcium intake data were collected in any of these studies, as these factors have also been shown to be important in determining adult bone health [171;190]. Body size may be important in determining the quantity (size) of the bone (BMC), however the quality of the bone (BMD), independent of body or bone size, may be dependant on other environmental factors such as physical activity and nutrition.

Pediatric studies have been able to take into account the influence of confounding factors, such as physical activity and nutrition, at an early age, on the effect of prenatal events on later bone mass. Early nutrition may be one important consideration in determining bone mass, however what is not clear is the contribution of the individual nutrients to growth and bone development. Fewtrell et al., [99] showed no difference in bone area, BMC and BMD in 244 children between the ages of 8 and 12 years who were born prematurely and who weighed <1850g at birth, and who received various methods of early nutrition (banked breast milk (33mg Ca^{2+}/100ml), pre-term formula (70 mg Ca^{2+}/100ml), term formula (35 mg Ca^{2+}/100ml) or expressed breast milk (35 mg Ca^{2+}/100ml) which consisted of varying amounts of energy, protein, calcium and phosphorus. Unfortunately, most studies that have compared the influence of low birth weight on bone parameters have selected preterm children to represent the sample of low birth weight. As prematurity is associated with many other complications, it may be more relevant to compare health parameters of term children born with different birth weights.

Children's studies may also help to determine the point at which "fetal programming", or intrauterine insult, begins to influence health parameters. A study by Jones et al., [167] investigating the effect of maternal smoking during pregnancy on the BMD of 330 8-year old

male and female children, found that after co-varying for current weight and height, there was a deficit in BMD in the children whose mothers had smoked during pregnancy. In addition, the children of mothers who smoked during pregnancy had lower birth weights as well as lower heights and weights at the age of 8 years. Similar findings in a study by Godfrey et al., [117] from the United Kingdom, reported significantly lower birth weights of new-born infants of mothers who reported smoking during pregnancy compared to those who did not, and whole body BMC was 11% lower in the infants of mothers who smoked during pregnancy compared to the infants of mothers who reported not smoking during pregnancy. Other maternal factors during pregnancy such as diet and physical activity were also shown to influence neonatal BMC in this study.

As mentioned above, several studies have shown that adult height and weight track from birth into adulthood [63;65;316]. From these studies it seems that the quantity of bone mass is greater in larger (height and weight) adults, however it does not tell us about the quality of the bone as determined by BMD which has also been shown to be a major predictor of osteoporotic fractures [154]. In addition, what happens when weight at birth does not track into adulthood ie. if body weight overshoots the growth potential and the adult weight of two individuals may be the same but they have very different birth weights? Research is necessary to determine the role of birth weight, environmental factors during childhood as well as adulthood and current weight and height in determining the quantity, and quality, of current bone. Studies comparing the BMD of adults matched for height and weight but with different birth weights may help to answer this question.

Although it is suggested in the literature that there is a relationship between intrauterine stress and intrauterine growth and development, the influence of these stresses may be modified by certain genetic factors. In a longitudinal study by Jones et al., [165] birth weight, birth length and length gain in the first month contributed significantly to current BMD of the LS and the FN

even when including current weight and height in the model. Adjusting for various environmental factors including breastfeeding and physical activity, did not alter this relationship. However, when maternal BMD was included in the model, these factors no longer made a significant contribution to current BMD. The model including early growth parameters (birth weight, weight gain in the first month, and subsequent weight gain), various environmental parameters (breastfeeding, maternal smoking, sports participation, sunlight exposure, and calcium intake) and maternal BMD was able to explain 45% and 40% of the variation in FN and LS BMD respectively, however only subsequent weight gain and maternal BMD made a significant contribution to the model. None of the early growth parameters contributed to BMAD, which is an approximation of the volumetric density of the bone [54].

Results from a study by Godfrey et al., [117] reported a significant relationship between whole body BMC in a sample of 112 newly-born infants and paternal height (r=0.22). Although the authors report a significant correlation between whole body BMC and maternal and paternal birth weights, only the significance levels (p=0.001 and p<0.0001, respectively) are reported with no correlation coefficient (r) value. These factors, together with gestational age, maternal triceps skinfold thickness, smoking during pregnancy and maternal walking speed and vigorous activity during late pregnancy, explained 45.3% of the variance in whole body BMC in this young infant sample. These factors were only able to explain 29.6% of the variance in whole body BMD.

In the study by Cooper et al., [64], tall maternal stature was associated with an increased risk of hip fracture in adulthood suggesting a strong genetic component in determining future bone health. It is not within the scope of this literature review to explore the extensive evidence that covers the genetic determination of bone mass and how it is influenced by environmental factors during one's lifetime.

The incidence of low birth weight in South Africa has been reported in a recent perinatal survey [249] of 73 South African state hospitals. Their results reported a low birth weight rate of 19.6% in the metropolitan hospitals, followed by 16.5% in the city and town hospitals and 13% in the rural hospitals surveyed. Within the Cape Province, the incidence of low birth weight has been reported as 14.7% according to a 3-year survey of all the provincial and province-aided hospitals and clinics in the Cape Province. The incidence of low birth weight in South Africa is significantly higher than developed countries such as the United Kingdom that has reported a low birth weight incidence of 7%, however it is lower than the 30% incidence reported in other developing countries such as India. Due to the long-term health consequences associated with low birth weight, and the relatively high incidence of low birth weight in South Africa that is largely associated with socio-economic factors, more research in this field needs to be encouraged in order to prevent an increased demand on the already struggling health care system.

1.7. CONCLUSION

Bone is a living tissue, the growth, maintenance and loss of which can be influenced by a number of variables including genetics, ethnicity, lifestyle and socio-demographic factors. Bone accretion during childhood and adolescence will influence the attainment of peak bone mass, and therefore the factors that are responsible for influencing this trajectory of growth, must be identified and optimized. These factors, including physical activity and diet, can also influence the maintenance of bone, and subsequent bone loss, in adulthood.

The role of physical activity during these various life stages is the main focus of this dissertation. Evidence supporting the beneficial effects of physical activity on the bone is extensive and the suggested mechanisms, although not explored in the studies included in this dissertation, include increased bone formation [205], as well as a reduction in the rate of resorption. Although it is unclear as to which of these mechanisms is influenced by bone loading, it is generally accepted that the response of various types of bone to physical activity, will depend upon the metabolic environment. Therefore, we have considered the other factors that appear to regulate bone, and their interaction with physical activity in influencing the bone.

In addition, it may be the interaction between these factors, or "regulators", which will have an additional influence on the bone. The South African population is unique in that it consists of a number of ethnic groups that represent various socio-demographic profiles, and who live in a country that is undergoing a transition from a developing to a developed country, but which is experiencing many of the disadvantages of both. The result is a relatively high incidence of low birth weight, a very different physical activity profile amongst children and adults of various ethnic groups due to limited resources among the previously disadvantaged communities, and a high consumption of alcohol and cigarettes among certain ethnic groups. The influence of these factors on the various ethnic groups of the South African population, and other populations, need

to be investigated closely in order to ensure adequate osteoporosis prevention strategies.

AIMS AND OBJECTIVES

The aims of this thesis, were to investigate the association between physical activity and bone through the various life-stages, and to examine the interaction between physical activity and other lifestyle, demographic and physiological factors in determining BMD and bone QUS parameters in the South Africa population. The specific objectives of this thesis were to:

1. Evaluate the relationship between lifetime physical activity patterns, both in terms of total physical activity and bone impact loading, and BMD in middle and older-age women of black or of mixed ancestral origin in the Western Cape of South Africa.
2. Examine the interaction between lifestyle factors, including physical activity, and ethnicity, on calcaneal QUS bone parameters in school-age girls from different ethnic groups in South Africa.
3. Examine the effects of the intrauterine environment and early life influences on calcaneal QUS measures, by characterizing various maternal, intrauterine and lifestyle factors in 7-9 year old children.
4. Examine the relationship between various lifestyle factors, including current physical activity, and adult bone properties as determined by QUS.
5. Determine the factors contributing to current BMD in a group of previously competitive ultramarathon runners, in order to investigate the long-term effects of menstrual dysfunction previously associated with physical activity.

CHAPTER TWO

BONE MINERAL DENSITY AND LIFETIME PHYSICAL ACTIVITY IN SOUTH AFRICAN WOMEN

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2.1. INTRODUCTION

Current physical activity levels have been positively associated with BMD in a variety of populations [11;45;173;294]. Further, in a small number of studies, particularly in older women, past activity has been linked to current BMD [73;180]. The most detailed study of historical physical activity [190] found a consistent dose-response relationship between increasing quartiles of historical physical activity, excluding walking, and bone area and density, in a sample of postmenopausal women. However, Swedish researchers were unable to find any associations between BMD and lifetime occupational and sport activities, as well as bed-rest. This may be due to the fact that the study sample included both men and women, and covered a wide range of ages. Few longitudinal cohort studies have assessed the relationship between lifetime physical activity and BMD, and none have extended through to middle age. In the Amsterdam Growth and Health Longitudinal Study [178], both LS and hip BMD (at different sites) measured at 28 years of age were significantly associated with physical activity measured during adolescence.

In addition to general physical activity, weight-bearing exercise and specifically, high impact loading, have been shown to be positively associated with increased BMD in men and women [24;71;83;268;317]. A range of peak strain scores for different physical activities have previously been developed [125], and for both adolescents and adults, peak strain scores were positively related to LS BMD. It may, therefore, be important to quantify weight-bearing activity in the assessment of historical physical activity in relation to BMD. Furthermore, previous studies that have focused on the relationship between physical activity patterns and BMD, have largely involved middle-aged, Caucasian groups.

The aim of the present study was to evaluate the relationship between lifetime physical activity patterns, both in terms of total physical activity and bone impact loading, and BMD in middle and older-age women of black and mixed ancestral origin in the Western Cape of South Africa.

2.2. METHODS

2.2.1. Subjects

The sample of 144 subjects comprised black women (n=17) and women of mixed ancestral origin (n=127) aged 22 to 59 years, resident in Cape Town, South Africa. All subjects had been participants in a case-control study of BMD and breast cancer conducted in 1998. Signed informed consent was obtained from all subjects and the study was approved by the Ethics Committee at the University of Cape Town. Cases (n=62) were women who presented with newly diagnosed incident primary cancer of the breast at Groote Schuur Hospital; only women who had not yet received treatment with chemotherapy or radiation were included. Controls (n=82) were women who had been admitted to the same hospital for non-gynecologic illnesses unrelated to risk of breast cancer or contraceptive use. Cases and controls with bone diseases were excluded. Physical activity data were not collected on 17 of the subjects because the physical activity questionnaire was incomplete. 76% of the cases and 53% of the controls were aged 40 years or older; the proportion of women of mixed ancestral origin was 90% among the cases and 87% among the controls.

2.2.2. Medical history and lifestyle factors

2.2.2.1. Medical history

All participants were administered standard questionnaires by a trained nurse-interviewer to obtain information on risk factors for breast cancer, including reproductive history, contraceptive

history, and family history of breast cancer. Other information collected included years of current or past cigarette smoking, as well as current and past alcohol and coffee consumption. Demographic and socioeconomic data collected included education, employment and marital status.

2.2.2.2. Physical activity history

Historical information was obtained on levels of physical activity for 4 age epochs, 14-21, 22-34, 35-50, and 50 or more years of age. The historical physical activity questionnaire was adapted and customized to this population of South African women from that of Kriska et al. [190] (see Appendix 6.1). A cue card with 34 activities divided into household, occupational, leisure-time and transport activities was used. Only activities over 3 METs (metabolic equivalents) according to the classification of activities by Ainsworth et al. [7] were included (one MET is defined as the energy expenditure for sitting quietly, which for the average adult is approximately 3.5 ml of oxygen.kg body weight^{-1}.min^{-1}). Each subject, under the guidance of the nurse-interviewer, selected activities in which she had participated for at least 6 months. Subjects then recalled the total number of years within each epoch that they engaged in this activity, as well as months per year, sessions per month, and average minutes per session. We assigned each activity a MET level according to the Compendium of Physical Activities [7]. Lifetime physical activity data were divided into 'absolute' MET hrs, as well as an average of MET hrs.wk^{-1} for each epoch. In addition, MET hrs and MET hrs.wk^{-1} were calculated for household, occupational, leisure-time and transport activities within each epoch. Walking was quantified by separate questions: total time walking, walking for leisure, and walking for transport. Peak strain score was calculated for each epoch, based on the peak strain score for each activity developed by Groothausen et al. [125]. These scores are based on ground reaction forces of different physical activities found in the literature. This scoring system reflects impact loading and allocates a score of 1 for non weight-bearing activities (<1 x body weight), 2

for weight-bearing activities (1-2 x body weight), 3 for activities including sprinting and turning actions (2-4 x body weight), and 5 for activities including jumping actions (>4 x body weight). All scores were then totaled to determine a Total Peak Bone Strain Score (TPBSS) for each epoch.

2.2.2.3. Milk intake

Milk/maas (a local sour milk drink) intake during the four epochs (adapted from Sandler et al. [278]) was used as a proxy for calcium intake (see Appendix 6.2). Subjects were asked whether they consumed milk with every meal; frequently/not with every meal; sometimes; rarely or never. Responses were categorized as 1 for every meal, 2 if frequently/not with every meal, 3 if sometimes, and 4 if rarely or never. Due to respondent burden and the homogeneity of the population, additional dietary information was not collected.

2.2.3. Bone Densitometry and anthropometric measures

On completion of the interview, each of the subjects had an osteodensitometry scan of the LS and the left PF. Scans were performed in the Department of Nuclear Medicine, Groote Schuur Hospital using a Hologic QDR-1000 (version 4.20) dual-energy x-ray bone densitometer (Hologic Inc., Waltham, MA). Average bone mineral densities ($g.cm^{-2}$) were determined for lumbar vertebrae 1 through 4 (LS) and the PF, Ward's triangle, FN, greater trochanter, and the intertrochanteric area. We used the World Health Organisation criteria [1] based on the T-score (the subject's BMD expressed in relation to the young adult reference mean) to classify osteoporosis and osteopenia.

The nurse interviewer measured waist and hip circumferences and weight and height, at the time of the DXA scan. We calculated body mass index (BMI) as weight (kg) divided by height (m) squared.

2.2.4. Statistical analysis

The Statsoft™ (Statistica v5.0, 1999) statistical package was used. Descriptive analyses were performed to determine mean values and standard deviations. Continuous data were compared between cases and controls using the one-way analysis of variance, covaried for age, weight and ethnicity. In addition, Chi-square tests were used to compare categorical data such as smoking and alcohol intake between the groups. Pearson correlation coefficients for the relation of BMD to the various lifestyle and physical activity variables were calculated for the total cohort (cases and controls combined). These variables were then entered into a multiple stepwise regression in order to determine the influence of possible confounders. Transformation of the energy expenditure data, which was not normally distributed, did not improve the distribution of data and therefore was not used. Intraclass correlation coefficients were calculated for all subjects who had physical activity data for epoch's 1, 2 and 3 (n=96) to determine the extent to which physical activity measures tracked over time.

2.3. RESULTS

The only significant difference in subject characteristics between the cases and controls was age (45.3 ± 8.7 vs. 40.5 ± 8.6 yrs, respectively; $p<0.01$). We found no differences between groups in physical characteristics such as weight, height and BMI; reproductive history such as parity and months of breastfeeding; and lifestyle factors, including past and present smoking and milk intake. All comparisons between groups were adjusted for ethnicity and age.

As shown in table 2.1., BMD of the LS, PF and FN were similar in the cases and controls. There was also no difference between the groups in the prevalence of osteoporosis and osteopenia. Because the cases and controls were similar in BMD measures and other

characteristics, we combined the groups for all further analyses. Subject characteristics of the group (n=144) are presented in Table 2.2.

Table 2.1. BMD measurements of cases and controls

BMD measurement		Cases (n=62)	Controls (n=82)	p values
Lumbar spine ($g.cm^{-2}$)	mean ± SD	1.030 ± 0.159	1.036 ± 0.119	p=0.81
T-score	mean ± SD	-0.14 ± 1.45	-0.10 ± 1.09	p=0.86
Osteopenic	%	22.6	18.3	
Osteoporotic	%	4.8	1.2	
Total proximal femur	mean ± SD	0.967 ± 0.143	0.973 ± 0.120	p=0.77
($g.cm^{-2}$)	mean ± SD	0.18 ± 1.13	0.21 ± 1.01	p=0.88
T-score	%	17.7	11	
Osteopenic	%	0	0	
Osteoporotic				
Femoral neck ($g.cm^{-2}$)	mean ± SD	0.867 ± 0.214	0.867 ± 0.124	p=0.98
T-score	mean ± SD	-0.04 ± 1.24	0.789 ± 1.18	p=0.55
Osteopenic	%	19.4	17.1	
Osteoporotic	%	1.6	1.2	

Data adjusted for age and ethnicity

Table 2.2. Relationship between subject characteristics and BMD (n=144)

	Mean ± SD	Correlation coefficient (r)		
Characteristic		LS BMD ($g.cm^{-2}$)	PF BMD ($g.cm^{-2}$)	FN BMD ($g.cm^{-2}$)
Age (yrs)	42.6 ± 8.9	-0.13	-0.13	-0.26**
Height (cm)	158.6 ± 6.2	0.29***	0.19*	0.20*
Weight (kg)	71 ± 15.4	0.38***	0.54***	0.37***
BMI ($kg.m^{-2}$)	28.3 ± 6.3	0.27***	0.47***	0.29***
Waist circumference (cm)	90.9 ± 3.6	0.25**	0.48***	0.29***
Hip circumference (cm)	109.3 ± 13.4	0.29***	0.49***	0.32***
Age at menarche (yrs)	13.8 ± 2	0.16	0.16	0.18*

*p<0.05, **p<0.01, ***p<0.001

PF and FN BMD were lower in current and ex-smokers (n=80) than in never-smokers (n=64); (PF BMD: 0.952 ± 0.132 vs. 0.996 ± 0.124 $g.cm^{-2}$, p<0.05; FN BMD: 0.838 ± 0.128 vs. 0.906 ± 0.202 $g.cm^{-2}$, p<0.01), after adjusting for weight, age and ethnicity (Figure 2.1.). No differences were found in BMD between women who consumed alcohol (N=44) and those who did not (N=100) or between women who were past or present coffee drinkers (N=133) and women who never drank coffee (N=11).

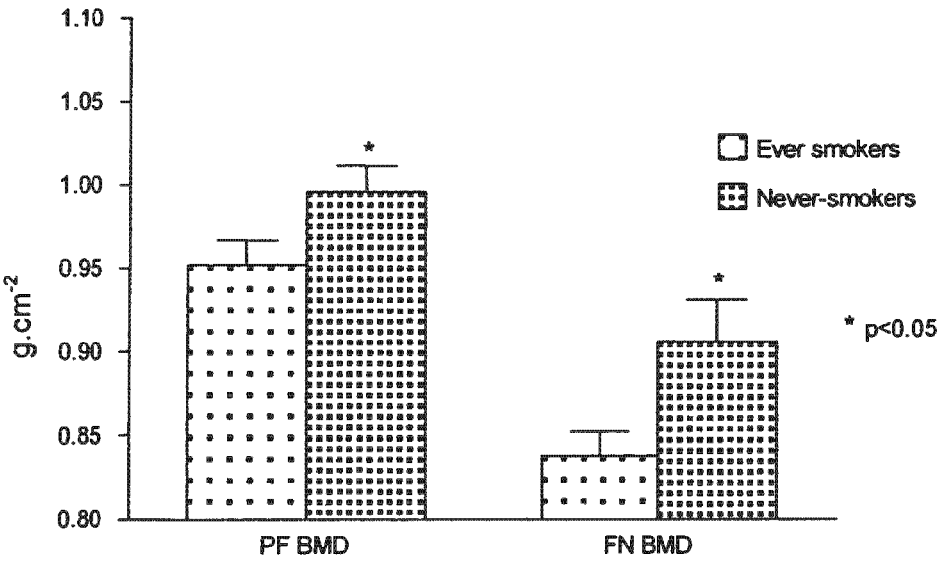

Figure 2.1. BMD of the total proximal femur (PF) and the femoral neck (FN), in ever smokers and never-smokers. The results are covaried for height, weight and ethnicity

Bivariate analysis was used to describe the relationship between the BMD measures and various subject characteristics (Table 2.2.). LS, PF and FN BMD were inversely correlated with age, but only the association with FN BMD was statistically significant ($r=-0.26$, $p<0.01$). All three BMD measures were significantly correlated with weight, height, BMI, waist and hip circumferences. The strongest correlation was seen with PF BMD, where 20-25% of the variance in BMD was explained by differences in these anthropometric measures. Age at menarche was significantly correlated with FN BMD ($r=0.18$; $p<0.05$) but not with any of the other BMD sites.

Physical activity parameters for epochs 1, 2, 3 and 4 are presented in Table 2.3. Using bivariate analysis, total physical activity (TPA) and household (HH) energy expenditure, in average MET hrs.wk^{-1}, between ages 14-21 years were both positively correlated with LS BMD (TPA: $r=0.18$, $p<0.05$; HH: $r=0.22$, $p<0.05$). Transport activity (TR), including walking and bicycling, and average time spent walking per day (W) were correlated with PF BMD at this age

(TR: r=0.19, p<0.05; W: r=0.22, p<0.05). TPBSS for activities between the ages of 14-21 years was also correlated with LS BMD (r=0.18, p<0.05).

Table 2.3. Physical activity parameters for epochs 1, 2, 3 and 4

Physical activity parameter	14-21 yrs (N=127)	22-34 yrs (N=127)	35-50 yrs (N=96)	> 50 yrs (N=28)
Energy expenditure (MET hrs.wk^{-1}):				
Total	87.8 ± 65.9	97.5 ± 70.1	60.3 ± 70.7	28.8 ± 38.9
Household	41.4 ± 31.1	45.1 ± 34.5	21.4 ± 27.2	12.1 ± 22.7
Occupational	29.4 ± 54.3	39.3 ± 61.7	30 ± 49.8	14 ± 30
Leisure-time	8.9 ± 16	5.1 ± 11.8	5.5 ± 28.5	0.56 ± 1.36
Transport	8.1 ± 11.8	8 ± 10.2	3.5 ± 7.25	2.08 ± 5.8
Walking (mins.day^{-1})	60.7 ± 49.9	56.9 ± 36.3	59.3 ± 74.6	58.9 ± 45.1
PSS (x 1000)	252.7 ± 180.7	483.4 ± 355.9	371.6 ± 410	139.2 ± 144.4

Values are presented as mean ± SD. PSS: peak strain score

Using multivariate analysis, the variables, age, total physical activity for epoch 1, and current weight were included in the model that accounted for 21% of the variance in LS BMD (p<0.0001, standard error of the estimate (SEE)=0.126, Table 2.4.). When total physical activity for epoch 1 was replaced with household energy expenditure for epoch 1, the same variables accounted for 23% of the variance in LS BMD (p<0.0001, standard error of the estimate (SEE)=0.124, Table 2.4.).

Table 2.4. Multivariate analysis for lumbar spine BMD

Variable	b	β	p level
Age (yrs)	-0.205	-0.003	0.013
Total physical activity* (Met hrs.wk^{-1})	0.215	0.0005	0.009
Weight (kg)	0.419	0.004	0.000
r=0.480, adjusted R^2=0.211, SEE=0.126, p<0.0001. β, parameter estimate; b, partial coefficient.			
With household energy expenditure* in place of total physical activity*			
Age (yrs)	-0.190	-0.003	0.0183
Household energy expenditure* (Met hrs.wk^{-1})	0.255	0.0012	0.0015
Weight (kg)	0.418	0.004	0.000
r=0.500, adjusted R^2=0.231, SEE=0.124, p<0.0001. β, parameter estimate; b, partial coefficient.			

* Between 14-21 years of age

The statistical model that best predicted PF BMD included age, transport activity including walking and bicycling between the age of 14 and 21 years, and current weight. These variables accounted for 33% of the variance in PF BMD (p<0.0001, standard error of the estimate (SEE)=0.109, Table 2.5.). When transport activity was replaced by average time spent walking per day between the ages of 14 and 21 years, 29% of the variance in PF BMD was accounted for (p<0.0001, standard error of the estimate (SEE)=0.105, Table 2.5.).

Table 2. 5. Multivariate analysis for total proximal femur (PF) BMD

Variable	b	β	p level
Age (yrs)	-0.177	-0.003	0.018
Transport activity* (Met hrs.wk^{-1})	0.183	0.002	0.014
Weight (kg)	0.548	0.005	0.000
r=0.588, adjusted R^2=0.330, SEE=0.109, p<0.0001. β, parameter estimate; b, partial coefficient.			
With average walking per day* in place of activity for transport*			
Age (yrs)	-0.192	-0.003	0.0213
Walking (mins.day^{-1})*	0.183	0.0005	0.027
Weight (kg)	0.497	0.004	0.000
r=0.552, adjusted R^2=0.285, SEE=0.105, p<0.0001. β, parameter estimate; b, partial coefficient.			

* Between 14-21 years of age

BMD was not significantly correlated to any measures of physical activity in epochs 2, 3 or 4.

Intraclass correlation coefficients were calculated to determine tracking of activity through epochs 1, 2 and 3. For total physical activity an intraclass correlation coefficient of 0.96 (95%CI: 0.94-0.97) was obtained. For household activity and occupational activity, the intraclass correlation coefficient was 0.98 (95%CI: 0.97-0.99) and 0.78 (95%CI: 0.71-0.84), respectively. Similarly, activity for transport had an intraclass correlation coefficient of 0.92 (95% CI: 0.89-0.94), however the intraclass correlation coefficient for leisure time activity was poor (-0.14; 95%CI: -0.22- -0.03).

2.4. DISCUSSION

In the present study we found physical activity between the ages of 14 and 21 years had the greatest influence on adult BMD, in this sample of women from working class communities. Total walking and activity for transport recalled during this period were positively associated with PF BMD. In addition, household activities, which were mostly weight-bearing tasks such as mopping and sweeping, were correlated with LS BMD during the same age period. Our results agree with earlier findings [73;180;190] that physical activity during early age periods is positively associated with BMD in later life.

The women in our study differed from those studied previously in that they were non-Caucasian and from socio-economically disadvantaged backgrounds, where leisure time physical activity was generally low, whereas the participants in previous studies were Caucasian women, generally from the middle class [43;190;304]. Another difference is that our study incorporated household and occupational activity, as well as walking for transport. Had we only captured leisure time activity, we may have failed to accurately characterize the relationship between physical activity in all domains, early in life, and adult bone mass. The finding that the relationship between physical activity and BMD was strongest in the earliest age period provides indirect evidence that mechanical loading resulting from physical activity during the adolescent years may result in a higher peak bone mass later in life. In addition, the relationship that we found between walking and PF BMD highlights the importance of impact loading and weight bearing activity on bone accretion. Our findings support the data from Ulrich et al. [304] who found that total weight-bearing exercise during childhood and the teenage years was associated with increased BMD in American women.

Although Ulrich et al. [304] obtained information on occupational physical activities and extensive household physical activities, as well as exercise, the method that they used to

classify weight-bearing activity did not take into account the impact loading of the activity, as all activities performed on the feet were equally weighted. In our study we used the classification system of Groothausen et al., [125] to calculate a total peak bone strain score (TPBSS) and we found that the TPBSS during the first epoch (14-21 years) was also significantly associated with LS BMD. In contrast, Groothausen et al., [125] found that peak strain physical activity during both adolescence and adulthood was positively related to LS BMD. These findings suggest that it is important to measure impact loading when investigating the relationship between BMD and physical activity.

Others have found that daily physical activity patterns track over time [303]. In our study, household activity accounted for most of the total physical activity in all four epochs and this, together with total physical activity, tracked throughout epochs 1, 2 and 3. Our study however, only found activity during adolescence to be related to current BMD. Therefore, although physical activity may be most effective for increasing BMD during the period of bone accretion, as shown by Kriska et al. [190], the relationship between past physical activity and current physical activity may actually confound this relationship.

With respect to factors other than physical activity, it is well known that body mass and BMI are associated with increased BMD [59;77]. Our findings are consistent with these studies, with body mass showing the strongest correlation with PF BMD in our study. However, we have also shown a strong relationship between waist and hip circumference, and LS BMD, which may just be a function of increased weight.

Another lifestyle factor which has been shown to influence adult BMD is calcium intake [95;171;260;294;299]. Sandler et al. [278] used milk consumption as a proxy for calcium intake and found that women who reported drinking milk with every meal during childhood and adolescence had significantly higher BMD than women who reported drinking milk less

frequently. Our findings were not able to confirm this relationship. As most of the women in our study reported that they consumed milk rarely, our failure to show an association between lifetime milk consumption and BMD may be due to decreased variability in the response to questions on milk consumption.

As the current study was part of a much larger study, it was impossible to collect sufficient detail on some important confounders such as nutrition history, and in particular calcium intake. Several studies have shown conflicting results with regard to the influence of previous calcium intake on current BMD. Fehily et al., [95] found no long-term effects of milk supplementation during a 2-year period of childhood, on adult BMD, however the findings of Teegarden et al., [299] suggested a tracking of milk consumption with age and showed a significant relationship between reported milk intake during adolescence and BMD in young women. In our study a more sensitive proxy for calcium intake may have minimized the likelihood of Type 2 error in characterizing the relationship between milk and/or calcium intake, and BMD. In addition, a semi-quantitative food frequency questionnaire would have provided insights into other sources of calcium that may have been more applicable to this population.

An unexpected finding was the significant positive correlation between age at menarche and FN BMD. Delayed menarche has previously been associated with reduced BMD [107]. We have no apparent explanation for this association in our study.

The present data were collected in a case-control study of breast cancer risk in relation to BMD. A criterion for subject selection was the absence of known or diagnosed bone disease. The successful application of this criterion is indicated by the low prevalence of osteoporosis in the cases and controls. The study was not primarily designed to assess correlates of BMD. However, insofar as the appropriate data for such an assessment were collected, and the BMD measures and variables related to physical activity were similar in the cases and controls, we

used the combined data for this purpose. The physical activity patterns of our study population generally included a large amount of household activity and daily walking as a means of transport and there was limited participation in leisure-time activity. The physical activity questionnaire that we used was specially adapted for this population. All BMD measurements were done at the same facility using internationally accepted procedures. Information bias was not a concern as the women were interviewed about their physical activity before their bone measurements were taken. However, there were undoubtedly recall errors in reporting physical activity, which if random could have weakened the relationship between BMD and physical activity. This questionnaire has also been found to be reproducible in a population of a similar age to ours [58].

To the best of our knowledge, the present study is the first to assess lifetime physical activity in relation to BMD in non-Caucasian women. These results suggest that the relationship between lifetime physical activity and BMD is robust as the positive effect of physical activity on BMD has even been shown in a low socio-economic population of women of mixed ancestral origin. In addition, our results add to the literature that indicates that physical activity during childhood and adolescence is related to BMD in later life. Our results also suggest that activities such as walking, provide significant impact loading, which positively influences BMD, and they highlight the importance of quantifying activity in all domains, not just with regard to leisure time activity. In addition, some aspects of physical activity early in life tracked over time. Further work is needed to define the separate contributions of physical activity at a young age and later on in life to adult BMD.

2.5. ACKNOWLEDGEMENTS

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CHAPTER THREE

CALCANEAL ULTRASOUND BONE MEASUREMENTS IN PRE-ADOLESCENT GIRLS – INTERACTION BETWEEN ETHNICITY AND LIFESTYLE FACTORS

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3.1. INTRODUCTION

Research has shown that eighty-six per cent of the adult bone mass of the spine is acquired by the skeletal age of 14 years, or the second year of menarche [276]. Therefore, bone mineral density in pre-adolescent girls significantly predicts peak bone mass achieved in early adulthood. Peak bone mass, as well as bone structure and microarchitecture, are important determinants of osteoporosis and fracture risk later in life [26;219], therefore careful evaluation of the factors that influence bone status in early life may yield valuable information regarding bone health in later life.

Bone health in later life, and in particular the incidence of osteoporosis and hip fracture, has also been associated with inter-ethnic differences [122]. To determine whether these inter-ethnic differences influence the attainment of peak bone mass, various studies have reported on the ethnic differences in BMD in children [29;152]. A study by Horlick et al. [152], comparing 336 Asian, black, and white prepubertal children, found the black children to have a significantly higher TB BMC, after adjusting for differences in total body bone area, age, height, and weight. Conversely, Patel et al. [247] found no difference in radial bone mass between age-matched black and white South African children between the ages of 6 and 20 years. After adjusting for height, bone mass parameters tended to be greater in black than white children, but these results were not significant.

There is some evidence that anatomical and genetic influences may also partially explain these ethnic differences in BMD [302]. Other studies suggest that modifiable lifestyle factors such as calcium intake [171] and increased physical activity [180] around the age of puberty, play an important role in determining adult bone mass. However, these studies were not designed to assess ethnic differences and all subjects were of the same ethnic background. The idea of a "window of opportunity" when bone is most responsive to physical activity has been previously

investigated by MacKelvie et al. [212]. Current physical activity in children has been investigated by Bass et al., [23] who reported an association between high impact physical activity and increased BMD in pre-pubescent children, as measured by DXA. In addition, a recent study by Lehtonen-Veromaa et al. [197] on peripubertal girls showed the mean ultrasound SOS values of the gymnasts and runners to be significantly higher than the controls. This study also reported a significant correlation between the calcaneal ultrasound parameters, SOS and BUA, and a measure of physical activity, the MET index, in the pre-pubertal and pubertal girls ($r=0.19$-0.31; $p<0.05$).

The impact of physical activity on the BMD of children from different ethnic backgrounds has only recently been addressed in a study comparing Asian and white children [213]. The various ethnic groups that make up the population of South Africa are unique in that they represent distinct dietary and lifestyle habits [271]. To our knowledge, no studies have been published comparing the QUS properties of bone between ethnic groups, and in particular between ethnically diverse South African children.

In order to further understand the possible interaction between lifestyle and environmental factors during childhood, and ethnicity, on bone development, the present study evaluates various factors associated with bone QUS parameters in pre-pubertal, school age girls within the different ethnic groups in South Africa.

3.2. METHODS

3.2.1. Subjects

Subjects included 198 pre-menarcheal girls [black, (n=80), white (n=41), mixed ancestral origin (n=77)] aged 7.5-11.7 years, from primary schools in the Cape Town metropolitan area. The

schools were selected using a stratified sampling technique designed to achieve a broad representation in measures of socioeconomic status between the participants. Eight schools were randomly selected from categories 1 (high socioeconomic group), 3 (medium socioeconomic group) and 5 (low socioeconomic group). This descriptive cross-sectional study was done in collaboration with a study relating body size to self-esteem and school performance in the same subject sample.

Permission was granted by the Department of Education and the school principals. Written informed consent was obtained from each of the participant's parent(s), and all the girls participated voluntarily. The study was approved by the Ethics and Research Committee of the University of Cape Town, and the Johns Hopkins University Institutional Review Committee.

3.2.2. Questionnaires

A pilot study of the physical activity and food frequency questionnaires was carried out prior to the start of the study, and all relevant changes were made thereafter. Testing took place over two visits to each school with a maximum of 20 girls participating in each visit. Subjects were divided into two groups with one half completing extensive self-report questionnaires with the guidance of one of the investigators, while anthropometric and QUS measurements were carried out on the other group. On the second visit testing was done on the alternate group.

The questionnaires were administered in the language that the children were taught in, by a field worker fluent in the respective language. Demographic, dietary intake and physical activity data was obtained by self-report and questionnaires were adapted to ensure cultural sensitivity. Demographic data included age, housing density (number of rooms in the house divided by the number of people living in the house), employment of parents and/or other breadwinners within the family.

3.2.2.1. Physical activity questionnaire

A physical activity questionnaire, adapted from the Modified Activity Questionnaire for Adolescents [3], was designed to assess year-round activity patterns (see Appendix 6.3). Subjects were asked to report on participation in activities at school as well as during the weekend, by season, times per week and time spent in each activity session. Questions regarding school transportation and physical education classes were also included. Each activity was then assigned a MET value [8], which was multiplied by the frequency and duration of the activity (hrs.wk^{-1}) to determine MET hrs.wk^{-1} [3]. All activities recorded in one year, for each individual, were totaled to calculate total physical activity. Activities were also assigned a peak strain score according to the guidelines set out by Groothausen et al., [125], and discussed in Chapter 2. The score for each activity was multiplied by frequency (hrs.wk^{-1}), and all activities recorded in one year, for each individual, were then totaled to determine a Total Peak Bone Strain Score (TPBSS), and also multiplied by body weight (TPBSS*WT). A separate set of questions on time spent walking as a means of transport, as well as intensity of walking, were included in the questionnaire. Walking energy expenditure (MET hrs.wk^{-1}), and walking energy expenditure as a percentage of total physical activity, was calculated. The number of hours spent watching television or playing computer games during the week and on weekends, was also reported by each child. These data were subsequently grouped according to whether the child watched 2 hours or less of television per weekday or weekend day, or more than 2 hours of television per weekday or weekend day.

3.2.2.2. Food frequency questionnaire

A semi-quantitative food frequency questionnaire to determine dietary calcium intake was modified from the Youth/Adolescent Food Frequency Questionnaire [269] (see Appendix 6.4.). Subjects were asked to recall their consumption of various food items which are high in calcium, using a four-point frequency scale – never (score=0); once a week (score=1); a few times a week (score=3) or every day (score=7). The frequency scores for each of the seven questions were added to calculate a composite calcium intake score.

3.2.2.3. Pubertal assessment

Pubertal stage was estimated by self-assessment using diagrammatic sketches of Tanner classifications of breast development and pubic hair growth [273]. Diagrammatic sketches were used in order to minimize cultural influences. As this classification system was only introduced after one of the schools had been tested, these data were not collected for 16 of the subjects. 12 girls (1 white and 3 black girls and 8 girls of mixed ancestral origin) reported having started menstruating. These girls and/or those who were classified as post-pubertal (Tanner breast stage 5) were excluded.

3.2.2.4. Quantitative ultrasound and anthropometric measures

The left calcaneus was measured by the ultrasonographic densitometer (Hologic Sahara Clinical Bone Sonometer, Waltham, MA, USA) to obtain the following measurements: Broadband Ultrasound Attenuation (BUA; dB/MHz) and Speed of Sound (SOS, m/s). Previous studies have demonstrated a good correlation between QUS measurements and BMD as measured by DXA, in children [235]. Subjects were required to sit barefoot in a stable chair facing the densitometer. The heel was then positioned within the foot support plate, between the transducers, and the leg was strapped onto the calf support to minimize motion. Quality

assurance was performed daily by calibrating the device using the phantom supplied by the manufacturer. Accurate measurements could not be obtained on 6 of the total 198 subjects.

Standing height (cm) was measured to the nearest 0.1 cm, using a stadiometer. Body weight was measured to the nearest 0.5 kg using a SECA scale, with subjects clothed in light-weight school uniform. Skinfold thickness of the triceps and subscapular regions were measured using Harpenden calipers. Three readings at each site were obtained and the average for each subject was recorded. All anthropometric measurements were conducted by the same fieldworker. Percentage body fat was calculated according to the equations of Slaughter et al., [284] and percentile for BMI (BMI%ILES) compared to US children between 5 and 17 years of age was obtained from Rosner et al., [272]. FFM (kg) and %FFM were calculated as fat mass subtracted from, and as a percentage of, total body mass (based on anthropometrical estimation of body fat percentage).

3.2.3. Statistical analysis

The Statsoft™ (Statistica v6.0, 2002) statistical package was used for data analysis. Girls who were currently menstruating and/or in stage 5 of Tanner breast stage development were excluded from data analysis. All descriptive data are presented as means ± standard deviations. The sample was divided according to ethnicity viz., black African (B; n=73), white (W; n=40) and mixed ancestral origin (MAO; n=64). Comparisons between the different ethnic groups were made using analysis of covariance, adjusting for age. Comparisons between the three groups for the QUS measurements, BUA and SOS, were made using analysis of covariance, with age, weight and height as covariates. In addition, a chi-square test was used to compare groups with reference to categorical data such as Tanner stage. Pearson's product-moment correlation coefficients were used to determine relationships between BUA and SOS, and all lifestyle data, excluding physical activity data, which were not normally distributed.

Spearman's rank-order correlation coefficients were calculated between the QUS measurements and physical activity data. The following predictor variables were then entered into a multiple linear regression analysis in order to determine the model which best predicted SOS: age, % FFM, calcium score, total physical activity, Tanner breast stage, ethnicity, and the interaction between Tanner breast stage and ethnicity. This was not completed for BUA as a significant model could not be found.

3.3. RESULTS

The subject characteristics of the three ethnic groups included in the study are presented in Table 3.1. There was no significant difference in age between the three groups. However, the black girls weighed significantly less ($p<0.05$) than the white girls, and were significantly shorter than both the girls of mixed ancestral origin ($p<0.001$) and the white girls ($p<0.001$). Although BMI was not significantly different between the three groups, the age standardized BMI centile (based on North American children of 5-17 years of age; BMI%ILES) was significantly lower in the black girls compared to the white girls ($p<0.01$). Percentage body fat, and triceps and subscapular skinfold measurement's were not different between the 3 groups. Fat-free mass (kg) was however significantly greater in the white girls compared to the black girls ($p<0.05$). However, there was no difference in %FFM between the ethnic groups. Housing density was significantly different between all the groups, and was highest in the black girls ($p<0.001$ vs. white girls; $p<0.01$ vs. mixed ancestral origin girls).

There was a significant difference in the breast stage development, which was used as an indicator of maturity, between the ethnic groups ($p<0.001$; Table 3.1.), with more black girls in the prepubertal stage. Tanner stage for pubic hair growth was not significantly different between the groups.

Table 3.1. Subject characteristics

	Black (n=73)	White (n=40)	Mixed ancestral origin (n=64)
Age (yrs)	9.7 ± 0.8	9.6 ± 0.6	9.8 ± 0.8
Weight (kg)	31.4 ± 7.8[a]	34.8 ± 7.5	33.6 ± 7.8
Height (cm)	1.29 ± 0.08[c,f]	1.35 ± 0.07	1.34 ± 0.07
BMI ($kg.cm^{-2}$)	18.6 ± 3	19 ± 2.7	18.5 ± 3.3
BMI%ILES	56.5 ± 25.9[b]	71.8 ± 22.2	62.9 ± 24.3
% body fat	19.9 ± 5.8	22.2 ± 5.7	21.4 ± 7.3
Triceps skinfold (mm)	13.4 ± 4.5	15.1 ± 4.3	14.6 ± 5.6
Subscapular skinfold (mm)	8.7 ± 4.7	10.2 ± 4.7	10 ± 5.9
Fat-free mass (kg)	24.8 ± 4.7[a]	26.7 ± 4.4	25.9 ± 4.3
Maturity (%) [μ]:	(n=63)	(n=39)	(n=59)
Prepubertal (%)	23.3	10	6
Early puberty (%)	39.7	87.5	51
Late stage of puberty (%)	23.3	2.5	32
Housing density	2.5 ± 1.4[c,e]	1.4 ± 0.7[d]	1.9 ± 0.8

[a] significantly different from white group ($p<0.05$); [b] significantly different from white group ($p\leq 0.01$); [c] significantly different from white group ($p<0.001$);[d] significantly different from mixed ancestral origin group ($p<0.05$);[e] significantly different from mixed ancestral origin group ($p<0.01$);[f] significantly different from mixed ancestral origin group ($p<0.001$);[μ] categories significantly different to each other for the 3 groups ($p<0.001$), Chi-square test; All ANOVA's co-varied for age

BUA and SOS were significantly lower in the white girls compared to the other two groups, even after adjusting for age, weight and height, or Tanner breast stage (Figure 3.1. and Figure 3.2.).

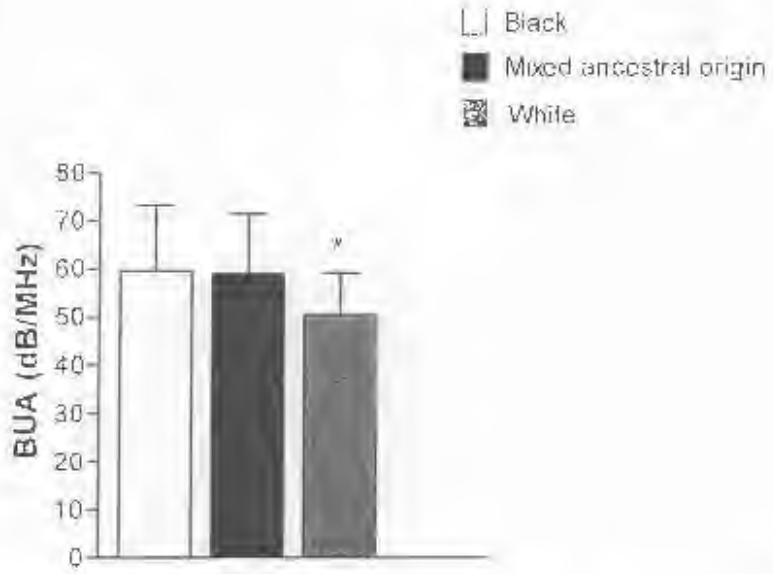

Figure 3.1. Graphic representation of the difference in BUA, between the different ethnic groups. * white group significantly different to the black group ($p<0.001$) and the mixed ancestral origin group ($p<0.01$). Data co-varied for age, weight and height. Results are presented as mean ± SD.

Figure 3.2. Graphic representation of the difference in SOS, between the different ethnic groups. * white group significantly different to the black group ($p<0.001$) and the mixed ancestral origin group ($p<0.01$). Data co-varied for age, weight and height. Results are presented as mean ± SD.

There was no difference between the ethnic groups for total physical activity (MET hrs.wk^{-1}) (Table 3.2.). Total peak bone strain score (TPBSS), a measure of weight-bearing physical activity, was significantly higher in white girls compared to black girls ($p<0.05$), and this relationship remained when TPBSS was multiplied by weight ($p<0.05$). Walking energy expenditure (MET hrs.wk^{-1}), and walking as a percentage of total physical activity, was significantly higher in the black girls compared to the other two groups ($p<0.001$), and significantly higher in the girls of mixed ancestral origin compared to the white girls ($p<0.05$).

Calcium score, used as a proxy for dietary calcium intake, was significantly lower in the white girls than the black girls ($p<0.01$; Table 3.2.).

Table 3.2. Physical activity and dietary parameters

Parameter	Black (n=73)	White (n=40)	Mixed ancestral origin (n=64)
Total physical activity (MET hrs.wk^{-1})	23.6 ± 21.2	27.8 ± 20.6	24.2 ± 19.4
TPBSS (arbitrary units)	5.0 ± 4.7	6.8 ± 4.8[a]	5.6 ± 4.7
TPBSS x weight	152.0 ± 150.0	229.1 ± 149.1[a]	185.9 ± 173
Walking (MET hrs.wk^{-1})	6.3 ± 6.4	0.6 ± 2[c,d]	3.0 ± 4.3[c]
Walking energy expenditure/ total physical activity (%)	32.2 ± 28.1	2.9 ± 10.8[c,d]	13.3 ± 19.5[c]
Calcium score	21.6 ± 11.1	16.1 ± 8.4[b]	19.7 ± 9.5

[a] significantly different from black group ($p<0.05$); [b] significantly different from black group ($p\leq 0.01$); [c] significantly different from black group ($p<0.001$); [d] significantly different from mixed ancestral origin group ($p<0.05$); [e] significantly different from mixed ancestral origin group ($p<0.01$); [f] significantly different from mixed ancestral origin group ($p<0.001$)

Bivariate analysis was used to describe the relationship between the QUS measures and various anthropometric and lifestyle factors. BUA and SOS were positively correlated with housing density (BUA: r=0.15; p<0.05, SOS: r=0.27; p<0.001). SOS was inversely correlated with weight (r=-0.30; p<0.001), height (r=-0.20; p<0.01), BMI (r=-0.30; p<0.001), triceps skinfold (r=-0.34; p<0.001), subscapular skinfold (r=-0.34; p<0.001) and % body fat (r=-0.36; p<0.001). SOS was positively correlated with % FFM (r=0.36; p<0.001). BUA was not correlated with any anthropometric measurements.

Calcium score was significantly correlated with SOS in the whole group (r=0.17; p<0.05), and there was a tendency for the calcium score to correlate with BUA (r=0.15; p=0.056).

Using Spearman correlations, walking energy expenditure (MET hrs.wk^{-1}) was significantly correlated with SOS (r=0.20; p<0.05). There were, however, no significant relationships between SOS and any of the other physical activity parameters, or between BUA and any of the physical activity parameters.

Bivariate analyses were completed between the QUS and anthropometric measurements, within the 3 ethnic groups (Table 3.3). In the black girls, SOS was inversely correlated with subscapular skinfold (r=-0.25; p<0.05), % body fat (r=-0.24; p<0.05), and positively correlated with % FFM (r=0.24; p<0.05). In the girls of mixed ancestral origin, SOS was inversely correlated with weight (r=-0.25; p<0.05), BMI (r=-0.30, p<0.05), triceps skinfold (r=-0.32; p<0.01); subscapular skinfold (r=-0.30; p<0.05), % fat (r=-0.31; p<0.05), and positively correlated with % FFM (r=0.31; p<0.05). In the white girls, SOS was inversely related to weight (r=-0.60; p<0.001), height (r=-0.40; p<0.05), BMI (r=-0.61; p<0.001), triceps skinfold (r=-0.55, p<0.001), subscapular skinfold (-0.56, p<0.001) and % body fat (r=-0.60, p<0.001). Also, in the white girls SOS was positively correlated with % FFM (r=0.60; p<0.001). Again, BUA was not

significantly correlated with any of the anthropometric measurements in any of the ethnic groups.

Table 3.3. Pearsons correlation coefficients (r) between SOS and anthropometric measures in the three ethnic groups

	SOS (m/s)		
	Black (n=73)	White (n=40)	Mixed ancestral origin (n=64)
Weight (kg)	-0.14	-0.60***	-0.25*
Height (cm)	-0.10	-0.40*	-0.07
BMI ($kg.cm^{-2}$)	-0.14	-0.61***	-0.30*
% body fat	-0.24*	-0.60***	-0.31*
Triceps skinfold (mm)	-0.20	-0.55***	-0.32*
Subscapular skinfold (mm)	-0.25*	-0.56***	-0.30*
% FFM	0.24*	0.60***	0.31*

* $p<0.05$; ** $p<0.01$; *** $p<0.001$

Calcium score was correlated with BUA in the black girls ($r=0.23$; $p<0.05$) but not in the white girls or the girls of mixed ancestral origin.

In the black girls, SOS was significantly correlated with total MET $hrs.wk^{-1}$ ($r=0.32$, $p<0.01$), TPBSS ($r=0.31$, $p<0.01$) and TPBSS*weight ($r=0.26$, $p<0.05$). BUA was not correlated with any of the physical activity parameters in any of the ethnic groups. There were no significant relationships between any of the QUS measurements and the physical activity parameters in the girls of mixed ancestral origin or the white girls.

Various factors including age, % FFM, calcium score, total physical activity, Tanner breast stage, ethnicity, and the interaction between Tanner breast stage and ethnicity were included in the multivariate analyses in order to determine the influence of factors on SOS. The model which best predicted SOS included the following variables: %FFM, Tanner breast stage, the interaction between ethnic group and Tanner breast stage, and ethnicity. These variables accounted for 22.2% of the variance in SOS ($p<0.0001$, standard error of the estimate (SEE) =21.9, Table 3.4.). It was not possible to develop a multivariate model for BUA due to fewer factors showing a relationship with this QUS measurement.

Table 3.4. Multivariate analysis for SOS

Variable	b	β	p level
% FFM	0.291	1.13	0.0002
Ethnic group	0.134	4.31	0.52
Tanner breast stage	0.403	10.00	0.024
Ethnic group x Tanner breast stage	-0.590	-5.44	0.034

$R=0.49$, adjusted $R^2=0.22$, SEE=21.9, $p<0.0001$. β, parameter estimate; b, partial coefficient.

3.4. DISCUSSION

In this study, we demonstrated an interaction effect between ethnicity and various lifestyle factors, including physical activity, both of which have an influence on bone quality, as determined by QUS, in a group of pre-pubertal South African school girls. Although various studies have compared the BMD, as measured by absorptiometry, of black and white children in America [29] and South Africa [247], to our knowledge, no studies have compared the QUS parameters, BUA and SOS, between different ethnic groups, and in particular how these may be related to various lifestyle factors.

Several studies have suggested that BUA and SOS may reflect the characteristics of bone that are associated with bone quality, such as architecture and elasticity [116;137], rather than bone quantity, which is assessed by BMD as measured by DXA. The relationship between BMD, and age, height and weight in children has been well explained [275], and is thought to be due to the increased strain on the skeleton resulting in increased bone formation. The correlation between BUA and age, height and weight [235;315] in children of various ages is further evidence that the QUS parameters are also measuring an important aspect of bone growth. There was no correlation between BUA and age, or any of the anthropometric measurements, in our study, however we did show an inverse relationship between SOS and weight, which has been shown in previous studies [315].

Studies have shown African American girls to be taller, heavier, and to mature earlier than Caucasian girls [113], and it is well recognized that African-American children have a higher BMD than white American children [29]. Although the black, age-matched, pre-pubertal girls in our study had significantly higher BUA and SOS measurements than the white girls, they were also shorter and weighed less. The significant difference in the QUS parameters remained after adjusting for the anthropmetric differences. Physical activity has previously been shown to explain a significant amount of the variation in calcaneal QUS values [197], and in our study in which the black girls participated in a significantly greater number of MET hours of walking per week, physical activity may play a more significant role than body weight in determining the bone parameters, BUA and SOS. In addition, for the whole group, %FFM shows the strongest correlation with SOS in comparison to the other soft tissue parameters, and explains the greatest amount of variance in SOS in the multiple regression equation. Therefore, as has been shown by previous studies [234], lean body mass may be the most important soft tissue component in determining and explaining bone quality in children.

Although all of the girls included in the study were from schools within Cape Town, the physical activity profiles of the three ethnic groups were very different. Walking energy expenditure (MET hrs.wk^{-1}), and walking energy expenditure as a percentage of total physical activity, were significantly higher in the black girls compared to the girls in the other ethnic groups. Walking as a means of transport is still very high amongst previously disadvantaged communities within South Africa, and this was clearly illustrated in these results. However, despite this, total peak bone strain scores (TPBSS) were higher in the white girls compared to the black girls ($p<0.05$). This may be due to a greater level of participation in leisure time activity, such as netball, that involves higher impact movements. This was further illustrated by the fact that only 17 of the 80 black girls (21%) and 22 of the 77 (29%) girls of mixed ancestral origin included netball on their list of activities at school or after school. This is in contrast to 29 of the 41 (71%) white girls who reported that they had participated in netball within the previous year.

It has been suggested that the benefits to BMD obtained by increasing calcium intake during childhood can be maintained into adulthood [171;278;299]. Although the calcium score used in our study did not calculate daily calcium intake, it was a proxy for calcium intake, and showed a significant relationship with SOS. These results suggest that lifestyle factors such as physical activity and diet, do influence bone quality in children, although the influence within different ethnic groups may vary. Our study is the first to compare lifestyle influences on bone QUS parameters in girls of different ethnic backgrounds.

The relationship between SOS and ethnicity is, in some way, influenced by maturational stage. As this study is cross-sectional and not longitudinal in design, we can only conclude that the impact of ethnicity on the QUS parameter, SOS, may be modified at different stages of pubertal development. This supports the data of Gilsanz et al., [111] who have shown ethnic differences in BMD at different stages of development.

A limitation of the study may be that we only used one method to assess bone in children, and it has been suggested that a combination of DXA and QUS may provide a more thorough assessment of bone quality and quantity. Although QUS is portable, inexpensive and involves no radiation, this method may not be sensitive enough to detect differences in bone quantity of the growing skeleton. In our study we did try to adjust for this by co-varying for body size ie. height and weight, when doing any comparison between the ethnic groups.

This study is unique in that it is the first to assess bone quality of the three ethnic groups in South Africa, and relate the differences to lifestyle factors. We conclude that ethnicity may modulate the effects of lifestyle factors on bone status in premenarcheal girls.

CHAPTER FOUR

MATERNAL AND EARLY LIFE INFLUENCES ON CALCANEAL ULTRASOUND PARAMETERS AND METACARPAL INDICES IN 7-9-YEAR-OLD CHILDREN

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4.1. INTRODUCTION

In the previous study, we were able to demonstrate strong associations between certain lifestyle factors, such as physical activity, ethnicity and bone QUS parameters in pre-pubescent girls. In the present study, we further examined early life influences, in particular, maternal factors and the intrauterine environment, as well that of lifestyle factors, on calcaneal QUS in children of a similar age to the previous study.

Extensive research has associated low birth weight with the development of various chronic diseases such as hypertension, impaired glucose tolerance and more recently, osteoporosis [19;63;132]. In general, birth weight has been used as a proxy for intrauterine stress on the fetus, however this does not take into account various genetic and intergenerational influences. Further, there are widely varying differences in mean birth weight reported by various developed and developing countries. Despite these differences the associated adult sequelae appear to distribute along a continuum of birth weight in the various communities studied, with their associated postnatal demographic and environmental stressors.

There are multiple factors including, amongst others, birth weight and the subsequent trajectory of bone growth throughout childhood, which determine adult bone mass. Several studies, with conflicting results, have investigated the relationship between birth weight and adult bone [63;65;106;134;316]. For example, Gale et al., [106] showed a significant increase in BMC with increasing tertiles of birth weight in their sample of 143 men and women between the ages of 70 and 75 years, after adjusting for current age. Conversely, no difference in BMD was found in a study of 230 women between the ages of 20 and 23 years who were either of normal weight at birth (>2500g) or low birth weight (≤ 2500g) [134]. None of these data were adjusted for current weight or height, and the low birth weight group included premature and small-for-gestational age subjects. These potential confounders complicate the interpretation of the data.

Studies which examine the relationship between birth weight and BMD in children are potentially useful as they are able to take into account the possible confounding effects of factors such as physical activity, nutrition and socio-economic status. At present there are few such reports. In a study of children aged 8 years [165], birth weight, subsequent weight gain in the first month, and weight gain up to their current age, together accounted for 35% and 40% of the variation in LS and FN BMD respectively, after adjusting for environmental factors including breastfeeding, maternal smoking, sports participation, sunlight exposure, and calcium intake. However the contribution of these variables, except for subsequent weight gain, to BMD, was reduced when data were adjusted for maternal BMD. Although this suggests a genetic association, the contribution of subsequent growth to the variance in BMD at the age of 8 years, was still significant. Therefore, the influence of early nutrition on growth may be an important consideration in determining bone mass. What is not clear, however, is the contribution of specific nutrients to growth and bone development. Fewtrell et al., [99] showed no difference in BMC or BMD in children between 8 and 12 years of age irrespective of whether they had received banked breast milk, pre-term formula, term formula or expressed breast milk, with varying amounts of energy (kcal), protein, calcium and phosphorus. However, all of these children were preterm at birth, which introduces a number of other confounding factors, which may not be relevant to full term, small-for-gestational age infants. Similarly, Jones et al., [168] did not report a difference in BMD, after adjusting for size, lifestyle factors and socioeconomic factors, in 8-year old preterm children who were breastfed for bottle-fed. However, lumbar spine, femoral neck and total body BMD was significantly higher in children born at term who were breastfed compared to those who were bottle-fed.

The influence of maternal factors such as smoking and alcohol consumption during pregnancy, on intrauterine stress and subsequent bone health has also been examined [117;167] in a small number of studies. In 8-year old male and female children, after co-varying for current weight

and height, BMD was lower in the children whose mothers had smoked during pregnancy [167]. In addition, these children had lower birth weights, heights and weights at the age of 8 years. Similarly, new-born infants of mothers who reported smoking during pregnancy had lower birth weights and 11% lower whole body BMC than the infants of mothers who reported not smoking during pregnancy [117]. Further, neonatal BMC was associated with other maternal factors during pregnancy, such as diet and physical activity, however it was not associated with maternal age, parity or social class.

To date, the relationship between BMD in children and birth weight, controlling for environmental and maternal factors, has been examined using procedures such as DXA [63;65;106;134;316]. Quantitative ultrasound (QUS), with it's parameters of BUA and SOS, has been associated with various mechanical properties of bone including elasticity and architecture, which are not evaluated by DXA [116;137]. In addition, the metacarpal index has been associated with bone mass of the appendicular skeleton [20] and fracture risk in children [210], and is another useful means of assessing bone characteristics in a community-based sample [305;306].

Thus, the aim of this study was to investigate the possible interactions between maternal and early life influences on the calcaneal QUS parameters, BUA and SOS, and the metacarpal index, in 7-9 year old children, and how these may be influenced by socio-demographic and environmental factors.

4.2. METHODS

4.2.1. Subjects

Subjects included 61 girls and 61 boys of mixed ancestral origin from a working class community which was previously financially and politically disadvantaged, in Cape Town, South Africa. Mixed ancestral origin is a distinct ethnic group in South Africa. The subjects' mothers had participated in a nutrition and pregnancy study at the time of the subject's birth, and data on birth weight and maternal nutritional status and anthropometry were collected. The subjects in this study were part of a larger follow-up study investigating the relationship between birth weight and various measures of growth and development in children. An original cohort of 287 mothers and their children was obtained. All of these children had a gestational age greater than 37 weeks. All twins were excluded (n=3). Tracking consisted of visiting the original addresses that were recorded at the child's birth. Of the original cohort, contact was made with 129, either with the mother themselves or a direct member of the family. For another 80, forwarding details were obtained. The remainder of the cohort were considered to be lost. Reasons for this included having moved with no forwarding details, death of the child or the mother, or incorrect address details. Approximately 4 months after the commencement of tracking a follow-up letter was sent to all the families who had been contacted or required follow-up. This was to re-establish contact with the families who had been tracked and to follow-up on forwarding details. Replies were received from 53 mothers, while 9 of the letters were returned to sender. Of this cohort, 1 mother refused participation in the study. 105 mothers and their children within this sample were tested as part of the larger study.

With the advice of a statistician, all the babies born with a birth weight < 2500 g were extracted from the larger list of 1975 subjects (n=115). Tracking commenced in order to make contact with these subjects. A letter was also sent out to all of the mothers. 17 replies were received, with 4 returned to sender. 1 woman refused participation in the study. 31 mothers and their children from this sample were tested.

Sampling continued by randomly selecting every 4th name and address from the whole sample of 1974 subjects. This resulted in a sample of 307 names.

QUS testing of this sample was random depending on the availability of equipment and the time constraints of the tester. QUS data was obtained on 122 children and 94 mothers.

Children who were peri-pubertal or pubertal, based on maternal reporting of axillary hair, hair in the genital region or on the face (boys only), were excluded from further analysis (n=10; 5 boys and 5 girls). None of the children were on any chronic steroid medication. All of the children were born at term (37-42 weeks). The sample was divided according to birth weight. Birth weights >2500g were classified as appropriate for gestational age (AFA; n=83) and birth weight ≤ 2500g was classified as underweight for gestational age (UFA; n=29). Data on placental weight was not available. On analysis, three children were identified as outliers for current body weight for their age, due to z-scores > 2.5 (NCHS/WHO reference curves; [135]), and excluded from further analysis.

The study was approved by the Ethics and Research Committee of the University of Cape Town, and informed consent was obtained from the mothers prior to testing of the children. Testing took place at the local community health centre.

4.2.2. Questionnaire data

Demographic and socioeconomic data were obtained by questionnaire and included age, parent's education, housing density and a detailed asset register (see Appendix 6.5.). Details regarding developmental milestones, the child's current health, health over the past year and in the first year of life, were collected from the mother or primary caretaker. The presence of body

hair on the child, as an indicator of pubertal development, was ascertained from the mother [46]. The mother, or primary caretaker, also provided details of alcohol consumption and smoking during pregnancy and currently, as well as total months of breastfeeding and early nutritional practices. All of the mothers were asked whether they had smoked during pregnancy, and whether they were currently smoking, however no data was obtained on number of cigarettes smoked. This maternal data was not obtained for one of the children as his mother was deceased. Details of physical activity during school and after school, as well as total hours of television viewing time during the week and over weekends, were collected. Children were asked what activities they were currently participating in during school time, and where children reported an activity additional questions regarding frequency per week and duration per session were asked. This was the same for activities after school. This is adapted from the Modified Activity Questionnaire for Adolescents [3], however only current activity, and no year-round activity patterns, were assessed. Only seven children in the whole sample reported participating in organized school activities, therefore this data was not included in further analysis.

4.2.3. Anthropometry

Standing height (cm) and body weight (kg) were measured as described in Chapter 3. Sitting height (cm) was also measured in this study. Skinfold thicknesses of the biceps, triceps, subscapular and suprailiac regions were measured using Harpenden skinfold calipers according to the techniques described by Deurenberg et al., [81]. Three readings at each site were obtained and the average for each subject was recorded. All anthropometric measurements were conducted by the same fieldworker. Only the skinfold measurements for the triceps and

subscapular regions were used to calculate % body fat using the equations of Slaughter et al., [284]. FFM (kg) and % FFM were calculated as described in Chapter 3. We were unable to obtain anthropometry data for two subjects.

4.2.4. Calcaneal quantitative ultrasound

Both calcanei were measured by the same ultrasonographic densitometer (Hologic Sahara Clinical Bone Sonometer, Waltham, MA, USA) according to the methods described in Chapter 3. The results of the nondominant calcaneus were used for analysis in the children, and the results of the left calcaneus were used for analysis in the mothers. In the case where leg dominance of the child was unknown or mixed, results of the left calcaneus were used. If a result could not be obtained from the relevant side for the mother or the child, the result from the alternative side was used. The coefficient of variation (CV) of the phantom supplied by the manufacturer for the period of testing was 6.7% for BUA and 0.5% for SOS. These measures are within acceptable limits according to previous ultrasound research.

4.2.5. Metacarpal morphometry

Radiographs of the left hand and wrist were obtained from 104 subjects and used to determine various metacarpal morphometric measurements, by one examiner. These measurements, which included the metacarpal diameter (D) and medullary cavity (d), were measured at exactly halfway up the second metacarpal using a digital caliper, which was calibrated to the nearest 0.01mm. To test intra-observer reliability, the average coefficient of variation for two measurements of 15 x-rays, measured on different occasions, was 0.08% for the metacarpal

length (l), 0.86% for the metacarpal diameter, and 2.21% for the medullary cavity. Similarly, to test inter-observer reliability, the average coefficient of variation of two different testers measuring the same six x-rays was calculated. The coefficients of variation were 0.25% for the metacarpal length, 1.1% for the metacarpal diameter, and 5.8% for the medullary cavity. The measures that were calculated included the combined cortical thickness (CT=D-d) and the Barnett-Nordin index (BN=C/D x 100) of the second metacarpal bone. The equations to calculate these measures were obtained from Barnett and Nordin [20].

4.2.6. Maternal data

Height, weight and bone parameters were also measured on a sub-sample of the mothers (n=94) at the same visit. Weight could not be obtained on one of the mothers as she had given birth two weeks previously. Baseline data of the mother's weight change during pregnancy was calculated as PSW (Percentage of Standard Weight).

4.2.7. Statistical analysis

The Statsoft™ (Statistica v5.0, 1999) statistical package was used. Data are described as means ± standard deviations. Pearson's product-moment correlation coefficients were used to determine relationships between the various bone measures, birth weight and the other continuous variables. Spearmans rank-order correlation coefficients were calculated for skewed and ordinal data. Subject's birth weights were classified as appropriate for gestational age (> 2500g; AFA; n=80) and underweight for gestational age (≤ 2500g; UFA; n=29). Continuous data were compared between AFA and UFA groups and between boys and girls, using two-way analysis of variance, covarying for weight and height. Continuous data were also compared between lifestyle categorical variables, such as smoking during pregnancy and alcohol

consumption during pregnancy, using analysis of variance, covarying for weight and height where applicable.

4.3. RESULTS

Birth and current morphological characteristics of boys and girls (Table 4.1.)

Although there were no differences in age, height or weight between the boys and girls, the boys had a significantly higher ponderal index at birth, compared to the girls. There was no difference in birth weight between the boys and girls, even after co-varying for current body weight and height. Current sitting height, weight-for-age z-scores (WAZ), height-for-age z-scores (HAZ) and %FFM were significantly lower in the boys compared to the girls. There was no significant difference in the weight-for-height z-scores (WHZ) between the boys and the girls.

Table 4.1. Birth and current morphological characteristics of boys and girls

	Boys			Girls		
	Total (n=55)	AFA (n=42)	UFA (n=13)	Total (n=54)	AFA (n=38)	UFA (n=16)
Birth parameters:						
Birth weight (g)	2996 ± 531	3201 ± 427	2335 ± 165	2894 ± 521	3142 ± 384	2305 ± 265
PI (g/cm^3)*	26.3 ± 3.8	27 ± 3.7	23.5 ± 3.3	24.6 ± 3.1	25.2 ± 3.4	23.3 ± 2
Current parameters:						
Age (yrs)	8.2 ± 0.6	8.1 ± 0.6	8.5 ± 0.6	8.1 ± 0.6	8.1 ± 0.6	8.2 ± 0.6
Weight (kg)	23.5 ± 3.5	23.9 ± 3.7	22.5 ± 2.4	24.6 ± 4.3	24.6 ± 4.5	24.5 ± 3.9
Height (cm)	123.4 ± 5.4	123.3 ± 5.9	123.7 ± 3.7	125.4 ± 6.1	125.1 ± 6.1	126.1 ± 6.3
Sitting height (cm)**	63.5 ± 4.5	63.4 ± 4.6	63.9 ± 4.6	66.1 ± 4.6	66 ± 3.7	66.2 ± 6.5
$WAZ **	-0.81 ± 0.91	-0.69 ± 0.9	-1.2 ± 0.7	-0.33 ± 0.88	-0.3 ± 0.9	-0.38 ± 0.8
$HAZ **	-0.93 ± 0.92	-0.87 ± 1.0	-1.1 ± 0.7	-0.36 ± 0.98	-0.4 ± 0.9	-0.20 ± 1.1
$WHZ	-0.22 ± 0.87	-0.09 ± 0.8	-0.6 ± 0.9	-0.05 ± 1.3	0.07 ± 1.3	-0.3 ± 1.3
Waist (cm)	54.5 ± 3.3	54.6 ± 3.4	54 ± 2.8	54.1 ± 4.7	54.2 ± 5.1	53.9 ± 3.9
Hip (cm)	64.4 ± 5.3	64.8 ± 5.8	62.9 ± 3.2	66.1 ± 6.1	66.2 ± 6.2	66 ± 6.1
% BF***	13.4 ± 3.0	13.6 ± 3.0	12.7 ± 3.2	10.4 ± 2	10.5 ± 1.9	10.2 ± 2.3
% FFM***	86.6 ± 3.0	86.4 ± 3.0	87.3 ± 3.2	89.6 ± 2	89.5 ± 1.9	89.8 ± 2.3

Mean ± SD ; PI =ponderal index; waist=waist circumference; hip=hip circumference; %BF=% body fat; %FFM=% fat-free mass

$ NCHS/WHO reference curves; WAZ=weight-for age z-score; HAZ=height-for-age z-score; WHZ=weight-for-height z-score.

* $p<0.05$; ** $p<0.01$; *** $p<0.001$ differences between boys and girls

Bone measures in children and their mothers (Table 4.2.)

Neither the QUS nor metacarpal measures differed significantly between the boys and girls. For the mothers, the mean BUA was 74.2 ± 14 dB/MHz and SOS was 1547.0 ± 26.7 m/s.

Table 4.2. Calcaneal QUS measurements and metacarpal morphometry of children and their mothers

	Children			Mothers
	Total	Boys	Girls	
QUS measurements:	(n=109)	(n=55)	(n=54)	(n=92)
BUA (dB/MHz)	56.0 ± 8.5	56.5 ± 7.6	55.5 ± 9.4	74.2 ± 14
SOS (m/s)	1565.3 ± 16.7	1566.5 ± 16.1	1564.1 ± 17.4	1547.0 ± 26.7
Metacarpal morphometry:	(n=104)	(n=53)	(n=51)	
D (mm)	5.82 ± 0.55	5.84 ± 0.56	5.80 ± 0.54	-
d (mm)	3.16 ± 0.54	3.23 ± 0.51	3.10 ± 0.56	-
CT (mm)	2.66 ± 0.42	2.61 ± 0.40	2.70 ± 0.44	-
BN (%)	45.77 ± 6.70	44.82 ± 6.17	46.77 ± 7.14	-

Mean ± SD; BUA=broadband ultrasound attenuation; SOS=speed of sound; D=metacarpal diameter; d=medullary diameter; CT=combined cortical thickness; BN=Barnett-Nordin index

Morphologic and demographic factors related to bone measures in children and their mothers (Table 4.3.)

Neither BUA nor SOS correlated significantly with weight, height, sitting height, %FFM, % body fat, or any of the circumference measurements in the children. The metacarpal diameter and combined cortical thickness of the second metacarpal were significantly correlated with weight ($p<0.001$ and $p<0.01$, respectively), height ($p<0.001$ and $p<0.001$, respectively) and sitting height ($p<0.01$ and $p<0.05$, respectively) of the child. The medullary diameter was only significantly correlated with weight ($p<0.05$). The Barnett-Nordin metacarpal index was not significantly correlated with any of the anthropometric estimates of body composition.

BUA of the child was associated with two measures of socio-economic status, housing density (negatively, $p<0.05$) and mother's education level (positively, $p<0.05$). Housing density and mother's education level were also significantly negatively correlated with each other ($r=-0.26$; $p<0.05$). None of the metacarpal measures were correlated with any demographic factors.

Table 4.3. Pearsons correlation coefficients (r) between bone measures, and morphologic and demographic factors

	BUA (dB/MHz)	SOS (m/s)	D (mm)	d (mm)	CT (mm)	BN (%)
Weight	0.114	-0.128	0.434***	0.209*	0.298**	0.029
Height	0.072	-0.081	0.439***	0.189	0.332**	0.060
Sitting height	0.148	0.007	0.315**	0.126	0.249*	0.067
Housing density#	-0.221*	-0.069	-0.128	-0.116	-0.119	0.020
Mother's education#	0.219*	0.044	-0.036	0.002	-0.022	-0.028

Spearman rank-order correlation coefficient; BUA=broadband ultrasound attenuation; SOS=speed of sound; D=metacarpal diameter; d=medullary diameter; CT=combined cortical thickness; BN=Barnett-Nordin index; * $p<0.05$; ** $p<0.01$; *** $p<0.001$

Maternal SOS was inversely correlated with maternal height ($r=-0.27$; $p<0.01$), however weight was not related to any of the QUS measures. In addition, differences in parity, alcohol consumption and smoking, maternal education or housing density were unrelated to maternal bone QUS parameters.

Relationship between early life influences and bone parameters in children (Table 4.4.)

BUA was significantly positively correlated with ponderal index ($r=0.25$; $p<0.05$). However, neither the QUS, nor the metacarpal, measurements were significantly correlated with birth weight, nor were there differences in the mean BUA, SOS or metacarpal measurements between the birth weight groups, UFA and AFA, even after co-varying for current body weight and height. None of the metacarpal measures were correlated with birth weight or ponderal index.

BUA of the child was, however, significantly correlated with maternal SOS ($r=0.22$; $p<0.05$). SOS of the child was not significantly correlated with BUA or SOS of the mother.

Table 4.4. QUS and metacarpal measures of appropriate for gestational age (AFA) and underweight for gestational age (UFA) children

	AFA	UFA
QUS measurements:	(n=80)	(n=29)
BUA (dB/MHz)	56.7 ± 8.9	54.2 ± 7.4
SOS (m/s)	1564.2 ± 17.5	1568.2 ± 14.1
Metacarpal morphometry:	(n=76)	(n=28)
D (mm)	5.80 ± 0.53	5.86 ± 0.59
d (mm)	3.15 ± 0.54	3.20 ± 0.52
CT (mm)	2.65 ± 0.41	2.67 ± 0.46
BN (%)	45.87 ± 6.72	45.50 ± 6.76

Mean ± SD

The various correlations between the QUS parameters, BUA and SOS, and the various body composition, socio-demographic and lifestyle parameters are presented in Fig 4.1.

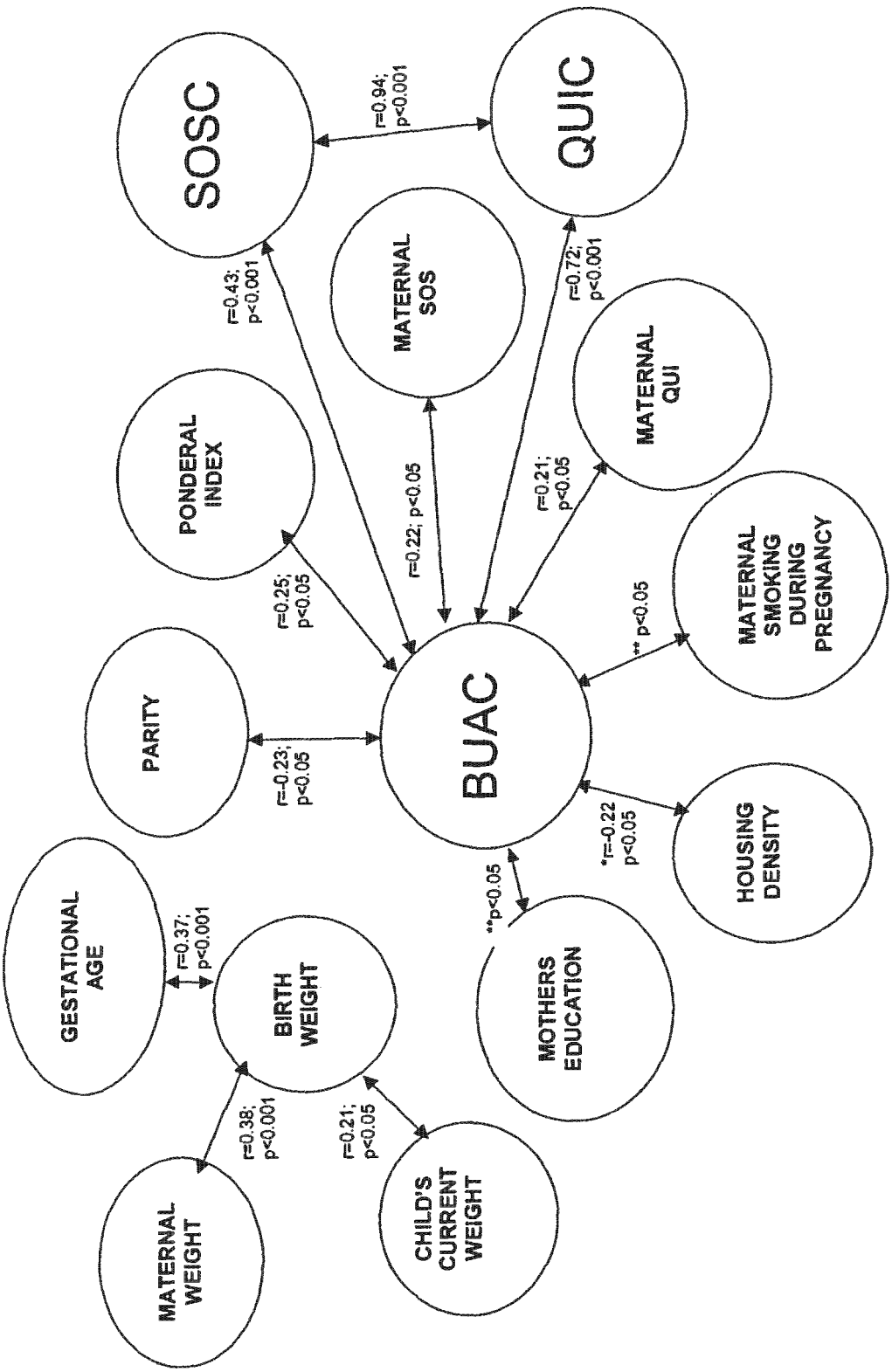

Figure 4.1. Significant correlations between BUA and other variables measured in this study

* Spearman's correlations; ** ANCOVA, co-varying for child's current weight and height

Forty-seven (43.5%) children were born to mothers who smoked during pregnancy. These children had a significantly lower BUA than the children of non-smoking mothers (54.2 ± 8.2 vs 57.4 ± 8.6 dB/MHz; $p<0.05$) (Figure 4.2). There were no differences in weight or height between these two groups of children. In addition, neither of the QUS parameters were correlated with weight, height or sitting height, therefore results were not co-varied for height or weight. There was, however, no association between maternal smoking and birth weight, or maternal smoking and any of the metacarpal measures.

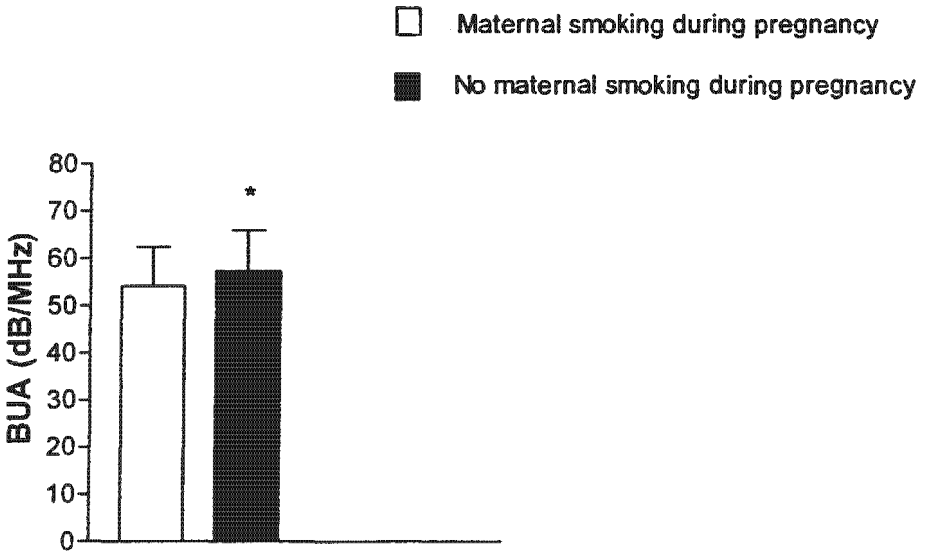

Figure 4.2. BUA of children whose mothers smoked or did not smoke during pregnancy.
* $p<0.05$

Eighteen (16.7%) of the mothers reported consuming alcohol during pregnancy. In their children, the Barnett-Nordin metacarpal index was significantly lower than in the children whose mothers did not consume alcohol during pregnancy (42.17 ± 4.41 vs 46.56 ± 6.90 %; $p<0.05$). There was no difference between the groups for weight or height. As the metacarpal measures were correlated with weight, height and sitting height, these results were co-varied for weight and height, however the relationship did not change.

For the whole group, the total television viewing time on a typical week-day was 122 ± 93.6 mins, and on a typical week-end day was 95 ± 134.1 mins. None of the children reported structured school-based physical activity, and there was little variability of respone in physical activity reporting. There were no associations between any of the QUS or metacarpal measurements, and physical activity or inactivity levels in this sample.

4.4. DISCUSSION

Calcaneal QUS in children has not been as extensively researched as it has in adults, however it has been postulated to measure important properties of bone. BUA signals depend on trabecular orientation and BUA is 50% higher along the axis of compressive trabeculae, the site at which weight-bearing is at its highest [115]. SOS has also been shown to be dependent on direction of the trabeculae and is associated with bone density and elasticity [137]. Previous studies have demonstrated a good correlation between QUS and BMD in children [235], and adults [119]. In addition, some studies have found relationships between growth parameters, age, height and weight, and BUA in children [235;315]. The BUA and SOS values for the girls included in our study were similar to those obtained from a previous study (Chapter 3) on pre-adolescent girls from the same community, as well as reference data for 9 year-old girls living in Germany (56.4 ± 10.2 dB/MHz and 1574.4 ± 24.8 m/s) [315]. However when the boys in our study were compared to the reference data for 9-year-old boys provided by Wunsche et al., [315], BUA of the boys of mixed ancestral origin was 7.8% lower than the reference database.

Birth weight and ponderal index have been traditionally used as measures of intrauterine stress in in the last decade, birth weight has been studied in relation to fetal programming and the adult onset of various diseases such as hypertension [18]. However, intrauterine events may cause programming independent of an effect on birth weight. Although our study did not find an association between birth weight and any of the QUS parameters, there was a significant

correlation between BUA and the ponderal index at birth. Indeed, ponderal index may be more helpful in examining prenatal events and their effect on bone development. As the long bones are formed during the third trimester, any insult during this time may jeopardize the development and quality of these bones. Therefore, ponderal index provides a proxy for reduced fetal growth, and it's association with BUA in 7-9 year old children suggests that intrauterine stress during pregnancy may play a role in determining bone properties, such as micro-architecture, in children.

Nearly half of the children in this sample were born to mother's who smoked and just less than 20% of the children were born to mother's who consumed alcohol during pregnancy. Previous research in this community has reported a similar prevalence of alcohol consumption [68], which is nearly double that reported in the USA (42.8% vs. 25%). Although we did not find a relationship between birth weight and maternal smoking during pregnancy, there was an association between maternal smoking during pregnancy and decreased BUA. These results support the findings of Jones et al., [167] and Godfrey et al., [117] in 8-year-old children and neonates, respectively. The effect of smoking during pregnancy on offspring bone has now been found using two different methods of measuring bone structure, namely, DXA and QUS.

The consistent relationship between maternal smoking and bone, despite the inconsistent relationship between smoking and birth weight, suggests that the influence of maternal smoking during pregnancy on bone in children may be independent of birth weight. Thus, maternal smoking during pregnancy may programme certain systems in the fetus that are particularly relevant to bone development, resulting in altered properties of bone in the offspring. Extensive literature has demonstrated lower BMD and a higher hip fracture incidence in smokers compared to non-smokers [11;32;66;108;222]. However, the mechanisms underlying these differences are not clearly understood, however it has been suggested that smoking may result in a reduction in calcium absorption [47;283], an increased turnover of estradiol [32] and reduced bone formation [32].

Alcohol consumption during pregnancy has also previously been associated with an adverse fetal environment, and consequently, low birth weight [311]. Similar to the effect of smoking, in our study there was no relationship between alcohol consumed by the mother during pregnancy, and birth weight of the child. However the Barnett-Nordin metacarpal index was lower in the children of mothers who consumed alcohol during pregnancy compared to the children whose mothers did not consume alcohol during pregnancy. This may again suggest some measure of intrauterine programming of the mechanisms responsible for decreased bone mass in 7-9 year old children, independent of birth weight, in response to alcohol consumption of the mothers during pregnancy. These mechanisms may include decreased bone formation, as well as disruption of vitamin D and calcium metabolism.

Smoking and alcohol consumption during pregnancy may influence different properties of bone in the offspring. The measurement of the metacarpal index from a standard x-ray film has been shown to be a reliable means of assessing appendicular cortical bone mass, and is also well correlated with absolute bone mass [20]. It appears that these properties of bone are different to those that have been shown to be measured by calcaneal QUS, which reflects trabecular orientation and structure, as well as elasticity and density of the bone [116;137]. In our study, there was no relationship between smoking and the metacarpal index, and in addition, the metacarpal and QUS measures were not correlated.

Smoking and increased alcohol consumption have also been associated with socioeconomic status [87;109]. Our finding of an association between BUA and housing density, as well as mother's education, suggest that socio-economic factors influence the mechanical properties of bone that are measured using QUS. A high housing density and a lower standard of education may be associated with a more challenging nutritional and lifestyle environment that is not conducive to optimal bone health. This may include lower intakes of calcium and protein in this

community as well as reduced physical activity due to the lack of safety of recreational play. No studies on the calcium intake of this South African community have been done, however Eyberg et al., [92] have reported low calcium intakes in rural black South African children. Contrary to the results of the previous chapter, there was no association between physical activity or inactivity, and the various bone measurements. This may be due to the very small variation in the structured school and after-school activities that were reported, as all of these children were in their first year of primary school and physical education and school sport is only introduced in the second school year. In addition, we did not show a significant relationship between %FFM and SOS, as was shown in the previous chapter. This may be due to the slightly younger age of this sample, as well as the inclusion of boys and girls.

Our findings of a relationship between maternal and child bone QUS parameters support those of Jones et al., [166]. They showed that when maternal BMD was included in a model to predict LS and FN BMD, in a sample of 8-year-old prepubertal children, the early growth factors including birth weight, birth length and length gain in the first month were no longer significant. Another study by Jones et al., [166] on the same sample of children showed that maternal osteopenia was associated with a significant reduction in bone mass at all the sites in the children.

This study provides evidence that maternal and early life factors influence the various bone properties measured and assessed by calcaneal QUS and metacarpal morphometry, in children between the age of 7 and 9 years. These findings also suggest that a number of socio-demographic factors may exacerbate these influences. Further research investigating the influence of smoking and alcohol intake on the various properties of bone in neonates, prior to the influence of other environmental factors, is recommended. This research has particular relevance in a community in which smoking and alcohol consumption is high, even in pregnancy. An improved understanding of underlying mechanisms may help in creating public awareness and motivate for behaviour change in this group of women.

CHAPTER FIVE

LIFESTYLE QUESTIONNAIRE TO EVALUATE RISK FOR REDUCED QUANTITATIVE ULTRASOUND MEASUREMENTS IN WOMEN

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5.1. INTRODUCTION

Our previous research in this dissertation has demonstrated that physical activity interacts with various demographic and lifestyle factors at an early age to influence QUS parameters in children. We have also shown that lifetime physical activity is associated with BMD in adults, however there is a paucity of data on the relative contributions of all of these factors on the bone health of adults. The prevalence of osteoporosis in South Africa is unknown, however by understanding the demographic determinants, early screening programmes may be implemented in order to increase education and reduce the risk of this lifestyle-related disease, which results in an increased risk of fracture, causing increased morbidity and mortality.

A single BMD measurement has been shown to be predictive of future fracture risk [154], however recent research has shown that a multidimensional approach to stratifying hip fracture risk is most effective [199]. The US National Osteoporosis Foundation has recommended all white women over the age of 65 years have a BMD measurement to assess their risk of osteoporosis [2]. The South African National Osteoporosis Guidelines support the recommendations of the World Health Organisation [1] that suggest screening of women over the age of 60 years. However, the high cost and low availability of BMD scans does not allow screening to be a practical means of assessing future risk of osteoporosis and fracture in many developing countries. Use of a questionnaire as a means of screening for compromised bone health takes into account the influence of various lifestyle factors on the bone, and may be helpful in identifying those individuals for whom it would be prudent and cost-effective to have a BMD scan.

For example, maternal and paternal history of osteoporosis is associated with a 1.5-2 fold greater risk of osteopenia in women [289]. In addition, the significant correlation in BMD between monozygotic (r=0.92) compared to dizygotic (r=0.36) twins [254], and the results of mother-daughter studies [97;208;218;282], are evidence that genetic inheritance makes a significant contribution to the development of peak bone mass. In the study by Ferrari et al., [97] the

proportion of the variance in BMD that was attributable to genetic factors, in a sample of 8-year-old pre-pubertal girls and their mothers, was 33 and 36% at the LS and the PF, respectively. These results suggest that a large portion of the unexplained variance in bone mass must be determined by other environmental factors, such as physical activity and nutrition. Although research has shown that a significant amount of adult bone mass is determined around the age of puberty [16;275;276], it is also important to evaluate factors that may influence bone during adulthood, at a time after which peak bone mass has been attained, in order to ensure optimal bone health in the future by controlling the factors that influence bone loss.

The lifestyle factors that have been shown to influence adult bone include current and previous physical activity, anthropmetric measures, calcium intake, smoking, caffeine and alcohol consumption. The role of current physical activity in promoting bone health in adults has been extensively investigated using DXA [11;67;173;255;263;296], often in combination with QUS [36;44;296]. In addition, the role of previous participation in physical activity has been investigated in a study by Fehily et al., 1992 [95], who found that both sports activity during adolescence and body weight explain 14% of the variance in radial BMD in their sample of 371 men and women between the ages of 20 and 23 years who were followed up over a period of 14 years. In a study by Mazess and Barden, [222] who measured BMD in a young adult population (20-39 years), height and weight significantly contributed to 4.9-17.6% of the variance in BMD of various sites. In that study [222] current calcium intake did not contribute to the variance in BMD in young adults, however in a similar study [294] in postmenopausal women, current calcium intake and physical activity contributed 20% and 25% of the variance in LS BMD, respectively. In addition, it has been consistently demonstrated [222;229] that smokers have a significantly lower BMD than non-smokers. Ilich et al., [157] investigated the influence of various lifestyle factors on BMD in a sample of 136 Caucasian women (mean age 68.6 ± 7.1 years) and showed smoking to have no effect on BMD, however the combination of alcohol, calcium intake, caffeine and smoking exposure accounted for 53.3% and 56.1% of the variance in TB and LS BMD, respectively. Although caffeine

showed a significant negative effect on BMD, the effect of alcohol was positive. Other studies have shown an inverse relationship between alcohol intake and BMD [95] and an age-adjusted relative risk of hip fracture of 2.35 (95% CI=1.02-5.41) in women who reported a moderate intake of alcohol compared to women who did not consume alcohol [147].

Combining these lifestyle factors into an assessment tool may be a useful and cost effective means of assessing risk of low BMD and possible risk of fracture. Several studies [34;49;51;76;118;188] have designed models to predict osteoporosis and future risk of fracture, however these have specifically focused on older adults due to the prevalence of osteoporosis increasing with age. Only one study [138] has attempted to identify younger women at risk of osteopenia, with the aim of early detection, in order to minimize the future risk of osteoporosis.

The capacity of QUS to quantify bone has been demonstrated by it's significant correlation with BMD, as measured by DXA, in adults [9;119;292;296]. Other studies have found QUS to be useful in predicting women with osteopenia and/or osteoporosis [26;27;40;41;136], and suggest that QUS may play a complementary role in determining an individuals risk of osteoporosis.

Therefore, the aim of this study was to examine the relationship between various lifestyle factors, including current physical activity, and adult bone properties, as determined by QUS, in a self-selected sample of South African women of various ages. In addition, the study aimed to design a tool that is applicable to younger, as well as older, adults in order to assess risk of low bone mass at an earlier age, to ensure sufficient time to incorporate preventative lifestyle measures.

5.2. METHODS

5.2.1. Subjects

The study evaluated the relationship between risk factors for osteopenia and measures of estimated bone parameters using calcaneal QUS. The sample comprised 220 volunteers who completed a questionnaire and underwent calcaneal QUS testing as part of a public health screening day at a local centre comprising a fitness centre, conference centre and administrative offices for various businesses and sporting codes.

5.2.2. Medical history and lifestyle factors

All participants completed a questionnaire designed to obtain information on risk factors for bone health (see Appendix 6.6). This questionnaire was either administered by the health professional on duty or completed by the subject themselves, under the supervision of the health professional. Questions included:

History of osteoporosis: personal and family history of osteoporosis, and personal history of hip, vertebra or wrist fracture over the age of 50 years.

Current physical activity: Subjects reported on frequency of current physical activity, as well as occupational physical activity (if applicable).

Historical physical activity: Historical information was obtained on levels of leisure-time physical activity for 4 age epochs, 14-21, 22-34, 35-50, and more than 50 years of age. The portion of the questionnaire was adapted from that of Kriska et al., [190]. Only activities over 3 METs (metabolic equivalents) according to the classification of activities by Ainsworth et al., [8], were included. The method used to convert the physical activity data to average Met hrs.wk^{-1} for each epoch has been described in more detail in Chapter 2. An impact score was also calculated for each epoch, based

on the peak strain score for each activity developed by Groothausen et al., [125], and is also described in Chapter 2. A lifetime MET score, as a measure of energy expenditure, and a lifetime IMP score, as a measure of impact activity, were calculated for each individual by weighting the epoch scores according to the number of years the individual spent in each epoch.

Additional questions were asked regarding occupational work activity and frequency of current physical activity patterns.

Calcium intake: Subjects recorded their use of all calcium supplements, as well as how regularly they consumed them and over what period of time they had been taking the supplement. Calcium supplements were then divided into those that contained less than 100mg of calcium, and those that contained 100mg of calcium or more. Subjects were also asked about their daily intake of dairy servings (1 dairy serving = glass of milk, 175ml carton of yoghurt).

Alcohol and caffeine intake: Subjects were asked whether they consumed alcohol on a daily basis, and if so, how many drinks (tots, beers or glasses of wine) per day. Information about daily intake of caffeinated coffee was also obtained.

Smoking: Smoking status was noted for all subjects, including the number of cigarettes smoked. If the subject reported having given up smoking within the last 10 years, they were considered a current smoker [66].

Menstrual and reproductive information: Subjects reported the age and number of years of any menstrual irregularities. Menopausal status, number of pregnancies and total months of breastfeeding were recorded. Current use, duration of use and type of oral contraceptive pill was recorded, and similarly, current use, duration of use and type of hormone replacement therapy (HRT) was recorded. The type of oral contraceptive was divided into 3 categories: (i) tri-phasic and

mono-phasic pills containing gestodene and ethinylestradiol; (ii) pills containing progesterone; (iii) pills containing cyproterone acetate and ethinylestradiol. However, to our knowledge no research has compared the effect of the different formulations of the oral contraceptive pill on BMD. Hormone replacement therapies were divided into 3 categories according to the literature on their therapeutic value with regard to preserving bone mass and preventing bone loss. Due to the lack of scientific literature on homeopathic therapy, these women were excluded from this analysis. The categories were: (i) transdermal oestrogen, oral oestrogen and combination oestrogen-progesterone [56]; and oestrogen gel [150]; (ii) progesterone cream [198]; (iii) progesterone [307].

Corticosteroids: Subjects were required to record long-term (> 6 months) use of corticosteroids.

5.2.3. Calcaneal ultrasound and anthropometric measures

The left calcaneus was measured by the ultrasonographic densitometer (Hologic Sahara Clinical Bone Sonometer, Waltham, MA, USA) to obtain the following measurements: Broadband Ultrasound Attenuation (BUA; dB/MHz) and Speed of Sound (SOS, m/s). The methodology used to measure calcaneal QUS has been described in detail in Chapter 3. BUA and SOS were used to calculate estimated bone mineral density ($g.cm^{-2}$), and the T-score. The World Health Organisation criteria [1] for the classification of osteoporosis and osteopenia, using DXA, is currently being applied to QUS, and has been used in this study. This method of classification is based on the T-score (the subject's BMD expressed in relation to the young adult reference mean). The main outcome measure for this study was osteopenia (≤-1 SD below the mean for young US women).

The interviewers measured weight and height and we calculated body mass index (BMI) as weight (kg) divided by height (m) squared.

5.2.4. Statistical analysis

Data were analysed using Statistica v5.0 (Statsoft™, 1999), Microsoft Excel XP and NCSS 2001 (Jerry Hintze, 2003). Means and standard deviations are presented for numerical variables. Frequency distributions of the categorical variables are given. Analysis of variance, covaried for age, was used to compare estimated BMD between categorical data. In addition, logistic regression analyses were used to determine the odds ratios (with 95% confidence intervals) of being osteopenic (T-score < -1SD) against not being osteopenic for all variables. Because age was the most important risk factor observed, and highly correlated with many of the other risk factors, age-adjusted odds ratios are also presented.

Pearson and Spearman correlations were also calculated to investigate the relationship between the variables and estimated BMD, however these results showed similar relationships to those using the odds ratio results and therefore are not included in this paper. Age, weight, height and body mass index were also categorized to calculate the odds ratio of being osteopenic, however as the results were similar to those reported when the data is expressed continuously, only odds ratios of being osteopenic for all the variables, and ANCOVA's of BMD for the categorical variables are included in this paper.

Logistic regression analyses were used to derive a simple algorithm to predict osteopenia based on age and current physical activity per week, the two variables which were most significantly associated with the QUS parameters, and which could be easily obtained from a questionnaire. The regression coefficients for age and physical activity were converted to whole numbers by multiplying them by 100. To calculate the score for each person, the regression coefficient (*100) was multiplied by the subject's response for each variable and added to the total. A receiver operating characteristic (ROC) curve was generated, and the area under the curve (AUC) was

inspected in order to determine the sensitivity, specificity and positive predictive value (PPV) at various cutoff points.

5.3. RESULTS

Subject characteristics

The original sample comprised 220 subjects who volunteered for calcaneal QUS measurements at a local centre, as part of a free health risk factor screening service. Nineteen subjects were excluded due to incomplete data. Members of ethnic backgrounds other than Caucasian (n=13), and women who had been diagnosed with osteoporosis (n=21), were excluded due to inadequate representation for purposes of statistical analysis. The final convenience sample consisted of 167 Caucasian women with an average age of 51.2 ± 12.8 (SD) years. As all women were volunteers for this screening procedure for osteoporosis, they are not likely to be representative of women in general, as they may be more proactive with regard to their health than other women of the same age in the general population.

Descriptive statistics

The physical characteristics of the sample are presented in Table 5.1. The age of the subjects ranged between 19 and 79 years. The mean weight of the sample was 64.8 ± 11.7 kg and the mean height was 165.1 ± 8.6 cm.

Thirty-seven (22.2%) of the subjects reported a family history of osteoporosis.

Table 5.1. Physical characteristics

Characteristics	Mean ± SD	Odds ratio (95% CI)	Age-adjusted OR (95% CI)
Age (years)	51.2 ± 12.8	1.031 (1.005-1.059)*	
Weight (kg)	64.8 ± 11.7	1.023 (0.995-1.052)	1.020 (0.992-1.050)
Height (cm)	165.1 ± 8.6	0.996 (0.958-1.035)	1.005 (0.966-1.045)
BMI ($kg.m^{-2}$)	24 ± 4.7	1.066 (0.992-1.145)	1.052 (0.976-1.133)

* statistically significant (5%) odds ratio for osteopenia; $p<0.05$

The reproductive characteristics of the sample are presented in Table 5.2. Age-adjusted odds ratios are presented for all continuous reproductive characteristics, and categories are only included to more clearly define the population. One hundred and twenty-eight of the women had been pregnant and had an average of 2.5 children. Total months of breastfeeding, only in those women who had given birth, are summarised in Table. 5.2. Twenty-five of the women had experienced menstrual irregularities, however the average age of the women who had experienced menstrual irregularities was 42 ± 12 years compared to the women who reported no history of menstrual irregularities (52.6 ± 12.3 years; $p<0.001$), therefore estimated BMD was partially confounded by age in this group. Five women did not record how many years they had experienced menstrual irregularity. Odds ratios are presented for menopausal and post-menopausal status, with pre-menopausal as the reference condition.

Table 5.2. Reproductive characteristics

Characteristic		No. of women	%	Age-adjusted OR (95% CI)
Number of pregnancies				0.847 (0.632-1.136)
	None	33	19.8	
	1-2	77	46.1	
	> 2	51	30.5	
Total months of breast-feeding				1.006 (0.973-1.040)
	<1	20	15.6	
	1-6	23	17.9	
	7-12	30	23.4	
	13-24	38	29.6	
	25-48	17	13.2	
Years on oral contraceptives				0.770 (0.220-2.690)
	0	149	89.2	
	≤ 10	12	7.0	
	>10	6	5.6	
Years on hormone replacement therapy				0.695 (0.339-1.423)
	0	110	69.6	
	≤ 10	38	24.0	
	> 10	10	6.3	
Total years of menstrual irregularity				0.403 (0.126-1.290)
	0	138	87.3	
	≤ 10 years	13	8.2	
	> 10 years	7	4.4	

Table 5.2. cont.

Characteristic		No. of women	%	Age-adjusted OR (95% CI)
Menopausal status	Pre-menopausal	65	38.9	Reference
	Menopausal	20	12.0	0.494 (0.144-1.692)
	Post-menopausal	78	46.7	0.783 (0.268-2.291)

* statistically significant (5%) odds ratio for osteopenia; $p<0.05$

Descriptive statistics of the lifestyle characteristics and physical activity data are presented in Table 5.3. As described above, the age-adjusted odds ratios are presented for the continuous data, which has only been categorized for descriptive purposes. The average MET score for the subjects was 11.7 ± 14.5, while the average IMP score was 2.9 ± 3.5. Seventy-four women (44.3%) reported consuming alcohol on a daily basis, and 35 women (21%) reported currently smoking, or had smoked within the last ten years. One hundred and fifty women (90%) reported consuming coffee on a daily basis. Sixty-one women (36%) reported consuming supplements, however only 23% were consuming supplements containing 100mg or more of calcium.

Table 5.3. Lifestyle and physical activity characteristics

Characteristics		No. of women	%	Age-adjusted OR (95% CI)
Current physical activity	No physical activity	29	17.4	Reference
(times/wk):	1-2	45	26.9	0.691 (0.266-1.796)
	≥ 3	92	55.1	0.290 (0.120-0.702)*
MET score:				0.967 (0.938-0.997)*
	0	25	15.0	
	1-5	45	26.9	
	6-14	49	29.3	
	>14	48	28.7	
IMPACT score:				0.933 (0.838-1.038)
	0	31	18.6	
	0-1	33	19.8	
	1-4	54	32.2	
	> 4	49	29.3	
Consumption of alcohol:	No	96	57.5	Reference
	Yes	71	42.5	0.765 (0.398-1.474)
Smoker:	No	144	86.2	Reference
	Yes	23	13.8	1.097 (0.499-2.413)

Table 5.3 cont.

Characteristics		No. of women	%	Age-adjusted OR (95% CI)
Current calcium supplementation	None	85	58.2	Reference
	Supplement < 100mg	22	15.1	0.589 (0.206-1.683)
	Supplement ≥ 100mg	39	26.7	0.871 (0.388-1.953)
No.of dairy servings/day	0	12	7.5	Reference
	1-2	98	60.9	2.202 (0.540-8.983)
	2-3	39	24.2	2.163 (0.487-9.608)
	>3	12	7.5	1.252 (0.188-8.361)

* statistically significant (5%) odds ratio for osteopenia; $p<0.05$

Two (1.2%) women reported a previous fracture of the wrist, hip or vertebrae over the age of 50 years, and 10 (6%) subjects reported previous use of inhaled steroids for more than 6 months.

Descriptive statistics for all the QUS parameters are presented in Table 5.4. Four women (2.4%) were classified as osteoporotic (T-score < -2.5 SD), while 55 women (32.9%) were classified as osteopenic (–2.5 SD < T-score < -1 SD), and 108 (64.7%) women had a T-score > -1SD.

Table 5.4. QUS parameters

	Mean ± SD	Range
Estimated BMD ($g.cm^{-2}$)	0.526 ± 0.135	0.255-0.921
QUI (%)	95.2 ± 21.4	52.4-157.7
BUA (dB/MHz)	73 ± 19.3	33.5-130.4
SOS (m/s)	1551.8 ± 35.3	1466.5-1646.8

BMD: bone mineral density; QUI: quantitative ultrasound index; BUA: broadband ultrasound attenuation; SOS: speed of sound

Estimated BMD and physical characteristics (Table 5.1.)

The only physical characteristic that significantly predicted an increased risk of osteopenia was age with an odds ratio of 1.03 (95% CI: 1.005-1.059; $p<0.05$). This suggests that the odds of being osteopenic are increased by 3% for every year of increasing age. The odds ratios for increased risk of osteopenia based on differences in weight, height and body mass index were not statistically significant.

Estimated BMD and reproductive factors (Table 5.2.)

Although estimated BMD was higher in subjects who reported previous menstrual irregularities compared to those who had not experienced menstrual irregularities (0.586 ± 0.132 vs. 0.512 ± 0.132 $g.cm^{-2}$; $p<0.01$), this relationship was no longer significant after co-varying for age. There was no significant difference in any of the bone parameters for subjects taking HRT or oral contraceptives compared to those who were not. With and without co-varying for age, estimated BMD was not significantly different between the three categories of current menopausal status. These conclusions were confirmed by the odds ratios, which were not statistically significant. We also found no relationships between parity, breastfeeding or any other reproductive factors and estimated BMD.

Estimated BMD and history of osteoporosis

After controlling for age, there was no statistically significant difference in estimated BMD between the subjects who reported a family history of osteoporosis and those with no family history. This is confirmed by the odds ratio of 1.202 (95% CI: 0.561-2.574). After controlling for age, the odds ratio was increased to 1.350 (95% CI: 0.619-2.945). However, neither of these odds ratios were statistically significant.

Estimated BMD and lifestyle and physical activity factors

Estimated BMD was significantly different with respect to the physical activity categories, even after co-varying for age. After post-hoc analysis, estimated BMD was lower in the group who reported participating in no current physical activity compared to the group who reported participating in physical activity three times a week or more (0.481 ± 0.023 vs. 0.544 ± 0.013 $g.cm^{-2}$; $p<0.05$). Further, the age-adjusted odds ratio for being osteopenic was significant for current physical activity ($\geq$ 3 times/wk) compared to no physical activity (OR: 0.290; 95% CI: 0.120-0.702; $p<0.05$). This suggests that if current physical activity is equal to, or greater, than 3 times a week the odds of being osteopenic are approximately 30% of what it would be if there was no current physical activity. After controlling for age, the odds ratio for lifetime MET score was also significant (OR: 0.967; 95% CI: 0.938-0.997; $p<0.05$). Odds ratios for energy expenditure (MET $hrs.wk^{-1}$) and lifetime IMP (peak strain) score during the various epochs were determined, however, only energy expenditure during epoch 3 was significantly associated with increased odds of being osteopenic (OR:0.950; 95% CI: 0.914-0.988; $p<0.05$). Occupational physical activity was not associated with estimated BMD.

No significant differences in estimated BMD were found between subjects who did and did not consume calcium supplements. Nor was there an association between any of the bone parameters and dairy intake of calcium. Although the odds ratio for the number of dairy servings consumed per day suggests an increased risk of osteopenia with increased consumption of dairy products, the

confidence intervals are large and therefore should not be over-interpreted. There was also no significant difference in any of the bone parameters between women who did or did not consume alcohol or coffee. Nor was there a significant difference in estimated BMD between smokers and non-smokers.

Score function for osteopenia

Age and current physical activity per week were included in a model to predict osteopenia. To calculate a basic osteopenia score, age (yrs) was multiplied by 3 (Beta = 0.0294*100). This is then added to the subject's response to frequency of physical activity. If current physical activity is once or twice a week, -37 is added (Beta = 0.3692*100), and if current physical activity is three or more times a week, -124 (Beta = -1.2377 * 100) is added to the total score.

A score of 50, or greater, correctly identified 60% (100/166) of women with or without osteopenia in this sample. The sensitivity for this model was 75% (44 women out of 59 obtained scores of 50 or more and were correctly classified as being osteopenic) and the specificity was 52% (56 women out of 107 were correctly classified as not being osteopenic).

If a cutoff score of 100, or greater, was selected, 67% (111/166) of women were correctly identified with or without osteopenia in this sample. The sensitivity for this model was 58% (34 women out of 59 were correctly classified as being osteopenic) and the specificity was 72% (77 women out of 107 were correctly classified as not being osteopenic).

5.4. DISCUSSION

In the present study we found that age and current physical activity were able to correctly classify the osteopenic status, based on calcaneal QUS measurement, of a significant number of women within a relatively small convenience sample. Although it is still unclear as to exactly when peak

bone mass is achieved [191;244;263;275], our finding that BMD is reduced by 3% with every year of increasing age in a group of women between the ages of 19 and 79 years corroborates the findings of other studies which show a reduction in BMD with increasing age [48;267]. Although the results of the study by Riggs et al., 1981 [267] also showed a reduction in BMD with increasing age, the rate of bone loss was 0.0092 g/cm^2 per year (approximately 0.6-1.2% per year), which is significantly less than the loss shown in the current study. The age of their subjects was similar to that of the present study (20-89 years), however men and women were included in their sample and the inclusion of men may attenuate the annual rate of bone loss when the sample is combined.

The attainment of peak bone mass, as well as the bone loss that occurs after peak bone mass has been achieved, may be influenced by several environmental factors. Several cross-sectional studies have shown a significant correlation between BMD and habitual physical activity as measured by activity monitors [11;263], questionnaires [67;173], and physical fitness [255]. There is now a substantial body of evidence showing a strong and significant relationship between current physical activity and BMD in adults, however there is limited information comparing physical activity to other lifestyle factors in assessing risk of osteopenia or osteoporosis. Koh et al., [188] included time spent in recreational physical activity as a potential variable in their development of a risk assessment tool for osteoporosis in an Asian population, however this variable was not included in the final model as it was not found to be related to BMD. Similarly, in the study by Goemaere et al., [118] the lifestyle variables, including frequency of physical activity, reduced the diagnostic performance of their self-administered questionnaire to identify subjects with postmenopausal osteoporosis. In contrast to these findings, a study by Hawker et al., [138] assessed risk factors for low bone mass in premenopausal women between the ages of 18 and 35 years, and found low body weight, menarche at age 15 years or later, and physical inactivity as an adolescent to be significant predictors of low bone mass. In our study we obtained extensive information on past and current physical activity including leisure-time activity, occupational activity and distance walked on average each week. A question regarding frequency of current activity per week, within

a self-administered questionnaire format included in our study, showed that any physical activity on a weekly basis was beneficial in reducing the risk of osteopenia, however participating in physical activity three or more times a week was significantly more beneficial. Further, we found that the risk of osteopenia in women who participated in physical activity on a regular basis was only 30% of the risk in women who did not participate in any physical activity. These data were confirmed by the significant odds ratio for the average lifetime energy expenditure score, which can be interpreted as a reduced risk of osteopenia of 3% for every increase in energy expenditure of one MET hour per week. A 30 minute moderately-paced walk, twice per week is equivalent to an energy expenditure of approximately 4 MET hours.wk^{-1}.

In Chapter 2 we reported a relationship between historical physical activity and current BMD in a group of socio-economically disadvantaged women, however this relationship was not significant in the present study. This may be due to the different demographic profiles of the populations sampled in the two studies. Most women (85%) in the present study reported that they had participated in leisure time activity at some time in their life. This is in contrast to the women in the previous study (Chapter 2) who reported participating in a significant amount of walking as a means of transport during adolescence, and very little leisure time activity at any time during their life. In addition, in the previous study historical physical activity information was obtained by a trained interviewer. This may be necessary to ensure the accuracy of the information obtained, and may not be applicable to a self-administered questionnaire format.

Several studies have combined other lifestyle risk factors into a tool which can be used to assess various risk factors for osteoporosis [51;118;188] and hip fracture [34;70;76]. The variables included in the model developed by Black et al., [34], called the FRACTURE index, include total hip BMD, weight < 57kg, history of fracture after age 50 years, history of maternal hip fracture after age 50 years, use of the arms to stand up from a chair, and current smoking. The sensitivity of this model was 78.6% and the specificity was 61.7%. Cadarette et al., [51] have designed a 3-item

Osteoporosis Risk Assessment Instrument (ORAI) with a sensitivity of 93.3% and specificity of 46.4%. Although the algorithm is only based on age, weight and current estrogen use, it has been designed to help clinicians identify women at risk of osteoporosis, and a score of 9 or greater identified 90% of women with a BMD T score ≥ 2SD below the mean. In addition, all of these studies, with the exception of one [138], were designed for older adults. Although the prevalence of osteoporosis does increase with age, the risk of osteopenia may also be associated with future risk of osteoporosis, and should ideally be assessed at a young age. We included many of the previously associated risk factors for reduced BMD in our model however, only age and physical activity made a significant contribution to the variance in estimated BMD. Therefore, this study provides a tool that only consists of 2 variables which is easily accessible to women of all ages, and stresses the importance of considering physical activity when assessing osteoporosis or osteopenia risk.

Several studies that have developed tools for assessing osteoporosis and hip fracture risk have found body weight to be a significant contributor to the variance in BMD as measured by DXA [34;49;51;118;138;188;209;281]. Other studies have reported a significant positive relationship between QUS parameters and body weight [120], and body mass index [36]. Our study did not find a relationship between body composition and estimated BMD, which may be a consequence, in part, of a smaller sample size. It may also be due to the homogeneous nature of the sample as they presented with a mean body weight of 64.8 ± 11.7 kg, and therefore provided very little variation with regard to body weight.

The self-assessment evaluation developed by Goemaere et al., [118] also included questions on caffeine, calcium and alcohol intake within their final risk assessment tool, however the inclusion of these variables reduced the power of the other well known risk factors, such as age and weight, in predicting BMD. These lifestyle factors were not included [49], or did not make any significant contribution [34;51;76;138;188], to BMD in the other assessment studies. With regard to smoking

in particular, all of the risk assessment studies only looked at current smoking. It is not clear why the results of some studies found smoking to be important when assessing risk of osteoporosis [34;49;118], while others did not [51;76;138;188]. Unfortunately it is difficult to compare these studies, and the reasons for their inconsistent findings, as the outcome BMD measures for identifying women at an increased risk were very different and ranged between a T-score of ≤ –3.5 and a Z-score of –1.0. Although no studies report obtaining information on calcium intake from dietary recall, most obtained the information via self-report or interview ([34;51;118;188] as was done in our study, or by food frequency questionnaire [76;138]. However in these studies, calcium intake has not been shown to be a significant predictor of BMD. Similarly, our results corroborate those of other studies [34;51;76;138;188], which did not show any relationship between BMD and smoking, caffeine, calcium or alcohol intake. Although the odds ratios presented may suggest a relationship, it is important to take note that these are observed odds ratios with wide confidence intervals, therefore the non-significant odds ratios need to be interpreted with caution.

Many cross-sectional studies that have found an association between menstrual irregularity and reduced BMD have compared eumenorrheic athletes to oligomenorrheic and/or amenorrheic athletes [85;216;230]. However, there is limited literature looking at the association between menstrual irregularity and BMD in a general population of women. The results of our study suggest that menstrual irregularity is protective against osteopenia, however this result is likely to be confounded as the 25 women who reported menstrual irregularities were significantly younger (>10 years) than those women who reported no history of menstrual irregularities. In addition, women who present with menstrual irregularities have traditionally been placed on some kind of hormone replacement, most often in the form of the oral contraceptive pill, which has been shown to have a positive effect on BMD [263]. Unfortunately, although we obtained details on current oral contraceptive and hormone replacement therapy use in our questionnaire, we did not get details on history of oral contraceptive use. Despite this we were aware that 13 (52%) of the 25 women who

reported experiencing menstrual irregularities were currently on the oral contraceptive pill or hormone replacement therapy, which may also explain this unexpected finding.

It is well accepted that the loss of BMD that occurs after peak bone mass has been achieved is accelerated during, and for a short period after, menopause [127]. Although the results of our study suggest that the menopausal state is protective against osteopenia, this finding is also likely to be confounded by the fact that there were only 20 (12%) women who were menopausal in our sample, and only 2 of them were osteopenic. In addition, (60%) of the menopausal women were on HRT, which has been shown to have a protective effect on BMD [56].

Although the studies by Recker et al., [263] and Mazess and Barden, [222] investigated oral contraceptive use in samples of healthy adult women, their results showed a positive association and no association, respectively. Both studies quantified current oral contraceptive use, however the study by Mazess and Barden, [222] also included previous oral contraceptive pill use. Our study included a similar number of women to the study by Recker et al., [263], and significantly fewer subjects than Mazess and Barden, [222] (n=300) however we found no relationship between oral contraceptive use and estimated BMD in our sample. Further, the lack of association between lactation history and estimated BMD in our study is supported by the results of other studies which have also found no association when lactation history is quantified [187], or which show a return to baseline BMD values approximately one year after parturition [290].

Several studies suggest that the assessment of risk factors for osteoporosis should not be a substitute for bone mass measurements [285] or should be done in combination with BMD testing [49]. Although our assessment tool only correctly identified 60% of the sample, it's sensitivity was 75%, which is comparable to other previously validated osteoporosis assessment questionnaires [34;51]. For example, using our questionnaire, a score of over 50 is obtained for women over the age 16 years who do not exercise on a regular basis. Similarly, a woman of 58 years of age who

does exercise at least 3 times a week, will obtain a score of 50, suggesting that all women over the age of 58 years are at an increased risk of osteopenia due to their age, and it may be recommended that they are referred for a DXA assessment. However, although useful, this tool may not be sufficient to provide adequate clinical screening for osteoporosis, and should be combined with some assessment of BMD.

In addition, based on the results of our analyses, we would recommend that women with a score over 50 should be referred for a more intensive evaluation of other risk factors that could not be included in this self-administered assessment and which could be more thoroughly investigated by a physician. However, our results do confirm the importance of obtaining information on physical activity when assessing osteopenia risk. A limitation of the study is the self-selected nature of the sample, which may introduce bias, and therefore is not likely to be representative of the South African population. The high-risk nature of this sample is confirmed by the high occurrence of a family history of osteoporosis (22.2%). Therefore, the "2-question" assessment of osteopenic risk may not be sensitive or specific to a broader population of women. The applicability of this risk assessment needs to be assessed in other South African populations, in whom the additional questions included in our original questionnaire, which were not shown to alter risk in this population, may be more applicable.

CHAPTER SIX

LONGITUDINAL FOLLOW-UP OF ULTRAMARATHON RUNNERS

6.1. INTRODUCTION

Despite the extensive support in the literature, as well as this dissertation, for the role of physical activity in the accretion and maintenance of bone, some literature suggests that there may be a "cautionary tale". Most of the literature that has investigated the influence of exercise training on BMD has compared various athletes to each other, and to sedentary controls [31;83;143;297]. These studies have shown that exercise training is positively associated with BMD. However, recent research by Zanker et al., [320], confirms the findings of others [149] suggesting that decreased energy availability that occurs due to an imbalance between energy intake and energy expenditure, common in many athletes, will result in menstrual dysfunction and subsequent bone loss. Most of the early studies investigating the relationship between menstrual dysfunction and BMD have only considered current menstrual status, with a specific focus on amenorrhea [53;62;85;216]. The results of a more recent study by Micklesfield et al., [230] reported that ultramarathon runners, who had either current or prior oligomenorrhea or amenorrhea, had a LS BMD of only 87% of those women who had always had regular menstrual periods. This study and others [84;204;237] have illustrated the importance of quantifying menstrual history, as well as current menstrual status, when assessing bone health.

There is a paucity of literature that has provided follow-up information on the current bone status of women who have previously experienced menstrual dysfunction. A follow-up study by Drinkwater et al., [86] was the first to report on current BMD when regular menses are resumed. The results of their study showed a significant increase in vertebral BMD (6.3%) in the formerly amenorrheic athletes, while these measurements were maintained (-0.3%) in the eumenorrheic athletes. Similar follow-up studies over periods of between 15 months and 2 years have shown increases of between 6.6% and 17% in BMD in previously amenorrheic women who have regained regular menses [170;202;308]. However, in all of these studies, the BMD of the formerly amenorrheic subjects was still lower than the regularly menstruating subjects, and it is unclear

from studies of this length whether the initial increase in BMD that occurs with the resumption of menses, will continue. The results of a longer follow-up study by Keen and Drinkwater [176] showed that even when menses had been resumed for a significantly longer period of time (± 8 years) in previously amenorrheic athletes, their vertebral BMD was still significantly lower (84.4%) than those athletes who had always had regular menses (100%). Similar results in a study by Micklesfield et al., [231] found LS BMD of women with a history of menstrual irregularity to be 89.7% of regularly menstruating women after a follow up period of 3-5 years, and 85.6% of sedentary controls.

Therefore the aim of this study was to determine the factors contributing to current BMD in a group of previously competitive ultramarathon runners. This study investigated the change in BMD, on 3 separate occasions over a period of approximately seven years, in these runners, most of whom had gained weight and decreased their average weekly running distance.

6.2. METHODS

6.2.1. Subjects

We attempted to contact all forty women who had volunteered for the first study seven years previously (Test 1). Nineteen of these subjects, and an additional 3 subjects who had been involved in a similar study at the same time, participated in a follow-up study three years later (Test 2). Many of the women were lost to follow-up, and therefore, our final sample consisted of 14 women who had a total of 3 osteodensitometry scans each over the seven year period, and two women who had had 2 osteodensitometry scans each over the same period (Test 3). However, there were no differences in anthropometric, reproductive or BMD data at Test 1 between those women included in this study (n=13) and those lost to follow-up (n=27). These data are presented in Table 1.

6.2.2. Medical and weight history

Subject's details including age, height, highest and lowest adult body weight, as well as weight history over the last 5 years, were collected. An extensive menstrual and reproductive history was collected from the subjects. This included age at menarche, number of years of oral contraceptive use, age at pregnancy/ies, breast-feeding history, use of fertility drugs, hormone injections, hysterectomy and/or ovarectomy and history of miscarriage. In addition, the number of years that the subject had been eumenorrheic, oligomenorrheic and amenorrheic were calculated from the menstrual history calendar (see Appendix 6.7.). The menstrual data were used to calculate the following:

Menstrual history index [123] was calculated to determine the estimated number of periods per year since age at menarche:

Menstrual history index = (11.5*R + 7*O+1.5*A)/(C-M)

where: R=number of years of regular menstrual cycles (defined as 10-13 menstrual periods per year and assuming an average of 11.5 periods per year);

O=number of years of oligomenorrhea (defined as 4-9 menstrual periods per year and assuming an average of 7 periods per year);

A= number of years of amenorrhea (defined as 0-3 menstrual periods per year and assuming an average of 1.5 periods per year);

C= current age

M= age at menarche

6.2.3. Training and exercise history

Each subject provided details of their current exercise programme as well as their exercise history. These details included frequency/week, duration/week, distance/week, and months/years

of participation. Injury history, with specific reference to muscle, ligament, joint or tendon, as well as history of stress fracture was collected.

6.2.4. Milk intake

Reported lifetime milk intake for four epochs, namely, under the age of 14 years, 14-21 years, 22-34 years and 35-50 years, was used as a proxy for calcium intake (adapted from Sandler et al., 1985)[278]. Subjects were asked whether they consumed milk with every meal, frequently/not with every meal, sometimes, rarely or never. Responses were categorized as 1 for every meal, 2 if frequently/not with every meal, 3 if sometimes, and 4 if rarely or never.

6.2.5. Bone densitometry

Areal bone mineral density (BMD) of the LS and left PF were measured using a Hologic QDR-1000 (version 4.20) dual-energy x-ray bone absorptiometer (Hologic Inc., Waltham, MA). BMD's ($g.cm^{-2}$) were determined for lumbar vertebrae 1 through 4 and the PF, Ward's triangle, FN, greater trochanter, and the intertrochanteric area.

6.2.6. Statistical analysis

The Statsoft™ (Statistica v6, 2002) statistical package was used. Descriptive analyses were performed to determine mean values and standard deviations. Pearson's product-moment correlation coefficients were used to determine relationships between the current variables. A one-way ANOVA with repeated measures was performed to determine change in any of the bone, body weight or training variables over time. Variables that have previously been associated with BMD were entered into a multiple stepwise regression in order to determine what percentage of the variance in current LS BMD could be attributed to current and previous lifestyle

factors. This was not completed for the total proximal femur as a significant model could not be obtained.

6.3. RESULTS

Characteristics of the current study sample at test 1 and those subjects lost to follow-up are presented in Table 6.1. There were no significant differences between the women recruited to participate in Test 3 and the women lost to follow-up.

Table 6.1. Subject characteristics at Test 1

	Subjects included in follow-up (n=13)	Subjects lost to follow-up (n=27)
Age (yrs)	34 ± 4.9	32.1 ± 5.1
Weight (kg)	59.5 ± 7.2	58.0 ± 7.1
Height (cm)	166.8 ± 5.6	165.7 ± 6.7
BMI ($kg.cm^{-2}$)	21.3 ± 1.8	21.1 ± 2.2
Age at menarche	13.3 ± 1.3	13.1 ± 1.5
Menstrual History Index	10.4 ± 1.7	10.6 ± 1.4
LS BMD ($g.cm^{-2}$)	1.008 ± 0.110	1.045 ± 0.092
PF BMD ($g.cm^{-2}$)	0.923 ± 0.097	0.960 ± 0.131

Data are presented as mean ± SD

Current subject characteristics are presented in Table 6.2. At follow-up (Test 3) the mean age of the sample was 42.6 ± 4.9 years, and the range of ages of the women in the sample was 31.2-50.5 years. The current body weight of the sample was 59.7 ± 7.2 kg, with the range of weight of 46-72 kg. Of the sixteen subjects, 9 women had gained an average of 5.3 ± 4.1 kg (range: 0.8-14kg) since test 1, 6 women had lost an average of 2.2 ± 1.1 kg (range: 0.7-4kg), and one

woman's weight had remained the same. Ten of the sixteen subjects were parous with an average of 2.1 ± 0.9 pregnancies. Parity for the whole sample was inversely correlated with FN (r=-0.63; p<0.01) and trochanteric BMD (r=-0.51; p<0.05). One of the subjects had undergone a hysterectomy 3 years prior to test 3, and one of the subjects had commenced hormone replacement therapy 3 years prior to test 3. Including these two subjects, four subjects were currently irregular, with the remaining 12 subjects experiencing no current menstrual irregularity. There was no significant change in the mean menstrual history index since the baseline study. Only one subject was currently taking oral contraceptives.

Table 6.2. Measurements at test 1, 2 and 3

	Test 1 (n=16)	Test 2 (n=14)	Test 3 (n=16)
Age (yrs)	34.8 ± 4.7	39.2 ± 4.1	42.6 ± 4.9
Weight (kg)	57.9 ± 7.6	56.6 ± 6.4	59.7 ± 7.2
Menstrual History Index	10.3 ± 1.8	10.2 ± 1.8	10.2 ± 1.8
LS BMD ($g.cm^{-2}$)	1.007 ± 0.118	0.993 ± 0.108	1.019 ± 0.11
PF BMD ($g.cm^{-2}$)	0.932 ± 0.111	0.931 ± 0.121	0.931 ± 0.119
FN BMD ($g.cm^{-2}$)	0.845 ± 0.085	0.840 ± 0.088	0.827 ± 0.095
Trochanteric BMD ($g.cm^{-2}$)	0.696 ± 0.095	0.692 ± 0.095	0.701 ± 0.092
Inter-trochanteric BMD ($g.cm^{-2}$)	1.089 ± 0.135	1.019 ± 0.305	1.082 ± 0.145
LS T-score	-	-	-0.24 ± 1.04
PF T-score	-	-	-0.10 ± 0.98

Data are presented as mean ± SD

There were no significant differences in any of the BMD measurements between tests 1, 2 and 3. At test 3, five of the women had a LS T-score < -1, and were therefore considered to be osteopenic. There was no significant difference in age, weight or menstrual history between the

women who presented with osteopenia of the LS and those who did not. However, the osteopenic women had experienced significantly more pregnancies than the women were did not have osteopenia of the LS (2.4 ± 1.1 vs 0.8 ± 0.98 pregnancies; p=0.01). There was a trend towards significance in the association between duration of training per week (minutes.wk^{-1}) over the previous 5 years in the osteopenic women compared to the women without osteopenia (374 ± 83.5 vs 232.8 ± 139.1 mins.wk^{-1}; p=0.056). Two different women presented with osteopenia of the proximal femur (T-score<-1). None of the subjects was osteoporotic.

Lifetime calcium intake data for various life epochs is presented in Table 6.3. Lifetime calcium intake was not associated with current BMD. Using a repeated measures ANOVA, the current duration of running per week was significantly lower than duration of running in the past 5 years (178.6 ± 118 vs 276.9 ± 139 mins.wk^{-1}; p<0.05). Two subjects were no longer running, however both were walking on a regular basis. Only one subject had increased her weekly training distance since test 2, while 7 of the subjects were training less, and 6 of the subjects had maintained their mileage. For the whole sample of subjects, average frequency of running per week was 3.8 ± 2 times.wk^{-1}, duration of running per week was 178.6 ± 118 mins.wk^{-1} and the average distance run per week was 47.8 ± 38.5 km.wk^{-1}.

Table 6.3. Lifetime calcium intake at various life epochs

	<14 yrs	14-21 yrs	22-35 yrs	35-50 yrs
Every meal (%)	31.3	6.3	0	0
Frequently/not with every meal (%)	43.7	43.7	18.7	6.7
Sometimes (%)	12.5	31.3	43.7	53.3
Rarely or never (%)	12.5	18.7	3.7	40

The statistical model that best predicted current LS BMD in this group included age, age at menarche, current menstrual history index and duration of running in the last 5 years. These

variables accounted for 62% of the variance in LS BMD ($p<0.01$, standard error of the estimate (SEE)=0.0713, Table 6.4.).

Table 6.4. Multivariate analysis for lumbar spine BMD

Variable	b	β	p level
Age (yrs)	-0.29	-0.0007	0.099
Age at menarche	0.571	0.053	0.005
Menstrual history index	0.438	0.028	0.024
Running: $mins.wk^{-1}$ (last 5 yrs)	-0.460	-0.000	0.017

$R=0.845$, adjusted $R^2=0.611$, SEE=0.071, β, parameter estimate; b, partial correlation coefficient

6.4 DISCUSSION

These results suggest that physical activity is not protective of BMD in a select group of women long distance runners with a history of menstrual dysfunction and reduced BMD. Most cross-sectional studies comparing the BMD of athletes, participating in various sports, and sedentary controls, have found a positive association between exercise training and BMD [31;83;143;297]. However, competitive sport has been associated with an increased incidence of menstrual dysfunction [313], which has been shown to negatively influence BMD [53;85] [62;216;230;237]. Although our study did not include professional athletes, the occurrence of current and previous menstrual irregularity was 50%, which is similar to the incidence reported in other literature (23-59%) for various sports [28;50;184;266]. This may also have been due to the self-selection bias of the study participants.

However, our study did show that age, menstrual history index, age at menarche and average running duration in the last 5 years accounted for 61% of the variation in current LS BMD. The

menstrual history index is a proxy for menstrual function throughout the reproductive years and takes into account the total number of years of eumenorrhea, oligomenorrhea and amenorrhea. These results suggest that, in a sample of previous ultramarathon runners, most of whom have increased body weight and reduced training, that prior menstrual dysfunction will still account for a significant portion of current BMD, and that their exercise may not be able to prevent this. These findings support those of Keen et al., [176] and Micklesfield et al., [231] who showed that bone loss due to prior menstrual irregularity may not be reversed. In addition, the women in our study who presented with osteopenia of the LS trained for a significantly longer duration per week within the last 5 years, compared to women who were not osteopenic. Our study supports the findings of Burrows et al., [50] who showed a negative association between endurance running distance and BMD of the LS and FN. These data suggest that exercise is not beneficial in a certain population who present with an increased occurrence of menstrual dysfunction compared to the general population. Menstrual dysfunction that occurs as a result of an energy deficit created by the imbalance between energy intake and energy expenditure, is proposed to be due to the inhibition of the hypothalamic gonadotropin releasing hormone (GnRH) pulse generator resulting in a disruption of luitenizing hormone pulsatility [206]. However, although it has previously been proposed that it is the consequent decrease in oestrogen levels that results in bone loss, Zanker et al., [319] suggests that this disruption in the menstrual cycle occurs simultaneously with a reduced bone turnover and consequently bone formation, rather than an increased bone turnover that would be a result of decreased oestrogen. In this case, treatment of menstrual dysfunction should be more focused on correcting the energy imbalance, rather than increasing oestrogen levels.

There is conflicting evidence regarding the association between parity and BMD due to increased levels of estrogen during pregnancy, as well as the increased calcium demands of the fetus [6;175;291]. In addition, lactation and post-partum amenorrhea have been associated with bone loss [151], however LS BMD levels are restored within 12 months post-partum in women who did

not breastfeed for more than 6 months [175;233]. In our study the women who were osteopenic had been pregnant significantly more often than the women who did not present with osteopenia of the LS. In addition, the number of pregnancies for the whole sample was inversely correlated with neck and trochanteric BMD. These findings suggest that an increasing number of pregnancies are associated with a compromised BMD, however as we did not collect breast-feeding data from the subjects, it is not possible to suggest a mechanism for this finding. None of these women had been pregnant within the last 12 years (12-23 years).

The present study illustrates that there are a group of individuals in whom physical activity is not beneficial for bone health and does not protect against bone loss. Longitudinal studies over a significant period of time are needed to determine how much BMD is restored with increasing age, and whether these women are at an increased risk of osteoporosis in later life.

CHAPTER SEVEN

SUMMARY AND CONCLUSIONS

The aims of this thesis were to investigate the interaction between physical activity and bone health through various life stages, in order to better understand the determinants of adult bone mass and consequently, osteoporosis. In addition, the studies within this thesis explored the relationships and interactions between physical activity, and other lifestyle, physiological and demographic factors, on BMD and bone QUS parameters in the South African population.

In the first part of this thesis we investigated the relationship between lifetime physical activity and BMD in South African women using data collected in a case-control study of breast cancer in relation to BMD. BMD was measured using DXA and extensive demographic and lifestyle data were collected, including historical physical activity during 4 epochs. We found that the major determinants of LS and PF BMD included age, current weight, and a component of physical activity between the ages of 14 and 21 years. In addition, total physical activity and average time spent walking per day, between the ages of 14 and 21 years, were significantly correlated with LS and PF BMD, respectively. These findings suggest that walking or activities resulting in impact loading at a young age are associated with higher BMD in later years, and suggest that in some groups physical activity patterns track over time.

The second study attempted to determine whether there is a link between physical activity and bone early in life by investigating physical activity patterns in 198 pre-adolescent girls of various ethnic groups. Total peak bone strain scores (TPBSS) were higher in white compared to black girls, however, walking energy expenditure was higher in black girls compared to the other groups. Walking energy expenditure and calcium score were significantly correlated with SOS for the whole group. We also found that BUA and SOS were higher in the black girls compared to the white girls. These findings suggest that there is an interaction between ethnicity and lifestyle factors, which together will determine bone properties, as measured by QUS, in preadolescent girls. This study also provides additional evidence for the promotion of physical activity at a young age in optimizing future bone health.

In the third study, we collected demographic, anthropmetric and maternal data from 122 children of a similar age to those in the second study, of mixed ancestral origin, whose mothers had participated in a nutrition and pregnancy study at the time of their birth. We performed calcaneal QUS measures on all of the children, as well as a sub-sample of the mothers. Hand radiographs were used to measure metacarpal morphometry. Although we found an association between maternal and early life influences, and the various bone measures, these were influenced by socio-demographic and environmental factors.

In the fourth study, we examined the relationship between various lifestyle factors, including current physical activity, and adult bone properties, as determined by QUS. An osteoporosis risk questionnaire was designed and administered to the 167 women who volunteered for the study. Estimated BMD values were obtained from calcaneal BUA and SOS measurements using the Hologic Sahara Clinical Bone Sonometer. Significant odds ratios were obtained for age, current physical activity and lifetime energy expenditure score, and a model including age and weekly physical activity correctly predicted 60% of the women with or without osteopenia according to their QUS score.

In the fifth study, we introduced a caveat. We investigated the factors that contributed to current BMD in a group of 16 previously, competitive ultra-marathon runners, most of whom had gained weight and reduced their training over the previous 7 years. A model that accounted for 61% of the variance in LS BMD included age, age at menarche, current MHI and weekly running time over the previous 5 years. These data suggest that history of menstrual irregularity is an important determinant of current BMD, and that high levels of physical activity may further increase the risk of reduced BMD in these individuals, rather than protect against it.

Together, these studies provide evidence supporting the beneficial effect of physical activity on bone health, in South African preadolescent girls, adults and older adults. This is influenced by various socio-demographic factors.

Limitations and potential bias in interpretation: implications of study design

Measurement issues:

This thesis included a combination of various methods of measuring and estimating bone mineral density, including dual energy x-ray absorptiometry and quantitative ultrasound. These methods have been well described in the Literature Review (Section 1.3), however we are aware of the limitations of combining more than one technique when interpreting results.

Dual energy x-ray absorptiometry is considered to be the "gold standard" with regard to the measurement of bone mineral density, and has been included in Chapter 3 in the assessment of BMD in a cross-section of women of average age 42.6 ± 8.9 yrs and in the follow-up study in Chapter 7 of 13 female ultramarathon runners. The use of quantitative ultrasound in the remainder of the dissertation was due to the opportunistic nature of these studies which resulted either from financial constraints or because the research was considered ancillary to the main study being undertaken.

The use of quantitative ultrasound in children has not been well validated, however there is sufficient evidence to suggest that it does correlate well with other measures of bone mineral content, and may have a useful role for, in particular, the "field" assessment of bone quality. There have been a number of studies that have used ultrasound to approximate bone mineral density in children, one of which was published in Acta Paediatrica (Mughal et al., [235]) and concluded that the ultrasound measurement of broadband ultrasound attenuation (BUA) reflects bone mineral density in children and adolescents. This study compared BUA to total body bone mineral density as measured by dual-energy x-

ray absorptiometry (DXA), the "gold standard" for measuring bone mineral density. Their results on 58 children between the ages of 7 and 17 years, showed a significant relationship between calcaneal BUA and total body BMD ($r=0.74$; $p<0.001$).

A more recent study by Wunsche et al., [315] performed ultrasound measurements on 3299 children and teenagers between the ages of 6 and 18 years and have published their BUA and SOS (Speed of Sound) results for the various age groups to be used as quantitative ultrasound reference data. As mentioned in the thesis, the BUA and SOS results for Chapters 3 and 4 were similar to each other, as well as the reference data for 9 year-old girls living in Germany. However, when the boys in our study were compared to the reference data for 9 year-old boys provided by Wunsche et al., BUA of the boys of mixed ancestral origin was 7.8% lower than the reference database. Their results showed significant gender and age-related changes in the QUS measurements, as well as with height and weight. The relationship between these changes during childhood and bone mineral density as measured by DXA, have been extensively reported in the literature.

We understand that there are some limitations with the use of quantitative ultrasound, as mentioned in the literature review of the thesis. We are confident however that ultrasound does reflect bone mineral density in our study as the values are similar to those shown in other studies [315] in which QUS and DXA have been measured. Therefore, it is our impression that the study provides valuable and novel findings with regard to differences in bone parameters between pre-adolescent girls of different ethnic groups, and the important interaction of different lifestyle factors, such as physical activity.

More extensive research has been completed on ultrasound in adults. Most importantly, research has shown the ultrasound parameters, BUA and SOS, to be sensitive to exercise-related loading, and this has been confirmed by our study in Chapter 5 which showed physical activity to make a significant contribution to the variance in estimated BMD, in comparison to other well-known risk factors.

Convenience sampling and potential for bias:

As pointed out by the examiner, the limitations of convenience sampling include the potential for bias and the difficulty in applying the findings to the general population. Although all of the studies in this thesis have been completed on convenience samples, the populations that they represent have not been well studied and the results of these studies have provided a glimpse into the importance of lifestyle factors at various ages throughout the lifespan. The paucity of physical activity data in developing countries has made these findings particularly interesting, as although the relationship between physical activity and bone is the same as has been shown in more westernized populations, the quantification of physical activity is quite different due to low levels of leisure time activity and the high prevalence of walking for transport in various South African populations.

In the case-control study of Chapter 3 the sample of women was selected from working class communities living in the Cape Town metropole. As mentioned in the discussion, walking for transport is high in this community and leisure time activity is low, and the finding that physical activity between the ages of 14 and 21 years had the greatest influence on adult BMD is obviously particularly relevant to this community, and other similar communities. These findings further confirm the important relationship between BMD and lifetime physical activity.

The ethnic differences in QUS measurements shown in the findings of Chapter 3 confirm previous findings of American studies that have shown differences in BMD between ethnic groups, particularly at different stages of pubertal development. However South Africa, and in particular the Cape Province, provides a unique opportunity to include another important ethnic group, the people of mixed ancestral origin, and again to further investigate the relationship between physical activity and bone in samples with very different physical activity profiles. Schools were cluster-sampled from various socioeconomic categories in order to get a cross-section of girls with

various demographic and socioeconomic backgrounds. All of the girls within the classes selected were measured.

Subjects included in the study in Chapter 4 were children of similar age, of mixed ancestral origin from a working class community which was previously financially and politically disadvantaged. This has resulted in this sample being extremely homogenous, resulting in reduced variability and the potential for introducing Type 2 error with respect to between group comparisons. We have been careful not to generalize the results found in this study to the broader community, however the influence of smoking and alcohol intake on the various properties of bone, shown in this study, may be relevant to other communities in which the prevalence of alcohol consumption and smoking during pregnancy may be high.

We have adjusted the focus of Chapter 5 quite considerably in order to highlight the contribution of physical activity to QUS measurements in comparison to other risk factors, rather than the development of a risk questionnaire. We are aware of the limitations of the subject sample as all of the women volunteered for the study and therefore were more proactive with regard to their health, however the findings are interesting and provide further insight into the role of a physically active lifestyle in promoting positive bone health.

The follow-up study may be more correctly labeled a case series and we are aware of the limitations with the loss to follow-up, only managing to contact 13 of the original 40 subjects. Statistics were completed to compare the subjects included in the follow-up in comparison to those lost to follow-up. There were no significant differences between the samples. Most research on the relationship between menstrual dysfunction and bone mineral density in female runners is cross-sectional in nature, and there is very little data to show what happens when women reduce their running mileage and regain regular menses. The paucity of data may be due to the difficulty in finding these runners, which is a problem we had, however we are reasonably

confident that the data were representative of the original sample, although may not be generalisable to the broader community of active women.

Interaction effects:

In a multiple regression, it is assumed that the variables are contributing to the variance of the dependant variable independently. However, if there is a relationship between the variables, then an interaction effect, which may result in co-linearity, needs to be investigated. In addition, not only may these variables be correlated with each other, they may also have opposite effects on the outcome. For example, inactivity may explain the variance in obesity as well as in bone mineral density. However, if one is trying to determine what other factors may be contributing to the variance in bone mineral density, obesity and its interaction with inactivity may need to be considered, although their effects are opposite to each other.

There was a significant interaction effect between ethnicity and Tanner breast stage in Chapter 3 which suggests that the contribution of ethnicity to SOS is dependant on the contribution of Tanner breast stage. We understand that if the two variables are related to each other, as are ethnicity and Tanner breast stage, then their effectiveness in contributing to the variance in SOS is reduced. An interaction variable is created to investigate it's contribution to the dependant variable, and in this case it makes a significant contribution to SOS. We can therefore conclude that ethnicity is making a significant contribution to SOS, and this is altered by the stage of pubertal development.

Sample size:

With respect to the effects of birth weight on BMD, we modeled the sample size required to detect a 0.05 $g.cm^{-2}$ difference in BMD (similar to that shown in preterm vs. full term children between 8-12 years of age; Fewtrell et al., [99], with a standard deviation of 0.09 or 0.1 $g.cm^{-2}$. At an alpha level of 0.05 and a statistical power of 80%, we ideally would need 50-60 subjects per group. We

therefore cannot rule out the possibility that the study (Chapter 4) was underpowered with an increased likelihood of Type 2 error for the birth weight effect on BMD. In addition, we are not clear what role the methodological difference between QUS and DXA would play, however it is possible that there would be an increased likelihood of Type 2 error.

In adult studies [91] in which ethnicity effects on BMD were examined, a sample size of 16-20 for each group would theoretically be sufficient to detect a difference of 0.09-0.1 $g.cm^{-2}$ in either femoral neck or posterior-anterior lumbar spine, with a standard deviation of 0.1 and an alpha level of 0.05 (80% power). We cannot be certain whether we can extrapolate this to pre-menarcheal girls, however in the absence of comparable data it would seem unlikely that these results are spurious.

In summary, we accept that there are a number of limitations, as outlined above, in some of the studies included in this thesis. However, the findings have confirmed the role of physical activity in promoting bone health within various South African populations, and these studies have explored relationships which can be investigated further within specific hypothesis-driven research.

CHAPTER EIGHT

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CHAPTER NINE

APPENDIX

6.1 Historical Physical Activity Questionnaire

- Used to assess historical physical activity in Study 1 (Chapter 2).
- Adapted from Kriska et al., [190].

Historical Physical Activity Questionnaire

I am now going to ask you questions in order to measure your physical activity over your lifetime.

1. Do you think you were more physically active than your friends and other women of your age between the age of:

KEY 1=YES, 2=NO

14-21 yrs	☐
22-34 yrs	☐
35-50 yrs	☐
50+ yrs	☐

2. Now I am going to ask you specifically about walking. Walking includes time spent walking to and from school or work, the bus stop and the shops, as well as walking for pleasure. The walking that I am interested in must be at a moderate of brisk pace, and does not include strolling.

How much time did you, or do you normally walk each day between the age of::

14-21 yrs	☐☐	☐
22-34 yrs	☐☐	☐
35-50 yrs	☐☐	☐
50+yrs	☐☐	☐
	Amount	min/hrs/wks

NOTE ONLY ACTIVITIES FOR AT LEAST 6 MONTHS (ONCE A WEEK)

Household	**Occupational (time spent at work/job)**	**Leisure-time (time after work)**	**Transport**
1. Scrubbing floors	9. Moving, carrying boxes, stocking shelves (shop assistant)	20. Jogging/race walking	32 Walking to work
2. Cleaning the house – general	10. Factory work (lifting, tailoring, excluding sitting)	21. Brisk walking	33. Bicycling to work
3. Heavy house cleaning (washing windows, mopping, cleaning the garage)	11. Nursing/patient care (includes walking or lifting)	22. Walking for pleasure	34. Other
4. Sweeping garage, pavement, outside house	12. Char/domestic work	23. Tennis	
5. Childcare- active play	13. Farming – shovelling grain	24. Netball	
6. Childcare – standing, dressing, bathing, grooming, feeding	14. Farming – herding cattle	25. Bowling	
7. Washing clothes by hand	15. Farming – milking by hand	26. Volleyball	
8. Other	16. Carrying, loading or stacking wood	27. Aerobic dancing	
	17. Hoeing	28. Dancing – general	
	18. Gardening	29. Cycling	
	19. Other	30. Gardening	
		31. Other sports and leisure activities	

I am now going to ask you about activities that you participated in regularly (GIVE PATIENT THE CUE CARD). By this I mean activities that you have participated in once a week or more for at least 6 months.
Note: Interviewer to establish for each activity and to collect information for each time period for respondent's as follows: how many years, how many months of each year, how often – sessions per week and minutes per session

Activity		Period 14-21 (8yrs total)				Period 23-34 (13yrs total)				Period 35-50 (16 yrs total)				Period >50 (9 years total)			
	Y/N	yrs	mo/yrs	ses/wk	min/ses	yrs	mo/yrs	ses/wk	min/ses	yrs	mo/yrs	ses/wk	min/ses	yrs	mo/yrs	ses/wk	min/ses
1	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
2	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
3	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
4	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
5	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
6	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
7	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
8	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
9	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
10	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
11	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
12	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
13	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
14	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
15	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
16	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
17	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
18	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐

Activity	Period 14-21 (8yrs total)					Period 23-34 (13yrs total)				Period 35-50 (16 yrs total)				Period >50 (9 years total)			
	Y/N	yrs	mo/yrs	ses/wk	min/ses	yrs	mo/yrs	ses/wk	min/ses	yrs	mo/yrs	ses/wk	min/ses	yrs	mo/yrs	ses/wk	min/ses
19.....	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
20.....	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
21.....	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
22.....	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
23.....	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
24.....	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
25.....	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
26.....	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
27.....	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
28.....	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
29.....	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
30.....	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
31.....	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
32.....	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
33.....	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
34.....	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐
35.....	☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐	☐	☐☐	☐☐	☐☐

6.2 Milk intake questionnaire

- Used to assess milk intake in Study 1 (Chapter 2).
- Adapted from Sandler et al., [278].

MILK INTAKE QUESTIONNAIRE

I am now going to ask you some questions about your milk/maas consumption at various times in your life. This includes only milk that you drank, not milk that you might have with your cereal/porridge or with your tea and coffee.

I would like you to tell me, during (<14/14-21/22-34/35-50/>50 years of age) *did you drink milk:*

KEY:	1=Every meal
	2=Frequently/not with every meal
	3=Sometimes
	4=Never

1. Under the age of 14 years ☐
2. For the period 14-21 years ☐
3. For the period 22-35 years ☐
4. For the period 35-50 years ☐
5. For > 50 years ☐

6.3. Physical Activity Questionnaire

- Used to assess year round activity patterns in pre-adolescent girls in Study 2 (Chapter 3).
- Adapted from the Modified Activity Questionnaire for Adolescents [3].

Code ☐

Physical Activity Questionnaire

1. On an average night of the week, what time do you **go to sleep**? ☐ **PM**

2. And at what time do you **wake up**, the next morning? ☐ **AM**

3. During a normal **weekday**, how many TV shows do you **OR** how many hours do you spend watching TV or playing on your computer each day?
☐ **SHOWS**
☐ **HOURS**

4. During a normal **weekend**, how many TV shows do you **OR** how many hours do you spend watching TV or playing on your computer each day?
☐ **SHOWS**
☐ **HOURS**

5. How do you usually **get to** school? – Choose one of the following.

☐ **Walk to school** ☐ **Bus/Taxi** ☐ **Parents' car** ☐ **Other**

6. **If you do walk** to school, to the bus stop, or to the taxi rank, how many minutes does it take to walk there?

☐ **Less than 15 minutes** ☐ **15 to 30 minutes** ☐ **30 to 60 minutes** ☐ **More than 1 hour**

7. **If you do walk** to school, to the bus stop, or to the taxi rank, how fast do you walk?

☐ **I walk slowly** ☐ **I walk quickly** ☐ **I walk very quickly and sweat a lot!**

8. Do you take **physical education** (P.E./P.T./Gym) classes? – This does not include after school sport.

☐ Yes ☐ No

9. If you do take physical education classes, how many times per week do you attend?
☐ **TIMES PER WEEK**

Code

Activities at school:

Circle **all** the activities that you do at school. Place a tick next to each activity that you do at school, either in the autumn and winter or spring and summer or both. How many times each week do you do this activity? Put the number of times in the box. How long do you do this activity? Place a tick in the box which tells us how much time you spend doing this activity at school.

Activity	Time of Year		How many times per week?	For how long do you do this activity each time?				
	Autumn and Winter	Spring and Summer		Half hour	One hour	One and a half hours	Two hours	More than two hours
Example: Netball	✓		2		✓			
Athletics								
Ballet/Dancing								
Basketball								
Gymnastics								
Hockey								
Karate/Judo								
Netball								
Running								
Soccer								
Swimming								
Tennis								
Other:								

Code

Out of school/Weekend activities:

Circle all the activities that you do after school or on weekends.. Place a tick next to each activity that you do, either in the autumn and winter or spring and summer or both. How many times each week do you do this activity? Put the number of times in the box. How long do you do this activity? Place a tick in the box which tells us how much time you spend doing this activity after school or on weekends.

Activity	Time of Year		One or two days of the Weekend?	For how long do you do this activity each time?				
Example:	Autumn and Winter	Spring and Summer		Half hour	One hour	One and a half hours	Two hours	More than two hours
Netball		✓	Both		✓			
Aerobics								
Ballet/Dancing								
Bicycling								
Gymnastics								
Lifesaving/ Nippers								
Running								
Roller skating								
Skateboarding								
Surfing								
Swimming								
Volleyball								
"On-on", Kennetjie, Pegads, Drie Stokkies								
Other:								

6.4. Food frequency Questionnaire

- Used to determine dietary calcium intake in pre-adolescent girls in Study 2 (Chapter 3).
- Adapted from the Youth/Adolescent Food Frequency Questionnaire [269].
- Only questions related to calcium intake included below.

Food frequency Questionnaire

Please take note that when the word "WEEK" is used, it means during your usual week, including the weekend.

---

3. How often do you drink milk/maas, including with tea and coffee?

☐ NEVER ☐ ONCE A WEEK ☐ A FEW TIMES A WEEK ☐ EVERY DAY

---

4. How much milk/ maas do you drink?

☐ MORE THAN 2 GLASSES A DAY ☐ 2 GLASSES A DAY ☐ 1 GLASS A DAY ☐ NEVER

---

5. How much milk do you have with your cereal or porridge during the **WEEK?**

☐ Don't eat cereal/porridge ☐ None ☐ Just a little ☐ Quite a lot ☐ A lot

---

6. HOW OFTEN DO YOU HAVE ICE-CREAM/MILKSHAKES DURING THE **WEEK?**

☐ NEVER ☐ ONCE A WEEK ☐ A FEW TIMES A WEEK ☐ EVERY DAY

---

7. HOW OFTEN DO YOU HAVE YOGHURT/FROZEN YOGHURT DURING THE **WEEK?**

☐ NEVER ☐ ONCE A WEEK ☐ A FEW TIMES A WEEK ☐ EVERY DAY

---

8. HOW OFTEN DO YOU HAVE CHEESE DURING THE **WEEK?.** E. G._ON A SANDWICH (YELLOW CHEESE, WEDGES, CHEESE SPREAD).

☐ ☐ ☐ ☐

NEVER ONCE A WEEK A FEW TIMES A WEEK EVERY DAY

9.HOW OFTEN DO YOU HAVE PIZZA, LASAGNE, MACARONI AND CHEESE DURING THE **WEEK?**

☐ ☐ ☐ ☐

NEVER ONCE A WEEK A FEW TIMES A WEEK EVERY DAY

6.5. Bishop Lavis Growth and Nutrition study questionnaire

- Used to collect demographic and lifestyle data in Study 3 (Chapter 4).

Office use only

BISHOP LAVIS GROWTH AND NUTRITION STUDY QUESTIONNAIRE

Date: _____ Cohort number: _____

Interviewer name:_____ Reference number: _____

Are you the child's mother? YES/NO

If YES, Details of mother: Surname: _____ Name: _____

Original surname (if changed): _____

If NO, Details of primary caretaker (if different from above):

Surname: _____ Name: _____ N/A

Relationship to child: _____

Reason for mother not attending interview:

☐ Unavailable for interview because of work

☐ Unavailable because of another commitment

☐ Ill at home or in hospital

☐ Living in another area but in contact with child and caregiver

☐ Living in another area but not in contact with child and caregiver

☐ Deceased

Details of child: Surname: _____ Name: _____

Date of birth: ___/___/19___

Sex: Male/Female

Name of school: _____

Grade: _____

Hospital in which the child was born: _____

Office use only

DEMOGRAPHIC AND SOCIOECONOMIC DETAILS

1. Who is the household head? _____
2. Relationship to child: _____

3. Please list all the members of the household, with respect to the child, in which the child lives (people generally sharing the same main meal). If a sibling, please note date of birth and details of father.

Please complete from the oldest to the youngest person (including the child):

Name	Sex	Age	Date of birth (for siblings only)	Relationship to child	Details of father siblings only)

4. How many rooms do you have in your house (including kitchen, lounge, dining room, bedrooms)? ☐ rooms

5. In your home, how many rooms are there just for sleeping? ☐ rooms

6. How would you describe your home (tick the one that best describes it)?

Room/garage attached /not attached to house		House		Shared house	
Flat		Hostel		Other:	

7. Household water: do you have access to?

Indoor water		Only outside tap water		Other water source	

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8. What type of toilet do you have?

Flush inside		Only flush outside	
Outside toilet		Other:	

9. How do you dispose of your refuse?

Dump garbage away from home	
Burn garbage	
Burn garbage in yard	
Garbage get collected	
Take garbage to central place to be collected	

10. Which of the following do you have in your household at the present time?

	YES	NO
Electricity		
Television		
Radio		
Motor vehicle		
Fridge		
Stove		
Washing machine		
Telephone		
Video machine		
Microwave		
Computer		
Cellular telephone		
MNet		
DSTV		

11. Marital status of mother/primary caretaker:

Single		Divorced/separated	
Married		Widowed	
Living with partner, not married			

12. Is the child's biological father living with you? YES/NO

13. Does the child's biological father give you any financial assistance? YES/NO

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14. Does your current partner (if he is not the biological father) give you financial assistance for the child? YES/NO/N.A

15.Is the child currently covered by medical aid? YES/NO

16. Education (last standard passed):

	Mother	Father/ Current partner	Father (if not current partner)
No formal education			
Sub A/B (Grade 1-2)			
Std 1-3 (Grade 3-5)			
Std 4-5 (Grade 6-7)			
Std 6-7 (Grade 8-9)			
Std 8 (Grade 10)			
Std 9 (Grade 11)			
Matric (Grade 12)			
College or University			

17. Mother/primary caretakers job: _____

18. Father/current partners job: _____

19. Sources of income:

Employment		Informal income	
Disability grant		Support from parents	
Other			

RETROSPECTIVE HISTORY

20. Have any of the following changed since the birth of your child? If YES, please give details as to the age of the child at the time of the change and details of the change.

	YES	NO	0-2 yr	2-5 yr	5-7 yr	Details
Marital status of mother						
Moved house						
Mother's job						
Father's job						
Primary caretaker						
Household density						☐ Increased ☐ Decreased ☐ Family ☐ Non-family

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MEDICAL HISTORY OF CHILD

21. Would you say your child's health is:

Good		Fair		Poor	

If POOR please explain:

_____

_____

22. Does the child have, or has the child had, any serious medical or developmental problems (physical or mental) or any injuries during the past year? YES/NO

Details:

_____

_____

_____

23. Is the child on any chronic medication? YES/NO

If yes, what is the name and what has it been prescribed for?

_____

_____

24. Has the child ever broken any bones? YES/NO

If yes, which bone and how? _____

_____

25. How would you describe the child's health in their **first year of life** compared to other children of their age and/or their siblings at that age, in relation to the following:

	Less	Equal	More	N/A
Visits to the doctor				
Medication/antibiotics				
Hospitalisations				

26. Has there been any body hair growth on the child? YES/NO

If yes,

Girls:	Boys:
☐ Underarms	☐ Underarms
☐ Genital area	☐ Genital area
	☐ Facial area

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27. GIRLS ONLY. Has the child started menstruating? YES/NO/N.A

28. Do you have any growth data for your child taking part in the study? YES/NO

MEDICAL HISTORY OF BIOLOGICAL MOTHER

29. Has a doctor or nurse told you that you have/had:

	YES	NO	DON'T KNOW	* Please specify
High blood pressure				
Diabetes or "sugar"				
Heart attack/angina				
Stroke				
High blood cholesterol or "fat in the blood"				
Osteoporosis				
Other*				

30. Has medication for all of the following been prescribed to you by a doctor:

	YES	NO	DON'T KNOW	*Please specify
High blood pressure				
Diabetes or "sugar"				
Heart attack/angina				
High blood cholesterol or "fat in the blood"				
Osteoporosis/bone fractures				
Other*				

31. Has a doctor or nurse told the father of your child that he has/had:

	YES	NO	DON'T KNOW	* Please specify
High blood pressure				
Diabetes or "sugar"				
Heart attack/angina				
Stroke				
High blood cholesterol or "fat in the blood"				
Osteoporosis				
Other*				

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32. Does the child have any grandparent/s who has any of the following:

	YES	NO	DON'T KNOW	RELATIONSHIP TO THE CHILD	*Please specify
High blood pressure					
Diabetes or "sugar"					
Heart attack/angina					
Stroke					
High blood cholesterol or "fat in the blood"					
Osteoporosis/bone fractures					
Other*					

33. Do you currently drink alcohol

(i) Everyday
(ii) Only on the weekend
(iii) Once or twice a week
(iv) Only on social occasions
(v) Never

34. Did you drink alcohol during your pregnancy

(i) Everyday
(ii) Only on the weekend
(iii) Once or twice a week
(iv) Only on social occasions
(v) Never

35. Do you currently smoke? YES/NO

36. Did you smoke during your pregnancy? YES/NO

37. How many times have you been pregnant? ☐

38. Have you ever miscarried or had an abortion? YES/NO

39. How many children have you given birth to? ☐

40. Are they all healthy now? YES/NO

If no, please give more details: _____

_____

41. Did you breastfeed your child? YES/NO

42. How old was your baby when you stopped breastfeeding? ☐ years ☐☐ months

43. When did you introduce bottle/cup feeds? ☐ years ☐☐ months

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44. What type of milk did you feed your child?

	YES	NO	DON'T KNOW
Powder infant formula*			
Soya formula			
Powder milk			
Full cream cow's milk			
Skimmed cow's milk			
Combination*			
Other*			
General comments			

45. When did you introduce the child to solid foods? ☐☐ months

46. What food/s did you initially introduce?

	YES	NO	DON'T KNOW	*Please specify
Baby porridge/cereal				
Processed baby food				
Fruit/fruit juice				
Vegetables				
Mealie pap				
Eggs				
Tea (normal)				
Tea (rooibos)				
Other*				

PHYSICAL ACTIVITY OF THE CHILD

47. ACTIVITIES AT SCHOOL

47.1 Do you attend physical education (PE/PT/GYM/games) classes at school? YES/NO

47.2 If YES, how many classes do you have a week? ☐ classes

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47.3 Using this cue card of school sports, over the last 12 months which 3 do you participate in the most?

Activities	Months/yr	Times/week	Hours/time

48. **INFORMAL ACTIVITIES**

48.1 Using this cue card, what activities do you participate in the most outside school but not in a sports club or as part of your school sports, from MON-THURS. Only name a maximum of 3 activities that you engage in and how often and how long?

Activities	Times/week	Hours/time

48.2 Using this cue card, what activities do you participate in the most outside school but not in a sports club or as part of your school sports, on a WEEKEND. Only name a maximum of 3 activities that you engage in and how often and how long?

Activities	Times/week	Hours/time

49. **SEDENTARY ACTIVITIES**

49.1 During a normal weekday, how many TV shows do you watch OR how many hours do you spend watching TV each day? ☐ shows ☐ hours ☐☐ minutes

49.2 During a normal weekend, how many TV shows do you watch OR how many hours do you spend watching TV each day? ☐ shows ☐ hours ☐☐ minutes

50. **ACTIVITY FOR TRANSPORT**

50.1 How do you usually get to school? Choose one of the following:

Walk to school		Bus/taxi	
Car		Other	

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50.2 If you do walk to school, to the bus stop, or to the taxi rank, how many minutes does it take to walk there?

Less than 15 minutes		15-30 minutes		N/A	
30-60 minutes		More than 1 hour			

50.3 If you do walk to school, to the bus stop, or to the taxi rank, how fast do you walk?

I walk slowly and there is no change in my breathing		I walk quickly which makes me breathe harder than normal	
I walk very quickly and sweat a lot, and makes me breathe much harder than normal		N/A	

6.6 Osteoporosis Evaluation Sheet

- Used to obtain information on risk factors for bone health in Study 4 (Chapter 5).

Osteoporosis Evaluation Sheet Date:__/__/__

For office use only

1. Have either of your parents been diagnosed with osteoporosis?
   - ☐ Yes
   - ☐ No

2. Have you ever been diagnosed with osteoporosis?
   - ☐ Yes
   - ☐ No

3. How would you describe your physical activity pattern?
   - ☐ I do not exercise regularly
   - ☐ < 3 times a week
   - ☐ > 3 times a week

4. Have you ever participated in any of the activities listed below more than once a week for more than 6 months of the year? Please fill in the relevant details next to the particular activity. [Y=years; M=months/yr; S=sessions/week; M=minutes/session].

Y/N	Activity	14-21 years (8 yrs total)				22-34 years (13 yrs total)				34-50 years (16 yrs total)				>50 years			
		Y	M	S	M	Y	M	S	M	Y	M	S	M	Y	M	S	M
	Jogging																
	Walking for pleasure																
	Tennis																
	Netball/volleyball																
	Aerobic dance																
	Dancing - general																
	Hockey																
	Weight-training																
	Other																
	Other																

5. During work time, do you?
   - ☐ spend most of your time sitting
   - ☐ stand a lot
   - ☐ walk around a lot
   - ☐ lift heavy objects regularly
   - ☐ I do not work

6. Do you take a calcium supplement? If yes, please specify which one, how often you take it and for how long you have been taking it.
   - ☐ Yes Name:_____ How often:_____ How long:_____
   - ☐ No

7. How many dairy servings do you consume a day?
   (1 serving = glass of milk, 175ml carton of yoghurt)
   - ☐ 1-2 dairy servings
   - ☐ 2-3 dairy servings
   - ☐ more than 3 dairy servings/day
   - ☐ I do not eat dairy

8. Do you drink alcohol? If yes, how many tots, beers or glasses of wine per day?
   - ☐ Yes _____ per day
   - ☐ No

9. How many cups of caffeinated coffee do you drink a day?
   - ☐ > than 4 cups
   - ☐ 1-4 cups
   - ☐ I do not drink coffee

10. Do you smoke? If yes, how many cigarettes per day?
    - ☐ Yes _____ per day
    - ☐ No
    - ☐ Have given up If so, how long ago? _____

11. Have you experienced any menstrual irregularities in the past? If so, please specify at what age, how many cycles per year (0-3 cycles/yr or 4-9 cycles/yr), and for how many years.
    - ☐ Yes Age:____ Cycles per year:___ No. of years:___
    - ☐ No

12. Are you currently
    - ☐ pre-menopausal
    - ☐ menopausal
    - ☐ post-menopausal

13. Are you currently on Hormone Replacement Therapy? If yes, please specify what sort and for how long you have been taking it.
    - ☐ Yes Type:_____ Duration of use:_____
    - ☐ No

14. Are you currently taking any form of oral contraceptive? If yes, please specify what sort and for how long you have been taking it.
    - ☐ Yes Type:_____ Duration of use:_____
    - ☐ No

15. How many full-term pregnancies have you had?
    ☐

16. How many months did you breastfeed in total?
    ☐

17. What is your current age?
    ☐ years

18. What is your current weight?
    ☐ Kg

19. What is your height?
    ☐ cm

BMI = _____

20. Have you ever fractured any of the following bones, and at what age?
    - ☐ Hip Age:_____
    - ☐ Vertebra Age:_____
    - ☐ Wrist Age:_____
    - ☐ I have never fractured any of the bones listed above.

BMD measurements
Total BMD:
_____
T-score:
_____
QUI:
_____
BUA:
_____
SOS:
_____

21. Please mark next to the ethnic group to which you belong:
    - ☐ African
    - ☐ Coloured
    - ☐ Indian
    - ☐ White
    - ☐ Other

22. Have you ever taken corticosteroids for more than six months, and please indicate how this has been administered (ie. nasal, inhaler, injection)?
    - ☐ Yes Administered:_____
    - ☐ No

6.7 Longitudinal follow-up questionnaire

- Used to obtain medical and training history from ultramarathon runners, in Study 5 (Chapter 6).

BONE DENSITY FOLLOW-UP QUESTIONNAIRE

Name: _____ Age:_____ yrs

Tel no:_____

1. WEIGHT HISTORY

Present weight	1 yr ago	2 yrs ago	3 yrs ago	4 yrs ago	5 yrs ago

Highest weight as an adult (excl pregnancy): _____kg (Age:_____ yrs)

Lowest weight as an adult:_____ kg (Age:_____ yrs)

2. MEDICAL & MENSTRUAL HISTORY

2a. Age at menarche:_____

2b. Please complete the following table:

(i) Please mark in the appropriate column the approximate number of periods you had per year at each age?

(ii) Please indicate at which ages you were using oral contraceptives.

(iii) Please indicate at which ages you were pregnant.

(iv) Other: please indicate, at the relevant age, if you underwent any of the following: breastfeeding, fertilty drugs (type), hormone injections (type), hysterectomy, hysterectomy and ovarectomy, miscarriage (no.of weeks).

(v) Any additional comments (also include here any medications).

	(i)				(ii)	(iii)	(iv)	(v)
Age	**0**	**1-3**	**4-9**	**10-13**	**O/C**	**Pregnant**	**Other**	**Comments**
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								

Age	0	1-3	4-9	10-13	O/C	Pregnant	Other	Comments
25								
26								
27								
28								
29								
30								
31								
32								
33								
34								
35								
36								
37								
38								
39								
40								
41								
42								
43								
44								
45								
46								
47								
48								
49								
50								

3. EXERCISE HISTORY

3a. AT PRESENT:

Do you exercise regularly? Y/N

If yes, please complete the table below.

EXERCISE	FREQUENCY/ WEEK	DURATION/ WEEK	DISTANCE/ WEEK	MONTHS/YEARS PARTICIPATION
Running				
Aerobics				
Swimming				
Cycling				
Gym training				
Other				
Other				
Other				

3b. IN THE PAST 5 YEARS:

Did you exercise regularly? Y/N

If yes, please complete the table below.

EXERCISE	FREQUENCY/ WEEK	DURATION/ WEEK	DISTANCE/ WEEK	MONTHS/YEARS PARTICIPATION
Running				
Aerobics				
Swimming				
Cycling				
Gym training				
Other				
Other				
Other				

4. INJURIES

4a. Have you ever been injured? Y/N

4b. If yes, did you (please tick the relevant box):

(i) injure a muscle, ligament, joint or tendon ☐

(ii) fracture a bone due to a bad fall or accident ☐

(iii)fracture a bone due to a trivial fall ☐

(iv) gradually develop bone pain ☐

4c. Have you ever been diagnosed with a stress fracture? Y/N (When:_____)

4d. If yes,what was the site of the stress fracture: _____

5. CALCIUM INTAKE

The questions below concern your milk consumption at various times in your life. This includes only milk that you drank, not milk that you might have with your cereal/porridge or with your tea and coffee.

At the following ages, did you drink milk

1=Every meal
2=Frequently/not with every meal
3=Sometimes
4=Rarely or never

(Please enter the relevant number in the block below)

5a. Under the age of 14 years ☐

b. For the age 14-21 years ☐

c. For the age 22-35 years ☐

d. For the age 35-50 years ☐

6. GENERAL

6a. Has anyone in your family (including your grandparents) been diagnosed with osteoporosis? ☐ Yes ☐ No ☐ Don't know

6b. Do you ever experience lower back pain? Y/N