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Bone mass and bone size in 10 year-old South African children

by

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04 May 2012

Declaration

I declare that this thesis is my own unaided work. This thesis is being submitted for the degree of Doctor of Philosophy in the Faculty of Health Sciences at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other university.

_____

(Linda van der Lingen)

_____day of _____, 2012

I certify that the studies contained in this thesis have the approval of the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (See Clearance Certificate Protocol Number M050463 in Appendix 1).

_____ Date _____

Abstract

Osteoporosis has been described as a paediatric disease with geriatric consequences. This thesis explored the associations between proximal, historical and predictive genetic and environmental factors affecting bone mass and bone size in socio-economically- and environmentally-disadvantaged black and -advantaged white pre- and early-pubertal South African children. Data were collected from 476 children (182 black boys, 72 white boys, 158 black girls, 64 white girls) of mean age 10.6 years (range: 10.0-10.9), 406 biological mothers and 100 biological fathers. The main findings were that black children and their parents compared to white, had greater DXA-measured BMC at the femoral neck regardless of the way in which BMC was corrected for size (height, weight, BA and/or BA^{PC}) and greater bone strength. Lumbar spine BMC was greater or similar depending on which measures were used to correct BMC for size. At the whole body, mid radius and distal one third of the radius, BMC varied between children, and between their parents, and were dependent on which measures were used to correct BMC for size. Weight at 1 year (WT1), length at 1 year (LT1) and birth weight (BW), were significant predictors of BMC of the femoral neck (P<0.05-0.01) after correcting BA and BMC for race/ethnicity, gender, age, socioeconomic status, bone age, height and weight at 10 years. Maternal and paternal heritability was estimated to each be ~30% in both black and white subjects. The main conclusion was that ethnicity is the single most important proximal factor affecting bone mass and bone size in 10 year old South African children. Black children demonstrate a superior bone mass and bone strength at the femoral neck. Historical and predictive factors however indicate that black children have not been programmed for optimal bone health in utero and early life, nor are contemporary environmental factors favourable for

the maximisation of peak bone mass. This cohort may be at risk of developing osteoporosis as an elderly population, particularly at the lumbar spine and forearm.

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List of abbreviations and definitions of terms

Table 1-1. List of abbreviations and definitions of terms

Abbreviation/Term	Definition
½h^2	Heritability estimate (%), by maternal or paternal descent, defined as the proportion of trait / phenotypic variation that is accounted for by genetic factors.
25(OH)D	Serum 25-hydroxyvitamin D
AA	African American
ANCOVA	Analysis of covariance
BA	Bone area (cm^2)
BD	Bone density (mg/cm^3)
Bone mass	Noun. The mineral content of bone. (Source: The American Heritage® Stedman's Medical Dictionary. Houghton Mifflin Company. http://dictionary.reference.com/browse/bone mass. Accessed 23-Dec-2011). In this thesis, refers to BMC or BMD.
Bone size	Refers to BA
BMAD	Bone mineral apparent density (g/cm^3)
BMC	Bone mineral content (g)
BMD, aBMD	Bone mineral density (g/cm^2), areal bone mineral density (g/cm^2), Bone density refers to areal bone density (g/cm^2) throughout the thesis
BMI	Body mass index (kg/m^2)
BTT, Bt20	Birth to Ten, Birth to Twenty
BW	Birthweight
Current factors	Refers to genetic factors, environmental factors, height, weight, skeletal maturation, pubertal status, SES, nutrition, physical activity.
cm	Centimetre
DPA	Dual-beam photon absorptiometry
DR	Distal 1/3rd of radius

Abbreviation/Term	Definition
DXA	Dual energy X-ray absorptiometry
Environmental factors	Refers to lifestyle factors (SES, nutrition, physical activity)
Ethnicity	Identity with or membership in a particular racial, national, or cultural group and observance of that group's customs, believes, and language. (Source: The American Heritage® Stedman's Medical Dictionary. Houghton Mifflin Company. http://dictionary.reference.com/browse/ethnicity. Accessed 23-Dec-2011).
F	Female
FN	Femoral neck
g	Grams
Genetic factors	Refers to gender and race/ethnicity
Heredity	Noun. The transmission from one generation to another of genetic factors that determine individual characteristics: responsible for the resemblances between parents and offspring. (Source: Collin's English Dictionary – Complete and Unabridged 10th Edition., http://dictionary.reference.com/browse/heredity. Accessed 23-Dec-2011).
Hereditary	Adjective. Of, relating to, or denoting factors that can be transmitted genetically from one generation to another. (Source: Collin's English Dictionary – Complete and Unabridged 10th Edition., http://dictionary.reference.com/browse/hereditary. Accessed 23-Dec-2011).
Heritability	Noun. (1) The quality or state of being heritable. (2) The proportion of observed variation in a particular trait that can be attributed to inherited genetic factors in contrast to environmental ones. (Source: Merriam-Websters Medical Dictionary. Merriam-Webster,

Abbreviation/Term	Definition
	Inc., http://dictionary.reference.com/browse/heritability. Accessed 23-Dec-2011).
Heritable	Adjective. Capable of being inherited; inheritable. (Source: Collin's English Dictionary – Complete and Unabridged 10th Edition., http://dictionary.reference.com/browse/heritable. Accessed 23-Dec-2011).
Historical factors	Refers to birthweight, length at 1 year, weight at 1 year.
Ht	Height (m)
LS	Lumbar spine
LT, LT1	Length, length at 1 year
M	Male
MR	Mid radius
P	Probability-value
PBM	Peak bone mass
PC	Power coefficients, derived from the linear-regression analyses of ln(BMC) on ln(BA)
Predictive factors	Refers to bone mass and bone size of parents, and the heritability of bone mass and bone size in children
pQCT	Peripheral quantitative computed tomography
Race[1]	A local geographic or global human population distinguished as a more or less distinct group by genetically transmitted physical characteristics. (Source: The American Heritage® Stedman's Medical Dictionary. Houghton Mifflin Company. http://dictionary.reference.com/browse/race. Accessed 23-Dec-2011).
B, W	black, white

[1] Statistics South Africa (Stats SA) has deprecated the term 'race' and advocated the use of Population Group which defines groups with common characteristics (in terms of descent and history), particularly in relation to how they were (or would have been) classified before the 1994 elections. The following categories are used: Black African, coloured, Indian or Asian, white, other. (Source: Statistics South Africa, http://www.statssa.gov.za/inside_statssa/standardisation/Concepts_and_Definitions_%20StatsSAV3.pdf, accessed 31-Oct-2011)

Abbreviation/Term	Definition
RDA	Recommended dietary allowance
SAT	Subcutaneous adipose tissue
SD	Standard deviation
SE	Standard error of the mean
SES	Socioeconomic status
SPA	Single photon absorptiometry
VAT	Visceral adipose tissue
WB, WBLH	Whole body, whole body less head Total body, total body less head
Wt, WT1	Weight, weight at 1 year

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- To all participants, without whom this project would not have been possible. Working with the subjects from the metropolitan area of Johannesburg-Soweto on a daily basis for 2 years, was a life-changing experience.
- All those who worked on the project. In particular, I'd like to thank:-
    - Sr Saeeda Mohamed for running the "DXA room" as efficiently as she did, for her DXA measurements and superior paediatric phlebotomy skills.
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    - Ms Thabile Sibiya for being extremely willing and helpful with all aspects of the day-to-day running of the project: DXA measurements, data collection, capturing and cleaning, and problem-solving.

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Dedication

To my supervisors

Preface

In 2000, I was afforded the incredible opportunity of working on the Bone Health sub-study of the formerly known Birth to Ten (BTT) project. Since extended and now called Birth to Twenty (Bt20), it is Africa's largest and longest running observational study of children and adolescent growth, development and health, and one of the few large-scale longitudinal studies in the world. Bt20 children and their families are based in Soweto-Johannesburg, South Africa's largest and densest metropolitan area, extending over 200km^2, with ~3 million inhabitants [Statistics South Africa, www.statssa.gov.za/community_new/content.asp, accessed 31-Oct-2011].

In 1990, shortly after Nelson Mandela was released from Victor Verster Prison on 11 February 1990, BTT was launched and continues to date. Within a seven week period from 23 April 1990 to 08 June 1990, 5460 singleton births were recorded in the metropolitan area of Johannesburg-Soweto. The source of the population data was the official birth notifications, governed by a local ordinance, which were completed by delivery staff at the time of every birth in the area. [273] This information was subsequently recorded in the registers maintained by each of the three local health authorities comprising most of the metropolitan area of Soweto-Johannesburg. [273] To be included in the original BTT cohort, mothers had to reside in the study catchment area for at least the first six months of the child's life. [272] A total of 3273 children met these inclusion criteria. The demographics of the BTT cohort closely approximated national

demographics at the time which were 74% black, 12.5% white, 10% coloured and 3.5% Indian. [273] The aim of BTT when initiated was to investigate prenatal risk factors, mortality, morbidity, growth, nutritional status, psychological development, environmental and household air pollution, family composition and child health associated with rapid urbanization. [273]

In 1999, researchers interested in bone health in childhood initiated a Bone Health sub-study (approved by the Committee for Research on Human Subjects, University of the Witwatersrand: M980810). The main aims of the study were to investigate a multitude of environmental and hereditary factors on bone mass acquisition. Comprehensive sets of longitudinal data (weight at birth as well as weight and length/height at 1, 2, 4, 5, 7 and 8 years of age) were available on 1200 black children from which 623 were randomly enrolled onto the Bone Health sub study. Cross checks were performed to ensure that there were no significant differences between the Bone Health subset and BTT cohort for key demographic variables such as residential area at birth, maternal age at birth, gravidity, gestational age and birth weight. All white children with longitudinal data were enrolled into this Bone Health sub study (n=65). To increase the number of white children on the study, children of the same age from schools in the greater Johannesburg metropolitan area were asked to volunteer. An additional 71 white children (boys = 38; girls = 33) were recruited onto the study. Subjects with chronic illness (juvenile rheumatoid arthritis, epilepsy or asthma) on medication known to affect growth or bone mass development were excluded from the study (n=4). I, together with an incredible team of individuals, collected data from these 476 healthy South African children (182 black boys, 72

white boys, 158 black girls, 64 white girls) of median age 10.6 years (range: 10.0-10.9) from 23 April 2000 to the 07 June 2001.

In this cohort, I investigated the relationship between bone mass, bone size, and genetic- and environmental factors during the 10th year of life. This thesis presents the data which I was involved in collecting, capturing, and which I cleaned, analysed, published, and am submitting for the degree of Doctor of Philosophy. A series of the three published manuscripts form the core of the thesis, which aimed to answer key research questions:-

1) What **proximal** factors contribute to bone mass and bone size of 10-year old pre- and early pubertal, black and white South African children?
2) Do **historical** factors contribute to the current status of bone mass? More specifically, (1) Do weight and/or length in infancy predict bone mass in 10 year old children? (2) If there is a relationship is it because weight and/or length in infancy are related to bone size or bone mass?
3) Is parental bone size and bone mass **predictive** of bone size and bone mass in 10-year old children? More specifically, what is the heritability of bone size and bone mass?

Chapters 1 and 2 describe the relevant background to the subsequent chapters. Chapter 1 briefly highlights at the outset the relevance of studying bone health in this population, and the

potential value this thesis has to add to the current knowledge and understanding of factors affecting bone health in the setting of a developing country. Chapter 2 reviews literature relating to the measurement and interpretation of bone mass and bone size in children, black-white ethnic differences, infant programming and heritability thereof. The chapter gives rise to three key research questions and hypotheses which the rest of the thesis addresses.

Chapters 3 to 5 present in the three published papers the data, analyses, results and discussion, as they were published hence the format: introduction, materials and methods, results and discussion. The following papers were published and a poster was presented in support of this thesis. As first author, I contributed substantially to the acquisition, analyses and interpretation of data. I drafted the manuscript and submitted it for publication. In the case of the poster, I gave an oral presentation of the poster at the conference.

1. Vidulich,L., Norris,S., Cameron,N. and Pettifor,J. Differences in bone size and bone mass between black and white 10-year-old South African children. *Osteoporos Int* **17**, 433-440 (2006). [320] Impact factor: 4.695. (See Appendix 2).

2. Vidulich,L., Norris,S., Cameron,N. and Pettifor,J. Infant programming of bone size and bone mass in 10-year-old black and white South African children. *Paediatr Perinat Epidemiol* **21**, 354-362 (2007). [321] Impact factor 2.110. (See Appendix 3).

3. Vidulich,L., Norris,S., Cameron,N. and Pettifor,J. Bone mass and bone size in pre- or early pubertal 10-year-old black and white South African children and their parents. *Calcif Tissue Int* **88**, 281-293 (2011). [322] Impact factor 2.893. (See Appendix 4).

4. Vidulich, L; Norris, SA; Pettifor, JM. 2002. The relationship between birth weight and weight at 1 year with bone mass variables in 10 year old South African children. 2nd *International Conference on Child Bone Health*, Sheffield, England, June 2002: Poster Presentation.

Chapter 6 consolidates the findings from Chapters 3-5. A summary of this thesis' findings, hypotheses tested, key results, common research themes that emerged and contributions to the body of knowledge that were made, are presented. It was beyond the scope of the thesis to address in detail a number of limitations and answer the questions that emerged from this body of work. The limitations to be kept in mind when interpreting the results of this thesis, and to be considered for future research in this field, are documented in this chapter, as are unanswered questions and data that could still remains to be analysed. I finally end with what the findings of this thesis conclude.

CHAPTER 1 - Why study bone health in South African children?

Osteoporosis, the most common of bone diseases, is defined as "a disease characterised by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk". [338] The disease is associated with significant morbidity, mortality and financial costs. In 2002, full-blown osteoporosis cost the US economy as much as 18 billion dollars, exclusive of the indirect costs of billions more amounting from the loss of productivity of patients and caregivers. [318] Once diagnosed, osteoporosis is not curable. By 2020, it is predicted that 50% of Americans over the age of 50 years, will have or be at risk of developing osteoporosis of the hip. [318] The figures were calculated for all ethnic groups though the fracture incidence is usually highest for whites. [176,218] Although as per the National Osteoporosis Foundation of South Africa (NOFSA) [www.osteoporosis.org.za/downloads/NOFSAguide.pdf, accessed 31-Oct-2011], data on the incidence of fracturing and osteoporosis in South Africa are not available, it appears to be similar to developed countries for white, Asian and mixed-race/ethnic populations. The black population is however different, in that osteoporosis and fracturing at the hip are less for both African Americans and black South Africans.

The International Osteoporosis Foundation has predicted that the largest increase in bone fractures worldwide is expected to occur in developing countries. The proportion of worldwide hip fractures from Africa has been projected to increase from 0.2 to 0.6% by the year 2050, based on fracture rates and population growth. [59] Indeed, South African experts are offering anecdotal evidence that the incidence of fractures is on the increase. [26] NOFSA predicted 1 in 3

South African women and 1 in 5 men (altogether potentially 4-6 million people) would be afflicted by the disease. [26] Osteoporosis and associated fragility fractures are not currently considered the most concerning health problem in South Africa. The health system is currently burdened by diseases related to HIV/AIDS, poverty and chronic diseases of lifestyle, highlighting the need for prevention strategies. [70]

Osteoporosis has been described as "a paediatric disease with geriatric consequences". There are two key concepts core to understanding the proposal that osteoporosis has its origins in childhood, namely, (1) Barker's foetal origins hypothesis and (2) the attainment of peak bone mass (PBM) during childhood and adolescence. The purpose of this section is to highlight at the outset the relevance of studying bone health in this population, and the potential value this thesis has to add to the current knowledge and understanding of factors affecting bone health in the setting of a developing country.

(1) Barker's foetal origins hypothesis of osteoporosis

Epidemiological studies have suggested that size at birth and later growth patterns are associated with chronic diseases in later life: early onset of cardiovascular, metabolic and endocrine disease in adult life, including coronary heart disease, hypertension, type 2 diabetes, hypercholesterolemia, [20,124] stroke, [17] obesity, [240] chronic lung disease, [174] psychological outcomes, [123] characteristic changes in fingerprint patterns [337] and most important to this thesis, osteoporosis. [12] That is, the bone size and mass attained in childhood and adolescence may be

limited by programming having occurred during pre- and early postnatal life. The literature is reviewed in more detail in Chapter 2.

The reasons for studying the foetal origins hypothesis in a South African population are motivated by Adair and Prentice (2004) [2] who argue that the foetal origins hypothesis is most applicable to developing countries in which most of the world's low birthweight babies are born. [73] Almost a quarter of South Africa's infants weigh less than 2.5kg, [248] and are at greatest risk of chronic disease in later life.

Socio-economically and environmentally-disadvantaged black babies are smaller at birth when compared to -advantaged white children. [248] It has been hypothesized, that smaller babies result from (1) their failure as foetus' to thrive in utero and reach their genetic potential, (2) their mothers' failure to thrive during their life and reach their genetic potential, which in turn impose uterine restraint on their babies and / or (3) generations of deprivation resulting in the evolutionary selection of a thriftier genotype and phenotype. [2] In response to undernutrition in early development, the foetus is programmed to reduce its demand for nutrients, and although adaptation may be beneficial for short-term survival, it has been linked to permanent and negative changes in the body's structure, physiology and metabolism. [13,196] Lower birthweight babies have lower bone mass and less muscle mass, both of which are reported to persist for life, and are thought to be mediated by changes to the hypothalamic-pituitary-adrenal axis and the two associated bone mass-influencing hormones: growth hormone and cortisol.

The foetal origins hypothesis is addressed in Chapter 2 (literature review), Chapter 4 (publication) [321] and Chapter 6 (discussion and conclusions).

(2) Peak bone mass (PBM)

Bone mass at any point in life reflects the balance between the activity of bone-resorbing cells (osteoclasts) and bone-forming cells (osteoblasts), with osteoclasts being more active during the process of ageing and osteoblasts being more active during the years of growth and development. [266] This bone remodelling process is regulated by the complex interaction of endogenous factors (genetic) and exogenous factors (environmental) which affects bone cell function both directly and indirectly by altering the production of local and systemic hormones that modulate bone cell activity. [264] The bone size and mass attained in childhood and adolescence is an important determinant of lifelong skeletal health and is critical in determining the individual's fracture risk in later life. [206] The greater the peak bone mass achieved at the end of the bone-growing years, the greater the protection against the inevitable bone loss and the risk of osteoporosis in later life. The vast majority of adult bone mass is attained around the time of puberty. [279] Data from the Australian-based Saskatchewan Pediatric Bone Mineral Accrual Study Peak estimated total body PBM was reached by 18.8y in females and 20.5y in males, given the evidence that bone area plateaus around 5 years after peak height velocity and bone mineralization, around 7 years. [27] While much is understood about bone loss and its mechanisms, less is known about optimising the attainment of PBM, and the complex interaction of genetic factors (heredity, body size, gender) and environmental factors (nutrition, physical activity, pubertal development) on bone acquisition during the period from birth through puberty to

adulthood. [56,122,230] More longitudinal data are required to advance our current knowledge and understanding of the factors affecting the acquisition of bone between birth to puberty, especially in different ethnic groups from developed and developing countries.

This study forms part of the greater longitudinal Bt20 study which aims to provide these data. This thesis presents cross-sectional data on the bone size and bone mass status of 10 year old children, as they entered adolescence and are covered in Chapter 2 (literature review), Chapter 3 (publications) [320] and Chapter 6 (discussion and conclusions).

CHAPTER 2 - Literature review

Measuring and interpreting bone mass and bone size in children

Over the years, paediatric studies have measured bone mass using various scanning technologies and processing software and established paediatric normative datasets. These datasets served both clinical and research applications in the diagnosis and monitoring of diseases affecting bone as well as studying the effect of genetic and environmental factors on bone mineralization and skeletal growth. The different techniques have included radiogrammetry, photon absorptiometry, single-beam photon absorptiometry (SPA), dual-beam photon absorptiometry (DPA), computed tomography, neutron activation, dual energy x-ray absorptiometry (DXA), quantitative computed tomography (QCT), quantitative ultrasound (QUS) and magnetic resonance imaging (MRI). Of these, DXA is the most widely used technique for the measurement of bone mass in paediatric populations [32,156] and recommended by the International Society for Clinical Densitometry (ISCD) as the preferred method of measuring BMC and BMD in children. [119]

DXA can acquire data from the whole body (head, arms, ribs, thoracic and lumbar spine, pelvis, legs) as well as from specific skeletal regions of interest such as the hip (total hip and femoral neck), spine (lateral, midlateral, and anteroposterior spine) and forearm (radius and ulna). [32,261,300] DXA measures bone mineral content (BMC, g) and bone area (BA, cm^2) from

which areal bone mineral density (BMD or aBMD, g/cm^2) is calculated (BMD = BMC ÷ BA), [32,36]

The advantages of using DXA technology is that it measures quickly (2-3 minutes per site), and its radiation doses are low (0.001-0.005 mSv), both of which are particularly important for children. Comparative radiation doses are tabulated below.

Table 2-1 Examples of comparative radiation doses to which people are exposed.

Radiation dose	Exposure
<0.005 mSv	The radiation dose received by a DXA scan
0.01 mSv	The radiation dose received by a patient having his/her teeth x-rayed
0.01 mSv	The radiation dose received by a patient having his/her lungs x-rayed
2 mSv	The annual dose of cosmic radiation received by a person working in an aeroplane
2.4 mSv	The global background radiation is measured at 2.4 mSv and South Africa's average is close to this.
4 mSv	The average annual radiation dose for South Africans caused by indoor radon, X-ray examinations etc.
100 mSv	The highest permitted dose for a radiation worker over a period of five years.
1000 mSv	The dose which may cause symptoms of a radiation sickness (e.g. tiredness and nausea) if received within 24 hours.
6000 mSv	The dose which may lead to death when received all at once.

Source: http://www.necsa.co.za/Necsa/Nuclear-Technology/Nuclear-Waste-442.aspx, accessed 24-Apr-2012)

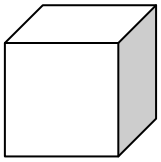
DXA is easy to use, patient-friendly and less costly than most other methods making it widely available and more accessible to patients. [230] In addition, its measurements of BMC and

BA are accurate and precise. BMC measurements are within 7-9% of BMC as determined by ash weight, the current gold standard for measuring BMC in the laboratory, [136,326] as well as concurring with Quantitative Computed Tomography (QCT) which measures true density in mg/cm^3 of either cortical or trabecular bone. [90] BMD measurements have been shown to be predictive of future fracture risk in adults [36,261] and children. [56,120,121] In children, for each standard deviation decrease in size-adjusted BMC, the risk of fracture increased by 89%, [56] which is comparative to the risk of fracture in adults which increases 1.5-2.5 times for each standard deviation decrease in BMD (or 10-12% BMD in the spine). In children, a smaller skeleton relative to overall body size, or being overweight, increases the risk of fractures than in those of health body weight. [56,120] The relationship between BMD and risk of fracture is stronger than the relationship between cholesterol and risk of heart attack, and as strong as the relationship between blood pressure and risk of stroke. [202] BMD however does not fully explain bone strength. Since 50-75% of fragility fractures occur in women who have BMD within the normal range, the prediction of fracture is improved by measuring additional parameters together with BMC to better account for bone strength. [76,185]

There are disadvantages in using DXA to measure bone mass. The first concern is related to variability in BMD measurements between different types of machines, even those made by the same manufacturer. Measured BMDs between different machines (by type or location) cannot be accurately compared due to machine and software variability, [168] over and above technologist-measuring and physician-interpretive variability, a limitation applicable to most medical technologies.

The second main concern in using DXA to measure bone mass, particularly in children is that it remains a two-dimensional projection technique of a three-dimensional structure. The third dimension of bone depth determining volume cannot be measured as it lies in the same direction as the x-ray beam. What this means is that the larger of two bones, each known to have the same volumetric BMD (for example 1g/cm^3), will be measured by DXA to have a greater areal (aBMD) (See Figure 2-1).

Figure 2-1. The larger of two bones, each known to have a volumetric BMD of 1g/cm^3, will be measured by DXA to have a greater areal BMD (2 g/cm^2 vs. 1 g/cm^2)

	Cube A	Cube B
Dimensions (cm)	1 x 1 x 1 cm	2 x 2 x 2 cm
		
BA (cm^2)	1	4
BMC (g)	1	8
aBMD (g/cm^2)	1	2

Source: Binkovitz and Henwood (2007) [32]

Another disadvantage to DXA is that it is a composite measurement of bone, lean- and fat tissue mass. Body composition can confound results. This is particularly relevant given the increasing prevalence of obesity.

DXA measurements (BMC and BA) are therefore **directly** dependent on size-related variables such as body size (height), bone size (short bones or narrow bones), [227] and **indirectly** dependent on age of the child, ethnicity and body size and composition. [9,333] In adults, this is not of significant concern since bone volume remains fairly constant but is of major concern to those working with children as they undergo great changes in body and skeletal size during growth

which is not uniform in all dimensions. [111,148] Thus, the comparison, analyses and interpretation of BMC and BMD in children need to be corrected for differences in bone or body size so that BMD is not underestimated in smaller children and overestimated in larger ones.

A number of statistical ways to address the effect of size on BMC and BMD have been proposed. Each manufacturer (Hologic, Lunar and Norland) has developed for each scanner its own normative database, each making use of their own combination of physiological size-related variables (race/ethnicity, gestational age, weight, height, maturity, and / or surface area) for which their data should be corrected. Binkovitz *et al.* (2007) [32] have summarized how these databases differ (Table 2-2). The table shows just how complex, confusing and potentially erroneous the interpretation of DXA can be. [178]

Table 2-2. Normative paediatric DXA databases (C/B/H/A/O Caucasian/black/Hispanic/ Asian/other, GA gestation age, (L) longitudinal study, SA surface area)

Reference	Year	Scanner	No. of patients (M/F)	Age range	Ethnicity (C/B/H/A/O)	Input	Output
25	1992	Hologic 1000 PB	29/28	Newborn		GA + weight + height + SA	Lumbar BMD and BMC
25	1992	Hologic 1000 PB	22 total	1–24 months		GA + weight + height + SA	Lumbar BMD and BMC
29	1996	Hologic 1000 PB	82/68	GA 27–42 weeks		Weight	Total BMD, BMC and BA
35	1991	Hologic 1000 PB	84/134	2–17 years	162/56/0/0/0	Weight + Tanner stage	Lumbar BMD
56	1990	Lunar DP-3	184 total	5–11 years		Weight	Lumbar BMD
57	1993	Norland XR-26	86/68	5–18 years		Gender + Tanner stage	Total BMC and % fat
46	1997	Hologic 1000 PB	142/201	4–19 years (L)	343/0/0/0/0	Gender + Tanner stage	Total BMD, BMC and BA
58, 59	2002	Lunar DPXL/ PED	188/256	4–20 years (L)	444/0/0/0/0	Gender + age	Lumbar BMD and apparent BMD or total BMC, % fat and lean body mass
						Gender + Tanner stage	Lumbar BMD and apparent BMD or total BMD and BMC
62	2002	Hologic 4500 FB	107/124	5–22 years	226/0/0/3/2	Gender + age	Total BMC and BA
						Gender + height	Total BA
						Gender + total BMC	Total BMD
61	2005	Hologic 4500 FB	up to 1948	3–20 years		Gender + age	Lumbar, total hip and total BMD
21	1991	Hologic 1000 PB	109/98	9–18 years	207/0/0/0/0	Gender + Tanner stage or age	Lumbar BMC, BA and BMD, femoral neck BMD
22, 33	1996	Hologic 2000 FB	110/124	8–17 years (L)	220/0/0/0/0	Gender + age	Lumbar and total BMC and BMD
							Femoral neck BMC and BMD
40[a]	1999	Hologic 1000 PB	193/230	9–25 years (L)	103/114/103/103	Gender + age + ethnicity	Lumbar, femoral neck, and total hip BMD and BMAD
							Total BMD and BMC/Ht
10	2004	Hologic 4500 FB	0/422	12–18 years	153/264/0/0/5	Age + weight + ethnicity	Lumbar and femoral neck BMD, femoral neck apparent BMD
60[b]	2001	Hologic 2000 PB	0/151	9–14 years (L)	151/0/0/0/0	Breast stage + age	Lumbar, femoral neck, trochanter and forearm BMC and BMD
54	2003	Lunar DPX PB	210/249	3–30 years	459/0/0/0/0	Gender + height or age	Total lean body mass and total BMC/lean body mass

[a]Further data available at http://www.stat-class.stanford.edu/pediatric-bones
[b]Further data available at http://www.bcm.edu/bodycomplab

Source: Binkovitz *et al.* (2007) [32]

There is no standard way to correct BMC or areal BMD data for changes in skeletal size; BMC and BMD have been corrected for varying combinations of body size (height, weight), bone size, bone area, pubertal stage, skeletal maturity, and body composition (BMI, lean body mass). [115] In the case of children, BMC or BMD data may be expressed as Z-scores. [119,343] (See

Table 2-3 below). A Z-score is a standardized score that indicates how many standard deviations a data point is from the population mean. A z-score is calculated by subtracting the population mean from the individual score, and dividing the difference by the population standard deviation. Z is negative when the raw score is below the mean and positive when it is above. BMC or areal BMD Z-scores less than or equal to 2.0 in children and adolescents are defined as low bone mineral content or bone mineral density for chronologic age, by the The Society for Clinical Densitometry (ISCD). [119]

The many different methods used, together with a number of factors may influence bone mass such as race/ethnicity, growth, nutritional status, lifestyle, and pubertal development, [323] make the interpretation of uncorrected and corrected DXA data, and the objective comparisons between studies, populations and age groups very complex, confusing and potentially erroneous. [32]

Table 2-3. Different statistical corrections used to adjust DXA measurements for bone size and / or body size

Measure	Statistical corrections	Reference
BA	Height	Buison *et al.* (2005) [44]
BMC	Weight	Leonard *et al.* (2004) [179]
	Height	Patel *et al.* (1992); [247] Zemel *et al.* (2010) [343]
	Weight, height & age	Bedogni *et al.* (2002) [30]
	Bone area	Horlick *et al.* (2000) [142]
	Bone area & height	Mølgaard *et al.* (1997) [227]
	Bone area, weight & height	Prentice *et al.* (1994) [261]
	BMC / height	Cundy *et al.* (1995); [67] Ellis *et al.* (2001) [88,89]
BMD(=BMC/BA)	Weight	McCormick *et al.* (1991) [208]
	Height	Mølgaard *et al.* (1997) [227]
	Weight & height	Prentice *et al.* (1994) [261]
	Lean body mass	Robinson *et al.* (1995) [276]
	Body mass index	Luckey *et al.* (1989) [197]
	BMD / (√height)	Cundy *et al.* (1995) [67]
BMAD	See Table 2-4.	Katzman *et al.* (1991) [161]; Carter *et al.* (1992) [51]
WB BMC	Stepwise corrections:-	Mølgaard *et al.* (1997) [227]
	(1) BMC adjusted for BA	
	(2) BA adjusted for height	
	(3) Height adjusted for age	
WB BMD	Stepwise corrections:-	Högler *et al.* (2003) [137]
	(1) BMD or BMC/age	
	(2) height/age, then	
	(3) LTM/height, then	
	(4) BMC/LTM ratio for height	
WB or LS BMC	LTM for height Z-scores	Crabtree *et al.* (2003) [65]
	BMC for LTM Z-scores	

To address the confounding effect of bone size on bone mass Katzman *et al.* (1991) [161] and Carter *et al.* (1992) [51] proposed measurements less dependent on size by mathematically converting BMC to a three-dimensional estimate of volumetric BMD (bone mineral apparent density (BMAD)). Bones were assumed to be shaped as cubes, and the formulae in Table 2-4 were applied to calculate BMAD.

Table 2-4. Formulae used to calculate BMAD

Site	Formula
Whole body	$BMAD = BMC / (BA^2 \div height)$
Mid-forearm	$BMAD = BMC / BA^2$
Femoral neck	$BMAD = BMC / BA^2$
Lumbar spine (L1-L4)	$BMAD = BMC / BA^{1.5}$

Kröger *et al.* (1992) [171] applied a similar concept assuming bones (vertebral bodies, femoral shaft and neck) to be shaped as cylinders and applied the formula BMAD=(BMC)(4/[π{bone width}]). Similarly, Lu *et al.* (1996) [195] assumed the femoral neck, mid-third of the femoral shaft, and the four lumbar vertebral bodies to be cylinders and used bone width (d) and height (h) to calculate bone volume: $[(\pi(d/2)^2 \times h]$. All methods however calculate coefficients by assuming bones are shaped as cubes or cylinders which do not necessarily hold true in different ethnic groups, ages and sex. [261]

Prentice *et al.* (1994) [261] proposed a method that calculated population-specific power coefficients (PCs) for specific skeletal sites. Power coefficients linearise the relationship between BMC and BA and allow BMC to be custom-corrected for size for each ethnic and sex group and each skeletal site.

Given our study was conducted in two ethnic groups (black and white), both sexes, and in children and their parents, the greatest confounder of bone size needed to be addressed. Therefore, the first concern of this thesis was to study the effect of correcting BMC for various combinations of height, weight, BA or BA^{PC} in black and white children, and with appropriately justified corrections, explore the associations of BMC and BA between black and white children and their parents. The different methods which were used to correct BMC for size are addressed in Chapters 3 and 5 (publications) and Chapter 6 (discussion and conclusion).

Black-white ethnic differences in bone mass and bone size

Introduction

This literature review summarises black-white ethnic differences or similarities that have been found in bone mass in children and adults. Bone mass, which may be reported as BMC, BMD, or BMAD, has been reviewed at the whole body, the femoral neck, lumbar spine and / or forearm, measured using different technologies (DXA, SPA and QCT), in females, males or both genders combined, spanning the age range from 0-65y and from different parts of the world. Table 2-5 to Table 2-8 presents studies which have reported black-white ethnic differences or similarities. Data shaded in yellow highlight those measurements which were greater in blacks than whites (B>W). Data shaded in red highlight those measurements which were greater in whites than blacks (W>B). The studies are presented per skeletal site studied, in alphabetical order of author, and includes whether DXA-, or SPA-derived BMC or BMD was corrected for size. Corrections for size were not required by QCT-derived bone density (BD). The tabulated literature summary is followed by a summary of common findings and by a discussion of what are known to be favourable and unfavourable factors contributing to black-white differences in bone mass, and what is known about the bone mass profile of South African women and children compared to their white counterparts.

Table 2-5. Summary of studies which have explored ethnic differences at the whole body (in alphabetical order of author)

Reference	N	M/F	Age	Country	Measure	Result unadjusted for size	Result adjusted for size
			Children & adolescents				
Bachrach *et al.* (1999) [10]	29	F	9-11y	USA	BMC	B>W	-
					BMD	B>W	-
	25	M	9-11y		BMC	B=W	-
					BMD	B=W	-
Ellis *et al.* (1997) [89]	245	F	3-18y	USA	BMC	-	B>W (age, wt & ht)
Ellis *et al.* (1997) [88]	297	M	3-18y	USA	BMC	-	B>W (age, wt & ht)
Horlick *et al.* (2000) [142]	336	M&F	6-11y	USA	BMC	-	B>W (BA, age, wt & ht)
Micklesfield *et al.* (2007) [220]	156	F	9y	SA	BMC	B=W	B>W (age, wt & ht)
	180	M	9y		BMC	B=W	B>W (age, wt & ht)
	172	F	9y	USA	BMC	B>W	B>W (age, wt & ht)
	239	M	9y		BMC	B>W	B>W (age, wt & ht)
Nelson & Barondess (1997) [231]	773	M&F	9y	USA	BMC	B>W	-
					BMD	B>W	-
Wang *et al.* (1997) [329]	39	F	10.2 ± 1.1y [1]	USA	BMC	B=W	B>W (wt, ht, diet & activity)
					BMD	B=W	B=W (wt, ht, diet & activity)
					BMAD	B=W	B=W (wt, ht, diet & activity)

Reference	N	M/F	Age	Country	Measure	Result unadjusted for size	Result adjusted for size
	81	F	13.5 ± 1.8y [2]		BMC	B=W	B=W (wt, ht, diet & activity)
					BMD	B=W	B=W (wt, ht, diet & activity)
					BMAD	B=W	B=W (wt, ht, diet & activity)
	54	M	10.9 ± 1.3y [1]		BMC	B=W	B>W (wt, ht, diet & activity)
					BMD	B=W	B>W (wt, ht, diet & activity)
					BMAD	B=W	B>W (wt, ht, diet & activity)
	65	M	14.4 ± 2.4y [2]		BMC	B=W	B=W (wt, ht, diet & activity)
					BMD	B=W	B=W (wt, ht, diet & activity)
					BMAD	B=W	B=W (wt, ht, diet & activity)
			Adults				
Barondess *et al.* (1997) [25,25]	79	M	33-64y	USA	BMC	B>W	B>W (BMC/ht)
Chantler *et al.* (2011) [53]	427	F	18-45y	SA	BMD	B=W (age)	Not-shown
Ettinger *et al.* (1997) [92]	402	M&F	25-36y	USA	BMD	B>W	B>W (clinical & biochemical variables)
Henry & Eastell (2000) [134]	103	M&F	20-37y	UK	BA	B=W	-
					BMD	B>W	-
Wang *et al.* (1997) [329]	109	F	20.2 ± 3.2y [3]		BMC	B>W	B=W (wt, ht, diet & activity)
					BMD	B>W	B=W (wt, ht, diet & activity)
					BMAD	B>W	B=W (wt, ht, diet & activity)

Reference	N	M/F	Age	Country	Measure	Result unadjusted for size	Result adjusted for size
	75	M	20.3 ± 3.1y [3]		BMC	B>W	B=W (wt, ht, diet & activity)
					BMD	B>W	B=W (wt, ht, diet & activity)
					BMAD	B>W	B=W (wt, ht, diet & activity)

[1]Pre-/early pubertal; [2]Pubertal; [3]Mature. Data shaded in yellow highlight those measurements which were greater in blacks than whites (B>W).

Table 2-6. Summary of studies which have explored ethnic differences at the femoral neck (in alphabetical order of author)

Reference	n	M/F	Age	Country	Measure	Result unadjusted for size	Result adjusted for size
				Children and Adolescents			
Bachrach *et al.* (1999) [10]	29	F	9-11y	USA	BMC	B>W	-
					BMD	B>W	-
	25	M	9-11y		BMC	B=W	-
					BMD	B>W	-
Bell *et al.* (1991) [31]	53	F	7-12y	USA	BMD	B>W	B>W (wt, age)
	53	M	7-12y		BMD	B>W	B>W (wt, age)
Wang *et al.* (1997) [329]	39	F	10.2 ± 1.1y[1]	USA	BMC	B=W	B>W (wt, ht, diet & activity)
					BMD	B=W	B=W (wt, ht, diet & activity)
					BMAD	B=W	B=W (wt, ht, diet & activity)
	81	F	13.5 ± 1.8y[2]		BMC	B=W	B=W (wt, ht, diet & activity)
					BMD	B=W	B=W (wt, ht, diet & activity)
					BMAD	B>W	B=W (wt, ht, diet & activity)
	54	M	(10.9 ± 1.3y[1]		BMC	B=W	B>W (wt, ht, diet & activity)
					BMD	B=W	B>W (wt, ht, diet & activity)
					BMAD	B>W	B>W (wt, ht, diet & activity)
	65	M	14.4 ± 2.4y[2]		BMC	B=W	B=W (wt, ht, diet & activity)
					BMD	B=W	B=W (wt, ht, diet & activity)
					BMAD	B=W	B=W (wt, ht, diet & activity)
				Adults			
Aspray *et al.* (1996) [8]	134	F	44+y	UK & The Gambia	BMC	?B=W	?B=W (age, ht & wt)
					BMD	?B=W	?B=W (age, ht & wt)

Reference	n	M/F	Age	Country	Measure	Result unadjusted for size	Result adjusted for size
Chantler *et al.* (2011) [53]	427	F	18-45y	SA	BMD	B>W (age)	B>W (all combinations of age, wt, fat mass, waist circumference, ht, VAT & FFSTM)
Daniels *et al.* (1995) [70]	364	F	20-64y	SA	BMD	-	B>W (wt & ht)
Daniels *et al.* (1997) [69]	294	F	20-64y	SA	BMC		B>W (wt, ht & BA)
					BMD		B>W (wt, ht)
					BMAD	B>W	
Henry & Eastell (2000) [134]	103	M&F	20-37y	UK	BA	B=W	-
					BMD	B>W	-
					BMAD	?B=W	-
Wang *et al.* (1997) [329]	109	F	20.2 ± 3.2y[3]		BMC	B>W	B=W (wt, ht, diet & activity)
					BMD	B>W	B=W (wt, ht, diet & activity)
					BMAD	B>W	B=W (wt, ht, diet & activity)
	75	M	20.3 ± 3.1y[3]		BMC	B=W	B=W (wt, ht, diet & activity)
					BMD	B=W	B=W (wt, ht, diet & activity)
					BMAD	B=W	B=W (wt, ht, diet & activity)

[1]Pre-/early pubertal; [2]Pubertal; [3]Mature. VAT visceral adipose tissue, FFSTM fat-free soft tissue mass.
Data shaded in yellow highlight those measurements which were greater in blacks than whites (B>W). Results preceded by a '?' were not explicitly reported, but deduced.

Table 2-7. Summary of studies having explored ethnic differences at the lumbar spine (in alphabetical order of author)

Reference	n	M/F	Age	Country	Measure	Result unadjusted for size	Result adjusted for size
				Children & Adolescents			
Bachrach *et al.* (1999) [10]	29	F	9-11y	USA	BMC	B=W	-
					BMD	B>W	-
	25	M	9-11y		BMC	B=W	-
					BMD	B=W	-
Bell *et al.* (1991) [31]	53	F	7-12y	USA	BMD	B>W	B>W (wt, age)
	53	M	7-12y		BMD	B=W	B>W (wt, age)
Gilsanz *et al.* (1998) [114]	294	M&F	8-18y	USA	Volumetric BD	B>W	N/A since adjustment for size not needed for QCT-derived volumetric bone density.
Southard *et al.* (1991) [299]	218	M&F	1-19y	USA	BMD	-	B=W (wt & tanner)
Wang *et al.* (1997) [329]	39	F	10.2 ± 1.1y[1]	USA	BMC	B=W	B=W (wt, ht, diet & activity)
					BMD	B=W	B=W (wt, ht, diet & activity)
					BMAD	B=W	B=W (wt, ht, diet & activity)
	81	F	13.5 ± 1.8y[2]		BMC	B=W	B=W (wt, ht, diet & activity)
					BMD	B=W	B=W (wt, ht, diet & activity)
					BMAD	B=W	B=W (wt, ht, diet & activity)
	54	M	10.9 ± 1.3y[1]		BMC	B=W	B>W (wt, ht, diet & activity)
					BMD	B=W	B>W (wt, ht, diet & activity)
					BMAD	B>W	B>W (wt, ht, diet & activity)
	65	M	14.4 ± 2.4y[2]		BMC	B=W	B=W (wt, ht, diet & activity)
					BMD	B>W	B=W (wt, ht, diet & activity)

Reference	n	M/F	Age	Country	Measure	Result unadjusted for size	Result adjusted for size
					BMAD	B>W	B=W (wt, ht, diet & activity)
				Adults			
Aspray *et al.* (1996) [8]	370	F	44+y	UK & The Gambia	BMC BMD	W>B W>B	W>B (age, ht & wt) W>B (age, ht & wt)
Chantler *et al.* (2011) [53]	427	F	18-45y	SA	BMD	B=W (age)	W>B (combinations of age, fat mass, waist circumference & ht)
Daniels *et al.* (1995) [70]	364	F	20-64y	SA	BMD	B>W	B=W (wt & ht)
Daniels *et al.* (1997) [69]	294	F	20-64y	SA	BMC BMD BMAD	 B>W	B=W (wt, ht & BA) B=W (wt & ht)
Henry & Eastell (2000) [134]	103	M&F	20-37y	UK	BA	B=W	-
					BMD	B>W	-
					BMAD	B>W	-
Wang *et al.* (1997) [329]	109	F	20.2 ± 3.2y3		BMC BMD BMAD	B>W B>W B>W	B=W (wt, ht, diet & activity) B=W (wt, ht, diet & activity) B=W (wt, ht, diet & activity)
	75	M	20.3 ± 3.1y3		BMC BMD	B=W B=W	B=W (wt, ht, diet & activity) B=W (wt, ht, diet & activity)

Reference	n	M/F	Age	Country	Measure	Result unadjusted for size	Result adjusted for size
					BMAD	B=W	B=W (wt, ht, diet & activity)

[1]Pre-/early pubertal; [2]Pubertal; [3]Mature.

Data shaded in yellow highlight those measurements which were greater in blacks than whites (B>W). Data shaded in red highlight those measurements which were greater in whites than blacks (W>B).

Table 2-8. Summary of studies which have explored ethnic differences at the forearm (in alphabetical order of author)

Reference	n	M/F	Age	Country	Measure	Result unadjusted for size	Result adjusted for size
				Children & Adolescents			
Bell *et al.* (1991) [31]	53	F	7-12y	USA	BMD	B>W	B>W (wt, age)
	53	M	7-12y		BMD	B=W	B>W (wt, age)
Li *et al.* (1989) [183]		M&F	1-6y		BMC	B>W	
Lohman *et al.* (1984) [191]					BMC	B>W	
					BMC/BW	B>W	
Patel *et al.* (1992) [247]	580	F	6-20y	SA	BMC	W>B	B>W (ht)
					BMC/BW	W>B	B>W (ht)?
		M	6-20y		BMC	W>B	B=W (ht)
					BMC/BW	W=B	B=W (ht)
Prentice *et al.* (1990) [260]	377	M&F	0-36m	UK & The Gambia	SPA-derived BMC	W>B	W>B (ht, wt, bone width)
Slaughter *et al.* (1990) [294]	108	F	8-18y	USA	BMD	B>W	
				Adults			
Aspray *et al.* (1996) [8]	386	F	44+y	UK & The Gambia	BMC	W>B	W>B (age, ht & wt)
					BMD	W>B	W>B (age, ht & wt)

Daniels *et al.* (1995) [70]	364	F	20-64y	SA	BMD	B=W	B=W (wt & ht)
Daniels *et al.* (1997) [69]	294	F	20-64y	SA	BMC	-	B=W (wt, ht & BA)
					BMD	-	B=W (wt & ht)
					BMAD	B=W	

[1]Pre-/early pubertal; [2]Pubertal; [3]Mature.
Data shaded in yellow highlight those measurements which were greater in blacks than whites (B>W).
Data shaded in red highlight those measurements which were greater in whites than blacks (W>B).
Results preceded by a '?' were not explicitly reported, but deduced.

From the above summary of the various studies conducted which investigated black and white ethnic differences in SPA-, DXA- and QCT-derived bone mass at different ages, there is evidence that bone mass before and after adjusting for body size differences (if applicable) has been found to be equivalent or greater at all sites and at all ages in African Americans than whites. Studies of black-white differences in other countries are much fewer and thus it is difficult to comment on whether these findings can be generalized across countries.

At the hip, a superior bone mass was evident in both the black population from South Africa and in African Americans both before and after making appropriate corrections for differences in size between black and white ethnic groups.

In South Africa, BMD at the proximal femur in black 20-64y old premenopausal, perimenopausal and postmenopausal women has been found to be respectively 7%, 10%, and 13% greater than in their white peers, after adjusting for differences in height and weight. [70] Limited research had been done on children in South Africa prior to 2000 when data collection for this thesis began. Since then, greater BMD at the hip has also been reported. [212,222]

In the US, the difference in femoral neck BMD between African American and white females in late premenopausal and early perimenopausal period has been reported to be of a similar magnitude (6–9%) to that reported in black and white South Africans, after selected anthropometric and lifestyle factors (which included weight) were adjusted for. [100] Greater bone mass at the hip has also been reported in African American children of all ages, when compared to white children. [31,92,183,208,329]

Given that bone mass is greater at the hip in blacks than whites, a similar ethnic difference would be expected at the lumbar spine and other skeletal sites, as there is 90-97% concordance of the WHO osteoporosis classification between the hip and lumbar spine using DXA derived T-scores in the same subject. [194] This is true at the lumbar spine in most US studies of adults, in which a greater bone mass has indeed been observed in African American adults [99,100,208,339] when compared to US white peers both before and after correcting for size. BMD was 7-12% [100] and 18% greater in African Americans and 11% higher in Somali immigrants living in the USA, than white women. [219] In African American children compared to their white US peers, both greater [10,31,208,219,329] and similar bone mass [10,31,69,70,143,183,257,277,299,329,339] have been reported before and after correcting for differences in size.

In South Africa, the results are less conclusive. In one adult study, BMD at the lumbar spine was similar in premenopausal, perimenopausal and postmenopausal black and white women after adjusting for differences in height and weight, [70] while in another, BMD at the lumbar spine was lower after correcting for ethnic differences in body composition. [53] In children, a greater BMC at the lumbar spine has been observed in prepubertal 9 year old South African girls before and after correcting for differences in height and weight [212] and age. [220]

Very few other studies have been conducted in Africa, however in The Gambia black Gambian women have been shown to have similar or lower BMD to white British women after correcting for differences in body size. [53] Lumbar spine BMC was 31% lower (and 24% after correcting for age, height and weight) than in white British women. [8]

At the forearm, black Gambian women were shown to have 16% less BMC at the midshaft of the radius than in their white British counterparts (and 10% less after adjustment for age, height, and weight). [8] No differences in forearm bone mass between South African blacks and whites were found before or after corrections. [70]

A study in children aged 0-36 months reported a greater BMC in 134 British children (123 Caucasian, 11 mixed, mostly Eurasian) when compared to 243 Gambian children both before and after correcting for height, weight and bone width. [261]

Factors influencing bone mass in black populations

Black-white differences in bone mass at the hip especially, have been proposed to result from differences in the macro- and microarchitecture of bone, as a result of biological/physiological and social/behavioural factors directly or indirectly influence bone.

Macroarchitecture of the hip

In relation to the macroarchitecture of the hip, data from the proximal femur analyzed with a hip structural analysis program, have shown that both South African and African American women have narrower marrow cavities, thicker cortices, and lower buckling ratios (ratio of outer radius to cortical thickness), yet non-significant differences in outer bone diameter. [232,233,290] Estimates of greater bone strength in the neck were also reported. [222,233] Interestingly, direct comparisons between South African blacks and African Americans and their

white counterparts showed intercountry black-black differences to be less than intracountry black-white differences, [233] suggestive of similar genetic influences. Support for this hypothesis comes from studies which suggest that the South African black population and the African-American population (originating from West Africa) had similar genetic pools, as the South African Bantu-speaking ethnic groups migrated from West Africa. [93,233,238]

Microarchitecture of the iliac crest

At the iliac crest, the microarchitecture as determined from bone histomorphometric analyses, has been established to be better in black South Africans and African American adults and children than their white peers in the few studies in which it has been assessed. Blacks had thicker cortical bone, have less porous cortices, greater endocortical wall thickness, and greater osteoid thickness. Adults in addition had fewer canals in the cortical bone and thicker trabeculae than whites. [244,286-288]

Socioeconomic status (SES)

There are a number of controllable lifestyle factors known to directly or indirectly positively influence bone mass, key of which is socioeconomic status (SES).

South Africa is a country classified by the World Bank as a developing country of upper-middle income status (http://data.worldbank.org/country/south-africa, accessed 23-Dec-2011). In 2011, South Africa officially joined the group of top emerging markets, now known as BRICS

(Brazil, Russia, India, China and South Africa). Despite this development status, the vast majority of South Africans remain poor and remain socioeconomically and environmentally disadvantaged compared to whites, with regards to income, education, employment, property ownership and access to health care. [80] As per Bloomberg (http://www.bloomberg.com/apps/news?pid=newsarchiveandsid=aoB7RbcZCRfU, accessed 23-Dec-2011), a quarter of the population is estimated to be unemployed, and as per the United Nations Development Programme (http://hdr.undp.org/en/media/HDI_2008_EN_Tables.pdf, accessed 23-Dec-2011), they survive on less than the equivalent of US $1.25 a day. There is a strong correlation between SES and health; the lowest socioeconomic groups having higher deaths and illness rates. [157] SES has also been linked to bone mass. For example, data from the UK's the Avon Longitudinal Study of Parents and Children (ALSPAC), show social position of mothers in pregnancy was related to bone area and bone mass of children aged 9.9 years, which was mediated by opposing actions of height and weight. [55] Norris *et al.* (2008) reported indicators of SES such as social support and disposable income as ascertained by caregiver's marital/cohabiting status and the presence of television in the home, were associated with whole body BMC in black and white pre-early pubertal children of the BTT cohort through its impact on BA. [237] Chantler *et al.* (2011) suggested socioeconomic and lifestyle factors of black and white South Africans contribute to BMD by different magnitudes. [53]

Fat body mass (FBM) / adiposity

The SES-adipose relationship is complex, and whether SES positively or negatively influences adipose tissue seems to depend on individual countries and on their levels of SES

development, as well as whether the studies were conducted in adults or children. It is generally accepted that in women and men from countries with medium and low levels of SES development, such as South Africa, high-SES is associated with greater adiposity, particularly in urban areas. [98,211,301] Rapid urbanisation and the associated nutrition transition are key determinants of this relationship. In contrast, countries with a high level SES development, such as the US, low-SES is associated with higher adiposity in both adults and children. [292,332] There are however always exceptions such as high-SES African American adolescent girls for examples, are more likely to be overweight than their medium-SES counterparts. [332]

Both South African black and African American populations have higher obesity rates, greater peripheral fat and less visceral fat than whites. [162,263] According to the International Association for the Study of Obesity [http://www.iaso.org/iotf/obesity, accessed 31-Oct-2011] and Puoane *et al.* (2002), [263] the obesity rates in South Africa are of the highest in Africa with around 27% of women and 9% of men being obese (BMI $\geq$30kg/m^2), 57% of women and 29% of men, 18% of girls and 14% of boys being overweight or obese (BMI $\geq$25kg/m^2).

FBM is generally considered to have a positive influence on bone mass, and be protective against osteoporosis in both adults and children. DXA and pQCT data from ALSPAC showed fat mass to be a positive predictor of bone mass, independent of LBM. [57,283,312] Fat mass was suggested to stimulate radial bone growth by increasing the rate of periosteal growth. [57]

Potential mechanisms proposed to mediate the fat-bone relationship include the mechanical loading of the skeleton and the endocrine activities of adipose tissue. Adipocytes

secrete fatty acids and peptide hormones or cytokines, known as adipocytokines (leptin, adiponectin, resistin and visfatin). [270] These hormones have been linked to both bone formation and bone resorption. The latest systematic review and meta-analysis showed leptin to be positively associated to BMD and adiponectin, negatively associated with BMD. [34] The nett effect of adiposity on bone mass, and on the growing skeleton however remains controversial. [50]

Adiposity has also been shown to negatively influence bone mass, and that increased FBM has also been associated with low total BMD and BMC. [50,278] In white and African American adults, BMD was inversely related to abdominal visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) after adjustment for LBM. [162] Associations between LBM or FBM and bone mineral to vary depending on the bone site study, the bone indices used, the technical problems with scanning technique, the sample size, and statistical analyses employed. [57,305] It has also been suggested that these results stem from total and central adiposity (visceral adipose tissue, VAT, and subcutaneous adipose tissue, SAT) which influence bone differently. In prepubertal children, Pollock *et al.* (2011) reported total body BMC to be 4% lower in overweight children with pre-diabetes than those without pre-diabetes, after controlling for sex, race, height, and weight. [256] Whereas total fat mass had a positive association with total body bone mass, the central adiposity measurements of abdominal visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) had negative relations with total bone mass, supporting that central, rather than total, adiposity may be deleterious for developing bone. In adolescents and young adult women, Gilsanz *et al.* (2009) [112] reported VAT to negatively influence QCT-measured bone structure and strength of the midshaft of the femur, though SAT positively

influenced the same parameters. Much research is still needed to fully understand the pattern of adipose tissue deposition and its influences on bone mass.

Physical activity / weight-bearing / mechanical loading

There is a strong association between SES and participation in physical activity. SES may mediate the relationship that has been found between race/ethnicity and physical activity which in turn mediates the relationship between race/ethnicity and disease. [66,165] In the US, socioeconomically disadvantaged groups are least likely to participate in physical activity, [317] the lack of which is strongly associated with being overweight or obese in adults. [172,291] Indeed, black South African and African American adults and children are less physically active, and more obese than their white counterparts. [75,172,212,213,259,291] Weight-bearing physical activity has been positively correlated with bone BMD outcomes from childhood onwards. [135,167,212,214,224,237] A study in South African women of black African or mixed ancestral origin from socioeconomically disadvantaged backgrounds showed that the influence of physical activity on adult BMD was most influential between the ages of 14 and 21 years. [224] Data from longitudinal studies spanning between 6.5 months and 6 years support that physical activity is beneficial to bone mineral accrual throughout the bone-growing years. [45] The bone response to physical activity depends on the child's maturity level, sex, skeletal site measured, and intensity and length of the exercise intervention. [39,45] The most significant changes to bone mass and bone strength have been observed in response to weight-bearing exercise during the pre- and peripubertal periods [45,228] The more intense the exercise, the longer its duration, the greater the osteogenic response and bone mass accrued. [45] The endocrine changes and rapid bone growth

associated with the pre- and peripubertal periods, support bone adaptation in response to weight-bearing exercise more so than during adolescence or adulthood. [45] That said, McVeigh *et al.* (2004) however found that 9-year old black children of the Bt20 cohort were significantly less physically active than their white counterparts, and there was no association between the level of physical activity of black children and their bone mass. [212]

Greater weight-bearing has been proposed to explain the greater femoral neck bone mass in black South African women. [69,70] Indeed, black women are of greater body weight, walk long distances, carry loads on their heads, babies on their backs; and the men are employed largely as labourers. The mechanostat theory proposes that muscles exert the greatest loads on the skeleton not just through body weight by dynamic loading of bone. These forces cause bones to adapt by altering their strength, to accommodate the load. [106] Mechanical loading has been suggested to stimulate bone formation by decreasing apoptosis and increasing the proliferation and differentiation of osteoblasts and osteocytes, [87] and so protect against bone loss and osteoporosis. This is one mechanism through which greater body weight and lean mass are proposed to be predictive of higher bone mass, lower rates of bone loss and fracture incidence. Lean-, fat- and bone mass are highly correlated, which makes the relative contributions of LBM and FBM difficult to determine. However, lean body mass (LBM) more so than fat body mass (FBM) have been independently linked to bone mass as measured by DXA and pQCT. In children (5-18y), LBM was the strongest single predictor for DXA-measured whole body BMC (r^2=0.945) and lumbar spine BMC (r^2=0.887). [65] Högler *et al*. (2003) also observed close associations between LBM and whole body BMC in girls (r=0.975) and boys (r=0.984). [137] Schönau *et al.* (2002) used pQCT and showed a linear muscle-bone relationship between muscle cross-sectional area and

BMC of the radial diaphysis in girls (r=0.89) and boys (r=0.92). [289] In South Africa, LBM was the most significant contributor to BMD at the lumbar spine and hip sites in black premenopausal women and at the hip in white women. [53] South African black women have less LBM than their white counterparts, [82] in contrast to African Americans who have more LBM. [177,234,265,336]

Diet

The socioeconomically disadvantaged tend to consume cheap energy-dense foods that are typically low in nutrient density. For black South Africans, a healthy diet is mostly unaffordable. [309] The resultant diet consumed by adults and children is higher in fat, and lower in macro- and micro-nutrients including those needed to directly or indirectly optimize bone growth and mineralization: calcium, vitamin D, phosphorus, copper, iron, magnesium, manganese, protein and zinc. [118,199,214] Black Bt20 children consume almost half the calcium intake (boys 453 mg/day; girls 494 mg/day) [214] than white children (boys 822 mg/day; girls 885 mg/day), [214] as assessed by using a validated Food Frequency Questionnaire. [199,214] Black South African women also consume a diet which is approximately 25% lower in calcium (mean 436 vs. 577 mg/day), [54] than their white peers. Despite the marked differences in calcium intake between black and white children, only a marginal effect of dietary calcium intake on BMC was found. [199,214] African American children have been shown to have higher intestinal calcium absorption and lower urine calcium excretion than white children, however these parameters have not been studied in the cohort. Serum 25-hydroxyvitamin D [25(OH)D] concentrations, the best indicator of vitamin D nutritional status, were significantly lower in black children of this cohort than white children

when aged 10 years and (93 ± 34 nmol/l vs. 120 ± 37 nmol/l). [258] The following categories were used to define vitamin D status: vitamin D deficiency (<50 nmol/), insufficiency (50-74 nmol/l) and sufficiency (>75 nmol/l). [138-140] Based on this, the vitamin D status of both groups was adequate. In addition to calcium, the dietary intakes of 18 other micronutrients were investigated and the means were found to fall below the Recommended Dietary Allowance (RDA). Recommended Dietary Allowances (RDAs) are quantities of nutrients in the diet that are required to maintain good health in people. Each nutrient has its own RDA, the actual amounts of which are required to maintain good health in specific individuals, may differ from person to person. Table 2-9 below shows that more than 75% of the Bt20 cohort sampled in 2000, at age 10 years, had intakes >75% below the RDA for calcium, potassium, zinc, copper, vitamin A, riboflavin, vitamin B_6, ascorbic acid, pantothenic acid and biotin.[200] Given the nutritional profile of black South Africans, especially children, a compromised bone mass could be expected.

Table 2-9. Nutrients by quartile according to the percentage of urban black children below the Recommended Dietary Allowance (RDA) (n=143)

% below the RDA	Year 2000	Year 2003
0-25	Protein, vitamin B_{12}, magnesium, folic acid	Folic acid
26-50	Phosphorus, manganese, vitamin E	Protein, vitamin B_{12}, magnesium, magnesium, potassium, ascorbic acid, vitamin E
51-75	Energy, iron, thiamine, nicotinic acid	Energy, iron, phosphorus, thiamine, riboflavin, nictonic acid, manganese
>75	Calcium, potassium, zinc, copper, vitamin A, riboflavin, vitamin B_6, ascorbic acid, pantothenic acid, bioton	Calcium, zinc, copper, vitamin A, vitamin B6, pantothenic acid, biotin

Source: Mackeown *et al.* (2003) [199]

Puberty

Menarcheal age in girls

Mean menarcheal age is considered a measure of socioeconomic standing, health, nutritional status of a population, and has also been associated with bone mass. [262] Early menarche has been associated with higher bone mass, [110] and a late menarche with low bone mass at the forearm, spine and proximal femur as well as an increased risk of fracture. [86,104] The relationship has been suggested to be mediated by the extended exposure (as with early menarche) or shortened exposure (as with delayed menarche) to endogenous oestrogens. [86] There is also evidence supporting that age at menarche and adult BMD are not associated, [37,217] and that menarche, body composition and bone mass might all be pre-programmed in utero or near the time of birth. [1]

SES is linked to the onset of puberty with higher SES being associated with earlier menarche. [242] Declines in menarcheal age are attributed to improvements in socioeconomic circumstances, and to the associated increase in the percentage of the population that is overweight and obese. [152] Menarcheal ages differ between developed and developing countries, and between racial/ethnic groups. African Americans girls achieve menarche significantly earlier than whites (mean age of 12.1 years for 330 black girls and 12.7 years for 419 white girls), [340] independent of select social economic factors (family size, family income level, urban residence). [340] Regarding South Africa's Bt20 cohort, menarcheal age was similar to that of African Americans, but there were no racial/ethnics differences between 188 black and 99 whites

girls: 12.4 years (95% confidence interval (CI 12.2, 12.6) in blacks and 12.5 years (95% CI 11.7, 13.3) for whites, [152] probably because of sample size (n=749 vs. 287). Menarcheal age in both black and white South Africans has declined at a rate of 0.5 years and 0.22 years per decade. [152] From another part of Africa, Gambian girls enter menarche and about of 2 years later than African Americans and South Africans; 14.90 years (95% CI 14.52-15.28). Their average rate of decline of median menarcheal age was however far more rapid at 0.65 years of age per decade. [262]

Peak height velocity in boys

Pubertal timing, as estimated by age at peak height velocity (PHV), predicted both cortical and trabecular volumetric BMD and previous fractures in young adult Swedish men from the population-based Gothenburg Osteoporosis and Obesity Determinants (GOOD) study. Late puberty proved a risk factor for low BMD and previous fractures in young adult men. [164]

Other factors

A number of additional biological/physiological factors have been documented to positively influence bone mass. Those reported in black populations include lower bone remodelling rates, [100] longer bone apposition and formation rates, [287,288] more efficient calcium economy, [3,69] and genetics. [129,235]

What was the aim of our study?

In the above section, we have discussed the bone mass profile of black South African women and children (where data were available) and compared the findings to their white counterparts. We have also reported on ethnic differences between African American and white Americans. We have discussed biological/physiological and social/behavioural factors previously proposed to influence racial/ethnic differences in bone mass. Black South Africans, children in particular, are exposed to a number of environmental factors known to impact negatively on bone mass. Given this, it was hypothesized that black children would have lower bone mass than white children. In contributing to the understanding of the complex interaction between genetic and environmental factors on bone acquisition before and in early puberty in black South Africans, the research questions this thesis therefore aimed to answer were:-

1. What **proximal** factors contribute to bone mass and bone size of 10-year old pre- and early pubertal, black and white South African children?
2. Do **historical** factors contribute to the current status of bone mass? More specifically, (1) Do weight and/or length in infancy predict bone mass in 10 year old children? (2) If there is a relationship is it because weight and/or length in infancy are related to bone size or bone mass?
3. Is parental bone size and bone mass **predictive** of bone size and bone mass in 10-year old children? More specifically, what is the heritability of bone size and bone mass?

The background and literature review related to the 2nd and 3rd research questions are discussed in the following sections:

Infant programming of bone mass and bone size

Introduction

Of all the factors affecting bone growth in childhood, perhaps the least investigated is the influence of early life on later bone mass and bone size. This section summarises what is understood about foetal and infant programming of bone mass and bone size. It begins with definitions followed by a description of the Barker's foetal origins hypothesis, measure of compromised intrauterine growth and developing and this is all applicable to bone size and bone mass. The literature review includes data from developed countries, adults and children, and males and females, as well as various measures of early life and bone mass. Table 2-10 presents studies which have linked early life events to bone mass status in children and adults. The studies are presented chronological order, and the table includes a comment on whether or not BMC or BMD was corrected for size. The tabulated literature summary is followed by a summary of common findings and a discussion of how the literature explains these findings. The relationship between Barker's foetal origins hypothesis to the bone mass profile of South African children remains an unknown and is addressed by way of a research question, the second of this thesis.

Definition of programming and metabolic imprinting

Environmental stimuli or insults during critical periods of intrauterine development are known to result in adaptations that permanently change the structure and physiology of a foetus. This is known as programming. [12,196] Programming stimuli may be endogenous (e.g. hormonal)

or exogenous (e.g. environmental) in origin, the most important of which is undernutrition.[196] Tissues that are compromised during periods of rapid cell division adapt by slowing their rates of cell division thereby protecting the foetus from relative undernutrition in late gestation.[16] In response to undernutrition in early development, the foetus is programmed to reduce its demand for nutrients, and although adaptation may be beneficial for short-term survival, it has been linked to permanent and negative changes in the body's structure, physiology and metabolism.[13,196] Lower birthweight babies have lower bone mass and less muscle mass both of which are reported to persist for life. These consequences are thought to be mediated by changes to the hypothalamic-pituitary-adrenal axis and the two associated bone mass-influencing hormones: growth hormone and cortisol.

The term 'metabolic imprinting' is related to programming.[334] The difference between metabolic imprinting and programming is in the details of their definitions.[334] Metabolic imprinting has 4 characteristics "1) a susceptibility limited to a critical ontogenic window in development 2) a persistent effect lasting through adulthood, 3) a specific and measurable outcome (that may differ quantitatively among individuals), and 4) a dose-response of threshold relation between a specific exposure and outcome."[334]

Barker's foetal originals hypothesis

Barker was the first to suggest that adult diseases may originate during foetal development. He and his colleagues observed regions in England which had the highest rates of mortality from cardiovascular disease, also had the highest rates of infant mortality in the early

20th century. [18] A strong relationship was shown between compromised intrauterine growth and development (as reflected by decreasing birthweight, head circumference and ponderal index), and death from coronary heart disease. [19,20] It was later found that not only birthweight, but also weight at one year of age were related to coronary heart disease, even if birthweight was unrelated to weight at one year of age. [105] Low birthweight has been linked to the early onset of cardiovascular, metabolic and endocrine disease in adult life, including coronary heart disease, hypertension, type 2 diabetes, hypercholesterolemia, [20,124] stroke, [17] obesity, [240] chronic lung disease, [174] psychological outcomes, [123] characteristic changes in fingerprint patterns [337] and most important to this thesis, osteoporosis. [12] Larger birthweights have also been associated with increased risk of polycystic ovarian disease and the hormone-related cancers of the breast, prostate and testicles. [281]

Measures of compromised intrauterine growth and development

Socio-economically and environmentally disadvantaged black babies are smaller at birth than advantaged white children. [248] It has been hypothesized, that smaller babies result from (1) their failure as foetus' to thrive in utero and reach their genetic potential, (2) their mothers' failure to thrive during their life and reach their genetic potential which in turn imposes uterine restraint on their babies and/or (3) generations of deprivation resulting in the evolutionary selection of a thriftier genotype and phenotype. [2]

Birthweight is determined by both the genome (~40%) and intrauterine environment (~60%), the quality of the latter depending on the supply of nutrients and oxygen, which is

influenced by among other factors maternal age, maternal height, parity, social class, the presence of pre-eclampsia and maternal smoking. [62,107,126] Birthweight is not a perfect indicator of the quality of intrauterine life, but it is the most commonly used and is one of the measures used in this thesis, together with height and weight at 1 year of age. There are additional measures reflecting the quality of intrauterine life and their association with disease patterns seen in later life, namely:-

- *Body proportions:* Intrauterine growth retardation/restriction may affect weight and length proportionately or disproportionately. [133] Altered birth proportions more so than birthweight are associated with adult coronary heart disease and type 2 diabetes. [103,188] A neonate who is of low birthweight, yet proportionate (weight and length have been equally affected), is likely to have been undernutritioned in early gestation, and this has been linked to the risk of developing high blood pressure but not coronary heart disease. [14] A neonate who is of low birthweight and disproportionate at birth (weight and length have not been equally affected) may be short or thin. A short neonate is likely to have been affected for some time in utero. A short body in relation to head size in particular has been linked to a greater risk of developing high levels of cholesterol and clotting factors. [14,21,205] A thin neonate suggests recent undernutrition. Both short and thin neonates have been linked to a greater risk of developing hypertension in adult life. [14] Thin neonates have been linked to a greater risk of developing insulin resistance in adulthood. [251]
- *Ponderal index (ratio of birthweight to birth length (weight/length^3):* A low ponderal index reflects that the foetus has recently been undernutritioned. Both animal and human

studies have shown that undernutrition late in pregnancy is commonly a consequence of an inadequate maternoplacental blood supply set up earlier in gestation. [116]

- *Abdominal circumference:* Abdominal circumference is an indicator of foetal growth, which when compromised, is predictive of high serum LDL-cholesterol and plasma fibrinogen concentrations in adult life. [21]
- *Birthweight:placenta ratio:* Placental weight and size (area) have been used to measure foetal growth. Infants born with low birthweight in relation to placental weight, or low placental weight are at increased risk of developing hypertension; [15,49] while those born with low placental weight in relation to birthweight are at increased risk of developing hypertension in combination with type 2 diabetes. [91] Also, low birthweight in relation to placental weight has been associated with failure to thrive and poor catch-up growth for the first 18 months. [128]
- *Crown-heel length:* Birth length reflects the foetus' trajectory of growth, which is set at an early stage in development, provided that the maternoplacental unit is able to supply sufficient nutrients to maintain that trajectory. Crown-heel length is an indicator of lean body mass and skeletal growth, which is most important to skeletal health in later life. There is a strong relationship between paternal birthweight and crown-heel length, [117] and this may reflect paternal imprinting of genes important for skeletal growth, such as those regulating the concentrations of insulin-like growth factor (IGF). [74]

Barker's foetal origins hypothesis in relation to bone mass and bone size

It has been suggested that Barker's foetal origins hypothesis may apply to the skeletal system and that growth during prenatal and early postnatal life are related to bone status in later life. Epidemiological studies have indeed shown retarded growth in infancy is associated with lower bone mass in adults and children, independent of environmental influences known to negatively impact bone, such as calcium, nutrition and physical activity. [64] A tabulated review of the literature in chronological order, a summary of common findings and a discussion of how the literature explains these findings follows.

Table 2-10. Studies linking early life events to bone mass status in adults and children, in chronological order

Reference	n	M/F*	Age (y)	From	Measures of early life	Outcome (with no adjustment)	Outcome (with adjustments)
Hamed *et al.* (1993) [126]	230	F	20-23	UK	Birthweight	Not associated with either lumbar spine or femoral neck BMD	-
Cooper *et al.* (1995) [60]	153	F	21-22	UK	Weight at 1 y; Short height at 5 & 10 y (height at 1 y not available)	Associated with lumbar spine & femoral neck BMC (not BMD, BMAD)	After adjusting for weight results remained significant, but not when adjusting for height & weight concurrently.
					Birthweight	Not associated with any measure of bone mass at any site	-
Cooper *et al.* (1997) [62]	189 F & 223 M	M&F	61-73	UK	Weight at 1 yr	Females: Weakly associated with lumbar spine & femoral neck BMC (not BMD) Males: Weakly associated with lumbar spine BMC (not BMD)	After adjusting for height & weight, results no longer significant.
					Birthweight, breastfeeding & social class	Not associated with any measures of bone mass	-
Düppe *et al.* (1997) [83]	7	M&F	Mean 15	UK	Weight at 4 & 6 y	Associated with total body BMC (not BMD, & not femoral neck BMC or BMD)	After adjusting for weight & height, results strengthened.
Jones *et al.* (1999) [150]	30	M&F	8	Aus-tralia	Birthweight & growth in infancy	Associated with lumbar spine & femoral neck BMD.	After adjusting for height & weight, results still significant.

Reference	n	M/F*	Age (y)	From	Measures of early life	Outcome (with no adjustment)	Outcome (with adjustments)
Jones *et al.* (2000) [151]	30	M&F	8	Aus-tralia	Breastfeeding > 3 months	Associated with higher BMD at the whole body, lumbar spine & femoral neck.	-
Fewtrell *et al.* (2000) [97]	44	M&F	8-12	UK	Weight & height at 18 m & 7.5 y	-	After correcting for current age & sex, associated with whole body, lumbar spine, femoral neck & radial BA, BMC & BMD
					Birthweight	-	After adjusting for gestational age, associated with lumbar spine BA
					Birth length	Associated with lumbar spine & whole body BA.	After adjusting for gestational age, current BA, height & weight, sex, age, pubertal status, birth length was negatively related to whole body & lumbar spine BMC.
					Length of gestation	Not associated with any measures of bone mass	-
Yarbrough *et al.* (2000) [342]	305	F	47-89	USA	Birthweight	Associated with lumbar spine, total hip & forearm BMC (not BMD)	After adjusting for weight, BW associated with lumbar spine BMC only.
Gale *et al.* (2001) [107]	143	M&F	70-75	UK	Birthweight	Associated with whole body, lumbar spine & femoral neck BMC	After adjusting for age, sex & height results remained significant, but not when adjusting for height & weight concurrently. Associations also

Reference	n	M/F*	Age (y)	From	Measures of early life	Outcome (with no adjustment)	Outcome (with adjustments)
							remained significant after adjusting for lifestyle factors.
					Head circumference : abdominal circumferences (measure of brain-sparing)	Not associated with bone size or density.	-
McGuigan *et al.* (2002) [209]	460	M&F	22	Northern Ireland	Birthweight	Not associated with BMD	Not associated after adjusting for parental height & weight
Weiler *et al.* (2002) [335]	5	M&F	Mean 17.3	Canada	Premature births of birthweight <1500g	Bone mass is appropriate for body size.	-
Antoniades *et al.* (2003) [5]	2822	M&F	47.5	UK	Birthweight	Associated with BMD at the femoral neck, lumbar spine, but not at the forearm	Not associated after adjusting for height & weight
Dennison *et al.* (2004) [77]	205 M & 132 F	M&F	61-73	UK	Weight at 1 y	Associated with GH1 genotype associated with bone loss	-
te Velde *et al.* (2004) [308]	261	M&F	36	The Netherlands	Birthweight	Associated with BMC at the whole body, femoral neck & lumbar spine	After adjusting for weight, no longer associated.
Arden *et al.* (2005) [6]	29	F	66	UK	Birthweight	Negatively associated with serum 1,25 $(OH)_2D$	-

Reference	n	M/F*	Age (y)	From	Measures of early life	Outcome (with no adjustment)	Outcome (with adjustments)
					Weight at 1 year	Negatively associated with serum 1,25 $(OH)_2D$, more so than birthweight.	-
Dennison *et al.* (2005) [78]	966	M&F	70's	UK	Birthweight	Correlated with femoral neck & lumbar spine BMC	-
					Weight at 1 year	Correlated with femoral neck & lumbar spine BMC	-
Laitinen *et al.* (2005) [173]	1099	M&F	31	Finland	Birthweight	-	Standardised BW correlated with standardised distal radius BMC
					Growth retardation at birth	Associated with distal & ultradistal radius BMC & distal BMD	Associated with distal & ultradistal radius BMC after adjusting for body size
					Gestational age, ponderal index	Not associated with distal & ultradistal radius BMC or BMD	
Pearce *et al.* (2005) [250]	389	M&F	49-41	UK	Birthweight	Not associated with hip or lumbar spine BMD	Not associated with hip or lumbar spine BMD after adjusting for height & weight
					Childhood growth	Associated with BA (total BA of lumbar spine & hip)	Not associated with BA (total BA of lumbar spine & hip) after correcting for height & weight
Saito *et al.* (2005) [280]	86	F	18-21	Japan	Birthweight	Associated with lumbar spine & total hip BMC	-
					Weight gain from birth to 1.5 y	Associated with lumbar spine & total hip BMC & lumbar	-

Reference	n	M/F*	Age (y)	From	Measures of early life	Outcome (with no adjustment)	Outcome (with adjustments)
						spin BMD.	
					Weight gain from 9 to 12 y	Associated with femoral neck BMC	-
Tobias *et al.* (2005) [314]	451	M&F	9	UK	Maternal intake of folate in pregnancy at 32 weeks	Folate intake associated with spinal BMC	Folate intake associated with spinal BMC (adjusted for BA, height, weight & other factors
					Maternal intake of K^+ in pregnancy at 32 weeks	K^+ intake associated with spinal BMC	Association no longer significant
					Maternal intake of Mg^{2+} in pregnancy at 32 weeks	Mg^{2+} intake associated with total body BMC	Association no longer significant
Dalziel *et al.* (2006) [68]	174	M&F	31	US	Birthweight	BW corrected for gestational age was associated with lumbar spine, total body & femoral neck BMC.	BW Z-score associated with BMC & BMD of the proximal femur.
						Associated with narrowing of the upper shaft & narrow neck regions	Other associations no longer significant.
					Prematurity, betamethasone exposure	No effect on PBM or femoral geometry	
Leunissen *et al.* (2009) [181]	312	M&F	18-24	The Netherlands	Birthweight & birth length	Z-score associated with LS BMD	Association no longer significant after correcting for age, gender & adult body size

* Gender: M = males; F = females

Summary of common findings

The relationship between birthweight, growth in infancy and bone mass has been explored in several epidemiological studies; in adults, [5,60,62,63,78,107,126,196,308,342] young adults, [60,280] adolescents[83] and children, [97,150,314] which Table 2-10 summarises. Regardless of the different populations, genders, age groups and statistical analyses employed, three common findings emerged:-

- A heavier birthweight or weight at 1 year was positively and consistently associated with higher BMC in adulthood.
- Size in infancy was a better predictor of BMC than birthweight.
- By adjusting BMC for current size (height and/or weight), or by using BMD, relationships between birthweight or infant size and bone mass were generally rendered insignificant, at the hip and lumbar spine (in women).

Birthweight is associated with BMC

A heavier birthweight was positively and consistently associated with higher BMC in adulthood especially at the lumbar spine and hip, and less consistently at the forearm. A pooled meta-analysis-estimate showed that for every 1 kg increase in birthweight, lumbar spine BMC increased by 1.49g and hip BMC by 1.41g. [11]

Size in infancy is a better predictor of BMC than birthweight.

All studies having explored the relationship between weight at 1 year and adult bone mass, have reported weight at 1 year to be significantly associated with bone mass. [60,62,78]

Childhood weight, especially weight at 1 year (WT1), more so than birthweight (BW) was predictive of bone mineral content (BMC) before adjusting for the confounding variables of current height and weight, but generally, not after. The evidence supports the hypothesis that skeletal development is programmed in utero and tracks to infancy and adulthood, but that it is the tracking of body or bone size that is the major factor accounting for differences in bone mass in adulthood. Low birthweight and slow growth in height during childhood have been shown to be directly associated with the adult risk of hip fracture. [61]

Size at birth and infancy are not associated with BMC corrected for size (height and / or weight), or BMD

Meta-analyses and systematic reviews of studies revealed no associations between birthweight and BMD of the lumbar spine or hip. [11,285] By adjusting BMC for size (height and/or weight), or by using BMD, relationships between infant size and bone mass were generally rendered insignificant. [11,285]

How does the literature explain these findings?

Evidence from animal studies

Studies in animals support the principle that early environment has long-term consequences on health. [7] The features of metabolic syndrome have been shown in the offspring of rats fed calorie-restricted diets, [109] protein-restricted diets, [125] iron-restricted diets, [182] high-fat diets, [163] or have been subjected to intrauterine growth retardation following intrauterine artery ligation [293] or have been overexposed to glucocorticoids. [186] The common outcome despite the range of insults suggests a common pathway.

Evidence from these animal studies indicates that the growth trajectory is likely to be set at an early stage of foetal development. [187] In sheep, alterations in maternal diet and plasma progesterone, around the time of conception and embryo implantation, have been shown to change the foetal growth trajectory. [166,327] Progesterone may alter the growth trajectory by changing the allocation of cells between the embryoblast which develops into the foetus and the trophoblast which develops into the placenta. [166,327]

Rats fed a protein-restricted diet have offspring with altered skeletal growth and bone biochemistry. [175] BMC and BA are lower in the experimental animals than in controls, there are changes in appearance to the growth plates, and the formation of osteogenic precursors within the bone marrow compartment is delayed. [215,241]

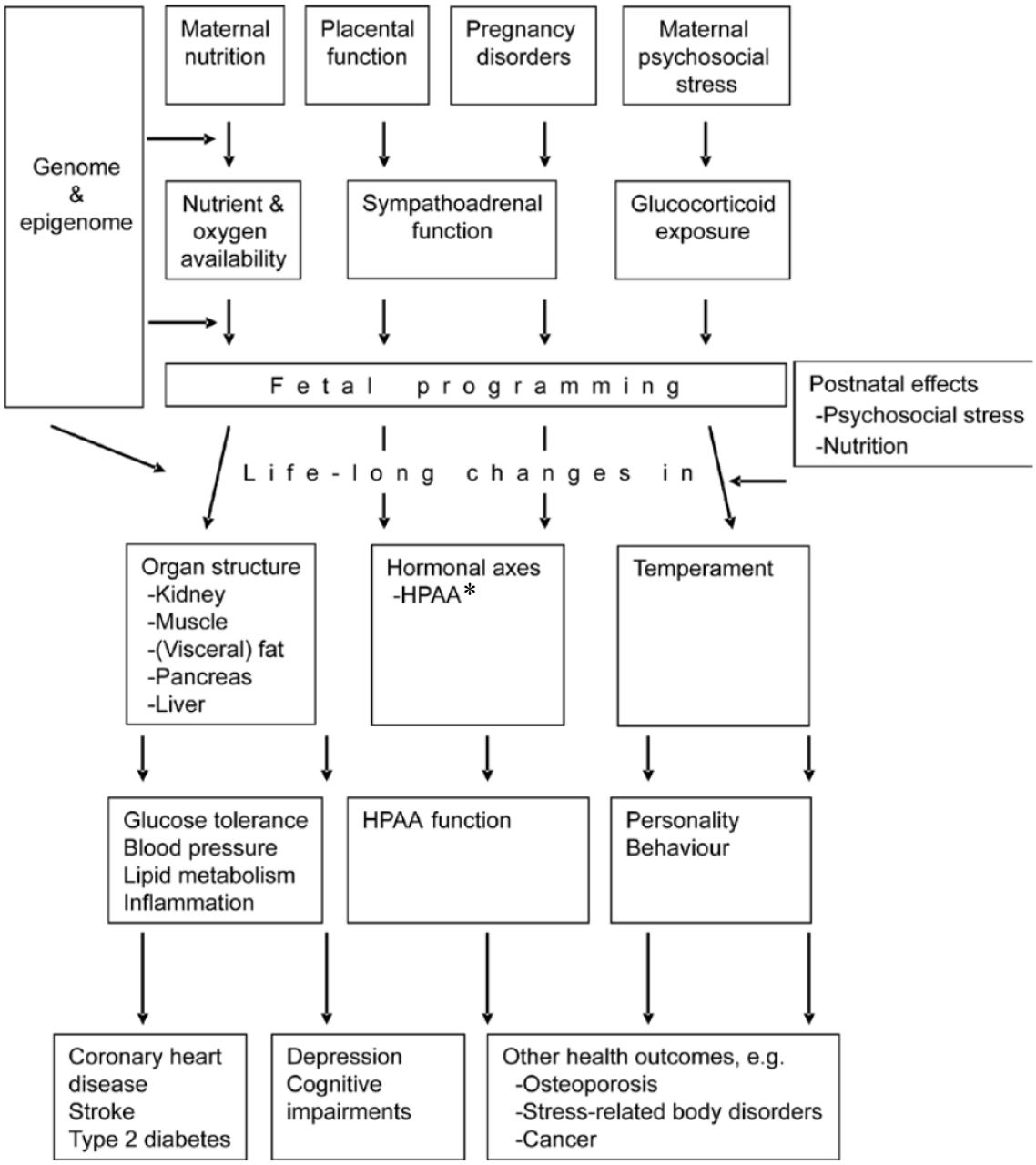
In addition, in response to calcium deficiency, the size of the trabecular envelope is increased and that of the cortical envelope, reduced. This is thought to be mediated by parathyroid hormone (PTH) and PTH-related peptide (PTHrP). [313]

Evidence from studies in humans

In humans, skeletal size, BMC and BMD have been shown to track, from as early as 19 weeks gestation. [43,78,107,130] Harvey *et al.* (2010) showed femur length from 19 to 34 weeks gestation was predictive of postnatal skeletal size at age 4 years, and foetal abdominal circumference was predictive of volumetric bone density. [130] Tobias *et al.* (2005) showed constituents of maternal diet (magnesium, potassium and folate) were related to total body and spinal DXA measures in pre- and early pubertal boys and girls aged 9 years, supporting the hypothesis that early life factors program the trajectory of bone development in childhood. [314]

Kajantie (2008) [155] proposed a conceptual framework of possible pathways of the foetal programming of adult diseases. This framework summarises the potential stimuli or insults a foetus may experience and the resultant lifelong metabolic changes. See Figure 2-2 below.

Figure 2-2. Conceptual framework of possible pathways of foetal programming of adult diseases



* HPAA, hypothalamic-pituitary-adrenocortical axis

Source: Kajantie (2008) [155]

Altered foetal nutrition and/or excess foetal glucocorticoid exposure during critical periods of development are the environmental stimuli or insults that cause metabolic adaptations and/or modification of epigenetic gene regulatory mechanisms. [72] In any tissue at any given stage, only a few of the 30 000 genes are expressed. The inhibition/activation of this "epigenetic code" is what determines our phenotype. [108] Epigenetics may be defined as the inheritance of information based on gene expressed levels rather than gene sequence (genetics). [144]

The metabolic adaptations are thought to result from metabolic imprinting by way of 1) induced variations in organ structure, 2) alterations in cell number, 3) clonal selection, 4) metabolic differentiation and 5) hepatocyte polyploidization. [334] In relation to the skeletal system and growth trajectory, the candidate mechanism through which programming occurs is through the regulation of mesenchymal stem cell and osteoblastic activity. [282] The regulatory hormones which are implicated include vitamin D, insulin-like growth factor (IGF)-1, [6,94,145,146] growth hormone (GH), [77] PTH and PTHrP [313] and leptin. [160]

Osteoblast function has been shown to be positively influenced by IGF-1 [225] which is mainly secreted by the foetal liver. Leptin, which is mainly secreted by the foetal adipose tissue, [180] is thought to affect bone formation via the hypothalamic action on the sympathetic nervous system, [160] by stimulating the differentiation of stromal cells into osteoblasts and inhibiting the differentiation to adipocytes. [311] There are leptin receptors on bone mass stomal cells, as well as on osteoblasts and chondrocytes. [127]

The reason size in infancy is a better predictor of BMC than birthweight is proposed to be because of accelerated or catch-up growth which may occur in the first 2 years following compromised growth *in utero*. [306] This catch-up growth is thought to restore the genetically determined growth trajectory [306]

The reason why birthweight is associated with BMC but not BMC corrected for height and/or weight, or BMD, is partly because of (1) BMC's strong dependence on current bone or body size, to which birthweight and weight at 1 year are associated and (2) the inability of DXA to distinguish between the contributions by size and density. It remains unknown to what extent associations seen between size at birth and in infancy reflect the tracking of weight and height during childhood.

What was the aim of our study?

In the above section, I reviewed studies which have explored the relationship between Barker's foetal origins hypothesis and bone mass. These studies were conducted in the developed countries of the UK, Australia, Canada and Finland in adult Caucasian populations. There is a need for Barker's foetal originals hypothesis to be supported by replicating findings in other settings, such as in a developing country and in other races/ethnicities, to assess whether this hypothesis manifests in children and whether the relationship between size in infancy and bone mass is mediated by the tracking of infant size, on which bone mass is dependent. Also, very few studies have addressed the influence of post natal growth (e.g. height and weight at 1 year) on BMC. Thus we have explored Barker's foetal originals hypothesis by studying the relationship

between growth in infancy and bone mass, by examining how birthweight and weight at 1 year relate to bone size and bone mass, as well as body size (height and weight) in 10 year old black and white South African boys and girls. This is the first study to be conducted in a developing country with a research sample of 10 year old children from both black and white ethnic groups. I asked the research question **“Do historical factors contribute to the current status of bone mass? More specifically, (1) Do weight and/or length in infancy (birth and at 1 year) predict bone mass in 10 year old children? (2) If there is a relationship is it because weight and/or length in infancy are related to bone size or bone mass?"**

Heritability of bone mass and bone size

Introduction

The preceding chapters have discussed the variation in the bone mass phenotype in black and white populations. Phenotypic variation may be due to environmental factors, random chance and genetic factors, the last of which accounts for the major proportion (50-80%) of variation in BMD. [24,122,132,192,253]

The bone mass phenotype is classified as a quantitative trait, which is measured on a continuous scale (as opposed to a discrete scale), shows variability in the population, is affected by the environment, and for which no specific gene has been identified. Geneticists use quantitative genetics (which includes expertise from the fields of genetics, genomics, physiology, statistics, bioinformatics and computational biology) to study the link between continuous traits and their underlying genetic basis, and to predict patterns of trait variations among relatives. Genetic components of various continuous traits may be estimated by using simple statistical methods. In this chapter, I review and discuss the familial resemblance and heritability of bone mass and bone density, parameters which I used to analyse my data.

Familial resemblance

Familial resemblance relates to traits, such as bone mass, that appear similar between family members more so than unrelated pairs of individuals. Familial resemblance may be

estimated statistically using correlations or covariances among family members. [271] These measures may not necessarily be entirely due to genetic transmission of a trait given the common environments families are exposed to, such as lifestyle habits, and in the case of bone mass, calcium intakes, physical activity, smoking and alcohol. The strength of familial resemblance may be quantified by estimating heritability.

Heritability

Heritability has two definitions; (1) the quality or state of being heritable (Source: Merriam-Websters Medical Dictionary. Merriam-Webster, Inc., http://dictionary.reference.com/browse/heritability. Accessed 23-Dec-2011) and (2) a statistical one that defines heritability as the genetic variance divided by the total variance. Heritability estimates (0-100%) are used to help identify genetic and/or environmental causes of differences between individuals, by using variance. A high heritability estimate implies a strong resemblance between parents and their children with regards to a specific trait and a low heritability implies a low level of resemblance. Heritability of bone density has been estimated to be 50-80% [141,153,169,192,255,295,316] and as high as 80-90% in twin studies. [81,253,295]

Heritability indices that are estimated from full siblings and include non-additive genetic variance are termed "heritability in the broad sense" (H^2). Heritability in the broad sense (h^2) can be estimated as twice the intraclass correlation coefficient of the sons and daughters from analyses of variance of BMD Z-scores. [169]

Heritability indices that include only additive genetic variance (e.g. parent-offspring comparisons) in the numerator are termed "heritability in the narrow sense" (h_N^2). Heritability in the narrow sense (h_N^2) can be estimated from the regression of offspring phenotypic value (e.g. BMD z-scores of offspring, BMD_o) on the average parent phenotypic values (e.g. BMD z-scores of parents (BMD_P). The regression coefficient (β_p), by definition, is the ratio of covariance between parent and offspring to variance of the parents, which represents total variance in the phenotype i.e. $BMD_o = \beta_i + \beta_p (BMD_p)$. [169]

The non-heritable portion (1-h_N^2 or 1 -h^2) may be attributed to measurement error and environmental influences specific to an individual. Studies aim to adjust BMC or BMD measurements for lifestyle and reproductive factors, accounting for the co-variance between BMC or BMD of parents and their children due to non-genetic factors. [169] Table 2-11 to Table 2-13 summarise studies which have explored familial resemblance and the heritability of bone mass. All studies chose BMD as their primary variable in which to study heritability or familial resemblance of bone mass.

y of studies (presented in alphabetical order of author) having explored familial resemblance and

Country	Race/ Ethnicity	Relations	Age ± SD (y)	n	Adjust-ments	Statistics	Authors' conclusion	Reference
Switzerland	Caucasian	Daughter Mother	8.1 ± 0.7 40.0 ± 4.0	138 138	Age-adj z-scores	(1) Pearson's (2) Heritability	Familial resemblance of bone mineralisation was clearly detectable in prepubertal girls, particularly at sites of prevailing trabecular bone.	**Ferrari *et al.* (1998)** [95]
Australia	Not specified	Daughter Son Mother	8.3 8.2 33.5	105 186	(1) z-scores (2) BMD adj for height & weight	(1) Pearson's (2) Logistic regression (3) Heritability	Heritability consistently higher in mother-daughter than mother-son pairs. [Adjusting for bone size did not alter results]	**Jones & Nguyen, (2000)** [149]
France	Caucasian	Daughter Son Mother Father	18.1 ± 2.0 18.4 ± 2.4 41.9 ± 3.6 44.0 ± 3.8	98 85 129 129	BMD adj for age & sex	(1) Pearson's (2) Logistic regression (3) Stepwise multiple regression	The BMD in children in healthy families was related to the BMD of their parents, as well as to environmental factors (weight, height, percentage body fat, BMI, daily calcium intake & physical activity, but not alcohol consumption & smoking).	**Jouanny *et al.* (1995)** [153]
USA	European ancestry	Daughter Son Mother Father	31 ± 6 32 ± 5 60 ± 6 63 ± 6	40 40 40 40	Age-, wt- & ht-adj BMD z-scores; MR adj for lifestyle factors	(1) Pearson's (2) Forward stepwise multiple regression	Heredity contributes significantly to bone density	**Krall & Dawson-Hughes (1993)** [169]

Sites	Technology	Measures of bone mass	Country	Race/ Ethnicity	Relations	Age ± SD (y)	n	Adjust-ments	Statistics	Authors' conclusion	Reference
R	SPA	BA, BMC, vBMD	Australia	Not specified	Daughter Son Mom Father	11-17 11-17 44.6 ± 4.8 47.1 ± 5.6	52 54 99 78	Adj for age, gender, sexual maturity, ht & wt	(1) Percentage of parent (2) Pearson's (3) Heritability (4) Stepwise multiple regression	After adjusting for age, gender, sexual maturity & body size, heritability account for the greatest variation in bone mass	**Magarey *et al.* (1999)** [201]
Metacarpal **LS (L1-L4)** **R (distal)**	RG SPA DPA	BL, BM, BMD	USA	Caucasian	Daughter Mother Father	14 42 ± 4 43 ± 4	31 24 24		(1) Pearson's (2) Stepwise multiple regression (3) Canonical-correlations	Genetic info from mothers & fathers strongly influences bone size, mass & density in young women	**Matkovic *et al.* (1990)** [207]
FN, Tot hip, LS (L1-L4)	DXA	BMC, BMD	Canada	Not specified	Daughter Mother Son Mother	11.8 ± 2.1 38.8 ± 4.4 12.7 ± 2.0 40.9 ± 5.0	41 41 42 42	Age-derived BMD z-scores	(1) Percentage of mother's BMD (2) Pearson's	Strong BMD familial resemblance between mother-daughter & mother-grandmother pairs. [Hip bone mass accumulates before that of the spine]	**McKay *et al.* (1994)** [210]
FN, LS, WB & head		BMD	Sweden	Not specified	Son Mother Father	17.0 ± 0.4 44.5 ± 4.4 47.1 ± 4.4	50 pairs	Midparent & offspring z-scores	(1) Heritability (2) Principal component analysis	Heritability is a main determinant of the variance in BMD in young men	**Nordström & Lorentzom (1999)** [236]

Sites	Technology	Measures of bone mass	Country	Race/ Ethnicity	Relations	Age ± SD (y)	n	Adjust-ments	Statistics	Authors' conclusion	Reference
R (mid & distal)	SPA	BW, BMC, BMD	USA	Not specified	Daughter Mother	18.6 ± 0.1 44.2 ± 0.4	84 84	Both adj for weight & BMI & non adj values used	(1) Pearson's (2) Heritability (3) Backward stepwise multiple regression	There is a strong maternal genetic influence on the accrual of bone mass in 18-22y olds.	**Tylavsky *et al.* (1989)** [316]

Sites: FN, femoral neck; LS, lumbar spine; R, radius (distal unless specified); WB, whole body.
Technology: DXA, dual x-ray absorptiometry, RG, radiogrammetry; SPA, single-photon absorptiometry.
Measures of bone mass: BL (mm), bone length; BA (cm^2) bone area; BW (cm), bone width; BM (g), bone mass; BMC (g), bone mineral content; BMD (g/cm^2), bone mineral density.
Statistics: Pearson's, Pearson product moment correlation; Heritability, heritability (regression coefficient, ß).
Unless specified, all associations mentioned are positive and significant. Outcomes (with adjustments) when not made, was left blank
PS Midradius = 95% cortical, distal radius = 38-50% trabecular [284]

Table 2-12. Summary of genetic studies (presented per skeletal site) using percentage of parents' bone mass

Sites	Measures of bone mass	% Daughter of Mother	% Son of Mother	Age	Adjustments	Reference
			Femoral neck (FN)			
FN	BMC	33-43	-	8y	Age-adjusted z-scores	Ferrari *et al.* (1998) [95]
	BMD	59-78	-			
	BMAD	75-105	-			
FN	BMD	75	92	Pre-puberty	Age-derived BMD z-scores	McKay *et al.* (1994) [210]
FN	BMD	85	111	Early-puberty		
			Total Hip (Tot hip)			
Tot hip	BMD	67	81	Pre-puberty	Age-derived BMD z-scores	McKay *et al.* (1994) [210]
Tot hip	BMD	80	100	Early-puberty		
			Lumbar spine (L)			
L2-L4	BMC	33-43	-		Age-adjusted z-scores	Ferrari *et al.* (1998) [95]
	BMD	59-78	-			
	BMAD	75-105	-			
L1-L4	BMD?	90-97	-	16y		Matkovic *et al.* (1990) [207]
L1-L4	BMD	62	65	Pre-puberty	Age-derived BMD z-scores	McKay *et al.* (1994) [210]
L1-L4	BMD	74	80	Early puberty	Age-derived BMD z-scores	

Sites	Measures of bone mass	% Daughter of Mother	% Son of Mother	Age	Adjustments	Reference
			Radius (R)			
R	BW	99	97	17y	Adjusted for age, gender, sexual maturity, ht & wt	Magarey *et al.* (1999) [201]
	BMC	95	85			
	vBMD	98	89			
Distal (1/10th) R	BMD	90-97	-			
Distal(1/5th) R	BMD	90-97	-			
Mid R	BMC	90	-	19y		
	BMD	95	-			
Distal R	BMC	90	-			
	BMD	95	-			

D, daughter; M, mother; S, son; F, father; MP, midparent.
* Both parents and not midparent
Measures of bone mass: BL (mm), bone length; BA (cm^2) bone area; BW (cm), bone width; BM (g), bone mass; BMC (g), bone mineral content; BMD (g/cm^2), bone mineral density.
PS Midradius = 95% cortical, distal radius = 38-50% trabecular [284]
Results preceded by a '?' were not explicitly reported, but deduced.

Table 2-13. Summary of genetic studies (presented per skeletal site) using Pearson's moment product correlation coefficients (r)

Sites	Measures of bone mass	D-M	D-F	D-MP	S-M	S-F	S-MP	Adjustments	Reference
					Femoral neck (FN)				
FN	BA	0.34	-	-	-	-	-	Age-adjusted z-scores	Ferrari *et al.* (1998) [95]
	BMC	0.35	-	-	-	-	-		
	BMD	0.34	-	-	-	-	-		
	BMAD	0.34	-	-	-	-	-		
FN	BMD	0.40	-	-	0.16	-	-	(1) z-scores (2) BMD adjusted for height & weight	Jones & Nguyen (2000) [149]
FN	BMD	0.40	-0.12	0.22	0.47	0.31	0.58	Age-, wt- & ht-adjusted BMD z-scores	Krall & Dawson-Hughes (1993) [169]
FN	BMD	0.31	-	-	0.22	-	-	Age-derived BMD z-scores	McKay *et al.* (1994) [210]
					Total hip				
Total hip	BMD	0.36	-	-	0.26	-	-	Age-derived BMD z-scores	McKay *et al.* (1994) [210]
					Lumbar spine (LS)				
L2-L4	BA	0.36	-	-	-	-	-	Age-adjusted z-scores	Ferrari *et al.* (1998) [95]
	BMC	0.65	-	-	-	-	-		
	BMD	0.31	-	-	-	-	-		
	BMAD	0.24	-	-	-	-	-		
LS	BMD	0.30	-	-	0.30	-	-	Z-scores	Jones & Nguyen (2000) [149]

Sites	Measures of bone mass	D-M	D-F	D-MP	S-M	S-F	S-MP	Adjustments	Reference
L2-L4	BMD	0.30	0.16	0.34	0.28	0.24	0.37	Age-, wt- & ht-adjusted BMD z-scores	Krall & Dawson-Hughes (1993) [169]
L1-L4	BM	0.43	0.53	0.65	-	-	-		Matkovic *et al.* (1990) [207]
	BMD	0.46	0.45	0.60	-	-	-		
L1-L4	BMD	0.34	-	-	0.25	-	-	Age-derived BMD z-scores	McKay *et al.* (1994) [210]
					Whole body (WB)				
WB	BMD	0.22	-	-	0.06	-	-	(1) z-scores (2) BMD adjusted for height & weight	Jones & Nguyen (2000) [149]
WB	BMD	0.24	0.29	0.29*	0.30	0.18	0.18*	BMD adjusted for age & sex	Jouanny *et al.* (1995) [153]
WB	BMD	0.54	0.11	0.46	0.57	0.24	0.54	Age-, wt- & ht-adjusted BMD z-scores	Krall & Dawson-Hughes (1993) [169]
					Radius (R)				
R	BMD	0.35	0.40	0.47	0.27	0.23	027	Age-, wt- & ht-adjusted BMD z-scores	Krall & Dawson-Hughes (1993) [169]
R	BW	0.36	0.18	0.34	0.57	.24	0.52	Adjusted for age, gender, sexual maturity, ht & wt	Magarey *et al.* (1999) [201]
	BMC	0.37	0.22	0.37	0.50	026	0.47		
	vBMD	0.33	0.12	0.32	.32	0.25	0.36		
Distal (1/10th) R	BMD	0.46	0.57	0.72	-	-	-		
Distal (1/5th)	BMD	0.46	0.28	0.52	-	-	-		

Sites	Measures of bone mass	D-M	D-F	D-MP	S-M	S-F	S-MP	Adjustments	Reference
R									
Mid R	BW	0.44	-	-	-	-	-	Both adjusted for wt & BMI & non adjusted values used	Tylavsky *et al.* (1989) [316]
	BMC	0.47	-	-	-	-	-		
	BMD	0.34	-	-	-	-	-		
Distal R	BW	0.33	-	-	-	-	-		
	BMC	0.37	-	-	-	-	-		
	BMD	0.32	-	-	-	-	-		

D, daughter; M, mother; S, son; F, father; MP, midparent.
*Both parents and not midparent
Measures of bone mass: BL (mm), bone length; BA (cm^2) bone area; BW (cm), bone width; BM (g), bone mass; BMC (g), bone mineral content; BMD (g/cm^2), bone mineral density.
Midradius = 95% cortical, distal radius = 38-50% trabecular [284]

Discussion of common findings

The studies which explored familial resemblance and heritability of bone mass had the following common findings:-

1. Associations: Heritability was the main determinant of variance in BMD. The BMD of children was associated with that of their parents as determined by (1) percentage of parents' bone mass, (2) Pearson's moment product correlation coefficients, (3) heritability estimates, (4) logistic regression, (5) stepwise multiple regression, (6) canonical-correlations and (7) principal component analyses, all of which strongly suggest a genetic contribution. The extent to which genetic factors were shown to influence bone mineral mass depended on the choice of methodology, bone mass variables studied as well as skeletal sites studied, family relationships compared, gender involved, population studied, number of participants in study and statistics used.

2. Timing of associations: Studies showed that genetic factors governing bone mass are expressed before the pubertal growth spurt in girls, [95] peaked between the ages of 13 and 26 years, [122,141] and decreased with increasing age. [71,101,198] That is, the major genetic effect was on the attainment of peak bone mass rather than on bone loss in later life. [198] Also, the prepubertal period was the period during which environmental influences appear to have a much larger effect than during adulthood. [149] Given this, interventions should be targeted in prepubertal children having been identified directly or indirectly as being at higher genetic risk of low bone mass.

3. Gender-specificity: Osteoporosis affects more women than men (~30% vs. ~12%). [268] Several studies in adults have found gender differences in the heritability of BMD, with heritability being greater in men than women. For example, heritability of femoral neck BMD was ~67% in men vs. ~47% in women; [158] lumbar spine BMD was 72% in men vs. 55% in women; [269] forearm BMD was 89% in men vs. 74% in women. [229] In children, the bone density in girls was more strongly associated with that of their parents, especially their mothers suggesting genetic factors are more influential in girls. [153,210] The genetic variation observed including between the genders was not thought to be the cause of the complex disease but was rather thought to influence a person's susceptibility to the detrimental effects of negatively bone-influencing environmental factors. [159]

4. Site-specificity. Most studies report on the heritability of bone mass at the clinically important skeletal sites of femoral neck and lumbar spine. Fewer studies have investigated other skeletal sites, but those that have, have shown that besides heritability being gender-specific, it is also site-specific. It has been proposed that individual skeletal sites may respond differently to genetic and environmental influences. [253] The head, for example, a skeletal region relatively independent of external forces such as weight-bearing, is thought to be the region best preserving genetic effects on bone mass, and has been shown in adult monozygotic twins to be the most highly heritable region of the human skeleton. [315] Quantifying the degree to which BMD is heritable at different sites is important. By so doing, the degree to which low bone mass can be treated with environmental modifications such as diet, exercise and medication (vitamin D, hormone replacement therapy/oestrogen and bisphosphonates) can be better predicted. Sites of predominantly trabecular bone (38-50%) such as the distal radius have been shown found to be

more influenced by genetics than sites of predominantly cortical bone (95%) such as the mid radius. [95] More evidence is however needed to support the finding that heritability of BMD is site-specific and the reasons for this. [255]

There are a number of genes that have been identified to be associated with bone mineral density and bone turnover, the most recent of which have been confirmed by way of genome-wide association (GWA) studies. [302] GWA studies compare genomes between two groups and look for genetic variation associated with a particular trait. Identified variants are linked to traits which are investigated at the molecular level in a cell or organism. A number of osteoporosis susceptibility loci have been identified and validated. The following genes have been identified to be associated with bone mass and bone turnover:-

	Gene symbol	**Gene name**
1.	VDR:	Vitamin D receptor
2.	ESR1:	Oestrogen receptor α
3.	ESR2:	Oestrogen receptor β
4.	LRP4:	Low density lipoprotein receptor-related protein 4
5.	LRP5:	Low density lipoprotein receptor-related protein 5
6.	SOST:	Sclerostin
7.	GFP177:	Green fluorescent protein 177
8.	RANKL:	Receptor activator of NF-κB ligand

9.	RANK / TNFRSF11A:	Tumour necrosis factor receptor superfamily, member 11A
10.	OPG / TNFRSF11B:	Osteoprotegerin / Tumour necrosis factor receptor superfamily, member 11B
11.	COLIA1:	Collagen type I alpha 1
12.	SPP1:	Secreted phosphoprotein 1
13.	ITGA1:	Integrin alpha 1
14.	SP7:	SP7 transcription factor
15.	SOX6:	SRY (Sex determining region Y)-box 6

The above-listed genes are all associated with three biological pathways: (1) the oestrogen endocrine pathway, (2) the RANKL/RANK/OPG signalling pathway, and (3) the Wnt (Wingless and int-1)/β-catenin signalling pathway. [184]

The oestrogen endocrine pathway

Oestrogen binds to two types of oestrogen receptors (ESR1 and ESR2) and positively influences bone through three modes of action by: (1) inhibiting bone remodelling by suppressing the self-renewal of osteoblasts and osteoclasts progenitors, (2) prolonging the life of the osteoblasts and (3) inhibiting bone resorption by down regulation of the RANK/RANKL/OPG pathway (see section below). [40,249]

The RANKL/RANK/OPG signalling pathway

The RANKL/RANK/OPG signalling pathway regulates osteoclasts formation and activation. Osteoblasts and stromal cells express RANKL activators and secrete OPG. Osteoclasts and their precursors have RANK receptors on the surface. RANKL binds to RANK and by so doing regulates the differentiation of precursors into multinucleated osteoclasts. The RANKL/RANK interaction may be blocked by OPG which acts as a decoy receptor and binds to RANKL Thus it is suggested that the RANKL/OPG ratio is important in determining levels of bone mass in normal and diseased individuals. [40]

The Wnt/β-catenin signalling pathway

Wnts are secreted glycoproteins key to the regulation of cell growth, differentiation, function and death. Signalling through the Wnt/β-catenin pathway positively influences bone through the renewal of stem cells, stimulation of pre-osteoblast replication, induction of osteoblastogenesis and the inhibition of osteoblasts and osteocyte apoptosis. [170] Mutations in this Wnt-signalling pathway and altered expression of LRP5, a co-receptor, result in both high- and low (osteoporosis pseudoglioma syndrome) bone mass traits depending on whether the mutation is one related to loss-of or gain-of function. [325] These mutations are thought to alter the response of the skeleton to mechanical loading by increasing/decreasing production of OPG by osteoblasts. [147]

Although differences in the expression of the genes listed above have the potential to explain black-white differences in bone mass, none have unequivocally been proven to do so, [85,96,235,267] and is thus an area on which future research should be focused.

The relationship between the familial resemblance and heritability of bone mass can be summarized as follows:-

1. *Associations:* Heritability was the main determinant of variance in BMD.
2. *Timing of associations:* The major genetic effect was on the attainment of PBM rather than on bone loss in later life.
3. *Gender-specificity:* Heritability was consistently higher in mother-daughter than mother-son pairs.
4. *Site-specificity:* Genetics may have a greater effect on sites consisting mainly of trabecular bone than cortical bone.
5. *Conclusion:* Offspring of parents with low BMD also have a low BMD. Those at higher genetic risk can be identified either directly or indirectly and targeted for the optimisation of environmental factors leading up to attainment of PBM.

What was the aim of our study?

The variation in BMD is accounted for by mostly genetic factors (50-80%), with the remainder (20-50%) owing to environmental and random factors. In this chapter, the familial resemblance and heritability of bone mass in children and their parents have been reviewed. The

findings of studies conducted in Caucasian populations of Australia, Canada, Europe, US and Sweden support the possibility of being able to directly or indirectly identify children at a higher genetic risk of low bone mass so that they may be targeted for programs optimizing environmental factors in the years leading up to attainment of PBM. Similar studies assessing heritability of bone mass and bone size by way of parent-child associations have not been previously explored in developing countries and/or black populations. Populations subjected to environmental factors known to negatively influence bone mass are expected to have lower bone mass, higher fragility fracture rates and lower heritability estimates. Black South Africans, children in particular, are exposed to a number of environmental factors known to impact negatively on bone mass, as previously discussed. Given the environmental variability, it could be hypothesized that heritability estimates of bone mass would be less in black than in white children. In contributing to the understanding of the complex interaction between genetic and environmental factors on bone acquisition before and in early puberty in black South Africans the third research question this thesis aimed to answer was **"Is parental bone size and bone mass predictive of bone size and bone mass in 10-year old children? More specifically, what is the heritability of bone size and bone mass in 10-year old pre-and early pubertal South African children?"**

Research questions and hypotheses

The review of the literature in the preceding sections raised three key research questions. The key research questions and proposed hypotheses are summarized below, and are answered in the succeeding three chapters which are presented as a series of three publications. In order to answer these questions, it is important we explored how best to adjust DXA-derived measures of BMC for body- and bone size in South Africa's pre- and early pubertal children and their parents.

Research question 1:	**What <u>proximal</u> factors contribute to bone mass and bone size of 10-year old pre-and early pubertal, black and white South African children?**
Hypothesis:	Body size (height and weight), genetic factors (gender, race/ethnicity), lifestyle factors (SES, nutrition, physical activity), sexual and skeletal maturity influence bone mass and bone size.
Research question 2:	**Do <u>historical</u> factors contribute to the current status of bone mass?" More specifically, (1) Do weight and/or length in infancy predict bone mass in 10 year old children? (2) If there**

is a relationship, is it because weight and/or length in infancy are related to bone size or bone mass?

Hypothesis: Size in infancy is related to 10 year old bone size and mass, mainly through the tracking of body size, which influences bone mass.

Research question 3: **Is parental bone size and bone mass <u>predictive</u> of bone size and bone mass in 10-year old children? More specifically, what is the heritability of bone size and bone mass?**

Hypotheses: (3a) Bone mass is likely be heritable.

(3b) Heritability estimates of bone mass are less in black than in white children

CHAPTER 3 - Differences in bone size and bone mass between black and white 10-year old South African children

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Introduction

The incidence of osteoporosis and fracturing, a late manifestation of the disease, is significantly lower in African-American than Caucasian US populations [114,193,216] and has resulted in considerable research into ethnic differences in bone mass. The lower incidence of fracturing has in part been explained by a greater bone mass in African-Americans. [4,24,143,232] Although fracture rates are also low in Africans living in Africa, few studies have investigated bone mass in communities in Africa. [8,69,70,319]

A greater bone mass in African-Americans than in Caucasian Americans has been explained by advantageous differences in key bone-influencing factors. [143,303] Black South Africans, children in particular, are exposed to a number of environmental factors known to impact negatively on bone mass, such as poor nutrition, [46] low calcium intake, [199] little physical activity, [212,213] patterns of compromised growth, and delayed onset of puberty, [47,48] thus bone

mass could be expected to be reduced when compared to South African whites or African-Americans.

Studies of bone mass in adult South African ethnic groups have found that pre-, peri- and postmenopausal black women have a greater bone mass at the hip than white women (as had been found in African-Americans), but their bone mass at the radius and lumbar spine is similar to that of whites (unlike African-Americans). [69,70] Radial bone mass is greater in black than white children [246] but little is known of the factors influencing bone mass in children of different ethnic groups in developing countries. This study describes the ethnic differences in bone mass in pre- and early pubertal children in South Africa.

Materials and methods

Subjects

We collected data on 476 healthy children (182 black boys, 72 white boys, 158 black girls, 64 white girls) of median age 10.6 years (range: 10.0-10.9) who formed part of the Birth to Twenty (BT20) longitudinal cohort of children born in the greater Johannesburg metropolitan area within a six-week period (23 April - 8 June 1990). [102,273,341] Comprehensive sets of longitudinal data were available on 1200 black children from which 340 were randomly enrolled onto the Bone Health Study. Cross checks were performed to ensure that there were no significant differences between the Birth to Twenty and Bone Health cohort for key demographic

variables (residential area at birth, maternal age at birth, gravidity, gestational age and birth weight). All white children with longitudinal data were enrolled into this bone health study (n=65). To increase the number of white children on the study, children of the same age from schools in the greater Johannesburg metropolitan area were asked to volunteer. An additional 71 white children (boys = 38; girls = 33) were recruited onto the study. Subjects with chronic illness (juvenile rheumatoid arthritis, epilepsy or asthma) on medication known to affect growth or bone mass development were excluded from the study (n=4). This study protocol was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand, Johannesburg and the Ethical Advisory Committee of Loughborough University, UK. Both children and guardians gave written informed consent to be studied.

Anthropometry

Height was measured to the last completed 1 mm using a wall-mounted stadiometer (Holtain, UK) and weight to the nearest completed 0.1 kg using a digital electronic instrument (Dismed, USA). [190] Both instruments were regularly calibrated and subjects wore minimal clothing when being weighed. Forearm length, needed for DXA analyses, was measured as elbow-wrist length taken between the most posterior point of the olecranon and the most distal palpable point of the styloid process of the radius. [190]

Maturity

Sexual maturity was self-assessed by pubic hair development in boys and girls, using the Tanner scaling technique. [203,204] Children were divided into two stages of development, namely, pre-/early pubertal (Tanner stages 1-2) and midpubertal (Tanner stages 3-4). In addition, skeletal maturity was assessed by scoring bone age from hand radiographs using the Tanner-Whitehouse bone-specific scoring technique (TWII20). [307]

Dual-energy X-ray absorptiometry (DXA)

Bone area (BA) and bone mineral content (BMC) of the whole body, left total hip, femoral neck, lumbar spine (anteroposterior, L1-L4) and distal third of the radius were measured by DXA in array mode, using an Hologic QDR-4500 (Hologic, Inc., Waltham, MA, USA). Lean and fat tissue masses were also measured. The data were analysed with the software supplied by the manufacturer, version 11.2. A lumbar spine phantom was scanned daily to determine the machine's measurement precision, expressed as the coefficient of variation (CV) which for BA and BMC were 0.47% and 0.78% respectively. All measurements were performed and analysed by the same person.

Lumbar vertebral heights

Anterior, middle and posterior heights of lumbar vertebrae L1-L4 were measured at sites determined by the DXA operator. Vertebral heights were calculated as the mean of the heights of

the anterior, middle and posterior portions of lumbar vertebrae L1-L4, which were measured (in mm) from a lateral DXA scan, using provided software. [114]

Radiogrammetry

In addition to DXA measurements, radiogrammetry was used to measure cortical thickness of the second metacarpal from anteroposterior radiographs of the left hand. Using digital callipers calibrated to the nearest 0.01 mm, measurements were made to the nearest 0.1 mm of the length of the metacarpal (L), outer cortical diameter (D) and inner cortical diameter (d) at the midpoint of the shaft. Combined cortical thickness (C=D-d), cortical cross-sectional area (π/4[D2-d2]), % cortical cross-sectional area to total area ([(D2-d2)/D2]x100) and the Barnett-Nordin index ((C/D)x100) were calculated. The Barnett-Nordin index is a parameter of relative cortical thickness that compensates for differences in skeletal size and variations in tube-to-film and hand-to-film distance. [23] Measurement precision, expressed as the coefficient of variation (CV) was determined between two observers (LV and SN) which for metacarpal length, outer and inner diameters were 0.34%, 1.65% and 1.81% respectively.

Socioeconomic questionnaire

Primary caregivers answered questions about their social and economic status. This questionnaire had been modified appropriately for a South African population and previously validated. [42] The socioeconomic score was formulated from the presence or absence of 13 asset indicators, namely, house type, electricity, indoor flushing toilet, indoor running water, refuse

removal, television, digital satellite television, motor vehicle, refrigerator, microwave, washing machine, video machine and telephone).

Statistics

STATISTICA (data analysis software system) version 6 (StatSoft, Inc., 2001) was used to perform univariate and multivariate analyses to determine ethnic differences. Parametric data were analysed using univariate analyses (age, bone age, height, weight, body mass index (BMI), BMC and BA). Lean and fat mass, corrected for height were analysed using an ANCOVA. Stepwise multiple regressions analyses were used to determine predictors (gender, pubertal development, current height and weight) of the dependent variables (BMC or BA). A positive β meant BMC or BA in black children was greater than it was in white children. Nonparametric data were analysed using Fisher's exact test (pubertal development) and Mann-Whitney U-Test (socioeconomic status). Probability values <0.05 were considered significant for all tests. Numerous statistical comparisons were made, thus more cognisance was placed on differences with $p \leq 0.01$.

Results

Cohort characteristics

Characteristics of the cohort which took part in this study are shown in Table 3-1. Black children lived in households which scored significantly lower on the socioeconomic scale

(median = 7, range: 0-13) than white children (median = 12, range: 6-13) ($p<0.05$, Mann-Whitney U Test). Most of our cohort was prepubertal or in early puberty (black boys: 99%, white boys: 99%, black girls: 98%, white girls: 97%) as determined by pubic hair development, and there were no ethnic differences in sexual maturity (Fisher's exact test). Skeletal maturity as determined by bone age, was similar between the ethnic groups within each gender, even though black boys and girls were slightly younger than their white counterparts at the time of their visit. Black children were significantly shorter than their white peers (boys: $p<0.0001$; girls: $p<0.01$), black boys weighed significantly less than white boys ($p<0.001$) and had less lean tissue mass ($p<0.0001$). After correcting for differences in height, both ethnic groups had similar lean tissue masses, however black girls had higher fat mass ($p<0.05$) than white girls.

Table 3-1. Descriptive characteristics (mean ± SD (n)) of black and white children aged 10 years.

	Males			Females		
	White (n=72)	**Black (n=182)**	***P***	**White (n=64)**	**Black (n=158)**	***P***
Age (y)	10.65 ± 0.24	10.55 ± 0.27	<0.01	10.62 ± 0.25	10.53 ± 0.27	<0.05
Bone age (y)	10.31 ± 1.04 (71)	10.13 ± 1.05 (179)	ns	10.41 ± 1.23 (62)	10.38 ± 1.27 (156)	ns
Pre-/early puberty (Tanner hair 1and2)	99% (65)	99% (170)	ns	99% (65)	98% (154)	ns
Mid-puberty (Tanner hair 3and4)	1% (1)	1% (2)		1% (1)	2% (3)	
Height (cm)	143.5 ± 7.5	137.4 ± 6.2	<0.0001	142.6 ± 7.8	139.2 ± 6.3	<0.001
Weight (kg)	36.0 ± 6.4	32.6 ± 6.6	<0.001	35.6 ± 7.8	34.8 ± 8.3	ns
Lean mass (kg)*	26.9 ± 3.6	24.1 ± 3.2	<0.0001	25.0 ± 4.2	23.9 ± 3.9	ns
Fat mass (kg)*	8.2 ± 3.4	7.4 ± 4.0	*ns	9.8 ± 4.3	10.1 ± 5.1	*<0.05
Body mass index (kg/m^2)	17.4 ± 2.1	17.2 ± 2.6	*ns	17.3 ± 2.6	17.8 ± 3.4	*ns

*After corrections for ethnic differences in height**.**

DXA results

Table 3-2 summarises ethnic differences in BA and BMC of the whole body, femoral neck, total hip, lumbar spine and distal third of the radius, as determined by DXA. The data and statistics presented in Table 3-2 are not corrected for current body size. Table 3-3 shows the results from multiple regression analyses where BA and BMC were corrected for gender, pubertal development, height and weight.

Whole body

Black boys had significantly less whole body BA and BMC than white boys ($p<0.0001$) but after correcting for gender, puberty, height and weight, there were no significant differences in BA or BMC (Table 3-3).

Femoral neck

Black children had a smaller BA at the femoral neck (both genders: $p<0.0001$) but similar BMC. However, after correcting for gender, puberty, height and weight, there was no difference in BA and BMC was greater in black than white children ($\beta =0.20$, $p<0.0001$) (Table 3-3). BMC was 6% and 5% greater in black boys and girls respectively than their white peers when adjusted means were compared.

Total hip

Before corrections, black children had a smaller BA at the total hip (both genders: $p<0.0001$) (Table 3-2). After corrections, despite BA remaining smaller in black children ($\beta = -0.13$, $p<0.0001$), BMC was greater in black than white children. ($\beta = 0.07$, $p<0.05$) (Table 3-3). BMC was 6% greater in black boys than white boys when adjusted means were compared, and was no different in girls.

Lumbar spine

Black boys had less BA and BMC at their lumbar vertebrae than white boys (both $p<0.0001$) (Table 3-2) which was explained by differences in height and weight (Table 3-3). That is, after corrections, there were no ethnic differences at the lumbar spine in BA or BMC.

Radius

At the mid radius, before corrections, black children had similar BA but less BMC than white children (boys: $p<0.05$). After corrections, BA and BMC were significantly greater in black than white children (BA: $\beta=0.26$, $p<0.0001$; BMC: $\beta=0.13$, $p<0.0001$) (Table 3-3). That is, black boys and girls had 6% more BMC at the mid radius than white boys and girls respectively.

At the distal one third of the radius, before corrections, black boys had less BMC than white boys ($p<0.01$) (Table 3-2). After corrections, black children had a greater BA ($p<0.05$) but there were no ethnic differences in BMC.

Table 3-2. Bone area (BA) and bone mineral content (BMC) comparisons between ethnic groups within each gender. Values are unadjusted means (± SD)

	Males			Females		
	White	**Black**	***P***	**White**	**Black**	***P***
	n=72	n=182		n=64	n=158	
Whole body BA (cm^2)	1312.22 ± 163.87	1217.08 ± 140.59	<0.0001	1286.75 ± 187.92	1248.58 ± 171.87	ns
Whole body BMC (g)	1084.94 ± 164.78	995.13 ± 140.83	<0.0001	1036.84 ± 196.41	992.95 ± 179.03	ns
	n=71	n=180		n=64	n=158	
Femoral neck BA (cm^2)	4.32 ± 0.33	4.13 ± 0.31	<0.0001	4.21 ± 0.30	4.05 ± 0.31	<0.0001
Femoral neck BMC (g)	3.03 ± 0.42	3.06 ± 0.38	ns	2.70 ± 0.46	2.77 ± 0.41	ns
	n=71	n=180		n=64	n=158	
Total hip BA (cm^2)	22.36 ± 2.68	20.42 ± 2.43	<0.0001	23.18 ± 3.42	20.69 ± 2.50	<0.0001
Total hip BMC (g)	16.23 ± 2.73	15.52 ± 2.58	ns	15.39 ± 3.67	14.57 ± 3.00	ns
	n=72	n=182		n=64	n=158	
L1-L4 BA (cm^2)	46.00 ± 4.96	43.02 ± 4.26	<0.0001	43.99 ± 4.26	42.99 ± 4.34	ns
L1-L4 BMC (g)	26.72 ± 4.66	19.09 ± 3.69	<0.0001	25.54 ± 5.10	25.33 ± 5.24	ns
	n=69	n=180		n=64	n=158	
Mid radius BA (cm^2)	4.52 ± 0.81	4.48 ± 0.80	ns	4.24 ± 0.80	4.37 ± 0.84	ns
Mid radius BMC (g)	1.88 ± 0.38	1.75 ± 0.34	<0.05	1.70 ± 0.37	1.68 ± 0.41	ns
	n=69	n=180		n=64	n=158	
Distal $^1/_3{}^{rd}$ radius BA (cm^2)	2.32 ± 0.19	2.32 ± 0.22	ns	2.19 ± 0.20	2.18 ± 0.20	ns
Distal $^1/_3{}^{rd}$ radius BMC (g)	1.14 ± 0.12	1.09 ± 0.13	<0.01	1.06 ± 0.15	1.04 ± 0.15	ns

Table 3-3. Ethnic differences in bone area (BA) and bone mineral content (BMC) at the whole body, femoral neck, total hip, lumbar spine (L1-L4) and mid- and distal 1/3rd of the radius after correcting for gender, puberty, height and weight

Measure of bone mass	Ethnicity (β*)	± SE	*P*	R^2	Predictors (*P*<0.001)	Puberty
Whole body BA (cm^2)	0.04	0.02	ns	0.86	Height, weight	ns
Whole body BMC (g)	0.02	0.03	ns	0.70	Height, weight, gender	ns
Femoral neck BA (cm^2)	-0.07	0.04	ns	0.44	Height, weight, gender	ns
Femoral neck BMC (g)	0.20	0.03	<0.0001	0.50	Height, weight, gender	ns
Total hip BA (cm^2)	-0.13	0.03	<0.0001	0.59	Height	ns
Total hip BMC (g)	0.07	0.04	<0.05	0.50	Height, weight, gender	ns
L1-L4 BA (cm^2)	0.04	0.03	ns	0.57	Height, gender	ns
L1-L4 BMC (g)	0.02	0.04	ns	0.47	Height, weight	ns
Mid radius BA (cm^2)	0.26	0.03	<0.0001	0.63	Height, weight, gender	ns
Mid radius BMC (g)	0.13	0.03	<0.0001	0.61	Height, weight, gender	ns
Distal $^1/_3{}^{rd}$ radius BA (cm^2)	0.11	0.04	<0.05	0.27	Height, gender	ns
Distal $^1/_3{}^{rd}$ radius BMC (g)	0.12	0.04	ns	0.36	Height, weight, gender	ns

* A positive β means BA or BMC was greater in black than white children

General

Correcting BA and BMC for ethnicity, gender, puberty, height and weight accounted f between 27% and 86% of variance in BA and between 36% and 70% of variance in BMC measurements at different sites in black and white South African children (Table 3-3). Puberta development was not a significant predictor of BA or BMC.

Lumbar vertebral heights.

Lumbar vertebral heights were less in both black boys (L1-L4: $p<0.0001$) and girls (L L4: $p<0.01$ to $p<0.0001$) than in their white peers before and after correcting for ethnic differences in height (Table 3-4 and Table 3-5).

Table 3-4. Vertebral heights* (unadjusted means ± SD) of lumbar spine vertebrae (L1-L comparisons between ethnic groups within each gender

	Males		*P*	Females		
	White (n=70)	Black (n=179)		White (n=64)	Black (n=155)	
L1 (mm)	18.12 ± 1.50	16.97 ± 1.20	<0.0001	18.54 ± 1.33	17.95 ± 1.48	
L2 (mm)	18.83 ± 1.35	17.38 ± 1.27	<0.0001	19.34 ± 1.57	18.53 ± 1.55	<
L3 (mm)	18.98 ± 1.32	17.46 ± 1.19	<0.0001	19.49 ± 1.73	18.43 ± 1.60	<
L4 (mm)	19.14 ± 1.53	17.65 ± 1.27	<0.0001	19.80 ± 1.49	18.69 ± 1.74	<

*Vertebral heights were calculated as the mean of the heights of the anterior, middle and posterior portions of the first four lumbar vertebrae (mm) as did Gilsanz *et al.* (1998). [114]

Table 3-5. Ethnic differences in lumbar spine vertebral heights (L1-L4) after correcting for gender, height and puberty

	Ethnicity (β*)	± SE	*P*	R^2	Predictors (*P*<0.001)	Puberty
L1 (mm)	-0.09	0.04	<0.01	0.51	Height, gender	<0.05
L2 (mm)	-0.14	0.03	<0.0001	0.55	Height, gender	<0.01
L3 (mm)	-0.20	0.03	<0.0001	0.54	Height, gender	ns
L4 (mm)	-0.19	0.04	<0.0001	0.44	Height, gender	ns

*A negative β means vertebral heights are greater in white than black children.

Radiogrammetry results

Before corrections, the inner diameter of the 2nd metacarpal was greater in black children than white (boys: $p<0.001$; girls $p<0.05$). (Table 3-6.) Black boys also had a greater combined cortical thickness ($p<0.0001$) than white boys but a smaller Barnett-Nordin index ($p<0.001$) and % cortical area to total area ratio ($p<0.0001$). After corrections, black children had greater metacarpal length ($\beta=0.26$, $p<0.0001$), outer ($\beta=0.25$, $p<0.0001$) and inner diameters ($\beta=0.27$, $p<0.01$), as well as the cortical cross sectional area ($\beta=0.11$, $p<0.05$). However, this translated to a greater Barnett-Nordin index ($\beta=-0.20$, $p<0.0001$) and % cortical area to total area ($\beta=-0.21$, $p<0.0001$) in white children. (Table 3-7)

Table 3-6. Radiogrammetric comparisons between ethnic groups within each gender. Values are unadjusted means (± SD)

	Males			Females		
	White (n=71)	Black (n=178)	*P*	White (n=61)	Black (n=153)	*P*
Length (mm)	54.75 ± 3.49	54.41 ± 3.40	ns	55.32 ± 4.07	56.08 ± 3.93	ns
Outer diameter (mm)	6.98 ± 0.64	7.10 ± 0.67	ns	6.74 ± 0.59	6.91 ± 0.63	ns
Inner diameter (mm)	4.00 ± 0.71	4.36 ± 0.72	<0.001	3.69 ± 0.61	3.91 ± 0.66	<0.05
Combined cortical thickness (mm)	2.98 ± 0.42	2.75 ± 0.41	<0.0001	3.05 ± 0.49	3.00 ± 0.45	ns
Cortical cross-sectional area (mm^2)	25.61 ± 4.26	24.66 ± 4.40	ns	25.00 ± 4.73	25.45 ± 4.66	ns
% cortical area to total area	67.03 ± 7.59	62.29 ± 7.49	<0.0001	69.73 ± 7.56	67.79 ± 7.24	ns
Barnett-Nordin index (%)*	42.98 ± 6.80	38.91 ± 6.27	<0.0001	45.41 ± 6.90	43.62 ± 6.49	ns

Table 3-7. Ethnic differences in metacarpal indices after correcting for gender, height and puberty

	Ethnicity (β*)	SE	*P*	R^2	Predictors (*P*<0.001)	Puberty
Length (mm)	0.26	0.03	<0.0001	0.62	Gender, height	ns
Outer diameter (mm)	0.25	0.04	<0.0001	0.24	Gender, height	ns
Inner diameter (mm)	0.27	0.05	<0.01	0.18	Gender, height	ns
Combined cortical thickness (mm)	-0.07	0.05	ns	0.13	Gender, height	ns
Cortical cross-sectional area (mm^2)	0.11	0.04	<0.05	0.20	Height	ns
% cortical cross-section area to total cross-sectional area	-0.21	0.05	<0.01	0.13	Gender	ns
Barnett-Nordin index (%)*	-0.20	0.05	<0.0001	0.13	Height	ns

* A positive β means the respective metacarpal indices are greater in black than white children

Discussion

Ethnic differences in bone mass (BMC) between black and white 10 year old South African children, as measured by DXA and corrected for gender, pubertal development, current height and weight, were most apparent at the femoral neck and total hip. That is, black children had a greater BMC at the femoral neck (boys: 6%; girls: 5%), total hip (boys: 6%) and mid radius (boy and girls: 6%) than white children, despite black children being more exposed to environmental factors known to impact negatively on bone mass, such as living in poorer households and having poorer nutrition, compromised growth and development as reflected by their lower birth weights, shorter statures, lighter body weights and later onset of pubertal development, [46] lower calcium intake (estimated to be approximately 400 mg/day) [199] and less physical activity. [212] Black children had similar whole body and lumbar spine bone masses to white children. These data suggest that ethnic differences are site-specific in our 10 year old cohort of black and white South Africans which are not as a result of differences in current height or weight (for which statistical corrections were made), bone age and pubertal stage (which did not differ between ethnic groups), but are more likely as a result of differences in genetic factors.

The finding that bone mass at the femoral neck, total hip and mid radius was greater in 10-year-old South African black than white children is consistent with national and international studies which have explored black-white ethnic differences in both adults and children. Before correcting for differences in height and weight, pre- and early pubertal African-American children had greater femoral neck bone mass (BMC and / or BMD) than white children. [10,31]

Wang *et al.* (1997), after correcting for differences in both height and weight, found BMAD to be greater in African-American pre-/early pubertal girls than white girls. [329] Our results in children are also consistent with studies conducted in South African adult women (20-64y) where BMC of the femoral neck was greater in blacks than whites, before and after having corrected for body and bone size. [69,70] Greater weight-bearing was proposed to explain the greater femoral neck bone mass in black South African women. However, given that black 10 year old children, who are lighter or of similar weights to white children, also have a greater femoral neck bone mass, other reasons, such as genetics, are likely to account for a greater bone mass at the femoral neck and total hip in South Africa's black population.

Forearm BMC has also been found to be greater in black than white American children before and after corrections for weight and age in 7-12 year olds [31] and before corrections in 1-6 year old children. [183,191] In a previous study using single photon absorptiometry, South African blacks aged 6-20y were found to have greater BMC at the midshaft radius than white children, after correcting for differences in height. [247]

At the lumbar spine and whole body, ethnic differences in bone mass were absent. The results are similar to those found in South African pre-, peri- and postmenopausal women. [69,70] Although the majority of studies from the US have demonstrated greater bone masses in African-Americans, [31,113,183,231,234,328] there are indeed US studies comparable to ours where no differences in bone mass have been found; uncorrected lumbar spine BMC and BMD have been reported to be similar in African-American and Caucasian children, [10,31,257,329,339] as have results after correcting for ethnic differences in size or maturity. [143,299] Adult Somalis, living in the USA,

have also been reported to have a similar lumbar spine BMD to Caucasian Americans. [219] At the whole body, a site on which there is less literature in children to make comparisons, two studies did not find ethnic differences between African-American and Caucasian children. [10,329]

In addition to bone density, ethnic differences in bone architecture and geometry have more recently been studied. Histomorphometric analysis of iliac crest biopsies has shown that South African black adults have thicker trabeculae than whites. [193,232,233] At the proximal femur, both US and South African black populations have been shown to have a narrower marrow cavity, thicker cortex and a lower buckling ratio despite non-significant differences in outer bone diameter, characteristics consistent with greater bone strength and lower fracture rates in blacks at this region. [290] Geometrically, wider bones are stronger bones, which African-American populations have been found to have. [114,193] We found black children had shorter lumbar vertebral heights for the same BA before and after correcting for differences in height, suggesting that the vertebrae are wider. Further, DXA-measured BA at the mid shaft of the radius was consistently greater in black than white children after correcting for differences in height. And lastly, our radiogrammetry results support that black children had greater outer and inner diameters of their metacarpal, thinner cortices and less cortical bone both before and after correcting for height, gender and puberty, and differences in skeletal size and variations in tube-to-film and hand-to-film distance (i.e. the Barnett-Nordin index), it translates to greater bone strength. This architecture is consistent with greater polar strength-strain indices which are more resistant to bending and torsional forces. “In mechanics, however, it is well known that resistance of tubular structures to flexion can be maintained with a lower wall-area/total area ratio provided that the total diameter of the tube is large.” [296]

A number of candidate gene polymorphisms have been linked to bone mass, such as of the vitamin D receptor gene (VDR), calcium-sensing receptor gene (CASR), alpha2HS-glycoprotein gene (ASHG), oestrogen receptor alpha gene (ESR1), calcitonin gene, parathyroid hormone gene (PTH), collagen I alpha 1 gene, transforming growth factor beta (TGF-beta) gene, interleukin-1 (IL-1) gene, interleukin-6 (IL-6) and LDL receptor-related protein 5 (LRP5) apolipoprotein E gene. Ethnic differences have been found in allelic and genotypic differences in a number of these polymorphisms in various populations including between black and white ethnic groups. [85,96,235,267] Though these genes have the potential to explain ethnic differences in bone mass, none have unequivocally been proven to do so.

In conclusion, black children in South Africa have greater bone mass at the femoral neck, total hip and mid radius than their white peers, and similar bone mass at the lumbar spine and whole body. This bone mass pattern, at the femoral neck in particular, is similar to that reported in US children, yet our black South African children, unlike their African-American peers, are comparatively disadvantaged. These findings suggest that the femoral neck, total hip and mid radius bone mass patterns described in our black children are likely to be under similar genetic influences as those of African-American children rather than due to environmental influences. Support for this hypothesis comes from studies which suggest that the South African black population and the African-American population (originating from West Africa) had similar genetic pools, as the South African Bantu-speaking ethnic groups migrated from West Africa. [93,233,238] It is unclear at this stage, whether improvement in the adverse environmental factors in our black children would greatly change the bone mass findings at other sites. However it does

raise an intriguing question around how the genetic influences maintain bone mass in the face of what are generally considered to be adverse environmental factors. Not only do these genetic influences have a positive effect on bone mass during childhood, but these are maintained through adult life and are associated with a very low incidence of femoral neck and vertebral fractures in the elderly.

CHAPTER 4 - Infant programming of bone size and bone mass between black and white 10-year old South African children

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Introduction

In developed countries, the earliest of factors shown to identify those at a high risk of having low bone mass and so be prone to osteoporosis in later life, is that of quality of intrauterine and early life reflected by low birthweight and size in infancy. The relationship between birthweight, growth in infancy and bone mass has been explored in several epidemiological studies; in adults, [5,60,62,63,78,107,126,196,308,342] young adults, [60,280] adolescents [83] and children.[97,150,314] Childhood weight, especially weight at 1 year (WT1) has been shown to be predictive of bone mineral content (BMC) before adjusting for the confounding variables of current height and weight, but often, not after. Supporting studies are needed to confirm that the relationship between size in infancy and bone mass is not entirely mediated by the tracking of infant size to adulthood, on which bone mass is dependent. In addition, since most studies were conducted in Caucasian populations from developed countries such as the UK, Australia, Canada

and Finland, it is unclear whether such relationships exist in black populations and in developing countries.

We studied the relationship between growth in infancy and current bone mass in South African children by investigating how birthweight, weight and length at 1 year related to bone area (BA) and bone mass (BMC) before and after adjustment for current height and weight in a population of 10 year old black and white children born in Johannesburg during 1990. The questions asked were: (1) Do birthweight, or weight and / or length at 1 year of age predict bone size and bone mass in 10-year-old children? (2) If there is a relationship between infant weight and / or length in infancy and bone mass in 10-year-old children, is it because of its relationship to bone size or bone mass?

Materials and Methods

Subjects

The subjects were 473 healthy children stratified by race [2]/ethnicity and gender (182 black boys, 72 white boys, 158 black girls, 64 white girls) of median age 10.6 years (range: 10.0-10.9) who formed part of a longitudinal cohort of children born in Johannesburg during 1990 and whose growth and development have been tracked since birth. Weight had been recorded at birth as well as weight and length/height at 1, 2, 4, 5, 7, 8, 9 and 10 years of age. The source of the

[2] "Race does not refer to any biological attributes but rather to the compulsory classification of people into the Population Registration Act".[344] Although the act has been repealed, these categories are still powerful and commonly used by the South African government and statistical services.

population data was the official birth notifications, governed by a local ordinance, and completed by delivery staff at the time of every birth in the area. This information was subsequently recorded in the registers maintained by each of the three local health authorities comprising most of the metropolitan area of Soweto-Johannesburg. [273] Subjects with chronic illness (juvenile rheumatoid arthritis, epilepsy or asthma) or on medication known to affect growth or bone mass development were excluded from the study (n=4). The study protocol was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand, Johannesburg and the Ethical Advisory Committee of Loughborough University, UK. Both children and guardians gave written informed consent to be studied.

Anthropometry

Height was measured to the last completed 1mm using a wall-mounted stadiometer (Holtain, UK) and weight to the nearest completed 0.1kg using a digital electronic instrument (Dismed, USA), using standardised protocols.[190] Both instruments were regularly calibrated and subjects wore minimal clothing when being weighed.

Maturity

Sexual maturity was self-assessed as pubic hair development in boys and girls, using the Tanner scaling technique. [203,204] In addition, skeletal maturity was assessed by scoring bone age from hand radiographs using the Tanner-Whitehouse bone-specific scoring technique (TWII20). [307]

Dual-energy X-ray absorptiometry (DXA)

BA and BMC of the whole body, excluding the head (WB), femoral neck (FN) and lumbar spine (LS; L1-L4) were measured by DXA using an Hologic QDR-4500 (Hologic, Inc., Waltham, MA, USA). A lumbar spine phantom was scanned daily to determine its measurement precision. The coefficients of variations (CV), calculated from no less than 240 repeat scans, were 0.47% and 0.78% for BA and BMC respectively.

Socioeconomic questionnaire

Primary caregivers answered questions about their social and economic status. This questionnaire had been modified appropriately for a South African population and previously validated. [42] The socioeconomic score was formulated from the presence or absence of 13 asset indicators, namely, house type, electricity, indoor flushing toilet, indoor running water, refuse removal, television, digital satellite television, motor vehicle, refrigerator, microwave, washing machine, video machine and telephone).

Statistics

Univariable and multivariable relationships between weight (birth, 1y), length (1y) vs. BA and BMC (10y), and the extent to which these relationships were dependent on, or independent of measures of height and weight (10y) were analysed using STATISTICA (data analysis software system) version 6 (StatSoft, Inc., 2001). Student *t*-tests were used to compare

means (Table 4-1 and Table 4-2). ANCOVAs analysed the relationship between 10-year-old BA or BMC when BW, WT1 or LT1 were categorised into tertiles and age was treated as a continuous predictor. (Table 4-3). [62,107] Multiple regression models determined the predictive power of BW, WT1 and LT1 on BA and BMC when BA and BMC were in addition adjusted for race/ethnicity, gender, age, socioeconomic status, bone age, height and weight at 10 years (Table 4-4). The regression coefficient (β) was interpreted as the magnitude of the contribution infant size made to current BA and BMC variables. [226,245,261] Residual plots of all regression models showed no outlying or influential points, no deviation from the assumptions of linear relationships and constant variances. Probability values <0.05 were considered significant for all tests.

Results

Cohort characteristics

Anthropometry

Descriptive statistics for the 10-year-old subjects who took part in this study are shown in Table 4-1. Black children lived in households which scored significantly lower on the socioeconomic scale (median = 7, range: 0-13) than white children (median = 12, range: 6-13) (P<0.05, Mann-Whitney U Test).

At birth black males were lighter than white males ($P<0.01$). At age 1 year, black males were shorter than white males ($P<0.01$). Even though all subjects were aged between 10.0 and 10.9 years, black children were on average one month younger at the time of their visit and significantly shorter than their white counterparts (males: 6.3 cm shorter, $P<0.0001$; females: 3.4 cm shorter $P<0.001$). Black males were on average 3.4 kg lighter than white males at 10 years of age ($P<0.001$).

Sexual maturity

Most of our cohort was prepubertal or in early puberty (black boys: 99%, white boys: 99%, black girls: 98%, white girls: 97%) as determined by pubic hair development (Table 4-1). There were no ethnic differences in the distribution of sexual maturity (Fisher's exact test). Skeletal maturity as determined by bone age, was similar between the ethnic groups within each gender ($P>0.05$). Bone age, the measure of maturity included in the multiple regression analyses, was neither a significant predictor of BA nor BMC.

e characteristics (mean ± SE (n)) of black and white children aged 10 years

	Males		*P*	Females		*P*
	White	**Black**		**White**	**Black**	
	10.65 ± 0.03 (72)	10.55 ± 0.02 (182)	<0.01	10.62 ± 0.03 (64)	10.53 ± 0.02 (158)	<0.05
	10.31 ± 0.12 (71)	10.13 ± 0.08 (179)	ns	10.41 ± 0.15 (62)	10.38 ± 0.10 (156)	ns
ir 1) % (n)	51% (34)	65% (113)	ns	67% (43)	59% (92)	ns
nair 2) % (n)	48% (32)	34% (58)		30% (19)	39%(62)	
air 3 & 4) % (n)	1% (1)	1% (1)	ns	3% (2)	2% (3)	ns
	143.5 ± 0.9 (72)	137.3 ± 0.5 (182)	<0.0001	142.6 ± 0.9 (64)	139.2 ± 0.5 (158)	<0.001
	36.0 ± 0.8 (72)	32.6 ± 0.5 (182)	<0.001	35.5 ± 1.0 (64)	34.8 ± 0.7 (158)	ns
	17.4 ± 2 (72)	17.2 ± 3 (182)	ns	17.3 ± 3 (64)	17.8 ± 3 (158)	ns
	3.35 ± 0.53 (70)	3.16 ± 0.50 (181)	<0.01	3.12 ± 0.37 (64)	3.03 ± 0.53 (157)	ns
	9.79 ± 1.12 (15)	9.66 ± 1.40 (131)	ns	8.96 ± 0.85 (16)	9.28 ± 1.46 (104)	ns
	76.7 ± 3.5 (18)	74.3 ± 3.3 (127)	<0.01	74.0 ± 3.7 (16)	72.7 ± 3.1 (104)	ns

are means

Dual-energy X-ray absorptiometry (DXA)

Bone size (BA) and bone mass (BMC) measurements at the whole body, femoral neck and lumbar spine are shown in Table 4-2. Data and statistics presented in this table were not adjusted for any variables.

General

Table 4-1 and Table 4-2 provide anthropometric and DXA data respectively, reported per gender and race/ethnicity. Racial differences in anthropometry, BA and BMC, as well as the effect of socioeconomic status in this cohort have been reported elsewhere. [212,213,239,320]

Table 4-2. Bone area (BA) and bone mineral content (BMC) comparisons between race/ethnic groups within each gender. Values are unadjusted means (± SE)

	Males		*P*	Females		*P*
	White	Black		White	Black	
	n=71	n=180		n=64	n=158	
Whole body less head BA (cm^2)	1099.74 ± 18.75	1009.22 ± 10.06	<0.0001	1087.33 ± 22.64	1047.95 ± 13.41	ns
Whole body less head BMC (g)	786.35 ± 17.04	715.38 ± 9.36	<0.001	766.71 ± 21.93	738.75 ± 13.14	ns
	n=72	n=182		n=64	n=158	
Femoral neck BA (cm^2)	4.32 ± 0.04	4.13 ± 0.02	<0.0001	4.21 ± 0.04	4.05 ± 0.02	<0.0001
Femoral neck BMC (g)	3.03 ± 0.05	3.06 ± 0.03	ns	2.70 ± 0.06	2.77 ± 0.03	ns
	n=72	n=182		n=64	n=158	
L1-L4 BA (cm^2)	46.00 ± 4.96	43.02 ± 4.26	<0.0001	43.99 ± 4.26	42.99 ± 4.34	ns
L1-L4 BMC (g)	26.72 ± 4.66	19.09 ± 3.69	<0.0001	25.54 ± 5.10	25.33 ± 5.24	ns

Data and statistics presented in this table were not adjusted for any variables. Student *t*-tests were used to compare means.

Infant size (weight and length) vs. BA and BMC at 10 years

Mean BA and BMC values which are tabulated for each group (black and white boys and girls) according to tertiles of BW, WT1 or LT1, were positively and significantly associated with weight (BW, WT1) and length (LT1) at most sites and more so in males than females (Table 4-3).

After correcting BA and BMC for race/ethnicity, gender, age, socioeconomic status, bone age, height and weight at 10 years, WT1 and LT1 were still predictive of 10-year-old whole body BA and BMC (between 6% and 10% for a 1% change in predictor) and femoral neck BMC (between 8% and 17% for a 1% change in predictor) (Table 4-4). When BMC was in addition adjusted for BA, BW, WT1 and LT1 continued to be predictive of BMC at the femoral neck, but not at the whole body.

Table 4-3. BA and BMC means (± SE) adjusted for age at the whole body, femoral neck and lumbar spine, within each third of the distribution of Birthweight (BW, kg), and weight at 1 year (WT1, kg) and length at 1 year (LT1, cm).

Size	Category	n	Whole body		n	Femoral neck		n	Lumbar spine	
			BA	BMC		BA	BMC		BA	BMC
Black males										
BW	<3.00kg	59	961.5 ± 16.5	670.6 ± 15.4	59	4.08 ± 0.04	2.94 ± 0.05	59	41.36 ± 0.53	22.19 ± 0.46
	3.00-3.36kg	62	1007.5 ± 16.1	711.9 ± 15.0	62	4.10 ± 0.04	3.02 ± 0.05	62	43.06 ± 0.52	23.22 ± 0.45
	>3.36kg	60	1058.1 ± 16.3	763.9 ± 15.3	58	4.21 ± 0.04	3.22 ± 0.05	60	44.51 ± 0.52	25.07 ± 0.45
	***P* trend**		**<0.001**	**<0.001**		**<0.05**	**<0.001**		**<0.001**	**<0.0001**
WT1	<9.0kg	45	928.2 ± 17.4	648.3 ± 17.0	43	4.03 ± 0.04	2.87 ± 0.05	45	40.75 ± 0.62	21.64 ± 0.51
	9.0-10.0kg	44	1001.5 ± 14.7	704.1 ± 14.3	44	4.09 ± 0.04	3.03 ± 0.05	44	43.32 ± 0.63	23.62 ± 0.52
	>10.0kg	42	1097.7 ± 16.4	799.6 ± 15.9	42	4.28 ± 0.04	3.31 ± 0.05	42	44.99 ± 0.64	25.49 ± 0.53
	***P* trend**		**<0.0001**	**<0.0001**		**<0.001**	**<0.0001**		**<0.0001**	**<0.0001**
LT1	<73.0cm	34	928.3 ± 20.2	647.1 ± 19.4	33	4.01 ± 0.05	2.85 ± 0.06	34	40.39 ± 0.72	21.31 ± 0.60
	73.0-76.0cm	60	1021.2 ± 17.1	722.7 ± 16.4	59	4.17 ± 0.04	3.12 ± 0.05	60	43.83 ± 0.54	24.01 ± 0.45
	>76.0cm	33	1082.2 ± 17.3	787.0 ± 16.7	33	4.27 ± 0.05	3.26 ± 0.06	33	44.81 ± 0.73	25.61 ± 0.61
	***P* trend**		**<0.0001**	**<0.0001**		**<0.01**	**<0.0001**		**<0.0001**	**<0.0001**
White males										
BW	<3.20kg	23	1014.4 ± 31.1	705.0 ± 28.5	24	4.22 ± 0.06	2.82 ± 0.08	24	44.44 ± 1.03	24.65 ± 1.01
	3.20-3.50kg	23	1091.9 ± 30.5	781.3 ± 28.0	25	4.26 ± 0.06	2.99 ± 0.08	25	45.86 ± 1.01	27.08 ± 0.99
	>3.50kg	24	1186.9 ± 29.7	866.5 ± 27.3	23	4.52 ± 0.06	3.29 ± 0.08	24	48.57 ± 0.99	29.44 ± 0.97
	***P* trend**		**<0.001**	**<0.001**		**<0.01**	**<0.001**		**<0.05**	**<0.01**
WT1	<9.2kg	3	892.3 ± 60.0	596.5 ± 63.3	3	3.86 ± 0.14	2.34 ± 0.25	3	38.48 ± 0.98	19.01 ± 1.02
	9.2-9.9kg	7	1050.5 ± 39.2	751.1 ± 41.3	7	4.21 ± 0.09	3.01 ± 0.16	7	45.99 ± 0.64	27.61 ± 0.66
	>9.9kg	5	1265.3 ± 48.1	907.4 ± 50.7	5	4.47 ± 0.11	3.38 ± 0.20	5	50.82 ± 0.79	28.46 ± 0.82
	***P* trend**		**<0.01**	**<0.05**		**<0.05**	**<0.05**		**<0.0001**	**<0.0001**
LT1	<74.8cm	5	969.0 ± 92.0	671.5 ± 89.1	5	4.05 ± 0.14	2.54 ± 0.20	5	41.24 ± 1.19	21.66 ± 1.26
	74.8-78.5cm	9	950.3 ± 56.7	653.9 ± 54.9	9	4.32 ± 0.10	3.16 ± 0.14	9	46.30 ± 0.86	27.58 ± 0.91
	>78.5cm	4	1205.7 ± 44.2	863.6 ± 42.9	4	4.55 ± 0.16	3.34 ± 0.22	4	51.20 ± 1.32	27.61 ± 1.39
	***P* trend**		**<0.05**	**<0.05**		**ns**	**<0.05**		**<0.001**	**<0.01**

Size	Category	n	Whole body		n	Femoral neck		n	Lumbar spine	
			BA	BMC		BA	BMC		BA	BMC
					Black females					
BW	<2.86kg	50	1004.1 ± 22.5	701.6 ± 22.5	50	4.00 ± 0.04	2.69 ± 0.06	50	42.19 ± 0.60	24.60 ± 0.73
	2.86-3.21kg	55	1067.3 ± 21.4	754.8 ± 21.4	55	4.06 ± 0.04	2.81 ± 0.05	55	43.27 ± 0.57	25.69 ± 0.70
	>3.21kg	52	1069.9 ± 22.0	758.1 ± 22.0	52	4.07 ± 0.04	2.79 ± 0.06	52	43.44 ± 0.58	25.66 ± 0.72
	***P* trend**		**ns**	**ns**		**ns**	**ns**		**ns**	**ns**
WT1	<8.5kg	27	982.2 ± 21.3	677.0 ± 21.0	27	3.97 ± 0.06	2.60 ± 0.07	27	41.66 ± 0.76	23.15 ± 0.88
	8.5-9.5kg	43	1061.2 ± 24.9	758.0 ± 24.6	43	4.04 ± 0.04	2.81 ± 0.06	43	43.01 ± 0.60	25.82 ± 0.70
	>9.5kg	34	1147.7 ± 28.0	820.3 ± 27.6	34	4.18 ± 0.05	2.87 ± 0.06	34	44.39 ± 0.68	26.83 ± 0.78
	***P* trend**		**<0.0001**	**<0.001**		**<0.05**	**<0.05**		**<0.05**	**<0.01**
LT1	<71.0cm	28	1014.7 ± 21.3	705.8 ± 20.7	28	4.01 ± 0.06	2.61 ± 0.07	28	42.30 ± 0.76	24.34 ± 0.89
	71.0-74.0cm	50	1041.9 ± 24.3	733.8 ± 23.6	50	4.05 ± 0.04	2.79 ± 0.05	50	42.85 ± 0.57	25.33 ± 0.67
	>74.0cm	26	1163.8 ± 36.4	845.0 ± 35.4	26	4.17 ± 0.06	2.92 ± 0.07	26	44.31 ± 0.79	26.81 ± 0.92
	***P* trend**		**<0.01**	**<0.01**		**ns**	**<0.05**		**ns**	**ns**
					White females					
BW	<3.0kg	20	1095.3 ± 40.5	779.0 ± 39.5	19	4.26 ± 0.07	2.71 ± 0.11	19	43.82 ± 0.92	24.80 ± 1.07
	3.0kg-3.28kg	24	1052.5 ± 36.9	734.9 ± 36.0	22	4.17 ± 0.06	2.66 ± 0.10	22	43.78 ± 0.84	25.11 ± 0.97
	>3.28kg	20	1121.2 ± 40.3	792.6 ± 39.4	20	4.19 ± 0.07	2.71 ± 0.11	20	43.78 ± 0.92	25.79 ± 1.06
	***P* trend**		**ns**	**ns**		**ns**	**ns**		**ns**	**ns**
WT1	<8.3kg	4	944.6 ± 50.1	636.3 ± 49.8	4	3.96 ± 0.11	2.37 ± 0.17	4	40.71 ± 1.99	21.11 ± 2.19
	8.3-9.2kg	7	1079.0 ± 43.4	770.4 ± 43.2	7	4.00 ± 0.09	2.41 ± 0.13	7	42.11 ± 1.56	22.60 ± 1.72
	>9.2kg	5	1448.6 ± 86.7	1133.3 ± 86.3	5	4.28 ± 0.10	3.02 ± 0.16	5	47.37 ± 1.85	31.38 ± 2.04
	***P* trend**		**<0.01**	**<0.01**		**ns**	**<0.05**		**ns**	**<0.05**
LT1	<72.5cm	5	944.4 ± 63.7	633.1 ± 65.1	5	3.87 ± 0.09	2.30 ± 0.15	5	37.78 ± 1.21	19.50 ± 1.86
	72.5-74.0cm	7	1094.6 ± 58.5	777.9 ± 59.8	7	4.17 ± 0.08	2.64 ± 0.13	7	45.33 ± 1.05	25.80 ± 1.62
	>74.0cm	4	1311.3 ± 90.1	991.9 ± 92.1	4	4.31 ± 0.09	3.04 ± 0.17	4	47.22 ± 1.32	30.93 ± 2.03
	***P* trend**		**<0.05**	**<0.05**		**<0.05**	**<0.05**		**<0.001**	**<0.01**

ANCOVAs analysed the relationship between 10-year-old BA or BMC when BW, WT1 or LT1 were categorised into tertiles and age was treated as a continuous predictor.

Table 4-4. The predictive power (β ± SE) of birthweight (BW), weight at 1 yr (WT1) and length at 1 year (LT1) of BA and BMC at the whole body, femoral neck and lumbar spine when BA and BMC were adjusted for race/ethnicity, gender, age, socioeconomic status, bone age, height and weight at 10 years

Measure	Birthweight				Weight (1y)				Length (1y)			
	n	β	± SE	*P*	n	β	± SE	*P*	n	β	± SE	*P*
BA adjusted for race/ethnicity, gender, age, socioeconomic status, bone age, height (10y) and weight (10y)												
Whole body BA	460	0.03	0.02	ns	258	0.10	0.03	<0.001	257	0.06	0.03	<0.05
Femoral neck BA	457	-0.04	0.04	ns	256	0.10	0.06	ns	255	0.02	0.06	ns
Lumbar spine BA	460	0.02	0.03	ns	258	0.08	0.05	ns	257	0.01	0.05	ns
BMC adjusted for race/ethnicity, gender, age, socioeconomic status, bone age, height (10y) and weight (10y)												
Whole body BMC	460	0.03	0.02	ns	258	0.10	0.04	<0.01	257	0.08	0.04	<0.05
Femoral neck BMC	457	0.05	0.04	ns	256	0.14	0.05	<0.05	255	0.17	0.06	<0.01
Lumbar spine BMC	460	0.04	0.04	ns	258	0.08	0.05	ns	257	0.04	0.06	ns
BMC adjusted for race/ethnicity, gender, age, socioeconomic status, bone age, height (10y) and weight (10y) and BA												
Whole body BMC	460	-0.004	0.01	ns	258	-0.02	0.02	ns	257	0.01	0.02	ns
Femoral neck BMC	457	0.07	0.03	<0.05	256	0.11	0.05	<0.05	255	0.16	0.05	<0.01
Lumbar spine BMC	460	0.02	0.03	ns	258	0.03	0.04	ns	257	0.04	0.05	ns

Multiple regression models determined the predictive power of BW, WT1 and LT1 on BA and BMC when BA and BMC were in addition adjusted for race/ethnicity, gender, age, socioeconomic status, bone age, height and weight at 10 years. The regression coefficient (β) was interpreted as the magnitude of the contribution infant size made to current BA and BMC variables.

Discussion

In both black and white South African 10-year old children, size in infancy was predictive of BA and BMC at all sites before adjustments were made for confounding variables. After adjustments for race/ethnicity, gender, age, socioeconomic status, bone age, height and weight at 10 year, size in infancy remained predictive of whole body BA and BMC, and femoral neck BMC. This relationship was observed despite black children being exposed to a multitude of environmental factors known to impact negatively on bone mass, such as living in poorer households and poor nutrition, [46] low calcium intake (estimated to be approximately 400 mg/day), [199] little physical activity, [212,213] patterns of compromised growth and development as reflected by their shorter statures, lighter body weights and delayed onset of puberty. [47,48] It is well established that body size "tracks" through childhood, and that foetal and adult size lie on a continuum of body size and that the closer any two points are, the higher the correlation between them.[33] The relationship between infant weights and BMC was not entirely mediated by the tracking of skeletal size: infants of a lower birthweight and a smaller size at 1 year grow to develop smaller bones (as reflected by BA) and/or bones of lower BMC at the femoral neck (lower BMC with similar BA).

Meta-analyses and systematic reviews support a relationship between birthweight (and infant size) and lumbar spine BMC in adults both before and after adjustment for body size. [285] Possible reasons explaining why we did not observe the same in our 10 year old children include (1) categorising birthweight and infant weight data resulted in a decrease in statistical power. (2) There may be have been confounding variables relating to lumbar spine BMC or birthweight for

which our analyses did not correct (e.g. bone geometry or quality, pubertal status, hormonal status, physical activity, smoking habits, alcohol consumption, calcium intake). We did not adjust for lifestyle determinants as gestational age at birth, physical inactivity, low dietary calcium intake and cigarette smoking of parents have not been shown to affect relationships between infant size and current bone mass. [22,62,107,245] (3) The relationship between birthweight and infant size and lumbar spine BMC is indeed mediated by the tracking infant size through to childhood. These reasons may also explain possible gender differences observed, but not analysed, in Table 4-3.

The number of white subjects with data at 1 year was small, which should be borne in mind when interpreting any results in this race/ethnicity.

The statistical methods employed in this study were similar to those used by other researchers so that comparisons could be made between studies and populations. We therefore have reported results that were both unadjusted and adjusted for the confounding variables of height and weight (in addition to race/ethnicity, gender, age, socioeconomic status, bone age), so as to counter the effect of tracking of skeletal size.

Unadjusted BMC was positively and significantly related to birth- and infant weights and lengths and unadjusted BMC as has been observed in adults [5,60,62,107,196,280,308] and in the elderly, [62,78] at the whole body, lumbar spine and / or femoral neck (Table 4-2).

In addition to using similar categorising methods as those used by others, [60,62,107,126] we used multiple regression techniques, and made simultaneous adjustments for race/ethnicity, gender, age, socioeconomic status, bone age, [60,62,107] height and weight, which are analyses particularly recommended for use in children, [226,245,261] and showed that size in infancy, especially at 1 year, was correlated with, and was predictive of BA and BMC of the whole body and BMC at the femoral neck at age 10 years, independent of current size, which is a finding that has not been consistently observed in adults. In adults, after adjusting BMC for height and weight simultaneously, it was reported that birthweight and weight or length at 1 year were no longer associated with bone mass. [5,62,308]

The mechanisms through which *in utero* growth translates into compromised bone health have been suggested to be mediated by the development of fewer cells and / or the altered programming of stem cell function and regulatory hormones [282] such as vitamin D, IGF-1 [6,94,145,146] and GH. [77] The multitude of BMC-modifying factors to which adults are exposed over a lifetime have been suggested to mask the relationship between early growth and adult bone mass, which we suggest explain why this relationships was observed in pre- and peripubertal children aged 10 years.

The present findings support the hypothesis that growth and development both intrauterine and up until 1 year of age, which are measures of genetic, intrauterine and postnatal environmental factors, may have long-term consequences when compromised, and may be associated with the risk of osteoporosis in later life.

CHAPTER 5 - Bone mass and bone size in pre- or early pubertal 10-year old black and white South African children and their parents

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Introduction

Populations subjected to environmental factors known to negatively influence bone mass are expected to have lower bone mass, higher fragility fracture rates and lower heritability estimates. Black South Africans are subject to poor growth and nutrition, [46] low dietary calcium intake [199] and little physical activity, [212,213] yet have higher bone mass at the femoral neck [70,320] and lower fracture rates. [79,297,310] Genetic factors which account for a major proportion of bone mass variance in adults, [252] adolescents [207] and children [149] are thought to maintain bone mass in black South Africans in the face of these adverse environmental factors. Yet assessment of heritability of bone mass and bone size by way of parent-child associations has not been previously explored in this population.

Dual energy X-ray absorptiometry (DXA) technology remains the most widely used technique for the measurement of bone mass in both adult [32] and paediatric populations [32,156] and

was used in this study. DXA measurements, their analyses and interpretation are dependent on size-related variables such as age, body size (height and weight), and bone volume [227] and on skeletal maturity, ethnicity and body composition. [9,115]

There is no standard way to correct BMC or areal BMD data for changes in skeletal size; they have been corrected for varying combinations of body size, bone size, bone area, pubertal stage, skeletal maturity, and body composition [115]. The many different methods used make the interpretation of uncorrected and corrected DXA data and the objective comparisons between studies, populations and age groups very complex, confusing and potentially erroneous. [32]

To address these concerns Katzman *et al.* (1991) [161] and Carter *et al.* (1992) [51] proposed measurements less dependent on size by mathematically converting BMC to a three-dimensional estimate of volumetric BMD or bone mineral apparent density (BMAD). Bones were assumed to be shaped as cubes, and the following formulae were applied to calculate BMAD at whole body ($BMC/(BA^2 \div height)$), femoral neck and mid-forearm (BMC/BA^2) and lumbar spine ($BMC/BA^{1.5}$). Kröger *et al.* (1992) [171] applied a similar concept assuming bones (vertebral bodies, femoral shaft and neck) to be shaped as cylinders and applied the formula $BMAD=(BMC)(4/[\pi(\text{bone width})])$. Similarly, Lu *et al.* (1996) [195] assumed the femoral neck, mid-third of the femoral shaft, and the four lumbar vertebral bodies to be cylinders and used bone width (d) and height (h) to calculate bone volume $(\pi(d/2)^2 \times h$. All methods however calculate coefficients by assuming bones are shaped as cubes or cylinders which do not necessarily hold true in different ethnic groups, ages and sex. [261]

Prentice *et al.* (1994) [261] proposed a method that calculated population-specific power coefficients (PCs), and then corrected BMC for BA^{PC}, height and weight. This method allows BMC to be custom-corrected for size for each ethnic and sex group and each skeletal site.

Therefore, the first aim of this study was to compare BMC corrected for BA^{PC}, height and weight [261] against BMC corrected for other combinations of height, weight and / or BA in black and white children and their parents. The second aim of the study was to explore the associations of BMC and BA between black and white children and their parents in order to obtain an estimate of hereditability.

Materials and Methods

Subjects

The subjects were 419 healthy children stratified by ethnicity and gender (135 black girls, 63 white girls, 154 black boys, 67 white boys) of mean age 10.6 years (range: 10.0-10.9) who formed part of a longitudinal cohort of children born in Johannesburg during 1990 (the Bone Health sub-cohort of the Birth to Twenty study), and whose growth and development have been tracked since birth. Subjects with chronic illness (such as juvenile idiopathic arthritis, epilepsy or asthma) or on medication known to affect growth or bone mass development were excluded from the study (n=4). Of the parents of the 419 children, we collected maternal data from 406 biological mothers (280 black mothers, 126 white mothers) of median age 37 years and paternal

data from 100 biological fathers (53 black fathers, 47 white fathers) of median age 42 years. Many children were no longer living with their fathers whilst other fathers were not able to make themselves available for DXA scans because of a number of reasons, including work commitments. Both maternal and paternal data were available for 88 children. The study protocol was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand, Johannesburg and the Ethical Advisory Committee of Loughborough University, UK. Guardians gave written informed consent and the children written assent to be studied.

Anthropometry

Height was measured to the last completed 1 mm using a wall-mounted stadiometer (Holtain, UK) and weight to the nearest completed 0.1 kg using a digital electronic instrument (Dismed, USA), using standardised protocols. [190] Both instruments were regularly calibrated and subjects wore minimal clothing when being weighed.

Maturity

Sexual maturity was self-assessed as pubic hair development in boys and girls, using the Tanner scaling technique. [203,204] In addition, skeletal maturity was assessed by scoring bone age from hand radiographs using the Tanner-Whitehouse bone-specific scoring technique (TWII20). [307]

Socioeconomic questionnaire

Primary caregivers answered questions about their social and economic status. This questionnaire had been modified appropriately for a South African population and previously validated. [42] The socioeconomic score was formulated from the presence or absence of 13 asset indicators, namely, house type, electricity, indoor flushing toilet, indoor running water, refuse removal, television, digital satellite television, motor vehicle, refrigerator, microwave, washing machine, video machine and telephone.

Dual-energy X-ray absorptiometry (DXA)

Children's and their parents' BA and BMC of the whole body including and excluding the head (WB), femoral neck (FN), lumbar spine (LS; L1-L4), mid-radius (MR) and distal one-third of the radius (DR) were scanned using a fan beam densitometer in array mode (model Hologic QDR-4500A (Hologic, Inc., Bedford, MA, USA)). Lean body mass (LBM) and fat body mass (FBM) were determined from the DXA whole body scan. Adult and children's data were analyzed using adult software supplied by the manufacturer, version 11.2. (Hologic, Inc). To determine the densitometer's measurement precision, a lumbar spine phantom was scanned daily. The coefficients of variations (CV) were 0.47% and 0.78% for BA and BMC respectively. To determine operator measurement precision, 15 subjects were scanned twice and the resultant CV was <1% for both BA and BMC. Precision of measurement in the children was not assessed because of radiation concerns.

Structural geometry of the femoral neck

A series of standard formulae developed by Beck and colleagues [28,29] were used to calculate cross sectional area (CSA; cm^2) and section modulus (Z; cm^3) of the femoral neck from DXA-measured BMC and BA. Assumptions were made that the fixed length of the femoral neck area was 1.5 cm, the effective density of bone in fully mineralized bone tissue was ~1.05 g/cm^3 and the proportion of cortical mass was 0.6. The standard formulae included estimating femoral neck width, cross-sectional moment of inertia, endosteal diameter, cross sectional area, trabecular porosity, mean cortical thickness and buckling ratio.

Statistics

STATISTICA (data analysis software system) version 6 (StatSoft, Inc., 2001, Tulsa, OK, USA) was used to analyse data sets of children and their parents and the associations between them. Data sets included age, bone age (in children), height, weight, BA and BMC of the whole body, femoral neck, lumbar spine, mid- and distal one-third of the radius, and whole body LBM and FBM. All data are reported as means and standard errors of the mean. Probability values <0.05 were considered significant for all tests.

Power coefficients (PC) were derived from the linear-regression analyses of ln(BMC) on ln(BA). These regression coefficients were used as the PC to which BA were raised to correct for bone size and were determined for each skeletal site for each of the 8 groups in this study (black

and white boys and girls, mothers and fathers). BMC was then corrected for the size-related predictors of height, weight, and/or BA^{PC} or BA. BMC was also corrected ln(LBM) or ln(FBM).

To allow for comparisons between size-adjusted BMC of children and those of their parents, BMC was corrected for height, weight, BA^{PC} and age (the latter in adults only) and then converted to Z-scores. Z-scores were calculated from the means and standard deviations of each of the 8 groups. The associations between children's and parents' Z-scores were assessed by way of Pearson's correlation coefficients (r) and the calculation of heritability estimates ($½h^2$, %). Heritability by maternal ($½h^2$) and paternal descent ($½h^2$) was estimated by regressing children's Z-scores on mother's or father's Z-scores. The resulting regression coefficient gives the appropriate heritability estimate. [149,201,236]

Lastly, the predictors of children's BA and BMC were assessed by way of multiple regression analyses. Mother's or father's BA^{PC} were included separately as predictors of children's BA in addition to ethnicity, gender, child's height, weight and BA^{PC}. Mother's or father's size adjusted BMC (corrected for height, weight and BA^{PC}) were included separately as predictors of children's BMC in addition to ethnicity, gender, child's height, weight and BA^{PC}. Residual plots of all regression models showed no outlying or influential points, no deviation from the assumptions of linear relationships and constant variances.

Results

1. Descriptive characteristics of study population

Black families lived in households that scored significantly lower on the socioeconomic scale (median = 7, range: 0-13) than white families (median = 12, range: 6-13) ($P<0.05$, Mann-Whitney U Test).

Descriptive characteristics of the 10-year old black and white children and their parents are shown in Table 5-1. Ethnic differences in anthropometry in this cohort of children have been reported elsewhere.[212,239,320] Briefly, when compared to their white peers, black children and their parents were significantly shorter, and black boys and their fathers were significantly lighter. At the time of the study, the children had achieved ~80% of their parents' heights and ~50% of their parents' weights.

All the children were pre-or early pubertal (Tanner stages 1-2) as determined by pubic hair development. There were no ethnic differences in sexual maturity (Fisher's exact test) or skeletal maturity (independent *t*-test) within each sex.

2. BA and BMC

Uncorrected BA

Uncorrected BA data are shown in Table 5-1. Bone area was generally smaller in black children and their parents, or at the most, similar but never bigger. At 10 years of age, children had achieved ~80% of their parental BA at the femoral neck and distal one-third of the radius, ~75% at the lumbar spine, ~60% at the whole body and ~55% at the mid radius.

Uncorrected BMC

Uncorrected BMC data are shown in Table 5-1. When compared to their white peers, uncorrected BMC was lower in black boys and their fathers at all sites except the femoral neck. Uncorrected BMC was similar in black and white girls but less in black mothers at all sites except the mid-radius. Uncorrected BMC in children had reached ~65% of parental values at the femoral neck, ~50% at the distal one-third of the radius, ~45% at the whole body, ~40% at the lumbar spine and ~35% at the mid radius.

Power coefficients

The calculated power coefficients (PC) are shown for each of the 8 groups (black and white boys and girls, mothers and fathers) at each skeletal site in Table 5-2. Calculated PCs ranged from 0.87 to 1.83 in children and 0.43 to 1.58 in adults. They differed between blacks and

whites in both children and adults at the femoral neck, between black and white adults at the distal one-third of the radius, and only between black and white mothers at the mid-radius. In addition, for the most part, PCs were significantly different from 1, 1.5 and/or 2, which have been used by different authors to adjust for differences in size.

Table 5-1. Descriptive characteristics (± SE) of a) 10-year old black and white girls and boys and b) their parents. BA and BMC reported in this table are not corrected size. *P*-values indicate ethnic differences

a)

	Girls					Boys				
	Black		White			Black		White		
	Mean ± SE	n	Mean ± SE	n	*P*<	Mean ± SE	n	Mean ± SE	n	*P*<
Age (y)	**10.53 ± 0.27**	**135**	**10.61 ± 0.25**	**63**	**0.05**	**10.55 ± 0.27**	**154**	**10.65 ± 0.24**	**67**	**0.05**
Skeletal age (y)	10.26 ± 1.09	135	10.30 ± 1.21	63	ns	10.19 ± 1.15	154	10.36 ± 1.23	67	ns
Height (cm)	**139.2 ± 6.40**	**135**	**142.7 ± 7.61**	**63**	**0.01**	**137.3 ± 6.2**	**154**	**143.5 ± 7.4**	**67**	**0.0001**
Weight (kg)	34.8 ± 8.3	135	35.7 ± 8.0	63	ns	**32.7 ± 6.5**	**154**	**35.9 ± 6.2**	**67**	**0.001**
Lean mass (kg)	23.9 ± 4.0	135	25.1 ± 4.3	63	ns	**24.1 ± .31**	**154**	**26.9 ±3.6**	**67**	**0.0001**
Fat mass (kg)	10.1 ± 5.0	135	9.8 ± 4.4	63	ns	7.5 ± 4.0	154	8.1 ± 3.2	67	ns
Whole body less head BA (cm^2)	1049 ± 173	135	1081 ± 183	63	ns	**1010 ± 133**	**154**	**1095 ± 151**	**67**	**0.0001**
Whole body less head BMC (g)	741 ± 170	135	759 ± 172	63	ns	**717 ± 126**	**154**	**781 ± 139**	**67**	**0.001**
Femoral neck BA (cm^2)	**4.05 ± 0.32**	**135**	**4.20 ± 0.29**	**63**	**0.01**	**4.13 ± 0.32**	**153**	**4.33 ± 0.33**	**66**	**0.0001**
Femoral neck BMC (g)	2.78 ± 0.42	135	2.68 ± 0.45	63	ns	3.05 ± 0.39	153	3.03 ± 0.43	66	ns
Lumbar spine BA (cm^2)	34.02 ± 3.49	135	34.79 ± 3.33	63	ns	**34.11 ± 3.26**	**154**	**36.40 ± 4.01**	**67**	**0.0001**
Lumbar spine BMC (g)	20.72 ± 4.32	135	20.66 ± 4.17	63	ns	**19.09 ± 3.02**	**154**	**21.40 ± 3.67**	**67**	**0.0001**
Mid radius BA (cm^2)	4.37 ± 0.87	135	4.27 ± 0.85	63	ns	4.49 ± 0.77	152	4.51 ± 0.80	64	ns
Mid radius BMC (g)	1.69 ± 0.43	135	1.71 ± 0.40	63	ns	**1.76 ± 0.34**	**152**	**1.87 ± 0.37**	**64**	**0.05**
Distal ⅓ rd radius BA (cm^2)	2.17 ± 0.20	135	2.19 ± 0.21	63	ns	2.32 ± 0.22	152	2.32 ± 0.19	64	ns
Distal ⅓ rd radius BMC (g)	1.04 ± 0.16	135	1.06 ± 0.15	63	ns	**1.09 ± 0.13**	**152**	**1.14 ± 0.12**	**64**	**0.01**
Cross sectional area (cm^2)	1.76 ± 0.27	126	1.70 ± 0.29	63	ns	1.94 ± 0.25	143	1.93 ± 0.27	66	ns
Cross sectional area (cm^2)*	**1.79 ± 0.02**	**126**	**1.66 ± 0.02**	**63**	**0.001**	**1.99 ± 0.02**	**143**	**1.82 ± 0.03**	**66**	**0.0001**
Section modulus (cm^3)	**1.12 ± 0.40**	**126**	**1.25 ± 0.44**	**63**	**0.05**	**1.28 ±0.43**	**143**	**1.51 ± 0.50**	**66**	**0.001**
Section modulus (cm^3)*	1.16 ± 0.03	126	1.16 ± 0.04	63	ns	1.24 ± 0.03	143	1.31 ± 0.04	66	ns

b)

	Mothers					Fathers				
	Black		White			Black		White		
	Mean ± SE	n	Mean ± SE	n	*P*<	Mean ± SE	N	Mean ± SE	n	*P*<
Age (y)	**35.6 ± 4.81**	**280**	**40.2 ± 6.07**	**126**	**0.0001**	42.5 ± 7.8	53	41.9 ± 5.5	47	ns
Height (cm)	**157.7 ± 5.7**	**280**	**165.2 ± 5.9**	**126**	**0.0001**	**169.6 ± 6.2**	**53**	**179.6 ± 6.2**	**47**	**0.0001**
Weight (kg)	71.7 ± 14.9	280	69.4 ± 14.6	126	ns	**71.4 ± 13.5**	**53**	**81.2 ± 12.0**	**47**	**0.001**
Lean mass (kg)	**30.5 ± 10.5**	**280**	**25.4 ± 10.1**	**126**	**0.0001**	**49.5 ± 6.4**	**53**	**58.3 ± 6.7**	**47**	**0.0001**
Fat mass (kg)	**37.3 ± 5.3**	**280**	**41.3 ± 6.4**	**126**	**0.0001**	17.2 ± 7.8	53	19.9 ± 8.0	47	ns
Whole body BA (cm^2)	**1917 ± 147**	**278**	**2003 ± 143**	**125**	**0.0001**	**2139 ± 146**	**53**	**2330 ± 131**	**47**	**0.0001**
Whole body BMC (g)	**2096 ± 276**	**278**	**2221 ± 278**	**125**	**0.0001**	**2498 ± 261**	**53**	**2716 ± 304**	**47**	**0.001**
Femoral neck BA (cm^2)	**4.83 ± 0.35**	**280**	**5.12 ± 0.34**	**126**	**0.0001**	**5.47 ± 0.36**	**53**	**5.91 ± 0.30**	**47**	**0.0001**
Femoral neck BMC (g)	**4.26 ± 0.65**	**280**	**4.09 ± 0.64**	**126**	**0.05**	4.81 ± 0.57	53	4.95 ± 0.81	47	ns
Lumbar spine BA (cm^2)	**42.6 ± 4.3**	**280**	**47.5 ± 4.2**	**125**	**0.0001**	**49.3 ± 4.3**	**53**	**55.2 ± 4.7**	**47**	**0.0001**
Lumbar spine BMC (g)	**44.4 ± 7.8**	**280**	**50.5 ± 9.1**	**125**	**0.0001**	**51.2 ± 6.5**	**53**	**56.3 ± 8.5**	**47**	**0.01**
Mid radius BA (cm^2)	7.16 ± 0.99	280	7.01 ± 0.99	126	ns	**9.31 ± 1.26**	**53**	**10.1 ± 1.18**	**47**	**0.01**
Mid radius BMC (g)	4.11 ± 0.68	280	4.12 ± 0.69	126	ns	**6.09 ± 1.07**	**53**	**6.73 ± 0.96**	**47**	**0.01**
Distal ⅓ rd radius BA (cm^2)	2.63 ± 0.28	280	2.65 ± 0.24	126	ns	3.06 ± 0.44	53	3.12 ± 0.24	47	ns
Distal ⅓ rd radius BMC (g)	**1.77 ± 0.19**	**280**	**1.83 ± 0.20**	**126**	**0.01**	**2.30 ± 0.28**	**53**	**2.46 ± 0.28**	**47**	**0.01**
Cross sectional area (cm^2)	**2.71 ± 0.41**	**280**	**2.60 ± 0.41**	**126**	**0.05**	3.05 ± 0.38	43	3.14 ± 0.51	47	ns
Cross sectional area (cm^2)*	**2.82 ± 1.01**	**280**	**3.52 ± 1.23**	**126**	**0.001**	**5.28 ± 1.97**	**43**	**7.58 ± 2.44**	**47**	**0.0001**
Section modulus (cm^3)	**2.73 ± 0.02**	**280**	**2.55 ± 0.04**	**125**	**0.05**	**3.24 ± 0.08**	**43**	**2.97 ± 0.07**	**47**	**0.05**
Section modulus (cm^3)*	**3.01 ± 0.06**	**280**	**3.11 ± 0.10**	**125**	**0.001**	6.15 ± 0.39	43	6.79 ± 0.37	47	ns

*Femoral neck geometry results when corrected for height and total lean mass (less head for children) (± SE).

Table 5-2. Power coefficients (PC ± SE)* at each skeletal site in black and white a) children and b) their parents

a)

	Girls				Ethnic differences	Boys				Ethnic differences	Gender differences Black	Gender differences White
	Black	**n**	**White**	**n**	***P***	**Black**	**n**	**White**	**n**	***P***	***P***	***P***
Whole body less head	1.34 ± 0.02	135	1.32 ± 0.03	63	0.5749	1.28 ± 0.03	154	1.27 ± 0.04	67	0.8490	0.1046	0.3276
Femoral neck	**1.18 ± 0.13[d]**	**135**	**1.83 ± 0.21[2]**	**63**	**0.0010**	**0.87 ± 0.11[a]**	**153**	**1.35 ± 0.16[4]**	**66**	**0.0160**	0.0696	0.0721
Lumbar spine	1.52 ± 0.10[1]	135	1.72 ± 0.15[1]	63	0.1750	1.21 ± 0.09[4c]	154	1.30 ± 0.11[3]	67	0.5689	**0.0220**	**0.0253**
Mid radius	1.16 ± 0.04[2a]	135	1.09 ± 0.05[1a]	63	0.3005	0.96 ± 0.04[a]	152	1.04 ± 0.05[a]	64	0.2499	**0.0004**	0.4825
Distal ⅓ rd radius	1.24 ± 0.09[3c]	135	1.17 ± 0.11[c]	63	0.6214	**0.81 ± 0.08[4a]**	**152**	**1.07 ± 0.05[a]**	**64**	**0.0439**	**0.0004**	0.5012

b)

	Mothers				Ethnic differences	Fathers				Ethnic differences
	Black	**n**	**White**	**n**	***P***	**Black**	**n**	**White**	**n**	***P***
Whole body	1.39 ± 0.06[1]	278	1.37 ± 0.10[2]	125	0.8585	1.26 ± 0.12[4d]	53	1.60 ± 0.18[3]	47	0.1107
Femoral neck	0.78 ± 0.11[4a]	280	0.94 ± 0.20[c]	126	0.4500	0.43 ± 0.25[1b]	53	1.10 ± 0.46	47	0.1898
Lumbar spine	**1.33 ± 0.06[1c]**	**280**	**1.58 ± 0.11[1]**	**125**	**0.0314**	0.90 ± 0.16[b]	53	1.09 ± 0.21[b]	47	0.4671
Mid radius	1.00 ± 0.04[a]	280	1.02 ± 0.06[a]	126	0.7810	1.07 ± 0.11[b]	53	1.06 ± 0.10	47	0.0591
Distal ⅓ rd radius	**0.80 ± 0.05[2a]**	**280**	**1.02 ± 0.07[a]**	**126**	**0.0111**	**0.70 ± 0.11[3a]**	**53**	**1.16 ± 0.14[d]**	**47**	**0.0114**

[1-4] Significantly different from 1**:** [1]*P*<0.0001, [2]*P*<0.001, [3]*P*<0.01, [4]*P*<0.05
[a-d] Significantly different from 1.5**:** [a]*P*<0.0001, [b]*P*<0.001, [c]*P*<0.01, [d]*P*<0.05
***** PCs were calculated from the linear-regression analyses of ln(BMC) on ln(BA). BA^{PC} was used as a correction for BMC together with height and weight in Figure 5-1 to Figure 5-4. *P* values indicate ethnic and gender differences. The superscripts indicate whether PC was different from 1 or 1.5.

Table 5-3. The associations between children's and parents' BMC Z-scores* assessed by Pearson correlation coefficients (r) and heritability estimates (½h²)

a)

	Children vs. Mothers															
	Girls						Boys						Ethnic differences		Gender differences	
	Black			White			Black			White			Girls	Boys	Black	White
	r	½h² ± SE	n	r	½h² ± SE	n	r	½h² ± SE	n	r	½h² ± SE	n	*P*	*P*	*P*	*P*
WB	**0.38**[a]	0.39 ± 0.08	128	**0.51**[a]	0.40 ± 0.13	59	**0.46**[a]	0.40 ± 0.07	149	**0 .46**[a]	0.45 ± 0.11	61	r: 0.313 ½h²: 0.946	r: 1.000 ½h²: 0.701	r: 0.426 ½h²: 0.925	r: 0.728 ½h²: 0.769
FN	**0.31**[a]	0.32 ± 0.09	128	**0.44**[a]	0.43 ± 0.12	60	**0.23**[d]	0.23 ± 0.08	151	**0.29**[d]	0.32 ± 0.12	62	r: 0.344 ½h²: 0.479	r: 0.184 ½h²: 0.540	r: 0.478 ½h²: 0.454	r: 0.352 ½h²: 0.518
LS	**0.20**[d]	0.22 ± 0.10	128	**0.27**[d]	0.29 ± 0.14	59	**0.42**[a]	0.38 ± 0.07	152	**0.29**[d]	0.34 ± 0.13	62	r: 0.645 ½h²: 0.685	r: 0.333 ½h²: 0.771	r: 0.543 ½h²: 0.181	r: 0.908 ½h²: 0.794
MR	**0.48**[a]	0.52 ± 0.08	128	**0.28**[d]	0.28 ± 0.13	60	**0.27**[d]	0.25 ± 0.08	150	**0.39**[c]	0.40 ± 0.12	60	r: 0.096 ½h²: 0.103	r: 0.388 ½h²: 0.311	**r: 0.044** **½h²: 0.018**	r: 0.509 ½h²: 0.499
DR	**0.40**[a]	0.46 ± 0.09	128	**0.36**[c]	0.35 ± 0.12	59	**0.15**[d]	0.14 ± 0.07	149	**0.34**[d]	0.38 ± 0.12	58	r: 0.772 ½h²: 0.483	r: 0.201 ½h²: 0.077	**r: 0.026** **½h²: 0.048**	r: 0.905 ½h²: 0.860

b)

	Children vs. Fathers															
	Girls						Boys						Ethnic differences		Gender differences	
	Black			White			Black			White			Girls	Boys	Black	White
	r	½h² ± SE	n	r	½h² ± SE	n	r	½h² ± SE	n	r	½h² ± SE	n	*P*	*P*	*P*	*P*
WB	0.11	0.12 ± 0.23	25	0.39	0.30 ± 0.15	24	0.28	0.31 ± 0.21	27	**0 .45**[d]	0.36 ± 0.15	23	r: 0.323 ½h²: 0.516	r: 0.515 ½h²: 0.846	r: 0.548 ½h²: 0.545	r: 0.816 ½h²: 0.779
FN	**0.49**[d]	0.52 ± 0.29	25	0.38	0.32 ± 0.17	24	0.33	0.39 ± 0.22	27	-0.21	-0.18 ± 0.18	23	r: 0.656 ½h²: 0.558	r: 0.066 **½h²: 0.049**	r: 0.513 ½h²: 0.722	**r: 0.050** **½h²: 0.051**
LS	**0.41**[d]	0.46 ± 0.22	25	-0.03	-0.03 ± 0.21	24	**0.47**[d]	0.60 ± 0.22	28	**0.49**[d]	0.33 ± 0.13	23	r: 0.127 ½h²: 0.114	r: 0.931 ½h²: 0.301	r: 0.799 ½h²: 0.654	**r:0.070** ½h²:0.154
MR	0.33	0.32 ± 0.19	25	-0.43	-0.06 ± 0.31	24	**0.43**[d]	0.46 ± 0.19	28	**0.47**[d]	0.34 ± 0.14	22	**r: 0.009** ½h²: 0.305	r: 0.869 ½h²: 0.616	r: 0.689 ½h²: 0.604	**r: 0.002** ½h²: 0.248
DR	**0.59**[d]	0.57 ± 0.20	25	-0.17	-0.20 ± 0.24	24	0.36	0.37 ± 0.18	28	**0.50**[d]	0.26 ± 0.10	22	**r: 0.005** **½h²: 0.070**	r: 0.571 ½h²: 0.592	r: 0.304 ½h²: 0.459	**r: 0.023** ½h²: 0.233

[a-d] Significantly correlated**:** [a]*P*<0.0001, [b]*P*<0.001, [c]*P*<0.01, [d]*P*<0.05

* Z-scores were calculated from the means and standard deviations of BMC adjusted, for height, weight, and BA^{PC} (PCs listed in Table 5-2.) and age in adults. Z-scores were used so that children and their parents' data were comparative.

Size-adjusted BMC

Figure 5-1 to Figure 5-4 illustrate how BMC values vary in black and white children and their parents when corrected for different combinations of height, weight, BA and/or BA^{PC} at the different skeletal sites. Correcting BMC for height, weight and BA^{PC} or BA accounted for the greatest proportion of the variance in BMC at most skeletal sites. However, ethnic differences in BMC were magnified when correcting for BA^{PC} versus BA. That is, BMC (corrected for BA^{PC}) was greater in black children and their parents than in their white peers at the femoral neck (all $P<0.0001$) and lumbar spine (all $P<0.0001$), and in black boys and fathers at the whole body (both $P<0.0001$). At the femoral neck, black girls had 7% more BMC than whites when corrected for BA, height and weight, which increased to 69% when corrected for BA^{PC}, height and weight. Similar increases were observed in black boys (from 8% to 64%), mothers (from 8% to 34%) and fathers (from 6% to 98%) as well as at the lumbar spine (black girls: from 4% to 85%; boys from 3% to 34%; mothers from 1% to 166% and fathers from 2% to 89%). BMC was less in black girls and their mothers at the whole body (both $P<0.0001$), mid radius (girls: $P<0.0001$, mothers: $P<0.001$) and distal one-third of the radius (girls only $P<0.0001$).

Figure 5-1. Whole body less head (for girls and boys) and whole body (for mothers and fathers) BMC (± SE) corrected for ln(height), ln(weight) or combinations of size-related predictors of BA, BA^{PC}, (BAx), height (ht) and / or weight (wt) in black and white girls and boys, mothers and fathers. Asterisk's indicate ethnic differences, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001

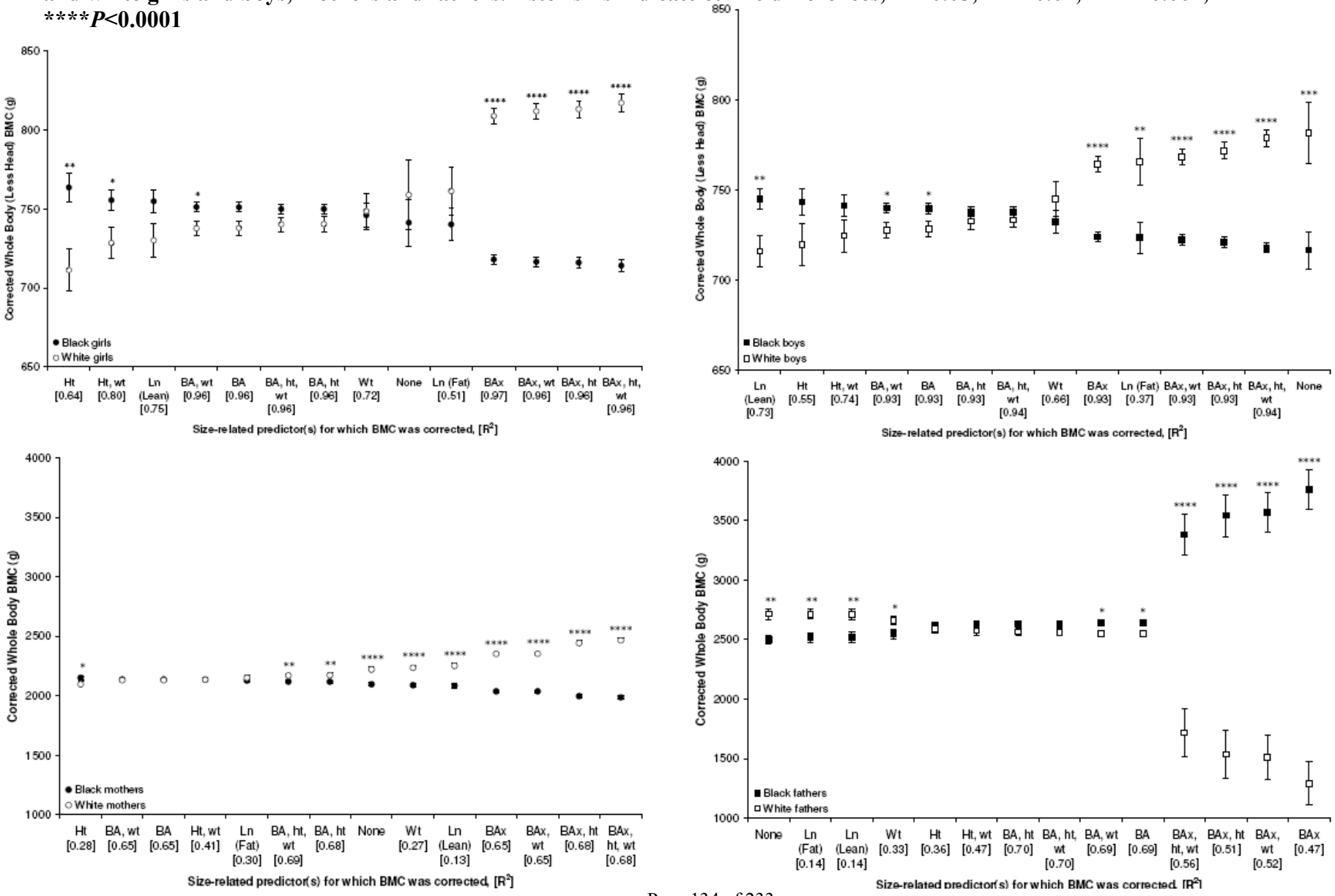
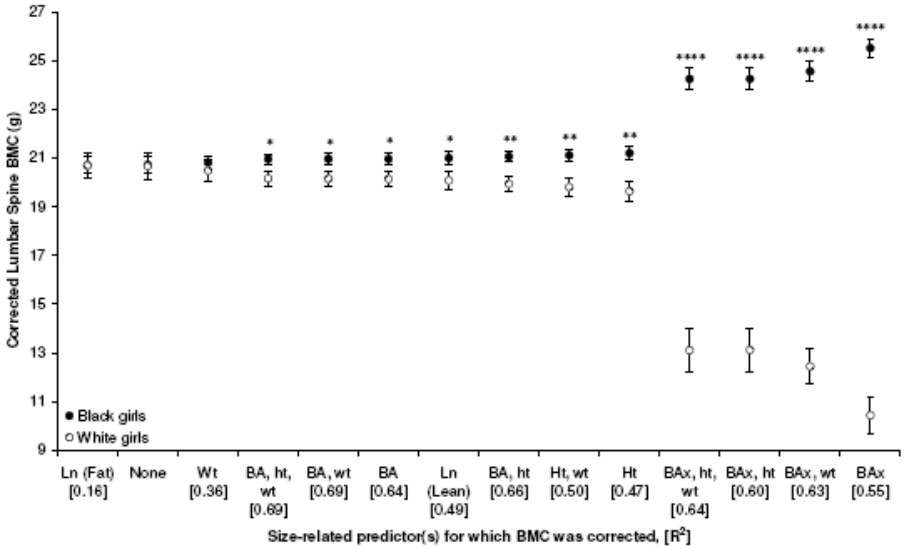
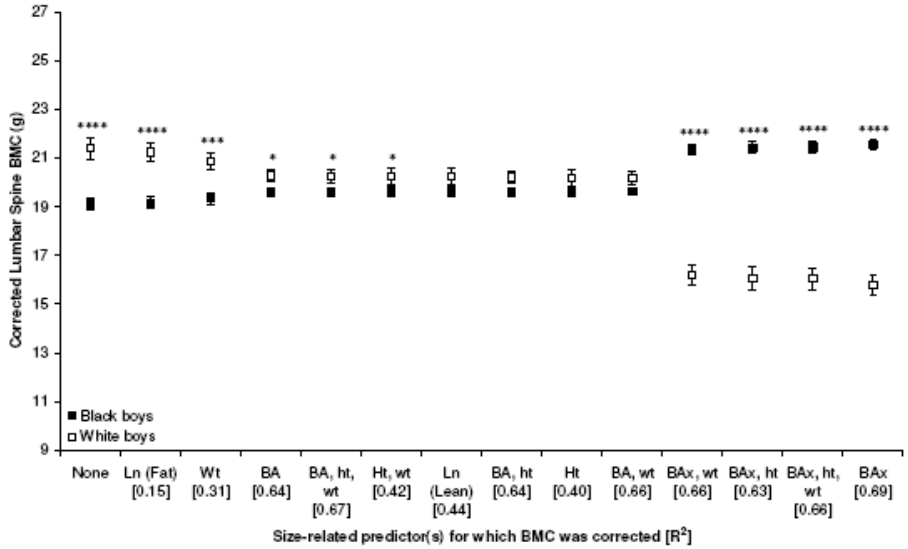
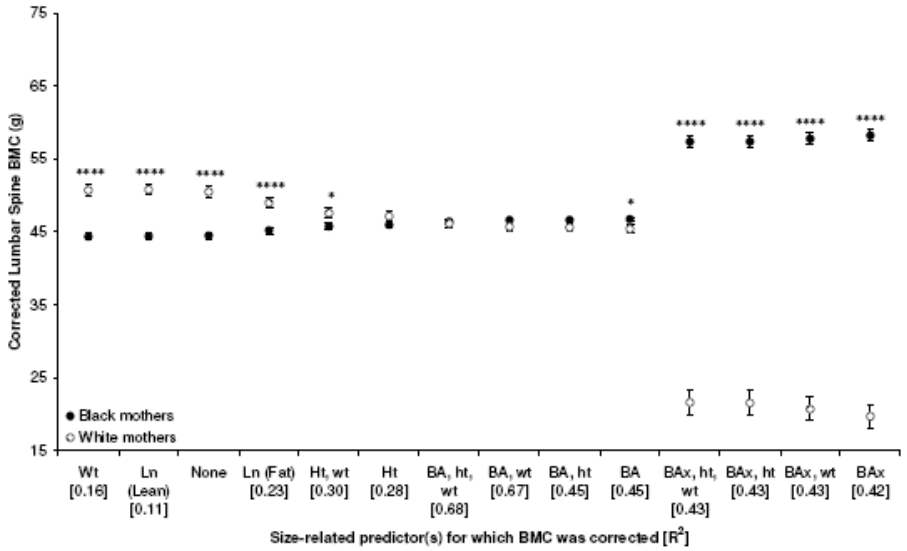


Figure 5-2. Lumbar spine BMC (± SE) corrected for ln(height), ln(weight) or combinations of size-related predictors of BA, BA^{PC}, (BAx), height (ht) and / or weight (wt) in black and white girls and boys, mothers and fathers. Asterisk's indicate ethnic differences, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001







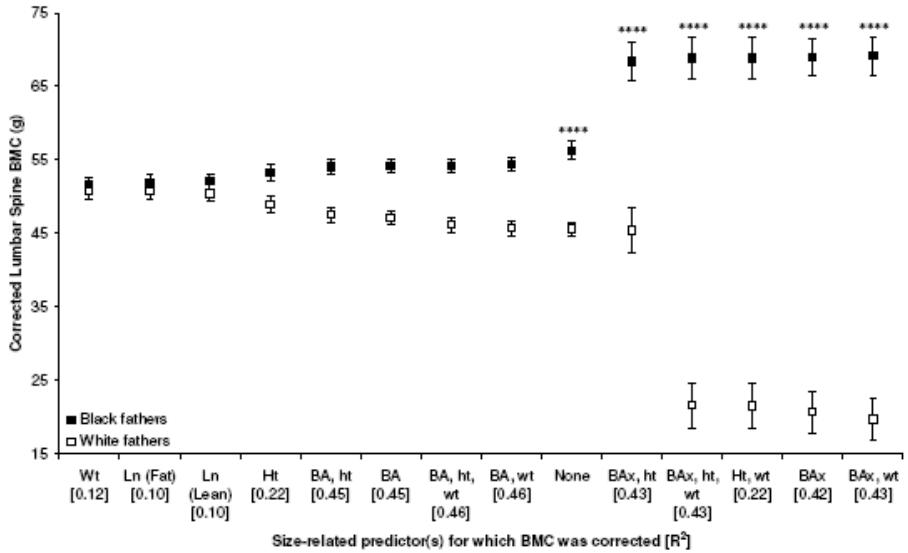


Figure 5-3. Femoral neck BMC (± SE) corrected for ln(height), ln(weight) or combinations of size-related predictors of BA, BA^{PC}, (BAx), height (ht) and / or weight (wt) in black and white girls and boys, mothers and fathers. Asterisk's indicate ethnic differences, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001 (not published)

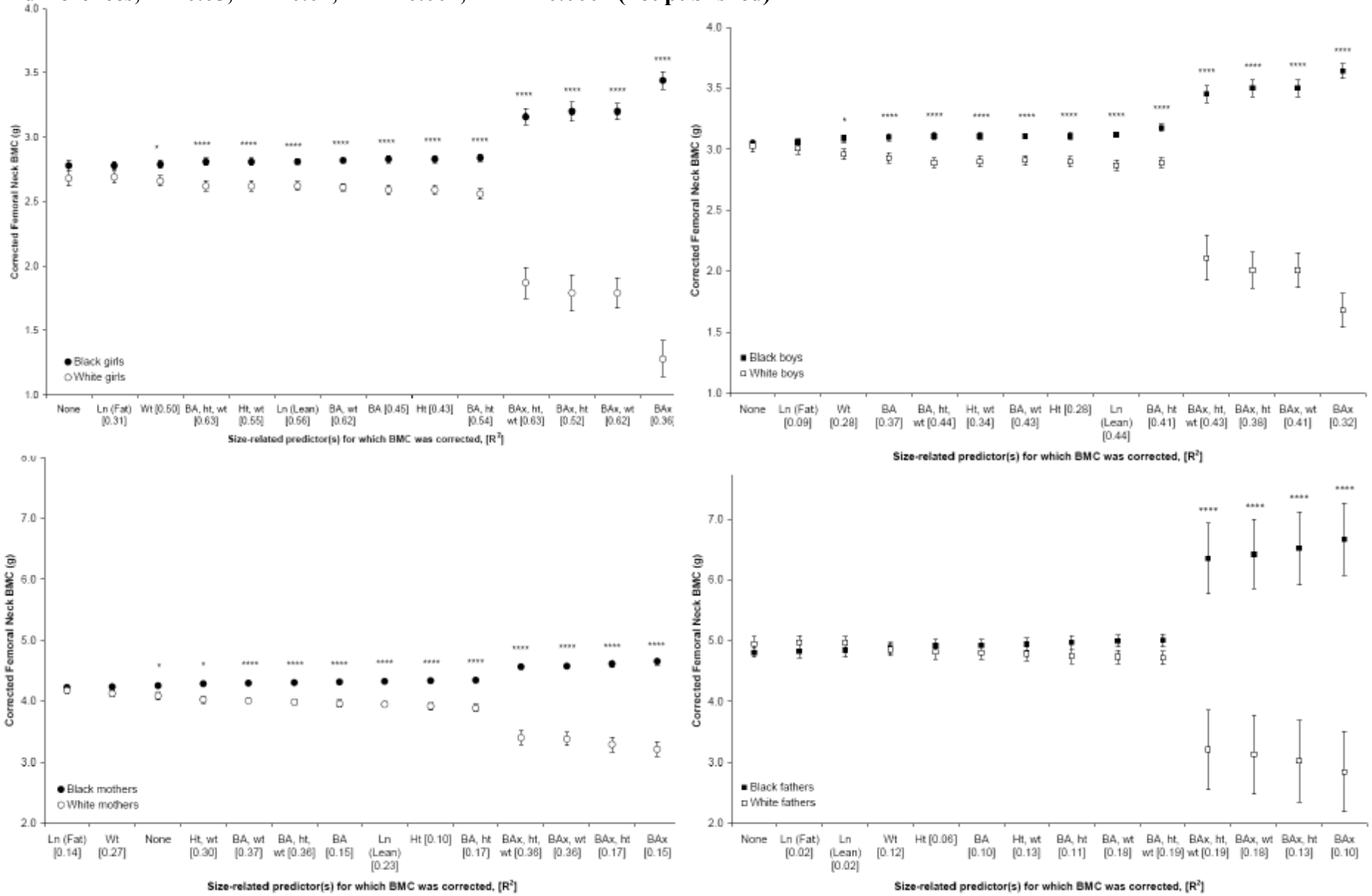
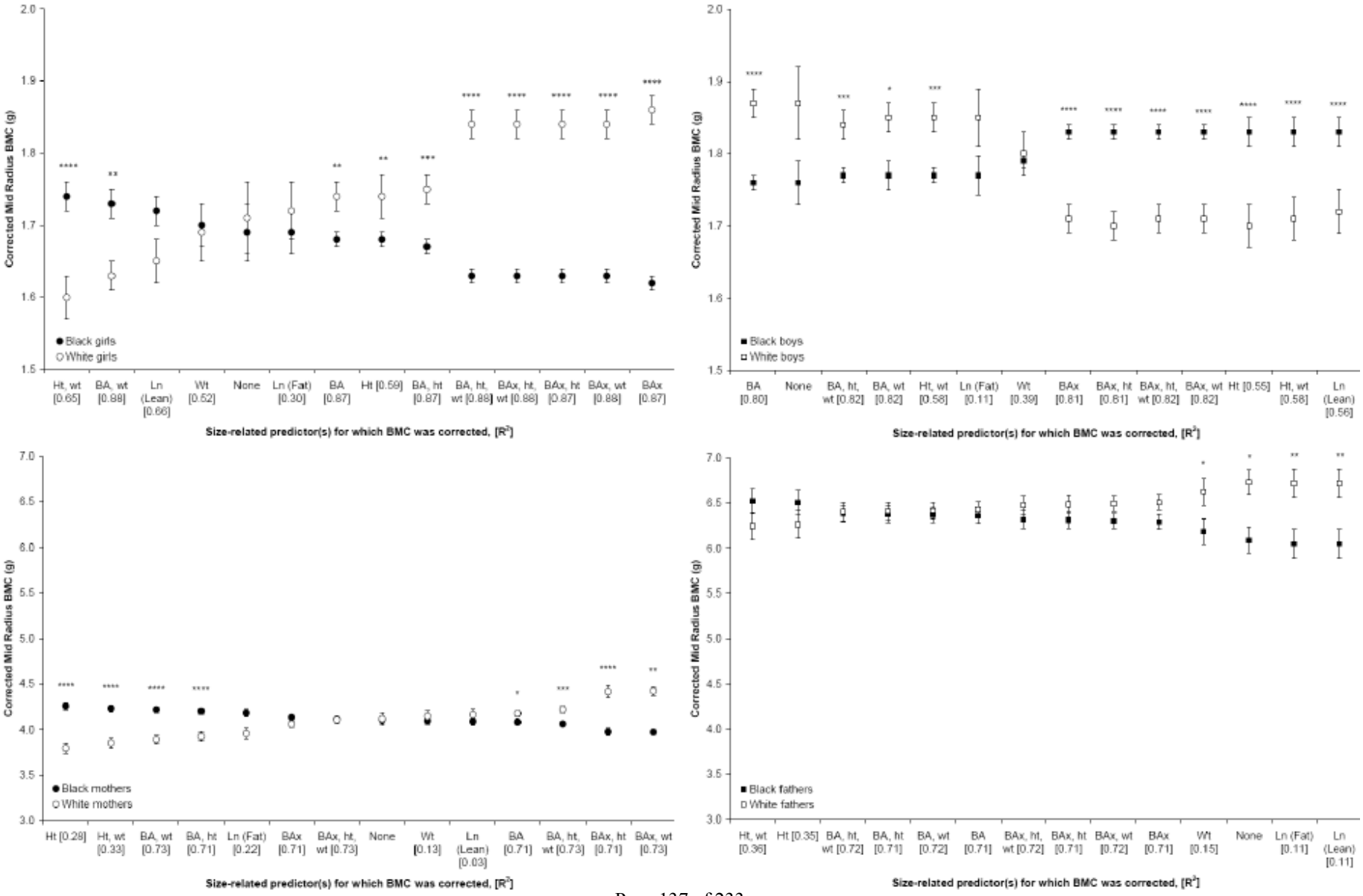


Figure 5-4. Mid radius BMC (± SE) corrected for ln(height), ln(weight) or combinations of size-related predictors of BA, BA^{PC}, (BAx), height (ht) and / or weight (wt) in black and white girls and boys, mothers and fathers. Asterisk's indicate ethnic differences, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 (not published)



3. Structural geometry of the femoral neck

There were no ethnic differences between children or parents in uncorrected cross sectional area (CSA). Uncorrected section modulus (Z) was significantly greater in black children and their parents (P<0.05-0.0001). Once corrected for height and total lean mass (less head for children), CSA was significantly greater in blacks (P<0.05-0.0001) and Z significantly smaller in black mothers (P<0.001). (Table 5-1)

4. Associations between children's and parents' BMC adjusted for height, weight and BA^{PC}

The associations between children's and parents' adjusted BMC Z-scores assessed by way of Pearson's correlation coefficients (r) and heritability estimates ($½h^2$, %) are presented in Table 5-3. BMC Z-scores of black and white children were significantly correlated with those of their mothers' at all skeletal sites. Heritability by maternal or paternal descent was estimated to be ~30%. There were no significant ethnic differences in correlation coefficients or heritability estimates.

Discussion

This study illustrates how various combinations of size-related adjustments influence DXA-measured BMC in black and white pre-pubertal children and their parents. Ethnic differences in BMC were dependent on ethnic differences in size. Correcting BMC for BA^{PC} (or

BA), height and weight proved to be the combination of size-related corrections that accounted for the greatest proportion of the variance in BMC at all skeletal sites. Size-adjusted BMC (adjusted for BA) at the different sites was greater in blacks by 2-8%, but by 34-166% when adjusted for BA^{PC}. Chapter 3 presented BMC corrected for height and weight only (excluding BA) in the same black children was ~6% greater at the femoral neck but not different at the lumbar spine. [320] In support of these latter findings, similar lumbar spine BMDs were shown in pre-, peri- and postmenopausal black and white South African women when corrected for height only. [69,70] Adjusting BMC for BA^{PC}, height and weight versus BA, height and weight increased ethnic differences in both adults and children at the femoral neck (in girls: from 7% to 69%; boys: 8% to 64%; mothers 8% to 34%; fathers: 6% to 98%) and unmasked ethnic differences at the lumbar spine (girls: 4% vs. 85%; boys: 3% vs. 34%; mothers 1% vs. 166%; fathers: 2% vs. 89%) and may in part explain the ten-fold lower prevalence of femoral neck fractures in adult black South Africans when compared to whites. [298,310]

DXA, histomorphometric and radiogrammetric evidence has accumulated supporting superior bone quality and strength in black South Africans and African-Americans when compared to their white counterparts; the macro-architecture of the proximal femur in blacks is characterised by narrower marrow cavities, thicker cortices and a lower buckling ratios (ratio of outer radius to cortical thickness), despite non-significant differences in outer bone diameter. [232,233,290] The micro-architecture of the iliac crest in South African blacks is characterised by thicker cortical bone, less porous cortices, greater endocortical wall thickness and greater osteoid thickness. Adults in addition have fewer canals in the cortical bone and thicker trabeculae than whites. [286-288] Estimates of strength as determined by cross-sectional geometry (cross-sectional

area and section modulus) at the femoral neck were greater in both South African blacks and African-Americans when compared to whites. [222,233] These macro- and micro- architectural features are consistent with greater bone strength and lower fracture rates. [290] Lastly, black South Africans have been shown to have greater bone apposition and formation rates. [287,288] Smaller bones with thicker cortices and trabeculae have also been found using high resolution pQCT in Chinese premenopausal women at the distal radius and tibia when compared to white women; [331] these findings are similar to that which has been found at the femoral neck in the comparison between our black and white South African children.

Structural differences in bone are suggested to originate in the peripubertal period because few ethnic differences in bone size and microarchitecture before puberty have been reported. [114] Differences in DXA-measurements are apparent by age 9 years in this cohort, suggesting that these differences had developed prior to puberty. [212,223]

It is possible that the better bone mass in black children and adults might have been due to greater weight-bearing or physical activity in which poorer people might need to engage. In fact, we had previously proposed this to be a mechanism for the greater femoral neck BMD in black South African women. [70] Correlations have been shown between weight of load carried on the head and lumbar spine BMD, as well as between years of load carrying and lumbar spine and total body BMD. [189] However, given that black 10 year old children, who are lighter than or of similar weight as white children, also have a greater femoral neck bone mass, other explanations must be sought. Physical activity is actually lower in our black than white children [212,213] thus excluding physical activity as a possible explanation Thus it appears that skeletal loading is

unlikely to have contributed to the higher bone mass and we now postulate that the differences are mainly genetically determined in otherwise unfavourable social and environmental conditions (poor growth and nutrition, [46] and low dietary calcium intake [199] of black children). In support of our findings, a study conducted in individuals of African descent in the West Indies, which analysed genetic and environmental factors influencing BMD measured by both DXA and QCT, found overall heritability of both areal and volumetric BMD to be substantial. [330]

Areal BMD remains an important predictor of fracture risk. The calculation of areal BMD or another measure of apparent density, BMAD, assumes PCs to be 1 (when calculating BMD), 1.5 (when calculating BMAD at the femoral neck and mid-radius) or 2 (when calculating BMAD at the whole body). PCs calculated in this study were for the most part significantly different from each of the three values in both children and their parents, confirming that neither BMD nor BMAD reflect true volumetric bone density. It is of interest to note that the calculated PCs were generally similar for the two ethnic groups at each of the different bone sites with the exception of the femoral neck in both boys and girls and at the distal third of the radius in boys. Similar PCs suggest that three dimensional size changes in bone associated with growth are similar at the whole body, lumbar spine and mid-radius in the two ethnic groups.

In the same cohort of children at age 9 years, higher power coefficients were found in white children when compared to black. [223] The difference between this study and Micklesfield *et al.* (2009) may be that we calculated power coefficients for black and white boys and girls as opposed to Micklesfield *et al.* (2009) who combined boys and girls in the calculation of their black and white PCs, thus increasing sample size and statistical power to detect ethnic

.differences in PCs, bone dimensions or bone distribution. These different approaches highlight the importance of calculating power coefficients specific to a sample, time point, and aim of the study.

Maternal BA and size-adjusted BMC significantly predicted their children's BA and adjusted BMC at all skeletal sites. Heritability by maternal descent was estimated to be ~30% and was similar for both black and white children. This is not the first study to demonstrate the influence of maternal genetics on the prepubertal acquisition of bone mass, [84,149] but it is the first to show similar genetic influences in BMC in both black and white prepubertal populations, despite their differences in body- and bone size, and environmental influences. Black South Africans, children in particular, are exposed to a number of environmental factors known to impact negatively on bone mass, such as poor growth and nutrition, [46] low calcium intake [199] and little physical activity. [212,213] Given the important contributions that diet and other environmental factors have on the phenotypic variance in bone mass or BMD, lower bone mass and heritability estimates in blacks would be expected. Lower heritability estimates have been shown before for stature in West African populations when compared to European populations, which were explained by the rigours of the traditional way of life in West African surroundings. [275]

In general, the possible genetic contribution to the variance of the bone mass phenotype is reported to be 50–80% at any age or in any group. [35] The bone mass phenotype in black South Africans is expressed even in pre-/early pubertal childhood. These heredity estimates in black children are comparable to those from environmentally-advantaged white South African children and Caucasians from other parts of the world.

Mother-daughter estimates of heritability of BMC are usually better than mother-son estimates. [149] In the current study, this was true only at mid- and distal one-third of the radii in black children, at all other sites no differences in heritability between male and female children were seen. It has also been suggested that estimates of maternal heritability are better than paternal estimates in both boys and girls. [149] Due to the small number of fathers, a major limitation in the current study, it was not possible to draw any conclusions from our data.

In conclusion, this study confirms that correcting BMC for height, weight and BA^{PC} was the combination of size-related adjustments that accounted for the greatest proportion of the variance of BMC at all skeletal sites. This combination increased ethnic differences in BMC 2.6 times greater at the femoral neck and unmasked ethnic differences at the lumbar spine in both adults and children and may in part explain the lower prevalence of fragility fractures at the hip in black South Africans when compared to whites. [298] Heritability by maternal descent, estimated by regressing children's Z-scores on parents Z-scores, was ~30%, and comparable between environmentally disadvantaged black and advantaged white South African children and similar to that found in Caucasians from other parts of the world. It is unclear at this stage, whether improvement in the adverse environmental factors in our black children would result in an increase in bone mass, even lower fracture rates and greater heritability. The intriguing question remains as to how genetic influences maintain bone mass in the face of what are generally considered to be adverse environmental factors. Not only do these genetic influences have a positive effect on bone mass during childhood, but these are maintained through adult life and are associated with a very low incidence of femoral neck fractures in the elderly.

CHAPTER 6 - Discussion and conclusions

Introduction

This thesis explored the associations between proximal, historical and predictive genetic and environmental factors affecting bone health in socio-economically- and environmentally-disadvantaged black and -advantaged white pre- and early-pubertal children from South Africa. Data presented in this thesis was collected in 2000 and 2001 when the understanding of DXA measurement and interpretation of BMC and bone mass in growing children was a relatively new field. This chapter serves to consolidate our three research publications over that time span. This chapter first presents a summary of this thesis' findings, hypotheses tested and key results. From the body of work, I discuss common research themes that emerged, and what contributions this thesis makes to the body of theoretical and contextual knowledge. I also discuss limitations and propose future research avenues to pursue, and finally, what the findings of this thesis conclude.

Summary of key findings

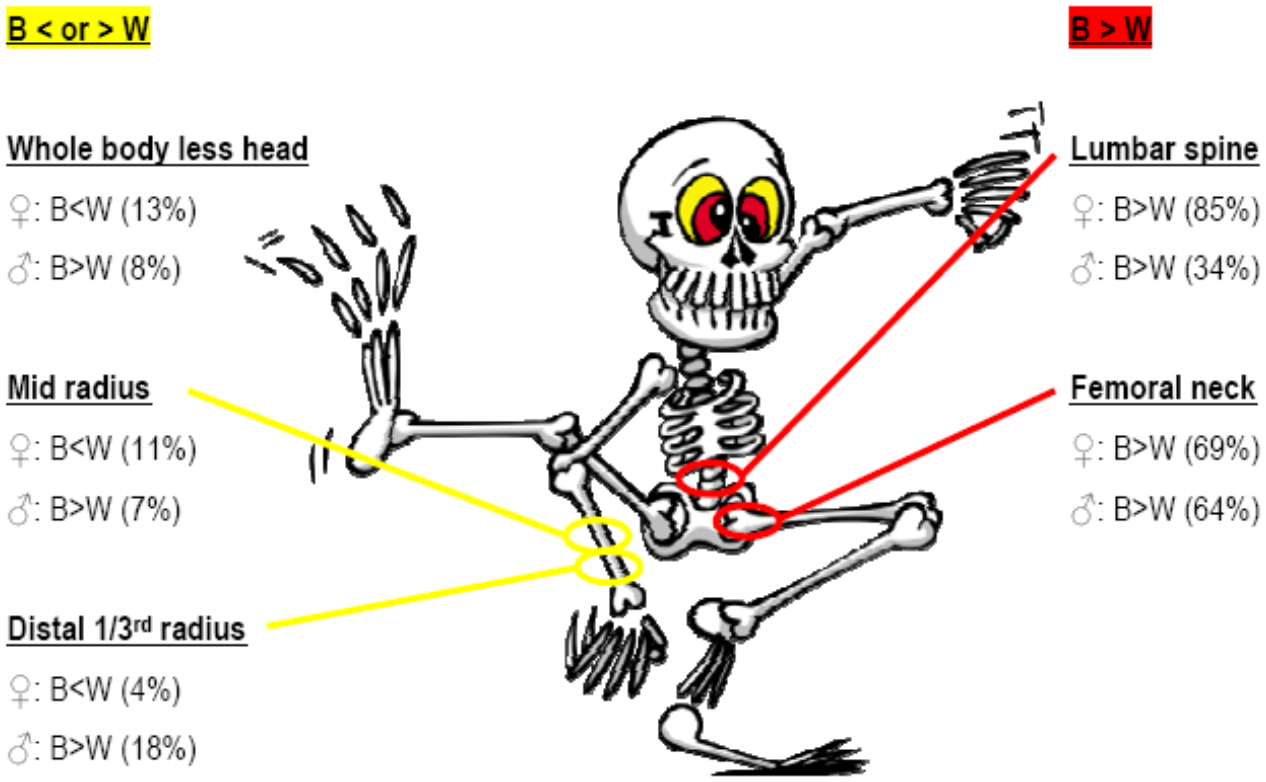
Table 6-1 below summarises the three key research questions, the hypotheses that were tested and the summary of key findings by chapter.

Table 6-1. Summary of key findings

Research questions	Hypotheses	Summary of chapter findings
(1) What proximal factors contribute to bone mass and bone size of 10-year old pre-and early pubertal, black and white South African children?	Body size (height, weight), genetic factors (gender, race/ethnicity), lifestyle factors (SES, nutrition, physical activity), sexual and skeletal maturity influence bone mass and bone size.	Socio-economically- and environmentally disadvantaged black children had a greater bone mass at the femoral neck, total hip and mid radius than white children which was neither explained by differences in current height or weight (for which statistical corrections were made) nor bone age and pubertal stage (which did not differ between ethnic groups).
(2) Do historical factors contribute to the current status of bone mass?" More specifically, (1) Do weight and/or length in infancy predict bone mass in 10 year old children? (2) If there is a relationship is it because weight and/or length in infancy are related to bone size or bone mass?	Size in infancy is related to 10 year old bone mass and bone size.	BMC at 10 years was independently associated with weight and length at 1 year, which was not completely mediated by the tracking of skeletal growth. Low BW and small size at 1 year resulted in smaller bones and bones of lower BMC at the whole body and lower BMC at the femoral neck.
(3) Is parental bone size and bone mass predictive of bone size and bone mass in 10-year old children? More specifically, what is the heritability of bone size and bone mass?	(3a) Bone mass is heritable. (3b) Heritability estimates of bone mass are less in black than in white children	(3a) Black children and their parents had a greater corrected BMC at the femoral neck, and lumbar spine, than their white counterparts. (3b) Maternal heritability of bone mass was estimated to be ~30%. That is, ~30% of the phenotypic variation is due to genetic variation in both blacks and whites at all sites and was comparable between socio-economically- and environmentally disadvantaged black children and advantaged white children.

The key findings in black and white 10-year black and white old children are schematically summarized in Figure 6-1 below.

Figure 6-1. Schematic skeletal figure summarising key findings in black (B) and white (W) children when DXA-measured BMC was corrected for BA^{PC}, height and weight (as in Chapter 5)



1) *At the femoral neck:* **BMC was consistently greater in both black children and their parents** when compared to their white peers, **regardless of the way in which BMC was corrected for size**. The percentages in brackets that follow refer to percentage black-white differences in BMC after correcting BMC for BA, height and weight (as in Chapter 3) versus **correcting BMC for BA^{PC}, height and weight** (as in Chapter 5). Percentages preceded by

'ns', indicate the black-white differences were 'not significant'. [Black girls (7% vs. **69%**), black boys (8% vs. **64%**), black mothers (8% vs. **34%**) and black fathers (ns 6% vs. **98%**)].

2) *At the lumbar spine:* **BMC was greater or similar in blacks** when compared to whites, **depending on which measures were used to correct BMC for size.** [Black girls (ns 4% vs. **85%**), black boys (ns 3% vs. **34%**), black mothers (ns 1% vs. **166%**) and black fathers (ns 2% vs. **89%**)].

3) *At the whole body, mid radius and distal one third of the radius:* **ethnic differences in BMC varied between boys and girls and their parents, as well as being dependent on which measures were used to correct BMC for size**. [Whole body: black girls (ns 1% vs. **-13%**), black boys (ns 1% vs. **8%**), black mothers (-2% vs. -**19%**) and black fathers (ns 3% vs. **97%**); mid radius: black girls (-11% vs. **-11%**), black boys (-4% vs. **7%**), black mothers (-4% vs. ns **0%**) and black fathers (ns 0% vs. ns **-2%**) and distal one third of the radius: black girls (ns 0% vs. **-4%**), black boys (ns -3% vs. **18%**), black mothers (-3% vs. **20%**) and black fathers (ns -3% vs. **24%**)].

Research themes

Three key and recurring factors or research themes emerged across the body of work in this study of proximal, historical and predictive genetic and environmental factors affecting bone mass and bone size in growing children, namely, the DXA measurement factor, the skeletal site factor and the genetic predisposition factor, which are discussed in more detail below.

The DXA measurement factor

In Chapter 3 when bone mass and bone size were compared between black and white **children only, BMC was corrected for BA, height and weight**. In Chapter 5, when bone size and bone mass were compared between black and white **children and their parents, BMC was corrected for BA^{PC}, height and weight,** after population-specific power coefficients (PCs) were calculated. This method allowed DXA-measured BMC to be custom-corrected for size for each group (black and white boys and girls and their parents) we studied and at each skeletal site. [261] Correcting BMC for BA^{PC} (or BA), height and weight proved to be the combination of size-related corrections that accounted for the greatest proportion of the variance in BMC at all skeletal sites. Chapter 5's Figure 5-1 to Figure 5-4 illustrated how BMC values varied in black and white children and their parents when corrected for different combinations of height, weight, BA and/or BA^{PC} and how different conclusions with regards to ethnic differences in bone mass at the different skeletal sites could be drawn: that BMC was greater in blacks than whites, greater in whites than blacks, or that there was no difference between the ethnic groups.

Very few studies from other parts of the world have reported greater bone mass (corrected or uncorrected for size) in white populations when compared to black, yet we found BMC (corrected and/or uncorrected for size) to be greater in whites at a few skeletal sites. That is, BMC was greater in whites at the whole body, and at the mid- and distal one-third of the radius in girls, boys, mothers and fathers, and at the lumbar spine in boys and mothers.

From South Africa, Patel *et al.* (1992), found a greater uncorrected BMC at the forearm in white South African women aged 6-20 years when compared to their black counterparts, but not after correcting for height. [247] Daniels *et al.* (1995) however found no differences in radial and spinal BMD between South African blacks and whites before or after corrections. [70]

From the UK and The Gambia, lumbar spine BMC was 31% higher in British women (44+ years) when compared to Gambian counterparts, and 24% higher after correcting for age, height and weight. Similarly, midshaft radial BMC was 16% higher before and 10% after adjustment for age, height, and weight. In 134 British children aged 0-36 months (123 Caucasian, 11 mixed, mostly Eurasian) BMC was greater when compared to 243 Gambian children both before and after correcting for height, weight and bone width. [260]

The questions to ask ourselves are: do these findings reflect a superior bone mass in whites, i.e. are we observing true site-specific ethnic differences in bone mass, or are we observing under- and/or overestimation of BMC? The International Society for Clinical Densitometry (ISCD) recommend that corrections for bone size and body composition must be factored into the interpretation of DXA measurements for children aged 5-19 years of age whose BMC and areal BMD are highly influenced by skeletal dimensions and body composition, which continuously changes in children. [119] The ISCD however does not recommend correcting DXA measurements in adults for skeletal dimensions and body composition, which remain relatively constant. In the clinical setting, uncorrected BMD is sufficient to diagnose and monitor bone mass in adults. That said, correcting adult BMC or BMD data for size would add value to the accuracy and precision of research data.

Using Figure 5-1 to Figure 5-4, it would be valuable to investigate which the most appropriate size-related corrections for DXA-measured BMC are by comparing corrected BMC values from these figures of both children and adults to BMC as determined by one of three possible methods; (1) ash weight which is the current gold standard for measuring BMC in the laboratory. DXA-measured BMC are within 7-9% of BMC as determined by ash weight; [136,326] (2) Quantitative Computed Tomography (QCT) which measures volumetric bone density in mg/cm^3 of either cortical or trabecular bone [90] or (3) true bone density which is wet bone weight divided by the actual volume of bone tissue. We may not know the answer yet but this thesis highlighted that black-white ethnic differences in BMC are dependent on the DXA measurement factor, and results must always be interpreted with this in mind.

The skeletal site factor

The femoral neck emerged as the site at which BMC was consistently greater in both black children and their parents when compared to their white peers, regardless of the way BMC was corrected for size. (Figure 5-3) Lumbar spine, BMC was however not consistently greater in black boys and mothers when compared to whites and depended on which measures were used to correct BMC for size. (Figure 5-2). There may be a few explanations for this apparent lack of agreement between skeletal sites.

The main difference between the femoral neck and lumbar spine is in the composition and relative proportions of cortical and trabecular bone. The femoral neck is predominantly

cortical (~75%) and lumbar spine predominantly trabecular (~66%). Table 6-2 below tabulates the relative proportions of cortical and trabecular bone at other skeletal sites too.

Table 6-2. Relative proportions of cortical and trabecular bone at the specific skeletal sites

Skeletal site	% Cortical Bone	% Trabecular Bone
PA spine	33%	66%
Ultra distal radius	33%	66%
Femoral neck	75%	25%
8mm radius	75%	25%
Total body	80%	20%
Mid radius	95%	5%

Source: Bonnick (1998) [38]

The turnover of cortical bone is slower than trabecular bone due to its predominantly mechanical function compared to the metabolic function of trabecular bone. The apparent discordance in ethnic differences in bone mass between skeletal sites may be because the rate of growth in varying dimensions differs between trabecular and cortical sites. Or trabecular bone may be more responsive, or responds quicker, to specific environmental influences than cortical bone. [254] For example, white girls might have had higher oestrogen levels which are associated with puberty before the development of secondary sexual characteristics, which were not yet apparent.

The incidence of vertebral fractures in South African women is significantly lower in rural and urban blacks (3% and 2%) when compared to whites (14%). [79] More recently Conradie (2008) published a thesis with updated data in which the prevalence of vertebral fractures was

reported to be similar between black (11.5%) and white (8.1%) women. [58] Small sample size cross-sectional study design were cited as concerns. Nevertheless, these data may suggest an increase in the prevalence of vertebral fractures in black South African women. These data in fracture incidence in together with the environmental and historical factors investigated in this thesis, suggest raise a potential concern relating to the bone health status of this cohort. That is, the children have not been programmed for optimal bone health in utero and early life. In addition, environmental factors are not favourable for maximisation of PBM as this cohort enters puberty, both of which are risk factors for the development of osteoporosis as an elderly population, particularly at forearm and lumbar spine. Despite disadvantages, black children fortunately demonstrate a superior bone mass- and strength at the femoral neck, to that of their white peers. This genetic advantage is seen in infancy, adults, and is associated with a very low incidence of femoral neck fractures in the black elderly.

The genetic predisposition factor

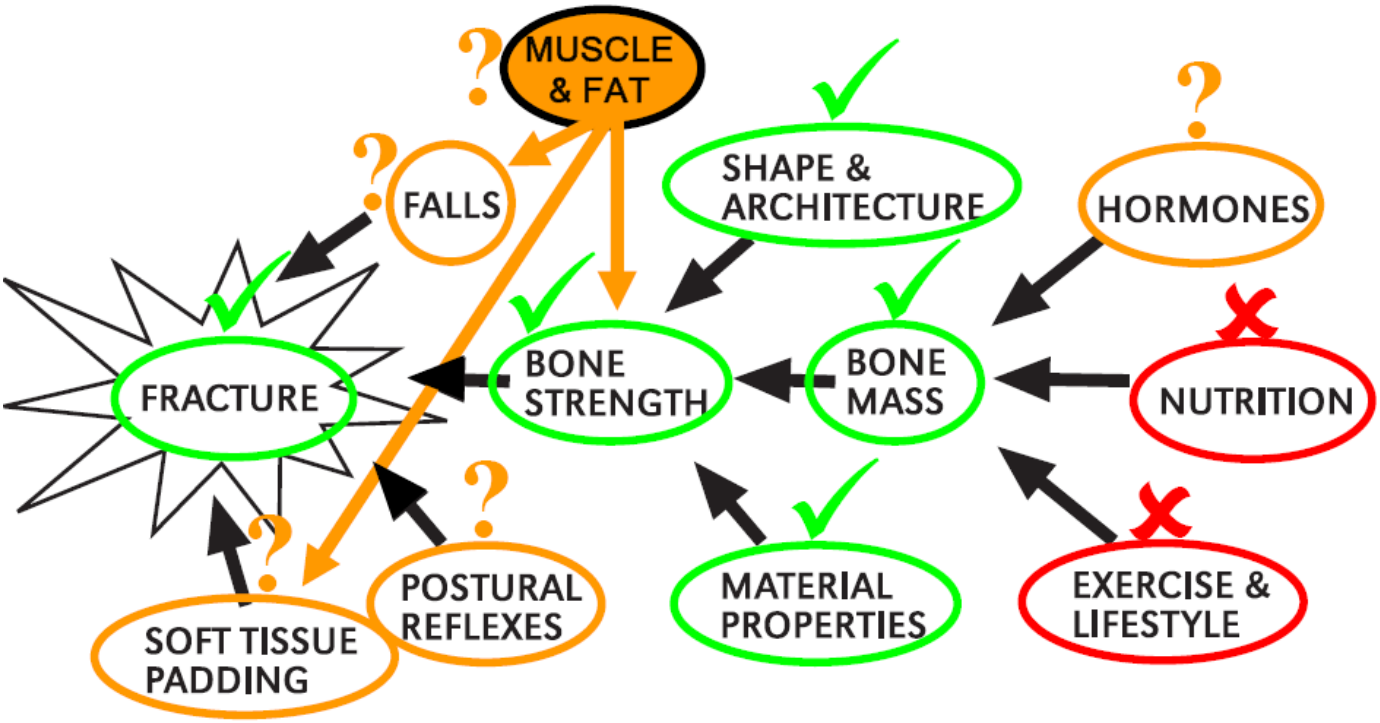
The third and final research theme consolidates the evidence from this thesis and related literature and argues that by inference, the superior bone mass observed in black children results from genetic predisposition rather than environmental factors. It is this genetic predisposition that holds the key to understanding ethnic differences in bone mass and bone size.

Genetic factors are documented as accounting for 50–90% of bone mass, and bone structure, the rate of bone loss, and the skeleton's response to environmental stimuli like nutrients and physical activity are inherited. [318] As was found in both black and white children,

maternal heritabilities were similar (~30%) between the two ethnic groups. This supports the findings of Visscher *et al.* (2008), [324] who noted that heritabilities were surprisingly constant across populations and species.

Figure 6-2 below from Heaney *et al.* (2003) [131] has been superimposed on what has been published on the Bt20 cohort together with the findings of this thesis. With regard to black children and their parents and factors influencing bone mass structure, a green tick (✓) indicates data which are favourable, a red cross (✗) indicates unfavourable data, and orange question mark (?) indicates data may not be available or if available, further research is needed.

Figure 6-2. Factors influencing bone mass structure and risk of fracture



Adapted from Heaney (2003) [131]

The favourable **bone mass** profile of the black Bt20 cohort, their parents and data from other South African studies are discussed at the beginning of this chapter, and will not be repeated here. A discussion of factors influencing the bone mass profile follows.

pQCT data has since been published on the Bt20 cohort when the children were aged 13 years.. Black children were shown to have greater trabecular and cortical densities at the radius (metaphysis, 4%) and tibia (diaphysis, 38%) respectively. [221] In addition, histomorphometric analyses of the iliac crest showed black South African adults to have thicker cortical bone, have less porous cortices, greater endocortical wall thickness, and greater osteoid thickness than whites. Adults in addition had fewer canals in the cortical bone and thicker trabeculae than whites, [244,286-288] all of which support favourable **material properties** of bone in black South Africans.

Favourable **shape & architecture** and **bone strength** are supported by pQCT data from the tibia at age 13 years, together with the radiogrammetry results from the 2nd metacarpal presented in chapter 3, which showed black children to have greater outer bone and inner marrow diameters. Despite thinner cortices, this architecture is consistent with greater polar strength-strain indices and more resistant to bending and torsional forces. Bending strength as determined by the strength-strain index, was calculated to be 10-20% greater in black children.

Favourable **fracture** rates were reported for black postmenopausal women at the femoral neck [297] and in these black children of Bt20 children at age 9 years. [310] The incidence of fracturing was half of what it was in black children (19%) compared to white (41.5%). The most

commons site fractured was the upper limb (57%), and the most common grade of trauma, moderate. [310] Thandrayen *et al.* (2009) did not include comparative analyses with bone mass but Clark *et al.* (2006) did and found that bone mass was predictive of fracture risk in UK children aged 9.9y from The Avon Longitudinal Study of Parents and Children (ALSPAC). [56] Fracture risk was reported to increase by 89% per SD decrease in size-adjusted BMC i.e. smaller skeletons relative to overall body size were at increased risk of fracturing. [56]

No **hormones** or bone turnover data was presented in this thesis; however, histomorphometric evidence suggests that black South Africans have higher rates of bone turnover than whites. Higher bone turnover rates in South African blacks have been suggested to minimise the volume of bone damaged by fatigue and stress fractures, resulting in better bone quality and consequently lower fracture risk in blacks than in whites. [288] However, no ethnic differences in biochemical markers of bone formation (serum alkaline phosphatase and osteocalcin) or bone resorption (urine hydroxyproline and pyridinoline), or in dietary calcium intake in either the pre- or postmenopausal groups have been found. [69] Serum 25-hydroxyvitamin D (25-(OH)D) was lower and 1,25-dihydroxyvitamin D (1,25-(OH)D) levels higher in blacks than whites and whites had higher ionized serum calcium, similar serum albumin, lower serum parathyroid hormone and higher urinary calcium excretion suggestive of nett skeletal calcium loss. [69] Subsequent to this thesis, no differences in menarcheal age between black and whites girls were found: 12.4 years (95% confidence interval (CI 12.2, 12.6) in blacks and 12.5 years (95% CI 11.7, 13.3) for whites. [152]

Given DXA-measured fat mass was no different between the black and white children at age 10 years (Chapters 3 and 5) and at age 13 years, [221] fat mass an unlikely **soft tissue padding** contributor in explaining ethnic differences in bone mass and the incidence of fracturing. Lean mass was significantly less in black boys when compared to white, as determined by DXA at age 10 years (Chapters 3 and 5) and DXA-measured whole body and pQCT-measured muscle cross-sectional area of the forearm and leg at age 13 years. Micklesfield *et al.* (2011) showed a significant positive relationship between muscle CSA and cortical area in black and white children. [221] In addition, South African black women have less LBM than their white counterparts, [82] in contrast to African Americans who have more LBM. [177,234,265,336] but LBM was the most significant contributor to BMD at the lumbar spine and hip sites in black premenopausal South African women and at the hip in white women. [53] These data on total lean mass however do not clarify the ethnic differences observed bone mass, and the incidence of fracture.

Fat and lean masses, are key contributing factors not just to 'soft tissue padding', 'fall prevention' but also to bone strength. Given that these were not included in Figure 6-2, they have been added. Given the attention body composition is receiving in the field of bone, these factors are important in future work relating to changes in bone in both ethnic groups.

With regard to **lifestyle** factors, black children of this cohort lived in households who scored significantly lower on the socioeconomic scale (median = 7, range: 0-13) than white children (median = 12, range: 6-13). Despite the poorer SES, Norris et al. (2008) [237] showed that SES had no influence on the bone mass of the hip and lumbar spine in the children in the Bt20

cohort; although there was a significant independent effect of SES on whole body bone size. Femoral neck BMC was shown to be influenced by the historical factors of birthweight, height at 1 year (1y) and weight at 1 year (1y), associations which were not entirely mediated by size. Infants of lower birthweight and a smaller size at 1 year grew to develop smaller bones (as reflected by BA) and/or bones of lower BMC at the femoral neck (lower BMC with similar BA).

With regard to **exercise**, McVeigh et al. (2004) [212] found that black children of this cohort at age 9 years were significantly less physically active than their white counterparts and that there was no association between the black children's level of physical activity and bone mass. However, a greater bone mass existed in black 10 year old children despite the fact that they were lighter or of similar weights. weight-bearing, greater weight-bearing was proposed to explain the greater femoral neck bone mass in black South African women. [69,70] Indeed, black women are of a greater body weight, walk long distances, carry loads on their heads, and babies on their backs; while men are employed largely as labourers. However, a greater bone mass existed in black than white 10 year old children despite the fact that the former were lighter or of similar weights. Nyati et al. (2006) [239] found black children to have longer forearms and black boys to have longer legs and humeri and shorter trunks than their white peers. These ethnic differences in anthropometry might indicate different centres of gravities and mechanical loads imposed on weight-bearing bones during physical activity.

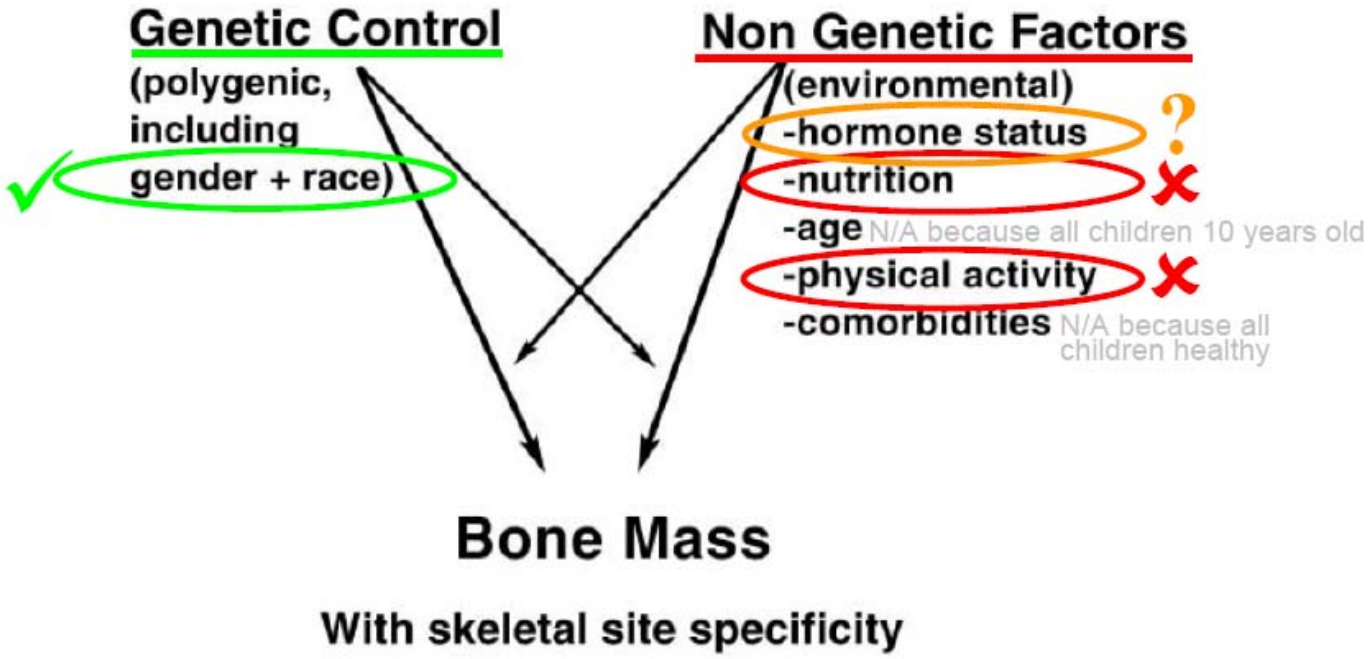
With regard to **nutrition**, the dietary calcium intake by black children of this cohort was almost half (~400 mg/day) of that in their white counterparts (~800 mg/day). This supports other

studies that have shown only limited evidence of an effect of dietary calcium intake on BMC. [199,214] Calcium absorption and renal calcium conservation has not been addressed in this cohort. 25(OH)D concentrations, were significantly lower in black children of this cohort than white children (93 ± 34 nmol/l vs. 120 ± 37 nmol/l). [258]

Therefore, the superior (✓) BMC, fracture rate, bone mass, shape and architecture, material properties and bone strength observed in South Africa's black children, adults and elderly cannot be explained by unfavourable (×) environmental factors (nutrition, exercise and lifestyle), or questionable or unknown (?) factors and are more likely to be explained by inherited traits that favourably determine bone mass. Based on Heaney's (2003) [131] adapted model, factors that warrant more research include body composition (muscle and fat) in particular, falls, soft-tissue padding and postural reflexes.

Figure 6-3 below illustrates the genetic and non-genetic control of bone mass. The superimposition of favourable (✓) versus unfavourable data (×) yields a striking picture which suggests that bone mass in our population must be under genetic control.

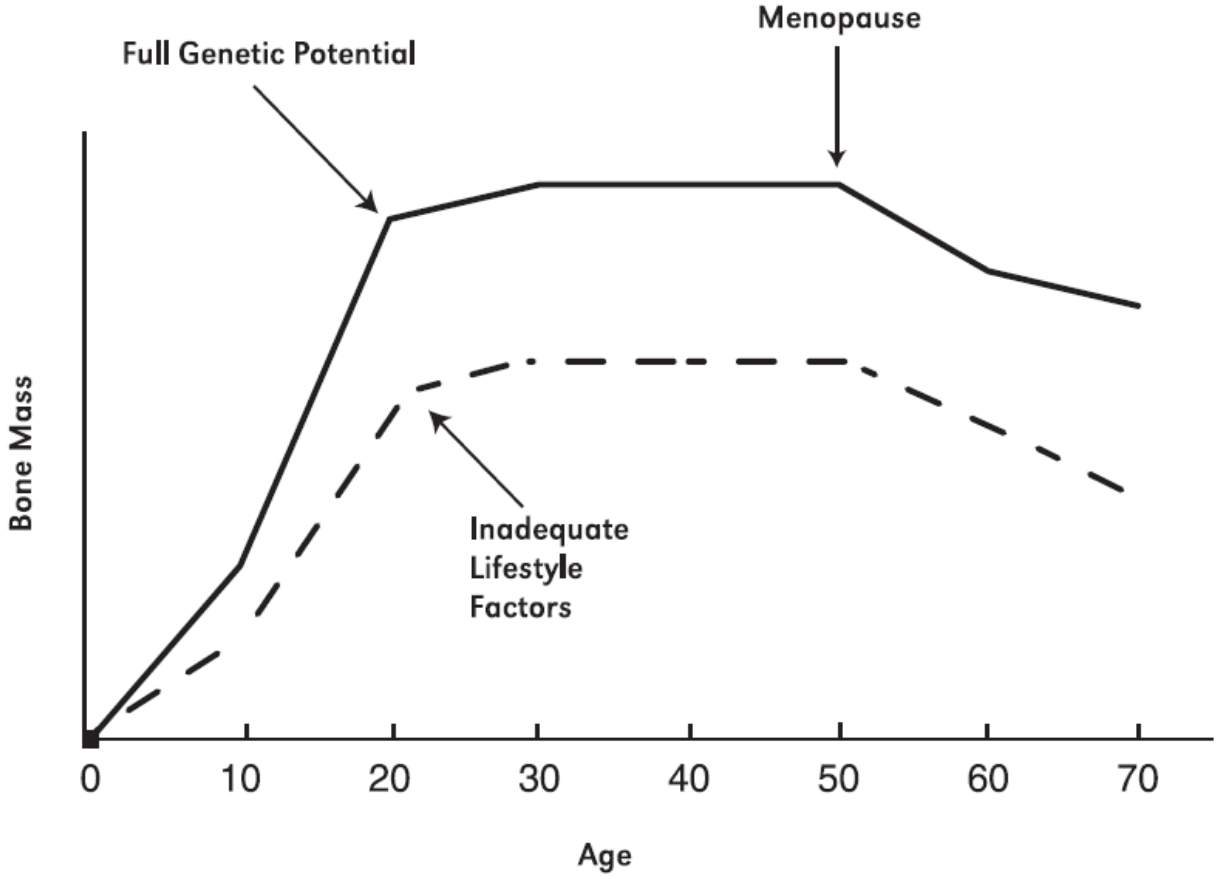
Figure 6-3. Interaction of genetic and non-genetic factors on bone mass



Source: Rizzoli *et al.* (2001) [274]

The last figure, Figure 6-4, supporting the hypothesis that bone mass is under predominantly genetic control is from Heaney *et al.* (2000). [132] Although this graph was not designed to compare different populations, we can hypothesise that *'Full Genetic Potential'* would be coupled with *'Optimal Bone'* bone acquisition (——) and '*Inadequate Lifestyle Factors*' would be coupled with '*Suboptimal*' bone acquisition (- - -). At the femoral neck especially, we see black children fitting the bone mass curve of *'Full Genetic Potential'*, despite having *'Inadequate lifestyle factors'* which are typically considered to result in '*Suboptimal'* bone acquisition.

Figure 6-4. Bone mass versus age with optimal and suboptimal bone acquisition



Source: Heaney *et al.* (2000) [132]

In support of the inference that a greater bone mass in South Africa's black children, adults and elderly is likely to be explained by genetic factors, a logic of arguments table is presented below. The table consists of 3 columns: resultant bone mass, environmental contribution and genetic contribution. Using the underlined row as an example, the argument across the 3 columns should be read as follows: "WHERE the resultant bone mass is shown to be less in blacks than whites (**B<W**), and assuming the environmental contribution to be less than the genetic contribution to bone mass, IF the environmental contribution is less favourable in blacks than whites (**B<W**) THEN the genetic contribution must be less than, equal to, or greater in blacks than in whites (**B<W, B=W or B>W**)". Every possible scenario is presented.

Table 6-3 Logic of arguments

WHERE*	**IF**	**THEN**
Resultant bone mass	**Environmental contribution**	**Genetic contribution**
	Assuming environmental contribution < genetic contribution	
B<W	**B<W**	**B<W, B=W or B>W**
	B=W	B<<W
	B>W	B<<W
B=W	B<W	B>>W
	B=W	B<W, B=W or B>W
	B>W	B<<W
B>W	B<W	B>>W
	B=W	B>W
	B>W	B<W, B=W or B>W
	Assuming environmental contribution = genetic contribution	
B<W	B=W	B<W
	B<W	B=W

WHERE* Resultant bone mass	IF Environmental contribution	THEN Genetic contribution
	B<<W	B>W
B=W	B>W	B<W
	B=W	B=W
	B<W	B>W
B>W	B>>W	B<W
	B>W	B=W
	B=W	B>W
	Assuming environmental contribution > genetic contribution	
B<W	B<W, B=W or B>W	B<W
	B<<W	B=W
	B<<W	B>W
B=W	B>>W	B<W
	B<W, B=W or B>W	B=W
	B<<W	B>W
B>W	B>>W	B<W
	B>W	B=W
	B<W, B=W or B>W	B>W

Figure 6-2, Figure 6-3 and Figure 6-4, together with the logic of arguments presented in Table 6-3 infer that greater bone mass in South Africa's black children, adults and elderly is likely to be explained by genetic factors.

This work is not the first to support a genetic basis for black-white differences, but the first with data from a population that demonstrates the relative non-contribution of environmental factors to a superior bone mass. Our arguments that the basis for black-white

differences in bone mass is predominantly genetic are also supported by evidence that different sets of genes control bone quantity and architecture at different skeletal sites [154] and by work done by Parfitt *et al.* (1997). [243] They argued the magnitude of ethnic differences in bone mass (10% to 40%) were not comparable to the differences of known non-genetic or environmental factors. And secondly, ethnic differences in bone mass were already evident in the foetus and continued to increase progressively during growth and PBM attained which appear to persist throughout life. [243] A recent study found that in a sample of multiethnic peripubertal children, BMC was associated with markers of genetic admixture and percent body fat but not with self-classified race/ethnicity categories, diet, or physical activity. [52]

Despite the genetic predisposition factor supporting a superior bone mass and geometry, at the femoral neck, this thesis highlights historical and predictive factors that may predispose the lumbar spine and other skeletal sites to the risk of developing osteoporosis. Osteoporosis, a "paediatric disease with geriatric consequences", can be addressed by way of optimising maternal health and the intrauterine environment, growth in early infancy, as well as by the optimisation of factors for the maximisation of PBM during childhood and adolescence. The following of a healthy diet, engagement in regular physical activity, and the avoidance of behaviours such as smoking and excessive alcohol consumption must be advocated and supported. The investment in such prevention programmes will have tremendous payoffs in the future for all chronic diseases of lifestyle, including osteoporosis.

Theoretical relevance

1. This work has contributed to the body of knowledge in the following ways:-
    1) This study has established that there are ethnic differences in bone size and bone mass (BMC) at the femoral neck, total hip and mid radius between black and white 10 year old South African children, as measured by DXA and corrected for gender, pubertal development, and current height and weight, which are covariants particularly relevant for use in children. [226,245,261] Black South African children had a greater BMC at the femoral neck (boys: 6%; girls: 5%), total hip (boys: 6%) and mid radius (boy and girls: 6%) than white children. Bone mass at the lumbar spine and whole body was similar in black and white children, but this depended on which method was used to correct for differences in body and bone size.
    2) It is the first study to illustrate how DXA-measured BMC varies in response to being corrected for different combinations of size-related corrections such as height, weight and BA or BA^{PC}. By calculating PC (power coefficients) for each ethnic-, gender-, and age-group, and correcting BMC for height, weight and BA^{PC}, ethnic differences in corrected BMC in both adults and children increased up to tenfold at the femoral neck and unmasked ethnic differences at the lumbar spine.
    3) It is also the first study in pre- and early pubertal black and white children to show a relationship between bone size and bone mass at 10 years of age and early life factors, which reflect the quality of growth in early life. By having made simultaneous adjustments for race/ethnicity, gender, age, socioeconomic status, bone age,[60,62,107] height and weight, historical factors as reflected by size in infancy, especially at 1 year, were

correlated with and predictive of BA and BMC of the whole body and BMC at the femoral neck at age 10 years, independent of the tracking of body size.

4) Bone size and bone mass in pre- and early pubertal black and white children and their parents have been assessed for the first time from a developing country and we have studied the heritability of bone mass and bone size by way of parent-child associations, establishing that maternal heritability estimates are ~30% for both environmentally disadvantaged black and advantaged white children. The magnitude of the estimate is similar to that found in Caucasians from other parts of the world.

Contextual relevance

The purpose of this section is to discuss the relevance of my findings to South Africans in relation to current bone health status in children and as future adults.

The three main findings of this these are:-

1) Black children have greater bone mass at the femoral neck, total hip and mid radius than white children which is neither explained by differences in current height or weight (for which statistical corrections were made) nor bone age and pubertal stage (which did not differ between ethnic groups). Estimates of bone strength as determined by cross-sectional geometry (cross-sectional area and section modulus) at the femoral neck were greater in black children. At the whole body, lumbar spine and distal one-third of the radius, there were no differences in BMC.

Thus socio-economically- and environmentally disadvantaged black children **currently** demonstrate a superior bone mass to that of their more advantaged white children. DXA findings and structural analyses of the femoral neck suggest black children have a superior shape and architecture, material properties and bone strength. Bone mass appears to be under genetic influences rather than environmental influences, findings which suggest reasons for the low prevalence of fragility fractures in adult black. It is unclear at this stage, whether improvement in the adverse environmental factors in our black children would greatly improve the bone mass findings at all sites. However it does raise an intriguing question around how the genetic influences maintain bone mass in the face of what are generally considered to be adverse environmental factors. For now, these genetic influences have a positive effect on bone mass during childhood, but the question is will they be maintained through adult life and continue to be associated with a very low incidence of femoral neck and vertebral fractures in the elderly?

2) BMC at 10 years of age is independently associated with weight and length at 1 year, which is not completely mediated by the tracking of skeletal growth. Low birthweight and small size at 1 year resulted in lower whole body BA and BMC and reduced BMC at the femoral neck.

Thus **historical** factors of the intrauterine environment and nutrition in early infancy, as indicated by birthweight and size in early infancy, were related to bone size and mass at age 10 years.

3) (3a) After correcting BMC for height, weight and BA^{PC}, black children and their parents had a greater corrected BMC at the femoral neck and lumbar spine than their white counterparts. (3b) Maternal heritability of BMC was estimated to be ~30%. In other words, ~30% of the phenotypic variation is due to genetic variation in both blacks and whites at all sites and is comparable between environmentally disadvantaged black and advantaged white children.

That is, parental bone size and bone mass were equally **predictive** factors of bone size and bone mass in their children.

Though black children of this cohort may currently demonstrate a superior bone mass status, all indications are that they have not been programmed for optimal bone health in utero and early life, nor are environmental factors favourable for the maximisation of peak bone health. In the years that lie ahead of them, lifestyle choice these children make, environmental factors to which they may be exposed, as well as an increase in life expectancy, may not continue to sustain the current genetic protection of their bone mass status that they currently demonstrate, and therefore they may be at risk of developing osteoporosis.

White children of this cohort, may have been programmed for better bone health in utero and early life than their black counterparts. However, they are probably less well protected by genetic factors and therefore must rely on optimizing environmental factors to ensure optimal bone health as do white children in developed regions such as Europe and North America, [273]

where authorities advocate the prevention of bone disease from birth and encourage individuals to choose to follow a bone-healthy diet, engage in regular physical activity, and avoid behaviours such as smoking and excessive alcohol consumption that can damage bone.

In South Africa, osteoporosis and associated fragility fractures are not considered the most concerning health problems. However, with increasing life expectancy in all developing countries including ours, these regions are where the greatest increase in the number of osteoporotic fractures worldwide are expected to occur. [304] Osteoporotic fractures are a major burden worldwide, because of the associated morbidity, mortality and financial costs. Currently, in South Africa, chronic diseases of lifestyle account for nearly 40% of adult mortality. [41] In the interests of ensuring bone health and reducing other chronic diseases of lifestyle in future generations of South Africans, this thesis supports the prevention of osteoporosis, a "paediatric disease with geriatric consequences", by the optimisation of maternal health and the intrauterine environment and growth in early infancy, as well as by the optimisation of factors for the maximisation of PBM during childhood and adolescence.

Limitations and future research

It is beyond the scope of the thesis to solve a number of limitations and address questions that emerged from this body of work. This section addresses these limitations so that they may be kept in mind when interpreting the results of this thesis, and so that they may be considered for future research in this field. Unanswered questions and data that could still be analysed are presented as potential future research avenues.

Limitations

The cross-sectional design of the studies presented in this thesis is but a snapshot of the bone health status and characteristics associated with the Bt20 cohort. Therefore, causality could not be inferred but should emerge from the longitudinal data set of which this cross-sectional study formed part.

The variation in presentation of BMC data across the chapters to explore ethnicity, environment and inheritance factors, is a potentially limiting factor of this thesis.

Also, the findings from our population may not apply to other populations (for example, do rural children in South Africa have similar bone mass to children in the Bt20 cohort?), and findings at one skeletal site may not apply to other skeletal sites (for example, why are findings at the femoral neck and lumbar spine different from those at the radius (mid-radius is ~95% cortical and distal radius ~33% cortical?).

With regard to the study of ethnic difference in bone mass, it would have been informative to include fracture risk in the analysing of all results. We have purposefully emphasized findings relating to black children and their mothers because of the small numbers of black fathers, white children and their parents. All results should be interpreted with this in mind.

With regards to the study of infant programming of bone mass, we have showed that birthweight, weight and length at 1 year continued to be predictive of BMC at the femoral neck after BMC had been corrected for race/ethnicity, gender, age, socioeconomic status, bone age, height (10y) and weight (10y) and BA (10y). Thus, in the case of black boys, in whom birthweight and length at 1y were significantly less than those of white boys, it might be expected that BMC at the femoral neck would be significantly less in black boys at 10 years of age, but this was not the case. Similarly, where there were no ethnic differences in size in infancy, there were ethnic differences in femoral neck BMC. Clearly, infant programming of bone mass differs between the ethnic groups and future research could explore this. There are other factors at play which I suspect may relate to the timing of genetic-environmental interactions, and which future research may consider addressing.

Regarding the study of heritability, heritability was estimated by way of parent-child associations and regressing children's Z-scores on mother's or father's Z-scores. The resulting regression coefficient gave the appropriate heritability estimate. [149,201,236] This method was chosen because of the statistical software that was available at the time, and because as a non-geneticist, it was statistical analyses, results and interpretation which I understood. There is a second way to estimate heritability by using ANOVAs and estimating variance components, statistical methods from which so much more about heritability in this population could still be learnt.

Future research

A number of questions emerged form this body of work which may be considered in future research.

With regard to black-white ethnic differences in bone mass

(1) What are the most appropriate size-related site-specific corrections for DXA-measured BMC in black and white South African children? The International Society for Clinical Densitometry (ISCD) have made the following general recommendations for measuring, and the clinical interpreting and reporting DXA findings in children and adolescents:-

- Use DXA-measured BMC and areal BMD. [119]
- Measure the posterior-anterior (PA) spine and total body less head (TBLH) as these skeletal sites yield the most accurate and reproducible data. [119]
- Adjust BMC and areal BMD for absolute height or height age, or compare them to appropriate paediatric reference data sets. [119]

There's a paucity of paediatric reference data in general. The ISCD calls for further research to expand upon and confirm their recommendations, which data from black South African children in particular, has the potential to do.

(2) How do genetic influences maintain bone mass (at the femoral neck, total hip and mid radius) in the face of what are generally considered to be adverse environmental factors?

(3) Will the improvement in the adverse environmental factors in our black children greatly change the bone mass findings at other sites (whole body and lumbar spine) and the incidence of fracturing?

(4) How do gene-environment interactions affect bone size and bone mass?

(5) Which genes are responsible for greater bone mass in South Africa's black population?

Although the technology of choice for future research should ideally be QCT, the availability, advantages of using DXA technology, as well as the broader range of applications e.g. body composition, DXA data most certainly have a place now and for years to come in clinical practice and research. Therefore, the models for improved analyses and interpretation of data should continue to be pursued.

With regard to infant programming of bone mass

A bank of data on antenatal, postnatal and early life exists for this cohort. The relationship between early life and bone mass at age 10 years could be researched further, with the intention of identifying factors in early life at which prevention strategies could be targeted.

Possible factors are listed below but there may be others:-.

- Antenatal:
    - Gravida
    - Gestational age at birth

  - Nature of mother's work when pregnant
  - Abnormalities or complications of pregnancy
  - Details of delivery
- Early life:
  - Growth and development (height, weight / length, body fat)
  - Feeding (breast-, bottle-, weaning and early nutrition)
  - Child's medical history and concomitant medication
  - Immunisations
- General:
  - Maternal and supporting family's:-
    - Education
    - Socioeconomic circumstances
    - Medical history and concomitant medication
    - Use of tobacco and alcohol

With regard to the heritability of bone mass

Heritability, a population parameter, depends on population-specific factors, such as allele frequencies, the effects of gene variants, and variation due to environmental factors. Given this, and the arguments presented in research theme 3 "the genetic predisposition factor", future research ought to focus on the genetics of bone mass, genetic-environmental interactions and timing thereof in black South Africans. Very little is known about the genetic inheritance of bone mass in black populations. There has been interest in a number of key gene groups associated

with BMD variation in European populations, namely genes associated with the receptor activator for nuclear factor κ B ligand (RANKL) / osteoprotegerin (OPG) signalling pathway, and the oestrogen receptor which have previously shown to be associated with BMD and should be explored in black South Africans.

Conclusion

This PhD thesis highlighted that ethnicity is the single most important proximal factor affecting bone mass and bone size in 10 year old South African children. Despite being socio-economically- and environmentally disadvantaged, black children fortunately demonstrate a superior bone mass- and strength at the femoral neck, to that of their white peers. This genetic advantage is seen in infancy, adults, and is associated with a very low incidence of femoral neck fractures in the black elderly. However, this thesis highlights that historical and predictive factors of bone mass indicate black children have not been programmed for optimal bone health in utero and early life, nor are contemporary environmental factors favourable for the maximisation of PBM. In the years that lie ahead, lifestyle choices this cohort makes, environmental factors to which they may be exposed, as well as an increase in life expectancy, may not continue to sustain the current genetic protection of their bone mass that they currently demonstrate at the femoral neck. This cohort may be at risk of developing osteoporosis as an elderly population, particularly at the lumbar spine and forearm. This is critically important to us as a nation and its policy makers, who in the midst of a profound health transition, are grappling with the management of a health system burdened by communicable, non-communicable, perinatal and maternal, and injury-related disorders. Those particularly affected are prominently poor people living in urban settings. Osteoporosis, a “paediatric disease with geriatric consequences”, must be addressed now. National health policies must focus on the optimisation of maternal health and the intrauterine environment, growth in early infancy, as well as by the optimisation of factors for the maximisation of PBM during childhood and adolescence. The following of a healthy diet, engagement in regular physical activity, and the avoidance of behaviours such as smoking and

excessive alcohol consumption must be advocated and supported. The investment in such prevention programmes will have tremendous payoffs in the future for all chronic diseases of lifestyle, including osteoporosis.

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APPENDIX 1 – Ethics Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Vidulich

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M050463**
PROJECT	Bone Mass in Black and White 10 Year Old South African
INVESTIGATORS	Ms L Vidulich
DEPARTMENT	Birth to Twenty/Paediatrics
DATE CONSIDERED	05.04.29
DECISION OF THE COMMITTEE*	Approved unconditionally

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

DATE 05.05.03 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Mr SA Norris

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX 2 – Pdf of "Vidulich,L., Norris,S., Cameron,N. and Pettifor,J. Differences in bone size and bone mass between black and white 10-year-old South African children. *Osteoporos Int* 17, 433-440 (2006)"

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ORIGINAL ARTICLE

Differences in bone size and bone mass between black and white 10-year-old South African children

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Abstract *Introduction:* Black and white South Africans hail from vastly disparate cultural and socio-economic backgrounds the result of which exposes black children to numerous factors known to impact negatively on bone mass. Thus, we studied ethnic differences in bone size and bone mass between 476 10-year-old black and white South African girls and boys (black boys n=182, white boys n=72, black girls n=158, white girls n=64) who formed part of a longitudinal cohort of children born in Johannesburg, South Africa, during 1990. *Methods:* Bone area (BA) and bone mineral content (BMC) were measured at the whole body, total hip, femoral neck, lumbar spine (L1–L4) and mid- and distal radii by dual energy X-ray absorptiometry (DXA). Vertebral heights and metacarpal indices were measured. Anthropometry, skeletal maturity and pubertal development were also assessed. *Results:* After correction for height, weight, gender and puberty, black children had greater BMC at the femoral neck (P<0.0001), total hip (P<0.05) and mid-radius (P<0.001) than white children.. At the whole body, lumbar spine, and distal one-third of the radius, there were no differences in BMC between black and white children after correction for differences in body size. After correction for height and puberty, vertebral heights were less in black children than white children, and cortical areas at the second metacarpal were greater in black children. *Conclusion:* These findings suggest that, at the femoral neck, total hip and mid-radius, these differences are not a result of differences in anthropometry, bone age or pubertal stage, or environmental factors but are most likely to result from genetic differences.

Keywords BA · BMC · Children · Dual-energy X-ray absorptiometry · Ethnic differences

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Introduction

The incidence of osteoporosis and fracturing, a late manifestation of the disease, is significantly lower in African–American populations than in Caucasian US populations [1–3] and has resulted in considerable research into ethnic differences in bone mass. The lower incidence of fracturing has, in part, been explained by a greater bone mass in African–Americans [4–7]. Although fracture rates are also low in Africans living in Africa, few studies have investigated bone mass in communities in Africa [8–11].

A greater bone mass in African–Americans than in Caucasian Americans has been explained by advantageous differences in key bone-influencing factors [4, 12]. Black South Africans, children in particular, are exposed to a multitude of environmental factors known to impact negatively on bone mass, such as poor nutrition, [13] low calcium intake, [14] little physical activity, [15, 16], patterns of compromised growth, and delayed onset of puberty, [17, 18]; thus bone mass could be expected to be reduced when compared with that of South African whites and African–Americans.

Studies of bone mass in adult South African ethnic groups have found that pre-, peri- and postmenopausal black women have a greater bone mass at the hip than white women (as had been found in African–Americans),

but their bone mass at the radius and lumbar spine is similar to that of whites (unlike African–Americans) [8, 9]. Radial bone mass is greater in black children than in white children [19], but little is known of the factors influencing bone mass in children of different ethnic groups in developing countries. This study describes the ethnic differences in bone mass in pre- and early pubertal children in South Africa.

Materials and methods

Subjects

We collected data on 476 healthy children (182 black boys, 72 white boys, 158 black girls, 64 white girls) of median age 10.6 years (range: 10.0–10.9 years) who formed part of the Birth-to-Twenty (BTT) longitudinal cohort of children born in the Greater Johannesburg metropolitan area within a 6-week period (23 April 1990−8 June 1990) [20–22]. Comprehensive sets of longitudinal data were available on 1,200 black children from which 340 were randomly enrolled into the Bone Health Study. Cross checks were performed to ensure that there were no significant differences between the Birth-to-Twenty and Bone Health cohort for key demographic variables (residential area at birth, maternal age at birth, gravidity, gestational age and birth weight). All white children with longitudinal data were enrolled into this bone health study (n=65). To increase the number of white children on the study, children of the same age from schools in the Greater Johannesburg metropolitan area were asked to volunteer. An additional 71 white children (boys n=38; girls n=33) were recruited into the study. Subjects with chronic illness (juvenile rheumatoid arthritis, epilepsy or asthma) on medication known to affect growth or bone mass development were excluded from the study (n=4). This study protocol was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand, Johannesburg, and the Ethical Advisory Committee of Loughborough University, UK. Both children and guardians gave written informed consent to be studied.

Anthropometry

Height was measured to the last completed 1 mm using a wall-mounted stadiometer (Holtain, UK) and weight to the nearest completed 0.1 kg using a digital electronic instrument (Dismed, USA) [23]. Both instruments were regularly calibrated, and subjects wore minimal clothing when being weighed. Forearm length, needed for dual energy X-ray absorptiometry (DXA) analyses, was measured as elbow–wrist length taken between the most posterior point of the olecranon and the most distal palpable point of the styloid process of the radius [23].

Maturity

Sexual maturity was self-assessed as pubic hair development in boys and girls, using the Tanner scaling technique [24, 25]. Children were divided into two stages of development, namely, pre-/early pubertal (Tanner stages 1–2) and mid-pubertal (Tanner stages 3–4). In addition, skeletal maturity was assessed by the scoring of bone age from hand radiographs using the Tanner–Whitehouse bone-specific scoring technique (TWII20) [26].

Dual-energy X-ray absorptiometry

Bone area (BA) and bone mineral content (BMC) of the whole body, left total hip, femoral neck, lumbar spine (anteroposterior, L1–L4) and left mid-radius (halfway between the styloid process and the tip of the olecranon of the elbow) and distal third of the radius (one-third of the distal distance between the styloid process and the tip of the olecranon of the elbow) were measured by DXA in array mode, using an Hologic QDR–4500 (Hologic, Waltham, Mass., USA). The data were analyzed with the software supplied by the manufacturer, version 11.2. A lumbar spine phantom was scanned daily to determine the machine's measurement precision, expressed as the coefficient of variation (CV), which, for BA and BMC, were 0.47% and 0.78%, respectively. All measurements were performed and analyzed by the same person.

Lumbar vertebral heights

Anterior, middle and posterior heights of lumbar vertebrae L1–L4 were measured at sites determined by the DXA operator. Vertebral heights were calculated as the mean of the heights of the anterior, middle and posterior portions of lumbar vertebrae L1–L4, which were measured (in millimeters) from a lateral DXA scan, using provided software [2].

Radiogrammetry

In addition to DXA measurements, radiogrammetry was used to measure cortical thickness of the second metacarpal from anteroposterior radiographs of the left hand. With digital callipers calibrated to the nearest 0.01 mm, measurements were made to the nearest 0.1 mm of the length of the metacarpal (L), outer cortical diameter (D) and inner cortical diameter (d) at the midpoint of the shaft. Combined cortical thickness (C=D−d), cortical cross-sectional area $\left(\pi/4[D^2 - d^2]\right)$, percent cortical cross-sectional area to total area ($[(D^2-d^2)/D^2]\times100$) and the Barnett–Nordin index $[(C/D)\times100]$ were calculated. The Barnett–Nordin

Table 1 Descriptive characteristics [mean ± SD (*n*)] of black and white children aged 10 years

Characteristic	Boys		*P*	Girls		*P*
	White (*n*=72)	Black (*n*=182)		White (*n*=64)	Black (*n*=158)	
Age (years)	10.65±0.24	10.55±0.27	<0.01	10.62±0.25	10.53±0.27	<0.05
Bone age (years)	10.31±1.04 (71)	10.13±1.05 (179)	NS	10.41±1.23 (62)	10.38±1.27 (156)	NS
Pre-/early puberty (Tanner hair 1 and 2)	99% (65)	99% (170)	NS	99% (65)	98% (154)	NS
Mid-puberty (Tanner hair 3 and 4)	1% (1)	1% (2)	NS	1% (1)	2% (3)	NS
Height (cm)	143.5±7.5	137.4±6.2	<0.0001	142.6±7.8	139.2±6.3	<0.001
Weight (kg)	36.0±6.4	32.6±6.6	<0.001	35.6±7.8	34.8±8.3	NS
Lean mass (kg)[a]	26.9±3.6	24.1±3.2	<0.0001	25.0±4.2	23.9±3.9	NS
Fat mass (kg)[a]	8.2±3.4	7.4±4.0	NS	9.8±4.3	10.1±5.1	<0.05
Body mass index (kg/m^2)	17.4±2.1	17.2±2.6	NS	17.3±2.6	17.8±3.4	NS

[a]After correction for ethnic differences in height
NS not significant

index is a parameter of relative bone mass that compensates for differences in skeletal size and variations in tube-to-film and hand-to-film distance [27]. Measurement precision, expressed as the coefficient of variation (CV) was determined between two observers (L.V. and S.N.), which, for metacarpal length, outer and inner diameters was 0.34%, 1.65% and 1.81%, respectively.

Socioeconomic questionnaire

Primary care givers answered questions about their social and economic status. This questionnaire had been modified appropriately for a South African population and previously validated [28]. The socioeconomic score was formulated from the presence or absence of 13 asset indicators, namely, house type, electricity, indoor flushing toilet, indoor running water, refuse removal, television, digital satellite television, motor vehicle, refrigerator, microwave oven, washing machine, video-machine and telephone).

Statistics

STATISTICA (data analysis software system) version 6 (StatSoft, 2001) was used to perform univariate and multivariate analyses to determine ethnic differences. Parametric data were analyzed by univariate analyses [age, bone age, height, weight, body mass index (BMI), BMC and BA]. Lean and fat mass, corrected for height, were analyzed by ANCOVA. Stepwise multiple regressions analyses were used to determine predictors (gender,

Table 2 Bone area and bone mineral content comparisons between ethnic groups within each gender. Values are unadjusted means (± SD)

Parameter	Boys		*P*	Girls		*P*
	White	Black		White	Black	
	n=72	*n*=182		*n*=64	*n*=158	
Whole body BA (cm^2)	1312.22±163.87	1217.08±140.59	<0.0001	1286.75±187.92	1248.58±171.87	NS
Whole body BMC (g)	1084.94±164.78	995.13±140.83	<0.0001	1036.84±196.41	992.95±179.03	NS
	n=71	*n*=180		*n*=64	*n*=158	
Femoral neck BA (cm^2)	4.32±0.33	4.13±0.31	<0.0001	4.21±0.30	4.05±0.31	<0.0001
Femoral neck BMC (g)	3.03±0.42	3.06±0.38	NS	2.70±0.46	2.77±0.41	NS
	n=71	*n*=180		*n*=64	*n*=158	
Total hip BA (cm^2)	22.36±2.68	20.42±2.43	<0.0001	23.18±3.42	20.69±2.50	<0.0001
Total hip BMC (g)	16.23±2.73	15.52±2.58	NS	15.39±3.67	14.57±3.00	NS
	n=72	*n*=182		*n*=64	*n*=158	
L1–L4 BA (cm^2)	46.00±4.96	43.02±4.26	<0.0001	43.99±4.26	42.99±4.34	NS
L1–L4 BMC (g)	26.72±4.66	19.09±3.69	<0.0001	25.54±5.10	25.33±5.24	NS
	n=69	*n*=180		*n*=64	*n*=158	
Mid-radius BA (cm^2)	4.52±0.81	4.48±0.80	NS	4.24±0.80	4.37±0.84	NS
Mid-radius BMC (g)	1.88±0.38	1.75±0.34	<0.05	1.70±0.37	1.68±0.41	NS
	n=69	*n*=180		*n*=64	*n*=158	
Distal 1/3rd radius BA (cm^2)	2.32±0.19	2.32±0.22	NS	2.19±0.20	2.18±0.20	NS
Distal 1/3rd radius BMC (g)	1.14±0.12	1.09±0.13	<0.01	1.06±0.15	1.04±0.15	NS

NS not significant

Table 3 Ethnic differences in bone area and bone mineral content at the whole body, femoral neck, total hip, lumbar spine (L1–L4) and mid- and distal 1/3rd of the radius after correction for gender, puberty, height and weight

Measure of bone mass	Ethnicity β[a]	± SE	*P*	R^2	Predictors *P*<0.001	Puberty
Whole body BA (cm^2)	0.04	0.02	NS	0.86	Height, weight	NS
Whole body BMC (g)	0.02	0.03	NS	0.70	Height, weight, gender	NS
Femoral neck BA (cm^2)	–0.07	0.04	NS	0.44	Height, weight, gender	NS
Femoral neck BMC (g)	0.20	0.03	<0.0001	0.50	Height, weight, gender	NS
Total hip BA (cm^2)	–0.13	0.03	<0.0001	0.59	Height	NS
Total hip BMC (g)	0.07	0.04	<0.05	0.50	Height, weight, gender	NS
L1–L4 BA (cm^2)	0.04	0.03	NS	0.57	Height, gender	NS
L1–L4 BMC (g)	0.02	0.04	NS	0.47	Height, weight	NS
Mid radius BA (cm^2)	0.26	0.03	<0.0001	0.63	Height, weight, gender	NS
Mid radius BMC (g)	0.13	0.03	<0.0001	0.61	Height, weight, gender	NS
Distal 1/3rd radius BA (cm^2)	0.11	0.04	<0.05	0.27	Height, gender	NS
Distal 1/3rd radius BMC (g)	0.12	0.04	NS	0.36	Height, weight, gender	NS

[a]A positive β means BA or BMC is greater in black children than in white children
NS not significant

pubertal development, current height and weight) of the dependent variables (BMC or BA). A positive β meant that BMC or BA in black children was greater than it was in white children. Non-parametric data were analyzed with Fisher's exact test (pubertal development) and Mann–Whitney U test (socioeconomic status). Probability values <0.05 were considered significant for all tests. Numerous statistical comparisons were made; thus, more cognisance was placed on differences with $P \leq 0.01$.

Results

Cohort characteristics

Characteristics of the cohort that took part in this study are shown in Table 1. Black children lived in households that scored significantly lower on the socioeconomic scale (median 7, range 0–13) than white children (median 12, range 6–13) ($P<0.05$, Mann–Whitney U test). Most of our cohort was prepubertal or in early puberty (black boys 99%, white boys 99%, black girls 98%, white girls 97%) as determined by pubic hair development, and there were no ethnic differences in the distribution of sexual maturity (Fisher's exact test). Skeletal maturity, as determined by bone age, was similar between the ethnic groups within each gender, even though black boys were significantly younger than white boys at the time of their visit ($P<0.01$). Black children were significantly shorter than their white peers (boys, $P<0.0001$; girls, $P<0.01$), and black boys weighed significantly less than white boys ($P<0.001$) and had less lean mass ($P<0.0001$). After correction for differences in height, both ethnic groups had similar lean masses; however, black girls had higher fat mass ($P<0.05$) than white girls.

DXA results

Table 2 summarizes ethnic differences in BA and BMC of the whole body, femoral neck, total hip, lumbar spine and mid and distal third of the radius, as determined by DXA. The data and statistics presented in Table 2 are not corrected for current size. Table 3 shows the results from multiple regression analyses, where BA and BMC were corrected for gender, puberty, height and weight.

Whole body

Black boys had significantly less whole body BA and BMC than white boys ($P<0.0001$), but after correction for

Table 4 Vertebral heights (unadjusted means ± SD) of lumbar spine vertebrae (L1–L4) comparisons between ethnic groups within each gender

Location	Boys		*P*	Girls		*P*
	White (*n*=70)	Black (*n*=179)		White (*n*=64)	Black (*n*=155)	
L1 (mm)	18.12±1.50	16.97±1.20	<0.0001	18.54±1.33	17.95±1.48	<0.01
L2 (mm)	18.83±1.35	17.38±1.27	<0.0001	19.34±1.57	18.53±1.55	<0.001
L3 (mm)	18.98±1.32	17.46±1.19	<0.0001	19.49±1.73	18.43±1.60	<0.0001
L4 (mm)	19.14±1.53	17.65±1.27	<0.0001	19.80±1.49	18.69±1.74	<0.0001

Vertebral heights were calculated as the mean of the heights of the anterior, middle and posterior portions of the first four lumbar vertebrae (in millimeters) as in the study by Gilsanz et al. [2]

Table 5 Ethnic differences in lumbar spine vertebral heights (L1–L4) after correction for gender, height and puberty

[a]A positive β means vertebral heights are greater in black children than in white children
NS not significant

Location	Ethnicity β[a]	± SE	*P*	R^2	Predictors *P* <0.001	Puberty
L1 (mm)	0.09	0.04	<0.01	0.51	Height, gender	<0.05
L2 (mm)	0.14	0.03	<0.0001	0.55	Height, gender	<0.01
L3 (mm)	0.20	0.03	<0.0001	0.54	Height, gender	NS
L4 (mm)	0.19	0.04	<0.0001	0.44	Height, gender	NS

gender, puberty, height and weight, there were no significant differences in BA or BMC (Table 3).

Femoral neck

Black children had a smaller BA at the femoral neck (both genders $P<0.0001$) but similar BMC. However, after correction for gender, puberty, height and weight, there was no difference in BA, and BMC was greater in black children than in white children (β=0.20, $P<0.0001$) (Table 3). BMC was 6% and 5% greater in black boys and girls, respectively, than in their white peers when adjusted means were compared.

Total hip

Before correction, black children had a smaller BA at the total hip (both genders $P<0.0001$) (Table 2). After corrections, despite BA remaining smaller in black children (β=−0.13, $P<0.0001$), BMC was greater in black children than in white children (β=0.07, $P<0.05$) (Table 3). BMC was 6% greater in black boys than in white boys, when adjusted means were compared, and was no different in girls.

Lumbar spine

Black boys had less BA and BMC at their lumbar vertebrae than white boys (both $P<0.0001$) (Table 2), which was explained by differences in height and weight (Table 3). That is, after correction, there were no ethnic differences at the lumbar spine in BA or BMC.

Radius

At the mid-radius, before corrections, black children had similar BA but less BMC than white children (boys $P<0.05$). After corrections, BA and BMC were significantly greater in black children than in white children (BA β=0.26, $P<0.0001$; BMC β=0.13, $P<0.0001$) (Table 3). That is, black boys and girls had 6% more BMC at the mid-radius than white boys and girls, respectively.

At the distal one-third of the radius, before correction, black boys had less BMC than white boys ($P<0.01$) (Table 2). After correction, black children had a greater BA ($P<0.05$), but there were no ethnic differences in BMC.

General

Correction of BA and BMC for ethnicity, gender, puberty, height and weight accounted for between 27% and 86% of variance in BA and between 36% and 70% of variance in BMC measurements in black and white South African children (Table 3). Puberty was never a significant predictor of BA or BMC.

Lumbar vertebral heights

Lumbar vertebral heights were less in both black boys (L1–L4 $P<0.0001$) and girls (L1–L4 $P<0.01$ to $P<0.0001$) than

Table 6 Radiogrammetric comparisons between ethnic groups within each gender. Values are unadjusted means (± SD)

Parameter	Boys		*P*	Girls		*P*
	White (*n*=71)	Black (*n*=178)		White (*n*=61)	Black (*n*=153)	
Length (mm)	54.75±3.49	54.41±3.40	NS	55.32±4.07	56.08±3.93	NS
Outer diameter (mm)	6.98±0.64	7.10±0.67	NS	6.74±0.59	6.91±0.63	NS
Inner diameter (mm)	4.00±0.71	4.36±0.72	<0.001	3.69±0.61	3.91±0.66	<0.05
Combined cortical thickness (mm)	2.98±0.42	2.75±0.41	<0.0001	3.05±0.49	3.00±0.45	NS
Cortical cross-sectional area (mm^2)	25.61±4.26	24.66±4.40	NS	25.00±4.73	25.45±4.66	NS
Percent cortical area to total area	67.03±7.59	62.29±7.49	<0.0001	69.73±7.56	67.79±7.24	NS
Barnett–Nordin index (%)	42.98±6.80	38.91±6.27	<0.0001	45.41±6.90	43.62±6.49	NS

NS not significant

Table 7 Ethnic differences in metacarpal indices after correction for gender, height and puberty

Parameter	Ethnicity β[a]	± SE	*P*	R^2	Predictors *P* <0.001	Puberty
Length (mm)	0.26	0.03	<0.0001	0.62	Gender, height	NS
Outer diameter (mm)	0.25	0.04	<0.0001	0.24	Gender, height	NS
Inner diameter (mm)	0.27	0.05	<0.01	0.18	Gender, height	NS
Combined cortical thickness (mm)	–0.07	0.05	NS	0.13	Gender, height	NS
Cortical cross–sectional area (mm^2)	0.11	0.04	<0.05	0.20	Height	NS
Percent cortical cross-section area to total cross-sectional area	–0.21	0.05	<0.01	0.13	Gender	NS
Barnett–Nordin index (%)[a]	–0.20	0.05	<0.0001	0.13	Height	NS

[a]A positive β means the respective metacarpal indices are greater in black children than in white children
NS not significant

in their white peers before and after correction for ethnic differences in height (Tables 4 and 5).

Radiogrammetry results

Before correction, the inner diameter of the 2nd metacarpal was greater in black children than in white (boys $P<0.001$; girls $P<0.05$). (Table 6). Black boys also had a greater combined cortical thickness ($P<0.0001$) than white boys but a smaller Barnett–Nordin index ($P<0.001$) and percent cortical area to total area ratio ($P<0.0001$). After correction, black children had greater metacarpal length (β=0.26, $P<0.0001$), outer (β=0.25, $P<0.0001$) and inner diameters (β=0.27, $P<0.01$), as well as the cortical cross sectional area (β=0.11, $P<0.05$). However, this translated to a greater Barnett–Nordin index (β=−0.20, $P<0.0001$) and percent cortical area to total area (β=−0.21, $P<0.0001$) in white children. (Table 7).

Discussion

Ethnic differences in bone mass (BMC) between black and white 10-year-old South African children, as measured by DXA and corrected for gender, pubertal development, current height and weight, were most apparent at the femoral neck, total hip and mid-radius. That is, black children had a greater BMC at the femoral neck (boys 6%; girls 5%), total hip (boys 6%) and mid-radius (boy and girls 6%) than white children, despite black children being more exposed to environmental factors known to impact negatively on bone mass, such as living in poorer households and having poorer nutrition, compromised growth and development, as reflected by their lower birth weights, shorter stature, lighter body weights and later onset of pubertal development, lower calcium intake (estimated to be approximately 400 mg/day) [14] and less physical activity [16]. Black children had similar whole body and lumbar spine bone masses to white children. These data suggest that ethnic differences are site-specific in our cohort of 10-year-old black and white South Africans, which are not the result of differences in current height or weight (for which statistical corrections were made), bone age and pubertal stage (which did not differ between ethnic groups), but are more likely the result of differences in genetic factors.

The finding that bone mass at the femoral neck, total hip and mid-radius was greater in 10-year-old South African black than white children is consistent with national and international studies, which have explored black–white ethnic differences in both adults and children. Before correction for differences in height and weight, pre- and early pubertal African–American children had greater femoral neck bone mass [BMC and/or bone mineral density (BMD)] than white children [29, 30]. Wang et al., after correcting for differences in both height and weight, found bone mineral apparent density (BMAD) to be greater in African–American pre-/early pubertal girls than in white girls [31]. Our results in children are also consistent with studies conducted in South African women (20–64 years), where BMC of the femoral neck was greater in blacks than in whites, before and after correction for body and bone size [8, 9]. Greater weight-bearing was proposed to explain the greater femoral neck bone mass in black South African women. However, given that black 10-year-old children, of similar weights to white children, also have a greater femoral neck bone mass, other reasons, such as genetics, are likely to account for a greater bone mass at the femoral neck and total hip in South Africa's black population.

Forearm BMC has also been found to be greater in black children than in white American children before and after correction for weight and age, in 7–12 year olds [30] and, before correction, in 1–6 year old children [32, 33]. In a previous study using single photon absorptiometry, South African blacks aged 6–20 years were found to have more BMC at the midshaft radius than white children, after correction for differences in height [34].

At the lumbar spine and whole body, ethnic differences in bone mass were absent. The results are similar to those found in South African pre-, peri- and postmenopausal women [8, 9]. Although the majority of studies from the USA have demonstrated greater bone masses in African–Americans [30, 32, 35–38], there are, indeed, US studies comparable to ours, where no differences in bone mass have been found; uncorrected lumbar spine BMC and BMD have been reported to be similar in African–American and Caucasian children [29–31, 39, 40], as have results after correction for ethnic differences in size or maturity [4, 41]. Adult Somalis, living in the USA, have also been reported to have a similar lumbar spine BMD to Caucasian Americans [42]. At the whole body, a site for which there is less literature in children to make com-

parisons, two studies did not find ethnic differences between their African–American and Caucasian children [29, 31].

In addition to bone density, ethnic differences in bone architecture and geometry have more recently received attention as a measure of bone strength. Histomorphometric analysis of iliac crest biopsies have shown that South African black adults have thicker trabeculae than whites [1, 5, 43] At the proximal femur, both US and South African black populations have been shown to have a narrower marrow cavity, thicker cortex and a lower buckling ratio, despite non-significant differences in outer bone diameter, characteristics that are consistent with greater bone strength and lower fracture rates in blacks at this region [44] Geometrically, wider bones are stronger bones, which African–American populations have been found to have [1, 2]. We that found black children had shorter lumbar vertebral heights for the same BA before and after correction for differences in height, suggesting that the vertebrae are wider. Further, DXA-measured BA at the mid-shaft of the radius was consistently greater in black children than in white children after correction for differences in height.

A number of candidate gene polymorphisms have been linked to bone mass, such as of the vitamin D receptor gene (VDR), calcium-sensing receptor gene (CASR), alpha2HS-glycoprotein gene (ASHG), estrogen receptor alpha gene (ESR1), calcitonin gene, parathyroid hormone gene (PTH), collagen I alpha 1 gene, transforming growth factor beta (TGF-beta) gene, interleukin-1 (IL–1) gene, interleukin-6 (IL–6) and LDL receptor-related protein 5 (LRP5) apolipoprotein E gene [45–47]. All these genes have the potential to explain ethnic differences in bone mass, but none has unequivocally been proven to do so.

In conclusion, black children in South Africa have greater bone mass at the femoral neck, total hip and mid-radius than their white peers, and similar bone mass at the lumbar spine and whole body. This bone mass pattern, at the femoral neck in particular, is similar to that reported in US children, yet our black South African children, unlike their African–American peers, are comparatively disadvantaged. These findings suggest that the femoral neck, total hip and mid-radius bone mass patterns described in our black children are likely to be under similar genetic influences to those of African–American children, rather than due to environmental influences. Support for this hypothesis comes from studies that suggest that the South African black population and the African–American population (originating from West Africa) had similar genetic pools, as the South African Bantu-speaking ethnic groups migrated from West Africa [43, 48, 49]. It is unclear, at this stage, whether improvement in the adverse environmental factors in our black children would greatly change the bone mass findings at other sites. However, it does raise an intriguing question about how the genetic influences maintain bone mass in the face of what are generally considered to be adverse environmental factors. Not only do these genetic influences have a positive effect on bone mass during childhood, but these are maintained through adult life and are associated with a very low incidence of femoral neck and vertebral fractures in the elderly.

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APPENDIX 3 – Pdf of "Vidulich,L., Norris,S., Cameron,N. and Pettifor,J. Infant programming of bone size and bone mass in 10-year-old black and white South African children. *Paediatr Perinat Epidemiol* 21, 354-362 (2007)"

Bone development in childhood

Infant programming of bone size and bone mass in 10-year-old black and white South African children

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Summary

Vidulich L, Norris SA, Cameron N, Pettifor JM. Infant programming of bone size and bone mass in 10-year-old black and white South African children. *Paediatric and Perinatal Epidemiology* 2007; **21:** 354–362.

In developed countries, the earliest of factors shown to identify those at a high risk of having low bone mass and so be prone to osteoporosis in later life is that of quality of early life reflected by low birthweight (BW) and size in infancy. It is unclear whether such relationships exist in developing countries and in black populations. Associations were studied between BW, weight (WT1) and length (LT1) at 1 year and bone size and bone mass in 476 children (boys: 182 black, 72 white; girls: 158 black, 64 white) aged 10 years, who formed part of a longitudinal cohort of children born in Johannesburg, South Africa, during 1990. Bone area (BA) and bone mineral content (BMC) measurements were made of the whole body, femoral neck and lumbar spine (L1-L4) by dual-energy X-ray absorptiometry (DXA).

After adjusting BA and BMC for race, gender, age, socio-economic status, bone age, height and weight at 10 years, on which BA and BMC in children are so dependent, WT1, LT1 and BW were significant predictors of whole body BA (WT1, $P < 0.0001$; LT1, $P < 0.01$; BW, $P < 0.05$) and BMC (WT1, $P < 0.01$; LT1, $P < 0.05$; BW, $P < 0.05$) and of BMC of the femoral neck (WT1, $P < 0.01$; LT1, $P < 0.05$). When BMC was in addition corrected for BA, then BW, WT1 and LT1 were predictive of femoral neck BMC (BW, $P < 0.05$; WT1, $P < 0.05$; LT1, $P < 0.01$) but not whole body BMC. Thus, BMC at 10 years appears to be independently associated with weight and length at 1 year, which is not completely mediated by the tracking of skeletal growth. Low BW and small size at 1 year resulted in smaller bones and/or bones of lower BMC at the femoral neck. The findings support the hypothesis that growth and development, both intrauterine and in the first year, which are measures of genetic, intrauterine and postnatal environmental factors, may have long-term consequences when compromised, and may be associated with the risk of osteoporosis in later life.

Keywords: *birthweight*, *infant growth*, *bone area*, *bone mineral content*, *longitudinal data*, *childhood*.

Introduction

In developed countries, the earliest of factors shown to identify those at high risk of having low bone mass and so be prone to osteoporosis in later life is that of quality of intrauterine and early life reflected by low birthweight (BW) and size in infancy. The relationship between BW, growth in infancy and bone mass has been explored in several epidemiological studies in adults,[1–10] young adults,[9,11] adolescents[12] and children.[13–15] Childhood weight, especially weight at 1 year (WT1) has been shown to be predictive of bone mineral content (BMC) before adjusting for the

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confounding variables of current height and weight, but often, not after the adjustment. Supporting studies are needed to confirm that the relationship between size in infancy and bone mass is not entirely mediated by the tracking of infant size to adulthood, on which bone mass is dependent. In addition, as most studies were conducted in Caucasian populations from developed countries such as the UK, Australia, Canada and Finland, it is unclear whether such relationships exist in black populations and in developing countries.

We studied the relationship between growth in infancy and current bone mass in South African children by investigating how BW, WT1 and length at 1 year (LT1) related to bone area (BA) and bone mass before and after adjustment for current height and weight in a population of 10-year-old black and white children born in Johannesburg during 1990. The questions asked were: (1) Do birthweight, or weight and/or length at 1 year of age predict bone size and bone mass in 10-year-old children? (2) If there is a relationship between size in infancy and bone mass in 10-year-old children, is it because of its relationship to bone size or bone density?

Methods

Subjects

The subjects were 476 healthy children stratified by race* and gender (182 black boys, 72 white boys, 158 black girls, 64 white girls) of median age 10.6 years (range: 10.0–10.9) who formed part of a longitudinal cohort of children born in Johannesburg during 1990 and whose growth and development have been tracked since birth. Weight had been recorded at birth as well as weight and length/height at 1, 2, 4, 5, 7, 8, 9 and 10 years of age. The source of the population data was the official birth notifications, governed by a local ordinance, and completed by delivery staff at the time of every birth in the area. This information was subsequently recorded in the registers maintained by each of the three local health authorities comprising most of the metropolitan area of Soweto-Johannesburg.[17] Subjects with chronic illness (juvenile rheumatoid arthritis, epilepsy or asthma) or on medication known to affect growth or bone mass development were excluded from the study ($n = 4$). The study protocol was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand, Johannesburg and the Ethical Advisory Committee of Loughborough University, UK. Both children and guardians gave written informed consent to be studied.

*'Race does not refer to any biological attributes but rather to the compulsory classification of people into the Population Registration Act'.[16] Although the act has been repealed, these categories are still powerful and commonly used by the South African government and statistical services.

Anthropometry

Height was measured to the last completed 1 mm using a wall-mounted stadiometer (Holtain, UK) and weight to the nearest completed 0.1 kg using a digital electronic instrument (Dismed, USA), using standardised protocols.[18] Both instruments were regularly calibrated and subjects wore minimal clothing when being weighed.

Maturity

Sexual maturity was self-assessed as pubic hair development in boys and girls, using the Tanner scaling technique.[19,20] In addition, skeletal maturity was assessed by scoring bone age from hand radiographs using the Tanner–Whitehouse bone-specific scoring technique (TWII20).[21]

Dual-energy X-ray absorptiometry

Bone area and BMC of the whole body, excluding the head, femoral neck and lumbar spine (L1-L4) were measured by duel X-ray absorptiometry (DXA) using Hologic QDR-4500 (Hologic, Inc., Waltham, MA, USA). A lumbar spine phantom was scanned daily to determine its measurement precision. The coefficients of variations, calculated from not less than 240 repeat scans, were 0.47% and 0.78% for BA and BMC respectively.

Socio-economic questionnaire

Primary caregivers answered questions about their social and economic status. This questionnaire had been modified appropriately for a South African population and was previously validated.[22] The socio-economic score was formulated from the presence or

absence of 13 asset indicators, namely, house type, electricity, indoor flushing toilet, indoor running water, refuse removal, television, digital satellite television, motor vehicle, refrigerator, microwave, washing machine, video machine and telephone.

Statistics

Univariable and multivariable relationships between weight (BW and WT1) and LT1 vs. BA and BMC (at 10 years), and the extent to which these relationships were dependent on, or independent of measures of height and weight at 10 years were analysed using STATISTICA (data analysis software system) version 6 (StatSoft, Inc. 2001, Tulsa, OK, USA). Student's *t*-tests were used to compare means. Analysis of covariance (ANCOVA) analysed the relationship between 10-year-old BA or BMC when BW, WT1 or LT1 were categorised into tertiles, and age was treated as a continuous predictor.[4,8] Multiple regression models determined the predictive power of BW, WT1 and LT1 on BA and BMC when BA and BMC were in addition adjusted for race, gender, age, socio-economic status, bone age, height and weight at 10 years. The regression coefficient (b) was interpreted as the magnitude of the contribution infant size made to current BA and BMC variables.[23–25] Residual plots of all regression models showed no outlying or influential points, no deviation from the assumptions of linear relationships and constant variances. Probability values <0.05 were considered significant for all tests.

Results

Cohort characteristics

Anthropometry

Descriptive statistics for the 10-year-old subjects who took part in this study are shown in Table 1. Black children lived in households that scored significantly lower on the socio-economic scale (median = 7, range: 0–13) than white children (median = 12, range: 6–13) ($P < 0.05$, Mann–Whitney *U*-test).

At birth girls were lighter than boys (blacks: $P < 0.01$; whites: $P < 0.01$), and black boys lighter than white boys ($P < 0.01$). At 1 year, girls were significantly lighter (blacks: $P < 0.001$; whites: $P < 0.05$) and significantly shorter (blacks: $P < 0.01$; whites: $P < 0.01$) than boys. At age 1 year, black boys were shorter than white boys ($P < 0.01$).

Even though all subjects were aged between 10.0 and 10.9 years, black children were on average one month younger at the time of their visit and significantly shorter than their white counterparts (boys: 6.3 cm shorter, $P < 0.0001$; girls: 3.4 cm shorter $P < 0.001$). Black boys were on average 3.4 kg lighter than white boys at 10 years of age ($P < 0.001$).

Sexual maturity

Most of our cohort was prepubertal or in early puberty (black boys: 99%, white boys: 99%, black girls: 98%, white girls: 97%) as determined by pubic

Table 1. Descriptive characteristics of black and white children aged 10 years

	Boys			Girls		
	White mean ± SE (*n*)	Black mean ± SE (*n*)	*P*	White mean ± SE (*n*)	Black mean ± SE (*n*)	*P*
Age (years)	10.65 ± 0.03 (72)	10.55 ± 0.02 (182)	<0.01	10.62 ± 0.03 (64)	10.53 ± 0.02 (158)	<0.05
Bone age (years)	10.31 ± 0.12 (71)	10.13 ± 0.08 (179)	ns	10.41 ± 0.15 (62)	10.38 ± 0.10 (156)	ns
Height at 10 years (cm)	143.5 ± 0.9 (72)	137.3 ± 0.5 (182)	<0.0001	142.6 ± 0.9 (64)	139.2 ± 0.5 (158)	<0.001
Weight at 10 years (kg)	36.0 ± 0.8 (72)	32.6 ± 0.5 (182)	<0.001	35.5 ± 1.0 (64)	34.8 ± 0.7 (158)	ns
Body mass index (kg/m^2)	17.4 ± 2 (72)	17.2 ± 3 (182)	ns	17.3 ± 3 (64)	17.8 ± 3 (158)	ns
Prepuberty (Tanner hair 1) % (*n*)	51% (34)	65% (113)	ns	67% (43)	59% (92)	ns
Early puberty (Tanner hair 2) % (*n*)	48% (32)	34% (58)		30% (19)	39% (62)	
Mid-puberty (Tanner hair 3 and 4) % (*n*)	1% (1)	1% (1)		3% (2)	2% (3)	
Birthweight (kg)	3.35 ± 0.53 (70)	3.16 ± 50 (181)	<0.01	3.12 ± 0.37 (64)	3.03 ± 0.53 (157)	ns
Weight at 1 year (kg)	9.79 ± 1.12 (15)	9.66 ± 1.40 (131)	ns	8.96 ± 0.85 (16)	9.28 ± 1.46 (104)	ns
Length at 1 year (cm)	76.7 ± 3.5 (18)	74.3 ± 3.3 (127)	<0.01	74.0 ± 3.7 (16)	72.7 ± 3.1 (104)	ns

ns, not significant.

Table 2. Bone area (BA) and bone mineral content (BMC) comparisons between race groups within each gender. Values are unadjusted as means ± SE

	Boys			Girls		
	White	Black	*P*	White	Black	*P*
Whole body	*n* = 71	*n* = 180		*n* = 64	*n* = 158	
BA (cm^2)	1100 ± 19	1010 ± 10	<0.0001	1087 ± 23	1048 ± 13	ns
BMC (g)	786 ± 17	715 ± 9	<0.001	767 ± 22	739 ± 13	ns
Femoral neck	*n* = 72	*n* = 182		*n* = 64	*n* = 158	
BA (cm^2)	4.32 ± 0.04	4.13 ± 0.02	<0.0001	4.21 ± 0.04	4.05 ± 0.02	<0.0001
BMC (g)	3.03 ± 0.05	3.06 ± 0.03	ns	2.70 ± 0.06	2.77 ± 0.03	ns
Lumbar spine	*n* = 72	*n* = 182		*n* = 64	*n* = 158	
L1-L4 BA (cm^2)	46.00 ± 4.96	43.02 ± 4.26	<0.0001	43.99 ± 4.26	42.99 ± 4.34	ns
L1-L4 BMC (g)	26.72 ± 4.66	19.09 ± 3.69	<0.0001	25.54 ± 5.10	25.33 ± 5.24	ns

ns, not significant.

hair development (Table 1). There were no ethnic differences in the distribution of sexual maturity (Fisher's exact test). Skeletal maturity as determined by bone age, was similar between the ethnic groups within each gender ($P < 0.01$). Bone age, the measure of maturity included in the multiple regression analyses, was neither a significant predictor of BA nor BMC.

Dual-energy X-ray absorptiometry

Bone area and BMC measurements of the whole body, femoral neck and lumbar spine are shown in Table 2. Data and statistics presented in Table 2 were not adjusted for any variables.

General

Tables 1 and 2 provide anthropometric and DXA data, respectively, reported per gender and race. Racial differences in anthropometry, BA and BMC, as well as the effect of socio-economic status in this cohort have been reported elsewhere.[26–29]

Infant size (weight and length) vs. BA and BMC at 10 years

Mean BA and BMC values which are tabulated for each group (black and white boys and girls) according to tertiles of BW, WT1 or LT1 were positively and significantly associated with weight (BW, WT1) and length (LT1) at most sites and more so in boys than in girls (Table 3).

After correcting BA and BMC for race, gender, age, socio-economic status, bone age, height and weight at 10 years, WT1 and LT1 were still predictive of 10-year-old whole body BA and BMC (between 6% and 10% for a 1% change in predictor) and femoral neck BMC (between 8% and 17% for a 1% change in predictor) (Table 4). When BMC was in addition adjusted for BA, then BW, WT1 and LT1 continued to be predictive of BMC at the femoral neck, but not at the whole body.

Discussion

In both black and white South African 10-year-old children, size in infancy was predictive of BA and BMC at all sites before adjustments were made for confounding variables. After adjustments for race, gender, age, socio-economic status, bone age, height and weight at 10 years, size in infancy remained predictive of whole body BA and BMC, and femoral neck BMC. This relationship was observed despite black children being exposed to a multitude of environmental factors known to impact negatively on bone mass, such as living in poorer households and poor nutrition,[30] low calcium intake (estimated to be approximately 400 mg/day),[31] little physical activity,[26,27] patterns of compromised growth and development as reflected by their shorter statures, lighter body weights and delayed onset of puberty.[32,33] It is well established that body size 'tracks' through childhood, and that fetal and adult size lie on a continuum of body size and that the closer any two points are, the higher the correlation between them.[34] The relationship between infant weights and BMC was not entirely mediated by the

Table 3. Bone area (BA) and bone mineral content (BMC) means ± SE adjusted for age at the whole body, femoral neck and lumbar spine, within each third of the distribution of birthweight (BW, kg), and weight at 1 year (WT1, kg) and length at 1 year (LT1, cm)

			Whole body			Femoral neck			Lumbar spine		
Group	Size	Category	*n*	BA	BMC	*n*	BA	BMC	*n*	BA	BMC
Black boys	BW	<3.00 kg	59	961.5 ± 16.5	670.6 ± 15.4	59	4.08 ± 0.04	2.94 ± 0.05	59	41.36 ± 0.53	22.19 ± 0.46
		3.00–3.36 kg	62	1007.5 ± 16.1	711.9 ± 15.0	62	4.10 ± 0.04	3.02 ± 0.05	62	43.06 ± 0.52	23.22 ± 0.45
		>3.36 kg	60	1058.1 ± 16.3	763.9 ± 15.3	58	4.21 ± 0.04	3.22 ± 0.05	60	44.51 ± 0.52	25.07 ± 0.45
		P trend		<0.001	<0.001		<0.05	<0.001		<0.001	<0.0001
	WT1	<9.0 kg	45	928.2 ± 17.4	648.3 ± 17.0	43	4.03 ± 0.04	2.87 ± 0.05	45	40.75 ± 0.62	21.64 ± 0.51
		9.0–10.0 kg	44	1001.5 ± 14.7	704.1 ± 14.3	44	4.09 ± 0.04	3.03 ± 0.05	44	43.32 ± 0.63	23.62 ± 0.52
		>10.0 kg	42	1097.7 ± 16.4	799.6 ± 15.9	42	4.28 ± 0.04	3.31 ± 0.05	42	44.99 ± 0.64	25.49 ± 0.53
		P trend		<0.0001	<0.0001		<0.001	<0.0001		<0.0001	<0.0001
	LT1	<73.0 cm	34	928.3 ± 20.2	647.1 ± 19.4	33	4.01 ± 0.05	2.85 ± 0.06	34	40.39 ± 0.72	21.31 ± 0.60
		73.0–76.0 cm	60	1021.2 ± 17.1	722.7 ± 16.4	59	4.17 ± 0.04	3.12 ± 0.05	60	43.83 ± 0.54	24.01 ± 0.45
		>76.0 cm	33	1082.2 ± 17.3	787.0 ± 16.7	33	4.27 ± 0.05	3.26 ± 0.06	33	44.81 ± 0.73	25.61 ± 0.61
		P trend		<0.0001	<0.0001		<0.01	<0.0001		<0.0001	<0.0001
White boys	BW	<3.20 kg	23	1014.4 ± 31.1	705.0 ± 28.5	24	4.22 ± 0.06	2.82 ± 0.08	24	44.44 ± 1.03	24.65 ± 1.01
		3.20–3.50 kg	23	1091.9 ± 30.5	781.3 ± 28.0	25	4.26 ± 0.06	2.99 ± 0.08	25	45.86 ± 1.01	27.08 ± 0.99
		>3.50 kg	24	1186.9 ± 29.7	866.5 ± 27.3	23	4.52 ± 0.06	3.29 ± 0.08	24	48.57 ± 0.99	29.44 ± 0.97
		P trend		<0.001	<0.001		<0.01	<0.001		<0.05	<0.01
	WT1	<9.2 kg	3	892.3 ± 60.0	596.5 ± 63.3	3	3.86 ± 0.14	2.34 ± 0.25	3	38.48 ± 0.98	19.01 ± 1.02
		9.2–9.9 kg	7	1050.5 ± 39.2	751.1 ± 41.3	7	4.21 ± 0.09	3.01 ± 0.16	7	45.99 ± 0.64	27.61 ± 0.66
		>9.9 kg	5	1265.3 ± 48.1	907.4 ± 50.7	5	4.47 ± 0.11	3.38 ± 0.20	5	50.82 ± 0.79	28.46 ± 0.82
		P trend		<0.01	<0.05		<0.05	<0.05		<0.0001	<0.0001
	LT1	<74.8 cm	5	969.0 ± 92.0	671.5 ± 89.1	5	4.05 ± 0.14	2.54 ± 0.20	5	41.24 ± 1.19	21.66 ± 1.26
		74.8–78.5 cm	9	950.3 ± 56.7	653.9 ± 54.9	9	4.32 ± 0.10	3.16 ± 0.14	9	46.30 ± 0.86	27.58 ± 0.91
		>78.5 cm	4	1205.7 ± 44.2	863.6 ± 42.9	4	4.55 ± 0.16	3.34 ± 0.22	4	51.20 ± 1.32	27.61 ± 1.39
		P trend		<0.05	<0.05		ns	<0.05		<0.001	<0.01

Black girls	BW	<2.86 kg	50	1004.1 ± 22.5	701.6 ± 22.5	50	4.00 ± 0.04	2.69 ± 0.06	50	42.19 ± 0.60	24.60 ± 0.73
		2.86–3.21 kg	55	1067.3 ± 21.4	754.8 ± 21.4	55	4.06 ± 0.04	2.81 ± 0.05	55	43.27 ± 0.57	25.69 ± 0.70
		>3.21 kg	52	1069.9 ± 22.0	758.1 ± 22.0	52	4.07 ± 0.04	2.79 ± 0.06	52	43.44 ± 0.58	25.66 ± 0.72
		P trend		ns	ns		ns	ns		ns	ns
	WT1	<8.5 kg	27	982.2 ± 21.3	677.0 ± 21.0	27	3.97 ± 0.06	2.60 ± 0.07	27	41.66 ± 0.76	23.15 ± 0.88
		8.5–9.5 kg	43	1061.2 ± 24.9	758.0 ± 24.6	43	4.04 ± 0.04	2.81 ± 0.06	43	43.01 ± 0.60	25.82 ± 0.70
		>9.5 kg	34	1147.7 ± 28.0	820.3 ± 27.6	34	4.18 ± 0.05	2.87 ± 0.06	34	44.39 ± 0.68	26.83 ± 0.78
		P trend		<0.0001	<0.001		<0.05	<0.05		<0.05	<0.01
	LT1	<71.0 cm	28	1014.7 ± 21.3	705.8 ± 20.7	28	4.01 ± 0.06	2.61 ± 0.07	28	42.30 ± 0.76	24.34 ± 0.89
		71.0–74.0 cm	50	1041.9 ± 24.3	733.8 ± 23.6	50	4.05 ± 0.04	2.79 ± 0.05	50	42.85 ± 0.57	25.33 ± 0.67
		>74.0 cm	26	1163.8 ± 36.4	845.0 ± 35.4	26	4.17 ± 0.06	2.92 ± 0.07	26	44.31 ± 0.79	26.81 ± 0.92
		P trend		<0.01	<0.01		ns	<0.05		ns	ns
White girls	BW	<3.0 kg	20	1095.3 ± 40.5	779.0 ± 39.5	19	4.26 ± 0.07	2.71 ± 0.11	19	43.82 ± 0.92	24.80 ± 1.07
		3.0–3.28 kg	24	1052.5 ± 36.9	734.9 ± 36.0	22	4.17 ± 0.06	2.66 ± 0.10	22	43.78 ± 0.84	25.11 ± 0.97
		>3.28 kg	20	1121.2 ± 40.3	792.6 ± 39.4	20	4.19 ± 0.07	2.71 ± 0.11	20	43.78 ± 0.92	25.79 ± 1.06
		P trend		ns	ns		ns	ns		ns	ns
	WT1	<8.3 kg	4	944.6 ± 50.1	636.3 ± 49.8	4	3.96 ± 0.11	2.37 ± 0.17	4	40.71 ± 1.99	21.11 ± 2.19
		8.3–9.2 kg	7	1079.0 ± 43.4	770.4 ± 43.2	7	4.00 ± 0.09	2.41 ± 0.13	7	42.11 ± 1.56	22.60 ± 1.72
		>9.2 kg	5	1448.6 ± 86.7	1133.3 ± 86.3	5	4.28 ± 0.10	3.02 ± 0.16	5	47.37 ± 1.85	31.38 ± 2.04
		P trend		<0.01	<0.01		ns	<0.05		ns	<0.05
	LT1	<72.5 cm	5	944.4 ± 63.7	633.1 ± 65.1	5	3.87 ± 0.09	2.30 ± 0.15	5	37.78 ± 1.21	19.50 ± 1.86
		72.5–74.0 cm	7	1094.6 ± 58.5	777.9 ± 59.8	7	4.17 ± 0.08	2.64 ± 0.13	7	45.33 ± 1.05	25.80 ± 1.62
		>74.0 cm	4	1311.3 ± 90.1	991.9 ± 92.1	4	4.31 ± 0.09	3.04 ± 0.17	4	47.22 ± 1.32	30.93 ± 2.03
		P trend		<0.05	<0.05		<0.05	<0.05		<0.001	<0.01

ns, not significant.

Table 4. The predictive power (b ± SE) of birthweight (BW), weight at 1 year (WT1) and length at 1 year (LT1) of BA and BMC at the whole body, femoral neck and lumbar spine when BA and BMC were adjusted for race, gender, age, socio-economic status, bone age, height and weight at 10 years

	BW			WT1			LT1		
Measure	*n*	b ± SE	*P*	*n*	b ± SE	*P*	*n*	b ± SE	*P*
BA adjusted for race, gender, age, socio-economic status, bone age, height (10 years) and weight (10 years)									
Whole body BA	460	0.03 ± 0.02	ns	258	0.10 ± 0.03	<0.001	257	0.06 ± 0.03	<0.05
Femoral neck BA	457	–0.04 ± 0.04	ns	256	0.10 ± 0.06	ns	255	0.02 ± 0.06	ns
Lumbar spine BA	460	0.02 ± 0.03	ns	258	0.08 ± 0.05	ns	257	0.01 ± 0.05	ns
BMC adjusted for race, gender, age, socio-economic status, bone age, height (10 years) and weight (10 years)									
Whole body BMC	460	0.03 ± 0.02	ns	258	0.10 ± 0.04	<0.01	257	0.08 ± 0.04	<0.05
Femoral neck BMC	457	0.05 ± 0.04	ns	256	0.14 ± 0.05	<0.05	255	0.17 ± 0.06	<0.01
Lumbar spine BMC	460	0.04 ± 0.04	ns	258	0.08 ± 0.05	ns	257	0.04 ± 0.06	ns
BMC adjusted for race, gender, age, socio-economic status, bone age, height (10 years) and weight (10 years) and BA									
Whole body BMC	460	–0.004 ± 0.01	ns	258	–0.02 ± 0.02	ns	257	0.01 ± 0.02	ns
Femoral neck BMC	457	0.07 ± 0.03	<0.05	256	0.11 ± 0.05	<0.05	255	0.16 ± 0.05	<0.01
Lumbar spine BMC	460	0.02 ± 0.03	ns	258	0.03 ± 0.04	ns	257	0.04 ± 0.05	ns

BA, bone area; BMC, bone mineral content; ns, not significant.

tracking of skeletal size: infants of a lower BW and a smaller size at 1 year grow to develop smaller bones (as reflected by BA) and/or bones of lower BMC at the femoral neck (lower BMC with similar BA).

We did not adjust for lifestyle determinants since gestational age at birth, physical inactivity, low dietary calcium intake and cigarette smoking of parents have not been shown to affect relationships between infant size and current bone mass.[4,8,23,35] The number of white subjects with data at 1 year was small, which should be borne in mind when interpreting any results in this race.

The statistical methods used in this study were similar to those used by other researchers so that comparisons could be made between studies and populations. We therefore have reported results that were both unadjusted and adjusted for the confounding variables of height and weight (in addition to race, gender, age, socio-economic status, bone age), so as to counter the effect of tracking of skeletal size.

Unadjusted BMC was positively and significantly related to birth and infant weights and lengths and unadjusted BMC as has been observed in adults[2–4,8,9,11,36] and in the elderly,[1,8] at the whole body, lumbar spine and/or femoral neck (Table 2).

In addition to using similar categorising methods as those used by others,[4,8–10] we used multiple regression techniques, and made simultaneous adjustments for race, gender, age, socio-economic status, bone age,[4,8,9] height and weight (these analyses are particularly recommended for use in children)[23–25] and showed that size in infancy, especially at 1 year, was correlated with, and was predictive of BA and BMC of the whole body and BMC at the femoral neck at age 10 years, independent of current size. This finding has not been consistently observed in adults. In adults, after adjusting BMC for height and weight simultaneously, it hs been reported that BW and weight or length at 1 year were no longer associated with bone mass.[2,3,8]

The mechanisms through which *in utero* growth translates into compromised bone health have been suggested to be mediated by the development of fewer cells and/or the altered programming of stem cell function and regulatory hormones[37] such as vitamin D, IGF-1[38–41] and growth hormone.[42] It has been suggested that the multitude of BMC-modifying factors to which adults are exposed over a lifetime mask the relationship between early growth and adult bone mass. We suggest that this explains why we did show such relationships in prepubertal and peripubertal children aged 10 years.

The present findings support the hypothesis that growth and development both intrauterine and up until 1 year of age, which are measures of genetic, intrauterine and postnatal environmental factors, may have long-term consequences when compromised, and may be associated with the risk of osteoporosis in later life.

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APPENDIX 4 – Pdf of "Vidulich,L., Norris,S., Cameron,N. and Pettifor,J. Bone mass and bone size in pre- or early pubertal 10-year-old black and white South African children and their parents. *Calcif Tissue Int* **88**, 281-293 (2011)"

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ORIGINAL RESEARCH

Bone Mass and Bone Size in Pre- or Early Pubertal 10-Year-Old Black and White South African Children and Their Parents

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Abstract Genetic factors are thought to maintain bone mass in socioeconomically disadvantaged black South Africans. We compared bone mass between environmentally disadvantaged black and advantaged white children and their parents, after determining the most appropriate method by which to correct bone mineral content (BMC) for size. We collected data from 419 healthy black and white children of mean age 10.6 years (range 10.0–10.9), 406 biological mothers, and 100 biological fathers. Whole-body, femoral neck, lumbar spine, and mid- and distal one-third of radius bone area (BA) and BMC were measured by dual-energy X-ray absorptiometry. Power coefficients (PCs) were calculated from the linear-regression analyses of ln(BMC) on ln(BA) and used to correct site-specific BMC for bone size differences. Heritability (½h^2, %) by maternal and paternal descent was estimated by regressing children's *Z* scores on parents' *Z* scores. Correcting BMC for height, weight, and BA^{PC} accounted for the greatest variance of BMC at all skeletal sites. In so doing, BMC in blacks was up to 2.6 times greater at the femoral neck and lumbar spine. Maternal and paternal heritability was estimated to be ~30% in both black and white subjects. These results may in part explain the lower prevalence of fragility fractures at the hip in black South African children when compared to whites. Heritability was comparable between environmentally disadvantaged black and advantaged white South African children and similar to that reported for Caucasians in other parts of the world.

Keywords Bone densitometry · Population study · Association · South Africa · Children · Ethnicity

Populations subjected to environmental factors known to negatively influence bone mass are expected to have lower bone mass, higher fragility fracture rates, and lower heritability estimates. Black South Africans are subject to poor growth and nutrition [1], low dietary calcium intake [2], and little physical activity [3, 4] yet have higher bone mass at the femoral neck [5, 6] and lower fracture rates [7–9]. Genetic factors, which account for a major proportion of bone mass variance in adults [10], adolescents [11], and children [12], are thought to maintain bone mass in black South Africans in the face of these adverse environmental factors. Yet, assessment of heritability of bone mass and bone size by way of parent–child associations has not been previously explored in this population.

Dual-energy X-ray absorptiometry (DXA) remains the most widely used technique for the measurement of bone mass in both adult [13] and pediatric [13, 14] populations and was used in this study. DXA measurements, their analyses, and interpretation are dependent on size-related variables such as age, body size (height and weight), and

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bone volume [15] and on skeletal maturity, ethnicity, and body composition [16, 17].

There is no standard way to correct BMC or areal BMD data for changes in skeletal size; they have been corrected for varying combinations of body size, bone size, bone area, pubertal stage, skeletal maturity, and body composition [16]. The many different methods used make the interpretation of uncorrected and corrected DXA data and the objective comparisons between studies, populations, and age groups very complex, confusing, and potentially erroneous [13].

To address these concerns, Katzman et al. [18] and Carter et al. [19] proposed measurements that are less dependent on size by mathematically converting bone mineral content (BMC) to a three-dimensional estimate of volumetric bone mineral density (BMD) or bone mineral apparent density (BMAD). Bones were assumed to be shaped as cubes, and the following formulae were applied to calculate BMAD at the whole body [$BMC/(BA^2/height)$], femoral neck, mid-forearm (BMC/BA^2), and lumbar spine ($BMC/BA^{1.5}$). Kröger et al. [20] applied a similar concept, assuming bones (vertebral bodies, femoral shaft and neck) to be shaped as cylinders and applying the formula $BMAD = (BMC)\{4/[\pi(\text{bone width})]\}$. Similarly, Lu et al. [21] assumed the femoral neck, mid-third of the femoral shaft, and the four lumbar vertebral bodies to be cylinders and used bone width (d) and height (h) to calculate bone volume [$\pi(d/2)^2 \times h$]. All methods, however, calculate coefficients by assuming that bones are shaped as cubes or cylinders, which does not necessarily hold true in groups differing in ethnicity, age, and sex [22].

Prentice et al. [22] proposed a method that calculated population-specific power coefficients (PCs) and then corrected BMC for bone area (BA^{PC}), height, and weight. This method allows BMC to be custom-corrected for size for each ethnic and sex group and each skeletal site.

Therefore, the first aim of this study was to compare BMC corrected for BA^{PC}, height, and weight [22] against BMC corrected for other combinations of height, weight, and/or BA in black and white children and their parents. The second aim of the study was to explore the associations of BMC and bone size between black and white children and their parents in order to obtain an estimate of heritability.

Materials and Methods

Subjects

Subjects were 419 healthy children stratified by ethnicity and gender (135 black girls, 63 white girls, 154 black boys, 67 white boys) of mean age 10.6 years (range 10.0–10.9) who formed part of a longitudinal cohort of children born in Johannesburg during 1990 (the Bone Health subcohort of the Birth to Twenty study) and whose growth and development have been tracked since birth. Subjects with chronic illness (such as juvenile idiopathic arthritis, epilepsy, or asthma) or on medication known to affect growth or bone mass development were excluded from the study ($n = 4$). Of the parents of the 419 children, we collected maternal data from 406 biological mothers (280 black mothers, 126 white mothers) of median age 37 years and paternal data from 100 biological fathers (53 black fathers, 47 white fathers) of median age 42 years. Many children were no longer living with their fathers, while other fathers were not able to make themselves available for DXA scans for a number of reasons, including work commitments. Both maternal and paternal data were available for 88 children. The study protocol was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand, Johannesburg, and the Ethical Advisory Committee of Loughborough University, UK. Guardians gave written informed consent and the children written assent to be studied.

Anthropometry

Height was measured to the last completed 1 mm using a wall-mounted stadiometer (Holtain, Crosswell, UK) and weight to the nearest completed 0.1 kg using a digital electronic instrument (Dismed), using standardized protocols [23]. Both instruments were regularly calibrated, and subjects wore minimal clothing when being weighed.

Maturity

Sexual maturity was self-assessed as pubic hair development in boys and girls, using the Tanner scaling technique [24, 25]. In addition, skeletal maturity was assessed by scoring bone age from hand radiographs using the Tanner–Whitehouse bone-specific scoring technique (TWII20) [26].

Socioeconomic Questionnaire

Primary caregivers answered questions about their social and economic status. This questionnaire had been modified appropriately for a South African population and previously validated [27]. The socioeconomic score was formulated from the presence or absence of 13 asset indicators: house type, electricity, indoor flushing toilet, indoor running water, refuse removal, television, digital satellite television, motor vehicle, refrigerator, microwave, washing machine, video machine, and telephone.

DXA

Children's and their parents' BA and BMC of the whole body including and excluding the head (WB), femoral neck (FN), lumbar spine (LS, L1–L4), mid-radius (MR), and distal one-third of the radius (DR) were scanned using a fan beam densitometer in array mode (model QDR-4500A; Hologic, Bedford, MA). Adult and children's data were analyzed using adult software supplied by the manufacturer, version 11.2 (Hologic). To determine the densitometer's measurement precision, a lumbar spine phantom was scanned daily. The coefficients of variations (CV) were 0.47 and 0.78% for BA and BMC, respectively. To determine operator measurement precision, 15 subjects were scanned twice and the resultant CV was <1% for both BA and BMC. Precision of measurement in the children was not assessed because of radiation concerns.

Structural Geometry of the Femoral Neck

A series of standard formulae developed by Beck and colleagues [28, 29] was used to calculate cross-sectional area (CSA, cm^2) and section modulus (Z, cm^3) of the femoral neck from DXA-measured BMC and BA. Assumptions were made that the fixed length of the femoral neck area was 1.5 cm, the effective density of bone in fully mineralized bone tissue was $\sim$1.05 g/cm^3, and the proportion of cortical mass was 0.6. The standard formulae included estimating femoral neck width, cross-sectional moment of inertia, endosteal diameter, cross-sectional area, trabecular porosity, mean cortical thickness, and buckling ratio.

Statistics

Statistica (data analysis software system), version 6 (StatSoft, Tulsa, OK), was used to analyze data sets of children and their parents and the associations between them. Data sets included age, bone age (in children), height, weight, and BA and BMC of the whole body, femoral neck, lumbar spine, mid- and distal one-third of the radius. All data are reported as means and standard errors of the mean. Probability values <0.05 were considered significant for all tests.

PCs were derived from the linear-regression analyses of ln(BMC) on ln(BA). These regression coefficients were used as the PCs to which BA were raised to correct for bone size and determined for each skeletal site for each of the eight groups in this study (black and white boys and girls, mothers and fathers). BMC was then corrected for the size-related predictors of height, weight, and/or BA^{PC} or BA.

To allow for comparisons between size-adjusted BMC of children and those of their parents, BMC was corrected for height, weight, BA^{PC}, and age (the latter in adults only) and then converted to Z scores. Z scores were calculated from the means and standard deviations of each of the eight groups. The associations between children's and parents' Z scores were assessed by way of Pearson's correlation coefficients (r) and the calculation of heritability estimates ($½h^2$, %). Heritability by maternal and paternal descent was estimated by regressing children's Z scores on mother's or father's Z scores. The resulting regression coefficient gives the appropriate heritability estimate [12, 30, 31].

Lastly, the predictors of children's BA and BMC were assessed by way of multiple regression analyses. Mother's and father's BA^{PC} values were included separately as predictors of children's BA in addition to ethnicity, gender, and child's height, weight, and BA^{PC}. Mother's and father's size-adjusted BMC values (corrected for height, weight, and BA^{PC}) were included separately as predictors of children's BMC in addition to ethnicity, gender, and child's height, weight, and BA^{PC}. Residual plots of all regression models showed no outlying or influential points, no deviation from the assumptions of linear relationships, and constant variances.

Results

Descriptive Characteristics of Study Population

Black families lived in households that scored significantly lower on the socioeconomic scale (median = 7, range 0–13) than white families (median = 12, range 6–13) ($P < 0.05$, Mann–Whitney U test).

Descriptive characteristics of the 10-year-old black and white children and their parents are shown in Table 1. Ethnic differences in anthropometry in this cohort of children have been reported elsewhere [3–5, 32]. Briefly, when compared to their white peers, black children and their parents were significantly shorter and black boys and their fathers were significantly lighter. At the time of the study, the children had achieved $\sim$80% of their parents' heights and $\sim$50% of their parents' weights.

All children were pre- or early pubertal (Tanner stages 1 or 2) as determined by pubic hair development. There were no ethnic differences in sexual maturity (Fisher's exact test) or skeletal maturity (independent t-test) within each sex.

BA and BMC

Uncorrected BA

Uncorrected BA data are shown in Table 1. BA was generally smaller in black children and their parents or, at the

Table 1 Descriptive characteristics (±SE) of 10-year old black and white girls and boys and their parents

	Girls				*P*	Boys				*P*
	Black		White			Black		White		
	Mean ± SE	*n*	Mean ± SE	*n*		Mean ± SE	*n*	Mean ± SE	*n*	
Age (years)	10.53 ± 0.27	135	10.61 ± 0.25	63	<0.05	10.55 ± 0.27	154	10.65 ± 0.24	67	<0.05
Skeletal age (years)	10.26 ± 1.09	135	10.30 ± 1.21	63	ns	10.19 ± 1.15	154	10.36 ± 1.23	67	ns
Height (cm)	139.2 ± 6.40	135	142.7 ± 7.61	63	<0.01	137.3 ± 6.2	154	143.5 ± 7.4	67	<0.0001
Weight (kg)	34.8 ± 8.3	135	35.7 ± 8.0	63	ns	32.7 ± 6.5	154	35.9 ± 6.2	67	<0.001
Lean mass (kg)	23.9 ± 4.0	135	25.1 ± 4.3	63	ns	24.1 ± .31	154	26.9 ±3.6	67	<0.0001
Fat mass (kg)	10.1 ± 5.0	135	9.8 ± 4.4	63	ns	7.5 ± 4.0	154	8.1 ± 3.2	67	ns
Whole body less head BA (cm^2)	1,049 ± 173	135	1,081 ± 183	63	ns	1,010 ± 133	154	1,095 ± 151	67	<0.0001
Whole body less head BMC (g)	741 ± 170	135	759 ± 172	63	ns	717 ± 126	154	781 ± 139	67	<0.001
Femoral neck BA (cm^2)	4.05 ± 0.32	135	4.20 ± 0.29	63	<0.01	4.13 ± 0.32	153	4.33 ± 0.33	66	<0.0001
Femoral neck BMC (g)	2.78 ± 0.42	135	2.68 ± 0.45	63	ns	3.05 ± 0.39	153	3.03 ± 0.43	66	ns
Lumbar spine BA (cm^2)	34.02 ± 3.49	135	34.79 ± 3.33	63	ns	34.11 ± 3.26	154	36.40 ± 4.01	67	<0.0001
Lumbar spine BMC (g)	20.72 ± 4.32	135	20.66 ± 4.17	63	ns	19.09 ± 3.02	154	21.40 ± 3.67	67	<0.0001
Mid-radius BA (cm^2)	4.37 ± 0.87	135	4.27 ± 0.85	63	ns	4.49 ± 0.77	152	4.51 ± 0.80	64	ns
Mid-radius BMC (g)	1.69 ± 0.43	135	1.71 ± 0.40	63	ns	1.76 ± 0.34	152	1.87 ± 0.37	64	<0.05
Distal one-third radius BA (cm^2)	2.17 ± 0.20	135	2.19 ± 0.21	63	ns	2.32 ± 0.22	152	2.32 ± 0.19	64	ns
Distal one-third radius BMC (g)	1.04 ± 0.16	135	1.06 ± 0.15	63	ns	1.09 ± 0.13	152	1.14 ± 0.12	64	<0.01
Cross-sectional area (cm^2)	1.76 ± 0.27	126	1.70 ± 0.29	63	ns	1.94 ± 0.25	143	1.93 ± 0.27	66	ns
Cross-sectional area (cm^2)[a]	1.79 ± 0.02	126	1.66 ± 0.02	63	<0.001	1.99 ± 0.02	143	1.82 ± 0.03	66	<0.0001
Section modulus (cm^3)	1.12 ± 0.40	126	1.25 ± 0.44	63	<0.05	1.28 ±0.43	143	1.51 ± 0.50	66	<0.001
Section modulus (cm^3)[a]	1.16 ± 0.03	126	1.16 ± 0.04	63	ns	1.24 ± 0.03	143	1.31 ± 0.04	66	ns
	Mothers				*P*	Fathers				*P*
	Black		White			Black		White		
	Mean ± SE	*n*	Mean ± SE	*n*		Mean ± SE	*n*	Mean ± SE	*n*	
Age (years)	35.6 ± 4.81	280	40.2 ± 6.07	126	<0.0001	42.5 ± 7.8	53	41.9 ± 5.5	47	ns
Height (cm)	157.7 ± 5.7	280	165.2 ± 5.9	126	<0.0001	169.6 ± 6.2	53	179.6 ± 6.2	47	<0.0001
Weight (kg)	71.7 ± 14.9	280	69.4 ± 14.6	126	ns	71.4 ± 13.5	53	81.2 ± 12.0	47	<0.001
Lean mass (kg)	30.5 ± 10.5	280	25.4 ± 10.1	126	<0.0001	49.5 ± 6.4	53	58.3 ± 6.7	47	<0.0001
Fat mass (kg)	37.3 ± 5.3	280	41.3 ± 6.4	126	<0.0001	17.2 ± 7.8	53	19.9 ± 8.0	47	ns
Whole-body BA (cm^2)	1,917 ± 147	278	2,003 ± 143	125	<0.0001	2,139 ± 146	53	2,330 ± 131	47	<0.0001
Whole-body BMC (g)	2,096 ± 276	278	2,221 ± 278	125	<0.0001	2,498 ± 261	53	2,716 ± 304	47	<0.001
Femoral neck BA (cm^2)	4.83 ± 0.35	280	5.12 ± 0.34	126	<0.0001	5.47 ± 0.36	53	5.91 ± 0.30	47	<0.0001
Femoral neck BMC (g)	4.26 ± 0.65	280	4.09 ± 0.64	126	<0.05	4.81 ± 0.57	53	4.95 ± 0.81	47	ns
Lumbar spine BA (cm^2)	42.6 ± 4.3	280	47.5 ± 4.2	125	<0.0001	49.3 ± 4.3	53	55.2 ± 4.7	47	<0.0001
Lumbar spine BMC (g)	44.4 ± 7.8	280	50.5 ± 9.1	125	<0.0001	51.2 ± 6.5	53	56.3 ± 8.5	47	<0.01
Mid-radius BA (cm^2)	7.16 ± 0.99	280	7.01 ± 0.99	126	ns	9.31 ± 1.26	53	10.1 ± 1.18	47	<0.01
Mid-radius BMC (g)	4.11 ± 0.68	280	4.12 ± 0.69	126	ns	6.09 ± 1.07	53	6.73 ± 0.96	47	<0.01
Distal one-third radius BA (cm^2)	2.63 ± 0.28	280	2.65 ± 0.24	126	ns	3.06 ± 0.44	53	3.12 ± 0.24	47	ns
Distal one-third radius BMC (g)	1.77 ± 0.19	280	1.83 ± 0.20	126	<0.01	2.30 ± 0.28	53	2.46 ± 0.28	47	<0.01
Cross-sectional area (cm^2)	2.71 ± 0.41	280	2.60 ± 0.41	126	<0.05	3.05 ± 0.38	43	3.14 ± 0.51	47	ns
Cross-sectional area (cm^2)[a]	2.82 ± 1.01	280	3.52 ± 1.23	126	<0.001	5.28 ± 1.97	43	7.58 ± 2.44	47	<0.0001
Section modulus (cm^3)	2.73 ± 0.02	280	2.55 ± 0.04	125	<0.05	3.24 ± 0.08	43	2.97 ± 0.07	47	<0.05
Section modulus (cm^3)[a]	3.01 ± 0.06	280	3.11 ± 0.10	125	<0.001	6.15 ± 0.39	43	6.79 ± 0.37	47	ns

BA and BMC reported in this table are not size-corrected. *P* values indicate ethnic differences

[a] Femoral neck geometry results cross-sectional area and section modulus are corrected for height and total lean mass (less head for children)

most, similar but never larger. At 10 years of age, children had achieved ~80% of their parental BA at the femoral neck and distal one-third of the radius, ~75% at the lumbar spine, ~60% at the whole body, and ~55% at the mid-radius.

Uncorrected BMC

Uncorrected BMC data are shown in Table 1. When compared to their white peers, uncorrected BMC was lower in black boys and their fathers at all sites except the femoral neck. Uncorrected BMC was similar in black and white girls but less in black mothers at all sites except the mid-radius. Uncorrected BMC in children had reached ~65% of parental values at the femoral neck, ~50% at the distal one-third of the radius, ~45% at the whole body, ~40% at the lumbar spine, and ~35% at the mid radius.

PCs

The calculated PCs are shown for each of the eight groups (black and white boys and girls, mothers and fathers) at each skeletal site in Table 2. Calculated PCs ranged from 0.87 to 1.83 in children and 0.43 to 1.58 in adults. They differed between blacks and whites in both children and adults at the femoral neck, between black and white adults at the distal one-third of the radius, and only between black and white mothers at the mid-radius. In addition, for the most part, PCs were significantly different from 1, 1.5, and/or 2, which have been used by different authors to adjust for differences in size.

Size-Adjusted BMC

Figures 1 and 2 illustrate how BMC values vary in black and white children and their parents when corrected for different combinations of height, weight, BA and/or BA^{PC} at the different skeletal sites (radial and femoral neck BMC graphs not shown). Correcting BMC for height, weight, and BA^{PC} or BA accounted for the greatest proportion of the variance in BMC at most skeletal sites. However, ethnic differences in BMC were magnified when correcting for BA^{PC} vs. BA. That is, BMC (corrected for BA^{PC}) was greater in black children and their parents than in their white peers at the femoral neck (all $P < 0.0001$) and lumbar spine (all $P < 0.0001$) and in black boys and fathers at the whole body (both $P < 0.0001$). At the femoral neck, black girls had 7% more BMC than whites when corrected for BA, height, and weight, which increased to 69% when corrected for BA^{PC}, height, and weight. Similar increases were observed in black boys (from 8 to 64%), mothers (from 8 to 34%), and fathers (from 6 to 98%) as

Table 2 Power coefficients (PC ± SE) at each skeletal site in black and white children and their parents

	Girls				Ethnic differences *P*	Boys				Ethnic differences *P*	Gender differences *P*	
	Black	*n*	White	*n*		Black	*n*	White	*n*		Black	White
Whole body less head	1.34 ± 0.02	135	1.32 ± 0.03	63	0.5749	1.28 ± 0.03	154	1.27 ± 0.04	67	0.8490	0.1046	0.3276
Femoral neck	1.18 ± 0.13^{d}	135	1.83 ± 0.21^{2}	63	0.0010	0.87 ± 0.11^{a}	153	1.35 ± 0.16^{4}	66	0.0160	0.0696	0.0721
Lumbar spine	1.52 ± 0.10^{1}	135	1.72 ± 0.15^{1}	63	0.1750	1.21 ± 0.09^{4c}	154	1.30 ± 0.11^{3}	67	0.5689	0.0220	0.0253
Mid-radius	1.16 ± 0.04^{2a}	135	1.09 ± 0.05^{1a}	63	0.3005	0.96 ± 0.04^{a}	152	1.04 ± 0.05^{a}	64	0.2499	0.0004	0.4825
Distal one-third radius	1.24 ± 0.09^{3c}	135	1.17 ± 0.11^{c}	63	0.6214	0.81 ± 0.08^{4a}	152	1.07 ± 0.05^{a}	64	0.0439	0.0004	0.5012

	Mothers				Ethnic differences *P*	Fathers				Ethnic differences *P*
	Black	*n*	White	*n*		Black	*n*	White	*n*	
Whole body	1.39 ± 0.06^{1}	278	1.37 ± 0.10^{2}	125	0.8585	1.26 ± 0.12^{4d}	53	1.60 ± 0.18^{3}	47	0.1107
Femoral neck	0.78 ± 0.11^{4a}	280	0.94 ± 0.20^{c}	126	0.4500	0.43 ± 0.25^{1b}	53	1.10 ± 0.46	47	0.1898
Lumbar spine	1.33 ± 0.06^{1c}	280	1.58 ± 0.11^{1}	125	0.0314	0.90 ± 0.16^{b}	53	1.09 ± 0.21^{b}	47	0.4671
Mid-radius	1.00 ± 0.04^{a}	280	1.02 ± 0.06^{a}	126	0.7810	1.07 ± 0.11^{b}	53	1.06 ± 0.10	47	0.0591
Distal one-third radius	0.80 ± 0.05^{2a}	280	1.02 ± 0.07^{a}	126	0.0111	0.70 ± 0.11^{3a}	53	1.16 ± 0.14^{d}	47	0.0114

PCs were calculated from the linear-regression analyses of ln(BMC) on ln(BA). BA^{PC} was used as a correction for BMC together with height and weight in Figs. 1 and 2. *P* values indicate ethnic and gender differences

Superscripts indicate whether the PC is different from 1 or 1.5. $^{1-4}$ Significantly different from 1: ^{1} $P < 0.0001$, ^{2} $P < 0.001$, ^{3} $P < 0.01$, ^{4} $P < 0.05$; $^{a-d}$ significantly different from 1.5: ^{a} $P < 0.0001$, ^{b} $P < 0.001$, ^{c} $P < 0.01$, ^{d} $P < 0.05$

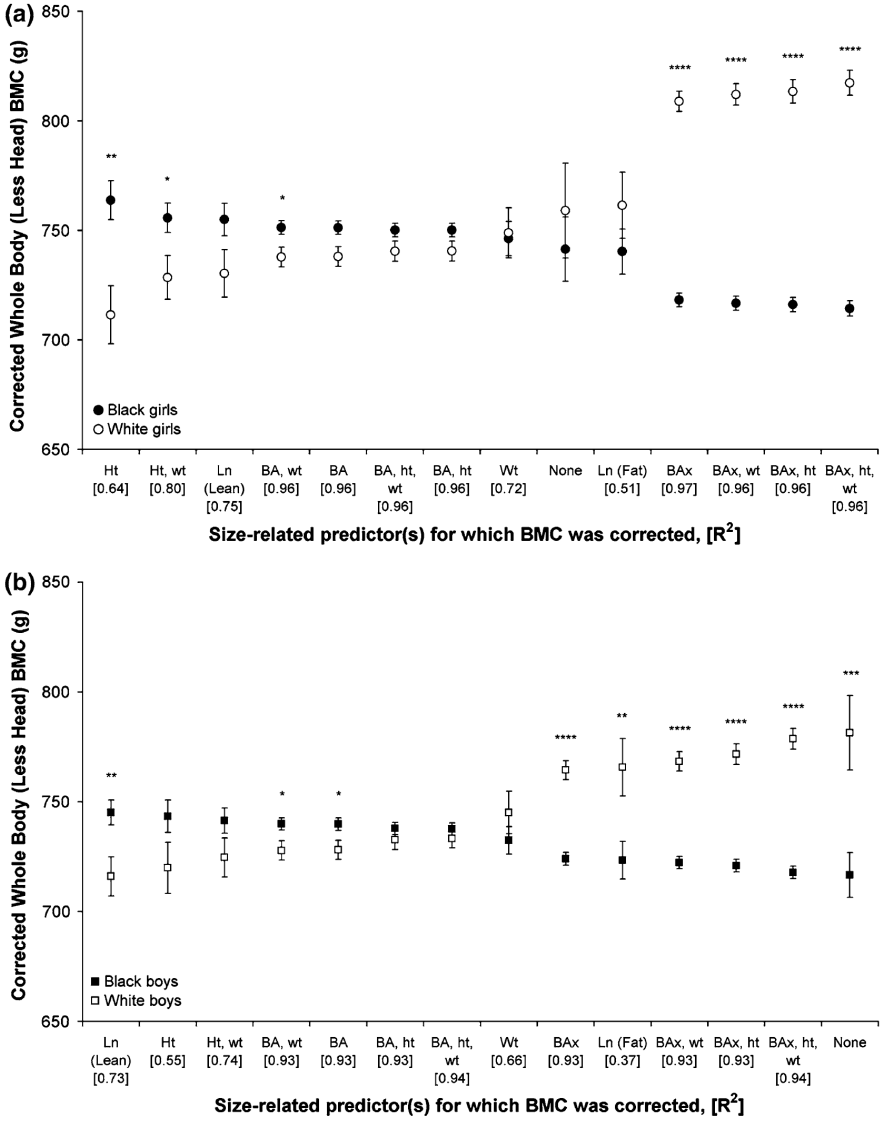


Fig. 1 Whole-body less head (for girls and boys) and whole-body (for mothers and fathers) BMC (±SE) corrected for ln(height), ln(weight), or combinations of size-related predictors of BA, BA^{PC}, (BAx), height (ht), and/or weight (wt) in black and white girls (**a**) and boys (**b**), mothers (**c**), and fathers (**d**). *Asterisks* indicate ethnic differences: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

well as at the lumbar spine (black girls, from 4 to 85%; boys, from 3 to 34%; mothers, from 1 to 166%; fathers, from 2 to 89%). BMC was less in black girls and their mothers at the whole body (both $P < 0.0001$), mid-radius (girls $P < 0.0001$, mothers $P < 0.001$), and distal one-third of the radius (girls only $P < 0.0001$).

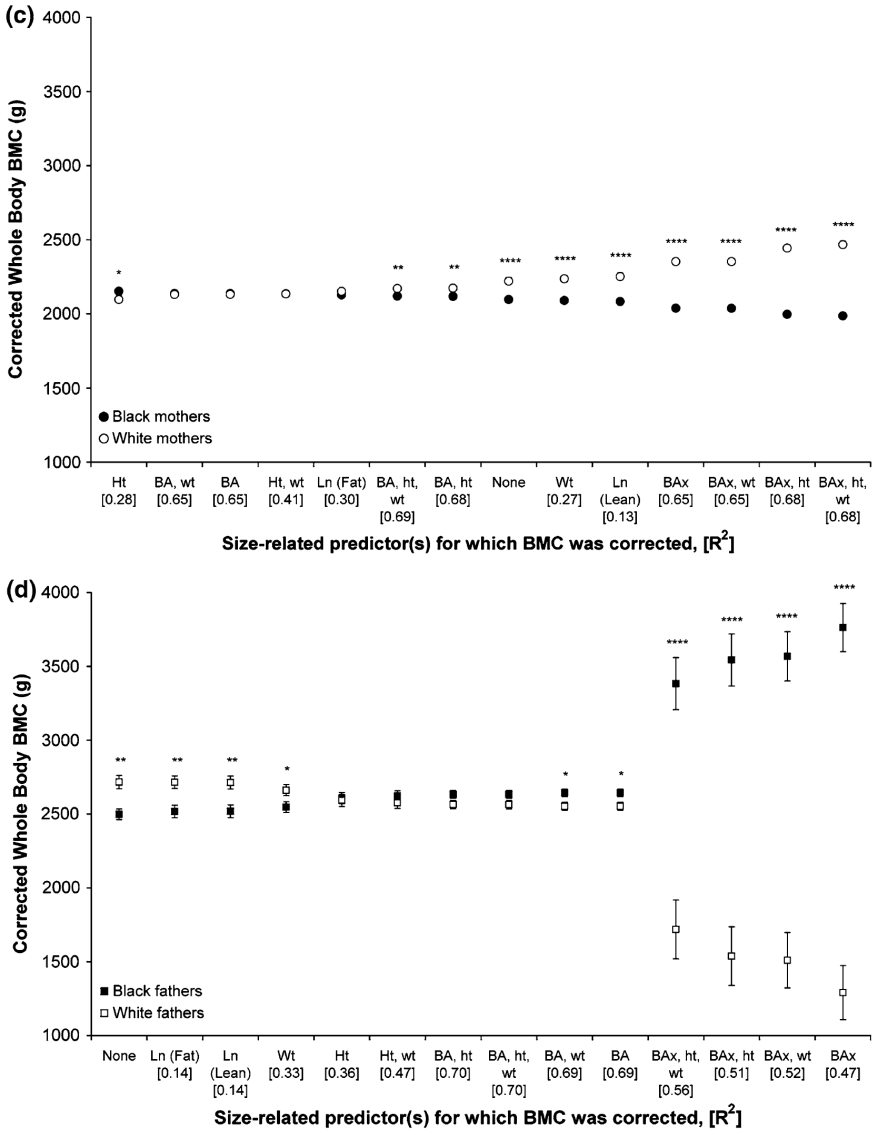
Structural Geometry of the Femoral Neck

There were no ethnic differences between children or parents in uncorrected CSA. Uncorrected section modulus (Z) was significantly greater in black children and their parents ($P < 0.05$–0.0001). Once corrected for height and total lean mass (less head for children), CSA was significantly greater in blacks ($P < 0.05$–0.0001) and Z was significantly smaller in black mothers ($P < 0.001$) (Table 1).

Associations Between Children's and Parents' BMC Adjusted for Height, Weight, and BA^{PC}

The associations between children's and parents' adjusted BMC *Z* scores assessed by way of Pearson's correlation coefficients (*r*) and heritability estimates ($½h^2$, %) are presented in Table 3. BMC *Z* scores of black and white children were significantly correlated with those of their mothers at all skeletal sites. Heritability by maternal or

Fig. 1 continued



paternal descent was estimated to be ~30%. There were no significant ethnic differences in correlation coefficients or heritability estimates.

Discussion

This study illustrates how various combinations of size-related adjustments influence DXA-measured BMC in black and white prepubertal children and their parents. Ethnic differences in BMC were dependent on ethnic differences in size. Correcting BMC for BA^{PC} (or BA), height, and weight proved to be the combination of size-related corrections that accounted for the greatest proportion of the variance in BMC at all skeletal sites. Size-adjusted BMC (adjusted for BA) at the different sites was greater in blacks by 2–8% but by 34–166% when adjusted for BA^{PC}. We previously reported that BMC corrected for height and weight only (excluding BA) in the same black children was ~6% greater at the femoral neck but not different at the lumbar spine [5]. In support of these latter findings, similar lumbar spine BMDs were shown in pre-, peri-, and postmenopausal black and white South African women when corrected for height only [6, 33]. Adjusting

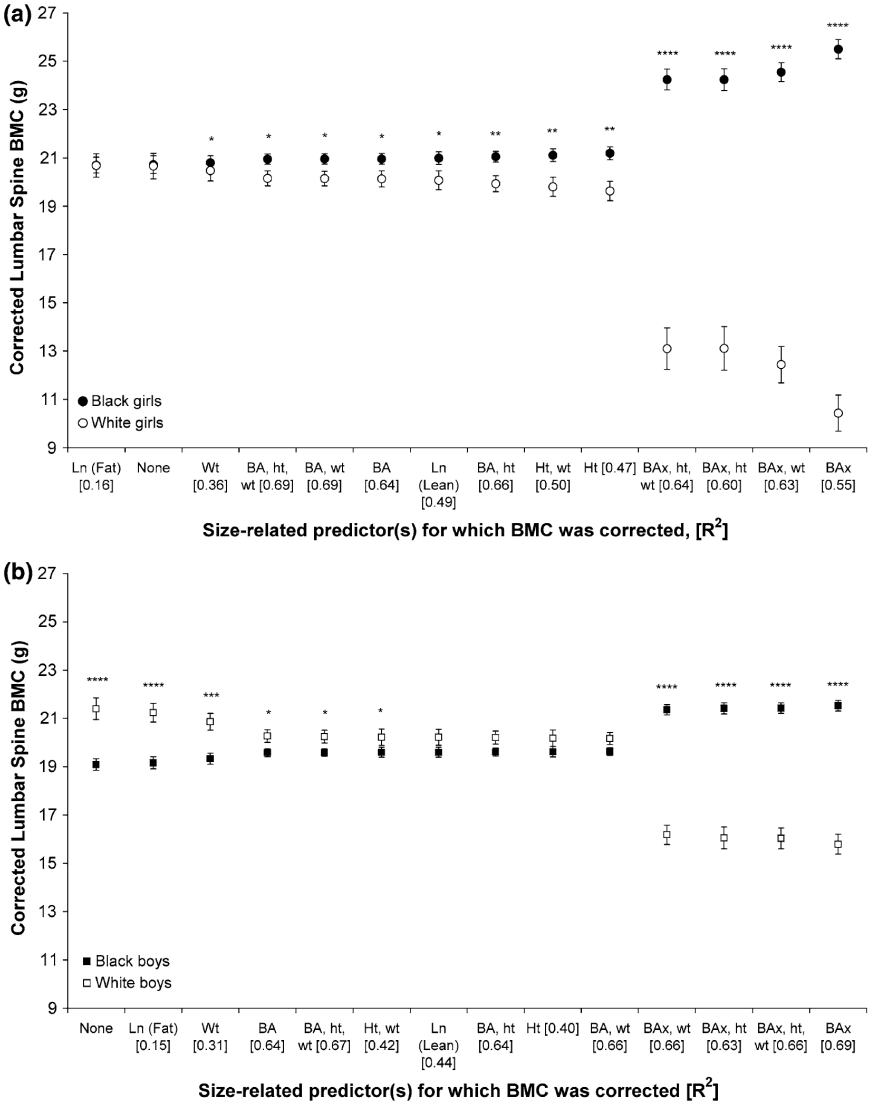
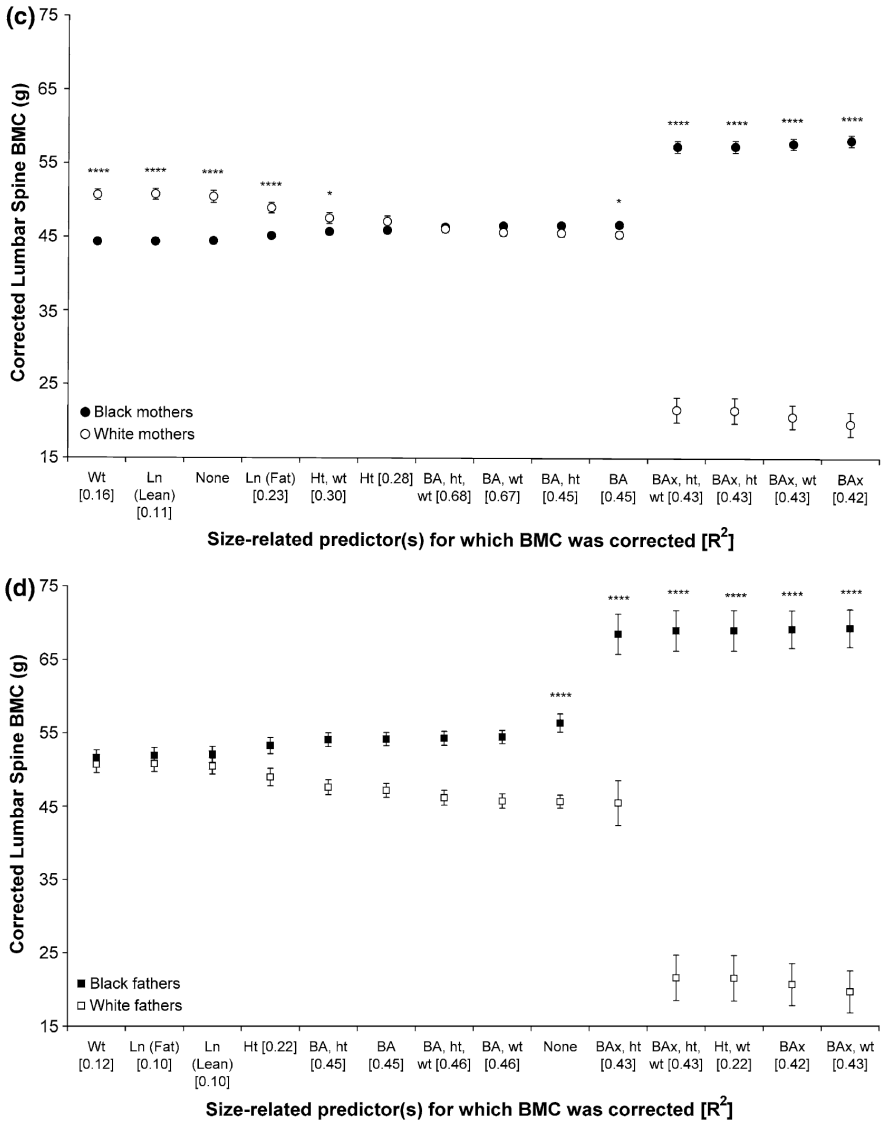


Fig. 2 Lumbar spine BMC (±SE) corrected for ln(height), ln(weight), or combinations of size-related predictors of BA, BA^{PC}, (BAx), height (ht), and/or weight (wt) in black and white girls (**a**), boys (**b**), mothers (**c**), and fathers (**d**). *Asterisks* indicate ethnic differences: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

BMC for BA^{PC}, height, and weight vs. BA, height, and weight increased ethnic differences in both adults and children at the femoral neck (in girls, from 7 to 69%; boys, 8 to 64%; mothers, 8 to 34%; fathers, 6 to 98%), unmasked ethnic differences at the lumbar spine (girls, 4% vs. 85%; boys, 3% vs. 34%; mothers, 1% vs. 166%; fathers, 2% vs. 89%), and may in part explain the 10-fold lower prevalence of femoral neck fractures in adult black South Africans compared to whites [9, 34].

DXA, histomorphometric, and radiogrammetric evidence has accumulated supporting superior bone quality and strength in black South Africans and African Americans compared to their white counterparts; the macroarchitecture of the proximal femur in blacks is characterized by narrower marrow cavities, thicker cortices, and lower buckling ratios (ratio of outer radius to cortical thickness), despite nonsignificant differences in outer bone diameter [35–37]. The microarchitecture of the iliac crest in South African blacks is characterized by thicker cortical bone, less porous cortices, greater endocortical wall thickness, and greater osteoid thickness. Adults in addition have fewer canals in the cortical bone and thicker trabeculae than whites [38–40]. Estimates of strength as determined by cross-sectional geometry (CSA and section modulus) at the femoral neck were greater in both South African blacks and African Americans compared to whites [35, 41]. These macro- and microarchitectural features are consistent with greater bone strength and lower fracture rates [37]. Lastly,

Fig. 2 continued



black South Africans have been shown to have greater bone apposition and formation rates [38, 39]. Smaller bones with thicker cortices and trabeculae have also been found using high-resolution pQCT in Chinese premenopausal women at the distal radius and tibia compared to white women [42]; these findings are similar to those at the femoral neck in the comparison between our black and white South African children.

Structural differences in bone are suggested to originate in the peripubertal period because few ethnic differences in bone size and microarchitecture before puberty have been reported [43]. Our findings demonstrate that differences in various DXA measures are present between children of African and European descent by age 10, suggesting that these differences had developed prior to puberty.

It is possible that the better bone mass in black children and adults might have been due to greater weight-bearing or physical activity in which poorer people might need to engage. In fact, we had previously proposed this to be a mechanism for the greater femoral neck BMD in black South African women [6]. However, given that black 10-year-old children, who are lighter than or of similar weight as white children, also have a greater femoral neck bone mass, other explanations must be sought. Physical activity is actually lower in our black than white children [3, 4], thus excluding physical activity as a possible

Table 3 Associations between children's and parents' BMC Z scores assessed by Pearson correlation coefficients (r) and heritability estimates ($½h^2$)

	Children vs. mothers																			
	Girls						Boys						Ethnic differences						Gender differences			
	Black			White			Black			White			Girls			Boys			Black		White	
	r	$½h^2 \pm SE$	n	r	$½h^2 \pm SE$	n	r	$½h^2 \pm SE$	n	r	$½h^2 \pm SE$	n	P			P			P		P	
Whole body	0.38[a]	0.39 ± 0.08	128	0.51[a]	0.40 ± 0.13	59	0.46[a]	0.40 ± 0.07	149	0.46[a]	0.45 ± 0.11	61	$r = 0.313$	$½h^2 = 0.946$		$r = 1.000$	$½h^2 = 0.701$		$r = 0.426$	$½h^2 = 0.925$	$r = 0.728$	$½h^2 = 0.769$
Femoral neck	0.31[a]	0.32 ± 0.09	128	0.44[a]	0.43 ± 0.12	60	0.23[d]	0.23 ± 0.08	151	0.29[d]	0.32 ± 0.12	62	$r = 0.344$	$½h^2 = 0.479$		$r = 0.184$	$½h^2 = 0.540$		$r = 0.478$	$½h^2 = 0.454$	$r = 0.352$	$½h^2 = 0.518$
Lumbar spine	0.20[d]	0.22 ± 0.10	128	0.27[d]	0.29 ± 0.14	59	0.42[a]	0.38 ± 0.07	152	0.29[d]	0.34 ± 0.13	62	$r = 0.645$	$½h^2 = 0.685$		$r = 0.333$	$½h^2 = 0.771$		$r = 0.543$	$½h^2 = 0.181$	$r = 0.908$	$½h^2 = 0.794$
Mid-radius	0.48[a]	0.52 ± 0.08	128	0.28[d]	0.28 ± 0.13	60	0.27[d]	0.25 ± 0.08	150	0.39[c]	0.40 ± 0.12	60	$r = 0.096$	$½h^2 = 0.103$		$r = 0.388$	$½h^2 = 0.311$		$r = 0.044$	$½h^2 = 0.018$	$r = 0.509$	$½h^2 = 0.499$
Distal one-third radius	0.40[a]	0.46 ± 0.09	128	0.36[c]	0.35 ± 0.12	59	0.15[d]	0.14 ± 0.07	149	0.34[d]	0.38 ± 0.12	58	$r = 0.772$	$½h^2 = 0.483$		$r = 0.201$	$½h^2 = 0.077$		$r = 0.026$	$½h^2 = 0.048$	$r = 0.905$	$½h^2 = 0.860$
	Children vs. fathers																			
	Girls						Boys						Ethnic differences						Gender differences			
	Black			White			Black			White			Girls			Boys			Black		White	
	r	$½h^2 \pm SE$	n	r	$½h^2 \pm SE$	n	r	$½h^2 \pm SE$	n	r	$½h^2 \pm SE$	n	P			P			P		P	
Whole body	0.11	0.12 ± 0.23	25	0.39	0.30 ± 0.15	24	0.28	0.31 ± 0.21	27	0.45[d]	0.36 ± 0.15	23	$r = 0.323$	$½h^2 = 0.516$		$r = 0.515$	$½h^2 = 0.846$		$r = 0.548$	$½h^2 = 0.545$	$r = 0.816$	$½h^2 = 0.779$
Femoral neck	0.49[d]	0.52 ± 0.29	25	0.38	0.32 ± 0.17	24	0.33	0.39 ± 0.22	27	−0.21	−0.18 ± 0.18	23	$r = 0.656$	$½h^2 = 0.558$		$r = 0.066$	$½h^2 = 0.049$		$r = 0.513$	$½h^2 = 0.722$	$r = 0.050$	$½h^2 = 0.051$
Lumbar spine	0.41[d]	0.46 ± 0.22	25	−0.03	−0.03 ± 0.21	24	0.47[d]	0.60 ± 0.22	28	0.49[d]	0.33 ± 0.13	23	$r = 0.127$	$½h^2 = 0.114$		$r = 0.931$	$½h^2 = 0.301$		$r = 0.799$	$½h^2 = 0.654$	$r = 0.070$	$½h^2 = 0.154$
Mid-radius	0.33	0.32 ± 0.19	25	−0.43	−0.06 ± 0.31	24	0.43[d]	0.46 ± 0.19	28	0.47[d]	0.34 ± 0.14	22	$r = 0.009$	$½h^2 = 0.305$		$r = 0.869$	$½h^2 = 0.616$		$r = 0.689$	$½h^2 = 0.604$	$r = 0.002$	$½h^2 = 0.248$
Distal one-third radius	0.59[d]	0.57 ± 0.20	25	−0.17	−0.20 ± 0.24	24	0.36	0.37 ± 0.18	28	0.50[d]	0.26 ± 0.10	22	$r = 0.005$	$½h^2 = 0.070$		$r = 0.571$	$½h^2 = 0.592$		$r = 0.304$	$½h^2 = 0.459$	$r = 0.023$	$½h^2 = 0.233$

Z scores were calculated from the means and standard deviations of BMC adjusted for height, weight, and BA^{PC} (PCs listed in Table 1) and age in adults. Z scores were used so that children and their parents' data were comparative

[a–d] Significantly correlated: [a] $P < 0.0001$, [b] $P < 0.001$, [c] $P < 0.01$, [d] $P < 0.05$

explanation. Thus, it appears that skeletal loading is unlikely to have contributed to the higher bone mass, and we now postulate that the differences are mainly genetically determined in otherwise unfavorable social and environmental conditions (poor growth and nutrition [1] and low dietary calcium intake [2] of black children). In support of our findings, a study conducted in individuals of African descent in the West Indies, which analyzed genetic and environmental factors influencing BMD measured by both DXA and QCT, found overall heritability of both areal and volumetric BMD to be substantial [44].

Areal BMD remains an important predictor of fracture risk. The calculation of areal BMD or another measure of apparent density, BMAD, assumes PCs to be 1 (when calculating BMD), 1.5 (when calculating BMAD at the femoral neck and mid-radius), or 2 (when calculating BMAD at the whole body). PCs calculated in this study were for the most part significantly different from each of the three values in both children and their parents, confirming that neither BMD nor BMAD reflects true volumetric bone density. It is of interest to note that the calculated PCs were generally similar for the two ethnic groups at each of the different bone sites with the exception of the femoral neck in both boys and girls and at the distal third of the radius in boys. Similar PCs suggest that three-dimensional size changes in bone associated with growth are similar at the whole body, lumbar spine, and mid-radius in the two ethnic groups.

Maternal BA and size-adjusted BMC significantly predicted their children's BA and adjusted BMC at all skeletal sites. Heritability by maternal descent was estimated to be $\sim$30% and similar for both black and white children. This is not the first study to demonstrate the influence of maternal genetics on the prepubertal acquisition of bone mass [12, 45], but it is the first to show similar genetic influences in BMC in both black and white prepubertal populations, despite their differences in body and bone size and environmental influences. Black South Africans, children in particular, are exposed to a number of environmental factors known to impact negatively on bone mass, such as poor growth and nutrition [1], low calcium intake [2], and little physical activity [3, 4]. Given the important contributions that diet and other environmental factors have on the phenotypic variance in bone mass or BMD, lower bone mass and heritability estimates in blacks would be expected. Lower heritability estimates have been shown before for stature in West African populations compared to European populations, which were explained by the rigors of the traditional way of life in West African surroundings [46].

In general, the possible genetic contribution to the variance of the bone mass phenotype is reported to be 50–80% at any age or in any group [47]. The bone mass phenotype in black South Africans is expressed even in pre-/early pubertal childhood. These heredity estimates in black children are comparable to those from environmentally advantaged white South African children and Caucasians from other parts of the world.

Mother–daughter estimates of heritability of BMC are usually better than mother–son estimates [12]. In the current study, this was true only at the mid- and distal one-third of the radius in black children; at all other sites no differences in heritability between male and female children were seen. It has also been suggested that estimates of maternal heritability are better than paternal estimates in both boys and girls [12]. Due to the small number of fathers, a major limitation in the current study, it was not possible to draw any conclusions from our data.

In conclusion, this study confirms that correcting BMC for height, weight, and BA^{PC} was the combination of size-related adjustments that accounted for the greatest proportion of the variance of BMC at all skeletal sites. This combination increased ethnic differences in BMC 2.6 times at the femoral neck, unmasked ethnic differences at the lumbar spine in both adults and children, and may in part explain the lower prevalence of fragility fractures at the hip in black South Africans compared to whites [34]. Heritability by maternal descent, estimated by regressing children's Z scores on parents Z scores, was $\sim$30%, comparable between environmentally disadvantaged black and advantaged white South African children, and similar to that found in Caucasians from other parts of the world. It is unclear at this stage whether improvement in the adverse environmental factors in our black children would result in an increase in bone mass, even lower fracture rates, and greater heritability. The intriguing question remains as to how genetic influences maintain bone mass in the face of what are generally considered to be adverse environmental factors. These genetic influences not only have a positive effect on bone mass during childhood but also are maintained through adult life and are associated with a very low incidence of femoral neck fractures in the elderly.

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