Ethnic and Gender Related Longitudinal Changes in Metacarpal Dimensions through Late Childhood and Adolescence, and their Associations with Bone Growth in a South African Cohort

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A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand

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DECLARATION

I, Ansuyah Magan, declare that this thesis titled “Ethnic and gender related longitudinal changes in metacarpal dimensions through late childhood and adolescence, and their associations with bone growth in a South African cohort” is my own work.

It is submitted for the degree of Doctor of Philosophy in the branch of Paediatrics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination to this or any other university.

Signature ……

……day of ....., 2018

DEDICATION

I dedicate this thesis to my dearest husband Mookesh Desai

ACKNOWLEDGEMENTS

"Everyone is my teacher.

Some I seek.

Some I subconsciously attract.

Often, I learn simply by observing others.

Some may be completely unaware that I'm learning from them,

Yet I bow deeply in gratitude."

Eric Allen

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Table of Contents

DECLARATION ..... **i**

DEDICATION ..... **ii**

ACKNOWLEDGEMENTS ..... **iii**

THESIS MATERIAL ..... **xiv**

LIST OF ABBREVIATIONS ..... **xvi**

PREFACE ..... **xviii**

ABSTRACT ..... **xix**

CHAPTER 1

Literature review **1**

1.1 Changes in bone through the life-stages 1

1.2 Bone's structural dimensions and strength 3

1.3 Bone mass 5

1.4 Determinants of bone mass 6

1.4.1 Genetics and heritability 7

1.4.2 Ethnicity 8

1.4.3 Puberty 11

1.4.3.1 Hormonal interactions during puberty 12

1.4.3.2 Linear bone growth 13

1.4.3.3 Bone growth in width 14

1.4.4 Nutrition 15

1.4.5 Body composition 19

1.4.5.1 Fat mass 19

1.4.5.2 Lean mass 21

1.4.6 Physical activity 22

1.4.7 Socio-economic status 24

1.5 Fracture epidemiology 27

1.6 Methods of bone mass assessment 29

1.6.1 Dual energy x-ray absorptiometry 31

1.6.2 Peripheral quantitative computed tomography 32

1.6.3 Radiogrammetry....33
1.7 Metacarpal Indices....36
1.7.1 Metacarpal Index....38
1.7.2 Bone Mineral Density....38
1.7.3 Polar Moment of Inertia....38
1.7.4 Section Modulus....38
1.7.5 Stress-Strain Index....39
1.7.6 Slenderness Index....39
1.8 The association between metacarpal indices and fracture....40
1.9 Bone health in South African children and adolescents....43
1.10 Summary and Gaps in the Literature....47
1.11 Aims, Hypotheses and Objectives of this study....47
CHAPTER 2
Study Methods....50
2.1 Study population....50
2.2 The Bone Health Cohort....51
2.3 Anthropometric measurements....51
2.4 Pubertal assessment....52
2.5 Radiography....52
2.6 Radiogrammetry....52
2.6.1 Intra-observer Reliability....53
2.6.2 Calculation of Metacarpal Indices....54
2.7 Peripheral Quantitative Computed Tomography Imaging....54
2.8 Stress-Strain Index....55
2.9 Fractures....57
2.10 Physical Activity....58
2.11 Data analyses used in Chapter 3....59
2.12 Data analyses used in Chapter 4....60
2.13 Data analyses used in Chapter 5....62
2.14 Summary....65

CHAPTER 3
Metacarpal growth during adolescence in a longitudinal South African cohort 66
3.1 Methods 66
3.2 Results 67
3.3 Discussion 87
3.4 Summary of major findings 91
CHAPTER 4
A longitudinal comparison of appendicular bone growth and markers of strength through adolescence in a South African cohort using radiogrammetry and pQCT 92
4.1 Methods 92
4.2 Results 93
4.3 Discussion 107
4.4 Summary of major findings 111
CHAPTER 5
Metacarpal indices and their association with fracture in South African children and adolescents 112
5.1 Methods 112
5.2 Results 112
5.3 Discussion 123
5.4 Summary of major findings 127
CHAPTER 6
Summary and Conclusion 128
6.2 Summary of thesis objectives, hypotheses and key findings 129
6.3 Emerging Themes 131
6.3.1 Methodological theme 131
6.3.2 Empirical theme 133
6.4 Strengths and limitations of the thesis 136
6.5 Implications of the study findings 138
6.6 Future Research 140

6.7 Conclusion 142
REFERENCES 143
Appendix A: Plagiarism Declaration 161
Appendix B: Plagiarism Report 162
Appendix C: Ethics Clearance Certificate 163
Appendix D: Year 17 Participant fracture questionnaire 164
Appendix E: Year 17 Caregiver fracture questionnaire 167
Appendix F: Year 17 Verification of fracture questionnaire 170
Appendix G: Year 15 Physical activity questionnaire 173
Appendix H: PDF of Heterogeneity of fracture pathogenesis in urban South African children: The Birth to Twenty cohort 187
Appendix I: Authors' contributions to publications 195
Appendix J: PDF of Metacarpal growth during adolescence in a longitudinal South African cohort 196
Appendix K: PDF of A longitudinal comparison of appendicular bone growth and markers of bone strength in a South African cohort using radiogrammetry and pQCT 205
Appendix L: PDF of Metacarpal indices and their association with fracture in South African children and adolescents 215

List of Tables

Table 1.1 Comparison of radiogrammetry, DXA and pQCT 30

Table 1.2 Indices derived using radiogrammetry 37

Table 1.3 Summary of studies that have used metacarpal indices to predict fracture 41

Table 1.4 Summary of key findings from studies of the Bt20 and Bone Health cohorts relevant to this thesis 44

Table 2.1 Coefficients of variation of radiogrammetry measures 53

Table 2.2 The grading system used to define the degree of trauma sustained at the time of fracture 58

Table 2.3 The ideal sample size for a given effect size in bone measure between fractured and non-fractured groups 64

Table 3.1 Summary of SITAR analyses of ethnic differences in the size, tempo and velocity of metacarpal length, bone width and medullary width growth during adolescence, as measured against chronological age 72

Table 3.2 Summary of SITAR analyses of sex differences in the size, tempo and velocity of metacarpal length, bone width and medullary width growth during adolescence, as measured against chronological age 73

Table 3.3 Unadjusted metacarpal dimensions in black and white females and males 74

Table 3.4 Metacarpal dimensions, adjusted for height, in black and white females and males 75

Table 3.5 Mean annual changes in bone width, medullary width and cortical thickness in black and white females 80

Table 3.6 Mean annual changes in bone width, medullary width and cortical thickness in black and white males 81

Table 3.7 Differences in bone width, cortical thickness and medullary width between the age at first measurement and age at maximal medullary width, and between age at maximal medullary width and age at last measurement 86

Table 4.1 Fixed-effects regression estimates of metacarpal bone measures as the outcome and radial and tibial bone measures as predictors in black and white females and males 95

Table 4.2 Results from mixed-effects growth models summarising differences in metacarpal, radial and tibial bone width growth 99

Table 4.3 Results from mixed-effects growth models summarising differences in metacarpal, radial and tibial cortical thickness growth 101

Table 4.4 Results from mixed-effects growth models summarising differences in metacarpal, radial and tibial medullary width change 104

Table 4.5 Results from mixed-effects growth models summarising differences in metacarpal, radial and tibial stress-strain index change 106

Table 5.1 Cohort fractures by site and grade of trauma 114

Table 5.2 Cohort anthropometry, bone measures and bone indices at 17 years of age, and physical activity at 15 years of age 115

Table 5.3 Sex and ethnic differences in mean Z-scores with standard deviations (SD) for anthropometric measures, bone measures, metacarpal indices and physical activity in 17-year old participants with and without a history of fracture 117

Table 5.4 Associations between anthropometric measures, bone measures, bone indices and physical activity, and history of fracture at age 17 years 118

Table 5.5 Correlation of metacarpal indices with height from ages 10 until 16 years 121

Table 5.6 GEE assessment of metacarpal bone measures and indices for the combined sex and ethnic groups from ages 10 until 16 years and odds ratios for a positive fracture history at age 17 years 122

Table 6.1 Summary of the thesis objectives, hypotheses and key findings 129

List of Figures

Figure 1.1 Arrangement of cortical and trabecular bone within a long bone ……1

Figure 1.2 Changes in cortical and trabecular bone within a long bone and the spine during the life-stages ……2

Figure 1.3 Galileo's illustration of how the dimensions of different sized bones need to vary to make them equally strong in relation to their function ……3

Figure 1.4 A schematic diagram showing how bone diameter relative to length varies among individuals ……4

Figure 1.5 Determinants of whole bone strength ……5

Figure 1.6 Bone mass across the lifespan with optimal and suboptimal lifestyle choices……6

Figure 1.7 Schematic representation of the non-modifiable and modifiable factors which influence peak bone mass attainment ……6

Figure 1.8 Hypothalamic-pituitary-gonadal-skeletal axis in girls ……12

Figure 1.9 Calcium homeostasis which is maintained by vitamin D, parathyroid hormone and calcitonin ……16

Figure 1.10 Fractures per year by age and sex distribution in South African children……28

Figure 1.11 Fracture rates per year by age and ethnicity in South African children……28

Figure 1.12 The effect of bone size on areal bone mineral density ……31

Figure 1.13 Radiogrammetry measurement of metacarpal bones ……34

Figure 1.14 Influence of bone's cross-sectional geometry on bone strength ……39

Figure 2.1 Points of measurement of metacarpal bone length, and bone width and medullary width at the midshaft ……53

Figure 2.2 Calculation of the stress-strain index ……56

Figure 3.1 Participant recruitment (N) and the number of participants (n) with their respective total number of radiographs over the study period ……68

Figure 3.2. Scatterplot of metacarpal length against chronological age, and individual curves adjusted for tempo and velocity, with the mean adjusted curve, for metacarpal length growth by ethnicity ……70

Figure 3.3 Scatterplot of metacarpal length against chronological age, and individual curves adjusted for tempo and velocity, with the mean adjusted curve, for metacarpal length growth by sex 71

Figure 3.4 Scatterplot of metacarpal bone width against chronological age, and individual curves adjusted for tempo and velocity, with the mean adjusted curve, for metacarpal bone width growth by ethnicity 77

Figure 3.5 Scatterplot of bone width against chronological age, and individual curves adjusted for tempo and velocity, with the mean adjusted curve, for bone width growth by sex 78

Figure 3.6 Scatterplot of bone width against years from peak metacarpal length velocity, and individual curves adjusted for tempo and velocity, for bone width growth by sex 79

Figure 3.7 Scatterplot of metacarpal medullary width against chronological age, and individual curves adjusted for tempo and velocity, with the mean adjusted curve, for metacarpal medullary width growth by ethnicity 83

Figure 3.8 Scatterplot of medullary width against chronological age, and individual curves adjusted for tempo and velocity, with the mean adjusted curve, for medullary width growth by sex 84

Figure 3.9 Scatterplot of medullary width against years from peak metacarpal length velocity, and individual curves adjusted for tempo and velocity, for medullary width growth by sex 85

Figure 4.1 Participant recruitment (N) and the number of participants (n) aged between 12 and 20 years with their respective total number of radiographs over the study period 94

Figure 4.2 Metacarpal stress-strain index in relation to radial muscle cross-sectional area in black and white females and males 97

Figure 4.3 Metacarpal stress-strain index in relation to tibial muscle cross-sectional area in black and white females and male 97

Figure 4.4 Mixed model growth curves of metacarpal, radial and tibial bone width against years from peak metacarpal length velocity in black and white females and males 98

Figure 4.5 Mixed model growth curves of metacarpal, radial and tibial cortical thickness against years from peak metacarpal length velocity in black and white females and males 100

Figure 4.6 Mixed model growth curves of metacarpal, radial and tibial medullary width against years from peak metacarpal length velocity in black and white females and males ……..103

Figure 4.7 Mixed model growth curves of metacarpal, radial and tibial stress-strain index against years from peak metacarpal length velocity in black and white females and males ……105

Figure 5.1 Participant recruitment (N) and the number of participants (n) with their respective total number of radiographs over the study period ……...113

Figure 5.2 Odds ratios for having a history of fracture based on bone measures and indices at 17 years of age, after covarying for height and formal physical activity ……..120

THESIS MATERIAL

The following publications and presentations have emanated from the research conducted for this thesis:

Peer Reviewed Publications

1. **Magan A, Nyati LH, Micklesfield LK, Norris SA and Pettifor JM** (2017) Metacarpal growth through puberty and adolescence in a longitudinal South African cohort. Journal of Bone and Mineral Research 32(9):1926-1934. DOI: 10.1002/jbmr.3179

2. **Magan A, Micklesfield LK, Nyati LH, Norris SA and Pettifor JM**. A longitudinal comparison of appendicular bone growth and strength through adolescence in a South African cohort using radiogrammetry and pQCT. Osteoporosis International. DOI: 10.1007/s00198-018-4761-9

3. **Magan A, Micklesfield LK, Norris SA, Thandrayen K, Munthali R and Pettifor JM**. Metacarpal indices and their association with fracture in South African children and adolescents. Calcified Tissue International. DOI: 10.1007/s00223-018-0467-4

For each of the abovementioned publications, I was responsible for data collection, data management including cleaning and statistical analysis, interpretation of the data, and drafting and editing of the manuscript. Please refer to Appendix I for more details regarding author contributions.

Conference proceedings

1. **Magan A**, **Micklesfield, LK, Norris SA and Pettifor JM** Ethnic and gender differences in metacarpal dimensions in black and white South African children from pre-puberty through adolescence 7th International Conference on Children's Bone Health, 27th - 30th June 2015, Salzburg, Austria

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LIST OF ABBREVIATIONS

Abbreviation/Term	Definition
aBMD/BMD	(Areal) bone mineral density
ALSPAC	Avon Longitudinal Study of Parents and Children cohort
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
aPHV/PHV	(Age at) peak height velocity
aPLV/PLV	(Age at) peak metacarpal length velocity
BHC	Bone Health Cohort
BIC	Bayesian Information Criterion
BMAD	Bone mineral apparent density
BMC	Bone mineral content
BMI	Body mass index
BMDCS	Bone Mineral Density in Childhood Study
BSI	Bone strength index
Bt20	Birth to Twenty cohort
cm	Centimetres
CV	Coefficient of variation
DXA	Dual energy x-ray absorptiometry
DXR	Digital (automated) x-ray radiogrammetry
g	Grams
GEE	Generalised estimating equations
GH	Growth hormone
HSA	Hip structural analysis
HPG	Hypothalamic-pituitary-gonadal
MCI	Metacarpal Index

Abbreviation/Term	Definition
IGF-1	Insulin-like growth factor-1
L	Litre
MCSA	Muscle cross-sectional area
mm	Millimetres
MRI	Magnetic resonance imaging
NHANES	National Health and Nutrition Examination Survey
nmol	Nanomoles
PBM	Peak bone mass
pQCT/QCT	(Peripheral) quantitative computed tomography
RDA	Recommended daily allowance
SA	South African
SD	Standard deviation
SEM	Standard error of the mean
SES	Socio-economic status
SITAR	Superimposition by Translation and Rotation
SLI	Slenderness index
SM	Section modulus
SSIp	Stress-strain index (polar)
TW3 RUS	Tanner-Whitehouse 3 radius, ulna and short bones of the carpus
US	United States
vBMD	Volumetric bone mineral density
WHO	World Health Organisation
Z-score	A statistical measurement of a score's relationship to the mean in a group of scores
25-(OH)D	25-hydroxyvitamin D

PREFACE

The seed for my interest in bone biology was planted in my teens when I had the privilege of observing my dad perform internal fixation of a radio-ulnar fracture. During my pursuit of a BSc. degree, I met Professor Ugo Ripamonti, who elevated my interest and enthusiasm in bone's remarkable ability to repair and regenerate, and with whom I continued my studies, culminating in postgraduate research on the influence of geometry of a hydroxyapatite substrate on osteogenesis in non-human primates.

As a dental practitioner, my interest in bone took another path. I now looked at craniofacial growth patterns and assessed skeletal maturity indicators on hand-wrist radiographs to determine when to implement orthodontic treatment, managed alveolar bone loss due to periodontal disease, and observed varied bone loss patterns in edentulous patients as a result of differing functional loads. I realised differences in maxillary and mandibular bone density and how this influenced implant osseointegration. I was intrigued by the factors which differentiate bone from dentine, both so similar in their organic and inorganic composition, yet so different in their structure and reparative capabilities. And, when treating paediatric patients, I speculated on ethnic differences and secular changes in tooth eruption times and differences in pubertal timing, all of which influence treatment.

My introduction to Professor John Pettifor and the Birth to Twenty (Bt20) project by my long-standing mentor Professor Beverley Kramer was pure serendipity. I realised the uniqueness of the longitudinal hand-wrist radiograph collection of South African children from two ethnic groups. Longitudinal data on bone growth are sparse, particularly in African children. I believe that this study of the metacarpal bones from the radiographs of children from the Bt20 Bone Health subcohort will provide further understanding of bone growth in South African children, and, that the outcomes could be applied to future research in bone health, and craniofacial and dental development relevant to our population.

ABSTRACT

Introduction

Attainment of peak bone mass in childhood influences skeletal fragility in late adulthood. Longitudinal studies on bone growth in South African (SA) children are sparse; however, ethnic differences in fracture rates have been reported in these children. This thesis presents unique longitudinal data on metacarpal bone development in two SA ethnic groups, spanning 12 years through puberty and adolescence, a period during which significant physiological changes are experienced.

Aims

The main aims of this thesis were to assess metacarpal bone growth longitudinally during adolescence in SA children of different ethnic and sex groups, and, to compare its growth and strength patterns (measured by radiogrammetry) to those of the non-weightbearing radius and the weight-bearing tibia (measured by pQCT). I further examined the association between radiogrammetric metacarpal bone measures and indices and fracture risk.

Hypothesis

There are ethnic and sex differences in appendicular bone growth and strength in South African children.

Objectives

1. To monitor the drift of the periosteal and endocortical bone surfaces during metacarpal growth longitudinally in black and white South African children and adolescents.
2. To compare the growth and strength of weight-bearing and non-weight bearing bones longitudinally in South African adolescents.

3. To determine whether metacarpal indices predict fracture risk in South African children and adolescents.

Design

Radiogrammetry measures of the second metacarpal bone form the basis of the three studies reported in this thesis. Radiogrammetry was performed at the midshaft of the second metacarpal bone on hand-wrist radiographs obtained from 572 children from the Birth to Twenty Bone Health Cohort (BHC) annually from ages 9 until 21 years. These radiographs represent the largest longitudinal collection in African children. Bone length, bone width and medullary width were measured in millimetres using digital vernier callipers on a total of 4730 radiographs. Superimposition by Translation and Rotation (SITAR) was used to obtain age at peak metacarpal length velocity (aPLV). Bone width and medullary width were modelled using SITAR against both chronological age and age from PLV.

In the second study of the thesis, radiogrammetry measures of the second metacarpal bone were compared to measurements obtained from peripheral quantitative computed tomography scans of the radius (at 65% of the length of the radius) and tibia (at both 38% and 65% of tibial length) taken annually on 372 black and 152 white participants from the BHC between the ages of 12 and 20 years. Measurements for individuals who had images of all 3 bones were aligned by age from PLV. Metacarpal, radial and tibial bone width (mm), cortical thickness (mm), medullary width (mm), the stress-strain index (SSIp, mm^3) and muscle cross-sectional area (MCSA, mm^2) were measured. Bivariate regression analysis was performed to determine the association between metacarpal bone width, cortical thickness and SSIp, and MCSA of the radius and tibia. Mixed-effects models were used to compare the growth patterns of bone width, cortical thickness, medullary width and SSIp of the metacarpal, radius and tibia, for each sex and ethnic group when aligned by age from PLV.

In the third study of the thesis, metacarpal bone length, bone width and medullary width were measured in 359 BHC children between 10 and 17 years of age, and the following proxies for bone strength calculated: Metacarpal Index (MCI), Bone Mineral Density (BMD), Section Modulus (SM), Stress-strain Index (SSIp) and Slenderness Index (SLI). Height and weight were measured annually. Self-reported physical activity was obtained at age 15 years for the preceding 12 months. Fracture history was obtained retrospectively at age 17 years.

Multivariate logistic regression analyses, adjusting for height and formal physical activity, were performed to examine the associations between anthropometric measures, bone measures and bone indices, and fracture history at 17 years separately for each sex and ethnic group.

Results

In the first study I showed that in black and white females, tempo and velocity of metacarpal length growth was similar. However, in males, black children attained PLV seven months later than whites ($p < 0.0001$), and had a longer second metacarpal ($p < 0.05$), greater bone width size ($p < 0.02$), later growth tempo ($p < 0.0009$) and reduced velocity ($p < 0.0001$) than white males. Medullary width growth velocity in black females and males peaked 2 years prior to attainment of PLV and exceeded that of their white peers ($p < 0.0001$) in whom it peaked 6-12 months after PLV attainment. Black adolescents therefore had wider bones with relatively thinner cortices and wider medullary cavities than their white peers. Ethnic and sex differences also occurred in the timing of medullary width contraction that accompanied expansion in bone width and cortical thickness. In black males, medullary width contraction commenced approximately 3 years later than in black females, whereas in white males this occurred a year later than in white females.

The second study showed that, from 12 until 20 years, the associations between metacarpal measures (bone width, cortical thickness and SSIp) and MCSA at the radius (males R^2: 0.33 - 0.45; females R^2: 0.12 - 0.20) were stronger than the tibia (males R^2: 0.01 - 0.11; females R^2: 0.007 - 0.04). In all groups, radial bone width, cortical thickness and medullary width accrual rates were similar to those of the metacarpal, except in white females who had lower radial cortical thickness (0.04 mm/year) and greater radial medullary width (0.06 mm/year) accrual. In all groups, except in white males for cortical thickness, tibial bone width and cortical thickness accrual rates were greater than at the metacarpal. Tibial medullary width (0.29 - 0.35 mm/year) increased significantly compared to metacarpal medullary width (-0.07 - 0.06 mm/year) in males only. In all groups, the SSIp increased in each bone throughout adolescence, except tibial SSIp in white females.

In the third study I found that, at age 17 years, 82 (23%) participants (black, 16%; white, 42%; $p < 0.001$) reported a previous fracture. None of the metacarpal bone measures or indices

were associated with fracture in black participants. In white females, after adjusting for physical activity, a 1 SD increase in SLI was associated with doubling the fracture risk (95% CI: 1.08; 3.98). In white males, indices usually associated with bone strength were associated with an increase in fracture risk; a 1 SD increase in BMD was associated with a 2.62-fold increase in fracture risk (95% CI: 1.22; 10.75), whilst a 1 SD greater SM (95% CI: 1.07; 4.89) and SSIp (95% CI: 1.11; 4.47) were associated with a more than twofold increase in fracture risk, after adjustment for height and physical activity.

Conclusions

This longitudinal African study on bone growth during adolescence revealed distinct ethnic and sex differences in bone acquisition, measured using radiogrammetry. Irrespective of sex or ethnicity, metacarpal growth was similar to the growth of the non-weight bearing radius but differed from that of the weight-bearing tibia, when aligned on a common biological maturity indicator. The local and systemic factors influencing site-specific differences require further investigation. No single metacarpal index consistently predicted fracture across the four groups which may be due to ethnic and sex differences in bone microstructure, muscle mass and skeletal loading. Thus, metacarpal radiogrammetry does not reliably predict fracture in SA children.

CHAPTER 1

Literature review

This chapter describes bone growth during childhood and adolescence, and the determinants of bone mass and strength in the context of international and South African (SA) studies. Methods of bone mass measurement are discussed, with a focus on radiogrammetry, which has been used in all the studies in this thesis. The chapter concludes with a synopsis of the literature review, a brief overview of the gaps in the literature, and, the aims and objectives of this study.

1.1 Changes in bone through the life-stages

Bone tissue is comprised of a 33% organic and a 67% inorganic matrix arranged in two specific configurations, namely cortical (compact) and trabecular (spongy) bone (Figure 1.1), which are separated at the endocortical interface.

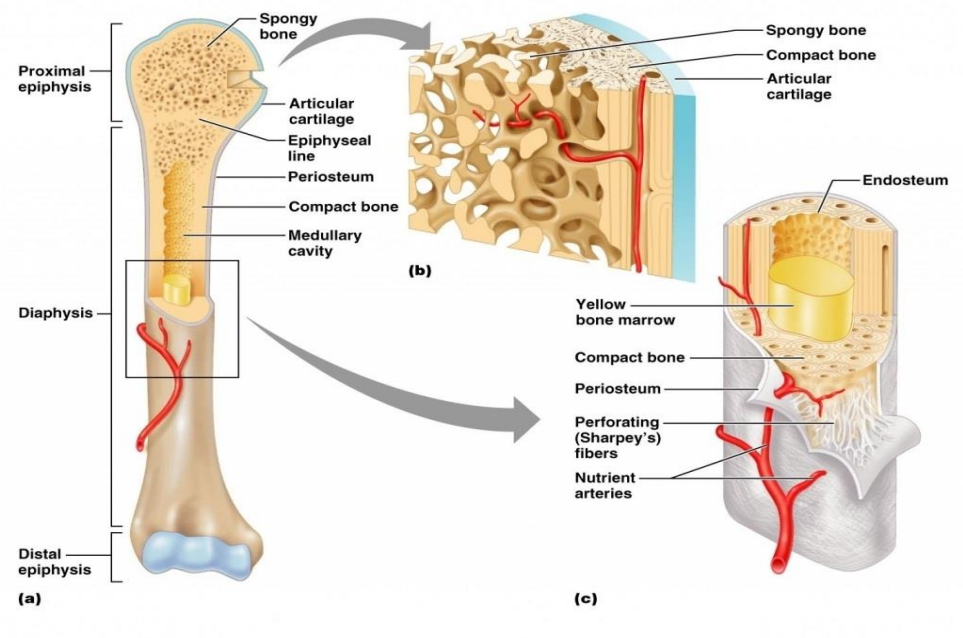


Figure 1.1 Arrangement of cortical and trabecular bone within a long bone [1]

Bone growth is a continuous process with expansion in its width occurring throughout life [2]. In the classic radiogrammetry study of 2799 adults from five population groups, Garn and co-workers [2] showed growth in the width of the second metacarpal bone to continue well into the eighth decade. Bone growth and repair occur via the processes of modelling and remodelling, respectively [3]. Long bones grow in width by bone apposition at the periosteal surface with modelling and remodelling at the endocortical surface. Changes at the periosteal and endocortical surfaces therefore determine a bone's diameter, its cortical mass and the distance of the cortices from the neutral axis (Figure 1.2), all of which influence bone strength [4].

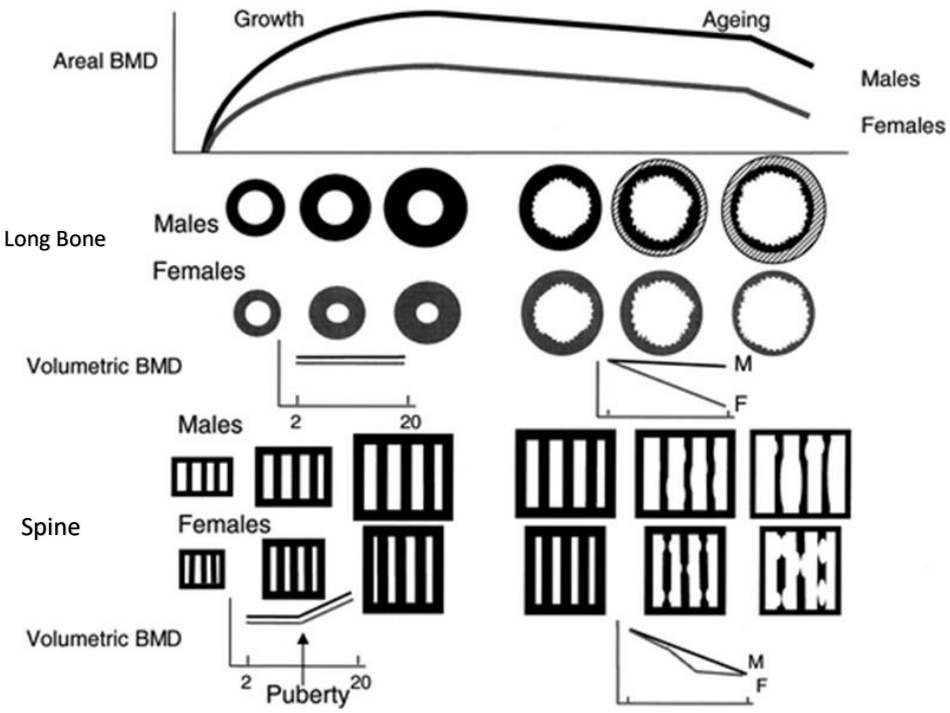


Figure 1.2 Changes in cortical and trabecular bone within a long bone and the spine during the life-stages. The x-axes on the left represent changes from 2 until 20 years, whilst those on the right represent young adulthood to elderly. During growth, areal BMD increases because bone size increases. There is no sex difference in peak volumetric BMD (M - males represented by bold line; F - females). During aging, men and women lose similar amounts of bone by endocortical resorption. Men have greater periosteal apposition than women, so that net bone loss is less in men than women. With age, there is trabecular thinning in men, but complete loss of trabeculae in women. Adapted from Seeman, 2001 [4].

As bone grows and increases in size, there is a corresponding increase in areal bone mineral density (aBMD) (Figure 1.2) [5]. Males, due to their greater bone size will have greater aBMD than females, but there may not necessarily be a sex difference in volumetric bone mineral density (vBMD) [5]. During aging, endocortical resorption is similar in both sexes; however, males have less net bone loss due to their greater apposition at the periosteal surface than females [4]. At the spine, aging females experience a decrease in the number of bony trabeculae whilst in males there is a thinning of trabeculae [6, 7] (Figure 1.2). Biomechanically, this translates to a loss in bone strength of 20% for trabecular thinning, 70% for trabecular loss and 77% for combined thinning and loss [8].

1.2 Bone's structural dimensions and strength

The relationship between the size and shape of a structure was first realised more than four centuries ago by Galileo (Figure 1.3). In all living organisms, the proportion of length increase is approximately two-thirds power of the diameter [9].

Figure 1.3 Galileo's illustration of how the dimensions of different sized bones need to vary to make them equally strong in relation to their function. The bones of large animals need to be proportionally thicker relative to their size than bones of smaller animals [10].

The long bones of the appendicular skeleton are adapted for their functions of reaching and grasping (upper limb), and, supporting the body and locomotion (lower limb) by differences in their length and cross-sectional geometry [11, 12] (Figure 1.4). Within the body, appendicular bones vary with respect to their cross-sectional geometry and length [13]. A non-weight bearing bone such as the radius has a small diameter relative to its length and relatively thick cortices, whilst the humerus, due to the influence of its associated musculature, and the femur which supports the body, have a wide diameter relative to bone length and relatively

thin cortices [11, 14]. There is also inter-individual variation in cross-sectional bone geometry, which in a longitudinal study of the metacarpal index (ratio of combined cortical thickness to bone diameter) was shown to be established by 2 years of age [15].

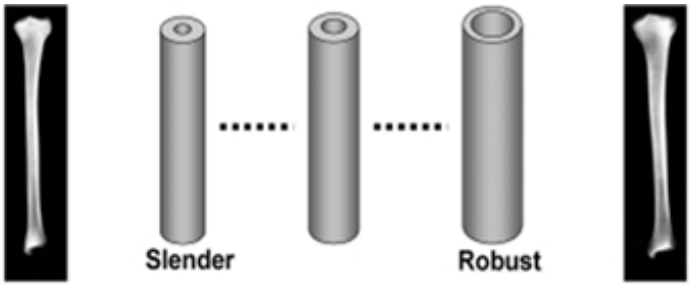


Figure 1.4 A schematic diagram showing how bone diameter relative to length varies among individuals. Although individuals may have bones of the same length, there can be variation in bone diameter and cortical thickness. Narrow bones have relative thick cortices (slender) compared to wide bones which have relatively thin cortices (robust). Modified from Jepsen 2011 [12].

The skeletal system functionally compensates for differences in external bone size, and it has been shown that in healthy individuals the cortical bone to trabecular bone ratio may be predicted from bone size [12, 16]. Wide diameter bones are more resistant to bending and torsional forces than narrow diameter bones as their relatively thinner cortices are further removed from the bone's neutral axis, thus conferring strength [17]. Under similar loading conditions wide and narrow bones fair similarly; however, under extreme or repetitive loading, narrow bones may be more susceptible to fracture due to their more highly mineralised cortices, which are also closer to the neutral axis of the bone [18]. Pandey and co-workers [15] suggested that bone modelling, and hence strength, may differ between narrow and wide bones. However, in a peripheral quantitative computed tomography (pQCT) study of the skeletal remains of adult African Americans, a strong correlation was reported between the cortical area, cortical density and strength of the second and third metacarpal bones with those of the major long bones of the appendicular skeleton [19]. It was suggested, therefore, that the metacarpal bones may be useful in longitudinal studies of bone biology [19].

1.3 Bone mass

Bone mass, a component of bone strength, is the quantity of bone tissue in the skeleton. Bone mass is a function of the bone size and its volumetric mineral density [20]. Bone mass and volume, its geometry or architecture, and matrix properties together determine strength, and, ultimately fracture risk [21, 22] (Figure 1.5). The relative contribution of each component to bone strength remains unanswered.

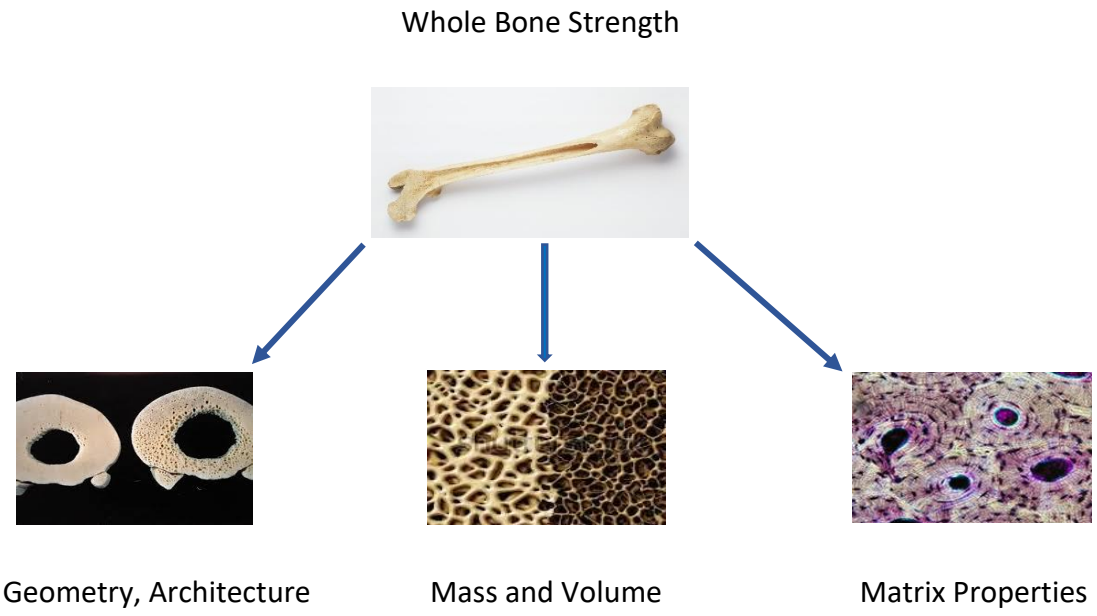


Figure 1.5 Determinants of whole bone strength. Geometry or architecture refers to the spatial distribution of cortical and trabecular components within the cross-sectional bone dimension. Mass and volume represent the quantity of bone, and, bone's matrix properties are determined by its mineral content and tissue properties.

Bone mass accrues rapidly from infancy and reaches a peak between the end of the second decade or in the third decade of life, depending on the skeletal site [23, 24]. In a longitudinal study of Canadian women, peak bone mass (PBM) occurred between the ages of 16 and 19 years at the hip, and between 33 and 40 years at the lumbar spine [23]. The amount of bone accrued during growth influences the PBM achieved, and this may contribute to the aetiology of skeletal fragility in late adulthood [25-30] (Figure 1.6). Environmental factors such as lifestyle choices, which include diet and physical activity, may influence PBM by 20-40% [31]. An increase in PBM of 10% may possibly delay the onset of osteoporosis by an average of 13 years [32].

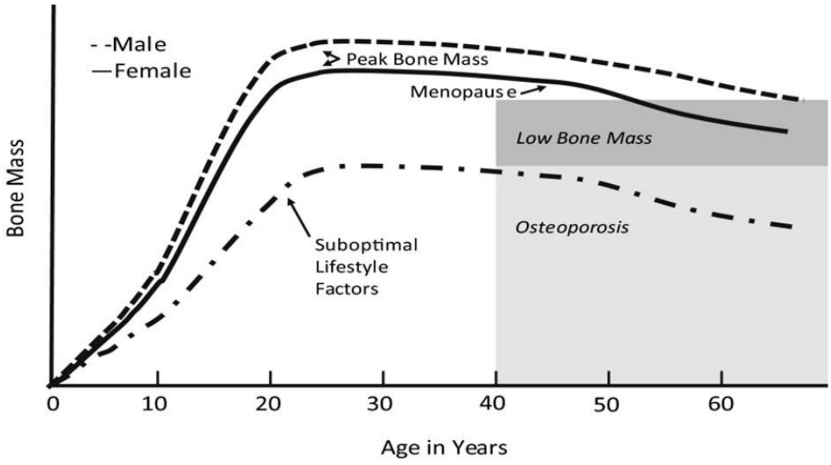


Figure 1.6 Bone mass across the lifespan with optimal and suboptimal lifestyle choices such as nutrition and physical activity. Note their influence on peak bone mass [31]. Optimal lifestyle factors in males (dashed line) and females (solid line).

1.4 Determinants of bone mass

Although bone mass is primarily genetically determined, several environmental factors may modulate an individual's genetic potential to attain their peak bone mass (Figure 1.7).

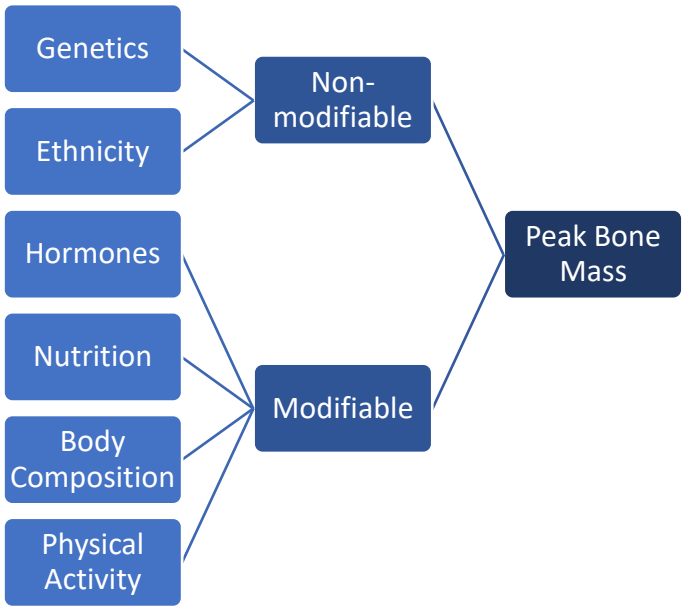


Figure 1.7 Schematic representation of the non-modifiable and modifiable factors which influence peak bone mass attainment.

1.4.1 Genetics and heritability

It is estimated that 60 - 80% of PBM attained is attributable to genetics [27]. Osteoporosis is associated with a heritability of approximately 90%, thus assessment of parental bone mass could identify at-risk children [33]. Wang and co-workers [34] showed that familial resemblance in bone mass and strength is established in the first year of life. They measured crown-heel length in 236 girls at 6 months of age. At ages 11, 13 and 18 years, whole-body, lumbar spine, and femoral neck size and mass were measured using dual energy x-ray absorptiometry (DXA), and the bone strength index (BSI) was calculated from radial and tibial pQCT scans. The girls' crown-heel length at 6 months of age correlated with their height, bone traits, and bone strength at all subsequent ages that they were measured [34]. At 18 years of age, there was a close correlation between the height, bone mass and strength of the girls with those of their parents [34].

Genetic factors may explain the greater bone mass at the hip seen in black SA children compared to white children of the same age [35, 36]. In a study of 151 pre-pubertal black participants from the Bone Health Cohort (BHC), a subcohort of the Birth to Twenty (Bt20) cohort in South Africa, the candidate genes ESR1 and TNFRSF11B were significantly associated with femoral neck and lumbar spine bone mineral content (BMC) when compared to Caucasian adults [37]. There are no studies in the literature which have examined the associations of these genotypes in white SA children. When pre-/early pubertal black and white SA children from the BHC and their parents were studied, DXA-derived bone area and BMC heritability by maternal lineage, determined by regressing children's Z-scores on parental Z-scores, was approximately 30% in both black and white children [36]. In the same children assessed at 15 years of age, the risk of adolescent fracture was inversely associated with current maternal lumbar spine aBMD, although there was no association between history of maternal childhood fracture and fracture prevalence in their adolescent offspring [38].

Site-specificity with respect to heritability is well documented [39-41]. In metacarpal bones, bone length and diameter have been shown to have the greatest heritability [40, 41] with variation in heritability also seen between such closely related sites as the medial and lateral cortices [40]. Furthermore, it has been suggested that trabecular bone is under stronger genetic control than cortical bone [27].

In South Africa, genetic factors may contribute to black children maintaining their bone mass even though their lifestyles are suboptimal compared to their white peers.

1.4.2 Ethnicity

Ethnic differences in bone structure and strength may contribute to the higher fracture rates reported in SA white than black children [42]. Studies in US and SA children have shown ethnic differences in bone growth [43, 44], bone mass [35, 45-47], the geometrical organisation of cortical and trabecular bone [48-50], bone strength [48, 50] and bone histology [51]. In a study of both US and SA children aged 9 years, black children had greater whole-body BMC than their white peers suggesting that ethnic differences in bone prevail irrespective of geographic location [52]. In the same study, SA black and white boys had significantly greater whole-body BMC than their US peers reflecting population differences in bone [52].

Gilsanz and co-workers [43] have shown that pre-pubertal US black children had shorter sitting heights and greater leg length to stature ratios than their white peers, after adjusting for height. Similarly, during the pre-pubertal period, the relative growth between appendicular and axial sites differed between black and white children from the BHC [44]. Black children from the BHC were shorter than their white peers, but had proportionally longer legs and humeri and shorter trunks. Differences in stature in pre-pubertal SA black and white children are therefore the result of differences in the upper body segment [44].

A DXA study of US infants aged between 1 and 18 months found that ethnic differences in bone mass are not apparent during infancy [53]. In 4- to 16-year old US children, whole-body BMC was higher in black than white children in all Tanner stages (see Section 1.4.3), suggesting that ethnic differences in bone mass are established in early childhood [54]. However, a shortcoming of the study by Hui and co-workers [54] was that within each Tanner stage, black children had a lower chronological age, and were therefore more sexually advanced, than white children. Thus, the independent contribution of age to the ethnic difference could not be assessed [54]. A further study of the same children by Hui and co-workers [55] showed that despite their earlier entry into each Tanner stage, black children spent an average of only 0.2 years longer in Tanner stages 2 through 4 than their white peers, and their overall gain in whole-body BMC between the ages of 5 to 15 years was not higher than in white children.

They concluded, therefore, that the higher bone mass in black children was due to a higher rate of bone mineral accrual during pre-puberty, and that ethnic differences in whole-body BMC velocity were due to differences in bone size [55].

In US children aged between 8 and 18 years, when participants from all Tanner stages were considered together, midshaft femoral cross-sectional area assessed by quantitative computed tomography (QCT) was 3% and 8.4% greater in black females and males, respectively, relative to their white peers [43]. In the same study, femoral cortical bone area and density were similar between black and white participants [43]. In South Africa, metacarpal radiogrammetry performed almost 50 years ago on 14-year old children reported lower cortical bone area in black relative to white children [45]. A pQCT study of the 65% radial and 38% tibial sites of 13-year old children from the BHC found that black children had greater diaphyseal bone area, diameter and periosteal circumference than white children [49]. Both black boys and girls also had thinner cortices and greater medullary cavity diameter than their white peers [49]. These ethnic differences were recently shown in the 65% radial and 38% tibial sites of the same children in a 10-year longitudinal pQCT study [50].

Ethnicity differentially affects appendicular and axial bone area and density in US children [43, 46, 56]. Using QCT, Gilsanz and co-workers [43] showed that in the appendicular skeleton, there were ethnic differences in femoral cross-sectional area but not in cortical density, whilst in the axial skeleton, cross-sectional area of the vertebral bodies was similar but trabecular bone density differed between US black and white children. In participants of the Bone Mineral Density in Childhood Study (BMDCS) in the US, DXA-derived whole-body BMC, and aBMD of the whole body, hip, and radius were greater in black compared to white, Hispanic and Asian children at all ages [46, 56]. Similarly, ethnic differences in bone mass were reported in 10-year old SA children from the BHC; DXA-derived BMC was higher at the femoral neck, hip and mid-radius of black children compared to their white peers, whilst lumbar spine BMC was similar in both groups [35]. When data were corrected for height and puberty, black children had decreased vertebral height by lateral vertebral assessment compared to white children. In the same study, cortical area of the second metacarpal bone, measured by radiogrammetry, was greater in black children [35]. This site-specificity with respect to ethnicity in SA children has been corroborated by other cross-sectional [36, 47] and longitudinal [50] studies.

Ethnic differences in bone dimensions could translate into differences in bone strength [48, 50, 57]. In a pQCT study of the 50% radial and 66% tibial sites in US white, black and Hispanic children aged 10.9 years, bone strength measured by the BSI, and, the stress-strain index (SSIp) which is derived using measures of bone geometry and volumetric bone density, was greater in black and Hispanic children than in their white peers [48]. At the tibia, increased bone strength was associated with greater bone area (larger bone size) and vBMD, whilst at the radius it was associated with greater cortical area (within a similar total bone area) and vBMD [48]. A cross-sectional study by Leonard and co-workers [57] showed that from Tanner stage 1 until Tanner stage 4, US black children had greater cortical density, size and strength at the 38% tibial diaphysis compared to their white peers. Tibial strength was greater in black than white children by 13.4% between Tanner stages 1 and 4, and by 2.5% in Tanner stage 5 [57]. In their longitudinal study of SA adolescents, Schoenbuchner and co-workers [50], controlling for biological maturity using age at peak height velocity (aPHV), showed that by four years after aPHV, radial (65% site) cortical cross-sectional area and cortical vBMD were greater in white than black children, whilst at the 38% tibia site the converse occurred. The influence of bone size on strength was consistent with the lower radial and higher tibial SSIp in black children compared to their white peers [50].

Ethnic differences in the bones of SA children have also been reported at a tissue level [51]. Histomorphometry of iliac crest trabecular bone in SA children aged between 6 weeks and 23 years showed that there was greater cortical porosity in white than black children until age 5 years [51]. Thereafter, there were diminutive changes in cortical porosity in black children, which commenced in white children only after age 15 years. Structurally, black children had greater endocortical wall thickness, Haversian osteoid thickness and decreased cortical porosity compared to white children [51].

The ethnic differences in bone structure at both a macro- and micro-anatomical level underlines the contribution of heritability in determining bone geometry and strength.

1.4.3 Puberty

Puberty is the period during which sexual maturation occurs. The development of secondary sexual characteristics during puberty has been classified into 5 stages commonly referred to as the Tanner stages [58]. A secular trend in sexual maturity has been observed in many countries with menarche (usually Tanner stage 4) occurring significantly earlier than in previous generations [59-62]. When the ages of onset of menarche in US girls during 1988 - 1994 were compared to those from 25 years previously, there was a change in black girls from 12.48 to 12.14 years and in white girls from 12.80 to 12.60 years [61]. In South Africa, no ethnic differences were found in the age of onset of menarche between urban black and white girls from the Bt20 BHC [62]. However, a comparison with historic data showed that between 1956 and 1977, the age of onset of menarche in SA black girls changed from 14.9 years to 12.4 years [62]. In white girls, the change was from 13.1 years in 1977 to 12.5 years in 2009 [62]. The positive secular change was attributed to an improvement in SES and nutrition [62].

The timing of pubertal initiation in SA adolescents has been investigated by assessing the age of transition from Tanner Stage 1 (pre-puberty) to Tanner Stage 2 (breast/genitalia or pubic hair development) [63]. Data from the SA Bt20 cohort and from studies undertaken in urban Johannesburg between 1988 and 2004 found the age of initiation of puberty ranged from 9.8 to 10.5 years and did not differ between black and white children of both sexes [63]. Furthermore, the age of onset of puberty remained stable over a 10- to 15-year period [63]. The constant age of initiation of puberty coupled with an earlier onset of menarche in SA girls suggests an increase in the rate of development [63]. Jones and co-workers [62, 63] speculated that environmental factors may have differentially influenced the onset of menarche via the hypothalamic-pituitary pathways. In a recent longitudinal study of the Bt20 cohort, from birth through adolescence, greater body mass index (BMI) in girls between the ages of 5 and 8 years was associated with an earlier initiation and faster progression through puberty, thus highlighting the influence of modifiable lifestyle factors during early life on the age of pubertal initiation [64].

1.4.3.1 Hormonal interactions during puberty

Puberty is characterised by a surge in the sex hormones and growth hormone (GH) and an increase in bone mass [65]. Oestrogen is a key regulator of bone metabolism in both females and males [66-69]. In males, testosterone also has direct effects on bone; however, its greater influence is via its aromatisation to oestradiol [69, 70]. Because of the complex interaction between reproductive hormones and bone metabolism, it has been proposed that the hypothalamic-pituitary-gonadal (HPG) axis should include the skeleton, and be referred to as the HPG-skeletal axis [65]. These interactions, in girls, are summarised in Figure 1.8.

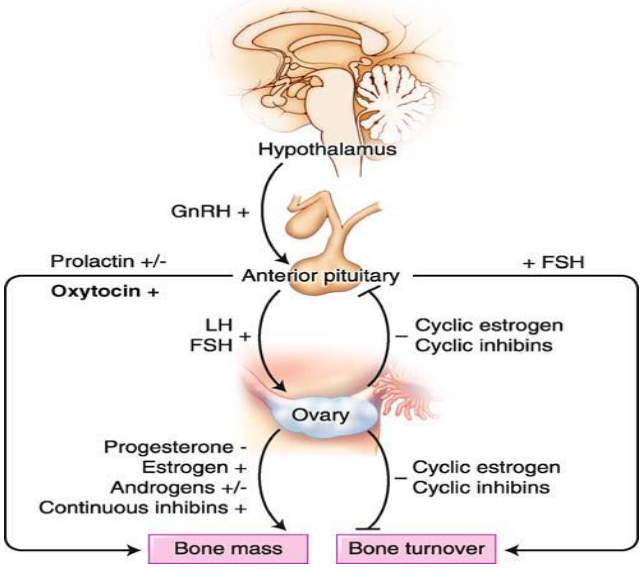


Figure 1.8. Hypothalamic-pituitary-gonadal-skeletal axis in girls. Gonadotropin-releasing hormone (GnRH) stimulates pituitary luteinising hormone (LH) and follicle stimulating hormone (FSH) secretion, which regulate ovarian function. FSH can stimulate osteoclast development, thereby increasing bone turnover. Oxytocin stimulates osteoblastogenesis/ bone formation; prolactin affects bone cells in an age-dependent manner. Ovarian oestrogen and inhibins negatively regulate LH and FSH, respectively, and modulate bone turnover and mass. Opposing effects of androgens have been observed, whereas progesterone has limited suppressive effects on bone mass accrual. Cyclic exposure of circulating inhibins and oestrogen suppresses bone turnover, and osteoblast and osteoclast differentiation. Continuous inhibins or oestrogen are anabolic and protect against gonadectomy-induced bone loss [65].

1.4.3.2 Linear bone growth

Linear bone growth occurs at epiphyseal growth plates at both the proximal and distal ends of long bones [71]. Sequential changes occur at the growth plates whereby chondrocytes proliferate, mature, hypertrophy and synthesise an extracellular matrix, which becomes calcified before being replaced by bone [71, 72]. From birth until puberty, a low concentration of oestrogen, via its action on the growth hormone-insulin growth factor1 (GH-IGF1) axis, stimulates cortical and trabecular bone development directly at the growth plate [73, 74]. During puberty, an increase in oestrogen levels results in linear bone growth velocity accelerating to reach a peak and then decelerating [75]. The high oestrogen concentration subsequently contributes to closure of the epiphyses, which marks skeletal maturity [73]. In a study of Turkish children at different stages of puberty, Yilmaz and co-workers [68] showed that oestrogen had a strong positive association with whole-body aBMD in both sexes. In addition, in boys, low testosterone levels during early puberty resulted in greater linear bone growth compared to bone mineral accrual; however, high testosterone levels at Tanner stage 4 promoted linear growth and bone mineral accretion [68].

The maximum rate of longitudinal growth during adolescence is known as peak height velocity (PHV), and it occurs at an earlier age in girls (approximately 11 years; Tanner stage 3) than in boys (approximately 13 years; Tanner stage 4) [75-77]. A recent mixed longitudinal study using Superimposition by Translation and Rotation (SITAR) analysis showed that age at peak height velocity (aPHV) was 13.1 years in African American boys and 13.4 years in white boys, whereas in African American and white girls it occurred at 11.0 and 11.6 years, respectively [77]. In a SITAR analysis of longitudinal bone maturity scores of children from the BHC measured using the TW3 RUS (Tanner-Whitehouse 3 radius, ulna, and short bones of the carpus) method, skeletal maturity occurred at age 15 years in black and white girls, and at ages 17 and 16.5 years in black and white boys, respectively [78]. In the same study, there was no difference in the velocity (rate) of skeletal maturation between black and white boys; however, skeletal maturation tempo (timing) in black boys occurred 7 months later than in white boys [78]. Whilst skeletal maturation tempo was similar in black and white girls, maturation velocity was 9% greater in black girls than their white peers [78]. Thus, there are ethnic and sex differences in the timing and velocity of skeletal maturation in SA children.

Studies of statural growth [77] and of the metacarpal [41, 79] and femoral bones [80, 81] have shown that the rate of linear bone growth is similar in both sexes in the pre-pubertal years. During pre-puberty, there is greater growth in the appendicular than axial skeleton [81]. Sexual dimorphism is established during puberty when a delayed onset of puberty in males allows for a longer duration of linear growth than in females [41, 77, 78]. In both sexes, during the period of PHV, linear bone growth precedes and progresses at a faster rate than the acquisition of bone mass [74, 77, 82]. It is during this period, at age 11 - 12 years in females and 13 - 14 years in males, when there is a relative weakness of bone, that individuals are more susceptible to fracture [74, 83]. In individuals aged between 2 and 50 years, whole-body BMC was highly correlated with height in both sexes and at all ages [84]. In both sexes, the timing of PHV preceded peak BMC velocity by almost a year [85]. In African American and white US children, the lag in age at peak velocity of whole-body BMC accrual relative to PHV was 0.6 - 0.9 years in boys and 0.8 - 1.2 years in girls [77]. Due to population differences in bone [52], BMC accrual relative to aPHV may differ in SA children and remains to be investigated. It would therefore be interesting to determine in SA children whether the pattern of BMC accrual mirrors the ethnic and sex differences in skeletal maturity, and how this contributes to differences in bone mass in SA adults.

1.4.3.3 Bone growth in width

Sex differences in bone width are established by 2 years of age [15, 41]. Studies of the metacarpal [41, 79, 86] and femoral [11, 81] bones have shown that, at all ages, bone diameter is approximately 5% larger in boys than in girls. However, more notable sex differences occur during puberty when the periosteal diameter in boys increases significantly compared to girls [41, 79, 81, 86].

The mechanism by which bone cortices grow during puberty differs between males and females [86, 87]. During early puberty, androgens, GH and IGF-1 stimulate cortical bone growth by apposition at the periosteal surface in males [70]. Low levels of oestrogen during early puberty also stimulate periosteal bone formation via its α-receptor in both sexes, and in females promote endocortical bone deposition [88]. Higher oestrogen levels during late puberty in females inhibit bone expansion at the periosteal surface via its β-receptor and

stimulate endocortical bone deposition [89, 90]. Whilst bone growth in width is primarily under hormonal control, there may be a differential pattern of cortical bone modelling and remodelling between weight-bearing and non-weight bearing bones due to functional loading [11, 91, 92]. In addition, modelling and remodelling within a bone may be heterogeneous [93]. In the tibia, for example, there is greater periosteal bone deposition in the antero-posterior region compared to the medio-lateral region due to the differential load of the associated musculature [93].

Single photon absorptiometry analysis of the distal third of the radius in SA children from pre-puberty through adolescence has shown that although bone width and BMC were significantly higher in males, when BMC was normalised for bone width, no differences were found between the sexes [94]. In a pQCT study of 13-year old SA children, although bone size was greater in boys than girls, the latter had a 5 - 7% higher cortical density [49]. These results corroborated those of other studies [15, 95] supporting the hypothesis that during puberty in girls, oestrogen secretion reduces bone turnover, thereby preserving cortical bone for future foetal bone growth [251].

The physiological events and accompanying changes in bone during childhood and adulthood are confounded by differing hormonal influences and growth rates in males and females experienced during the different stages of life. The interactions of hormones, in particular oestrogen, testosterone, growth hormone and IGF-1, in bone growth during puberty are intricately linked. Longitudinal assays of the hormones during the critical pubertal period would unravel their interplay in bone growth.

1.4.4 Nutrition

Micronutrient and macronutrient intakes are important determinants of bone health [96, 97]. Important micronutrients include inorganic minerals (calcium, magnesium, phosphorus, potassium, sodium and various trace elements) and vitamins (A, C, D, E, K, and certain B vitamins), whilst macronutrients that are important for bone health include protein and fatty acids [96]. In South Africa, an analysis of nutrient intake of children in the Bt20 cohort from its inception in 1990 until 2000 found the intake of eight micronutrients, including calcium, to be consistently below the Recommended Daily Allowance (RDA) [98].

Calcium and vitamin D are deemed the most important micronutrients for skeletal health [97]. Calcium deficiency in early life may result in a 5-10% decrease in PBM [28]. Calcium is stored in bone and is regarded as a "threshold" nutrient [99]. Whilst a regular intake of adequate calcium is necessary to maintain bone health [100], calcium consumed in excess of the body's requirements is excreted. Serum calcium is regulated by vitamin D, parathyroid hormone and calcitonin (Figure 1.9) [101].

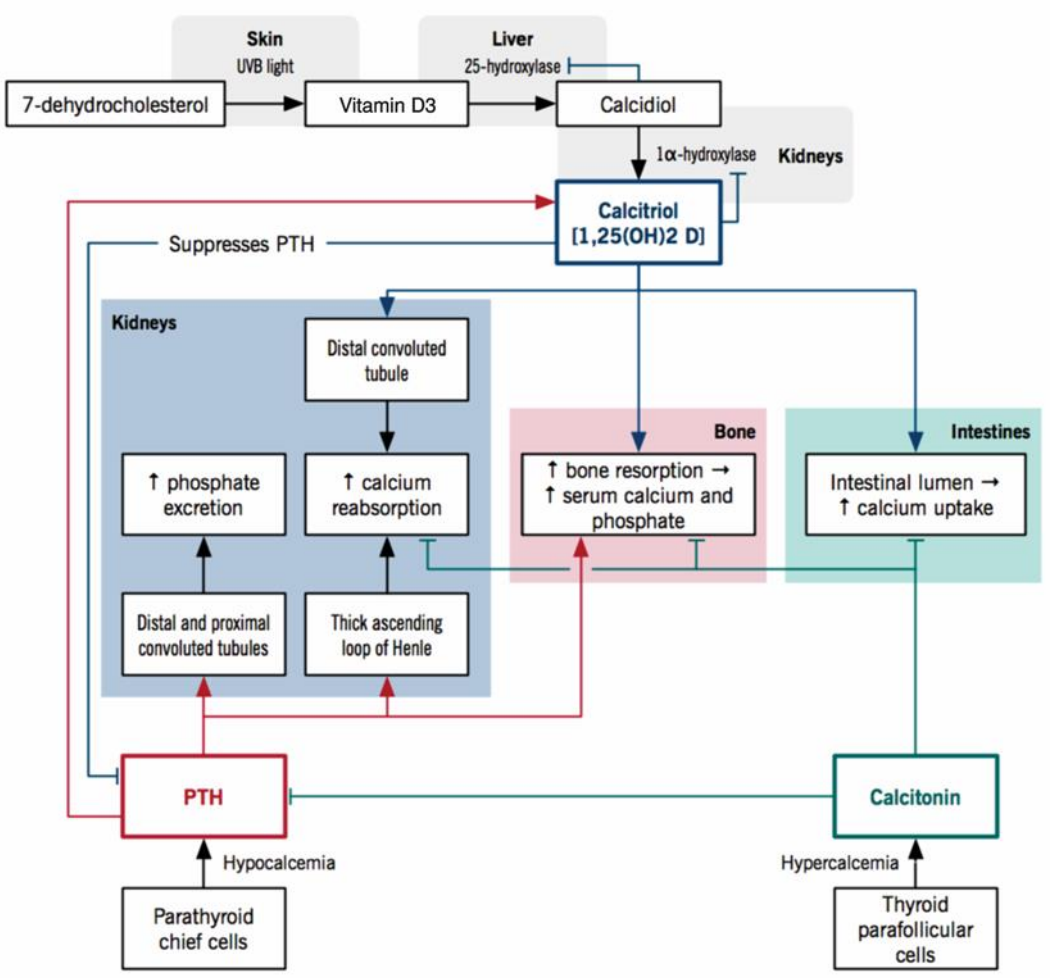


Figure 1.9 Calcium homeostasis which is maintained by vitamin D, parathyroid hormone and calcitonin [101].

The RDA for calcium in US children and adolescents between the ages of 9 and 18 years is 1300 mg [102]; however, its applicability in different population groups is uncertain [103] since calcium metabolism has been shown to be influenced by ethnicity [104]. In a US study, African American adolescent females were more efficient in absorbing and preserving calcium,

which was associated with their greater bone accrual, compared to their white peers [104]. In the SA Bt20 cohort, daily calcium intake of black and white children aged between 9 and 10 years was 25% (±314 mg) and 56% (±709 mg) of the RDA, respectively [105]. There is a high prevalence of hypocalcaemia in SA rural black children who have normal vitamin D levels, yet have similar bone mass to white children [105, 106], suggesting that bone mass is severely compromised only when low dietary calcium intakes are accompanied by a vitamin D deficiency [107].

In an intervention study where a 1000mg daily calcium supplement (5 days per week for 12 months) was administered to pre-pubertal Gambian children whose average daily calcium intake was 340 mg, final stature was compromised by up to 3cm in the boys who had received supplementation [108]. It was concluded that the calcium supplementation contributed to advanced growth during mid-adolescence, thus PHV occurred at a younger age and resulted in earlier closure of the epiphyseal plates [108]. In a randomised controlled trial of 195 Finnish girls aged between 10 and 12 years, an adequate daily calcium intake from food was found to be more beneficial for bone mass accrual than taking a supplement of 1000mg calcium [109]. In the same study, no changes in bone accrual were found in those participants who took 200 I.U. of vitamin D3 in addition to the 1000mg calcium supplement [109]. It was therefore suggested that increasing dietary calcium intake is more beneficial to bone health than taking daily supplements, as better intestinal absorption of calcium may occur in the presence of casein-phosphopeptides and lactose found in dairy products [109].

Vitamin D responds to a decrease in serum calcium by promoting the intestinal absorption of calcium and phosphorus, thereby providing minerals for the skeleton. Vitamin D is converted to 25-hydroxyvitamin D [25-(OH)D] in the liver, and the serum level of 25-(OH)D is measured to determine vitamin D status. There is no consensus as to the cut-off values for vitamin D deficiency, insufficiency and sufficiency [102, 110, 111]. Children with ≤25 nmol/L of serum 25-(OH)D are considered to be vitamin D deficient [110] and this may have adverse effects on bone health [102, 111]. Vitamin D insufficiency (27 - 50 nmol/L) may result in reduced intestinal calcium absorption, elevated blood concentrations of parathyroid hormone, and increased rates of bone resorption to maintain plasma calcium levels, which may contribute to bone fracture [100].

The role of vitamin D in childhood skeletal health is still unclear. Several studies have shown no significant change in BMC in vitamin D deficient children who received daily vitamin D supplementation [112-114]. However, a systematic review and meta-analysis showed that vitamin D supplementation was associated with greater bone density in children and adolescents who were vitamin D deficient, but not in those who had normal vitamin D status [115]. In a study of children aged between 10 and 17 years whose serum 25-(OH)D levels were 30 nmol/L (females) and 39 nmol/L (males), and who received daily vitamin D supplementation for 1 year, females experienced an increase in hip bone area and bone mass [116] and femoral neck geometry [117], but in males there was no change. The sexual dimorphism may have been due to higher baseline 25-(OH)D levels in males or to the differential effect of sex hormones on bone mass [117].

Serum 25-(OH)D levels have been assessed in 10-year old children from the Bt20 cohort by Poopedi and co-workers [118]. They found that although white children had higher serum 25-(OH)D levels than their black peers, 70% of black and 87% of white children were vitamin D sufficient, thus obviating the need for vitamin D supplementation [118]. When the same individuals were studied longitudinally until age 20 years, no association was found between a base-line measurement of serum 25-(OH)D obtained at age 11 years and serum 25-(OH)D measured in early and late adolescence [119]. It was concluded, therefore, that a single serum vitamin D measurement may not be an accurate indication of the vitamin D status of an individual over an extended period of time [119]. The influence of vitamin D status on bone between black and white SA children is yet to be explored.

Collectively, these studies have shown the importance of a healthy diet with adequate intake of both macro- and micronutrients for optimising bone health and decreasing fracture risk. Public awareness and education on nutrition is critical to modify behavioural patterns for optimal health.

1.4.5 Body composition

Both overweight and underweight impact on skeletal health [120-122]; however, the relative contributions of fat mass and lean mass to bone health in children are still unclear [123]. Overweight and obesity, previously associated with developed countries, have become a burgeoning problem even in low to middle income countries [124, 125], with an increase in prevalence in children and young adults [126-130]. The SA Health of the Nation Study showed that, between the ages of 6 and 13 years, the prevalence of overweight and obesity in SA black girls increased from 11.9% to 21.8% but declined in their white peers from 25.4% to 14.5% [131]. Amongst black and white SA urban youth assessed in 2002 and 2008 as part of the SA National Youth Risk Behaviour Survey, overweight increased from 6.3% to 11% in males and from 24.3% to 29.0% in females [127]. During the same period, obesity doubled in males from 1.6% to 3.3%, and in females increased from 5.0% to 7.5% [127]. A recent study of 7- to 15-year old rural SA children also showed a significantly higher prevalence of obesity in females compared to males [132]. In the Bt20 cohort, a 20% increase in overweight or obesity was documented between ages 9 - 10 and 16 years [133, 134]. A longitudinal study of the same children from birth until 20 years showed that overweight and obesity was not widely prevalent in boys; however, in girls obesity increased throughout childhood and adolescence with the increase greatest following puberty [135]. The interplay between body composition components such as fat and lean mass, and bone mineral content in children are described below.

1.4.5.1 Fat mass

Adipose tissue is a metabolically active tissue within which macrophages accumulate and secrete inflammatory mediators [136-138] that negatively influence bone mineral density [139-141]. In a 4-year prospective study of adolescent girls, overweight girls had higher serum levels of the inflammatory marker high-sensitivity C-reactive protein than girls of normal weight, and levels were inversely associated with forearm aBMD [140].

A useful indicator of over- or under-weight is the Body Mass Index (BMI), which although is unable to distinguish between fat mass and lean mass, adjusts for the wide variation in

average weight and height between different population groups [141-144]. In a cross-sectional prospective study of 2213 children between 2 and 17 years of age in the US, the odds for fracture were greater in underweight children (BMI less than the 5th percentile) than in normal weight children (BMI 5th to <85th percentile) [145]. However, several international studies in children have shown that obese children are at greater risk for fracture [146-152], in keeping with observations in South Africa that white girls from the Bt20 cohort with high BMIs have a higher fracture risk [42]. Thus, in SA children there appear to be ethnic and sex-related differences in the associations between BMI and fracture risk.

Bone strength, using the bone breaking resistance index as determined by digital radiogrammetry of the second metacarpal bone, was evaluated in overweight and obese children aged between 8 and 14 years by Longhi and co-workers [148]. They found that bone size was diminished and had lower resistance to fracture in obese children compared to those of normal weight [148]. In the same study, a high BMI was associated with reduced bone quality of the phalanges in females and lower bone strength in males, as measured by quantitative ultrasound [148].

Obese individuals require greater muscle force for functional movement which promotes bone mass accrual [153], thus weight-bearing and non-weight bearing bones may be influenced differentially by greater fat mass [149, 154, 155]. In a study of 6-year old children in the United Kingdom, DXA-derived whole-body fat mass was positively associated with bone area of the tibia, as determined by pQCT, but had a negative association with both cortical and trabecular vBMD [156]. Pollock and co-workers [154] assessed the 4% (mostly trabecular bone) and 20% (mostly cortical bone) sites of the non-weight bearing radius and weight-bearing tibia in adolescent females using pQCT. They showed that, at the 20% sites, fat mass was inversely associated with tibial cortical bone area, BMC and SSIp, and with radial total bone cross-sectional area and SSIp [154]. It was therefore suggested that greater body fat has a negative influence on bone area and strength independent of its weight-bearing functions, and that this affects predominantly cortical sites more than it does sites with a high trabecular content [154]. Leonard and co-workers [155] assessed bone strength using the section modulus and vBMD of the radius and tibia in obese (ages 10 to 15 years) and non-obese (ages 10 to 18 years) children. They found no significant differences in radial bone strength between the two groups; however tibial bone strength was greater in obese compared to non-obese

children and was associated with advanced skeletal maturation and greater calf muscle size and strength in the obese children [155]. Collectively, these studies suggest that whilst fat mass may contribute to greater bone size, it does not necessarily confer greater bone strength [149, 156].

The influence of fat mass on skeletal strength varies between childhood and adulthood and remains a contentious issue [157]. Conflicting results in the literature may be attributed to differences in statistical methodology such as inappropriately treating highly collinear variables as independent factors [158], and densitometry analysis such as a 2-dimensional representation by DXA versus 3-dimensional by pQCT [121]. Thickness of adipose tissue may also contribute to measurement inaccuracies; however, the error is smaller with QCT than with DXA [159, 160]. The identification of predisposing factors of obesity in childhood is fundamental in the management of the associated cardiometabolic and skeletal morbidities.

1.4.5.2 Lean mass

It is well documented that lean mass, through mechanical loading via muscle contraction [91, 161], has a greater positive effect on BMC, aBMD and bone strength than fat mass [162-165]. During growth, there is a linear relationship between lean mass and bone mass [166] with muscle development preceding and driving the development of bone [167]. This was shown in a longitudinal study of children aged between 8 and 14 years in whom peak accrual rate of lean mass was attained a few months prior to peak BMC [167], suggesting a role for muscle mass in determining peak bone mass.

In a 2-year longitudinal DXA study of 7-year old children, although children who had a higher proportion of their weight gain as fat mass had increased hip bone area, the increase in their aBMD was less than that of children who had weight gain primarily due to lean mass [163]. Using hip structural analysis (HSA) from DXA scans and step-wise regression analyses, Petit and co-workers [168] showed that the section modulus of the femoral shaft and femoral neck was greater in overweight compared to healthy children, and that lean mass, but not fat mass, was independently associated with bone strength. It was therefore suggested that bone strength is determined by muscle force, represented by lean mass, and not fat mass [168].

Numerous studies have shown that males have greater lean mass than females and that this sex difference is present in childhood [162, 164, 165, 167, 169-171]. In a DXA study of children (mean age 6.2 years) from the Generation R study in the Netherlands, boys had greater lean mass, femoral shaft and neck bone area and aBMD, and lower fat mass than their female peers [165]. Medina-Gomez and co-workers [165] showed that the sexual dimorphism in bone mass and strength was associated with differences in lean mass.

Ethnic differences in lean mass have been shown in a cross-sectional study of US children aged between 8 and 19 years, from the National Health and Nutrition Examination Survey (NHANES), with black children having greater lean mass than their white peers [171]. This is in contrast to studies in South Africa which have shown that 9-year old white children in the Bt20 cohort were heavier, taller and had higher lean mass but lower hip aBMD (boys) and hip and spine aBMD (girls), than their black peers [44, 172]. Peripheral QCT assessments of 13-year old SA children at different stages of puberty found that black children had larger tibial diameters with smaller muscle cross-sectional areas than white children [49]. The contribution of muscle size to bone growth has not been investigated longitudinally in African children.

1.4.6 Physical activity

Frost's Mechanostat Hypothesis for bone suggests that a bone's baseline conditions are genetically predetermined in utero, and that bone adapts to mechanical loading due to body weight and muscle contraction, the latter occurring during physical activity [173]. A close linear relationship between bone and muscle exists throughout life in both sexes [84, 167, 174, 175]. The type of physical activity undertaken has a site-specific influence on the skeletal site being loaded and on the extent of the effect on the bone [176]. High intensity and high magnitude activities such as jumping and racquet games elicit a greater positive osteogenic response compared to endurance training [176].

Numerous studies have shown that childhood and adolescent physical activity is positively associated with bone mass and strength [82, 105, 172, 177-186]. In the "Bounce at the Bell" intervention study, 10-year old children who performed 10 jumps three times daily (totalling 3 minutes per day) over an 8-month period, had greater increase in total proximal femur and

intertrochanteric DXA-derived BMC than the control group [187]. In a recent cross-sectional pQCT study on 5- to 29-year olds, in which maturity was aligned on aPHV, physical loading had the greatest positive effect on bone during the peri-pubertal and post-pubertal period compared to the pre-pubertal period [185]. A systematic review reported that interventional studies have shown weight-bearing exercise to have a significant positive effect on bone strength during pre-puberty in boys, and pre- and peri-puberty in girls [183].

Continued physical activity is important to maintain its skeletal benefits [176, 179, 184, 188]. In a 28-year follow up pQCT study of 3- to 18-year old children, those who had been habitually physically active had greater tibial bone size, BMC and bone strength in adulthood than their inactive peers [184]. However, an 8-year follow up study of 18-year old female soccer players showed that aBMD was lower in former athletes who no longer engaged in sporting activities than in those who had continued to participate in sport [189]. Physical exercise programmes implemented during the period of PBM accrual resulting in a greater PBM could therefore negate some of the hormonally induced bone loss experienced in later life [184].

Physical activity has sex- and site-specific influences on bone geometry and strength in the growing skeleton [180, 183, 185, 187, 190, 191]. In a recent pQCT study of pre-pubertal children, physical activity was significantly associated with cortical area and thickness at the 66% tibial site in boys, and total bone area in girls [190]. In addition, in physically active boys, greater cortical dimensions were found on the posterior and medial aspects of the tibia, whilst in physically active girls, greater tibial cortical mass only occurred postero-medially [190]. In the same study, although in both sexes SSIp was higher in the physically active compared to the inactive children, this was only significant in girls and was attributed to them having wider bones than their inactive peers [190].

The SA Youth Risk Behaviour Survey revealed that a third of the population of SA children are not afforded the opportunity to participate in physical activities at school [192]. Nationally, 42.8% of children had insufficient or no physical activity, and inactivity was more prevalent in females [192]. Several studies of SA children have shown ethnic differences in the response of bone to physical activity [105, 172, 181, 182, 186]. In 9-year olds from the BHC, physical activity was associated with bone mass only in white children, and it was therefore proposed that black children did not reach a high enough threshold of physical activity to induce an osteogenic stimulus [105]. Black children had low physical activity coupled with a low calcium

intake and a higher bone mass at the hip than white children, with similar aBMD values at other sites [105]. A 20-week weight-bearing physical activity intervention programme on pre- and early pubertal black SA children reported gains in tibial cortical and trabecular vBMD in the intervention group, while greater tibial cortical area was reported in the control group [182]. Furthermore, in pre- to early pubertal SA children, greater femoral neck BMC, radial circumference, and tibial bone area and SSIp occurred in black compared to white children partaking in low bone loading activities [181]. These ethnic differences were attenuated in children who participated in high bone loading activities [181].

The contribution of physical activity to reducing fracture risk is not fully understood. The associations between bone mass, physical activity and fracture risk were evaluated in children aged between 4.5 and 9 years from the Avon Longitudinal Study of Parents and Children cohort (ALSPAC) in the United Kingdom [193]. Fractures were more prevalent in children who engaged in vigorous physical activity regardless of whether they had a high or low aBMD [193]. This demonstrated that a higher bone mass does not necessarily confer protection against fracture and that physical activity contributed to fracture pathogenesis [193]. Similarly, when fracture history was assessed in the Bt20 participants at age 15 years, white males who had fractured engaged in greater physical activity and had greater bone size and area than their non-fracturing peers [42]. However, in a prospective 5-year intervention program in 6- to 9-year old Swedish children, although daily moderate intensity exercise contributed to greater radial and tibial bone mass, similar numbers of fractures were reported in physically active and inactive children [180].

To further our understanding of the response of bone to mechanical loading, bone's structure, mass and its associated musculature should be assessed, and the intensity, frequency and duration of physical activity considered.

1.4.7 Socio-economic status

Urbanisation and improved socio-economic status (SES) in developing countries are associated with a nutritional transition to a high fat diet and a decline in physical activity, which may result in changes in body composition and unintended health consequences [124,

125, 194]. South Africa, classified as a low to middle income country, is experiencing an increase in non-communicable diseases associated with urban lifestyles [195, 196]; however, malnutrition and concomitant stunting of growth still prevail [196, 197].

Griffiths and co-workers [133] measured SES via a questionnaire that captured both the social and economic aspects of SES at ages 0 - 2 years in the participants of the Bt20 cohort. They found that SES in infancy was positively associated with total body fat and lean mass at ages 9 - 10 years [133]. In a subsequent study of the same participants, whole-body bone area and BMC at ages 9 - 10 years were more strongly associated with SES measures taken during the same period compared to SES measures take in infancy [198]. It was therefore suggested that current SES, rather than that measured during infancy, is a stronger predictor of bone health [198].

Breastfeeding may be considered a proxy for SES. In developed countries mothers from a higher socio-economic background are more likely to breastfeed their infants than those with a lower SES [199, 200]. In high income countries affluent women breastfeed for a shorter time than poorer women, whilst in low to middle income countries, the pattern is reversed [200]. In South Africa, approximately 32% of infants are exclusively breastfed during the first six months of life [201, 202]. Ninety-seven percent of infants in the Bt20 cohort were breastfed for an average duration of 11.6 months with solid and semi-solid food introduced on average at 3.6 months [203]. Differences in the nutrient content between human breast milk and formula milk may differentially influence bone mass [204, 205].

The association between having been breastfed and later bone health is contentious [206-210]. Fewtrell and co-workers [208] found no significant differences in bone mass between 10-year old children who had been breastfed compared to those who had been formula-fed. In children from the Generation R study, at 6 years of age there was no positive association between bone mass and having been exclusively breast-fed for 4 months [209]. In contrast, in a study of Tasmanian children born at term and assessed at ages 8 and 16 years, children who had been breastfed had higher whole-body, femoral neck and lumbar spine aBMD at both time points, and reduced risk of adolescent fracture, compared to those who had not been breastfed [207]. Similarly, Chilean infants who had been exclusively breastfed for 6 months had higher whole-body aBMD at age 16 years than their peers who had been formula-fed [210]. In a study of 8-year old SA children a positive association was reported between a

history of having been breastfed and metacarpal diameter, highlighting the importance of early nutrition on the properties of bone [211].

Social status of pregnant mothers, measured by housing tenure and level of education, has been shown to differentially influence bone mass accrual in their offspring [212]. In participants from the ALSPAC cohort, greater bone mass due to longitudinal bone growth (gain in height) was found in children born to women with a high SES during pregnancy [212]. Mothers from a low SES had shorter and heavier children with wider bones, and similar bone area and BMC due to greater appositional bone growth [212]. Socio-economic status therefore did not affect bone area and BMC but influenced the direction of bone growth, that is, bone area and bone mass in high SES individuals were height-dependent, and in low SES individuals they were weight-dependent [212]. In SA pre-pubertal children of mixed ancestral origin, maternal education, housing density and assets showed no correlation with the second metacarpal cross-sectional area as assessed by radiogrammetry [211].

A recent study of Scottish children showed that SES influences fracture incidence, the mode of injury and the type of fracture sustained [213]. Children from lower SES backgrounds were more likely to sustain a fracture through a fall, blunt trauma or road traffic accident, compared to their less deprived peers who had a greater risk for fracture when playing sport [213]. Given the possible influence of SES factors on skeletal health through the lifecourse, maternal diet and behaviour may be important for the skeletal health and fracture risk of their offspring [214].

The influence of SES on phenotypic expression of bone in children remains to be fully elucidated, but may contribute to the ethnic differences in bone mass and fracture rates in the SA population. The nutritional transition to a high fat diet and a decline in physical activity that accompanies urbanisation and improved SES, as is currently occurring in South Africa, is associated with changes in body composition which may influence peak bone mass attainment. While it is important to consider the influence of factors such as hormones, SES, nutrition and physical activity on bone growth, their influence is dependent on the timing, duration and extent of exposure. In addition, their residual effects are difficult to determine. The complex interactions between these modifiable factors and bone may, however, lead to bias in the interpretation of results in longitudinal bone growth studies.

1.5 Fracture epidemiology

Lifetime fracture incidence is bimodal with the first peak occurring during adolescence [83, 215] and the second in late adulthood [216]. Several studies have shown that peak fracture incidence in children occurs at approximately age 13 - 14 years in boys and 10 - 11 years in girls [83, 215, 217, 218], which corresponds to the time of increased growth velocity in both sexes [28, 83]. Several European studies have shown that the probability of sustaining a fracture in the first 16 years of life is 42 - 61% for boys and 27 - 40% for girls [215, 219-221]. In children, the most common site of fracture is the distal forearm [83, 159, 215, 217, 218, 222-224]. Children with a history of fracture are more prone to fracture as adults in the same or similar site [20, 219, 225].

Epidemiological studies have shown secular changes in fracture incidence in children [218, 222, 226]. Secular trends vary depending on geographical location [227, 228], ethnicity [228, 229] and also according to anatomical site of fracture [218, 222]. Since bone mass is correlated with skeletal fragility, ethnic differences in BMD and environmental influences on BMD contribute to geographical variation in fracture incidence [230]. In a Finnish study, fracture incidence in children increased between 1960 and 1980 and then declined by 18% in the following two decades [218]. The decline was attributed to improved SES, urbanisation, decreased physical activity and an increase in overweight [218]. Although similar trends in improved SES and its associated consequences are occurring in SA, fracture incidence in SA white children is still high and is similar to that seen in European children [231]. In the Finnish study [218], despite the overall decrease in the number of fractures in children, forearm fracture incidence increased by a third, primarily during the pubertal growth spurt and due mainly to low-energy trauma. A similar increase in distal forearm fracture incidence occurred in US white children over a 30-year period [222].

In keeping with international studies in children, in the SA Bt20 children, peak fracture rate occurred during the time of peak bone mass acquisition, and upper limb fractures accounted for 57% of all fractures (Figure 1.10) [231]. Globally, individuals of African ancestry present with the lowest fracture rates [231-233], and in SA, the fracture incidence in white children is almost double that of black children (Figure 1.11) [231]. At ages 10 and 15 years, white males with a history of fracture were taller and more physically active than their black peers [42]. White males also presented with the highest fracture rates, despite them having higher bone

area and BMC compared to their non-fracturing peers, as well as black males, and black and white females [42]. A high BMI was the only contributory factor to fracture in white females [42].

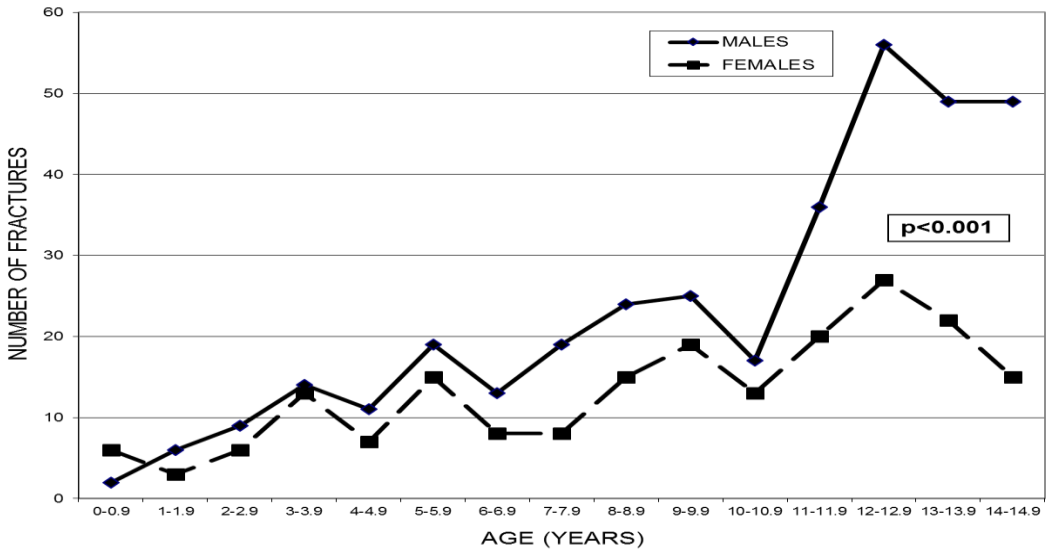


Figure 1.10 Fractures per year by age and sex distribution in South African children. The number of males and females in the study were similar [231].

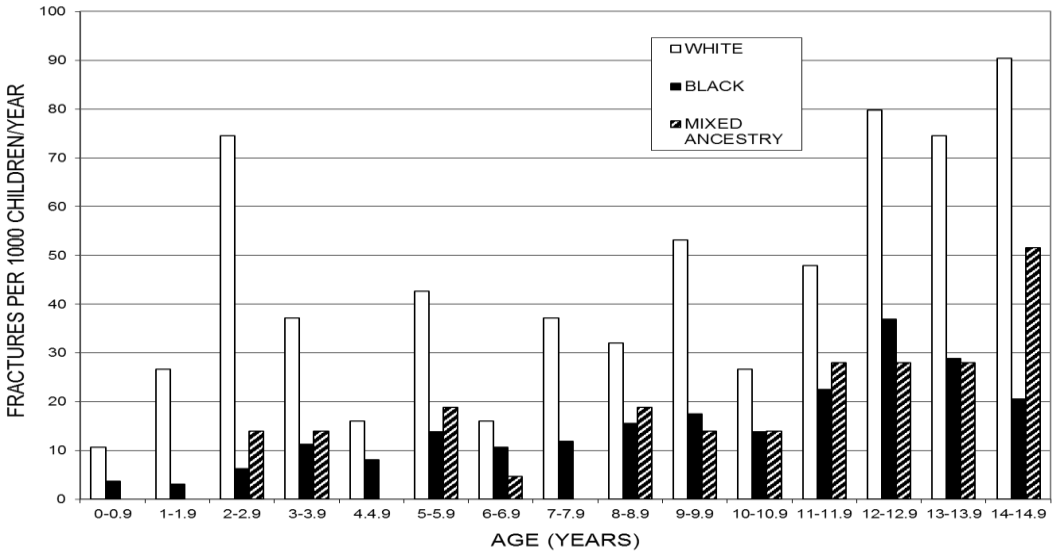


Figure 1.11 Fracture rates per year by age and ethnicity in South African children [231].

1.6 Methods of bone mass assessment

Prior to the advent of bone imaging, bone growth studies were limited to anthropometry/size measurements, and bone mass was determined from cadavers by bone's ash content [234]. The oldest method to quantify bone in vivo was performed in 1939 using radiographic absorptiometry [235]. In the 1960s, single photon absorptiometry [236] and radiogrammetry measurements from radiographs were introduced [237, 238]. This was followed by the development of two densitometric techniques, dual energy x-ray absorptiometry (DXA) and quantitative computed tomography (QCT) in the late 1970s and 1980s [239, 240]. The original QCT scanners were used at axial sites and later peripheral QCT (pQCT) scanners were developed for the measurement of appendicular bones. Two non-ionising methods of bone mass assessment quantitative ultrasound and magnetic resonance imaging (MRI) were also developed at approximately the same time [241, 242]. Magnetic resonance imaging assesses cortical and trabecular bone morphology in 3-dimensions [241, 243] and provides a quantitative bone measure. However, the scan time is long and costs prohibitive, rendering this mode of assessment unsuitable, especially for children [244].

Radiogrammetry, DXA and pQCT, which are currently the most commonly used methods for bone mass measurement, particularly in children, are compared in Table 1.1 below.

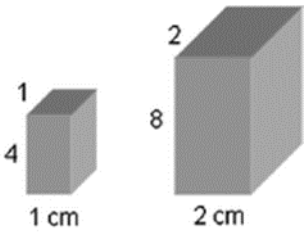
Table 1.1 Comparison of radiogrammetry, DXA and pQCT

	Radiogrammetry	**DXA**	**pQCT**
Bone Site	Metacarpal	Whole body, Lumbar spine, Femoral neck and other hip regions, Forearm	Radius, Tibia
Measured Parameters	Bone width, Medullary width, Cortical thickness	Bone area	Periosteal & endocortical circumferences
Calculated Parameters	Estimates of bone mass (MCI, RCA) and density Estimates of bone strength (CSMI, SSIp, SLI)	Areal BMD (aBMD), BMC Bone mineral apparent density (BMAD)	Volumetric BMD (total & cortical) Estimates of bone strength (CSMI, BSI, SSIp)
Effective radiation dose	Small 0.1-0.12 µSV	Depends on scan 1.4 to 13 µSV	Usually <1.5 µSV (varies with scan speed, voxel size and number of images)
Scan time	Fraction of a second	Varies depending on type of scan (<3 minutes per scan)	Varies depending on type of scan (<3 minutes for scout view and one slice)
Advantages	Low cost Minimal radiation Relatively fast	Low cost Minimal radiation Relatively fast	Measures true volumetric BMD Minimal radiation Measures bone size and geometry 3-D interpretation
Disadvantages	Measures cortical bone only 2-D interpretation	Cannot differentiate cortical and trabecular bone No measures of bone geometry Bone size will influence aBMD 2-D interpretation	Underestimates cortical vBMD when cortical thickness is <2 mm

Modified from Binkley and co-workers [245] *BMC* bone mineral content, *BMD* bone mineral density, *MCI* metacarpal index, *RCA* relative cortical area, *CSMI* cross-sectional moment of inertia, *BSI* bone strength index, *SSIp* stress-strain index, *SLI* slenderness index, µSV micro-Sievert

1.6.1 Dual energy x-ray absorptiometry

Dual energy x-ray absorptiometry, currently considered the gold standard for bone mass measurement, generates x-rays at two different energies to separate the mineralised and soft tissue compartments in the scanned region [246]. Bone area is measured in two dimensions, thus when used to determine bone mineral density, DXA gives an absolute measure in g/cm^2 (an areal density) which can be compared to a reference range by either a T-score in post-menopausal women and men older than 50 years, or a Z-score in children, pre-menopausal women and younger men [247]. Volumetric BMD cannot be measured using DXA. With growth, there is an increase in aBMD due to a greater bone size; however, vBMD remains relatively constant (Figure 1.12) [248].



Volumetric BMD (g/cm^3)	1.0	1.0
Projected Area (cm^2)	4.0	16.0
Volume (cm^3)	4.0	32.0
BMC (g)	4.0	32.0
BMD (g/cm^2)	1.0	2.0

Figure 1.12 The effect of bone size on areal bone mineral density (BMD). For two bones with known volumetric BMD of 1g/cm^3. Projected area (cm^2) is the region of interest. The bone mineral content (BMC) is the total amount of bone mineral (g) in the sample. Areal BMD (g/cm^2), is calculated as BMC divided by projected area. Both bone samples have identical volumetric densities; however, the areal BMD of the larger sample is twice that of the smaller sample [248].

In longitudinal studies using DXA, growth related changes in bone size and bone mass are accounted for by adjusting BMC for height, weight and bone area [249]. A method that is used

to adjust the two-dimensional aBMD to more closely reflect vBMD is to derive a bone mineral apparent density (BMAD, g/cm^3) [248, 250]. Bone mineral apparent density is BMC divided by the projected bone area (BA) raised to a power coefficient (PC), where PC is based on an assumed thickness of the tissue measured and is therefore site-specific [248, 250]. Prentice and co-workers [249] proposed that correction of BMC for height, weight and BA^{PC} would correct for bone size, sex and ethnic differences, and skeletal site. This was validated in a SA study of black and white children by Vidulich and co-workers [36] who showed that, at all skeletal sites, the greatest proportion of BMC variance was accounted for when BMC was corrected for BA^{PC}, height and weight.

A limitation of DXA is that cortical and trabecular bone cannot be differentiated [251]. In addition, soft tissue causes attenuation of the x-ray beam [252] which may result in inaccuracies in DXA measurements in overweight and obese individuals [157]. A further limitation of DXA is that scanners are programmed with a reference database which may not be applicable to all ethnic groups [46, 56, 253]. The most recently updated reference database for paediatric bone assessment is based on measurements of 3598 UK children, aged between 4 and 20 years, of white, South Asian, and black Afro-Caribbean/African descent who were enrolled in the Amalgamated Paediatric Bone Density (ALPHABET) study [254]. Dual energy x-ray absorptiometry can be safely used in paediatric populations because of the speed of the scan combined with a low radiation exposure [246]. The radiation imparted during a DXA scan is equivalent to a single day's natural background radiation [157].

1.6.2 Peripheral quantitative computed tomography

Peripheral QCT scanners were developed exclusively to measure cortical and trabecular bone, total vBMD, and associated fat and muscle cross-sectional areas of appendicular bones [255]. Compared with conventional QCT scanners, pQCT scanners are smaller and portable, less expensive and require less radiation exposure. The initial view, known as a scout view is used to locate the growth plate of the bone, which is used as a reference line [245]. Scans are acquired at the 4% distal site of the tibia or radius which comprises mostly trabecular bone, and either 20%, 38%, 50% or 65% sites which have higher cortical to trabecular bone volume ratios the further the distance of the slice is from the metaphysis [245, 256].

In longitudinal paediatric assessments replicating measurements at the same site is complicated by differing growth rates [245, 257]. This is overcome by using a specific anatomical landmark such as the distal endplate of the bone being measured as a reference from which to measure the midshaft and distal sites [258]. However, a single slice does not truly reflect the morphological changes taking place during growth and is one of the limitations of pQCT imaging in children and adolescents [256].

A disadvantage of pQCT is the partial volume effect [255]. This is when more than one tissue type is represented in a single voxel (a 3-dimensional volumetric representation of a pixel) and usually becomes a factor when cortical thickness is less than 2mm [255]. To prevent an underestimation of the vBMD of the cortex due to the partial volume effect, a threshold of 710 g/cm^3 is used, because this density is approximately midway between that of fully mineralised bone and soft tissue [259]. The partial volume does not influence the SSIp as the density of each individual voxel is used in its calculation [259].

1.6.3 Radiogrammetry

Radiogrammetry is the technique used to measure dimensions of appendicular bones from radiographs (Figure 1.13). The parameters measured are bone length, bone width, cortical thickness and medullary width, and it is from these measurements that indices for bone size, mass, volume, density and strength are calculated [237, 238]. The second metacarpal of the non-dominant hand is the bone most commonly measured as it is one of the largest bones of the hand, has a relatively circular midshaft and has a shape which is more consistent than the other metacarpal bones [2]. It is also a non-weight bearing bone with minor functional loading occurring during flexion of the hand [260].

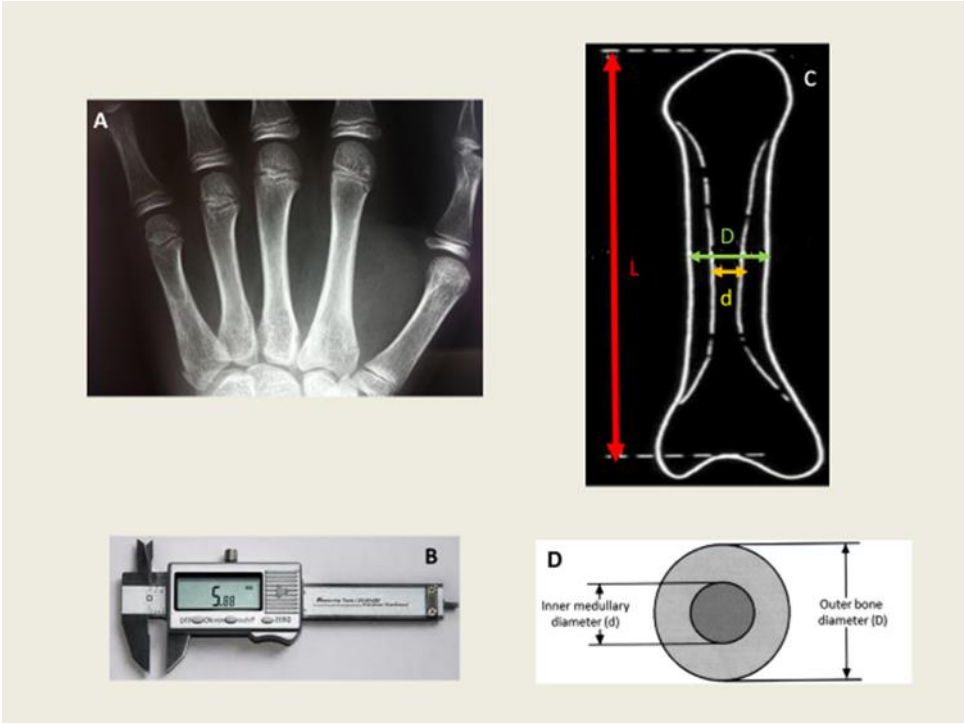


Figure 1.13 Radiogrammetry measurement of metacarpal bones. A – hand-wrist radiograph. B – Digital Vernier callipers used to measure metacarpal bone dimensions. C – Schematic representation of metacarpal bone illustrating points of measurements of total length (L), outer bone diameter (D) and inner medullary width (d); D – cross-sectional representation of a metacarpal bone at the diaphyseal midshaft showing the outer bone diameter (D) and inner medullary diameter (d). Modified from Adams, 2010 [261]

An advantage of radiogrammetry is that it requires the use of a single postero-anterior radiograph of the hand with minimal radiation exposure. It can be used in longitudinal studies because the film distance is standardised and therefore ensures reproducibility. The reliability of radiogrammetry is influenced by the quality of the processing of the x-ray film, operator reliability, and overlap of bones. These factors can be eliminated by the use of digital radiography [262]. Radiogrammetry measures cortical bone only, which is less susceptible to short-term changes in parameters than trabecular bone [263]. These measurements only provide quantitative information as the quality of bone and degree of mineralisation cannot be measured [237, 261].

As studies of bone health in children also include bone age measured from the carpal bones, a hand wrist radiograph is required [264]. With radiogrammetry, information for bone age and

bone size are obtained from a single image [264, 265]. Exposure times for hand-wrist radiographs are measured in fractions of a second, thus reducing movement artefacts in children [266].

The advent of digital radiography and automated programs for bone measurement has created a renewed interest in radiogrammetry. Radiogrammetry can be performed in three ways, either manually, semi-automated or automated (DXR). Manual radiogrammetry is the measurement of metacarpals directly on film radiographs using Vernier callipers and is an inexpensive process [261]. With the semi-automated method, measurements which are done manually are captured using specially designed software [267]. A fully automated system uses a specially devised algorithm to automatically locate and measure the regions of interest [268].

A challenge of radiogrammetry is the difficulty in distinguishing the cortico-medullary junction which is not always clearly delineated [261]. A meta-analysis of radiogrammetry precision showed the highest coefficient of variation (CV) for manual radiogrammetry and the lowest for automated measurements [262]. When an automated radiogrammetry system was used less precision was associated with measuring the outer cortical border than the inner border [269]. This was attributed to the well-defined outer margin being more susceptible to variability in the sharpness of the image [269]. Precision improved when more than one metacarpal bone was measured and the results averaged [262]. The precision of automated radiogrammetry (CV: 0.60 - 0.66%) [262] is similar to that of radio-absorptiometric techniques such as DXA which varies by site (CV: 0.38 - 1.12%) [270, 271].

Studies have shown that the robustness, that is the proportion of cortical bone to bone diameter, of the second metacarpal bone correlates well with other appendicular bones such as the radius and tibia [15, 272]. Radiogrammetry-derived BMD has been to shown to predict wrist, vertebral and hip fracture risk in adults as reliably as aBMD [273].

1.7 Metacarpal Indices

From the cortical and medullary measurements of the metacarpal bone obtained by radiogrammetry, several indices have been derived that provide information on bone geometry [86, 237, 262, 269, 274, 275] and bone strength [276-280] (Table 1.2). The metacarpal indices do not provide information on bone quality. A further limitation of the metacarpal indices is that they are obtained from measures of appendicular bone from a non-weight bearing site. Thus, only a measure of cortical bone dimensions is obtained, which may behave differently from cortical bone at sites with a high trabecular bone content such as the vertebral bodies, the proximal femur and the distal radius, which are the most common sites of fracture [281]. Since bone mass and height of an individual are interdependent, the ideal index should be independent of height [265] and should not be affected by scale and magnification. Metacarpal indices that have been used to determine bone geometry and strength are summarised in Table 1.2 and a brief description of those relevant to this thesis follows.

Table 1.2 Indices derived using radiogrammetry

Index	Formula	Units	Interpretation	References
Metacarpal Index*	$(D-d)/D$	Dimensionless	Bone size, cortical thickness	Barnett & Nordin 1960 [237] Virtama & Mahonen 1960 [238]
Exton-Smith Index	$\frac{(D^2-d^2)}{D\ x\ Bone\ length}$	Dimensionless	Bone size, cortical thickness as a function of bone length	Exton-Smith 1969 [274]
Relative Cortical Area	$\frac{(D^2-d^2)}{D^2}$	mm^2	Cortical area	Garn 1970 [86] Tothill 1989 [281]
Bone Mineral Density*	$\pi\ x\ (D-d) x\ (1-\frac{D-d}{D})$	mg/mm^2	Bone size, cortical area	Jorgensen 2000 [275] Rosholm 2001 [262]
Bone Breaking Resistance Index	$(D^4-d^4)/D\ x\ 100$	mm^3	Flexural strength	Gatti 2001 [277]
Polar Moment of Inertia*	$\pi\ x\ (D^4-d^4)/32$	mm^4	Flexural strength	Turner & Burr 1993 [282] Specker & Schoenau 2005 [283]
Section Modulus*	$Polar\ Moment\ of\ Inertia/0.5\ x\ D$	mm^3	Torsional strength	Beck 2000 [278] Franklyn 2008 [279]
Bone Strength Index	$Polar\ Moment\ of\ Inertia\ x\ vBMD$	mg^2/mm^4	Flexural strength	Ferretti 1996 [276]
Stress-Strain Index*	$Section\ Modulus\ x\ vBMD$	mm^3	Torsional strength	Kontulainen 2008 [284] Cointry 2014 [280]
Slenderness Index*	$\frac{Bone\ Length\ x\ Body\ weight}{Section\ Modulus}$	$1/mm^2/kg$	Torsional strength as a function of body size	Tommasini 2005 [18]
Paediatric Bone Index	$\frac{10\ x\ (D-d)\ x\ (1-\frac{D-d}{D})}{D^{0.33}\ x\ Bone\ length^{0.33}}$	$\mu m^{0.33}$	Cortical area as a function of bone length	Thodberg 2010 [269]

D – bone width; d – medullary width; vBMD – volumetric bone density

* indices relevant to this thesis

1.7.1 Metacarpal Index

The simplest and most widely used index is the Metacarpal Index, which is defined as the ratio of combined cortical thickness to bone diameter of a metacarpal bone as measured on a hand-wrist radiograph [2, 237]. It is not affected by magnification and is dimensionless.

1.7.2 Bone Mineral Density

Bone mineral density derived from radiogrammetry is not a true volumetric measure of bone density but is rather a calculation based on the volume of bone per projected area [262]. Radiogrammetry-derived BMD must be distinguished from DXA-derived BMD, the latter being a two-dimensional measure calculated from the projected area of bone mass [262, 275, 285, 286].

1.7.3 Polar Moment of Inertia

The polar moment of inertia is a surrogate measure of flexural strength, that is, the maximum bending stress that can be applied to a bone before it fractures. The polar moment of inertia is based on the distribution of cortical and trabecular bone around the central axis of a bone and is influenced by the diameter of the bone [282, 283]. A high polar moment of inertia relative to the cortical area requires less bone mass to resist torsion (twisting/rotation) and bending. A torque force, which is a rotational force relative to the long axis of the bone, when applied to a bone with a high polar moment of inertia will result in a small shear stress [15, 283]. A shear stress is caused by a force that creates internal deformation of a bone in a direction perpendicular to the plane of the applied force. Thus, shear stresses are created when a bone is subjected to compression and/or tension along its longitudinal axis.

1.7.4 Section Modulus

The section modulus is inversely related to the polar moment of inertia and reflects a bone's resistance to torsional stress [282, 283]. Figure 1.14 illustrates how bone's cross-sectional geometric properties influence its resistance to torsion.

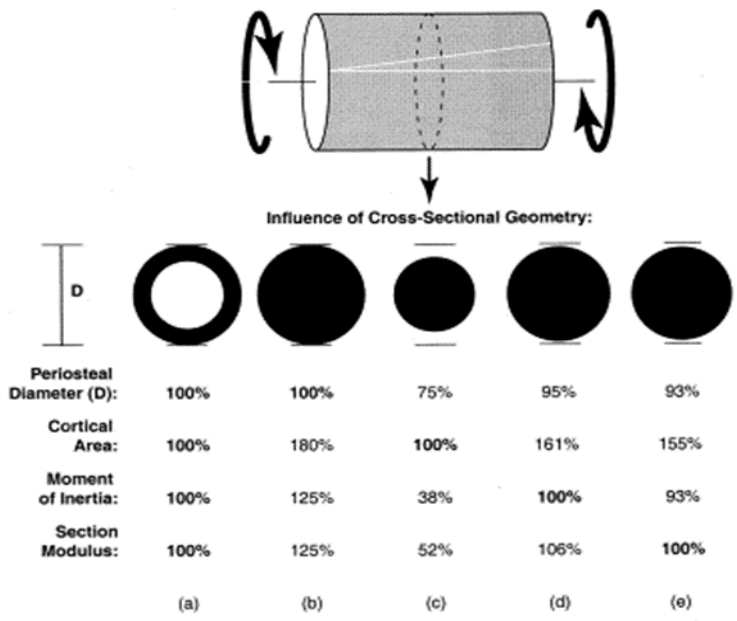


Figure 1.14 Influence of bone's cross-sectional geometry on bone strength. If all structures consist of the same material, the section modulus is proportional to the torsional failure strength and increases exponentially with outer diameter. The hollow cylinder (a) has a section modulus, and therefore strength, equivalent to a solid cylinder with only a 7% lower periosteal diameter (e). A solid cylinder of equal periosteal diameter (b) has 80% greater area, but only 25% greater torsional strength. A cylinder of equal area (c) has only 52% the structural strength. The cylinder of equal moment of inertia (d) is 6% stronger than the hollow cylinder. All values referenced to the hollow cylinder values [287].

1.7.5 Stress-Strain Index

The stress-strain index (SSIp) is a proxy for bone strength since it is correlated with maximal bone load at failure when assessed by a 3-point bending test [288]. It incorporates measures of bone geometry (the polar section modulus) and volumetric bone density to derive a proxy for flexural bone strength [280]. The SSIp is usually obtained from pQCT scans of the radius and tibia but it can also be calculated from conventional radiographs [280].

1.7.6 Slenderness Index

Variation in body mass and bone length have been shown to influence mechanical loading by their effect on moment arm lengths, that is, the distance from the line of action of the force

to the centre of rotation of the bone [13, 289]. The Slenderness Index (SLI) was therefore devised since it is based on moments of inertia but additionally factors in body weight and bone length [18].

1.8 The association between metacarpal indices and fracture

An individual's predisposition to wide or narrow bones is established early in life and is relatively consistent in postnatal growth; therefore, it was suggested that the metacarpal index of pre-pubertal children may provide a suitable indicator of fracture risk in later life [15]. The majority of studies that have explored the usefulness of metacarpal indices in predicting fracture risk have been undertaken in older adults [273, 290-297]. Although several studies have shown that metacarpal indices may be useful in identifying children with bone metabolic disorders [266, 285, 286, 298], a survey of the literature yielded only a few studies in children in which the association between metacarpal indices and fracture have been investigated [299-301]. The outcomes of these child and adult studies are conflicting and are summarised in Table 1.3.

Ma and Jones [299] assessed the association between MCI and upper limb fracture in 9- to 16-year old Tasmanian children. They found that children with fractures had thinner cortices and lower MCI than their age- and sex-matched peers without fractures [299]. In a subsequent study of 16-year old Tasmanian children, MCI was not associated with upper limb fracture [300]. The conflicting results between the two studies may be related to differences in the health status of the participants. The children in the study by Jones and Boon [300] were recruited as infants for being at greater risk for having sudden infant death syndrome, which was also associated with a lower SES, and may have influenced bone mass. A more recent study in 6- to 18-year old European children found that MCI and BMD measured using automated digital radiogrammetry (DXR) were lower in participants with a fracture than in their age- and sex-matched peers who had not fractured [301]. Renz et al [301] therefore suggested that, in children, MCI and metacarpal BMD are useful indicators for future fracture risk.

Table 1.3 Summary of studies that have used metacarpal indices to predict fracture

Study	Country	Age (years)	Participant number	Type of study	Duration of study	Index	Fracture site	Outcome	Conclusion
Ma & Jones 2003 [299]	Tasmania	9 - 16	215 (m) 106 (f) 321 (WF) 321 (NF)	Case-control	_	MCI	Upper limb	Children with fractures had thinner cortices and lower MCI than their age and sex-matched non-fractured peers.	MCI is associated with wrist and forearm fracture in children.
Jones & Boon 2008 [300]	Tasmania	16	265 (m) 150 (f)	Retrospective	_	MCI	All sites	MCI did not discriminate adolescents with a history of fracture from those with no fracture history.	MCI had no association with fracture history in adolescents.
Renz 2016 [301]	Germany	6 - 18	245 (m) 207 (f) 164 (WF) 288 (NF)	Retrospective	_	DXR-MCI DXR-BMD	Distal radius	DXR-MCI and DXR-BMD were lower in children with fractures than in age-matched controls.	DXR-MCI and DXR-BMD are useful indices to screen children for future fracture risk.
Cooper 1991 [290]	United Kingdom	65 - 85+	312 (m) 223 (f)	Prospective	14 years	MCI	Hip	Relative risk of fracture increased as MCI decreased but was not statistically significant.	MCI is not significantly associated with fracture.
Wishart 1993 [291]	Australia	63 (median)	239 (f)	Retrospective	_	Metacarpal RCA	All sites	RCA was low in the fractured compared to the non-fractured group.	Metacarpal RCA is useful for assessing fracture risk.
Kiel 2001 [292]	United States	61 (mean)	1394 (m) 1018 (f)	Prospective	30 years	Metacarpal RCA	Hip	Lower RCA increased hip fracture risk in males. Lower RCA only increased intertrochanteric fracture risk in females.	Metacarpal RCA is not useful for predicting fracture over the long-term.
Bouxsein 2002 [273]	United States	71-75 (mean)	975 (f)	Prospective	4-5 years	DXR-MCI DXR-BMD	Wrist, hip, vertebrae	DXR-MCI closely correlated to DXR-BMD. A 1 SD decrease in DXR-BMD was associated with a 1.6- to 1.9-fold increase in fracture risk.	DXR-BMD predicts wrist, hip and vertebral fracture risk.

Table 1.3 continued overleaf

Table 1.3 continued

Study	Country	Age (years)	Participants	Type of study	Duration of study	Index	Fracture site	Outcome	Conclusion
Bach-Mortensen 2006 [293]	Denmark	65 (mean)	1370 (f)	Prospective	±8 years	DXR-BMD	Upper limb, vertebrae, hip	A 1 SD decrease in DXR-BMD was associated with a 1.4- to 2.4-fold increase in upper limb and vertebral fracture risk. There was no significant association between DXR-BMD and hip fracture.	DXR-BMD may be used to identify women at risk for osteoporotic fracture.
Haara 2006 [294]	Finland	30 - 75+	1547 (m) 2014 (f)	Prospective	15 years	MCI	Hip	A 1 SD decrease in MCI increased the relative risk of fracture by 50%.	A low MCI strongly predicts hip fracture.
Vasireddy 2010 [295]	United Kingdom	80 (mean)	689 (f)	Prospective	4 years	DXR-MCI DXR-BMD	Vertebrae, hip	A 1 SD decrease in DXR-MCI and DXR-BMD increased vertebral fracture risk 1.81- and 1.56-fold, respectively. A 1 SD decrease in DXR-MCI and DXR-BMD increased hip fracture risk 1.72- and 1.79-fold, respectively.	Metacarpal indices are useful for vertebral and hip fracture prediction.
Wilczek 2013 [296]	Sweden	59 (mean)	2837 (m) 5420 (f)	Prospective	3 years	DXR-BMD	Hip	Men and women with hip fractures had lower BMD than the non-fractured group.	DXR-BMD predicts hip fracture risk in men and women.
Kalvesten 2016 [297]	United States	71 (mean)	5278 (f)	Prospective	10 years	DXR-BMD	Vertebrae, hip	DXR-BMD discriminated fractures similarly to FRAX and DXA-BMD.	DXR-BMD could be a useful method to identify patients who might benefit from an osteoporosis assessment.

DXR - automated digital radiogrammetry of metacarpal bones, MCI - metacarpal index; BMD - bone mineral density; RCA - Relative cortical area; FRAX – Fracture risk assessment tool; DXA - dual energy x-ray absorptiometry; m - male; f - female; WF - with fracture; NF - no fracture

1.9 Bone health in South African children and adolescents

Differences in body size, bone mass and body composition have been shown between US and SA white children confirming that it may not be possible to extrapolate results from other populations groups to SA individuals [52]. Our understanding of bone health in SA children is complicated by the heterogeneity of the SA population, the stark contrast between the SES of urban and rural population groups, and the lack of longitudinal data. Several studies have been undertaken on the children from the Bt20 cohort, the longest running longitudinal study of children and adolescents in Africa, and its Bone Health sub-cohort. The key findings of these investigations relevant to this thesis are summarised in Table 1.4 below.

Table 1.4 Summary of key findings from studies of the Bt20 and Bone Health cohorts relevant to this thesis

Factors Investigated	Studies	Main Findings	Interpretation/Conclusion
Bone Size	Vidulich 2006 [35]	Pre-pubertal black children had wider metacarpal bones with thinner cortices than white children.	Ethnic differences in bone size and geometry are present in pre-pubertal SA children.
Bone Mass	Vidulich 2006 [35]	Pre-pubertal black children had greater axial and appendicular bone mass than white children.	Ethnic differences in bone mass are present in pre-pubertal SA children.
Bone Strength	Micklesfield 2011 [49]	Thirteen-year old black children had greater radial and tibial bone size and strength than their white peers.	There are ethnic differences in bone size and strength in SA children.
Skeletal Maturity	Hawley 2009 [302]	Between 1962 and 2001, skeletal maturity advanced by 9.7 and 15.8 months in black males and females, respectively.	There has been a positive secular trend in skeletal maturity in black children.
	Demerath 2009 [303]	Children with rapid weight gain in infancy were taller, heavier and had more advanced skeletal maturity than those with slow weight gain.	Rapid weight gain in infancy is associated with advanced skeletal maturation in late childhood.
	Cole 2015 [78]	Girls reached skeletal maturity 1.9 years earlier than boys. Skeletal maturity in black boys occurred 7 months later than in white boys.	There are sex and ethnic differences in skeletal maturity.
Bone growth	Schoenbuchner 2017 [50]	Diaphyseal cortical CSA and SSIp at the radius were greater in white than black adolescents. At the tibia, white adolescents had lower diaphyseal CSA and SSIp.	There are ethnic differences in bone growth and strength in SA adolescents which are site-specific.
Puberty	Jones 2009 [63]	Age of pubertal initiation remained stable but age at menarche declined between 1998 and 2004 in urban SA girls.	The tempo of pubertal maturation in urban SA girls has increased.

Table 1.4 continued overleaf

Table 1.4 continued

Factors Investigated	Studies	Main Findings	Interpretation/Conclusion
Socio-economic status	Norris 2008 [198]	At 9 - 10 years whole-body bone area and BMC had a stronger association with SES measured at the same time compared to SES measured during infancy.	Current SES measures affect bone mass.
Nutrition	MacKeown 2007 [98]	Calcium, iron, zinc, copper and vitamins A and B6 intake were 70% below RDA in black children.	Micronutrient intake was consistently below the RDA in black children.
	McVeigh 2007 [105]	Pre-pubertal black children had higher hip bone area and BMC than white children, despite having a lower calcium intake.	Calcium intake did not influence BMC.
	Poopedi 2011 [118]	White children had higher 25-hydroxy vitamin D than black children. At age 10 years, very few children were vitamin D deficient.	There are ethnic differences in 25-hydroxy vitamin D levels. Vitamin D supplementation is not necessary in Johannesburg.
Overweight/Obesity	Lundeen 2015 [135]	Overweight and obesity was prevalent in girls from pre-puberty and increased throughout adolescence.	A healthy diet should be promoted early in life.
Physical Activity	McVeigh 2004 [172]	White children who engaged in physical activity had greater whole-body aBMD than less active children. There was no association between bone mass and exercise in black children.	Black children did not reach adequate levels of physical activity to have an osteogenic effect.
Population Differences	Micklesfield 2007 [52]	Pre-pubertal black and white SA children had higher whole-body BMC than their US peers.	Bone mass varies amongst different populations.
Genetics	May 2013 [37]	Candidate genes associated with bone mass in black children differed with respect to their association with adult Caucasian skeletal sites.	Genetic factors influence femoral neck bone mass in black children.

Table 1.4 continued overleaf

Table 1.4 continued

Factors Investigated	Studies	Main Findings	Interpretation/Conclusion
Fracture Incidence	Thandrayen 2009 [231]	Fracture incidence in white children was almost double that of black children.	There are ethnic differences in fracture rates in SA children.
	Thandrayen 2011 [42]	White males who fractured had greater bone area and BMC and participated in more physical activity than their non-fractured peers.	Physical activity increases fracture incidence despite a high aBMD.

CSA - cross-sectional area, SSIp - stress-strain index, BMC - bone mineral content, BMD - bone mineral density, SES - socioeconomic status

1.10 Summary and Gaps in the Literature

There is a genetic “landscape’’ which prevails in skeletal growth and development, with different genes influencing bone accrual and mineralisation, resorption and remodelling during childhood and adulthood [304]. The literature presents compelling evidence for the antecedents of bone mass in adulthood being established in early life. Key to our understanding of bone mass attainment is how changes in structure and function due to environmental influences at critical points during development affect skeletal growth.

There is consensus in the literature that little difference in bone mass exists between the sexes in the pre-pubertal phase and that differences arise during puberty. This thesis reports a longitudinal study, which tracks the changes that occur in appendicular bone development in a South African cohort from pre-puberty through adolescence. It shows the temporal changes in linear bone growth and bone width in black and white SA females and males, thus presenting unique data of longitudinal bone modelling.

A review of the literature reflects a paucity of studies on skeletal fragility in children from African populations. Internationally, most studies have been cross-sectional and do not present longitudinal data as is done in this thesis, particularly during the critical time of attainment of peak bone mass. These data are also presented for two ethnic groups and both sexes. The use of pQCT is still in its infancy and to my knowledge, there are no longitudinal studies comparing bone strength indices derived from pQCT to those obtained by conventional radiogrammetry. There are conflicting results in the literature regarding the reliability of these bone strength indices in predicting fracture risk.

1.11 Aims, Hypotheses and Objectives of this study

This thesis aims to address the following research objectives in 3 discrete chapters, the data from which have either been published or have been provisionally accepted for publication. Author contributions have been included in Appendix I.

Aims

The main aims of this thesis were to assess metacarpal bone growth longitudinally during adolescence in South African children of different ethnic groups, and to compare patterns of metacarpal bone growth and bone strength (measured by radiogrammetry) to those (measured by pQCT) of the non-weight bearing radius and weight-bearing tibia. I further examined the association between radiogrammetric metacarpal bone measures and indices and fracture risk.

Hypotheses

1. Puberty has a sex-specific effect on the periosteal and endocortical bone surfaces.
2. Black children have wider bones with thinner cortices than their white peers.
3. Metacarpal bone measures and strength correlate more closely with MCSA at the radius than at the tibia.
4. Irrespective of sex or ethnicity, the pattern of growth of the non-weight bearing metacarpal is similar to that of the radius, but different from that of the weight-bearing tibia.
5. Metacarpal bone dimensions and indices predict fracture risk.

Objectives

1. To assess the changes in metacarpal dimensions through puberty and adolescence in South African (SA) black and white children and adolescents.
2. To determine whether there are ethnic and sex differences in metacarpal bone development in SA children and adolescents.
3. To compare the growth pattern of the metacarpal bone with that of the radius and tibia through puberty and adolescence in SA black and white children and adolescents.
4. To compare a bone strength index derived from metacarpal radiogrammetry with those derived from radial and tibial peripheral quantitative computed tomography.
5. To determine whether there is an association between metacarpal indices and a history of fracture in SA adolescents.

6. To determine whether metacarpal indices can be used to predict fracture risk in SA children and adolescents.

This thesis consists of 6 chapters. Chapter 1 reviews the relevant background literature and describes the aims and objectives of the thesis. Chapter 2 describes the methods and statistical analyses performed to meet the aims and objectives above. Chapters 3, 4 and 5 are based on individual published manuscripts (Appendices J - L) and include the results, discussion and limitations of the findings. Minor modifications to the published chapters have been made in response to examiners' comments. These three chapters cover the following areas:

- Chapter 3: Metacarpal growth during adolescence in a longitudinal South African cohort (meets objectives 1 and 2 above)
- Chapter 4: A longitudinal comparison of appendicular bone growth and markers of strength through adolescence in a South African cohort using radiogrammetry and peripheral quantitative computed tomography (meets objectives 3 and 4 above)
- Chapter 5: Metacarpal indices and their association with fracture in South African children and adolescents (meets objectives 5 and 6 above)

Chapter 6 concludes the thesis. It integrates the findings of the research conducted as described in the 3 preceding chapters, and places them in context with what is known in the field and how they advance our understanding. Finally, there is a discussion on possible new studies in the field of bone growth and the directions that these should take.

CHAPTER 2

Study Methods

2.1 Study population

This longitudinal study is based on 3273 singleton children from the Birth to Twenty (Bt20) cohort and its Bone Health sub-cohort (BHC) born between April and June 1990 in Soweto, an urban area in Johannesburg, South Africa [305-307]. The broad aims of the longitudinal cohort were multidisciplinary, aiming to track the growth, health and well-being, and educational outcomes of the enrolled neonates from their early years and through puberty and adolescence.

Recruitment was performed at antenatal clinics and mothers who were predicted to deliver their babies during the enrolment period were selected. A criterion for inclusion into the cohort was that the mother and baby had intention to remain in the area until the child was at least six months old. Participants of the cohort were segregated geographically due to the Apartheid laws of South Africa at the time. Ethnic categorisation was based on the classification used in South Africa for demographic and restitution purposes at the time. Children were categorised as either Black (African descent), White (European descent), Coloured (mixed ancestry) or Indian. Although the black population in Soweto comprises several different tribal groups, genetic studies of the Bt20 black participants showed homogeneity within the group with a minimal contribution from various ancestral populations [37]. Ethnic classification was self-reported by the parents and only those children whose both parents belonged to the same ethnic group were included in this study. Thus, a total number of 498 children who were of either Coloured or Indian ancestry were excluded from the study.

2.2 The Bone Health Cohort

The participants of my study were from the BHC. The BHC consisted of 563 children enrolled at age 9 years from the large Bt20 cohort to investigate factors relating to the acquisition of bone mass during puberty and adolescence. Due to the small number of white children in the Bt20 cohort (being representative of the ethnic demographics in the region at that time), an additional 120 white children born in the same period were recruited into the BHC at 9 years of age from schools in other areas. They were born during the cohort enrolment dates and their birth weight, socioeconomic status, maternal age and education were similar to those of the initial white participants of the BHC [42]. At the time of recruitment into the BHC, height data of participants of the Bt20 cohort were used to determine the number of additional participants that would be required to ensure adequate power for the anticipated number of explanatory factors that would be used in the regression analyses. Participation in the BHC was offered to the entire Bt20 cohort; however, exclusion criteria included children suffering from chronic illnesses such as epilepsy and asthma because of possible effects of drug therapy on bone development and density. Informed assent from adolescent participants and consent from parents were obtained for inclusion in this study. Ethics clearance was obtained from the University of the Witwatersrand Committee for Research on Human Subjects (Ethics Clearance Number M130126 – Appendix C).

Annual measurements, taken at approximately the same time every year for each child, included weight, height, hand-wrist radiographs and pQCT images of the radius and tibia. Attrition of participants and missing observation points during the study were frequently due either to time constraints, challenges with transportation, or to the circular migration of individuals between their extended family in urban and rural areas [306].

2.3 Anthropometric measurements

Weight and height of participants were measured whilst wearing light clothing and no shoes using standard techniques [308]. Weight was measured to the nearest 100g using a digital scale (Dismed, Miami, Fl, USA) and height to the nearest millimetre using a stadiometer (Holtain, Crymych, UK). Quality control annual training and monitoring ensured a coefficient of variation between measurers of less than 2%.

2.4 Pubertal assessment

The ages of onset of puberty in the Bt20 cohort have previously been published [63]. However, due to the asynchrony between the timing and velocity of growth relative to sexual maturity [309], in this study, age at peak metacarpal length velocity (aPLV) was used as an indicator of pubertal development. The use of aPLV allowed females and males to be aligned on a common biological maturity indicator. Calculation of aPLV is described in Section 2.11. Unpublished data (provided by Lukhanyo Nyati) on aPHV were compared to aPLV using a Student t-test. In females, aPHV generally corresponds to Tanner stage 3, and in males to Tanner stage 4 [75].

2.5 Radiography

Postero-anterior hand-wrist radiographs of the left hand were taken annually from age 9 to 21 years by certified radiographers at the Charlotte Maxeke Academic Hospital in Johannesburg. The left hand was radiographed in all participants irrespective of hand dominance. The x-ray beam was focussed on the distal aspect of the third metacarpal of the left hand. Radiographs were taken using cassettes with single-emulsion film under standard conditions of tube to film distance of 76cm and exposure at 42 kV and 12 mA/s, and processed in an automatic developer in accordance with the optimal conditions described by Tanner and co-workers [310]. The effective radiation dose was 0.1 – 0.12 µSV.

2.6 Radiogrammetry

Second metacarpal dimensions were measured on a total of 4730 hand-wrist radiographs by a single reader (Ansuyah Magan). Measurements were carried out using a digital calliper calibrated to 0.01 mm [86]. The total length of the bone was measured by drawing a line from its most distal aspect, through the mid axis of the bone shaft, to the notch at the base of the bone (Figure 2.1). At the midpoint of the total length, a line was drawn perpendicular to the long axis of the bone to determine the midshaft of the bone. The following bone variables were measured at the midshaft in millimetres: outer bone width (D) and inner medullary width (d). The cortical thickness was calculated as the difference between the bone width and medullary width (D – d). The spatial relationship between cortical thickness and medullary width was referred to as bone architecture.

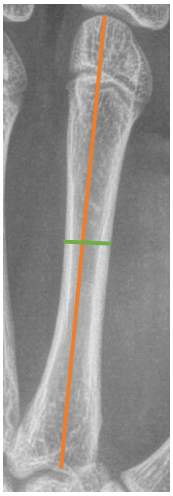


Figure 2.1 Points of measurement of metacarpal bone length, and bone width and medullary width at the midshaft.

2.6.1 Intra-observer Reliability

Measurements of 30 randomly selected radiographs were repeated by the same researcher 1 month after initial measurement (short-term) and again 16 months later (long-term) to assess intra-observer reliability and reproducibility. The coefficients of variation for the radiogrammetry measures and calculated indices are presented in Table 2.1.

Table 2.1 Coefficients of variation of radiogrammetry measures

Bone variable/Index	Short-term CV (%)	Long-term CV (%)
Bone length	0.13	0.20
Bone width	0.16	0.53
Medullary width	3.70	5.00
Cortical thickness	1.38	2.58
Metacarpal Index	2.13	3.02
Bone Mineral Density	1.41	2.04
Section Modulus	1.55	2.59
Stress-strain Index	2.50	5.28

CV – Coefficient of variation presented as a percentage (%)

2.6.2 Calculation of Metacarpal Indices

The metacarpal indices that were used in this study were calculated as follows, based on the assumption that the bone is a cylinder, and where D = bone width and d = medullary width:

- Metacarpal Index (MCI) = (D-d)/D [237]
- Bone Mineral Density (BMD; mg/mm^2) = π (D–d) x [1-((D-d)/D)] [275]
- Section Modulus (SM; mm^3) = π [(D^4-d^4) /32]/0.5D [279]
- Slenderness Index (SLI; $1/mm^2/kg$) = (Bone Length x Body Weight)/Section Modulus [18]
- Stress-Strain Index (SSIp; mm^3) = Section Modulus x volumetric BMD [280]. Calculation of the SSIp from radiogrammetry measurements is described further in Section 2.8.

2.7 Peripheral Quantitative Computed Tomography Imaging

The pQCT technology was only available for use in this study when the participants were 12 years old. Peripheral QCT imaging was carried out on the left forearm and lower leg on 524 participants of the BHC cohort annually from 12 until 20 years of age. At each visit, images were obtained from 2.3 mm thick diaphyseal slices taken at the tibia (38% and 65% sites) and the radius (65% site) (Stratec XCT-2000, Stratec Medical, Pforzheim, Germany). Forearm length was measured from the most distal end of the ulna styloid process to the tip of the olecranon process. The tibial length was measured from the distal end of the medial malleolus to the superior aspect of the medial tibial condyle. A scout view scan was performed and a reference line positioned at the distal end plate of the bone being measured. If epiphyseal fusion was incomplete, the reference line was positioned at the medial border of the growth plate instead. Scans locations were defined by a percentage of limb length.

The voxel size change over the study period. At the 65% radial site, voxel sizes were 0.4 mm in years 12 - 16 and 0.5 mm in years 17 - 20. At the 38% and 65% tibial sites, a 0.4 mm voxel size was used throughout the study; however, a few scans were acquired each year using a 0.5 mm voxel size. Scans were acquired at a speed of 25mm/s. Image processing and calculation of numerical values were performed using the manufacturer's software package

(version 6; Stratec Medical, Pforzheim, Germany) at thresholds of 710 mg/cm^3 for cortical bone and 180 mg/cm^3 for total bone.

Analyses at the 38% tibial site and the 65% radial sites were done using the Cort mode 1 (threshold = 710 mg/cm^3) for total cross-sectional area (TotA, mm^2) and cortical bone area (CoA, mm^2). Bone measures were calculated as follows [49]: Bone width = 2* √(TotA/π); Medullary width = 2*√(TotA-CoA)/π; Cortical thickness = Bone width – Medullary width. The Stress-strain Index (SSIp), measured in mm^3, was determined using a threshold of 280 mg/cm^3 [50].

The 65% radial and tibial sites are associated with the largest muscle belly and were assessed for MCSA (cm^2). Muscle CSA, calculated as the area with a density between 40 and 180 mg/cm^3, was analysed using contour mode 3.

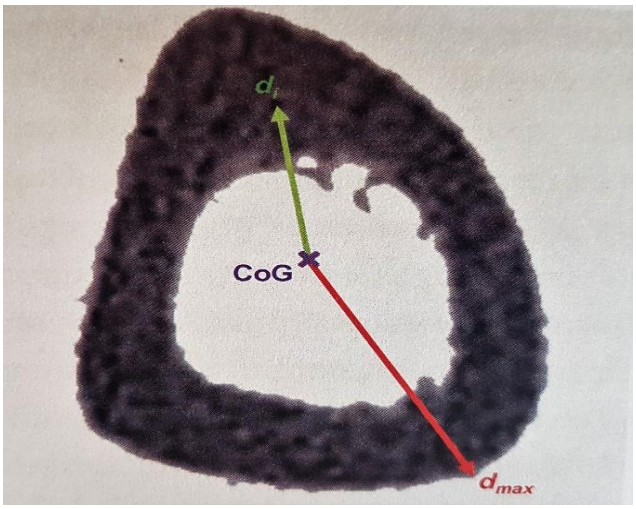
A scan of a phantom was performed daily for quality control. Throughout the study, all pQCT scans were performed by two trained operators. The inter-operator variation was less than 1%. All pQCT data were analysed by a single individual (Simon Schoenbuchner). Approximately 5% of images from each study year were assessed for distortion due to movement. Images that had been distorted by movement during scanning or incorrect limb placement were excluded from this study.

2.8 Stress-Strain Index

The stress-strain index (SSIp) is derived using measures of bone geometry and volumetric bone density to provide a proxy for bone strength [284].

Calculation of SSIp from pQCT images

The SSIp from pQCT scans of the radius and tibia were obtained from the algorithm of the manufacturer (Version 6; Stratec Medical, Pforzheim, Germany) at a threshold of 280 mg/cm^3 [50]. The formula used for calculation of the SSIp is described in Figure 2.2.



$$Stress\ Strain\ Index = \frac{a}{b_{norm}\ x\ d_{max}}\ x\ \sum_i b_i\ d_i^2$$

Figure 2.2 Calculation of the stress-strain index [311]. In the formula above, *a* is the voxel area (in this study, 0.25 mm^2), b_{norm} is a constant denominator of 1200 mg/cm^3, d_{max} is the distance of the furthest voxel from the centre of gravity (COG) of the bone's cross-section (mm), *i* indexes the individual voxels (above the inclusion threshold of 280 mg/cm^3), b_i is the density of voxel *i* (mg/cm^3) and d_i is the distance of voxel *i* from the COG (mm). The individual density of each voxel was used in the calculation of the SSIp to minimise the error due to the partial volume effect.

Calculation of SSIp from Radiographs

Radiographs are 2-dimensional, therefore, a volumetric BMD which is related to the volume of bone per projected area (VPA) was calculated, assuming that the bone has a cylindrical shape, as follows:

$$VPA = (\pi\ x\ Cortical\ thickness)\ x\ \left(1 - \frac{Cortical\ thickness}{Bone\ width}\right)$$

The VPA was multiplied by a constant of $1.2 mg/mm^3$ as this was the estimated BMD in the adult humerus when adjusting for porosities [312].

vBMD = VPA x constant of 1.2 mg/mm^3

$$Stress\ Strain\ Index\ (mm^3) = \frac{vBMD\ (mg/mm^3)}{1.2\ (mg/\ mm^3)}\ x\ \frac{Polar\ Moment\ of\ Inertia\ (\ mm^4)}{0.5\ x\ Bone\ width\ (mm)}$$

The polar moment of inertia was calculated as follows [282, 283]:

$$Polar\ Moment\ of\ Inertia\ (mm^4) = \pi\ x\ \frac{Bone\ width^4\ - Medullary\ width^4}{32}$$

2.9 Fractures

Fracture data was collected by a single individual (Kebashni Thandrayen). Fracture history was obtained retrospectively at age 17 years. Participants completed a questionnaire with the assistance of their parent or caregiver (Appendices C-D). Information obtained included age at fracture, cause and site of fracture (with the aid of a skeletal diagram), by whom the fracture was diagnosed, whether radiographs were taken to confirm the fracture, and, the treatment offered and performed.

As fracture data were collected retrospectively, fractures could not be verified radiographically. Between six months and a year after completing the initial fracture questionnaire, 51 (9%) of the original 565 participants in the Bt20 cohort who had reported a fracture were selected randomly and verified their fracture data telephonically (Appendix E). Forty-eight (94%) confirmed having had one or more fractures. Two individuals initially reported strains as fractures, but subsequently reported telephonically that no fractures had been observed by the attending health care worker following radiographic assessment. One individual who had reported no history of fractures in the initial questionnaire incorrectly reported a fracture on a follow-up questionnaire. On enquiring about details pertaining to the last fracture, 58% (28) of the individuals, or their caregivers, had 100% recall of the event including the age, site, side and trauma resulting in the fracture. Thirty seven percent had partial recall for the event as recorded in the initial questionnaire whilst in 4% the recollection did not correlate with that reported in previously completed questionnaires. Of the reported fractures, 46 (96%) were said to have been diagnosed by a doctor, one by a nursing sister and one was self-diagnosed. Of the last reported fracture, 89% had their fractures confirmed radiographically, 3 individuals had not had a radiographic assessment, two had no recollection whilst one fracture was self-diagnosed. Of the last reported fractures, management was as follows: 62% had plaster of Paris applied, 15% had a bandage applied, 11% had surgery, 6% had a splint or brace applied, 6% had a sling applied or were advised to rest.

The grades of trauma causing fracture were classified as slight (grade 1), moderate (grade 2) and severe (grade 3) (Table 2.2) [215, 313]. Only those participants who had sustained fractures as a result of mild to moderate trauma were included in the study.

Table 2.2 The grading system used to define the degree of trauma sustained at the time of fracture [231, 313]

Grade	Cause
Grade 1 (Slight)	Falling to the ground from standing on the same level Falling from less than 0.5 metres (falling from stools, chairs and beds)
Grade 2 (Moderate)	Falling from between 0.5 - 3 metres Falling down stairs, from a bicycle, roller blades, skateboard or swing Playground scuffles, Sport injuries
Grade 3 (Severe)	Falling from a height >3 metres (falls from windows or roofs) Motor vehicle or pedestrian accidents Injuries caused by heavy moving or falling objects (e.g. bricks or stones)

2.10 Physical Activity

Self-reported physical activity was obtained at age 15 years for the preceding 12 months via an interviewer administered questionnaire (Appendix G) which had been previously validated in a SA population [314]. Activities were categorised as formal (physical education, extra-mural school and club sport) and informal (leisure time activities outside of the school or club environment). The duration of activity was recorded in minutes per week (min/week) and capped at 1680 min/week for informal activity. School and club sport were both capped at 1200 min/week.

2.11 Data analyses used in Chapter 3

Statistical analyses that were used to assess growth of the metacarpal bone through puberty and adolescence are described below.

Cross-sectional Analyses

Participants were categorised into yearly age groups for cross-sectional analyses. For example, individuals ≥ 9 and <10 years were categorised as age group 9.5 years. Data were analysed cross-sectionally using Stata Version 14 (Stata Corporation, Inc., College Station, TX, USA) and SPSS Version 23 (IBM Corporation, Armonk, NY, USA). No imputation of missing data was undertaken. Normality of the data was established using skewness and kurtosis tests. A one-way ANOVA was performed on the data (means of the age groups) to determine statistical differences between the means of two or more independent groups. Sex and ethnic group differences were ascertained using a Bonferroni multiple comparisons test (alpha level 0.05, degrees of freedom = 3). To determine ethnic and sex differences in bone measures with height as a covariate, an ANCOVA with a Bonferroni multiple comparisons test (alpha level 0.05, degrees of freedom = 3) was performed. Mean annual differences for unadjusted bone measures were determined using a repeated measurements ANOVA followed by a pairwise comparison of marginal linear predictions for unbalanced data. A value of $p < 0.05$ was considered to be statistically significant.

Longitudinal Analyses

Longitudinal data analyses were performed by Lukhanyo Nyati. Data were modelled using Superimposition by Translation and Rotation (SITAR) in R (Version 3.2.2, R Foundation for Statistical Computing, Vienna, Austria; https://www.r-project.org/) [315]. SITAR is a shape invariant model with a single fitted curve that summarises individual growth patterns with three parameters – size, tempo and velocity [315]. Individual curves are modelled and matched to the mean curve by shifting vertically (to represent differences in individual size), or horizontally for differences in tempo (timing) of growth. Individual differences in velocity cause a stretching or shrinking of the age scale resulting in an increase or decrease of the slope [315].

Joint models were fitted for metacarpal length, bone width and medullary width data. These combined firstly the sexes by ethnic group, and secondly, the black and white participants by sex group. This allowed for group differences by sex or ethnicity to be tested. Distance curves for the bone dimensions of metacarpal length, bone width and medullary width plotted against chronological age were produced. The SITAR model used was:

$$E(y_{it}) = \alpha_i + h\left(\frac{t - \beta_i}{e^{-\gamma_i}}\right)$$

where y_{it} is the bone dimension of the ith participant at age t; α_i (size parameter), β_i (tempo parameter) and γ_i (velocity parameter) are random effects for participant i; $h(.)$ is a fitted cubic spline curve with degrees of freedom to minimise the Bayesian Information Criterion (BIC) [316].

The age at peak metacarpal length velocity (aPLV) was obtained for each participant. To assess sex and ethnic differences in metacarpal growth standardised on a biological maturity indicator, bone measures were plotted against age from PLV. Age from PLV for each bone measure was calculated by subtracting the aPLV from the chronological age at measurement. Growth distance curves for bone width and medullary width were modelled against age from PLV.

2.12 Data analyses used in Chapter 4

Statistical analyses that were used to compare bone growth and strength of the metacarpal, radius and tibia between ages 12 and 20 years are described below.

Analyses were conducted using Stata 14.0 (Stata Corporation Inc., College Station, Texas, USA).

Bivariate regression analysis

All analyses were performed separately for each sex and ethnic group due to known differences in their bone geometry. Bivariate regression analysis was performed to determine

the association between metacarpal bone width, cortical thickness and SSIp, and MCSA of the radius and tibia, and, between metacarpal SSIp, and radial and tibial SSIp. A Hausman test, which is appropriate for longitudinal data, was performed and the fixed effects model deemed to be appropriate for assessing the bone measures. The metacarpal outcome variables were log-transformed to approximately conform to normality. Fixed effects using the least squares dummy variable model (LSDV) removed the time-invariant characteristics of the data and assessed the net effect of the predictors on the outcome variable. The level of significance was set at $p < 0.05$.

Mixed-Effects modelling

Multivariate regression analyses showed that sex ($p < 0.0001$) and ethnicity ($p < 0.02$) were significantly associated with bone growth, therefore all analyses were conducted separately for each sex and ethnic group. Metacarpal, radial and tibial growth for each sex and ethnic group were standardised on aPLV. Years from aPLV for metacarpal, radial and tibial bone measures was calculated by subtracting the aPLV from the chronological age at measurement.

Linear mixed-effects models were used to compare the growth trajectories of bone width, medullary width and cortical thickness as measured by radiogrammetry and pQCT, between the four groups. No imputation of missing data was undertaken as the mixed-effects model handles missing data by using information from other participants to obtain an overall curve.

The regression model used was:

$$Y_{ij} = \beta_0 + \beta_1 Age_{ij} + \beta_2 Age^2_{ij} + \beta_3 Site_j + \beta_4 MCSA_j + \beta_5 Age_{ij} * Site_j + \beta_6 Site_j * MCSA_j + u_0 + u_1 Age_{ij} + \varepsilon_{ij}$$

where Y*ij* is the bone variable of individual *i* measured at time *j*.

In the fixed effect of the model β_0 and β_1 represent the average value of the bone variable for each individual and the overall slope of time (age from PLV), respectively. To adjust for the effect of bone site (metacarpal, radius and tibia) on the relationship between the bone variable and biological age, an interaction term for biological age and bone site (*Age*Site*) was included in the model. An additional interaction term *Site*MCSA* was included in the model to adjust for the effect of the associated musculature of each bone. Radial MCSA was used as

a proxy for metacarpal MCSA. A quadratic function for age was included in the model as it improved model fit by lowering the BIC.

In the random effect of the model, u_0 is the individuals' residual distance from the intercept of the overall trajectory, u_1 is the residual difference for individuals' slopes around the overall effect of time, and, ε_{ij} is the residual of the individual points around the subject-specific trajectory. Random intercepts and slopes were included in all models as they improved model fit by lowering the BIC. The random effects allowed each individual's profile to vary around the average curve, and for variation in the rate of growth between individuals. Maximum log likelihood (–2log likelihood) was used to determine the significance of random effect variances and covariances between nested models. The level of statistical significance was $p < 0.05$.

2.13 Data analyses used in Chapter 5

Statistical analyses that were used to determine the associations between metacarpal indices and fracture risk are described below.

Data were analysed using STATA version 14.0 (Stata Corporation Inc., College Station, TX, USA).

Cross-sectional Data

Data for black and white participants were analysed separately because of differences between the two ethnic groups in anthropometry and bone mass measures obtained using radiogrammetry and DXA [42]. Participants were categorised into yearly age groups for cross-sectional analyses. For example, age group 10 included participants aged between 10 and 10.9 years. A one-way ANOVA with a post-hoc Bonferroni multiple comparisons test (alpha level 0.05) was performed to determine sex and ethnic differences in fracture prevalence, and, in participant anthropometry, bone measures, bone indices and physical activity levels.

Participant height and BMI data were converted to Z-scores using the World Health Organisation Anthroplus software (http://www.who.int/growthref/tools/en). Black females were the largest group of non-fracturing participants and were therefore used as a reference

group against which to compare the other groups' bone measures, bone indices and physical activity scores after converting the variables to Z-scores based on black female data. The Z-scores were calculated by subtracting the reference mean from each individual score and dividing by the reference standard deviation. Student t-tests were used to determine differences in Z-score bone measures between participants with and without a history of fracture within the four ethnic and sex groups. Bivariate logistic regression analyses were used to determine the associations between anthropometric measures, bone measures and bone indices and fracture history at 17 years separately for each sex and ethnic group. Multivariate logistic regression analyses, adjusting for height and formal physical activity, were also performed to examine the same associations at 17 years. Statistical significance was set at $p < 0.05$ for all analyses.

A power calculation was performed to show the ideal sample sizes that would be required given the effect sizes observed in the bone measure between fractured and non-fractured participants in the 4 groups, and for the study to have a power of 80% (Table 2.3).

The calculations were done using the formula [317]:

$$sample\ size = \frac{r+1}{1}\frac{SD^2\left(Z_\beta + Z_{\alpha/2}\right)^2}{d^2}$$

d = Expected mean difference in bone measure between case (fracture) and control (nil fracture) groups

SD = Standard deviation of bone measure

r = Ratio of control (nil fracture) to cases (fracture)

Z_β = Standard normal variate for power = 0.84 for 80% power

$Z_{\alpha/2}$ = Standard normal variate = 1.96 at 5% type I error (p <0.05)

Table 2.3 The ideal sample size for a given effect size in bone measure between fractured and non-fractured groups

	This study sample size		Ideal sample size	
Group	Nil fracture	Fracture	Nil Fracture	Fracture
Black Female	108	15	1632	227
White Female	30	20	34	22
Black Male	116	29	430	107
White Male	**23**	**18**	16	13

The calculations in Table 2.3 show that there was sufficient power for the white males, despite the small sample size.

Longitudinal Data

Correlation analysis was performed on height at each year from 10 until 16 years with MCI, BMD, SM and SSIp to determine the influence of participant height on each index at different ages.

To determine the association between bone measures and indices prior to aPLV and a history of fracture at 17 years, logistic regression analysis was performed for each bone measure and index for the combined sex and ethnic groups of participants at 10 years of age to obtain the odds ratios for fracture at 17 years, after adjusting for height.

A Generalised Estimating Equations (GEE) model was used to analyse the correlated data of the bone measures and indices with the binary outcome of fracture. The combined data of participants from both ethnic and sex groups from ages 10 until 16 years were assessed against the cumulative fracture incidence between 10 and 17 years. A binomial family with a logit link was specified in the model. An autoregressive correlation structure was deemed the most appropriate to minimise loss of statistical power for the repeated measures. Odds ratios were calculated for the bone indices and the binary outcome of fracture. Statistical significance was set at $p < 0.05$.

2.14 Summary

The results of the three major studies of this thesis that were formulated using the objectives, methods and statistical analyses described above are discussed in the following 3 chapters. Chapter 3 describes the ethnic and sex differences in metacarpal growth from puberty through adolescence (addresses objectives 1 and 2). In Chapter 4, metacarpal growth and strength measured using radiogrammetry were compared to that of the radius and tibia obtained from pQCT (addresses objectives 3 and 4). Chapter 5 describes the association between metacarpal indices and fracture risk in children and adolescents (addresses objectives 5 and 6).

CHAPTER 3

Metacarpal growth during adolescence in a longitudinal South African cohort

This chapter describes the growth pattern of the second metacarpal bone of children from two ethnic groups through puberty and adolescence. The results are described and discussions pertaining to these findings are outlined together with the limitations. The principal findings and discussion in this chapter were published in the following journal (Appendix J:

Magan A, Nyati LH, Micklesfield LK, Norris SA and Pettifor JM. (2017) Metacarpal growth during adolescence in a longitudinal South African cohort. Journal of Bone and Mineral Research 32(9): 1926-1934. DOI: 10.1002/jbmr.3179

3.1 Methods

Manual radiogrammetry was performed on hand-wrist radiographs of 572 children from the Bone Health Cohort taken annually from ages 9 to 21 years. The second metacarpal bone length, and at the midshaft, bone width and medullary width were measured on a total of 4730 radiographs. The cortical thickness was calculated as the difference between the bone width and medullary width.

Superimposition by Translation and Rotation (SITAR) was used to obtain aPLV. Bone width and medullary width were modelled using SITAR against both chronological age and age from PLV. Cross-sectional analyses of the mean annual measurements for bone width, medullary width and cortical thickness were performed separately for each sex and ethnic group.

3.2 Results

Participant recruitment and range in number of observations are presented in Figure 3.1. Of the 683 participants recruited into the Bone Health Cohort, 111 participants elected to exit the study due either to time constraints or challenges with transportation. The 572 participants included in these analyses comprised 183 (32%) black females, 93 (16%) white females, 207 (36%) black males and 89 (16%) white males.

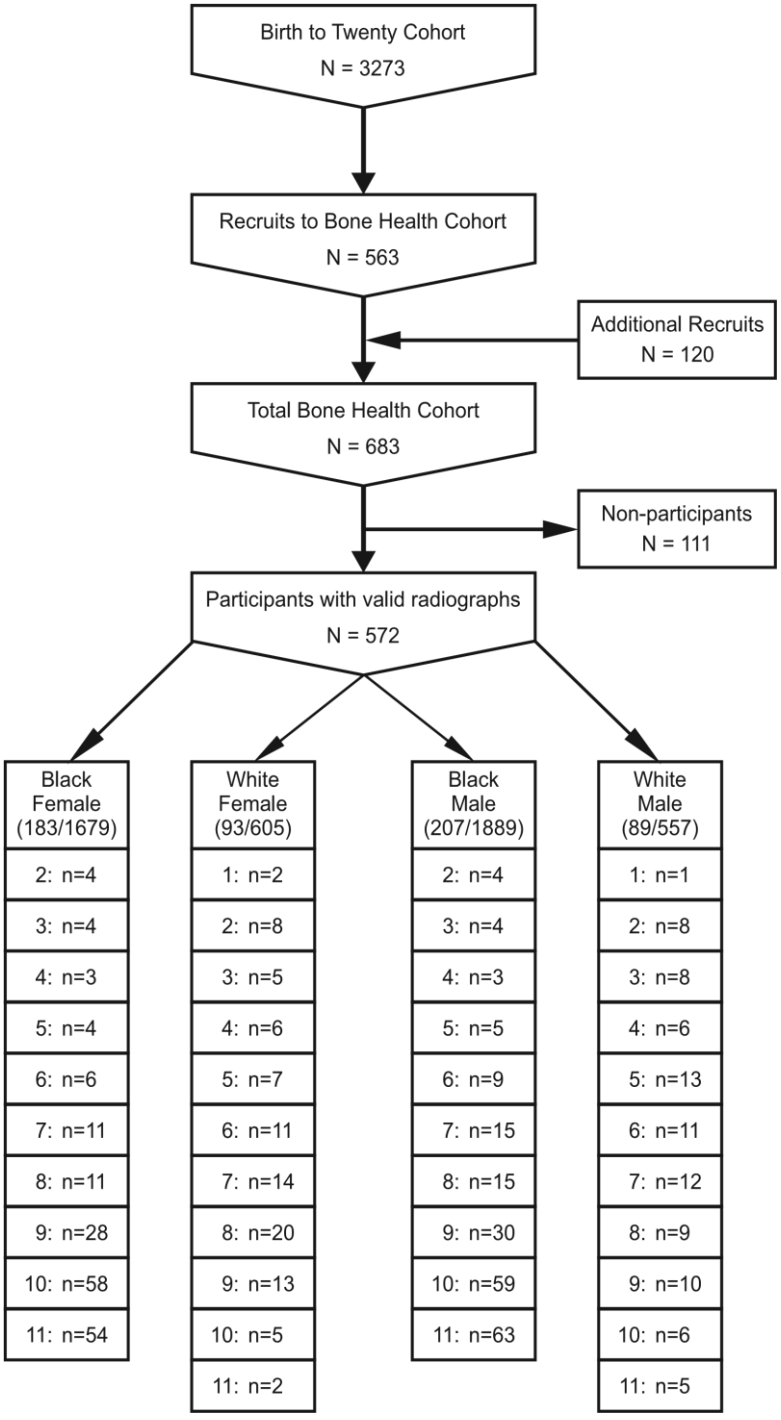


Figure 3.1 Participant recruitment (N) and the number of participants (n) with their respective total number of radiographs over the study period. (2: n=4 means that 4 participants had a total of 2 radiographs)

Metacarpal Length

The individual curves for metacarpal length growth of female and male individuals of both ethnic groups are presented in Figures 3.2A and 3.2B respectively. Peak metacarpal length velocity was attained at similar ages in black (11.7 ± 0.65 years) and white (12.0 ± 0.82 years) females (p = 0.27) (Figure 3.2A; Table 3.1). White males attained PLV significantly earlier than their black peers (13.4 ± 0.64 years vs 14.0 ± 0.89 years; p < 0.0001) (Figure 3.2B; Table 3.1). The tempo of the metacarpal growth spurt in black males lagged white males by 7 months (Figure 3.2B; Table 3.1). The tempo of the metacarpal growth spurt was similar between black and white females (Figure 3.2A; Table 3.1). In males, metacarpal length growth velocity was greater in white compared to black participants (p = 0.002).

In both ethnic groups, the metacarpal growth spurt occurred significantly earlier in females with black females attaining PLV 2.6 years earlier than their male peers, and white females attaining PLV 1.5 years earlier than white males (Table 3.2). In females, aPLV occurred earlier than aPHV (black 11.8 ± 0.94, p < 0.002; white 12.2 ± 0.86, p = 0.0001). Age at PLV coincided with aPHV (14.0 ± 0.92, p = 1.0) in black males, but occurred 0.1 year later than aPHV (13.3 ± 0.72, p < 0.03) in white males.

Significant ethnic differences were observed in metacarpal length in both sexes over the study period (Table 3.1). Adolescent black children had on average 1 mm longer second metacarpals than white children (p = 0.03) (Table 3.1). These ethnic differences were accentuated after adjusting data for height (Tables 3.3 and 3.4). The second metacarpal of black and white adolescent males was longer than that of their female counterparts following the pubertal growth spurt (p < 0.0001) (Figure 3.3; Table 3.2).

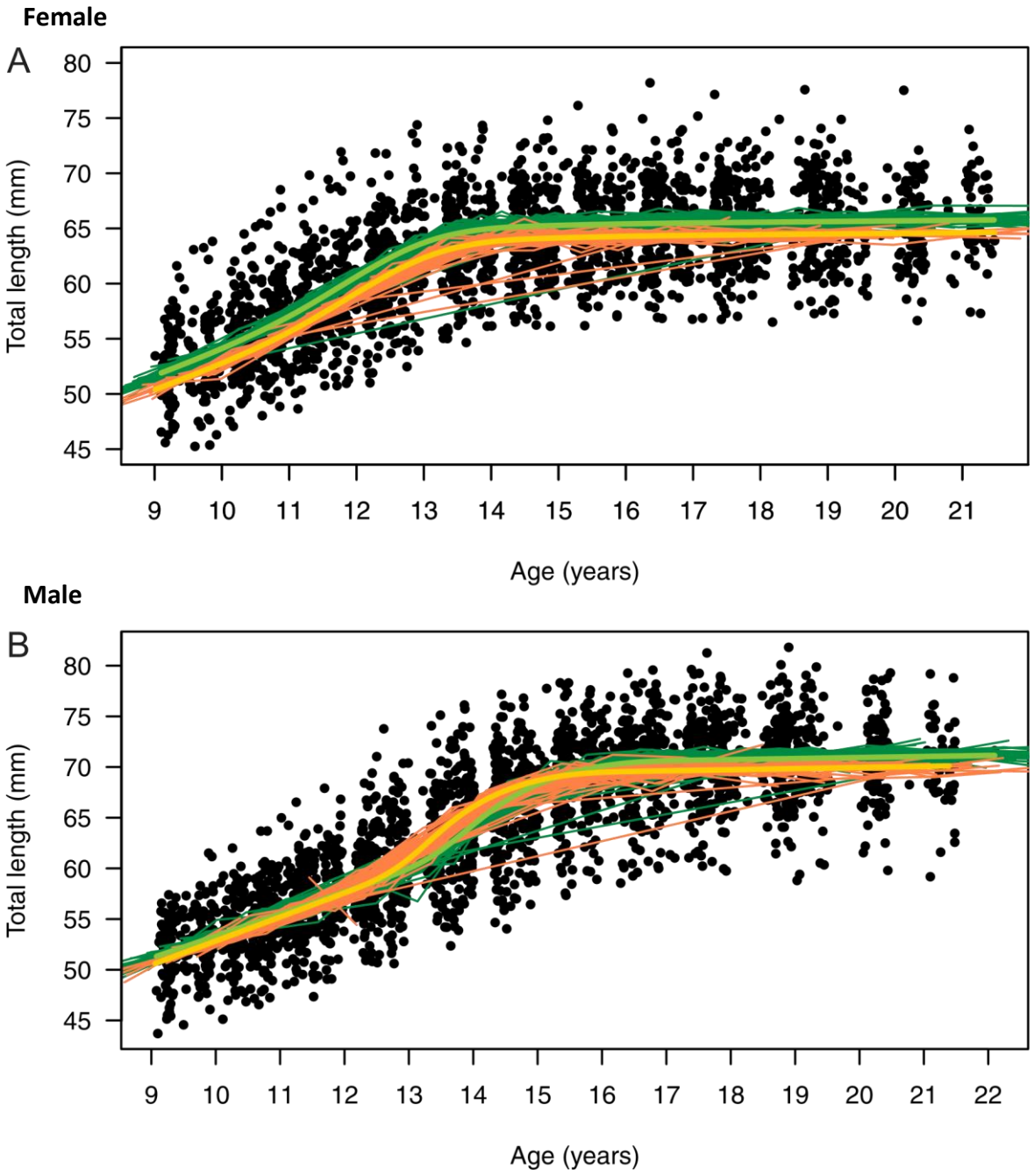


Figure 3.2 Scatterplot of metacarpal length against chronological age, and individual curves adjusted for tempo and velocity, with the mean adjusted curve, for metacarpal length growth by ethnicity, in female (A) and male (B) participants. Mean adjusted curves generated for black participants are in green and for white participants in orange colour. Note that the mean curves in black and white females are very similar, whilst the mean curve for black males deviated from that of white males during the pubertal growth spurt (13 – 16 years of age).

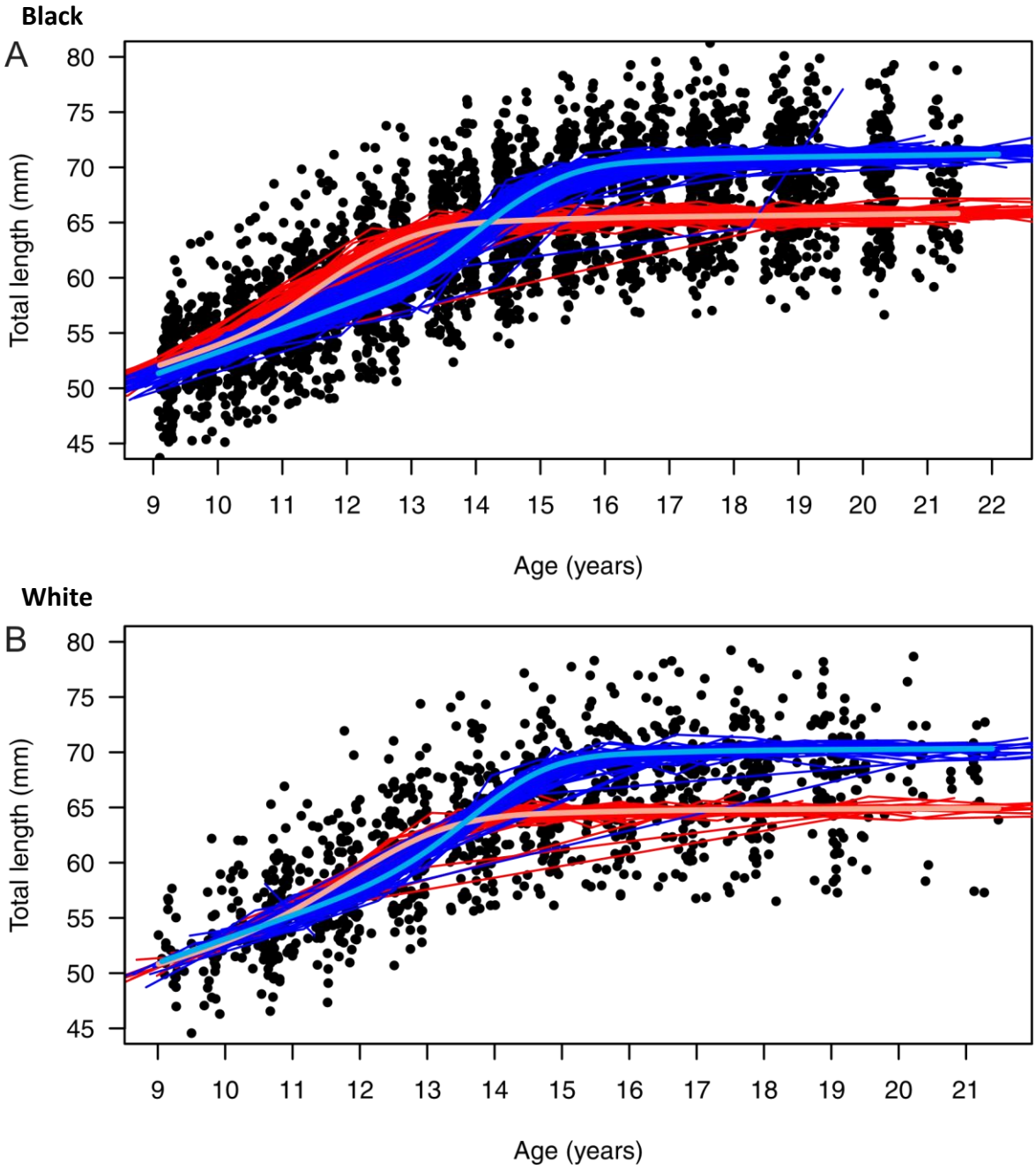


Figure 3.3 Scatterplot of metacarpal length against chronological age, and individual curves adjusted for tempo and velocity, with the mean adjusted curve, for metacarpal length growth by sex, in black (A) and white (B) participants. Mean adjusted curves generated for female participants are in red and for male participants in blue colour.

Table 3.1 Summary of SITAR analyses of ethnic differences in the size, tempo and velocity of metacarpal length, bone width and medullary width growth during adolescence, as measured against chronological age

	Metacarpal Length		Bone Width		Medullary Width	
	Females	Males	Females	Males	Females	Males
Participants/Observations	258/2099	275/2271	258/2099	275/2271	258/2099	275/2271
Degrees of freedom	5	5	5	6	8	5
Residual SD (mm)	0.51	0.66	0.10	0.11	0.19	0.25
Tempo-velocity correlation	-0.36	-0.47	-0.60	-0.01	-0.62	-0.01
Variance explained (%)	98.1	97.1	97.8	98.0	94.6	92.9

	Metacarpal Length				Bone Width				Medullary Width			
	Females		Males		Females		Males		Females		Males	
	Mean ± SE	*p*	Mean ± SE	*p*	Mean ± SE	*p*	Mean ± SE	*p*	Mean ± SE	*p*	Mean ± SE	*p*
Size (mm)	-1.04 ± 0.48	0.03	-1.10 ± 0.51	0.03	-0.13 ± 0.08	0.13	-0.24 ± 0.10	0.02	-0.40 ± 0.10	< 0.0001	-0.70 ± 0.12	<0.0001
Tempo (years)	0.15 ± 0.13	0.27	-0.58 ± 0.13	<0.0001	0.24 ± 0.19	0.23	-0.50 ± 0.15	0.0009	0.72 ± 0.27	0.008	-1.74 ± 0.29	<0.0001
Velocity (mm/year)	-0.01 ± 0.03	0.66	0.09 ± 0.03	0.002	0.02 ± 0.04	0.69	0.19 ± 0.05	<0.0001	0.06 ± 0.05	0.18	0.03 ± 0.04	0.4

Data are for white relative to black females and males. SD – standard deviation, SE – standard error of means

Table 3.2 Summary of SITAR analyses of sex differences in the size, tempo and velocity of metacarpal length, bone width and medullary width growth during adolescence as measured against chronological age

	Metacarpal Length		Bone Width		Medullary Width	
	Black	White	Black	White	Black	White
Participants/Observations	390/3568	182/1162	370/3349	163/1022	370/3349	163/1022
Degrees of freedom	6	4	6	4	6	5
Residual SD (mm)	0.61	0.63	0.10	0.13	0.05	0.22
Tempo-velocity correlation	-0.28	-0.62	0.01	0.21	0.39	-0.01
Variance explained (%)	98.0	97.9	98.6	97.0	94.7	92.8

	Metacarpal Length				Bone Width				Medullary Width			
	Black		White		Black		White		Black		White	
	Mean ± SE	*p*	Mean ± SE	*p*	Mean ± SE	*p*	Mean ± SE	*p*	Mean ± SE	*p*	Mean ± SE	*p*
Size (mm)	5.42 ± 0.38	<0.0001	5.38 ± 0.53	<0.0001	1.07 ± 0.07	<0.0001	0.99 ± 0.10	<0.0001	0.16 ± 0.02	<0.0001	0.48 ± 0.16	<0.0001
Tempo (years)	2.61 ± 0.10	<0.0001	1.53 ± 0.17	<0.0001	3.00 ± 0.11	<0.0001	2.51 ± 0.20	<0.0001	0.15 ± 0.01	<0.0001	0.06 ± 0.02	<0.0001
Velocity (mm/year)	0.13 ± 0.02	<0.0001	0.24 ± 0.04	<0.0001	0.26 ± 0.04	<0.0001	0.32 ± 0.06	<0.0001	0.22 ± 0.03	<0.0001	0.12 ± 0.05	0.03

Data are for males relative to females in black and white participants. SD – standard deviation, SE – standard error of means

Table 3.3 Unadjusted metacarpal dimensions in black and white females and males

		Black Female					White Female			
Age	N	L ± SD	D ± SD	d ± SD	CT ± SD	N	L ± SD	D ± SD	d ± SD	CT ± SD
9.5	131	53.09 ± 3.52	6.79 ± 0.64[b]	3.87 ± 0.64[b]	2.92 ± 0.41	28	52.31 ± 3.83	6.55 ± 0.53	3.49 ± 0.58	3.07 ± 0.50
10.5	156	55.95 ± 3.86[b]	7.10±0.64	3.96 ± 0.67[ab]	3.14 ± 0.48[b]	61	55.37 ± 4.02	6.92 ± 0.60	3.64 ± 0.69[d]	3.28 ± 0.53
11.5	156	58.94 ± 4.39[b]	7.38±0.68	4.09 ± 0.74[ab]	3.29 ± 0.50[b]	64	57.97 ± 4.45	7.18 ± 0.66	3.71 ± 0.73[d]	3.47 ± 0.60
12.5	161	61.74 ± 4.29[b]	7.63±0.68	4.12 ± 0.79[ab]	3.52 ± 0.54[ab]	68	61.39 ± 4.24	7.56 ± 0.65	3.75 ± 0.80[d]	3.82 ± 0.65
13.5	150	63.99 ± 3.86	7.90±0.69[b]	4.13 ± 0.82[b]	3.77 ± 0.54[b]	60	63.32 ± 3.97	7.75 ± 0.64[d]	3.80 ± 0.80[d]	3.93 ± 0.55
14.5	165	64.65 ± 3.62[b]	7.96±0.64[b]	4.04 ± 0.78[ab]	3.93 ± 0.52[ab]	72	64.01 ± 3.85[d]	7.83 ± 0.60[d]	3.61 ± 0.81[d]	4.24 ± 0.57
15.5	149	65.20 ± 3.56[b]	8.05±0.69[b]	4.05 ± 0.86[ab]	4.00 ± 0.52[a]	56	64.22 ± 3.72[d]	7.94 ± 0.64[d]	3.67 ± 0.86[d]	4.27 ± 0.63[d]
16.5	164	65.60 ± 3.56[b]	8.09±0.68[b]	4.02 ± 0.86[ab]	4.08 ± 0.56[a]	62	64.53 ± 3.67[d]	7.98 ± 0.57[d]	3.55 ± 0.87[d]	4.42 ± 0.62[d]
17.5	146	65.56 ± 3.60[b]	8.13±0.71[b]	3.97 ± 0.88[ab]	4.16 ± 0.56[a]	57	64.88 ± 3.96[d]	8.03 ± 0.61[d]	3.57 ± 0.93[d]	4.48 ± 0.59[d]
18.5	124	66.07 ± 3.65[b]	8.15±0.71[b]	3.96 ± 0.90[b]	4.18 ± 0.60[b]	30	65.08 ± 3.93[d]	8.03 ± 0.54[d]	3.49 ± 0.85	4.53 ± 0.68[d]
19.5	50	65.32 ± 3.51[b]	8.16±0.51[b]	3.93 ± 0.69[b]	4.23 ± 0.57	32	64.17 ± 3.94[d]	8.02 ± 0.60[d]	3.46 ± 1.01	4.55 ± 0.71[d]
20.5	127	65.69 ± 3.47[b]	8.11±0.69[b]	3.87 ± 0.88[b]	4.25 ± 0.59[b]	15	65.61 ± 4.62[d]	7.85 ± 0.47[d]	3.20 ± 0.84	4.64 ± 0.73[d]

		Black Male					White Male			
Age	N	L ± SD	D ± SD	d ± SD	CT ± SD	N	L ± SD	D ± SD	d ± SD	CT ± SD
9.5	146	52.30 ± 3.27	7.06 ± 0.67	4.23 ± 0.73[c]	2.83 ± 0.46[c]	26	51.64 ± 2.73	6.68 ± 0.74	3.54 ± 0.83	3.14 ± 0.43
10.5	177	54.45 ± 3.42	7.29 ± 0.69	4.39 ± 0.75[c]	2.89 ± 0.43[c]	62	54.69 ± 3.36	7.13 ± 0.65	4.00 ± 0.71	3.13 ± 0.44
11.5	179	56.82 ± 3.80	7.54 ± 0.73	4.54 ± 0.79[c]	3.00 ± 0.44[c]	69	56.96 ± 3.61	7.44 ± 0.70	4.12 ± 0.79	3.31 ± 0.52
12.5	183	59.47 ± 4.20	7.80 ± 0.76	4.61 ± 0.84[c]	3.19 ± 0.53[c]	63	60.86 ± 4.57	7.85 ± 0.72	4.25 ± 0.68	3.60 ± 0.65
13.5	170	63.61 ± 4.68	8.18 ± 0.79	4.75 ± 0.85[c]	3.44 ± 0.60[c]	49	64.43 ± 5.19	8.23 ± 0.87	4.29 ± 0.78	3.94 ± 0.60
14.5	184	66.02 ± 4.76	8.51 ± 0.84	4.85 ± 0.94[c]	3.65 ± 0.66[c]	53	67.32 ± 4.19	8.55 ± 0.79	4.34 ± 0.86	4.21 ± 0.68
15.5	167	68.74 ± 4.40	8.78 ± 0.81	4.87 ± 0.99[c]	3.91 ± 0.69[c]	55	69.51 ± 3.68	8.87 ± 0.85	4.26 ± 0.89	4.58 ± 0.61
16.5	183	69.89 ± 4.21	8.99 ± 0.81	4.90 ± 1.03[c]	4.08 ± 0.73[c]	53	70.36 ± 3.38	8.98 ± 0.82	4.22 ± 0.93	4.76 ± 0.57
17.5	172	70.55 ± 4.19	9.13 ± 0.79	4.84 ± 1.04[c]	4.30 ± 0.70[c]	46	70.30 ± 3.72	9.17 ± 0.81	4.20 ± 0.96	4.96 ± 0.55
18.5	137	71.05 ± 3.82	9.14 ± 0.79	4.67 ± 1.09[c]	4.46 ± 0.75[c]	36	70.65 ± 3.44	9.02 ± 0.81	4.03 ± 0.91	4.97 ± 0.48
19.5	55	71.23 ± 4.56	9.25 ± 0.68	4.90 ± 0.81[c]	4.35 ± 0.58[c]	28	69.57 ± 2.91	8.98 ± 0.77	3.93 ± 0.91	5.06 ± 0.66
20.5	136	70.86 ± 3.90	9.22 ± 0.79	4.69 ± 1.11[c]	4.53 ± 0.74	17	70.07 ± 4.10	8.95 ± 0.93	3.56 ± 0.85	5.39 ± 0.59

Data are presented as unadjusted means and standard deviations (SD). Bone Length (L), Bone width (D), Medullary Width (d), Cortical Thickness (CT). All measurements are in millimetres. Significant ethnic and sex differences ($p < 0.05$) are represented as follows: a - Black Female vs White Female, b - Black Female vs Black Male, c - Black Male vs White Male, d - White Female vs White Male

Table 3.4 Metacarpal dimensions, adjusted for height, in black and white females and males

	Black Females					White Females			
Age	N	L ± SE	D ± SE	d ± SE	CT ± SE	N	L ± SE	D ± SE	d ± SE	CT ± SE
9.5	131	53.26 ± 0.20[a]	6.81 ± 0.05[ab]	3.88 ± 0.06[ab]	2.93 ± 0.04	28	51.21 ± 0.42	6.42 ± 0.11	3.41 ± 0.13	3.01 ± 0.08
10.5	156	56.11 ± 0.18[ab]	7.12 ± 0.05[ab]	3.97 ± 0.06[ab]	3.14 ± 0.04[b]	61	54.10 ± 0.29[d]	6.79 ± 0.08	3.56 ± 0.09[d]	3.23 ± 0.06
11.5	156	58.95 ± 0.20[ab]	7.38 ± 0.05[ab]	4.10 ± 0.06[ab]	3.29 ± 0.04[b]	64	56.48 ± 0.32	7.03 ± 0.08	3.63 ± 0.09[d]	3.40 ± 0.06
12.5	161	61.87 ± 0.21[ab]	7.65 ± 0.05[ab]	4.12 ± 0.06[ab]	3.52 ± 0.04[b]	68	59.33 ± 0.33	7.35 ± 0.08	3.64 ± 0.10[d]	3.70 ± 0.07
13.5	150	64.90 ± 0.23[a]	8.00 ± 0.05[ab]	4.18 ± 0.07[ab]	3.82 ± 0.05[b]	60	61.67 ± 0.37	7.57 ± 0.09	3.70 ± 0.10	3.87 ± 0.07
14.5	165	66.25 ± 0.23[a]	8.13 ± 0.05[ab]	4.12 ± 0.07[ab]	4.00 ± 0.05[b]	72	63.27 ± 0.33	7.76 ± 0.08[d]	3.57 ± 0.10[d]	4.20 ± 0.07
15.5	149	67.65 ± 0.25[a]	8.32 ± 0.06[ab]	4.25 ± 0.08[ab]	4.07 ± 0.06[a]	56	64.45 ± 0.37	7.97 ± 0.09[d]	3.68 ± 0.12	4.29 ± 0.08
16.5	164	68.13 ± 0.27[a]	8.35 ± 0.06[ab]	4.25 ± 0.08[ab]	4.10 ± 0.06[a]	62	64.78 ± 0.37	8.00 ± 0.09[d]	3.58 ± 0.12	4.43 ± 0.08
17.5	146	68.73 ± 0.28[a]	8.43 ± 0.07[ab]	4.22 ± 0.09[ab]	4.21 ± 0.06	57	65.29 ± 0.37	8.07 ± 0.09[d]	3.60 ± 0.12	4.47 ± 0.08[d]
18.5	124	69.13 ± 0.30[a]	8.45 ± 0.08[b]	4.24 ± 0.11[a]	4.21 ± 0.07	30	65.36 ± 0.49	8.06 ± 0.13[d]	3.52 ± 0.17	4.54 ± 0.12
19.5	50	68.43 ± 0.49[a]	8.33 ± 0.11[ab]	4.05 ± 0.14[ab]	4.28 ± 0.11	32	65.20 ± 0.52	8.08 ± 0.11[d]	3.50 ± 0.15	4.57 ± 0.11
20.5	127	68.32 ± 0.32[a]	8.38 ± 0.08[b]	4.04 ± 0.11[ab]	4.34 ± 0.07	15	65.75 ± 0.75	7.86 ± 0.18[d]	3.21 ± 0.25	4.65 ± 0.17

	Black Males					White Males			
Age	N	L ± SE	D ± SE	d ± SE	CT ± SE	N	L ± SE	D ± SE	d ± SE	CT ± SE
9.5	146	52.57 ± 0.19[c]	7.09 ± 0.05[c]	4.25 ± 0.06[c]	2.84 ± 0.04	26	50.46 ± 0.44	6.54 ± 0.11	3.46 ± 0.13	3.08 ± 0.08
10.5	177	55.35 ± 0.17[c]	7.38 ± 0.04[c]	4.45 ± 0.05[c]	2.93 ± 0.03	62	52.98 ± 0.29	6.95 ± 0.08	3.89 ± 0.09	3.06 ± 0.06
11.5	179	57.98 ± 0.19[c]	7.66 ± 0.05[c]	4.61 ± 0.06[c]	3.05 ± 0.04[c]	69	55.32 ± 0.31	7.27 ± 0.08	4.03 ± 0.09	3.24 ±0.06
12.5	183	60.92 ± 0.20[c]	7.95 ± 0.05[c]	4.68 ± 0.06[c]	3.26 ± 0.04	63	58.55 ± 0.35	7.60 ± 0.08	4.13 ± 0.10	3.47 ± 0.07
13.5	170	64.33 ± 0.22[c]	8.26 ± 0.05[c]	4.79 ± 0.06[c]	3.47 ± 0.04[c]	49	61.16 ± 0.42	7.88 ± 0.10	4.09 ± 0.12	3.79 ± 0.08
14.5	184	65.94 ± 0.21[c]	8.50 ± 0.05[c]	4.85 ± 0.06[c]	3.66 ± 0.04[c]	53	63.60 ± 0.41	8.17 ± 0.10	4.13 ± 0.12	4.04 ± 0.09
15.5	167	67.91 ± 0.22[c]	8.69 ± 0.05	4.80 ± 0.07[c]	3.88 ± 0.05[c]	55	65.13 ± 0.43	8.39 ± 0.11	3.91 ± 0.14	4.48 ± 0.09
16.5	183	68.71 ± 0.23[c]	8.87 ± 0.05[c]	4.79 ± 0.07[c]	4.07 ± 0.05[c]	53	66.28 ± 0.47	8.56 ± 0.11	3.86 ± 0.14	4.70 ± 0.10
17.5	172	68.96 ± 0.23[c]	8.98 ± 0.06	4.71 ± 0.08[c]	4.27 ± 0.05[c]	46	65.66 ± 0.47	8.74 ± 0.12	3.83 ± 0.16	4.90 ± 0.11
18.5	137	69.33 ± 0.25[c]	8.97 ± 0.07[c]	4.52 ± 0.09[c]	4.46 ± 0.06[c]	36	66.42 ± 0.51	8.61 ± 0.13	3.65 ± 0.18	4.96 ± 0.13
19.5	55	69.79 ± 0.41[c]	9.17 ± 0.09	4.84 ± 0.12[c]	4.33 ± 0.09[c]	28	65.65 ± 0.64	8.77 ± 0.14	3.78 ± 0.19	5.00 ± 0.14
20.5	136	68.83 ± 0.29[c]	9.02 ± 0.07	4.56 ± 0.10[c]	4.46 ± 0.07[c]	17	66.58 ± 0.74	8.60 ± 0.18	3.34 ± 0.25	5.26 ± 0.17

Data are presented as means and standard error of means (SE). Bone Length (L), Bone width (D), Medullary Width (d), Cortical Thickness (CT) – All measurements are in millimetres. Significant ethnic and sex differences ($p < 0.05$) are represented as follows: a - Black Female vs White Female, b - Black Female vs Black Male, c - Black Male vs White Male, d - White Female vs White Male

Bone width

Black and white females had similar bone widths with no differences in tempo and velocity of growth throughout the study period (Figure 3.4A; Table 3.1). Black males had significantly greater bone width size ($p = 0.02$), a later growth tempo ($p = 0.0009$) and reduced velocity ($p < 0.0001$) than their white peers (Figure 3.4B and Table 3.1). Bone width in males of both black and white ethnic groups was approximately 1 mm greater than their respective female peers ($p < 0.0001$) (Table 3.2). Growth velocity in males also exceeded that of females in both ethnic groups ($p < 0.0001$) (Figure 3.5; Table 3.2). Peak velocity in bone width was attained at approximately similar ages relative to aPLV in both sexes and ethnic groups (Figure 3.6). The increase in bone width was greatest during the pubertal growth spurt and continued until a later age in males compared to females (Figure 3.6; Tables 3.3, 3.4, 3.5 and 3.6).

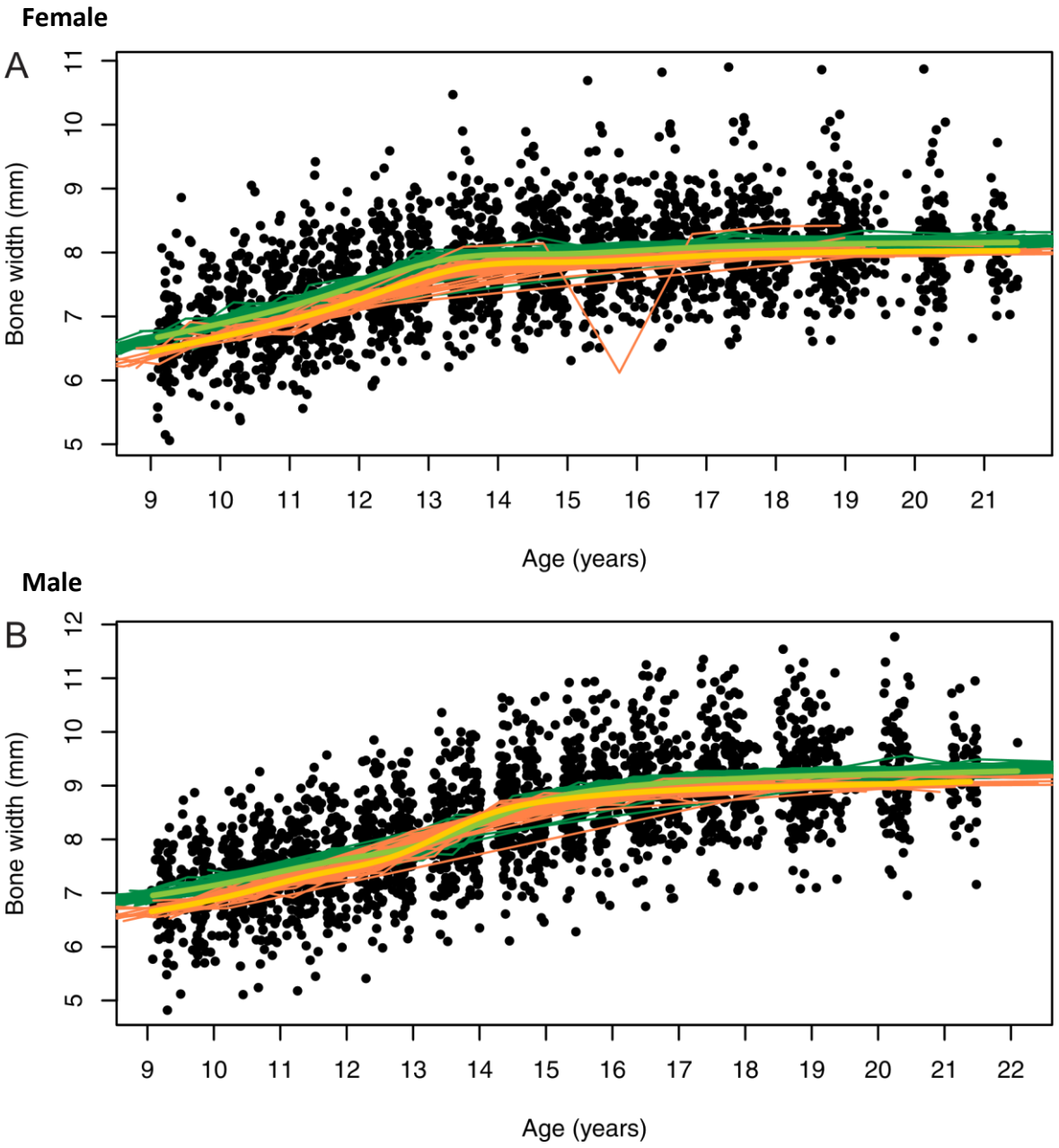


Figure 3.4 Scatterplot of metacarpal bone width against chronological age, and individual curves adjusted for tempo and velocity, with the mean adjusted curve, for metacarpal bone width growth by ethnicity, in female (A) and male (B) participants. Mean adjusted curves generated for black participants are in green and for white participants in orange colour.

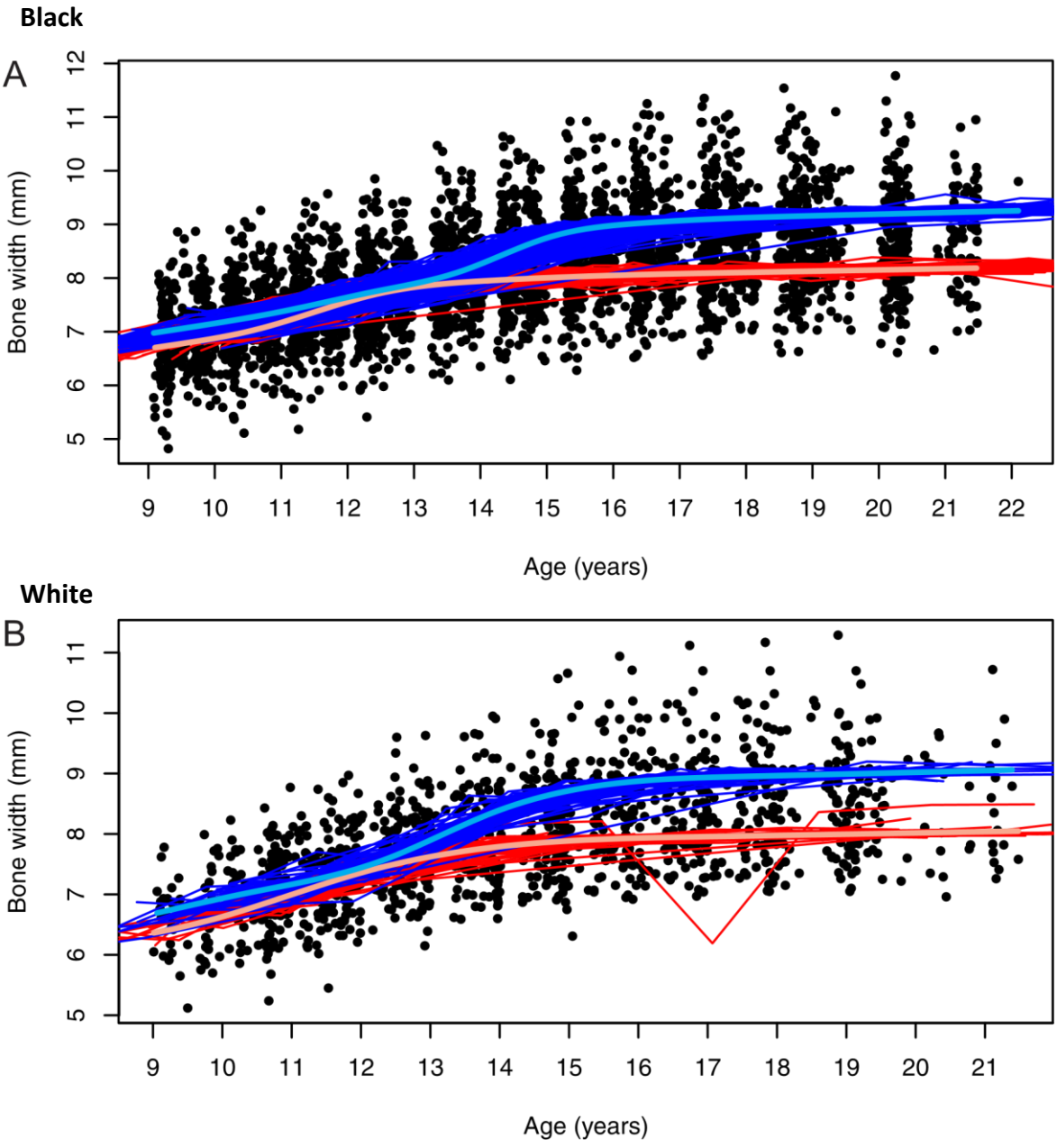


Figure 3.5 Scatterplot of bone width against chronological age, and individual curves adjusted for tempo and velocity, with the mean adjusted curve, for bone width growth by sex, in black (A) and white (B) participants. Mean adjusted curves generated for female participants are in red and for male participants in blue colour.

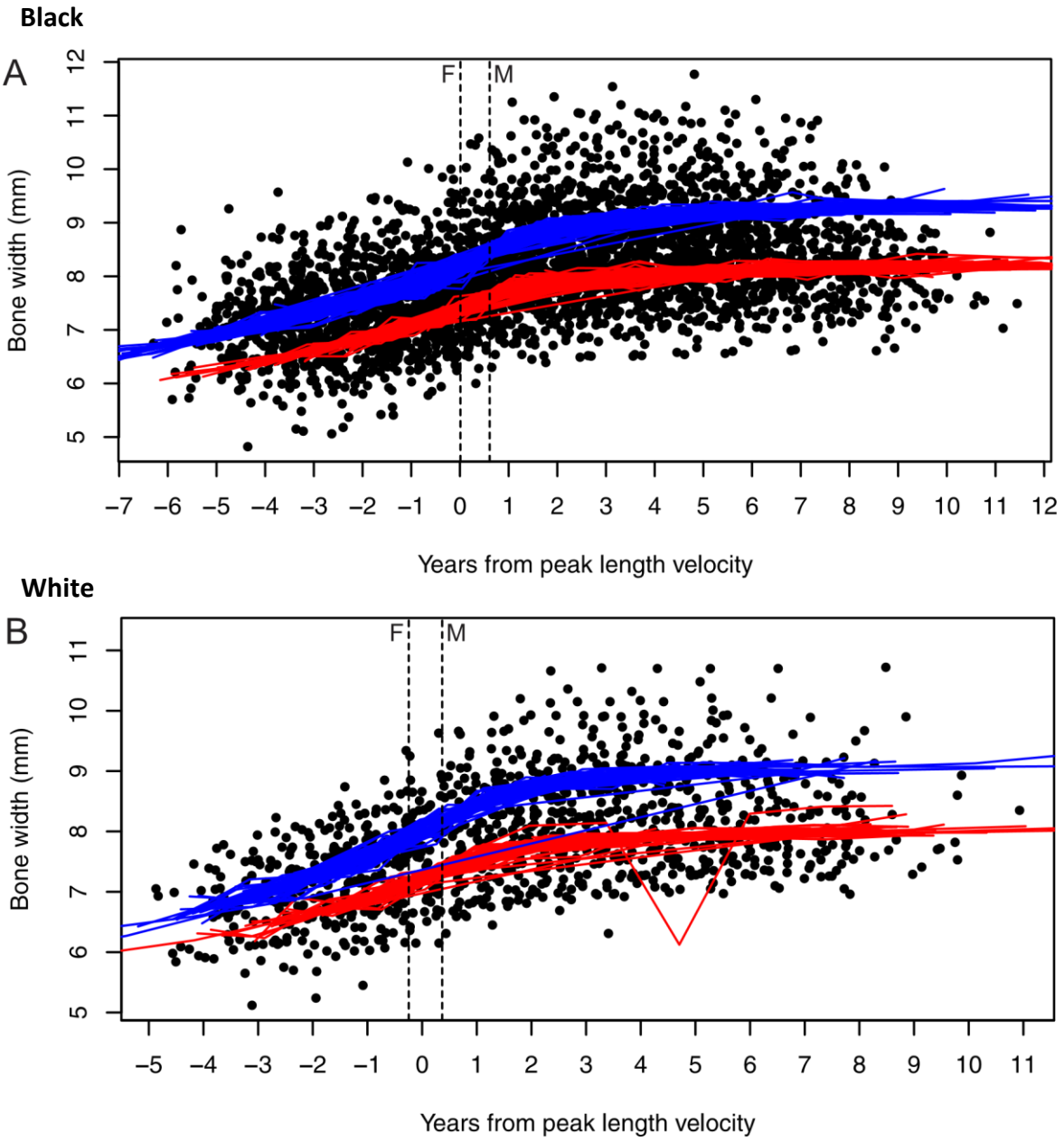


Figure 3.6 Scatterplot of bone width against years from peak metacarpal length velocity, and individual curves adjusted for tempo and velocity, for bone width growth by sex, in black (A) and white (B) participants. The vertical lines represent the age at peak bone width velocity in females (F) and males (M). Curves generated for female participants are in red and for male participants in blue colour.

Table 3.5 Mean annual changes in bone width, medullary width and cortical thickness in black and white females

BLACK FEMALE	Bone width (mm)		Medullary width (mm)		Cortical Thickness (mm)	
Age (years)	Mean ± SE	*p*	Mean ± SE	*p*	Mean ± SE	*p*
10.5 vs 9.5	0.29 ± 0.02	0.0001	0.15 ± 0.03	0.0001	0.13 ± 0.03	0.0001
11.5 vs 10.5	0.29 ± 0.02	0.0001	0.10 ± 0.03	0.0001	0.19 ± 0.03	0.0001
12.5 vs 11.5	0.27 ± 0.02	0.0001	0.06 ± 0.03	0.05	0.22 ± 0.03	0.0001
13.5 vs 12.5	0.24 ± 0.02	0.0001	-0.01 ± 0.03	0.6	0.25 ± 0.03	0.0001
14.5 vs 13.5	0.08 ± 0.02	0.0001	-0.06 ± 0.03	0.03	0.14 ± 0.03	0.0001
15.5 vs 14.5	0.06 ± 0.02	0.001	-0.02 ± 0.03	0.5	0.08 ± 0.03	0.006
16.5 vs 15.5	0.06 ± 0.02	0.003	-0.02 ± 0.03	0.4	0.08 ± 0.03	0.009
17.5 vs 16.5	0.04 ± 0.02	0.06	-0.04 ± 0.03	0.2	0.07 ± 0.03	0.01
18.5 vs 17.5	0.02 ± 0.02	0.3	-0.03 ± 0.03	0.3	0.06 ± 0.03	0.09
19.5 vs 18.5	-0.01 ± 0.03	0.7	-0.08 ± 0.04	0.09	0.06 ± 0.05	0.2
20.5 vs 19.5	0.04 ± 0.03	0.1	0.02 ± 0.04	0.6	0.02 ± 0.05	0.6

WHITE FEMALE	Bone width (mm)		Medullary width (mm)		Cortical Thickness (mm)	
Age (years)	Mean ± SE	*p*	Mean ± SE	*p*	Mean ± SE	*p*
10.5 vs 9.5	0.23 ± 0.05	0.0001	-0.01 ± 0.07	0.9	0.24 ± 0.07	0.001
11.5 vs 10.5	0.31 ± 0.04	0.0001	0.01 ± 0.05	0.8	0.29 ± 0.06	0.0001
12.5 vs 11.5	0.31 ± 0.04	0.0001	-0.02 ± 0.05	0.8	0.32 ± 0.06	0.0001
13.5 vs 12.5	0.18 ± 0.04	0.0001	-0.08 ± 0.05	0.1	0.26 ± 0.06	0.0001
14.5 vs 13.5	0.15 ± 0.04	0.0001	-0.09 ± 0.05	0.07	0.24 ± 0.05	0.0001
15.5 vs 14.5	0.05 ± 0.04	0.2	0.003 ± 0.05	0.9	0.04 ± 0.06	0.4
16.5 vs 15.5	0.08 ± 0.04	0.03	-0.06 ± 0.05	0.2	0.15 ± 0.06	0.01
17.5 vs 16.5	0.05 ± 0.04	0.2	0.01 ± 0.05	0.9	0.04 ± 0.06	0.4
18.5 vs 17.5	0.01 ± 0.05	0.9	-0.05 ± 0.06	0.5	0.05 ± 0.07	0.5
19.5 vs 18.5	-0.03 ± 0.05	0.6	-0.02 ± 0.07	0.7	-0.004 ± 0.08	1.0
20.5 vs 19.5	0.03 ± 0.07	0.6	0.02 ± 0.09	0.8	0.01 ± 0.10	0.9

SE - standard error of means

Table 3.6 Mean annual changes in bone width, medullary width and cortical thickness in black and white males

BLACK MALE	Bone width (mm)		Medullary width (mm)		Cortical Thickness (mm)	
Age (years)	Mean ± SE	*p*	Mean ± SE	*p*	Mean ± SE	*p*
10.5 vs 9.5	0.27 ± 0.03	0.0001	0.11 ± 0.04	0.002	0.16 ± 0.04	0.0001
11.5 vs 10.5	0.23 ± 0.02	0.0001	0.15 ± 0.03	0.0001	0.08 ± 0.04	0.02
12.5 vs 11.5	0.29 ± 0.02	0.0001	0.13 ± 0.03	0.0001	0.15 ± 0.04	0.0001
13.5 vs 12.5	0.39 ± 0.02	0.0001	0.14 ± 0.03	0.0001	0.25 ± 0.04	0.0001
14.5 vs 13.5	0.31 ± 0.02	0.0001	0.08 ± 0.03	0.02	0.23 ± 0.04	0.0001
15.5 vs 14.5	0.27 ± 0.02	0.0001	0.02 ± 0.03	0.5	0.25 ± 0.04	0.0001
16.5 vs 15.5	0.22 ± 0.02	0.0001	0.01 ± 0.03	0.8	0.21 ± 0.04	0.0001
17.5 vs 16.5	0.10 ± 0.02	0.0001	-0.08 ± 0.03	0.02	0.18 ± 0.04	0.0001
18.5 vs 17.5	0.08 ± 0.03	0.005	-0.07 ± 0.04	0.06	0.15 ± 0.04	0.0001
19.5 vs 18.5	0.03 ± 0.04	0.5	0.07 ± 0.05	0.2	-0.04 ± 0.06	0.5
20.5 vs 19.5	0.02 ± 0.04	0.5	-0.12 ± 0.05	0.03	0.14 ± 0.06	0.01

WHITE MALE	Bone width (mm)		Medullary width (mm)		Cortical Thickness (mm)	
Age (years)	Mean ± SE	*p*	Mean ± SE	*p*	Mean ± SE	*p*
10.5 vs 9.5	0.26 ± 0.05	0.0001	0.06 ± 0.08	0.4	0.20 ± 0.09	0.02
11.5 vs 10.5	0.29 ± 0.04	0.0001	0.09 ± 0.06	0.1	0.19 ± 0.06	0.002
12.5 vs 11.5	0.43 ± 0.04	0.0001	0.16 ± 0.06	0.004	0.27 ± 0.06	0.0001
13.5 vs 12.5	0.37 ± 0.05	0.0001	0.05 ± 0.06	0.5	0.33 ± 0.07	0.0001
14.5 vs 13.5	0.34 ± 0.05	0.0001	0.01 ± 0.06	0.8	0.32 ± 0.07	0.0001
15.5 vs 14.5	0.29 ± 0.05	0.0001	-0.09 ± 0.06	0.2	0.38 ± 0.07	0.0001
16.5 vs 15.5	0.15 ± 0.05	0.002	-0.0001 ± 0.06	1.00	0.15 ± 0.07	0.04
17.5 vs 16.5	0.09 ± 0.05	0.06	-0.11 ± 0.06	0.08	0.20 ± 0.07	0.005
18.5 vs 17.5	0.03 ± 0.05	0.6	0.003 ± 0.07	1.0	0.03 ± 0.08	0.8
19.5 vs 18.5	-0.02 ± 0.06	0.8	-0.08 ± 0.08	0.3	0.07 ± 0.09	0.5
20.5 vs 19.5	-0.001 ± 0.08	1.0	-0.10 ± 0.10	0.3	0.10 ± 0.11	0.4

SE - standard error of means

Medullary width

Adolescent black individuals of both sexes had larger medullary cavities than their white peers ($p < 0.0001$) (Figure 3.7; Table 3.1). Significant sex differences in medullary width size occurred in both ethnic groups with males having greater medullary cavities than their female peers throughout the study period ($p < 0.0001$) (Figure 3.8; Table 3.2). Peak growth velocity in medullary width in black participants occurred approximately 2 years prior to aPLV, whereas in white participants, this occurred 6 - 12 months after aPLV (Figure 3.9). Growth velocity of medullary width was significantly greater in black ($p < 0.0001$) and white ($p = 0.03$) males relative to their female peers (Table 3.2). In both ethnic groups, when assessed against aPLV, the timing of peak medullary width growth was similar between females and males, despite being different between the ethnic groups (Figure 3.9).

In all participants, there was an initial increase in medullary width followed by a decrease (Tables 3.3, 3.4, 3.5 and 3.6). Although annual changes in medullary width were not always significant, when the maximum medullary width attained (black and white females, 13.5 years; black males, 16.5 years; white males, 14.5 years) was compared to that at the last time of measure (20.5 years), the decrease was significant in both sexes and ethnic groups (Table 3.7).

Cortical thickness

In all participants, cortical thickness increased through adolescence (Tables 3.3, 3.4, 3.5 and 3.6). Ethnic differences were evident with white participants having thicker cortices than their black peers, although in females this difference was only present until 16.5 years (Table 3.4). Significant annual increases in cortical thickness occurred for 2 years longer in males compared to their female peers (Tables 3.5 and 3.6). Sex differences, with males having thicker cortices than their female peers, were more marked in white compared to black participants (Table 3.3).

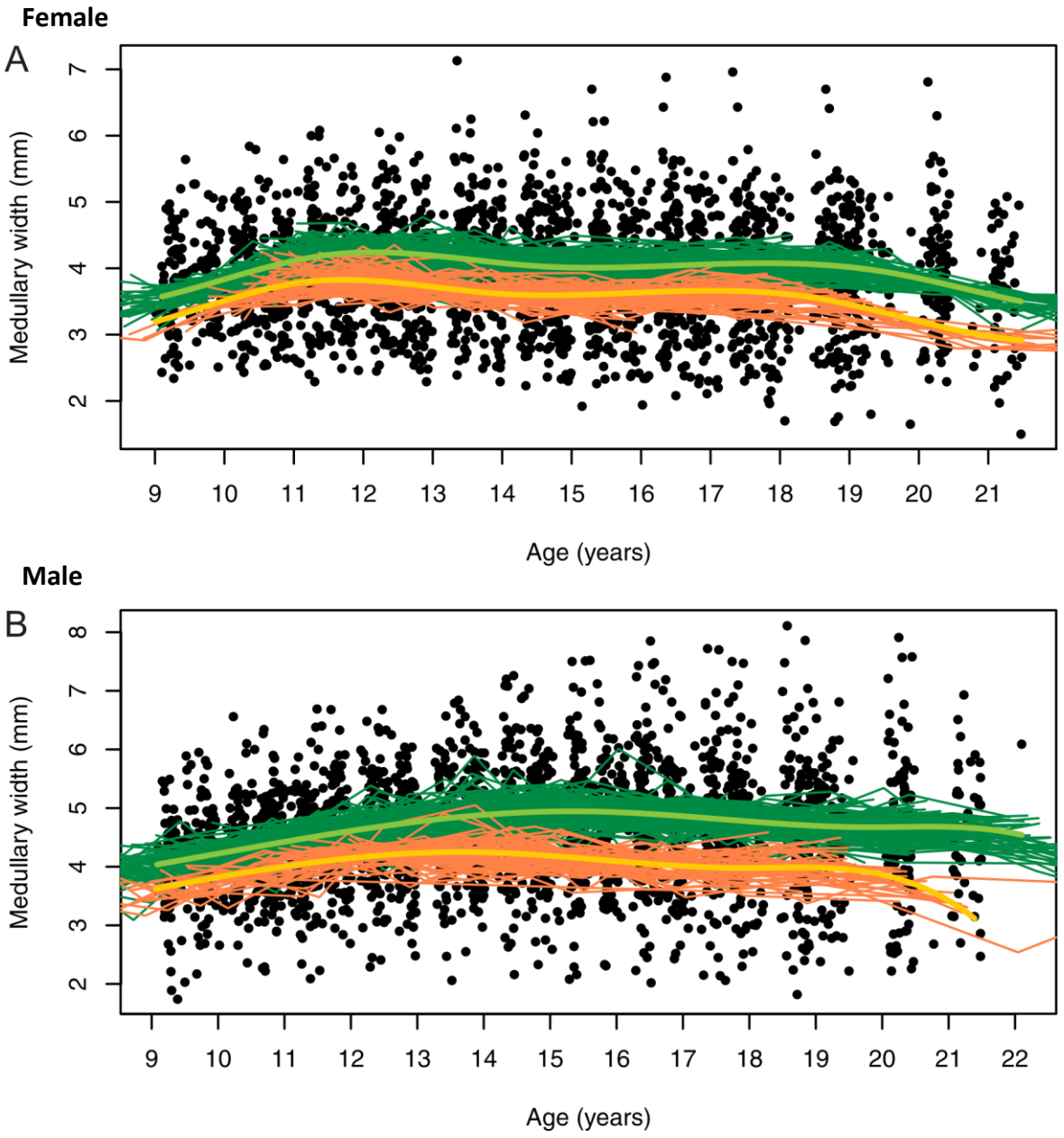


Figure 3.7 Scatterplot of metacarpal medullary width against chronological age, and individual curves adjusted for tempo and velocity, with the mean adjusted curve, for metacarpal medullary width growth by ethnicity, in female (A) and male (B) participants. Mean adjusted curves generated for black participants are in green and for white participants in orange colour.

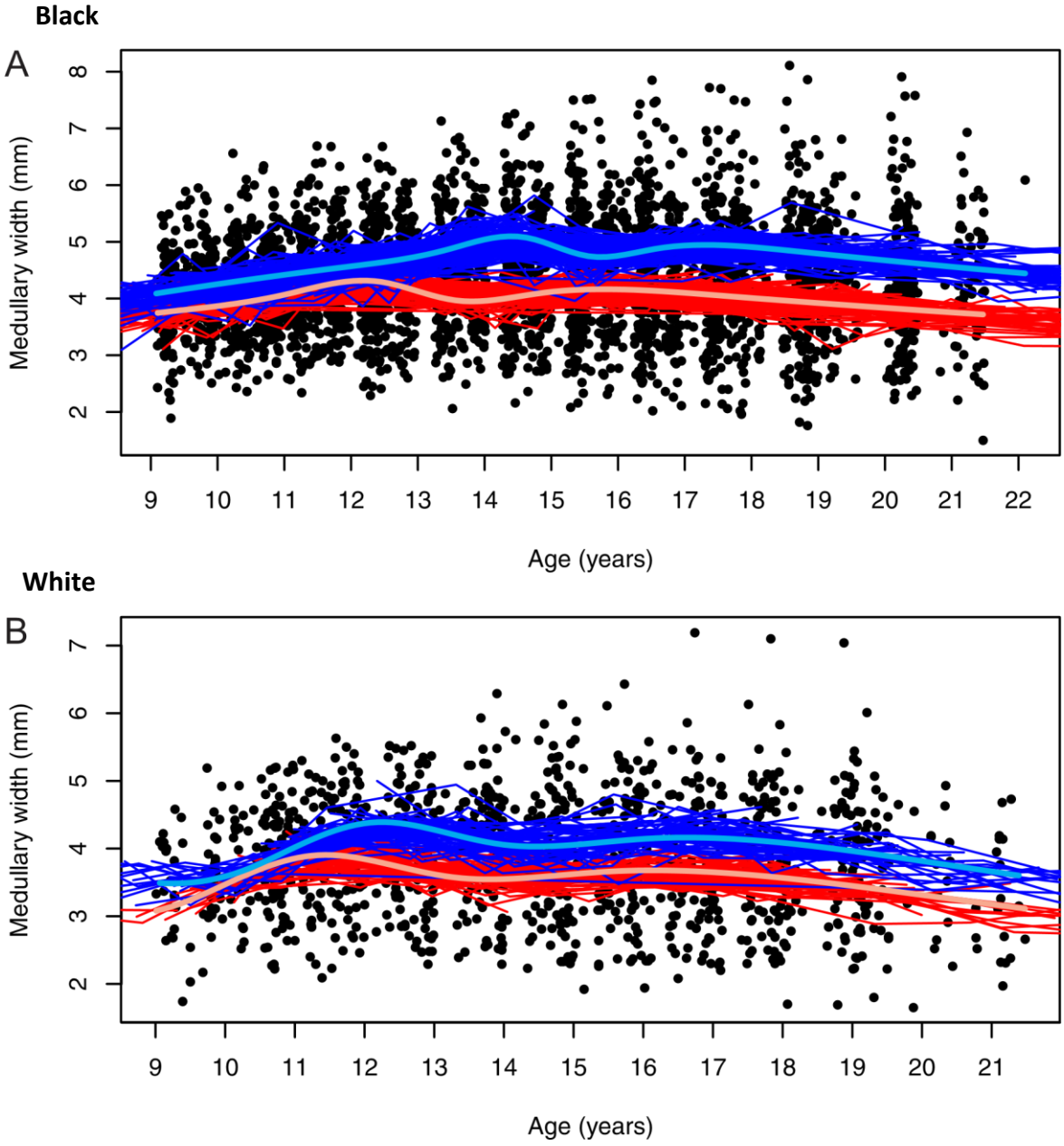


Figure 3.8 Scatterplot of medullary width against chronological age, and individual curves adjusted for tempo and velocity, with the mean adjusted curve, for medullary width growth by sex, in black (A) and white (B) participants. Mean adjusted curves generated for female participants are in red and for male participants in blue colour.

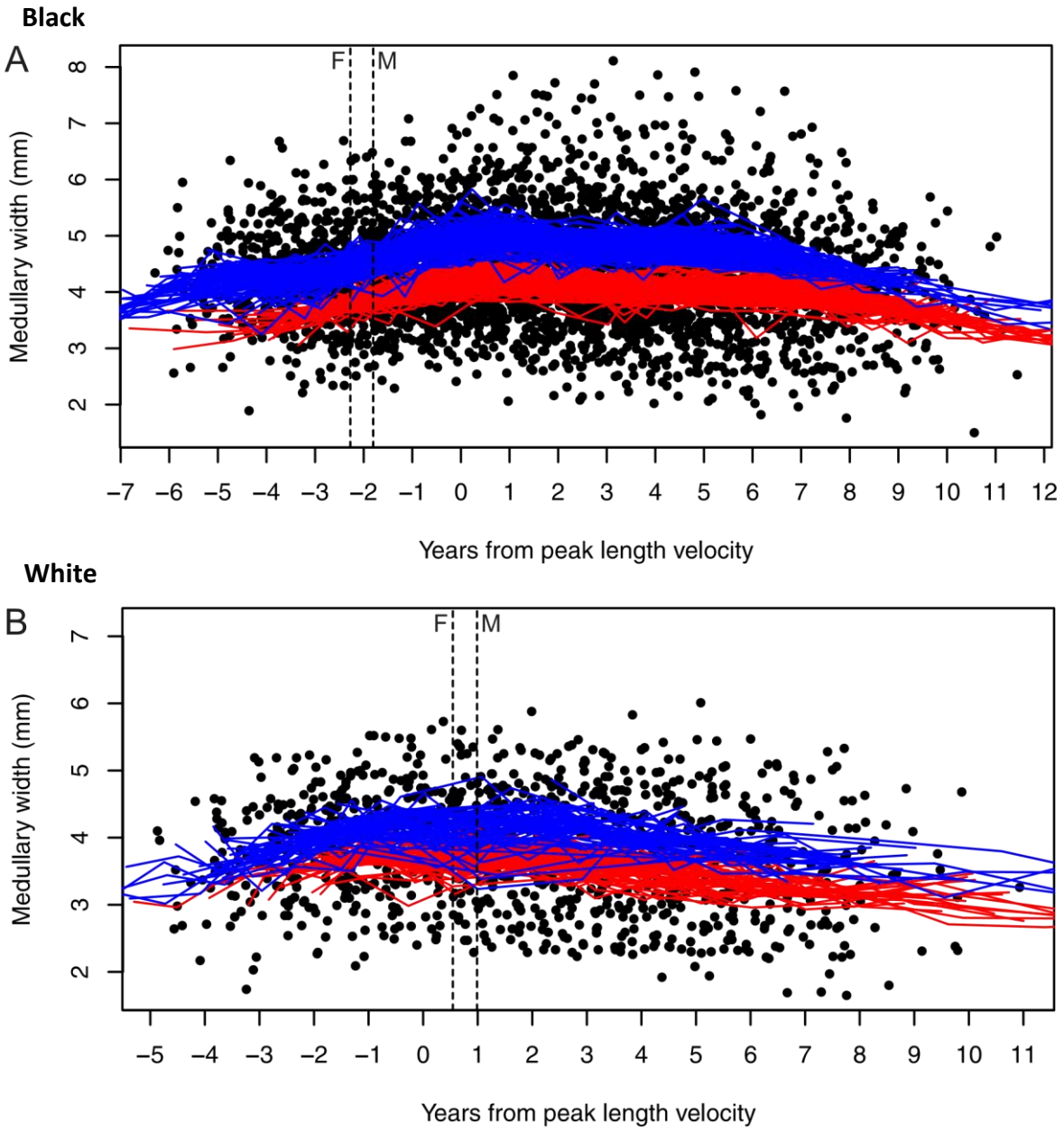


Figure 3.9 Scatterplot of medullary width against years from peak metacarpal length velocity, and individual curves adjusted for tempo and velocity, for medullary width growth by sex, in black (A) and white (B) participants. The vertical lines represent the age at peak medullary width growth in females (F) and males (M). Curves generated for female participants are in red and for male participants in blue colour.

Table 3.7 Differences in bone width, cortical thickness and medullary width between the age at first measurement and age at maximal medullary width, and between age at maximal medullary width and age at last measurement

Group	Age range (years)[a]	Bone width (mm) Mean ± SE	*p*	Medullary width (mm) Mean ± SE	*p*	Cortical thickness (mm) Mean ± SE	*p*
Black Female	9.5 - 13.5	1.09 ± 0.02	0.0001	0.30 ± 0.03	0.0001	0.79 ± 0.03	0.0001
	13.5 - 20.5	0.29 ± 0.02	0.0001	-0.23 ± 0.03	0.0001	0.52 ± 0.03	0.0001
White Female	9.5 - 13.5	1.03 ± 0.05	0.0001	-0.10 ± 0.07	0.16	1.12 ± 0.08	0.0001
	13.5 - 20.5	0.35 ± 0.06	0.0001	-0.19 ± 0.08	0.02	0.54 ± 0.09	0.0001
Black Male	9.5 - 16.5	1.97 ± 0.03	0.0001	0.64 ± 0.04	0.0001	1.33 ± 0.04	0.0001
	16.5 - 20.5	0.23 ± 0.03	0.0001	-0.20 ± 0.04	0.0001	0.43 ± 0.04	0.0001
White Male	9.5 - 14.5	1.69 ± 0.06	0.0001	0.38 ± 0.08	0.0001	1.31 ± 0.09	0.0001
	14.5 - 20.5	0.54 ± 0.07	0.0001	-0.38 ± 0.09	0.0001	0.91 ± 0.10	0.0001

[a]Age at first measurement (9.5 years); age at maximal medullary width (Black Female: 13.5 years; White Female: 13.5 years; Black Male: 16.5 years; White Male: 14.5 years); age at last measurement (20.5 years).

3.3 Discussion

This study presents unique longitudinal data on metacarpal bone development in an urban South African population spanning 12 years through puberty and adolescence, a period during which significant physiological changes are experienced. The results of this study using radiogrammetry to measure metacarpal dimensions revealed distinctly different ethnic and sex patterns of bone growth compared to previous studies [86, 318], as well as ethnic differences in size and cross-sectional architecture of adolescent bone.

Ethnic differences in bone length were accentuated when data were adjusted for height with black individuals of both sexes having longer metacarpals than their white peers at all ages. A previous study of this cohort also reported longer forearm and leg lengths relative to stature in black children compared to their white counterparts at 9 years of age [44]. In US children, a similar pattern of ethnic differences in metacarpal lengths were reported in black and white children although their data were not adjusted for height [319]. In contrast to SA black children, who are on average 5 - 6 cm shorter than their white peers (Lukhanyo Nyati, unpublished data), pre-pubertal US black children had taller stature compared to their white peers [320-322]. Ethnic differences in the US were attributed to US black individuals being more efficient than their white peers in absorbing and preserving calcium, particularly during adolescence [104, 323]. Calcium intake was not measured in my study; however, lower calcium intakes have been reported in black compared to white SA children [105]. Whilst low calcium intake may influence bone growth, it does not account for the longer metacarpals in black individuals in this study.

Ethnic differences in metacarpal width were reported in a South African study undertaken in 1971 in which 14-year old urban black males had significantly narrower metacarpals than white males [45]. My results do not support this finding. Comparing the results of the present study with those reported in 1971, the average metacarpal widths for the 14-year old black and white males from the BHC are 13.5% and 4.8% greater respectively, whilst for black and white females they are 7% and 4.7% greater, respectively. These differences likely reflect secular changes in bone growth and aPHV in urban SA children, particularly in black individuals. Improved socio-economic conditions and nutrition may have contributed to the

secular trend in black children. The delay in aPLV in black males has been suggested to be due to them being more sensitive to adverse environmental conditions [78].

The rate of bone growth was similar in both sexes prior to the pubertal growth spurt and consistent with those of other studies [79, 86, 318]. A divergence in bone length and width between females and males occurred during the pubertal growth spurt in response to the presumed differential expression of sex hormones. Although, the sex differences in which males are taller and have longer and wider bones are believed to be the result of the later onset of puberty in males compared to females [44, 81], my study also shows significantly greater peak bone length and bone width velocity during puberty in males.

In keeping with the findings of Garn [86], there were no significant sex differences in bone width prior to the onset of the pubertal growth spurt. However, during adolescence the degree of sexual dimorphism was similar in both ethnic groups and these differences diminished when height was factored in. In contrast, Garn and co-workers [324] reported less sexual dimorphism in adults of largely African ancestry compared to European subjects when raw data were analysed.

The present study showed that whilst sexual dimorphism emerged during the growth spurt, the pattern of bone growth in males was dissimilar to that of some previous metacarpal radiogrammetry studies [86, 318]. In males, the expansion in bone width and cortical thickening during puberty has been attributed primarily to periosteal bone apposition [4, 86] with endocortical expansion continuing until 16 years in US white boys [318]. However, in my study, in white males, the endocortical area ceased to expand at an earlier age and was followed by contraction, concurring with the results of Martin and colleagues [79]. The relative delay in endocortical contraction in black males may be a consequence of their later entry into puberty and/or related to their different bone architecture.

Previous studies in females reported dimensional changes in cortical thickness due to endocortical apposition and this was attributed to the pubertal rise in oestrogen [79, 86]. My results are consistent with these findings as greater endocortical contraction was observed in black and white adolescent females, and this occurred at similar ages to those previously reported [79, 86, 318]. Endocortical bone accrual is more closely associated with insulin-like growth factor-I in girls and with testosterone in boys [325]. It is therefore interesting that, in

the present study, endocortical contraction commenced only a year later in white males compared to white females and conflicts with Garn's observations in Ohio whites [86]. Longitudinal hormonal assays through adolescence may reveal the endocrine influence on the endocortical surface. In a recent longitudinal pQCT study of the tibia, it was proposed that in females, instead of increased endocortical bone deposition relative to males, there is less endocortical resorption [326]. The regulation of remodelling at the endocortical interface may therefore be region specific [86, 88] and/or genetically determined [324]. Given that in both sexes peak medullary width expansion in black participants occurred at least 2 years prior to attaining PLV compared to white participants, in whom it occurred at least 6 months after attainment of PLV, genetic influences at the endocortical surface may possibly override hormonal influences in bone development.

Distinct relationships of cortical thickness relative to medullary width and bone diameter were evident between black and white adolescents. Black adolescents had wider bones with thinner cortices and wider medullary cavities than white adolescents. These differences may be due to differences in calcium intake between the two ethnic groups [105]. A study comparing hypocalcaemic and normocalcaemic rural black SA children reported greater bone width and lower bone mineral density in the hypocalcaemic group [327]. It was suggested that secondary hyperparathyroidism contributed to increased endocortical bone resorption and periosteal bone deposition [327]. The outward displacement of the cortex from the central axis seen in black participants may therefore be a compensatory mechanism enhancing the bone's resistance to torsional and bending forces [328]. As the metacarpal is a non-weight bearing bone, the role of mechanical loading in determining bone size is unclear [15]. South African black children have smaller muscle cross-sectional area relative to bone size compared to white children and this may elicit a differential response to mechanical forces [49].

The initiation and duration of the growth spurt and the attainment of PLV were synchronised between black and white females. In males, ethnic differences were observed whereby the black males' delayed entry into puberty, which allowed for greater pre-pubertal appendicular growth [44], and their attainment of PLV later than white males is in keeping with the delayed skeletal maturity reported in the same cohort [78]. The lag in pubertal development in black males has been proposed to be a consequence of negative early life influences impacting growth [78]. An earlier onset of puberty is thought to be associated with an accelerated time

through puberty [78, 329]; however, in this study the duration of the growth spurt was similar in all groups regardless of the timing of the onset of the growth spurt.

The strengths of this study are its longitudinal nature spanning 12 years, and, its provision of information on a critical developmental period. Furthermore, it provides data based on ethnicity and sex and aligns these to biological maturity. SITAR has only been used to model stature [108] and skeletal maturity of short bones [78]; however, I have shown its applicability in modelling appendicular bone growth. I acknowledge that errors may occur with repeated measures, but as repeated measures are expected to vary with age, I sought to minimise these errors by using standardised protocols for radiography and radiogrammetry. Although radiogrammetry was performed manually using callipers, precision of the method was validated by intra-observer results. A digitised method may have been limiting in that it requires a minimum cortical thickness for recognition by an automated system, which may present a challenge in paediatric populations [301]. This study was standardised on the second metacarpal bone of the left hand irrespective of hand dominance. Previous radiogrammetry studies have shown greater bone width and cortical area of the right second metacarpal compared to the left regardless of hand dominance [330, 331]. Participants who may have experienced a forearm fracture were not excluded; however, I do not believe that this would have significantly impacted on the metacarpal dimensions. A limitation of the study was the relatively smaller number of white participants.

In conclusion, in South African children distinct ethnic and sex differences in bone acquisition emerge during puberty. The resultant bone morphology and cross-sectional architecture at the end of skeletal maturity differs from previous studies in high-income countries. These dissimilarities in bone accrual during adolescence may differentially influence bone mass in later life.

3.4 Summary of major findings

Ethnic and sex differences were observed in the pattern of bone acquisition in the second metacarpal bone in children going through puberty and adolescence. Black individuals of both sexes had longer metacarpals than their white peers at all ages. Black adolescents had wider bones with relatively thinner cortices compared to their white peers. Males had wider bones than females. Medullary width narrowing commenced at similar ages in all groups, except in black males in whom it continued to expand for an additional two years.

CHAPTER 4

A longitudinal comparison of appendicular bone growth and markers of strength through adolescence in a South African cohort using radiogrammetry and pQCT

This chapter compares the growth and strength of the second metacarpal bone (measured using radiogrammetry) to that of the non-weight-bearing radius and weight bearing tibia (measured using pQCT), when controlling for the associated musculature, in two ethnic groups during adolescence. The results are described and discussions pertaining to these findings are outlined together with the limitations. The principal findings and discussion in this chapter were published in the following journal (Appendix K):

Magan A, Micklesfield LK, Nyati LH, Norris SA and Pettifor JM (2018) A longitudinal comparison of appendicular bone growth and markers of strength through adolescence in a South African cohort using radiogrammetry and pQCT. Osteoporosis International DOI: 10.1007/s00198-018-4761-9

4.1 Methods

Radiogrammetry measures of the second metacarpal bone were compared to measurements obtained from pQCT scans of the radius (65% site) and tibia (38% and 65% sites) in 524 children from the BHC, between the ages of 12 and 20 years. The 65% site of the tibia was used to obtain MCSA only and no bone outcomes are reported at this site. Bone width (mm), cortical thickness (mm), medullary width (mm), stress-strain index (SSIp, mm^3) and muscle-cross-sectional area (MCSA, mm^2) were assessed. Bivariate regression analysis was performed to determine the association between metacarpal bone width, cortical thickness and SSIp, and MCSA of the radius and tibia. Mixed-effects models were used to compare the growth patterns of bone width, cortical thickness, medullary width and SSIp of the metacarpal, radius and tibia when adjusting for MCSA, for each sex and ethnic group when aligned by age from PLV.

4.2 Results

Participant recruitment, the number of participants in each sex and ethnic group, together with the total number of observations per group are shown in Figure 4.1. A total of 524 participants aged between 12 and 20 years had valid hand-wrist radiographs, and radial and tibial pQCT scans. They comprised 174 (33%) black females (BF), 79 (15%) white females (WF), 198 (38%) black males (BM) and 73 (14%) white males (WM). The mean number of visits over the study period was 5 for black participants and 3.5 for white participants. The number of participants who had pQCT data prior to aPLV were as follows: BF - 4, WF - 1, BM - 86 and WM - 16.

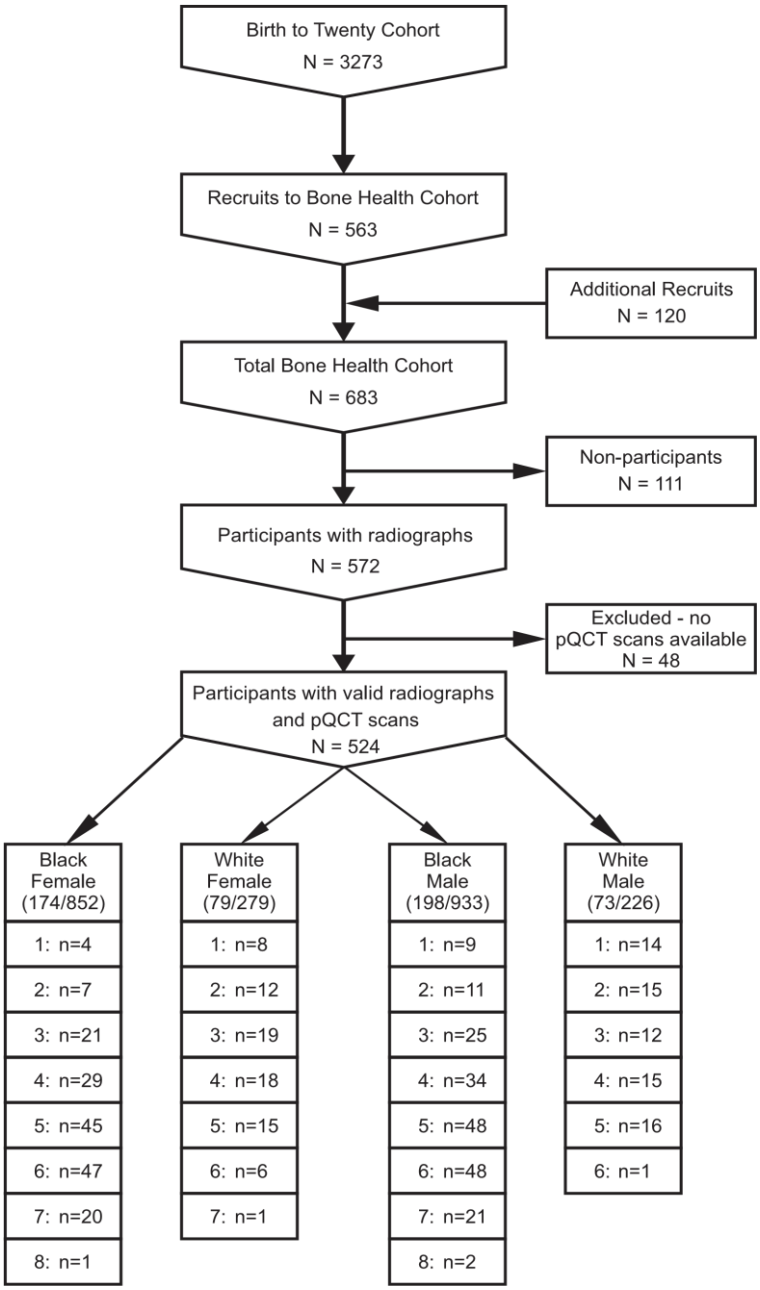


Figure 4.1 Participant recruitment (N) and the number of participants (n) aged between 12 and 20 years with their respective total number of radiographs over the study period. (1: n=4 means that 4 participants had a total of 1 radiograph)

Associations between metacarpal bone measures, and muscle cross-sectional area (MCSA) of radius and tibia

Metacarpal bone measures were associated with MCSA of the radius in all four groups (Table 4.1). The variance in metacarpal bone width and cortical thickness explained by radial MCSA was 34 - 45% in males (R^2: 0.34 - 0.45) and 12 - 19% in females (R^2: 0.12 - 0.19). There was no significant association between any of the metacarpal bone measures and tibial MCSA, except in black males (Table 4.1).

Table 4.1 Fixed-effects regression estimates of metacarpal bone measures as the outcome and radial and tibial bone measures as predictors in black and white females and males.

	Black Female (N=174)		White Female (N=79)	
	Coefficient [95% CI]	R^2	Coefficient [95% CI]	R^2
BW Met/MCSA radius	6.86E-05 [6.31E-05, 7.41E-0.5]*	0.19	8.36E-05 [6.89E-05, 9.83E-05]*	0.14
BW Met/MCSA tibia	1.71E-06 [-1.05E-06, 4.47E-06]	0.007	5.25E-07 [-4.74E-06, 5.79E-06]	0.03
CT Met/MCSA radius	2.23E-04 [2.01E-04, 2.44E-04]*	0.12	2.68E-04 [2.22E-04, 3.15E-04]*	0.13
CT Met/MCSA tibia	-8.71E-06 [1.91E-05, 1.71E-06]	0.01	-4.53E-06 [-2.06E-05, 1.15E-05]	0.01
SSI Met/ MCSA radius	2.92E-04 [2.66E-04, 3.18E-04]*	0.20	3.07E-04 [2.45E-04, 3.69E-04]*	0.15
SSI Met/ MCSA tibia	5.52E-06 [-7.27E-06, 1.83E-05]	0.009	1.07E-05 [-1.06E-05, 3.20E-05]	0.04
SSI Met/ SSI radius	1.97E-03 [1.77E-03, 2.16E-03]*	0.35	2.04E-03 [1.70E-03, 2.39E-03]*	0.20
SSI Met/ SSI tibia	1.35E-04 [8.35E-05, 1.87E-04]*	0.21	2.32E-04 [1.12E-04, 3.52E-04]*	0.11
	Black Male (N=198)		White Male(N=73)	
	Coefficient [95% CI]	R^2	Coefficient [95% CI]	R^2
BW Met/MCSA radius	7.68E-05 [7.39E-05, 7.96E-05]*	0.35	7.63E-05 [6.95E-05, 8.31E-05]*	0.33
BW Met/MCSA tibia	4.09E-05 [3.56E-05, 4.61E-05]*	0.09	8.92E-06 [7.81E-09, 1.78E-05]	0.06
CT Met/MCSA radius	1.83E-04 [1.73E-04, 1.93E-04]*	0.34	1.83E-04 [1.62E-04, 2.04E-04]*	0.45
CT Met/MCSA tibia	7.82E-05 [6.38E-05, 9.26E-05]*	0.06	9.04E-06 [-1.43E-05, 3.24E-05]	0.01
SSI Met/ MCSA radius	3.53E-04 [3.40E-04, 3.67E-04]*	0.44	3.31E-04 [2.98E-04, 3.64E-04]*	0.36
SSI Met/ MCSA tibia	1.85E-04 [1.60E-04, 2.09E-04]*	0.11	3.95E-05 [-2.56E-07, 7.92E-05]	0.02
SSI Met/ SSI radius	3.29E-03 [3.14E-03, 3.44E-03]*	0.51	2.49E-03 [2.17E-03, 2.80E-03]*	0.38
SSI Met/ SSI tibia	7.29E-04 [6.74E-04, 7.84E-04]*	0.30	7.41E-04 [6.04E-04, 8.79E-04]*	0.27

Log-transformed data are presented as β-coefficients with [95% confidence intervals] and coefficients of determination (R^2). BW, bone width (mm); CT, cortical thickness (mm); MCSA, muscle cross-sectional area (mm^2); SSI, stress-strain index (mm^3); Met - metacarpal

*$p < 0.0001$

Associations between metacarpal SSIp, and MCSA and SSIp of radius and tibia

The SSIp of the metacarpal bone was significantly associated with MCSA of the radius in all four groups although the variance explained in males was 36 - 44% (R^2: 0.36 - 0.44) and only 15 - 20% in females (R^2: 0.15 - 0.20) (Table 4.1; Figure 4.2). Metacarpal SSIp was associated with tibial MCSA in the black males only (R^2: 0.11) (Table 4.1; Figure 4.3).

Metacarpal SSIp was significantly associated with radial and tibial SSIp in all four groups (Table 4.1). The variance in metacarpal SSIp explained by the radial SSIp was 20 - 35% in females and 38 - 51% in males (females, R^2: 0.20 - 0.35; males, R^2: 0.38 - 0.51). Tibial SSIp explained only 11 - 21% variance in metacarpal SSIp in females but 27 - 30% in males (females, R^2: 0.11 - 0.21; males, R^2: 0.27 - 0.30).

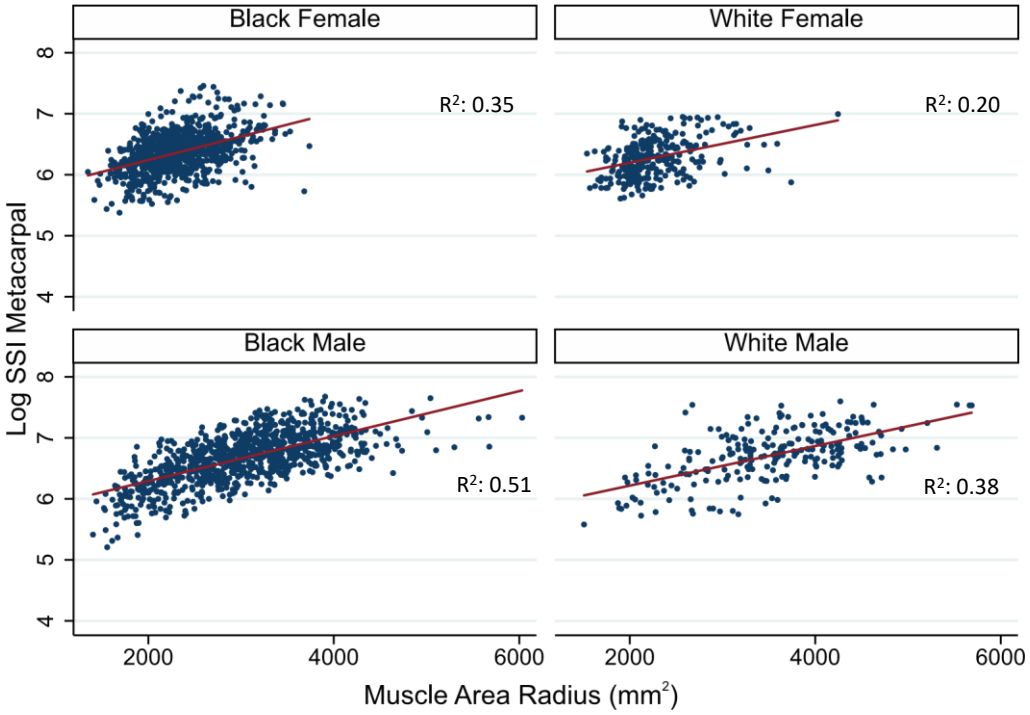


Figure 4.2 Metacarpal stress-strain index in relation to radial muscle cross-sectional area in black and white females and males

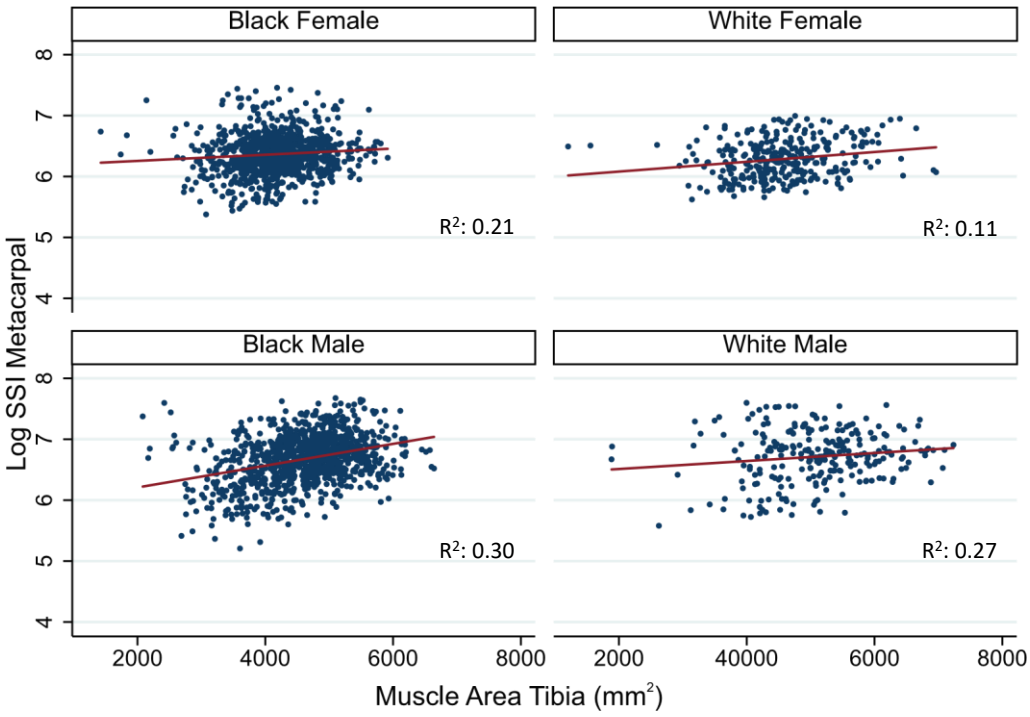


Figure 4.3 Metacarpal stress-strain index in relation to tibial muscle cross-sectional area in black and white females and males

Bone width

Metacarpal, radial and tibial bone width growth patterns are presented in Figure 4.4, and the average annual changes in bone width are shown in Table 4.2. Bone width over the period of study increased significantly in each of the three bones in all four groups, except for radial bone width in white females which did not increase significantly. Metacarpal and radial bone width growth rates were similar to each other in both sex and ethnic groups. However, comparisons between the tibia and metacarpal bone revealed that tibial bone width growth rate among black females and white females (0.23 and 0.31 mm/year, respectively) was double that of the metacarpal (0.12 and 0.14 mm/year, respectively), whilst tibial bone width growth rate among black males and white males (0.68 and 0.82 mm/year, respectively) was 3 - 4 times greater than that of the metacarpal bone (0.22 and 0.28 mm/year, respectively).

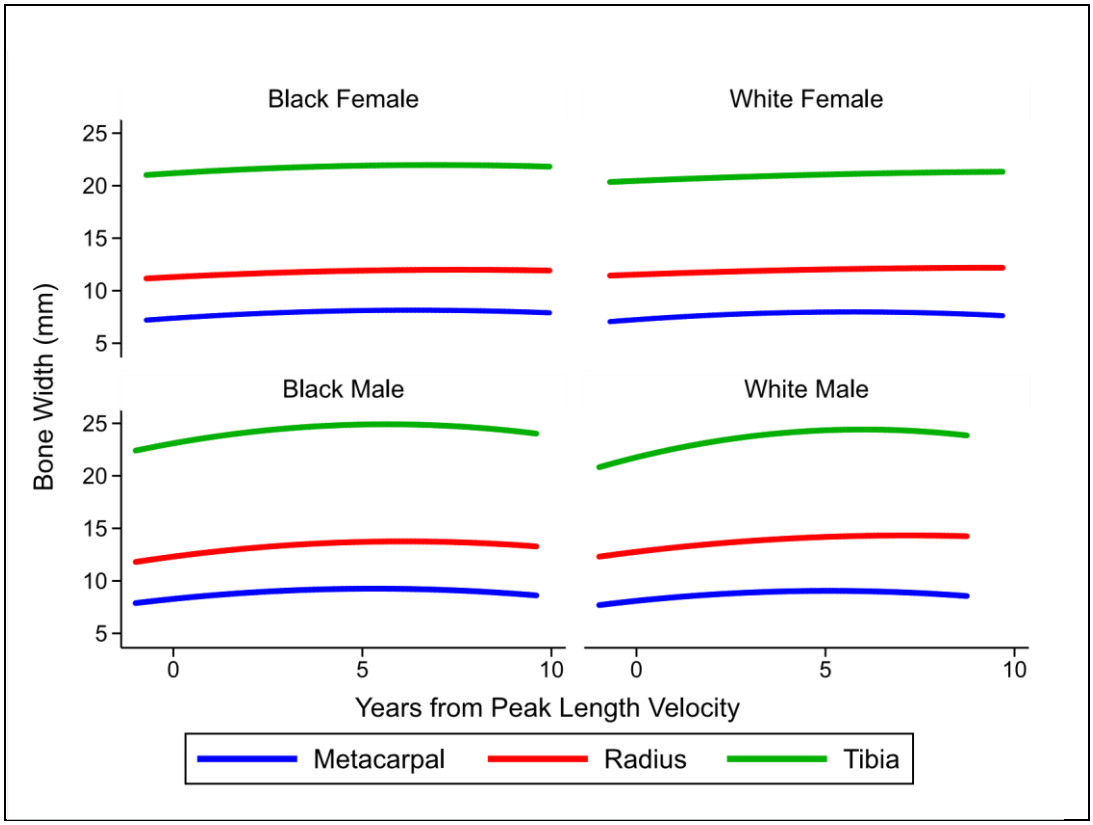


Figure 4.4 Mixed model growth curves of metacarpal, radial and tibial bone width against years from peak metacarpal length velocity in black and white females and males

Table 4.2 Results from mixed-effects growth models summarising differences in metacarpal, radial and tibial bone width growth

Outcome	Fixed Effects					Random Effects		
Bone width	YPLV	Bone	Intercept	Slope [CI]	Age*Site [CI]	Intercept	Slope	Residual
BF		Met	7.39	0.12 [0.11, 0.13]*				
	0.31 [0.25, 0.38]*	Rad	10.34	0.08 [0.04, 0.12]*	-0.004 [-0.03, 0.02]	0.72	0.0000	0.40
		Tib	21.14	0.23 [0.20, 0.26]*	0.10 [0.04, 0.15]*			
WF		Met	7.21	0.14 [0.12, 0.17]*				
	0.35 [0.23, 0.48]*	Rad	9.89	0.05 [-0.03, 0.12]	0.02 [-0.03, 0.07]	0.63	0.0000	0.40
		Tib	20.24	0.31 [0.25, 0.37]*	0.16 [0.06, 0.26]¥			
BM		Met	7.77	0.22 [0.20, 0.24]*				
	0.63 [0.55, 0.70]*	Rad	10.89	0.19 [0.15, 0.23]*	-0.01 [-0.06, 0.03]	0.87	0.001	0.71
		Tib	23.16	0.68 [0.64, 0.73]*	0.23 [0.17, 0.29]*			
WM		Met	7.35	0.28 [0.22, 0.33]*				
	0.94[0.77, 1.11]*	Rad	10.27	0.15[0.03, 0.27]¥	-0.02 [-0.12, 0.07]	1.04	0.005	0.65
		Tib	22.28	0.82 [0.73, 0.90]*	0.21 [0.10, 0.32]*			

BF – black female, WF – white female, BM – black male, WM – white male

YPLV – years from peak metacarpal length velocity

Met – metacarpal, Rad – radius, Tib – tibia

Fixed effects: The intercept is the mean value for the outcome variable. Slopes represent annual rates of accrual as assessed by YPLV and are presented with 95% confidence intervals [CI].

Age*Site represents the slopes of the radius and tibia relative to the metacarpal bone

Significant differences are given as * $p < 0.0001$, ** $p < 0.001$, ¥ $p < 0.01$

Random effects: Between-participant variation in intercepts and slopes with residual variances.

Cortical Thickness

The cortical thicknesses at the metacarpal, radial and tibial bone sites by years from peak metacarpal length velocity are shown in Figure 4.5. In all four groups, cortical thickness increased significantly over the period of study in the metacarpal, radius and tibia (Table 4.3). The average annual rate of growth in cortical thickness in the metacarpal and radius was similar to each other in all groups, except in white females in whom the radial cortical thickness growth rate was 0.04 mm/year less than that of the metacarpal ($p < 0.05$). In white males, the average annual cortical thickness growth rates of the three bones were similar. In the other three groups, the average annual increase in tibial cortical thickness growth rate was significantly greater than that of the metacarpal.

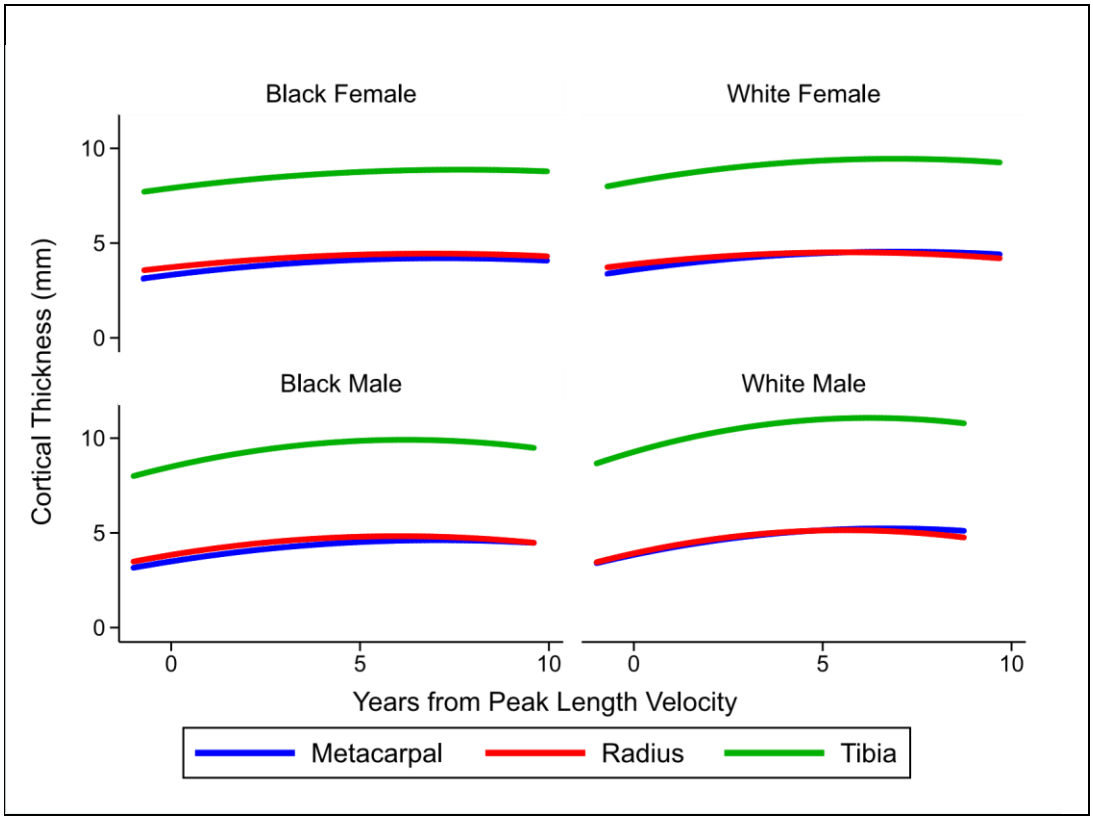


Figure 4.5 Mixed model growth curves of metacarpal, radial and tibial cortical thickness against years from peak metacarpal length velocity in black and white females and males

Table 4.3 Results from mixed-effects growth models summarising differences in metacarpal, radial and tibial cortical thickness growth

Outcome	Fixed Effects					Random Effects		
Cortical Thickness	YPLV	Bone	Intercept	Slope [CI]	Age*Site [CI]	Intercept	Slope	Residual
BF		Met	2.88	0.14 [0.11, 0.17]*				
	0.28 [0.23,0.34]*	Rad	3.60	0.19 [0.16, 0.23]*	-0.01 [-0.03, 0.02]	0.24	0.0000	0.29
		Tib	7.53	0.22 [0.20, 0.25]*	0.11 [0.06, 0.16]*			
WF		Met	2.94	0.22 [0.17, 0.27]*				
	0.33 [0.23, 0.43]*	Rad	3.45	0.19 [0.14, 0.24]*	-0.04 [-0.08, -0.01]ϑ	0.13	0.0004	0.23
		Tib	8.00	0.32 [0.27, 0.37]*	0.10 [0.03, 0.18]¥			
BM		Met	2.72	0.16 [0.12, 0.19]*				
	0.27 [0.22,0.32]*	Rad	3.44	0.25 [0.22, 0.28]*	0.01 [-0.02, 0.05]	0.28	0.0000	0.37
		Tib	8.08	0.39 [0.36, 0.42]*	0.07 [0.02, 0.11]¥			
WM		Met	3.08	0.32 [0.23, 0.41]*				
	0.59 [0.48,0.70]*	Rad	3.19	0.29 [0.22, 0.35]*	-0.05 [-0.11, 0.01]	0.27	0.0004	0.30
		Tib	8.92	0.46 [0.40, 0.52]*	0.04 [-0.03, 0.11]			

BF – black female, WF – white female, BM – black male, WM – white male

YPLV – years from peak metacarpal length velocity

Met – metacarpal, Rad – radius, Tib – tibia

Fixed effects: The intercept is the mean value for the outcome variable. Slopes represent annual rates of accrual as assessed by YPLV and are presented with 95% confidence intervals [CI].

Age*Site represents the slopes of the radius and tibia relative to the metacarpal bone

Significant differences are given as * $p < 0.0001$, ** $p < 0.001$, ¥ $p < 0.01$, ϑ $p < 0.05$

Random effects: Between-participant variation in intercepts and slopes with residual variances.

Medullary width

Metacarpal, radial and tibial medullary widths growth patterns are presented in Figure 4.6 and the average annual changes in medullary width are shown in Table 4.4. Black and white females experienced an average decrease in metacarpal medullary width growth rate of -0.03 and -0.09 mm/year, respectively. The average annual change in metacarpal medullary width in white males was -0.07 mm/year, while in black males a significant increase in the metacarpal medullary width of 0.06 mm/year was noted. At the radius, medullary width decreased significantly in all four groups, while at the tibia, both black and white males experienced significant increases in medullary width of 0.17 - 0.20 mm/year. In females, the average annual change in tibial medullary width was not significant.

When comparing the metacarpal medullary width change to that of the radius, there were no significant differences in any of the groups, except in white females who experienced a greater average increase in radial medullary width of 0.06 mm/year relative to the metacarpal. Tibial medullary width increased significantly relative to the metacarpal in males, but not in females.

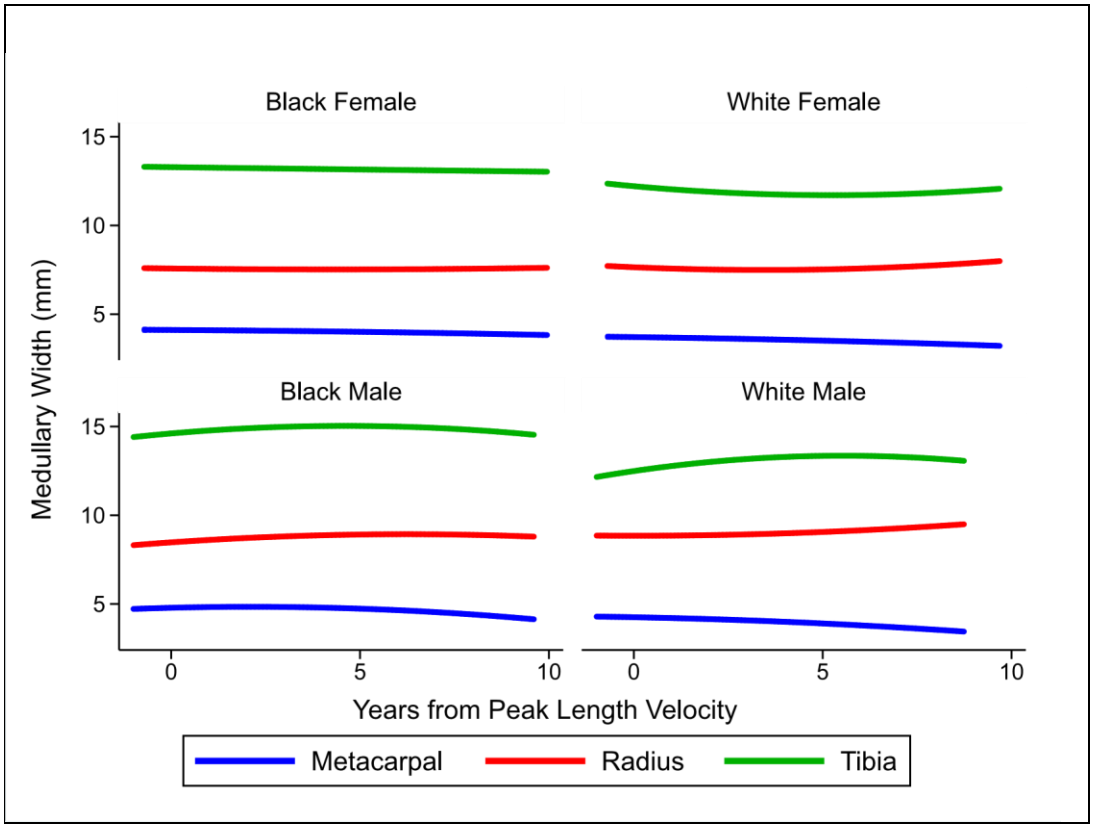


Figure 4.6 Mixed model growth curves of metacarpal, radial and tibial medullary width against years from peak metacarpal length velocity in black and white females and males

Table 4.4 Results from mixed-effects growth models summarising differences in metacarpal, radial and tibial medullary width change

Outcome	Fixed Effects					Random Effects		
Medullary width	YPLV	Bone	Intercept	Slope [CI]	Age*Site [CI]	Intercept	Slope	Residual
BF		Met	4.38	-0.03 [-0.06, -0.001]ϑ				
	0.03 [-0.05, 0.11]	Rad	6.56	-0.12 [-0.18, -0.06]*	0.004 [-0.03, 0.04]	0.80	0.0000	0.67
		Tib	13.61	0.01 [-0.02, 0.04]	0.005 [-0.07, 0.08]			
WF		Met	4.15	-0.09 [-0.14, -0.04]**				
	0.03 [-0.12, 0.18]	Rad	6.32	-0.15 [-0.26, -0.04]¥	0.06 [0.002, 0.12]ϑ	0.38	0.0000	0.56
		Tib	12.16	-0.02 [-0.08, 0.05]	0.12 [-0.001, 0.24]			
BM		Met	4.97	0.06 [0.02, 0.09]¥				
	0.33 [0.24, 0.43]*	Rad	7.41	-0.07 [-0.12, -0.01]ϑ	-0.03 [-0.09, 0.03]	1.30	0.0000	1.20
		Tib	15.10	0.29 [0.24, 0.34]*	0.17 [0.09, 0.25]*			
WM		Met	4.09	-0.07 [-0.16, 0.03]				
	0.26 [0.11, 0.42]*	Rad	6.94	-0.16 [-0.31, -0.02]ϑ	0.03 [-0.06, 0.12]	0.69	0.0000	0.63
		Tib	13.33	0.35 [0.27, 0.44]*	0.20 [0.09, 0.30]*			

BF – black female, WF – white female, BM – black male, WM – white male

YPLV – years from peak metacarpal length velocity

Met – metacarpal, Rad – radius, Tib – tibia

Fixed effects: The intercept is the mean value for the outcome variable. Slopes represent annual rates of accrual as assessed by YPLV and are presented with 95% confidence intervals [CI].

Age*Site represents the slopes of the radius and tibia relative to the metacarpal bone

Significant differences are given as * $p < 0.0001$, ** $p < 0.001$, ¥ $p < 0.01$, ϑ $p < 0.05$

Random effects: Between-participant variation in intercepts and slopes with residual variances.

Stress-Strain Index

The SSIp at the metacarpal, radial and tibial bone sites by years from peak metacarpal length velocity are shown in Figure 4.7. In all four groups, the SSIp increased significantly in each bone, except for tibial SSIp in white females in whom the average annual increase was not significant (Table 4.5). There was a greater average annual increase in radial than metacarpal SSIp in black females and white males. Tibial SSIp increased significantly compared to the metacarpal SSIp in white females and black males (Table 4.5).

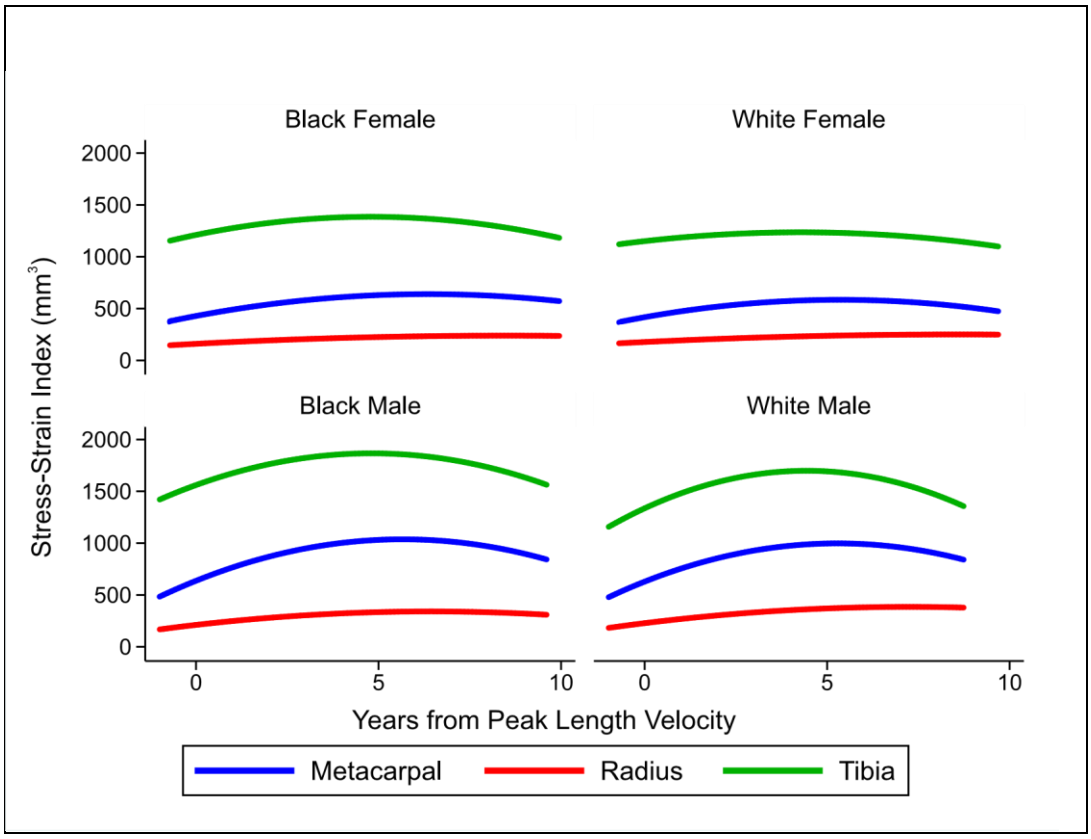


Figure 4.7 Mixed model growth curves of metacarpal, radial and tibial stress-strain index against years from peak metacarpal length velocity in black and white females and males

Table 4.5 Results from mixed-effects growth models summarising differences in metacarpal, radial and tibial stress-strain index change

Outcome	Fixed Effects					Random Effects		
Stress-strain index	YPLV	Bone	Intercept	Slope [CI]	Age*Site [CI]	Intercept	Slope	Residual
BF		Met	392.00	38.59 [33.49, 43.69]*				
	48.39 [35.65, 61.14]*	Rad	115.00	14.08 [11.80, 16.36]*	7.87 [2.30, 13.45]¥	1.53E+04	0.0000	1.61E+04
		Tib	915.00	41.69 [29.00, 54.38]*	2.90 [-8.22, 14.03]			
WF		Met	357.00	34.08 [25.44, 42.71]*				
	54.62 [29.68, 79.65]*	Rad	61.97	13.61 [8.95, 18.27]*	5.29 [-4.28, 14.85]	1.17E+04	0.0000	1.55E+04
		Tib	852.00	19.17 [-4.44, 42.78]	23.49 [3.75, 43.22]ϑ			
BM		Met	324.00	66.86 [57.23, 76.49]*				
	99.79 [84.14, 115.00]*	Rad	144.00	23.35 [20.20, 26.49]*	5.07 [-5.34, 15.49]	2.64E+04	40.94	3.42E+04
		Tib	1230.00	104.00 [91.68, 116.00]*	23.78 [10.28, 37.27]¥			
WM		Met	263.00	95.95 [68.29, 124.00]*				
	121.00 [84.83, 158.00]*	Rad	73.47	22.65 [12.62, 32.68]*	22.65 [1.31, 44.00]ϑ	3.37E+04	0.0000	3.50E+04
		Tib	1000.00	114.00 [90.19, 138.00]*	16.12 [-8.66, 40.89]			

BF – black female, WF – white female, BM – black male, WM – white male

YPLV – years from peak metacarpal length velocity

Met – metacarpal, Rad – radius, Tib – tibia

Fixed effects: The intercept is the mean value for the outcome variable. Slopes represent annual rates of accrual as assessed by YPLV and are presented with 95% confidence intervals [CI].

Age*Site represents the slopes of the radius and tibia relative to the metacarpal bone

Significant differences are given as * $p < 0.0001$, ** $p < 0.001$, ¥ $p < 0.01$, ϑ $p < 0.05$

Random effects: Between-participant variation in intercepts and slopes with residual variances.

4.3 Discussion

In this longitudinal study, the growth patterns of weight-bearing and non-weight bearing appendicular bones, measured by radiogrammetry or pQCT, were assessed in black and white SA children through puberty and adolescence. I have shown that metacarpal bone width and cortical thickness are strongly associated with radial MCSA but not with tibial MCSA. These findings are consistent with those of a pQCT study conducted in adults which showed a strong correlation between metacarpal bone mass and forearm muscle mass in both individuals with rheumatoid arthritis and healthy controls [332]. The stronger associations between metacarpal bone measures and radial MCSA are most likely due to them both being non-weight bearing bones which are in close proximity to each other and are subjected to similar mechanical strains. In the present study, the variance in metacarpal bone measures and strength explained by radial MCSA was 33 - 45% in males, compared to 12 - 20% in females. A similar sex difference has been reported in a study of young adults aged between 19 and 22 years in whom lean mass, measured by dual energy X-ray absorptiometry, explained 34.5% of the variability in whole-body bone mineral density in males but only 18.3% of the variability in females [333]. This sex disparity, which arises during puberty, is probably related to differences in muscle mass developing during this period [334]. It is of interest that peak muscle mass gain precedes peak bone mass gain by several months, as shown in a Canadian study in which peak muscle mass gain preceded peak bone mass gain by 0.36 years and 0.51 years in boys and girls, respectively [167], suggesting a role for muscle mass in determining peak bone mass.

This study showed that there were no differences in the growth of bone width between the non-weight bearing metacarpal and radial bones in all 4 groups when aligned on years from aPLV; however, bone width growth of the weight-bearing tibia was significantly greater than that of the metacarpal bone, even after adjusting for the influence of the associated musculature. The greater increases in cross-sectional bone dimensions in the tibia may have been the result of mechanical loading [335-337] due to body weight and muscle contraction, the latter creating the largest physiologic load on bone thus influencing bone strength [173].

In this study, cortical thickness of the three bones increased significantly during adolescence with a similar average annual rate in the metacarpal and radius in all groups, except in white females who had a lower rate of cortical thickness growth at the radius than the metacarpal.

Tibial cortical thickness growth rate exceeded that of the metacarpal in all groups, except in white males. In an anthropological study of cadavers of individuals aged between 1 and 30 years, Sumner and Andriacchi [335] found that cortical area as a function of bone length increased more rapidly in the weight-bearing femur than in the non-weight bearing humerus. The bone width and cortical thickness growth patterns that I observed in the weight-bearing tibia compared to the non-weight bearing metacarpal are analogous to those reported in the femur and humerus by Sumner and Andriacchi [335].

Sexual dimorphism, irrespective of ethnicity, was apparent in the velocities of bone width and cortical thickness growth of the participants in the present study, particularly in the tibia. Male participants experienced greater velocities which may be associated with higher rates of bone turnover [83, 325] and greater cortical porosity [338]. Whilst I did not analyse bone turnover or cortical porosity, it bears consideration, since the inverse relationship between cortical porosity and bone strength is exponential, with even a small increase in porosity resulting in a relatively large decrease in strength [339]. Changes in cortical porosity that accompany the greater growth rates in males may contribute to their higher fracture incidence [231] despite having superior strength, determined by SSIp, than females [326].

In this study, the medullary width growth patterns differed between the three bones. In the non-weight bearing bones there was an overall narrowing of the medullary cavity in all groups except for the metacarpal in black males in whom it expanded. At the tibia, the increase in medullary width was only significant in males. It has previously been shown that there are ethnic differences in medullary width growth between black and white males in this cohort [340]. The contraction in metacarpal medullary width in white males in this study differs from cross-sectional studies of white children in the United States [318] but concurs with a more recent longitudinal study in European children [79], which suggests population differences in medullary cavity growth. The overall contraction of radial medullary width in both male and female participants in my study, differs from that of a cross-sectional pQCT study at the 65% radius of white individuals between 6 and 40 years of age by Neu and co-workers [337]. In their study, from Tanner stage 3 onwards there was no change in medullary width in females, whilst in males it increased significantly which they attributed to modelling drift, that is, endocortical bone resorption in response to bone expansion as a result of periosteal bone deposition [337]. In contrast, in both sexes in the present study, radial medullary width

contraction was accompanied by bone width expansion indicating that bone deposition occurred at both the periosteal and endocortical surfaces. While influence of the associated musculature on bone growth was adjusted for in study, I did not assess hormonal influences which have been shown to differentially affect bone growth in the sexes [325]. Kirmani and co-workers [325] found that radial periosteal and endocortical bone accrual were driven by insulin-like growth factor-1 (IGF-1) in girls and by testosterone in boys. The greater expansion of tibial medullary width that I observed in male participants concurs with other longitudinal studies [326] and reflects the bone's compensatory mechanism to resist bending and torsional forces as the cortices move further from the bone's neutral axis. The site-specific differences at the endocortical surface that I observed suggest that in weight-bearing bones there is modelling drift and that this functional adaptation to increase bone strength may possibly override hormonal influences.

The SSIp, which is the product of the section modulus of the bone and the ratio of measured cortical density to physiologic bone density (1200 mg/cm^3), has been shown to be a strong proxy for bone strength and failure of loading [284]. There was a negligible change in tibial SSIp in the females in this study despite an increase in bone width and cortical thickness through adolescence. I observed greater average annual changes in SSIp of the metacarpal, radius and tibia in males than in females, which is in keeping with the greater increase in male bone width and cortical thickness over the period of measurement, and consistent with mixed longitudinal pQCT and high-resolution pQCT studies of the radius and tibia in Canadian adolescents [326, 341]. Whilst a lower SSIp might suggest that females may be more susceptible to fracture, childhood tibial fractures occur relatively infrequently. Up to the age of 12 years, tibial fracture incidence has been shown to be higher in British females compared to males [219]. Sex differences in proxies for tibial strength (section modulus and bone strength index), have previously been reported in a study of stress fracture in military recruits who averaged 19 years of age [278]. In male military recruits who had fractured, lower tibial strength indices were attributed to narrower bone widths, in contrast to female recruits who had fractured in whom lower tibial strength indices were a function of thinner cortices [278]. In the present study, I did not differentiate between participants with and without a history of fracture and am therefore unable to comment on the association between SSIp and tibial fracture in this cohort.

The study has a number of limitations. Although differences in somatic maturity were adjusted for using aPLV, this assumed that one year of biological age is equivalent to one year of chronological age. This only accounted for differences in the timing (tempo) of growth and not for potential differences in the rate (velocity) of growth [50, 342]. Furthermore, the timing of peak growth velocity varies according to anatomical site [50, 342]. The effects of physical activity on bone are probably mediated via muscle [183], and although physical activity was not adjusted for in these analyses, MCSA was included to try to account for this. My longitudinal data of bone growth of the metacarpal, radius and tibia in black and white children are unique; however, the number of white participants was relatively small which may have led to a type II error. This study only included radiographic and pQCT measurements from chronological age 12 years, by which time peak growth may have commenced or been completed in some individuals, particularly in females. The data were obtained using two different technologies and at different diaphyseal regions; radiogrammetry was done at the midshaft of the metacarpal bones, and pQCT at the 65% radial and 38% and 65% tibial sites. The ratio of cortical to trabecular bone, and the associated musculature of the three bones at these sites may differ. Although bone growth is region-specific, I do not believe that standardising measurements of all three bones at the diaphyseal midshaft would have changed the outcomes.

In conclusion, longitudinal analyses through adolescence showed that in both sex and ethnic groups, when aligned on a physiological maturity indicator, the growth patterns of the non-weight bearing metacarpal bone and radius were overall similar to each other but differed from that of the weight-bearing tibia. The extent of sexual dimorphism in bone width growth patterns was similar in both ethnic groups, suggestive of common biological mechanisms which regulate functional adaptation, despite different genetic backgrounds.

4.4 Summary of major findings

In both sex and ethnic groups, metacarpal measures correlated more closely with radial than tibial MCSA. The variance in metacarpal bone measures and strength explained by radial MCSA was 33 - 45% in males, compared to 12 - 20% in females.

Within each sex and ethnic group, the non-weight bearing metacarpal and radial bones had similar growth patterns for bone width, cortical thickness and medullary width. At the weight-bearing tibia, bone width and cortical thickness accrual rates were greater than at the metacarpal. Tibial medullary width increased significantly relative to metacarpal medullary width in males only. Bone strength increased significantly in each bone, except for tibial strength in white females.

CHAPTER 5

Metacarpal indices and their association with fracture in South African children and adolescents

This chapter describes the association between metacarpal indices derived using radiogrammetry, and fracture risk. The results are described and discussions pertaining to these findings are outlined together with the limitations. The principal findings and discussion in this chapter were published in the following journal (Appendix L):

Magan A, Micklesfield LK, Norris SA, Thandrayen K, Munthali RJ and Pettifor JM (2018) Metacarpal indices and their association with fracture in South African children and adolescents. Calcified Tissue International (published online).
DOI: 10.1007/s00223-018-0467-4

5.1 Methods

Metacarpal bone length, bone width and medullary width were measured in 359 BHC children between 10 and 17 years of age, and the following proxies for bone strength calculated: Metacarpal Index (MCI), Bone Mineral Density (BMD), Section Modulus (SM), Stress-strain Index (SSIp) and Slenderness Index (SLI). Height and weight were measured annually. Self-reported physical activity was obtained at age 15 years for the preceding 12 months. Fracture history was obtained retrospectively at age 17 years. Multivariate logistic regression analyses, adjusting for height and formal physical activity, were performed to examine the associations between anthropometric measures, bone measures and bone indices, and fracture history at 17 years separately for each sex and ethnic group.

5.2 Results

Participant recruitment and number of observations are shown in Figure 5.1. A total number of 359 participants, aged between 10 and 17 years, had valid hand-wrist radiographs and had completed the fracture and physical activity questionnaires.

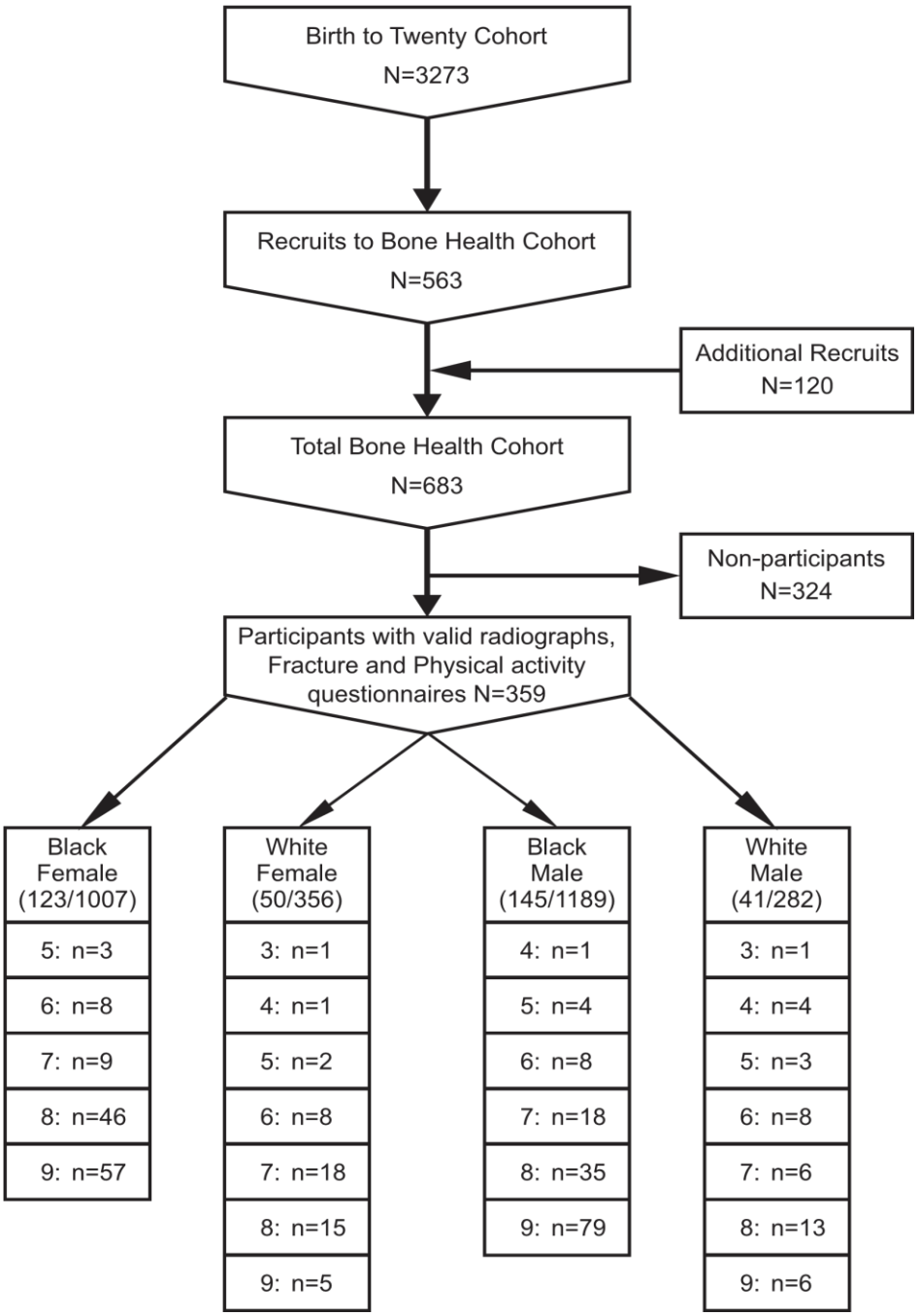


Figure 5.1 Participant recruitment (N) and the number of participants (n) with their respective total number of radiographs over the study period. (5: n=3 means that 3 participants had a total of 5 radiographs).

Fracture incidence

The mean age on completion of the fracture questionnaire was 17.69 ± 0.25 years. Eighty-two (23%) of these individuals had sustained one or more fractures during their lifetime. The percentage of white females who had fractured was more than triple that of black females (40% vs 12%), whilst the percentage of white males who had fractured was more than double that of black males (44% vs 20%). Within ethnic groups, there were no significant sex differences in fracture prevalence. The sites of fracture and grades of trauma are presented in Table 5.1. The five participants who had sustained a Grade 3 fracture were excluded from further analyses.

Table 5.1 Cohort fractures by site and grade of trauma

Site	Grade 1	Grade 2	Grade 3
Upper limb	10 (12%)	36 (44%)	0
Lower limb	6 (7%)	19 (23%)	4 (5%)
Other	0	6 (7%)	1 (1%)

Cross-sectional Data

Cohort characteristics at age 17 years

Characteristics of participants of the cohort at age 17 years are presented in Table 5.2. At 17 years of age, black participants were significantly shorter than their white peers. Black and white females were of similar weight; however, black males were lighter than white males. Black females and males had thinner cortices, greater medullary widths and hence lower MCI than their white peers. Black and white females had shorter and narrower bones with smaller medullary cavities than their respective male peers. Females therefore had lower BMD, SM and SSIp than males. Females also had more slender bones than males as reflected by their higher SLI. At 15 years, black participants engaged in significantly less formal physical activity than their white peers.

Table 5.2 Cohort anthropometry, bone measures and indices at 17 years of age, and physical activity at 15 years of age.

	Black Female (N=123)			White Female (N=50)		
Variable	Mean	SD		Mean	SD	
Height (cm)	158.20	6.13	ab	165.95	7.14	d
Weight (kg)	59.12	11.07		61.70	12.98	d
TL (mm)	65.38	3.70	b	65.56	3.85	d
BW (mm)	8.10	0.71	b	8.01	0.56	d
MW (mm)	3.98	0.87	ab	3.47	0.92	d
CT (mm)	4.12	0.53	a	4.54	0.59	d
MCI	0.51	0.08	ab	0.57	0.09	
BMD (g/mm^2)	6.21	0.61	b	5.97	0.71	d
SM (mm^3)	99.23	25.28	b	97.50	18.66	d
SSI (mm^3)	630.10	227.70	b	592.79	178.99	d
SLI ($1/mm^2/kg$)	40.69	10.39	b	42.84	11.91	d
FPA (min/wk)	11.18	22.17	ab	44.32	58.45	
lnFPA (min/wk)	16.36	30.50		14.51	28.54	

	Black Male (N=145)			White Male (N=41)	
Variable	Mean	SD		Mean	SD
Height (cm)	170.44	6.65	c	178.46	9.18
Weight (kg)	59.45	9.13	c	72.01	12.61
TL (mm)	70.53	3.87		70.94	3.74
BW (mm)	9.14	0.79		9.16	0.83
MW (mm)	4.83	1.07	c	4.20	0.94
CT (mm)	4.31	0.68	c	4.96	0.59
MCI	0.48	0.09	c	0.54	0.07
BMD (g/mm^2)	6.95	0.64		6.99	0.76
SM (mm^3)	138.89	32.43		146.04	36.44
SSI (mm^3)	982.58	312.23		1045.41	353.15
SLI ($1/mm^2/kg$)	31.41	7.32	c	36.78	10.26
FPA (min/wk)	26.21	31.60		33.42	34.67
lnFPA (min/wk)	21.12	35.30		22.82	27.02

a-d represent significant sex and ethnic differences: a - Black Female vs White Female, b - Black Female vs Black Male, c - Black Male vs White Male, d - White Female vs White Male ($p < 0.05$).

TL - Total Length, BW - Bone Width, MW - Medullary Width, CT - Cortical Thickness, MCI - Metacarpal Index, BMD - Bone Mineral Density, SM - Section Modulus, SSI - Stress-Strain Index, SLI - Slenderness Index, FPA - Formal Physical Activity, lnFPA - Informal Physical Activity

Comparison of fracture and non-fracture groups

Comparisons of anthropometry, bone measures and indices between the fractured and non-fractured groups at age 17 years are presented in Table 5.3. The only anthropometric difference between those who had and had not fractured was in white females, with those who had fractured having a significantly higher BMI Z-score than those who had not (p =0.04). Metacarpal length, bone width and cortical thickness did not differ between those who had and had not fractured in black females, black males and white females. However, white males who had fractured had wider bones (p = 0.03) and greater medullary widths (p = 0.02) than those without fractures. There were no differences in any of the bone indices between the fracture and non-fracture groups in black participants. In white participants, females who had fractured had a greater SLI than their non-fractured peers (p = 0.01), while males who had fractured had lower MCI and SLI but higher BMD, SM and SSIp than their non-fracturing peers.

Table 5.3 Sex and ethnic differences in mean Z-scores with standard deviations (SD) for anthropometric measures, bone measures, metacarpal indices and physical activity in 17-year old participants with and without a history of fracture.

	Black Female			White Female		
	No-fracture	Fracture		No-fracture	Fracture	
	(N=108)	(N=13)		(N=30)	(N=19)	
Variable	Mean (SD)	Mean (SD)	*p*	Mean (SD)	Mean (SD)	*p*
BMI	0.51 (1.14)	0.23 (0.84)	ns	-0.08 (0.95)	0.57 (1.24)	0.04
Height	-0.53 (0.92)	-0.63 (0.95)	ns	0.46 (1.04)	0.41 (1.16)	ns
TL	0.00 (1.00)	-0.05 (1.24)	ns	-0.11 (0.97)	0.28 (1.19)	ns
BW	0.00 (1.00)	0.34 (1.22)	ns	0.00 (0.83)	-0.21 (0.78)	ns
MW	0.00 (1.00)	0.21 (1.31)	ns	-0.39 (1.01)	-0.88 (1.17)	ns
CT	0.01 (1.00)	0.11 (0.84)	ns	0.61 (0.91)	1.09 (1.28)	ns
MCI	0.04 (0.97)	-0.03 (1.05)	ns	0.55 (1.02)	1.09 (1.29)	ns
BMD	0.00 (1.00)	0.25 (0.96)	ns	-0.12 (1.12)	-0.72 (1.18)	ns
SM	0.00 (1.00)	0.34 (1.33)	ns	0.05 (0.81)	-0.14 (0.72)	ns
SSI	0.00 (1.00)	0.33 (1.39)	ns	-0.01 (0.86)	-0.31 (0.77)	ns
SLI	0.00 (1.00)	-0.54 (0.56)	ns	-0.18 (0.79)	0.60 (1.36)	0.01
FPA	0.02 (0.94)	-0.03 (0.52)	ns	1.59 (2.69)	1.04 (1.85)	ns
InFPA	0.00 (1.08)	-0.16 (0.62)	ns	-0.05 (1.13)	-0.11 (0.69)	ns
	Black Male			White Male		
	No-fracture	Fracture		No-fracture	Fracture	
	(N=116)	(N=27)		(N=23)	(N=18)	
Variable	Mean (SD)	Mean (SD)	*p*	Mean (SD)	Mean (SD)	*p*
BMI	-0.59 (1.11)	-0.71 (0.90)	ns	-0.04 (0.89)	0.52 (0.95)	ns
Height	-0.61 (0.89)	-0.64 (1.07)	ns	0.09 (1.22)	0.64 (1.17)	ns
TL	1.41 (1.05)	1.47 (1.19)	ns	1.44 (1.18)	1.67 (0.84)	ns
BW	1.54 (1.11)	1.60 (1.27)	ns	1.22 (1.12)	2.04 (1.18)	0.03
MW	1.01 (1.27)	1.15 (1.28)	ns	-0.07 (1.14)	0.74 (0.95)	0.02
CT	0.41 (1.20)	0.26 (1.48)	ns	1.66 (1.04)	1.46 (1.16)	ns
MCI	-0.40 (1.07)	-0.55 (1.15)	ns	0.70 (0.99)	0.10 (0.72)	0.04
BMD	1.24 (1.00)	1.23 (1.28)	ns	0.87 (1.31)	1.88 (0.91)	0.01
SM	1.67 (1.29)	1.74 (1.56)	ns	1.53 (1.27)	2.55 (1.63)	0.03
SSI	1.65 (1.38)	1.75 (1.71)	ns	1.43 (1.36)	2.64 (1.73)	0.02
SLI	-0.94 (0.66)	-0.91 (0.80)	ns	-0.33 (1.11)	-0.56 (0.73)	ns
FPA	0.57 (1.27)	0.85 (1.34)	ns	0.35 (0.92)	1.53 (1.61)	0.02
InFPA	0.11 (1.15)	0.30 (1.36)	ns	0.24 (1.12)	0.16 (0.67)	ns

Non-fracturing black females were used as a reference group to develop Z-scores for the bone measures and indices. WHO reference standards were used for anthropometry. *p*-values represent significant differences between fracture and non-fracture groups. ns - no significant differences between fracture and non-fractured groups. TL - Total Length, BW - Bone Width, MW - Medullary Width, CT - Cortical Thickness, MCI - Metacarpal Index, BMD - Bone Mineral Density, SM - Section Modulus, SSI - Stress-Strain Index, SLI - Slenderness Index, FPA - Formal Physical Activity, InFPA - Informal Physical Activity

Associations between anthropometric characteristics and history of fractures at age 17 years

Bivariate logistic regression analyses showed that in white females a 1 SD greater BMI Z-score was associated with a 93% increase in fracture risk (OR: 1.93; 95% CI: 1.01, 3.71) (Table 5.4). None of the anthropometric measures were associated with fracture risk in the other 3 groups (Table 5.4).

Table 5.4 Associations between anthropometric measures, bone measures, bone indices and physical activity, and history of fracture at age 17 years.

	Black Female (N=121)				White Female (N=49)			
Variable	OR	SE	95% CI	*p*	OR	SE	95% CI	*p*
Height	0.89	0.27	[0.50; 1.61]	0.71	0.95	0.26	[0.56; 1.62]	0.85
BMI	0.71	0.21	[0.40; 1.28]	0.26	1.93	0.64	[1.01; 3.71]	**0.047**
TL	0.96	0.26	[0.56; 1.63]	0.87	1.43	0.41	[0.82; 2.50]	0.21
BW	1.35	0.34	[0.82; 2.23]	0.23	0.71	0.27	[0.34; 1.48]	0.36
MW	1.22	0.32	[0.73; 2.05]	0.45	0.65	0.19	[0.37; 1.13]	0.13
CT	1.11	0.31	[0.64; 1.93]	0.71	1.56	0.46	[0.88; 2.78]	0.13
MCI	0.93	0.27	[0.53; 1.62]	0.79	1.56	0.43	[0.91; 2.68]	0.11
BMD	1.29	0.36	[0.75; 2.24]	0.35	0.63	0.17	[0.37; 1.07]	0.09
SM	1.32	0.31	[0.83; 2.08]	0.24	0.72	0.28	[0.33; 1.55]	0.40
SSI	1.29	0.30	[0.82; 2.02]	0.26	0.63	0.24	[0.30; 1.32]	0.22
SLI	0.51	0.18	[0.26; 1.00]	**0.049**	2.00	0.60	[1.11; 3.61]	**0.02**
Formal PA	0.93	0.32	[0.48; 1.82]	0.84	0.90	0.13	[0.68; 1.19]	0.45
Informal PA	0.82	0.30	[0.40; 1.68]	0.58	0.94	0.31	[0.49; 1.79]	0.84
	Black Male (N=143)				White Male (N=41)			
Variable	OR	SE	95% CI	*p*	OR	SE	95% CI	*p*
Height	0.97	0.22	[0.62; 1.50]	0.87	1.49	0.42	[0.85; 2.59]	0.16
BMI	0.78	0.25	[0.42; 1.45]	0.44	2.16	0.98	[0.89; 5.24]	0.09
TL	1.06	0.21	[0.72; 1.55]	0.78	1.26	0.40	[0.68; 2.33]	0.47
BW	1.05	0.19	[0.73; 1.50]	0.80	1.96	0.64	[1.03; 3.72]	**0.04**
MW	1.10	0.18	[0.79; 1.51]	0.58	2.22	0.85	[1.05; 4.69]	**0.04**
CT	0.91	0.15	[0.65; 1.27]	0.57	0.84	0.25	[0.47; 1.50]	0.55
MCI	0.88	0.17	[0.59; 1.29]	0.51	0.45	0.18	[0.20; 0.99]	**0.047**
BMD	0.99	0.19	[0.67; 1.45]	0.95	2.45	0.93	[1.16; 5.17]	**0.02**
SM	1.04	0.16	[0.77; 1.41]	0.78	1.68	0.43	[1.02; 2.76]	**0.04**
SSI	1.05	0.15	[0.79; 1.38]	0.75	1.70	0.41	[1.06; 2.72]	**0.03**
SLI	1.07	0.32	[0.60; 1.93]	0.82	0.77	0.27	[0.39; 1.53]	0.46
Formal PA	1.17	0.18	[0.87; 1.58]	0.30	2.17	0.80	[1.05; 4.48]	**0.04**
Informal PA	1.12	0.17	[0.83; 1.52]	0.45	0.90	0.36	[0.41; 1.99]	0.80

Data are presented as Odds ratios (OR) with standard error of means (SE) and 95% Confidence Intervals (CI). BMI - Body Mass Index, TL - Total Length, BW – Bone width, MW - Medullary Width, CT - Cortical Thickness, MCI - Metacarpal Index, BMD - Bone Mineral Density, SM - Section Modulus, SSI - Stress-Strain Index, SLI - Slenderness Index, PA - Physical Activity. Significant *p*-values < 0.05 are in bold.

Associations between bone measures and history of fractures at age 17 years

Bivariate logistic regression analyses showed that in white males, a 1 SD greater bone width was associated with a 96% increase in fracture risk (OR: 1.96; 95% CI: 1.03, 3.72) (Table 5.4). When adjusted for height and physical activity (Figure 5.2), the odds ratio increased to 2.77 (95% CI: 1.09, 7.09). In the same group, the odds for fracture increased 2.2-fold with a 1 SD greater medullary width (OR: 2.22; 95% CI: 1.05, 4.69) (Table 5.4); however, the association was no longer significant when adjusted for height and physical activity (Figure 5.2). In the other 3 groups, none of the bone measures were associated with fracture risk.

Associations between bone indices and history of fractures at age 17 years

Bivariate logistic regression analyses showed that in white females a 1 SD greater SLI was associated with a twofold increase in odds for fracture (OR: 2.00; 95% CI: 1.11, 3.61) (Table 5.4). After adjusting for physical activity, the odds ratio did not change (OR: 2.08; 95% CI: 1.08, 3.98) (Figure 5.2). In contrast, greater SLI in black females was associated with a reduced risk of fracture (OR: 0.51; 95% CI: 0.26, 1.00) (Table 5.4); however, this was no longer significant when adjusted for physical activity (Figure 5.2).

In white males, a 1 SD greater MCI was associated with a 55% decreased risk of fracture (OR: 0.45; CI: 0.20, 0.99) (Table 5.4) which was no longer significant after adjusting for height and physical activity (Figure 5.2). The bone indices BMD, SM and SSIp were associated with fracture risk in white males before and after adjusting for height and physical activity (Tables 5.4 and Figure 5.2). When adjusted for height and physical activity, a 1 SD greater BMD increased the risk of fracture threefold (OR: 3.62; 95% CI: 1.22, 10.75), and a 1 SD greater SM and SSIp increased the odds of fracture by 129% (OR: 2.29; 95% CI: 1.07, 4.89) and 123% (OR: 2.23; 95% CI: 1.11, 4.47), respectively (Figure 5.2).

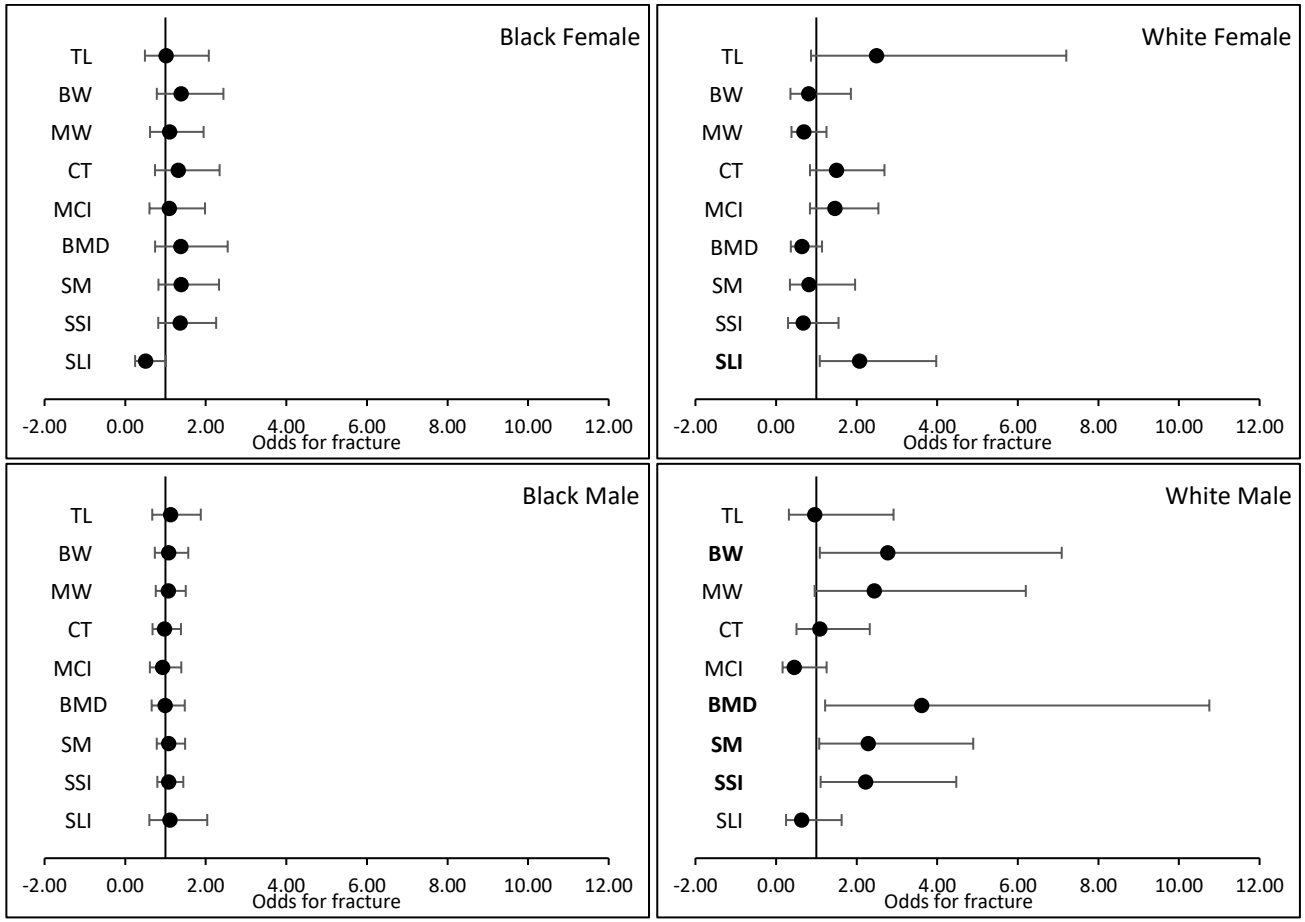


Figure 5.2 Odds ratios for having a history of fracture based on bone measures and indices at 17 years of age, after covarying for height and formal physical activity.

Data are presented as odds ratios with 95% Confidence Intervals (CI). TL - Total Length, BW - Bone Width, MW - Medullary Width, MCI - Metacarpal Index, CT - Cortical Thickness, BMD - Bone Mineral Density, SM - Section Modulus, SSI - Stress-Strain Index, SLI -Slenderness Index. SLI covaried for formal physical activity only. Significant *p*-values < 0.05 in bold

Longitudinal Data

The bone indices BMD, SM and SSIp were moderately correlated with height at each year from age 10 until 16 years (r = 0.32 - 0.55) (Table 5.5). There was no consistent relationship between MCI and height across the age range (Table 5.5).

Table 5.5 Correlation of metacarpal indices with height from ages 10 until 16 years.

	Age group (years)						
	10	11	12	13	14	15	16
MCI	0.07	0.11*	0.17*	0.07	0.01	-0.08	-0.12*
BMD	0.37*	0.41*	0.47*	0.41*	0.36*	0.36*	0.32*
SM	0.42*	0.45*	0.49*	0.48*	0.48*	0.55*	0.52*
SSI	0.42*	0.45*	0.48*	0.48*	0.47*	0.54*	0.51*

Data are presented as Pearson's correlation coefficients (r values). MCI - Metacarpal Index, BMD - Bone Mineral Density, SM - Section Modulus, SSI - Stress-Strain Index. * denotes significance at $p < 0.05$

When the association between bone indices prior to aPLV and adolescent fracture history were assessed, logistic regression showed that for the combined groups of participants, none of the bone indices measured at age 10 years significantly predicted fracture history at age 17 years (data not shown).

Longitudinal analyses of the bone measures and indices from ages 10 until 16 years and the odds of a positive fracture history by 17 years of age, before and after adjusting for height, using GEE for the combined groups are presented in Table 5.6. Increases in cortical thickness, MCI and BMD were associated with decreases in fracture risk when unadjusted, but after adjusting for height, the relationships were reversed. When unadjusted, a 1 mm increase in cortical thickness between 10 and 16 years was associated with a 1% lower risk of fracture history at age 17 years (p = 0.03). A unit increase in MCI between ages 10 and 16 years resulted in a 9% decrease risk of fracture risk by age 17 years (p = 0.02), whilst a unit increase in BMD was associated with a 1% decreased risk of fracture by 17 years of age (p = 0.03) using unadjusted data. After adjusting for height, there was a slight increase in fracture risk by age 17 years for a unit increase in CT (p = 0.01), MCI (p = 0.02) and BMD (p = 0.01) between ages 10 and 16 years.

Table 5.6 GEE assessment of metacarpal bone measures and indices for the combined sex and ethnic groups from ages 10 until 16 years and odds ratios for a positive fracture history at age 17 years.

Variable	Odds Ratio	SE	95% CI	*p*
BW	1.004	4.75E-03	[0.99; 1.01]	0.44
Ht	1.00003	1.80E-04	[0.9997; 1.0004]	0.85
BWxHt	1.000002	2.11E-05	[0.99996; 1.00004]	0.92
CT	0.99	3.93E-03	[0.98; 0.9989]	**0.03**
Ht	1.00001	9.07E-05	[0.9998; 1.0002]	0.92
CTxHt	1.00006	2.29E-05	[1.00002; 1.0001]	**0.01**
MCI	0.91	3.81E-02	[0.84; 0.99]	**0.02**
Ht	0.99997	1.32E-04	[0.9997; 1.0002]	0.83
MCIxHt	1.0006	2.51E-04	[1.0001; 1.001]	**0.02**
BMD	0.99	3.64E-03	[0.99; 0.999]	**0.03**
Ht	0.99998	9.50E-05	[0.9998; 1.0002]	0.84
BMDxHt	1.00005	2.12E-05	[1.00; 1.0001]	**0.01**
SM	1.00006	1.68E-04	[0.9997; 1.0004]	0.71
Ht	1.0001	8.64E-05	[0.9999; 1.0003]	0.27
SMxHt	1.00	7.99E-07	[0.999999; 1.000002]	0.92
SSI	1.00	2.06E-05	[0.99996; 1.00004]	0.98
Ht	1.0001	8.29E-05	[0.99996; 1.0003]	0.13
SSIxHt	1.00	1.02E-07	[0.9999998; 1.00]	0.73

Data are presented as odds ratios with standard error of means (SE) and 95% Confidence Intervals (CI). BW - Bone Width, Ht - Height, CT - Cortical Thickness, MCI - Metacarpal Index, BMD - Bone Mineral Density, SM - Section Modulus, SSI - Stress-Strain Index. Significant *p*-values < 0.05 are in bold.

5.3 Discussion

This study of SA children and adolescents from a longitudinal cohort comprising two ethnic groups assessed metacarpal bone measures and indices and their association with fracture risk during childhood and adolescence. There were ethnic differences in fracture prevalence in the first 17 years of life, with substantially higher incidences in both white females and males relative to their black peers, consistent with results obtained at 10 and 15 years in the Bt20 cohort [42]. Similarly, ethnic differences in fracture incidence have previously been reported in SA adults in whom black individuals had a lower incidence of femoral neck fractures [232] even though their metacarpal BMD was lower than that of their white peers [343]. Whilst these studies taken together suggest that genetics may play a role in protecting against fracture, extrinsic factors which affect the attainment of peak bone mass may also contribute to the aetiology of skeletal fragility in late adulthood [29].

Fracture prevalence has been shown to be a function of bone geometry [344], thus geometric indices obtained by radiogrammetry such as MCI and BMD, both of which reflect bone size and cortical thickness, have been the most widely used metacarpal indices to determine fracture risk [294, 299, 345]. Studies on both adults [292] and children [299] have shown a low MCI to be associated with a greater fracture risk. Even though I have previously shown that black children have thinner cortices relative to bone width [340], and hence a lower MCI than white children, I have also shown that they fractured less. In keeping with the above findings, the white males at 17 years of age who had a history of fracture had greater bone widths and wider medullary cavities, reflective of a lower MCI, than their non-fracturing peers. They also had a 55% decrease in fracture risk associated with a 1 SD gain in MCI, but this was negated when height and physical activity were considered. The group of white males with a history of fractures participated in more formal physical activities, thus any biomechanical advantage provided by the geometrical organisation of their bones may have been overridden by greater force at the time of injury. The finding that white males who fractured were taller, played more formal sport and had greater DXA-derived bone mass has been previously reported from this cohort [42]. I have also shown that white males who had fractured had a higher BMD than their non-fracturing peers. In contrast, in Europe, a lower BMD was reported in adolescent males who had fractured compared to those who had not [301]. My results indicate that a higher BMD does not entirely compensate for higher risk-taking activities.

In the present study, although there were no sex differences in fracture prevalence (possibly because of the small numbers), black males in both the fracture and non-fracture groups had a lower MCI than their female counterparts, whereas white males and females who had not fractured had a similar MCI. In contrast, 17- to 18-year old females in Europe with no history of fracture had a higher MCI than their male peers [301]. Collectively, in addition to sex differences, these results also reflect population differences in bone geometry. In both the fractured and non-fractured participants, I also observed sex differences in radiogrammetry-derived BMD, which reflects the greater bone widths and cortices in males compared to females. These sex differences are consistent with the greater DXA-derived BMD at 15 years in males compared to females in this cohort [42]. A shortcoming of both radiogrammetry- and DXA-derived BMD is that they represent bone size rather than volumetric BMD which may be obtained by more advanced high-resolution computed tomography. The latter technique may also give insight into factors such as cortical porosity that may contribute to fracture.

Long bone fracture is primarily the result of bending or torsional stresses [346]. When SM and SSIp which are surrogate strength indices based on moments of inertia were assessed, these indices were associated with fracture only in white males. A greater SM and SSIp increased fracture risk significantly, even after adjusting for height and physical activity. The white males who fractured had greater bone width and medullary width, and hence reduced cortical thickness, resulting in a higher SM and SSIp compared to their non-fractured peers. This outward displacement of the cortex from the neutral axis of the bone should have conferred a greater resistance to bending and torsional forces, and, a reduced propensity to fracture [328]. This incongruity may be attributed to the overriding influence of higher levels of physical activity participation.

My study and others have shown that metacarpal indices are influenced by height [265] which is a major determinant of bone size and mass [347]. In this cohort, at 17 years of age there were no differences in height or metacarpal length between participants who had fractured and those who had not. This finding differs from results obtained at 15 years of age where white males of this cohort who fractured were taller than their non-fracturing peers [42], and may be related to differences in age of pubertal development and peak height velocity. An additional influencing factor may have been related to my sample size which was 23% smaller than that at 15 years. Taller individuals are at a greater risk for fracture despite their longer

and wider bones with relatively thinner cortices [348]. In a twin study, the taller twins had increased fracture risk due to increased porosity of the cortex, despite their bone geometry offering greater resistance to torsional and bending forces [348].

Since height influences metacarpal indices, I assessed the SLI, which is also a proxy for bone strength, for its association with a history of fracture. The SLI, is an index based on moments of inertia which factors in body size, that is, the product of weight and bone length [18]. I showed that the SLI was associated with a history of fracture in white females only. Whilst the white females with a history of fracture had a greater SLI and hence more slender bones than their non-fractured peers, I also showed that after adjusting for physical activity, a 1 SD increase in SLI doubled the risk of fracture. Since body weight is used in the calculation of the SLI, the greater weight in white females who fractured may account for the association between the SLI and fracture in this group only. At both 15 and 17 years, white females in this cohort who fractured had greater fat mass [42] which supports the findings of Goulding and co-workers in New Zealand [349].

Bone mass measures in 8-year old children obtained by DXA have been reported to predict upper limb fracture during puberty [350]. In this study, metacarpal indices of the combined sex and ethnic groups evaluated at 10 years, which is prior to the age of peak metacarpal length velocity in these participants [340], were of no value in predicting adolescent fracture. This suggests that external risk factors may play a greater role than measures of bone mass in the association with fracture. Bone mass measures have been shown to track from childhood through adolescence [351]. In the longitudinal analyses I have shown that an increase in bone width and/or cortical thickness, as reflected by the MCI, is associated with a 9% reduction of fracture history risk at age 17 years in SA children. However, it is unclear for how many years from the time of measurement that the index has predictive value and prospective studies would be needed to elucidate this. Radiogrammetry-derived BMD, when assessed longitudinally, was only weakly associated with decreased adolescent fracture. I acknowledge a shortcoming of this study in that the timing of the fractures was not documented as they occurred; this data may have provided better correlation with the indices longitudinally.

A strength of this study is that it presents longitudinal data on radiogrammetry-derived metacarpal indices and their associations with fracture history in SA children of two ethnic groups. To the best of my knowledge there are no other such studies. The study was limited

by the overall small number of white individuals and, relatively few black participants with a history of fracture, which may have resulted in the loss of statistical power. In this study, I considered it essential to stratify the participants by sex and ethnicity due to known differences in their bone geometry. Another limitation is that radiogrammetry only provides quantitative rather than qualitative information. The metacarpal measurements were at the mid-diaphyseal region where the composition of bone differs from metaphyseal sites which have a higher trabecular content and are the most prevalent sites of fracture [352]. Fractures were not verified radiographically, though there was consistency with fracture reports obtained at ages 10, 13 and 15 years [42]. Self-reported fracture history has been shown to be relatively accurate, particularly for upper limb and hip fractures [353]. In this study, the cause, site and grade of fracture were not assessed separately due to the small participant numbers, nor were data adjusted for potential modifiers of skeletal health. Previous studies on this cohort have shown that despite ethnic disparities in nutrition, 25-hydroxy vitamin D levels and socio-economic status, black children had greater bone mass than their white peers at most sites [35, 118, 172]. I also did not adjust for lean mass or the possible influence of bone's microstructural properties. I acknowledge that self-reported physical activity may be prone to recall bias; however, it is the most practical method to assess physical activity in a large longitudinal cohort.

In conclusion, metacarpal indices obtained at age 10 years, that is prior to the age of peak bone length velocity, did not predict fracture risk in SA adolescents. When the combined sex and ethnic groups were assessed longitudinally throughout adolescence, greater cortical thickness, MCI and BMD were associated with a small decrease in fracture risk by age 17 years, but the opposite was seen when differences in height were adjusted for. At 17 years of age, the SLI was associated with fracture risk in white females, whereas greater bone width, BMD, SM and SSIp were associated with fracture risk in white males. There are ethnic and sex differences in the association of bone geometry and indices of moments of inertia with fracture in SA adolescents. This study was unable to show that metacarpal radiogrammetry was reliably able to predict fracture in SA children, but this may possibly be due to sample size limitations.

5.4 Summary of major findings

There were ethnic differences in the association between metacarpal indices and fracture risk. Metacarpal indices were associated with fracture risk only in adolescent white males and females, but not in their black peers, and are therefore not a reliable means of assessing fracture risk. The metacarpal indices of pre-pubertal children were not associated with a history of fracture at age 17 years. The results obtained using radiogrammetry are consistent with previously described associations between DXA-derived bone measures and fracture risk in SA children.

CHAPTER 6

Summary and Conclusion

This chapter is a summary of the key objectives, hypotheses and findings of the studies included in this thesis, and the strengths and limitations of these studies. The contextual relevance of the salient findings and how they pertain to future research are outlined. The chapter concludes with a discussion on the contribution of this thesis to our understanding of bone health in SA children.

6.2 Summary of thesis objectives, hypotheses and key findings

Table 6.1 Summary of the objectives, hypotheses and key findings

Objectives	Hypotheses	Summary of key findings
To assess metacarpal bone growth longitudinally in black and white South Africans from childhood through adolescence	Puberty has a sex-specific effect on the periosteal and endocortical bone surfaces. Black children have wider bones with thinner cortices than their white peers.	There were sex and ethnic differences in the pattern of metacarpal bone acquisition from childhood through adolescence. Males had wider bones than females due to greater periosteal bone deposition. Black adolescents had wider bones with relatively thinner cortices compared to their white peers. Medullary width narrowing commenced at similar ages in all groups, except in black males in whom it continued to expand for an additional two years.
To compare the growth and strength patterns of the metacarpal bone (measured using radiogrammetry) to those of the non-weight bearing radius and the weight-bearing tibia (measured using pQCT), in black and white South African children and adolescents	Metacarpal bone measures and strength correlate more closely with MCSA at the radius than at the tibia. Irrespective of sex or ethnicity, the pattern of growth of the non-weight bearing metacarpal is similar to that of the radius, but different from that of the weight-bearing tibia.	Metacarpal measures correlated more closely with radial than tibial MCSA. The variance in metacarpal bone measures and SSIp explained by radial MCSA was 33 - 45% in males, compared to 12 - 20% in females. In each sex and ethnic group, the metacarpal and radius had similar growth patterns for bone width, cortical thickness and medullary width.

Table 6.1 continued overleaf

Table 6.1 continued

Objectives	Hypotheses	Summary of key findings
		Tibial bone width and cortical thickness accrual rates were greater than at the metacarpal. Tibial medullary width increased significantly relative to metacarpal medullary width in males only. Bone strength increased significantly in each bone, except for tibial strength in white females.
To determine the association between metacarpal indices and fracture risk in a cohort of SA children and adolescents	Metacarpal bone dimensions and indices predict fracture risk.	Metacarpal indices were associated with fracture risk in white male and female adolescents, but not in their black peers. Metacarpal indices in pre-pubertal children were not associated with a history of fracture at age 17 years. The radiogrammetry-derived results are consistent with previously described associations between DXA-derived bone measures and fracture risk in SA children.

6.3 Emerging Themes

The two main themes that emanated from this thesis were the application of metacarpal radiogrammetry as a method to measure bone changes over time, and the ethnic and sex differences in bone growth that were revealed using this method.

6.3.1 Methodological theme

This thesis is based on the use of radiogrammetry of the second metacarpal bone, which is the largest of the metacarpal bones. The second metacarpal bone has a relatively uniform cylindrical shape that can be easily and reliably measured. Using radiogrammetry I have shown longitudinal changes in bone dimensions including its length and width, and the spatial organisation of its cortices within the bone diameter, in children and adolescents.

In this study, radiogrammetry was performed manually using digital callipers and its precision was comparable to that of other studies [262]. There is great variability in the precision of radiogrammetry between studies depending on the bone measurement or index used [237, 238]. Most studies have reported precision for the combined cortical thickness or indices such as the metacarpal index (MCI) or BMD, rather than for the individual bone measurements. In this study, bone length and bone width were the most reproducible measurements and the greatest variation occurred in the medullary width measurement due to the poorly delineated cortico-medullary junction. Although the coefficient of variation for medullary width in this study was relatively high, the overall patterns of metacarpal bone width, cortical thickness and medullary width growth observed were consistent with the respective radial growth patterns measured using pQCT in the same cohort [50]. Thus, any inaccuracies in the interpretation of metacarpal medullary width change in this study are most likely minor. In contrast, when using automated digital x-ray radiogrammetry (DXR), greatest variability has been reported in the measurements of the outer cortical border [269]. When using a digitised method, variation may also occur between different radiographic equipment, so there should be consistency in the equipment used in longitudinal studies [261].

Radiogrammetry-derived measures have been shown to be comparable to those obtained using DXA [262]. In this study I assessed the association between metacarpal indices and fracture risk, and showed that the radiogrammetry-derived results were consistent with

previously described associations between DXA-derived bone measures and fracture risk in SA children [42]. Similarly, Ma and Jones [299] showed that the risk for wrist and forearm fracture (per sd reduction) in children was similar for DXA and metacarpal measures. A high correlation has previously been shown between measures of BMD obtained from DXR of the forearm and hand with that of DXA of the distal forearm [262]. In addition, precision of BMD measures obtained using DXR is also lower than that of DXA, showing the reliability of radiogrammetry [262]. I have also shown in this thesis that radiogrammetry-derived bone measurements of the metacarpal bone are strongly associated with those derived using pQCT of the radius. The metacarpal bone and the radius are both non-weight bearing bones and anatomically are in close proximity to each other. This strong association validates the comparison of measures obtained using radiogrammetry with those from an advanced densitometric technique such as pQCT at similar sites.

Using radiogrammetry, I have provided reference data for metacarpal bone measures and indices for black and white South African children from age 9 to 21 years. These data are unique as they have been obtained longitudinally. Currently there are no such data available for South African children. Reference data that are available for use in forensic analyses have been obtained from anthropometric measurements of skeletal remains of SA adults [354].

Radiogrammetry yields information about the relationship of the cortices to the bone diameter, and from these measurements various indices are derived that may be used as proxies for bone strength. In the study investigating the association between metacarpal indices and fracture history, I found that section modulus (SM), stress-strain index (SSIp) and slenderness index (SLI) which are surrogate strength indices based on moments of inertia, had a strong association with fracture, whilst geometric bone measures such as bone width and cortical thickness had very limited associations. To my knowledge these indices have not previously been investigated for their association with fracture history in children. No single index consistently predicted fracture across both sex and ethnic groups in my study. Greater SM and SSIp were associated with an increased fracture risk in white males, whilst a greater SLI was associated with an increased fracture risk in white females. This was surprising as the outward displacement of the cortex from the neutral axis of the bone should have conferred a greater resistance to bending and torsional forces, and a reduced propensity to fracture. The inconsistency in the associations between the metacarpal indices and fracture history, in

addition to none of the indices having an association with fracture risk in black children, suggest that radiogrammetry may not be a useful method for assessing fracture risk in SA children. However, the inconsistent results may not have been due to the methodology employed, but may have been influenced by sample size and potential modifiers of bone health such as nutrition and SES that were not considered in this study. The associations between bone measures and fracture risk in this cohort should be further explored using radial bone indices obtained using pQCT.

Clinical applications of radiogrammetry are limited as the technique is insensitive to changes in the degree of mineralisation and may not reflect a high enough magnitude of change to determine the amount of bone that has been lost [355]. However, hand-wrist radiographs are routinely performed to determine bone age from the carpal bones when assessing skeletal growth and maturation disorders in children; thus, metacarpal indices and skeletal maturity indicators may be obtained from a single image. Radiography also requires a lower radiation dosage than other densitometric techniques and a scan time of only a fraction of a second, making it suitable for use in children. Radiogrammetry may therefore be useful to screen for possible bone disorders in the absence of expensive densitometric equipment in low resource settings; however more studies are required to confirm this. Radiogrammetry is a useful and easy method to use in longitudinal bone biology studies and in forensic anthropology and although it is a laborious method when performed manually, this can be overcome by using DXR. With film radiography becoming obsolete, manual measurements may be taken from radiographs that have been obtained digitally, in the absence of expensive software for the automatic calculation of bone measures and indices.

6.3.2 Empirical theme

The key outcomes of the longitudinal assessment of the second metacarpal bone were ethnic and sex differences in skeletal maturity and growth, and in skeletal phenotype.

Skeletal Maturity

In this thesis, metacarpal bone measurements of black and white children were aligned on a biological age (the age of peak metacarpal length velocity), since maturational status varies

between children at the same chronological age, especially during the adolescent period. The pioneering longitudinal studies on bone growth were performed in developed nations, namely the US [86] and Europe [79], on data collected on white individuals born more than 60 years ago, and they did not control for maturational status. In contrast, South Africa is a developing nation, the majority of whose citizens face social and environmental deprivation, which may adversely influence bone health.

This thesis showed that the initiation and duration of the growth spurt in metacarpal length and the attainment of PLV were synchronised in black and white females. In contrast, a previous study of the females from this cohort which assessed skeletal maturity using the TW3 RUS method showed that although the tempo (timing) of the growth spurt in black and white females was synchronised, the velocity of the growth spurt was 9% faster in black compared to white females [78]. This thesis also showed that black males entered into puberty and attained PLV later than in white males, which is in keeping with the delayed skeletal maturity measured using the TW3 RUS method previously reported in the same cohort [78]. It is not known whether factors such as body composition and SES may have influenced the differences in tempo and velocity of skeletal maturity observed. In addition, it has been suggested that growth in black males may be more sensitive to adverse environmental conditions [78]. The type, timing and extent of exposures which influence skeletal maturity are not known and require further investigation.

Skeletal phenotype

There was an ethnic difference in metacarpal bone length in both sexes with black individuals having a longer second metacarpal bone than their white peers at all ages. This is interesting because black SA children have significantly shorter stature compared to their white peers [44]. This is also in contrast to US black children whose longer metacarpal bones [319] were in keeping with them being taller than their white peers [322]. The purpose of this thesis was to provide a quantitative description of bone growth rather than to assess the factors influencing it, therefore nutrient intake was not considered. However, the intake of calcium, zinc, iron and biotin by the black children in this cohort have been shown to be less than 67% of the RDA [98]. South African studies have also previously reported lower serum calcium

concentrations in black compared to white children [105], which may explain the shorter stature in black individuals, but does not account for them having a longer second metacarpal bone.

This thesis reports distinct ethnic differences in cross-sectional diaphyseal bone geometry, which were present pre-pubertally and retained through adolescence. Black participants had wider bones with relatively thinner cortices compared to their white peers. These ethnic differences are also consistent with findings of greater femoral and tibial bone area in US black compared to white children [43, 48]; however, there are no comparative longitudinal studies in US children. Similarly, distinct ethnic differences in cross-sectional bone geometry have also been shown in a longitudinal pQCT study of the radius and tibia in adolescents from the BHC who had already attained PHV. This thesis adds to the pQCT study by Schoenbuchner and co-workers [50] by showing that ethnic differences in bone geometry were present pre-pubertally. These results are suggestive of a strong genetic role in determining bone's cross-sectional dimensions; however, this needs to be confirmed in future studies.

The wide bones with relatively thinner cortices in black individuals compared to their white peers, confer a biomechanical strength advantage, and this may contribute to the lower fracture rates in black children as previously reported in this cohort [42, 231]. The contribution of cortical mineralisation to the disparate fracture rates could not be investigated due to the limitation of radiogrammetry.

In US individuals, whole-body BMC was highly correlated with height in both sexes and aPHV preceded age of peak BMC velocity by almost a year [77]. In black and white US children, the lag in age at peak velocity of whole-body BMC accrual relative to PHV was 0.6 - 0.9 years in boys and 0.8 - 1.2 years in girls [77]. Whether there is a similar dissociation in the timing of peak BMC and peak linear growth in SA children is yet to be investigated and may also reveal a vulnerable period when there may be greater susceptibility to fracture.

There are ethnic differences in the timing of peak medullary width expansion in SA children in both sexes, which occurred approximately 2 years prior to attaining PLV in black participants and 6 to 12 months after aPLV in white participants. The pattern of bone changes at the endocortical surface of the second metacarpal bone in the white males of this study was consistent with the findings in males from the First Zurich Longitudinal study [79] but differed

from those in US males [318]. In US white males, endocortical expansion continued until 16 years of age [318], whereas in the white males in this thesis, the medullary area ceased to expand at an earlier age (14.5 years) and was followed by contraction. Although endocortical bone growth is influenced by hormones, it is unclear whether genetic factors override hormonal influences, thus accounting for the population differences described.

This thesis advances our understanding of bone growth further as it has shown that:

- there are ethnic differences in the skeletal maturity of appendicular bones in SA children;
- there are ethnic differences in bone's cross-sectional dimensions (bone width, cortical thickness and medullary width) which are evident pre-pubertally and retained through adolescence;
- there are ethnic differences in the biological markers of bone strength.

6.4 Strengths and limitations of the thesis

The longitudinal data, spanning 12 years, presented in this thesis are unique internationally and for the African continent, as the participants are representative of two ethnic/racial groups. Whilst cross-sectional studies conducted in US and SA children have shown ethnic differences in bone geometry [43, 48, 49], longitudinal studies on bone growth in children are sparse. Most longitudinal studies on bone growth have been conducted in white children from developed countries [79, 86, 326, 341]. The majority of the black children were from communities associated with poorer living conditions, and inadequate health access and nutrition, factors which have been shown to influence bone mass. However, the socio-economic status of the white children in this cohort at birth was higher than that of the black children (unpublished data, Lukhanyo Nyati).

To my knowledge, this study is the first to compare the bone growth and strength patterns of three different bones, measured using different technologies at non-weight bearing and weight-bearing appendicular sites, in two ethnic groups over time. In addition, I controlled for

the influence of the associated musculature, and aligned participants on a common biological maturity indicator. The influence of muscle size on bone growth during adolescence has not previously been investigated longitudinally in African children. The similar growth patterns observed between the metacarpal bone and the radius, both non-weight bearing bones which are located in close proximity to each other, validates the comparison of measurements obtained using radiogrammetry with those from pQCT at closely related sites.

This thesis is the first to report on the relationship between metacarpal indices and fracture in African children. Although there are significant differences in fracture incidence between black and white SA children, this thesis has shown that metacarpal indices do not reliably predict fracture risk.

There are some limitations associated with the studies conducted for this thesis. One of the major limitations of my study was the small number of white participants. Although this shortcoming was appreciated at the time of enrolling participants into the Bone Health Cohort, and an attempt was made to address this by further recruiting white participants, the numbers remained small. Despite this, the patterns of bone growth that occurred in white participants in this study are similar to those observed in studies conducted in European children [79]. The small participant numbers may also have contributed to a lack of association being found between metacarpal indices and fracture history, particularly in the group of black participants with fracture.

Another limitation of the thesis was that fracture data were obtained retrospectively through interviews with the care-givers and the children, and questionnaires. Documenting fractures as they occurred may have provided better correlation with the metacarpal indices longitudinally. Furthermore, the occurrence of fractures was not verified radiographically, although fracture reports were consistent with those obtained at two previous time points. Sustaining a fracture is an exact incident and any inaccuracies in reporting most likely relate to the precise timing or exact site of the fracture [293]. The use of a single measure of physical activity at age 15 years (for the preceding 12 months) in the analyses of fracture risk may be considered as a limitation; however, the benefits of physical activity have been shown to track through adolescence [179, 184].

While this study did not include potential modifiers of skeletal health such as nutrition, vitamin D status, socio-economic status, and physical activity, their influences in relation to children of the BHC have previously been reported on and this has been incorporated into my discussion. The analyses also did not include anthropometric measures and limb length. In addition, it was assumed that aPLV was synchronised with radial and tibial PLV. Functional measures such as muscle strength assessments were not performed, thus the functional muscle-bone relationship could not be explored. An additional limitation of this study was that when calculating the bone indices derived from pQCT scans of the radius and tibia, a cylindrical shape was assumed and this may have influenced the outcome.

Although the use of radiogrammetry in this study was limiting by its provision of quantitative rather than qualitative information, it has provided valuable information on bone growth patterns in SA children. The use of radiogrammetry enabled changes in bone at the periosteal and endocortical surfaces to be mapped longitudinally. In addition, metacarpal indices that were derived allowed for the underlying structural determinants of bone strength to be evaluated, and for these indices to be assessed for their ability to explain the different fracture rates between black and white SA children.

6.5 Implications of the study findings

This thesis has provided insight into appendicular bone growth patterns in black and white SA children which is relevant to a multitude of disciplines including auxology, anthropology, clinical medicine and dentistry, genetics, forensic science, nutrition and epidemiology.

The distinct sex and ethnic differences in bone geometry may differentially influence involutional changes in bone, bone strength in pathological conditions and fracture pathogenesis. In addition, the ethnic differences in bone geometry may influence bone strength during adolescence and in late adulthood, both times when peak fracture occurs. The low fracture rates in black compared to white SA children is in keeping with the lower fracture rates reported globally in individuals of African descent, suggestive of an underlying genetic protection in black individuals which is yet to be unravelled. The ideal time to identify genes associated with bone health is during childhood, as this is when peak genetic regulation of

bone occurs with the effects of environmental influences having been present for a relatively limited time [356].

Since linear growth is determined by genetic potential and nutrition [357], the peri-pubertal years are considered an optimal time to intervene to improve bone mass accrual. It is unclear whether the delayed puberty in black males, attributed to environmental constraints, affords them an opportunity to catch-up in linear growth. Similar delays in puberty have been reported in rural boys in the Gambia [108]. In an attempt to improve bone mass, calcium supplementation of pre-pubertal rural Gambian boys resulted in shorter adolescent height due to aPHV occurring at an earlier age and premature epiphyseal growth plate closure [108]. Thus, public health intervention to compensate for nutritional deficiencies should be approached with caution.

The metacarpal bones are considered suitable for sex and stature determination in forensic science [354, 358, 359]. Anthropometric and radiographic studies on sex discrimination using metacarpal length have produced conflicting results [358, 360] and it has been suggested that midshaft dimensions may provide a more reliable indicator of sex [361]. A recent anthropometric study of contemporary SA adults was not conclusive in its ability to correlate metacarpal bone length to other appendicular bones to reliably predict stature [354]. In addition, discriminant function formulae were derived for sex determination; however data for black and white individuals were pooled [354]. The distinct ethnic geometries that this thesis reports suggests that reference data should be obtained separately for black and white SA individuals, and that radiogrammetry-derived measures of the metacarpal bones may contribute to revealing not only sex but also ethnicity in forensic science.

In dentistry, the prognosis of orthodontic and orthognathic treatment is influenced by an individual's pubertal and maturational status [362, 363]. Functional orthodontic treatment is ideally undertaken during the pubertal growth spurt whilst orthognathic surgery is performed only once active growth has ceased [364]. Strong correlations have been reported between dental and skeletal maturation [365, 366]. Thus, ethnic differences in skeletal maturity and linear bone growth may translate into differences in the timing and velocity of growth of the craniofacial complex.

Although metacarpal indices have limited clinical applications, they may alert a practitioner to underlying bone disease. For example, in Marfan's syndrome, appendicular bones are disproportionately long compared to their width and this is easily identified on a hand-wrist radiograph [367]. An implication of the ethnic differences in bone geometry is whether black and white individuals have different compensatory mechanisms for bone strength in disease.

The ideal nutritional intake to attain one's genetic potential with respect to peak bone mass needs to be established. Public health policies should emphasise the importance of a balanced diet together with adequate and appropriate physical exercise to optimise bone health to reduce fracture risk. In addition, bone mass screening in childhood and diet and lifestyle assessments could assist with identifying those at risk for fracture. Bone growth and strength are products of complex interactions between genetic and environmental factors, including diet, hormonal influences, and mechanical stimulation. These factors need to be considered collectively to minimise future fracture risk.

6.6 Future Research

Whilst conducting this research, several questions have arisen with respect to optimising bone health in SA children to reduce future fracture risk. Having obtained and studied such valuable information on children and adolescents from the BHC, I would propose prospective studies on these individuals using the more advanced high-resolution pQCT technique to assess bone mass, its microstructure and strength. Such studies would allow us to track changes in cortical porosity and bone strength over the years into late adulthood when the next peak in fracture incidence occurs. Prospective studies would also provide insight into the patterns of age-related physiological bone changes such as cortical thinning and trabecular bone loss, which may differ between black and white individuals due to differences in their bone size and geometry.

The basis of this thesis was to understand the fundamentals of bone development longitudinally in SA children of different ethnic backgrounds. However, given the disparate socio-economic conditions in SA, future studies should include factors which influence bone health such as diet, SES, body composition and skeletal loading to determine whether they influence black and white individuals differentially, and how they can be modified to improve

bone health. With greater urbanisation and its associated lifestyle changes and influences on bone health, it would be interesting to see whether fracture incidence in black individuals begins to approximate that of their white peers. Further research should also consider the specific timing of fracture and assess fractures by their site and cause.

Comparative studies of bone growth in SA children with children from other cohorts in developed and developing countries would provide further insight into population-specific differences in bone growth and strength. Since heritability is population specific, more comprehensive studies of the children in the BHC and their underlying genetic traits remain to be investigated. Fundamental to our understanding of bone health is how an individual's genotype is modulated by environmental factors and influences phenotypic expression.

Some of the questions regarding bone health in SA individuals that have emanated from this study are outlined below.

1. How are the ethnic differences in bone geometry influenced by age-related physiological bone changes?

2. How do the longitudinal changes in hormones during puberty and adolescence relate to changes at the periosteal and endocortical surfaces in black and white individuals?

3. What are the influences of SES, nutrition, body composition and skeletal loading on bone growth and fracture risk in black and white children, and how can these factors be modified to improve bone health?

4. What is the relationship between peak BMC gain and peak gain in bone length, width and cortical thickness in black and white children?

5. Which genes are responsible for influencing bone growth and geometry in the different ethnic groups and can an individual’s genome be used to identify at-risk individuals for fracture?

6.7 Conclusion

My thesis is based on children born in 1990 in an urban area (Johannesburg) of South Africa, who were participants in the Bone Health cohort nested within the longitudinal Birth to Twenty cohort. The black children of this cohort show more favourable cross-sectional bone geometry that confers greater bone strength than white children.

The salient findings of my thesis are that there are distinct ethnic and sex differences in bone geometry, measured using radiogrammetry, in black and white SA children from pre-puberty through adolescence. Black children had wider bones with thinner cortices than their white peers. The geometric configuration of the bones of black children and adolescents confers a biomechanical advantage, which may contribute to a lower incidence of fracture compared to their white peers.

In South Africa, there is a disparity in the SES of its urban and rural citizens, and malnutrition and stunting still prevail. Since South Africa's inception as a democracy in 1994, there has been a shift in lifestyle for many of its citizens. Urbanisation and a higher SES are associated with a more sedentary lifestyle, a tendency towards an unfavourable diet, and an increase in the prevalence of overweight and obesity. The effects of these changes on peak bone mass attainment, the long-term consequences of such confounders on bone health, and whether these factors override a possible genetic protection against fracture that black individuals have, requires further investigation.

This thesis contributes to our understanding of bone growth in South African black and white individuals, the results of which may assist in formulating population-specific intervention strategies to promote bone health.

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Appendix A: Plagiarism Declaration

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I _____ (Student number: 8600398H) am a student registered for the degree of *Doctor of Philosophy* in the academic year 2018.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: _____ Date: _____

Appendix B: Plagiarism Report

turnitin Turnitin Originality Report

8600398h:Thesis.docx by Ansuyah Magan

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(ca654600-c0f7-4b28-9e93-c904e5efd056)

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sources:

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"Handbook of Growth and Growth Monitoring in Health and Disease", Springer Nature America, Inc, 2012

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Lisa K. Micklesfield. "Ethnicity and bone: a South African perspective", Journal of Bone and Mineral Metabolism, 04/26/2011

3 1% match (publications)
"2012 Annual Meeting of the American Society for Bone and Mineral Research Minneapolis, MN October 12-15, 2012", Journal of Bone and Mineral Research, 2012

4 1% match (publications)
Micklesfield, L.K.. "Determinants of bone size and strength in 13-year-old South African children: The influence of ethnicity, sex and pubertal maturation", Bone, 20110401

5 < 1% match (publications)
Nutritional Influences on Bone Health, 2016.

6 < 1% match (publications)
Kebashni Thandrayen. "Heterogeneity of fracture pathogenesis in urban South African children: The birth to twenty cohort", Journal of Bone and Mineral Research, 12/2011

7 < 1% match (publications)
Ansuyah Magan, Lukhanyo H Nyati, Lisa K Micklesfield, Shane A Norris, John M Pettifor. "Metacarpal Growth During Adolescence in a Longitudinal South African Cohort", Journal of Bone and Mineral Research, 2017

Appendix C: Ethics Clearance Certificate

R14/49 Dr Ansuyah Magan

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130126

NAME: (Principal Investigator)	Dr Ansuyah Magan
DEPARTMENT:	Developmental Pathways for Health Res. Unit CH Baragwanath Academic Hospital
PROJECT TITLE:	Longitudinal Changes in Metacarpal Indices through Late Childhood and Adolescent in a South African Cohort
DATE CONSIDERED:	25/01/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof John Pettifor
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 25/01/2013

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University

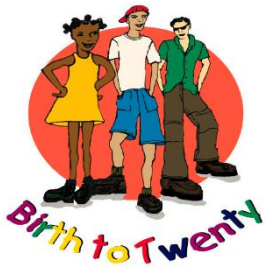
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**

Principal Investigator Signature

Date 08/02/2013

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix D: Year 17 Participant fracture questionnaire



University of the Witwatersrand

Department of Paediatrics and Child Health

Birth to Twenty: Year 17
ADOLESCENT FRACTURE QUESTIONNAIRE

Bt20 ID Number: **Date:**
Bone Health ID Number:

	If the box states **YES** please complete **Section A and C** If the box states **NO** please complete **Section B**

Section A – Verification

You told us you broke / fractured bones in your body _____ times. I would just like to make sure that we have the correct information:

Incident Number	Which side of your body? (Right / Left)	Which bone did you break / fracture? Please MARK the sites of fractures on the skeleton provided at the back of the page and number the fractures in order of occurrence with the year or age of occurrence next to the fracture.	When did this happen? (Year / Age)	How did this happen? Please choose from the options provided in the table on the next page and enter for e.g 2.2 and if not listed in the table then describe what happened
1.				
2.				
3.				
4.				

Section B – Please complete

Have you **ever** broken / fractured a bone in your body?

Yes	No

IF YES, how many **times in total** have you broken / fractured a bone in your body?

If Yes, please tell me about the different times this occurred.

Incident Number	Which side of your body (Right / Left)	Which bone did you break/fracture? Please MARK the sites of fractures on the skeleton provided at the back of the page and number the fractures in order	When did this happen? (Year / Age)	How did this happen? Please choose from the options provided in the table on the next page and enter for e.g. 2.2 and if not listed in the

		of occurrence with the year or age of occurrence next to the fracture.		table then describe what happened
1.				
2.				
3.				
4.				
5.				

Section C – Please complete

Did you break / fracture a bone in your body in the **past 12 or 24 months**?

Yes	No

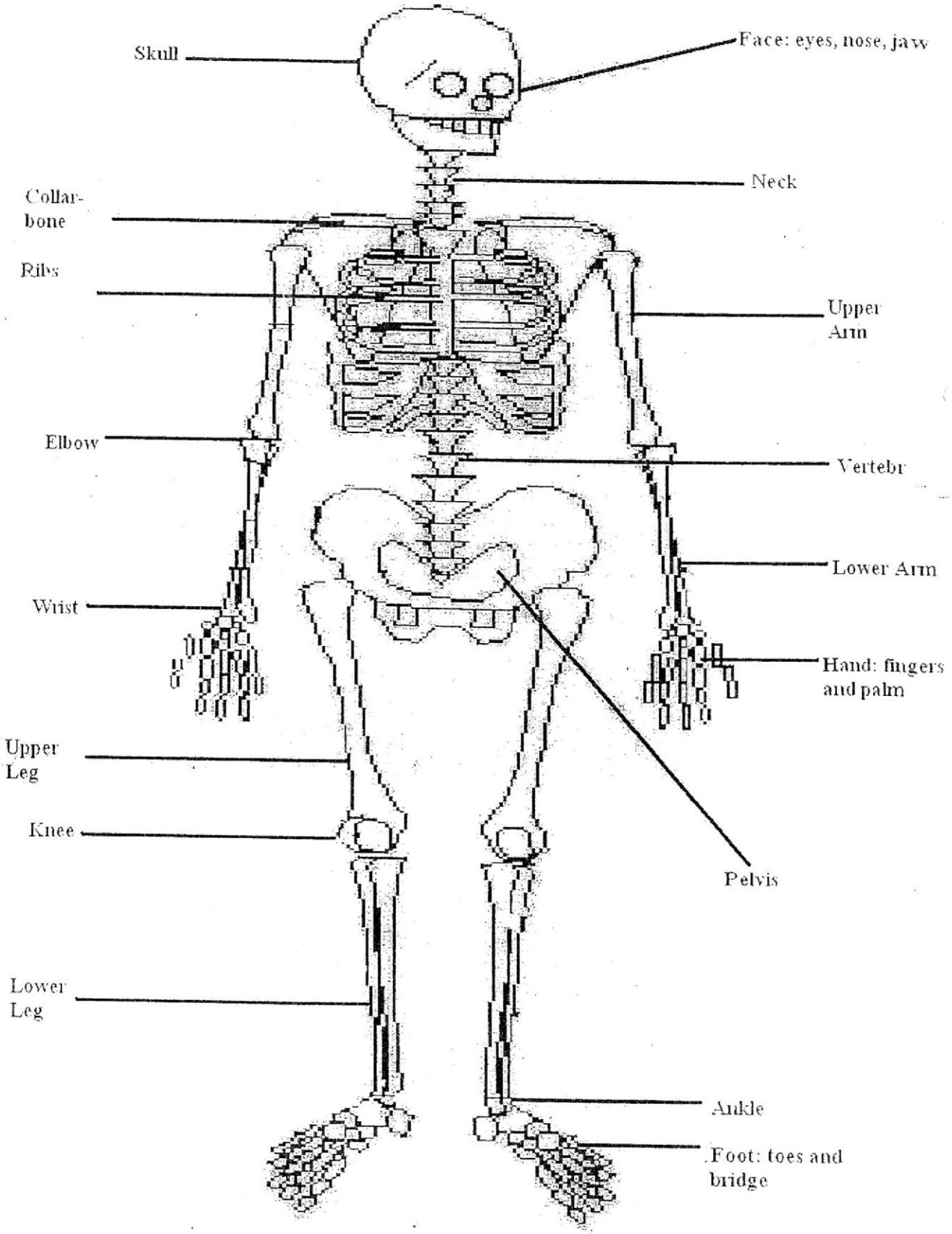
If Yes, please tell me about this event/s.

Incident Number	Which side of your body (Right / Left)	Which bone did you break / fracture? Please MARK the sites of fractures on the skeleton provided at the back of the page and number the fractures in order of occurrence with the year or age of occurrence next to the fracture.	When did this happen? (Year / Age)	How did this happen? Please choose from the options provided in the table on the next page and enter for e.g. 2.2 and if not listed in the table then describe what happened
1.				
2.				
3.				
4.				
5.				

GRADE 1 (Slight trauma)
1.1 Falling to the ground from standing on the same level (e.g. while walking you slipped and fell and had a fracture)
1.2 Falling from less than 0.5 metres (falling from stools, chairs and beds)
GRADE 2 (Moderate trauma)
2.1 Falling from between 0.5 – 3 metres (e.g. a wall or jungle gym or tree)
2.2 Falling down stairs, from a bicycle, roller blades, skateboard or swing
2.3 Playground scuffles (e.g. wrestling or boxing)
2.4 Sport injuries (e.g. soccer, rugby, netball, hockey etc)
2.5 Slamming fingers into a door or knocking against a solid object
GRADE 3 (Severe trauma)
3.1 Falling from a height > 3 metres (falls from windows or roofs)
3.2 Motor vehicle or pedestrian accidents
3.3 Injuries caused by heavy moving or falling objects (e.g. bricks or stones)
4. Do not recall or cannot remember

Quality checked by: **Date:**

Human Skeleton



Appendix E: Year 17 Caregiver fracture questionnaire

University of the Witwatersrand

Department of Paediatrics and Child Health

BIRTH TO TWENTY BARA SITE: 17TH YEAR CAREGIVER FRACTURE QUESTIONNAIRE

BTT ID NUMBER: ☐☐☐☐☐☐☐ **DATE**:

1. How many children do you have besides the child that is enrolled on the Birth to Twenty Study?
_____

2. Of these siblings, have any of them fractured or broken a bone/s?

YES ☐ NO ☐ I**f YES**, how many siblings have fractured? _____

AND please tell me about the gender and the different times this occurred in each sibling

SIBLING 1: Male ☐ Female ☐

Incident Number	Which side of your body (Right / Left)	Which bone did you break / fracture? Please MARK the sites of fractures on the skeleton provided at the back of the page and number the fractures in order of occurrence with the year or age of occurrence next to the fracture.	When did this happen? (Year / Age)	How did this happen? Please choose from the options provided in the table below and enter for e.g. 2.2 and if not listed in the table then describe what happened
1.				
2.				
3.				
4.				
5.				

SIBLING 2: Male ☐ Female ☐

Incident Number	Which side of your body (Right / Left)	Which bone did you break / fracture? Please MARK the sites of fractures on the skeleton provided at the back of the page and number the fractures in order of occurrence with the year or age of occurrence next to the fracture.	When did this happen? (Year / Age)	How did this happen? Please choose from the options provided in the table below and enter for e.g. 2.2 and if not listed in the table then describe what happened
1.				
2.				

3.				
4.				
5.				

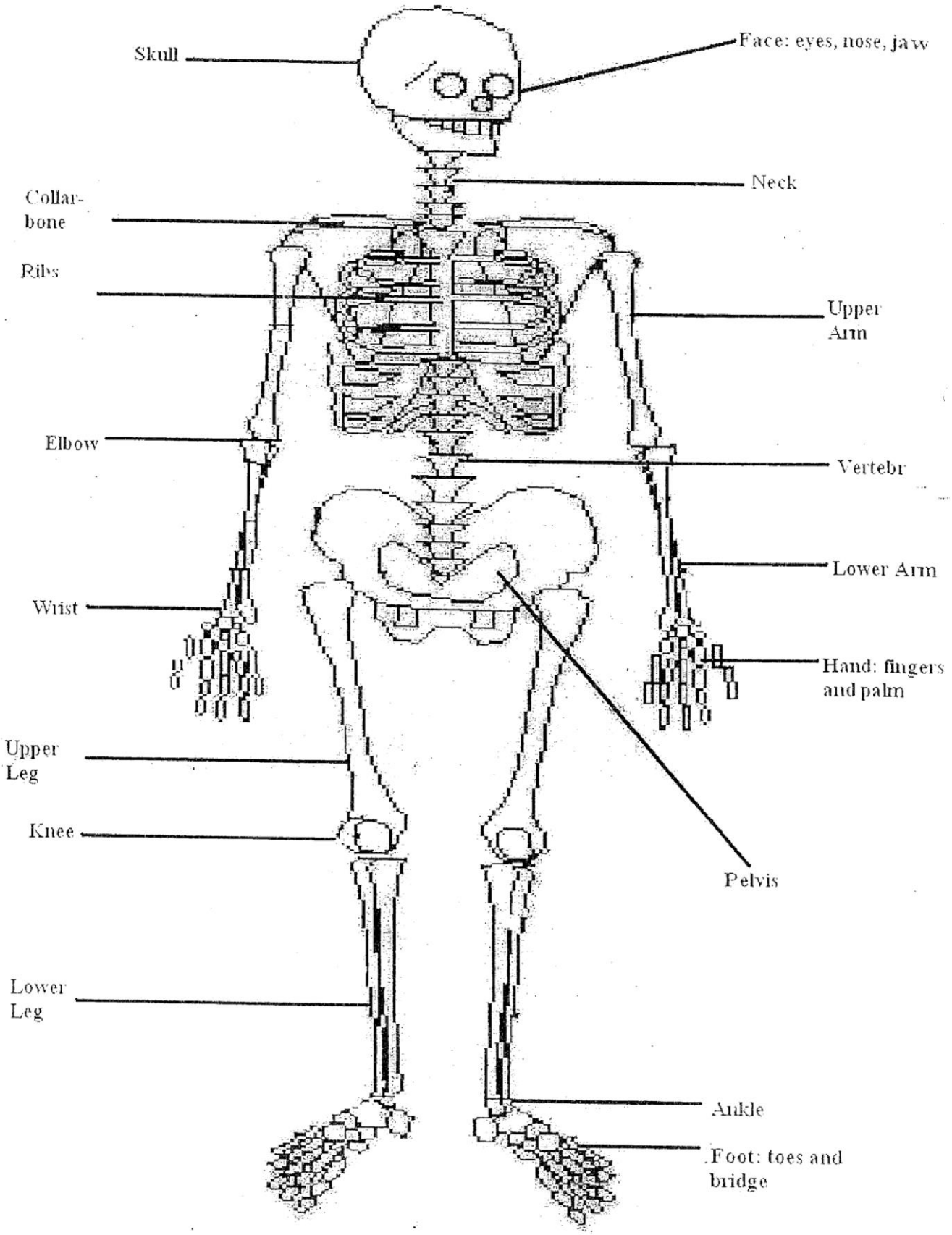
SIBLING 3: Male ☐ Female ☐

Incident Number	Which side of your body (Right / Left)	Which bone did you break / fracture? Please MARK the sites of fractures on the skeleton provided at the back of the page and number the fractures in order of occurrence with the year or age of occurrence next to the fracture.	When did this happen? (Year / Age)	How did this happen? Please choose from the options provided in the table below and enter for e.g. 2.2 and if not listed in the table then describe what happened
1.				
2.				
3.				
4.				
5.				

GRADE 1 (Slight trauma)
1.1 Falling to the ground from standing on the same level (e.g while walking you slipped and fell and had a fracture)
1.2 Falling from less than 0.5m (falling from stools, chairs and beds)
GRADE 2 (Moderate trauma)
2.1 Falling from between 0.5 – 3 m (e.g. a wall or jungle gym or tree)
2.2 Falling down stairs, from a bicycle, roller blades, skateboard or swing
2.3 Playground scuffles (e.g. wrestling or boxing)
2.4 Sport injuries (e.g. soccer, rugby, netball, hockey etc)
2.5 Slamming fingers into a door or knocking against a solid object
GRADE 3 (Severe trauma)
3.1 Falling from a height > 3 m (falls from windows or roofs)
3.2 Motor vehicle or pedestrian accidents
3.3 Injuries caused by heavy moving or falling objects (e.g. bricks or stones)
4. Do not recall or cannot remember

Quality checked by: ☐ **Date:** ☐

Human Skeleton



Appendix F: Year 17 Verification of fracture questionnaire

University of the Witwatersrand

Department of Paediatrics and Child Health

BIRTH TO TWENTY BARA SITE: 17TH YEAR CAREGIVER FRACTURE QUESTIONNAIRE

BTT ID NUMBER: | | | | | | | | **DATE**:

Dear Caregiver or Adolescent:

You have been randomly selected from those adolescents who have previously reported fractures. Please can you assist us with verifying the information that was previously given to us with regard to the number of fractures that your child or you, the adolescent has had since birth. To further verify that the fractures were correctly diagnosed we require information on whether the adolescent was seen by a doctor/hospital and whether x-rays were done and treatment was given.

- Please can you confirm with me, how many times your child or you (the adolescent) has fractured a bone/s: _____
- Please can you confirm the side and site of the most recent fracture and how your child or you (the adolescent) had fractured that bone?

Which side of your body (Right / Left)	Which bone did you break / fracture? .	When did this happen? (Year / Age)	How did this happen?

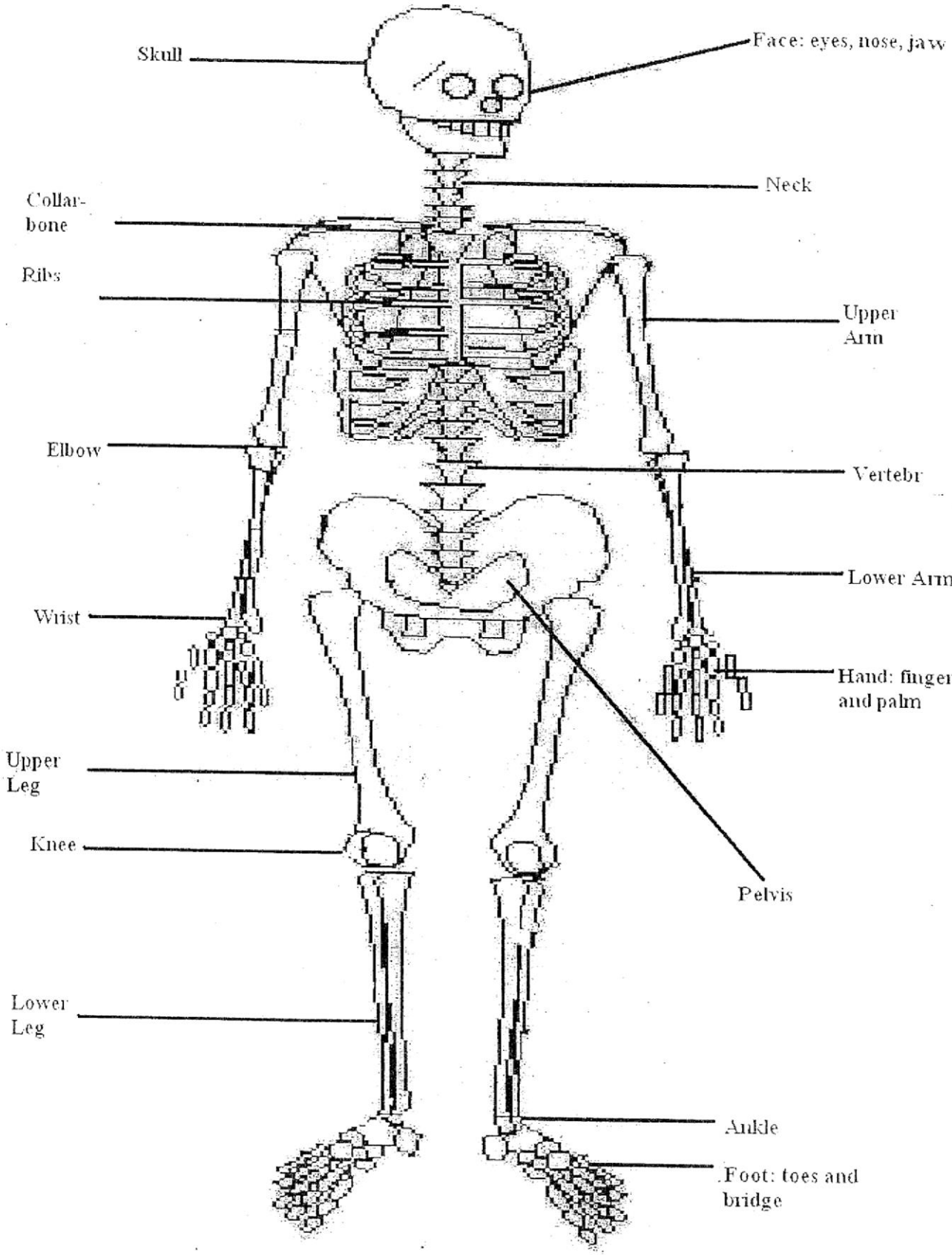
- For each fracture can you complete the following questions:

Fractures	Who diagnosed the fracture (doctor, nurse or self-diagnosed)?	Have you been to a hospital, clinic or private doctor?	Have you had an x-ray done?	Do you have the x-rays?	What was the treatment? (POP, sling, bandage, traction, operation)	Do you have any outpatient files or records of the fractures?
1.						

2.						
3.						
4.						
5.						

Quality checked by: **Date:**

Human Skeleton



Appendix G: Year 15 Physical activity questionnaire

University of the Witwatersrand
Department of Paediatrics and Child Health

BIRTH TO TWENTY MOBILE TEAM: 15TH YEAR ADOLESCENT QUESTIONNAIRE

DATE : Day ☐☐ **Month** ☐☐ **Year** ☐☐☐☐

BTT ID NUMBER : ☐☐☐☐☐☐☐

BONE STUDY ID NUMBER : ☐☐☐

There are 2 sections to this questionnaire and 4 additional components that we are going to work through together; it will take about 30 minutes

The FIRST section of the questionnaire is about EXERCISE
Let us talk about your activities at school

1. Do you attend physical education classes at school?
   (Exercise classes supervised by a teacher during school time)

Yes=1	No=0	

2. How often are classes held & how long are the classes?

Times / week	Hours / time

What are the three most frequent activities that you do during these classes?

Activities

3. Do your school teachers encourage you to participate in **physical activity**? **Y** **N**
4. Do your parents encourage you to participate in **physical activity**? **Y** **N**
5. Who (parent/caregiver or other) encourages you the most to participate in **physical activities? (This question MUST be answered**

Do you engage in any physical activity during **school breaks** or **outside school**, for example riding a bike, playing in the street or yard? **NOT** activity as part of a sports team or club. Tick the three most frequent activities that you do, and time spent on each activity.

Activity	Mon	Tue	Wed	Thu	Fri	Sat	Sun
Riding a bike							
Playing with a ball							
Skipping							
Hop scotch							
Dibeke (ball game)							
Bhati (tin game)							
Mgusha (panty hose game)							
Skateboarding							
Roller-skating							
Other (specify)							

Sedentary activities

Do you engage in any of the following activities before or after school, and if so, for how many hours?

Activity	Mon	Tue	Wed	Thu	Fri	Sat	Sun
Watching TV & videos & movies							
Reading, drawing, homework							
Playing a musical instrument - please detail what musical instrument?							
Playing video/ TV/ computer games							
Internet surfing							
Listening to radio/ music							

What time do you go to bed on a school night?

What time do you go to bed on a non-school night (on a weekend or on holiday)?

What time do you wake up on a school morning?

What time do you wake up on a non-school morning (on a weekend or on holiday)?

Transport

How do you get to school and how long does it take to get there and back?

1. By car, bus, taxi, train etc.

Yes=1	No=2			
There:_____minutes				
Back:_____minutes				

2. Walking

Yes=1	No=2			
There:_____minutes				
Back:_____minutes				

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

At a pace, that makes me breathe much harder than normal	1	
At a pace that makes me breathe somewhat harder than normal	2	
At a pace where there is no change in my breathing	3	

3. Bicycle

Yes=1	No=2			
There:_____minutes				
Back:_____minutes				

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

At a pace, that makes me breathe much harder than normal	1	
At a pace that makes me breathe somewhat harder than normal	2	
At a pace where there is no change in my breathing	3	

EXTRA MURAL ACTIVITIES AT SCHOOL (LAST 12 MONTHS)

	How many months?	Prac/Wk	Hrs/Prac	Comp/Wk
Athletics (running)				
Athletics (other)				
Cricket				
Swimming				
Tennis				
Hockey				
Netball				
Rugby				
Soccer				
Badminton				
Basketball				
Ballet				
Cycling				
Dancing				
Gymnastics				
Judo / karate				
Squash				
Volleyball				
Other				
Musical instrument				

PRIVATE EXTRA MURAL ACTIVITIES (LAST 12 MONTHS)

	How many months?	Prac/Wk	Hrs/Prac	Comp/Wk
Athletics (running)				
Athletics (other)				
Cricket				
Swimming				
Tennis				
Hockey				
Netball				
Rugby				
Soccer				
Badminton				
Basketball				
Ballet				
Cycling				
Dancing				
Gymnastics				
Judo / karate				
Squash				
Volleyball				
Other				
Musical instrument				

The SECOND section of this questionnaire is about your HOUSEHOLD CHORES and CASUAL WORK

What jobs do you do or instructed to do about the house to help with housework?

	YES or NO	How much time do you spend on this chore on average?	How many days a week do you normally do this chore?
Cooking (e.g. cooking food)			
Indoor cleaning (e.g. cleaning the house, vacuuming, dusting)			
Outdoor cleaning (e.g. gardening)			
Taking out the rubbish			
Washing clothes			
Washing dishes			
Looking after siblings (e.g. walking to or from school)			
Looking after pets (e.g. feeding, bathing)			
Looking after adult who is old, sick (washing, feeding)			
Other (provide details)			

1. Not counting jobs around the house, do you sometimes work for **pay** (money) such as deliveries, gardening, cleaning, and looking after children?

Y or N	

2. If **YES**, please complete the table

What do you do?	Where do you work?	What are your duties

3. When you do these kinds of jobs, how often do you usually work?
    a. About once a month
    b. A few times a month
    c. About once a week
    d. A few times a week
    e. 4 or more times a week
    f. Everyday

4. When you do these kinds of jobs, how many **hours** would you say you usually work per **week**?

5. Do you do any of these jobs before going to school? **Y or N**
    a. If YES, which ones?

6. On average, how much do you earn per week doing these things? **R**

The FIRST additional component to this questionnaire is a urine test to check for any health problems.

If NOT performed, why?		
Urine Test	**Result**	**Notes**
pH level		
Leucocytes		
Nitrite		
Protein		
Glucose		
Ketone		
Urobilinogen		
Bilirubin		
Blood		

Urine results referral note to family: *Y* *N*

Leucocytes / Nitrite notification to family: *Y* *N*

EATING HABITS AND PRACTICES OF ADOLESCENTS

SECTION A: Breakfast habits

Think about a **usual school week and weekend** and try to answer the following questions about your eating habits as truthfully as possible. There are no right or/ wrong answers so please feel free to give your answer.

1. On how many weekdays do you usually eat breakfast? **Mark one only**

Never	1	
1-2 days	2	
3-4 days	3	
Every weekday (5)	4	☐

2. How often do you usually eat breakfast on a weekend? **Mark one only**

Never	1	
Saturdays only	2	
Sundays only	3	
Saturdays and Sundays	4	☐

3.1 What best describes the way you usually eat during the week? **Mark one only**

3 or more meals a day	1	
2 meals a day	2	
1 meal a day	3	☐

3.2 What best describes the way you usually eat over a weekend? **Mark one only**

3 or more meals a day	1	
2 meals a day	2	
1 meal a day	3	☐

4. How many times do you eat snacks in a day? **Mark one only**

Just once a day	1	
Twice a day	2	
3 or more times a day	3	
Never	4	☐

SECTION B: Fastfoods

1. How often during the **past week** (past 7 days) did you eat any of the following takeaways? **Tick each item**

	0 x last week	1x last week	2x last week	3x last week	4x last week	5+ last week
Hamburger						
Chicken Burger						
Fried fish						
Fried chips						
Pizza						
Vetkoek						
Pies or sausage roll						
Samoosas						
Pita bread						
Hotdog						
Boerewors roll						
Doughnuts						
Sweets						
Cake						
Chocolates						
Chips e.g. nik naks						
Ice cream						
Soft drinks e.g. Coke						
Squash e.g. *Drink-o-pop/Oros*						
Diet drinks						
Other:						

2. How often do you usually eat at a friend's house? (In a week) Tick where applicable.

0 x per week	1x per week	2x per week	3x per week	4x per week	5+ x per week

SECTION C: School lunch box

Think about a typical school week and answer the following questions about your lunch box that you take to school.

1. How often do you generally take a lunch box to school? **Mark one only**

0 x per week	1x per week	2x per week	3x per week	4x per week	5 per week

2. Do you share or exchange what you have in your lunch box with friends?

Yes	No
1	2

3. Which foods do you often have in your lunch box? **Tick each item**

	0 x per week	Less than 2x per week	More than 2x per week
White bread or rolls			
Brown bread or rolls			
Fruit			
Chips			
Pap			
Meat or chicken			
Pie / sausage roll			
Cold drink			
Diet cold drinks			
Fruit juice			
Milk or sour milk			
Yoghurt			
Cheese			
Sweets or chocolates			
Biscuits or cookies			
Peanuts			
Other:			

4. Who prepares your school lunch box (yourself, mother, father etc)

_____

5. Do you get money to spend on food / snacks at school? **Mark one only**

Yes	No	Sometimes
1	2	3

6. How much money do you usually get to spend at school per week on food? **Mark one only**

R1 – R5	1
R6 – R10	2
R11 - R15	3
More than R15	4

7. Which of the following foods did you buy at school (tuck shop)? **Tick each item**

	Did not buy	Bought 1 time	Bought 2 times	Bought 3 times	Bought 4 times	Bought 5 times or more
White bread or rolls						
Brown bread or rolls						
Fresh fruit						
Chips						
Pap and Meat or chicken						
Fried chips						
Pie/sausage roll/samoosa						
Vetkoek						
Cold drink						
Diet cold drinks						
Fruit juice						
Milk or sour milk						
Yoghurt						
Cheese						
Sweets or chocolates						
Cakes/ donuts/ éclairs						
Popcorn						
Peanuts/nuts						
Other:						

1. How often do you snack when you are watching TV? **Mark one only**

Every day	1
More than three days a week	2
Less than 3 days a week	3
Never	4

2. Which snacks did you eat while watching TV last week (past seven days)? And how often? **Tick each item**

	Didn't eat	1 time	2 times	3 times	4 times	5 or more times
Fruit						
Popcorn						
Chocolates						
Bread (any type)						
Crisps e.g. nik-naks						
Biscuits						
Cakes/ donuts/ éclairs						
Drinks e.g. Coke						
Fries						
Other:						

4. Do TV adverts on foods influence you to buy those food items? **Mark one only**

Never	1
Hardly ever	2
Often	3
Very often	4

5. Which food and drinks that you see advertised on TV do you buy?

1.)

2.)

3.)

6. Where do you usually eat your main meal of the day? **Mark one only**

Kitchen at a table/counter	1
Dining room at a table	2
In front of the TV off your lap	3
Other	4

7. How many times do you eat dinner/supper with your family/parents/caregivers?

Never	1
Some Days	2
Most Days	3
Every Day	4

8. How much does your mother/caregiver/father control what you eat?

1.	Not at all	2.	Sometimes	3.	Mostly	4.	Completely

SECTION D: Acculturation

1. What is you favourite "soap opera" on television and why?

2. How many times a week do you watch it? ☐ time/s a week

The SECOND additional component is obtaining the GPS COORDINATES of your house
Perform GPS scan for coordinates

Verify that the coordinates are entered correctly

Y	N

The THIRD additional component is taking a PHOTOGRAPH of you

Take a photograph of the adolescent in front of his/her house.

Y	N

The LAST component is the SELF COMPLETION QUESTIONNAIRE

Has the adolescent completed the self completion questionnaire?

Y	N

NOTES

Research Assistant name:		**Date:**	
Quality checked by:		**Date:**	

Appendix H: PDF of Heterogeneity of fracture pathogenesis in urban South African children: The Birth to Twenty cohort

ORIGINAL ARTICLE

JBMR

Heterogeneity of Fracture Pathogenesis in Urban South African Children: The Birth to Twenty Cohort

Kebashni Thandrayen,[1] Shane A Norris,[1] Lisa K Micklesfield,[1,2] and John M Pettifor[1]

[1]MRC/Wits Developmental Pathways for Health Research Unit, Department of Paediatrics, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa
[2]UCT/MRC Research Unit for Exercise Science and Sports Medicine, Department of Human Biology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa

ABSTRACT

South African black children fracture less than white children. Differences in bone mass, body composition, and physical activity may be contributing risk factors. This study aimed to investigate the association between fracture prevalence, bone mass, and physical activity in South African children. Using the Bone Health cohort of the Birth to Twenty longitudinal study, we retrospectively obtained information of lifetime fractures until age 15 years in 533 subjects. Whole-body bone mineral content (BMC), bone area (BA), fat mass (FM), and lean mass (LM) (measured by dual-energy X-ray absorptiometry [DXA]), anthropometric data, physical activity scores, and skeletal maturity were obtained at ages 10 and 15 years. Nonfracturing black females were used as the control group and comparisons were made between those who did and did not fracture within the same sex and ethnic groups. Of the 533 subjects, 130 (24%) reported a fracture (black, 15%; white, 41.5%; $p < 0.001$). White males who fractured were significantly taller (10 years, $p < 0.01$), more physically active (15 years, $p < 0.05$) and had higher LM (10 years, $p = 0.01$; 15 years, $p < 0.001$), whereas white females who fractured were fatter (10 and 15 years, $p = 0.05$ and $p < 0.05$, respectively), than their nonfracturing peers. White males who fractured had greater BA and BMC at all sites at 10 and 15 years compared to their nonfracturing peers after adjusting for differences in height and weight; BA and BMC were similar in each of the other sex and ethnic groups. No anthropometric or bone mass differences were found between black children with and without fractures. The factor associated with fractures in white males appears to be participation in sports activities, while in white females obesity appears to play a role. No contributing factors in black males and females were found, and needs further elucidation. © 2011 American Society for Bone and Mineral Research.

KEY WORDS: FRACTURES; BONE DENSITOMETRY; PHYSICAL ACTIVITY; SOUTH AFRICA; CHILDREN; BONE MASS

Introduction

Adult studies have documented ethnic differences in bone mass and fracture risk.[1,2] African American adults fracture less and have greater areal bone mineral density (BMD) than white adults,[1] and similarly, South African black women have greater hip bone mass[3,4] and fracture less compared to South African white women.[5]

We have previously shown that South African black children fracture less than white children[6] and at various ages have greater BMD than their white peers at some sites.[7–10] Recently, Micklesfield and colleagues[11] have reported that South African black children, despite having a lower body weight than white children at age 13 years, have greater diaphyseal bone strength, as measured by peripheral quantitative computed tomography (pQCT). This could possibly explain the lower fracture incidence previously reported in South African black children. The role of lifestyle factors such as physical activity in determining bone mass differences has been investigated by McVeigh and colleagues,[10] who showed an association between physical activity and bone mineral content (BMC) and BMD only in South African white children at 9 years of age in the same cohort. No such relationship was found in black children of the same age.

In South African children, differences in bone mass and geometry, and physical activity between the ethnic groups may be contributing risk factors for the different fracture rates observed. Recent studies in the United Kingdom conclude that white children have significantly higher fracture rates than other ethnic groups but the reasons as to why have not been elucidated.[12,13] To our knowledge there are no studies that have investigated the contribution of bone mass and physical activity to ethnic differences in fracture risk. Therefore, the aim of this

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study was to investigate the associations between fracture prevalence, bone mass, body composition, and physical activity in urban South African children.

Methods

Study population

Data from 533 children from the Bone Health subcohort of the Birth to Twenty (Bt20) longitudinal study of child health and development were used. All eligible children (3273) born within a 7-week period (April 23 to June 8, 1990) in the greater Johannesburg metropolitan area in South Africa were originally recruited for the Bt20 study. The cohort profile has been previously described by Richter and colleagues.[14] The child's ethnic classification was defined by the race classification currently in use in South Africa for demographic and restitution purposes. Although the South African government currently classifies race into black (ethnic Africans), white (Europeans, Jews, and Middle Easterners), colored (mixed race), and Indian (Asian), only children for whom both parents were classified as either white or black were included in this study. The Bone Health study was constituted as a subcohort of Bt20 when the children were 9 years of age to investigate in more detail factors influencing bone mass accretion during puberty and adolescence. A supplementary sample of 120 white children born during the same period was recruited at the age of 10 years to increase the white sample size. These supplementary 120 white children were born during the cohort enrolment dates, but not in the area; however, there were no differences in birth weight, maternal age and education, and socioeconomic status between the supplementary children and the original white participants of the cohort.

Children who had chronic illnesses such as asthma and epilepsy were not included in the bone health subcohort, as the use of certain medications and immobility are associated risk factors for low bone mass and may increase the incidence of fractures. Participants were born prior to the human immunodeficiency virus (HIV) epidemic in South Africa and thus were unlikely to suffer from HIV or tuberculosis. The reported child mortality in the original Birth to Twenty cohort of some 3200 participants by age 10 years was 28 participants and this number had increased to 40 by age 15 years. The cause of death was mainly accident/trauma related. Thus the bone mass demographics were unlikely to be affected by chronic illnesses or death. All subjects provided assent and their parents/guardian provided written, informed consent; ethical approval was obtained from the University of Witwatersrand Committee for Research on Human Subjects.

Fracture questionnaire

A fracture questionnaire was completed by each adolescent with the help of his/her parent or guardian at age 15 years. The questionnaire included information about previous fractures, their sites with the aid of a skeletal diagram, and the causes and age at fracture. Due to the retrospective nature of the fracture data collection, the fractures could not be verified by X-ray.

Anthropometric measurements, skeletal maturity, and dual-energy X-ray absorptiometry–derived parameters

Anthropometric measurements, skeletal maturity, and bone mass data were obtained annually but data at age 10 years (pre- or early puberty) and 15 years (mid- or late puberty) were used for this study. Height was measured to the nearest millimeter using a stadiometer (Holtain, Crosswell, UK). Weight was measured to the last 100 g using a digital scale (Dismed, Halfway House, South Africa) with participants wearing light clothing and no shoes. Skeletal maturity was assessed by a trained expert by scoring bone age from hand radiographs using the Tanner-Whitehouse bone-specific scoring technique (TWIII 20).[15]

Total body less head bone area (TBLH BA), total body less head bone mineral content (TBLH BMC), whole body composition (fat mass and lean mass), and site-specific measurements of bone area (BA) and BMC at the radius (R), hip (H), hip neck (HN), and lumbar spine (LS) were performed using an Hologic QDR 4500A dual-energy X-ray absorptiometer (DXA) (Hologic, Inc., Waltham, MA, USA) according to standard procedures (software version 11.2; Hologic).

Assessment of physical activity levels

Questionnaires quantifying total physical activity (PA) for the previous 12 months were administered at ages 10 and 15 years via interview. The questionnaire was modified from previous studies to be appropriate for South African children and results obtained on this cohort at 9 years of age have been published previously.[10] The intensity, frequency, and duration of all physical activities (physical education, extramural school and club sport, informal physical activity, and active commuting to and from school) and sedentary activities were recorded. Formal activities were inclusive of sporting activities at school and club level, and informal activities included play activities at home or in the neighborhood outside of school. Physical activity was scored in minutes per week multiplied by metabolic equivalents (MET, 1 MET is defined as the energy expenditure for sitting quietly, which for the average adult is approximately 3.5 mL of oxygen/kg body weight/min) according to the classification of Ainsworth and colleagues,[16] to obtain a measure of physical activity related energy expenditure (METmins/week). The total, formal, and informal physical activity scores were thereafter converted to *Z*-scores by using the largest nonfracture group (black females) as the reference group for comparison between those who did and did not sustain a fracture.

Statistical analyses

Data were analyzed using the Statsoft (Statistica v7.0, 2006; Statsoft, Inc., Tulsa, OK, USA) package. Individual anthropometric measurements (height-for-age *Z*-score [HAZ] and body mass index [BMI]-for-age *Z*-score [BAZ]) were calculated using the World Health Organization (WHO) Anthroplus software (http://www.who.int/growthref/tools/en). Using logistic regression analyses, ethnicity and sex were found to be important factors predicting fracture risk in the entire cohort; therefore, analyses were performed for each sex and ethnic group separately. Data

were summarized as means (standard deviations [SDs]) or medians (interquartile range), depending on the distribution. Comparisons were made between those who had and had not fractured within the same sex and ethnic group using chi-square analysis. A p value of <0.05 was considered to be statistically significant. The largest nonfracturing group (black females) were used as the control or reference group to compare with other groups for whole-body composition and bone mass measurements and physical activity scores. Individual whole-body composition measurements were compared with the control group (nonfracturing black females) by calculating Z-scores, by subtracting the control mean from the participant's specific measurement and dividing by the control SD. Bone mass, height, and weight variables were log-transformed, then BA and BMC were adjusted for height and weight of the whole cohort using multiple regression analyses. Thereafter, Z-scores for BA and BMC of each of the sexes and ethnic groups were derived using data from nonfracturing black females as the control. Unadjusted physical activity Z-scores were derived from the control group. Fracture rates were calculated as the number of subjects who reported a fracture or the number of fractures divided by total person-time of observation.

Results

Fracture patterns

Of the 533 subjects in the cohort at 15 years of age, 186 (35%) were white (W) and 347 (65%) were black (B). The total number of children who sustained fractures over the first 15 years of life was 130 (24%). Of the 130 children reporting fractures, 70% had sustained a single fracture and 30% had had more than one fracture.

The percentage of white children who reported a fracture in the first 15 years of life was 41.5% ($n = 78$) compared to 15% ($n = 52$) in black children ($p < 0.001$). The proportions of white males (WM) and females (WF) who had fractured were significantly higher than for black males (BM) and females (BF), respectively (WM 47% versus BM 37%; $p < 0.001$ and WF 18% versus BF 11%; $p < 0.001$).

The overall fracture rate for the first 15 years of life was 23 per 1000 children per annum. White males had the highest fracture rates per annum followed by white females and then black males and females (Table 1).

The fracture incidence rates for all sites except the lower limb were significantly higher in the white population (Fig. 1). The most common site of fracture for the entire cohort was the upper limb (48%).

Table 1. Fracture Rates Over the First 15 Years of Life

Ethnic group	Fracture rate per 1000 children per annum	95% Confidence intervals
White males	52.4*	28.3–76.5
Black males	14.4	7.6–21.3
White females	37.1**	24.6–49.5
Black females	8.4	4.1–12.7

*$p < 0.01$ between white and black males.
**$p < 0.001$ between white and black females.

Anthropometric and whole body composition characteristics

Ethnicity ($p < 0.001$) and sex ($p = 0.024$) were shown by logistic regression (data not shown) to be important predictors of fracture risk in the entire cohort, thus further analyses were performed within the same sex and ethnic groups. Complete anthropometric and whole body composition measurements were available on 304 of the 347 black children with fracture data at mean age of 10.5 years and on 332 of the 347 children at mean age of 15.5 years. Seven of the 43 black children (16%) at age 10 years and 2 of the 15 (13%) at age 15 years with incomplete data had reported a fracture (proportions very similar to those with complete data, suggesting no selection bias in those used in the analyses).

Comparing the same ethnic and sex groups with and without fractures, there were no statistically significant differences in anthropometry or body composition measurements at age 10 or 15 years between black males or females with and without a history of fractures at age 15 years (Table 2). Comparing body mass variables of black males with those of nonfracturing black females, black males at 10 years and 15 years had a lower fat mass ($p < 0.001$) and at 15 years had a greater lean mass ($p < 0.001$) (Table 2).

Complete anthropometric and whole-body composition measurements were available from 101 of the 186 white children with fracture data at a mean age of 10.6 years and from 116 of the 186 white children at a mean age of 15.7 years. The major reason for the lack of complete data at the two time points was an unwillingness of parents or children to take time off from school for the DXA and radiological studies. Thirty-four of the 85 white children (40%) at age 10 years and 34 of the 70 (48.5%) at age 15 years with incomplete data had reported a fracture (proportions very similar to those with complete data, suggesting no selection bias in those used in the analyses).

Comparing the same ethnic and sex groups with and without fractures, white males who had fractured in the first 15 years of life were significantly taller ($p < 0.01$) and had a higher lean body mass ($p = 0.01$) at the age of 10 years, and remained with greater lean mass ($p < 0.001$) at age 15 years compared to their nonfracturing peers (Table 2). White females who had fractured had a higher lean body mass at age 15 ($p < 0.05$) and a higher BMI at age 10 years ($p = 0.05$) and 15 years ($p < 0.05$) compared to the white females who had not fractured (Table 3).

Comparing body mass variables of the different ethnic and sex groups with those of nonfracturing black females, white males at 10 and 15 years and white females at 15 years had a greater lean mass (Table 3).

Associations between fractures, bone mass characteristics, and physical activity

TBLH BA and TBLH BMC Z-scores adjusted for height and weight at 10 and 15 years of age were compared between the controls and the other sex and ethnic groups; and within the same sex

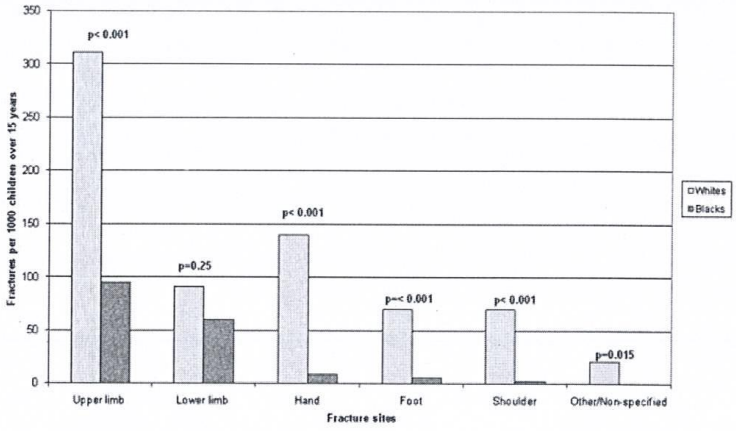

Fig. 1. Fracture incidence rates per annum at the different fracture sites in white and black children.

and ethnic groups for those who did and did not report a fracture during the first 15 years of life (Tables 4 and 5).

Comparing the same ethnic and sex groups with and without fractures, white males who reported a previous fracture/s during the first 15 years of life had greater BA and BMC at all sites at age 10 years (Table 4), and at 15 years (Table 5) than their nonfracturing peers. There were no significant differences in either BA or BMC at age 10 or 15 years in black or white females or in black males between those who did or did not fracture at age 10 and 15 years (Tables 4 and 5).

Comparing bone mass variables of the different ethnic and sex groups with those of nonfracturing black females, white males with fractures and white females with and without fractures at 10 years had greater BA and BMC at most sites and black males without fracture had lower BA and BMC at all sites (Table 4). The number of black males with fractures was too small to make a meaningful comparison. At 15 years, all white males and females and black males had greater BA and BMC compared to the nonfracturing black female controls (Table 5).

Table 6 compares total physical activity *Z*-scores (combining formal and informal activities) at ages 10 and 15 years between children with and without a history of fractures at age 15 years. White males who reported a fracture during the first 15 years of life participated in more physical activity at age 10 years (formal only; $p < 0.05$) and at 15 years (total and informal; $p < 0.05$ and $p < 0.01$, respectively) compared to white males with no fracture history. Fracture risk was positively associated with physical activity; at age 15 years for every 1 SD increase in formal physical activity (*Z*-score of METmins/week) the odds ratio for fracturing was 2.2 (95% CI, 1.22–3.97; $p < 0.01$) and for every 1 SD increase in total physical activity (*Z*-score METmins/week) the odds ratio for fracturing was 1.78 (95% CI, 1.06–3.00; $p < 0.05$). There was no

Table 2. Anthropometric and Body Composition Measurements at 10 and 15 Years of Age of Black Males and Females With and Without a History of Fractures at 15 Years of Age

	Black females at 10 years		Black females at 15 years		Black males at 10 years		Black males at 15 years	
	Without fractures ($n = 128$)	With fractures ($n = 16$)	Without fractures ($n = 140$)	With fractures ($n = 17$)	Without fractures ($n = 131$)	With fractures ($n = 29$)	Without fractures ($n = 142$)	With fractures ($n = 33$)
Chronological age (years)	10.5 (0.26)	10.5 (0.28)	15.6 (0.24)	15.5 (0.25)	10.6 (0.27)	10.5 (0.28)	15.6 (0.26)	15.5 (0.28)
Bone age (years)	9.9 (1.10)	9.8 (0.88)	14.7 (0.62)	14.7 (0.50)	9.9 (0.70)	9.9 (0.76)	14.8 (1.30)	14.73 (1.16)
HAZ score[a]	−0.41 (0.95)	−0.67 (0.65)	−0.48 (0.89)	−0.52 (0.99)	−0.52 (0.90)	−0.41 (1.04)	−0.13 (0.97)	0.04 (1.01)
BAZ score[a]	0.16 (1.21)	−0.28 (0.91)	0.60 (1.10)	0.16 (0.94)	0.07 (1.12)	−0.11 (0.94)	0.02 (1.13)	−0.14 (1.05)
Lean mass *Z*-score[b]	0.00 (1.00)	−0.46 (0.43)	0.00 (1.00)	−0.37 (0.48)	0.02 (0.80)	−0.08 (0.72)	1.51 (1.35)*	1.38 (1.16)*
Fat mass *Z*-score[b]	0.00 (1.00)	−0.33 (0.53)	0.00 (1.00)	−0.29 (0.49)	−0.52 (0.82)*	−0.61 (0.48)*	−1.06 (0.93)*	−1.09 (0.79)*

Data presented as mean (SD). There were no significant differences within the same sex and age groups.
HAZ = height age *Z*-score; BAZ = BMI age *Z*-score.
[a]Computed using WHO Anthroplus software.
[b]The *Z*-scores of lean and fat masses of each ethnic and sex groups were derived from the values obtained from the black nonfracturing females.
*$p < 0.001$ significant differences in lean and fat mass *Z*-scores comparing nonfracturing black females to other ethnic and sex group.

Table 3. Anthropometric and Body Composition Measurements at 10 and 15 Years of Age of White Males and Females With and Without a History of Fractures at 15 Years of Age

	White females at 10 years		White females at 15 years		White males at 10 years		White males at 15 years	
	Without fractures ($n = 28$)	With fractures ($n = 20$)	Without fractures ($n = 44$)	With fractures ($n = 19$)	Without fractures ($n = 29$)	With fractures ($n = 24$)	Without fractures ($n = 30$)	With fractures ($n = 23$)
Chronological age (years)	10.6 (0.27)	10.6(0.27)	15.7(0.28)	15.7 (0.26)	10.6 (0.22)	10.7 (0.25)	15.7 (0.24)	15.74 (0.25)
Bone age (years)	10.0 (1.24)	10.2 (1.26)	14.6 (0.71)	14.8 (0.48)	10.1 (0.66)	10.2 (0.72)	15.3 (1.06)[c]	15.8 (0.86)
HAZ score[a]	0.17 (1.16)	0.20 (1.17)	0.51 (1.03)	0.25 (1.01)	−0.08 (1.15)[d]	0.88 (1.11)	0.43 (1.11)	0.90 (1.08)
BAZ score[a]	−0.12 (1.06)[c]	0.52 (1.11)	0.13 (0.94)[c]	0.73 (1.07)	0.01 (0.86)	0.29 (1.06)	0.02 (0.76)	0.49 (1.02)
Lean mass *Z*-score[b]	0.34 (1.04)	0.63 (1.15)[g]	0.51 (0.96)[c,f]	1.18 (1.30)[h]	0.36 (0.83)[d,f]	1.12 (0.77)[h]	2.78 (1.60)[e,h]	4.05 (1.51)[h]
Fat mass *Z*-score[b]	−0.14 (0.77)[f]	0.27 (0.90)[h]	−0.27 (0.71)	0.15 (1.17)	−0.53 (0.50)[g]	−0.28 (0.66)	−1.09 (0.78)[h]	0.87 (0.75)[h]

Data presented as mean (SD).
HAZ = height age *Z*-score; BAZ = BMI age *Z*-score; BMI = body mass index; WHO = World Health Organization.
[a]Computed using WHO Anthroplus software.
[b]The *Z*-score of lean and fat masses of each ethnic and sex groups were derived from the values obtained from the black nonfracturing females.
[c]$p \leq 0.05$.
[d]$p < 0.01$.
[e]$p < 0.001$: significant differences within the same sex and age groups.
[f]$p < 0.05$.
[g]$p < 0.01$.
[h]$p < 0.001$: significant differences in lean and fat mass *Z*-scores comparing nonfracturing black females to other ethnic and sex groups.

Table 4. Bone Area and Bone Mineral Content *Z*-Scores (Adjusted for Height and Weight) at 10 Years of Age With and Without a History of Fractures at Age 15 Years

	Females at 10 years				Males at 10 years			
	Blacks		Whites		Blacks		Whites	
Bone mass measurements (Z scores)	Without fractures ($n = 128$)	With fractures ($n = 16$)	Without fractures ($n = 28$)	With fractures ($n = 19$)	Without fractures ($n = 130$)	With fractures ($n = 29$)	Without fractures ($n = 29$)	With fractures ($n = 24$)
TBLH BA	0.00 (1.00)	−0.36 (0.52)	0.32 (1.01)	0.59 (1.14)[c]	−0.30 (0.88)[d]	−0.33 (0.72	**0.11 (0.93)[b]**	**0.78 (0.79)[e]**
R 1/3 BA	0.00 (1.00)	−0.33 (0.53)	0.45 (1.07)[c]	0.63 (1.19)[d]	−0.31 (0.92)[d]	−0.30 (0.84)	**0.21 (1.02)[b]**	**0.95 (0.90)[e]**
R mid BA	0.00 (1.00)	−0.30 (0.55)	0.54 (1.11)[d]	0.65 (1.21)[d]	−0.32 (0.94)[c]	−0.28 (0.92)	**0.27 (1.06)[b]**	**1.05 (0.97)[e]**
R ultra distal BA	0.00 (1.00)	−0.28 (0.57)	0.59 (1.13)[d]	0.66 (1.22)[d]	−0.32 (0.96)[c]	−0.26 (0.98)	**0.31 (1.09)[b]**	**1.11 (1.02)[e]**
R total BA	0.00 (1.00)	−0.30 (0.55)	0.54 (1.11)[d]	0.65 (1.21)[d]	−0.32 (0.94)[c]	−0.28 (0.93)	**0.27 (1.06)[b]**	**1.05 (0.97)[e]**
H BA	0.00 (1.00)	−0.26 (0.59)	0.63 (1.15)[d]	0.66 (1.22)[d]	−0.31 (0.97)[c]	−0.25 (1.01)	**0.33 (1.11)[b]**	**1.15 (1.06)[e]**
HN BA	0.00 (1.00)	−0.31 (0.54)	0.50 (1.10)[c]	0.65 (1.20)[d]	−0.32 (0.93)[d]	−0.29 (0.89)	**0.24 (1.04)[b]**	**1.01 (0.94)[e]**
LS BA	0.00 (1.00)	−0.21 (0.64)	0.69 (1.16)[d]	0.66 (1.21)[d]	−0.30 (1.00)[c]	−0.21 (1.09)	**0.39 (1.13)[b]**	**1.22 (1.13)[e]**
TBLH BMC	0.00 (1.00)	−0.36 (0.53)	0.31 (1.00)	0.58 (1.14)[c]	−0.30 (0.87)[d]	−0.33 (0.71)	**0.10 (0.92)[b]**	**0.76 (0.78)[e]**
R 1/3 BMC	0.00 (1.00)	−0.35 (0.52)	0.38 (1.04)	0.61 (1.17)[d]	−0.31 (0.89)[d]	−0.32 (0.77)	**0.15 (0.96)[b]**	**0.85 (0.83)[e]**
R mid BMC	0.00 (1.00)	−0.33 (0.53)	0.45 (1.08)[c]	0.63 (1.19)[d]	−0.31 (0.92)[d]	−0.31 (0.84)	**0.20 (1.02)[b]**	**0.95 (0.89)[e]**
R ultra distal BMC	0.00 (1.00)	−0.36 (0.53)	0.29 (1.00)	0.57 (1.13)[c]	−0.30 (0.87)[d]	−0.33 (0.70)	**0.09 (0.91)[a]**	**0.75 (0.78)[e]**
R total BMC	0.00 (1.00)	−0.34 (0.53)	0.41 (1.06)[c]	0.62 (1.18)[d]	−0.31 (0.90)[d]	−0.31 (0.81)	**0.18 (0.99)[b]**	**0.90 (0.86)[e]**
H BMC	0.00 (1.00)	−0.33 (0.53)	0.45 (1.07)[c]	0.63 (1.19)[d]	−0.31 (0.91)[d]	−0.30 (0.83)	**0.20 (1.01)[b]**	**0.94 (0.89)[e]**
HN BMC	0.00 (1.00)	−0.37 (0.53)	0.26 (0.99)	0.56 (1.12)[c]	−0.29 (0.86)[d]	−0.34 (0.68)	**0.07 (0.89)[a]**	**0.70 (0.76)[e]**
LS BMC	0.00 (1.00)	−0.31 (0.54)	0.50 (1.10)[c]	0.64 (1.21)[d]	−0.32 (0.93)[d]	−0.30 (0.88)	**0.24 (1.04)[b]**	**1.00 (0.93)[e]**

Data presented as mean (SD). Bold values denote significant differences in BA and BMC Z-scores comparing nonfracturing and fracturing white males.
BA = bone area; BMC = bone mineral content; TBLH = total body less head; R = radius; H = total hip; HN = hip neck; LS = lumbar spine.
[a]$p < 0.05$.
[b]$p < 0.01$, significant differences within the same sex and age group.
[c]$p < 0.05$.
[d]$p < 0.01$.
[e]$p < 0.001$: significant differences in BA and BMC *Z*-scores comparing nonfracturing black females to fracturing and nonfracturing white males, white females, and black males, respectively.

Table 5. Bone Area and Bone Mineral Content *Z*-Scores (Adjusted for Height and Weight) at 15 Years of Age With and Without a History of Fractures at Age 15 Years

	Females at 15 years				Males at 15 years			
	Blacks		Whites[a]		Blacks[b]		Whites[c]	
Bone mass measurements (*Z*-scores)	Without fractures ($n = 147$)	With fractures ($n = 19$)	Without fractures ($n = 45$)	With fractures ($n = 20$)	Without fractures ($n = 146$)	With fractures ($n = 33$)	Without fractures ($n = 30$)	With fractures ($n = 25$)
TBLH BA	0.00 (1.00)	−0.20 (0.65)	0.60 (0.92)	0.90 (1.23)	0.64 (1.13)	0.72 (0.94)	**1.61 (1.13)**[e]	**2.43 (1.01)**
R 1/3 BA	0.00 (1.00)	−0.01 (1.02)	1.03 (1.02)	1.05 (1.25	1.19 (1.19)	1.38 (1.25)	**2.33 (1.23)**[e]	**3.12 (1.21)**
R mid BA	0.00 (1.00)	−0.04 (0.98)	0.99 (1.01)	1.05 (1.26)	1.14 (1.19)	1.31 (1.22)	**2.27 (1.23)**[e]	**3.08 (1.19)**
R ultra distal A	0.00 (1.00)	−0.08 (0.90)	0.92 (1.00)	1.03 (1.26)	1.04 (1.19)	1.20 (1.16)	**2.16 (1.22)**[e]	**2.98 (1.16)**
R total BA	0.00 (1.00)	−0.04 (0.97)	0.99 (1.01)	1.05 (1.26)	1.13 (1.19)	1.30 (1.22)	**2.26 (1.23)**[e]	**3.07 (1.19)**
H BA	0.00 (1.00)	0.04 (1.10)	1.10 (1.03)	1.05 (1.23)	1.29 (1.19)	1.49 (1.30)	**2.41 (1.22)**[d]	**3.16 (1.23)**
HN BA	0.00 (1.00)	−0.04 (0.97)	0.99 (1.01)	1.05 (1.26)	1.13 (1.19)	1.31 (1.22)	**2.26 (1.23)**[e]	**3.07 (1.19)**
LS BA	0.00 (1.00)	0.02 (1.07)	1.08 (1.03)	1.05 (1.24)	1.26 (1.19)	1.46 (1.29)	**2.39 (1.22)**[d]	**3.15 (1.23)**
TBLH BMC	0.00 (1.00)	−0.21 (0.61)	0.55 (0.91)	0.87 (1.22)	0.58 (1.12)	0.65 (0.91)	**1.52 (1.11)**[e]	**2.33 (0.99)**
R 1/3 BMC	0.00 (1.00)	−0.12 (0.81)	0.81 (0.97)	0.99 (1.26)	0.91 (1.17)	1.03 (1.09)	**1.99 (1.20)**[e]	**2.82 (1.11)**
R mid BMC	0.00 (1.00)	−0.16 (0.74)	0.72 (0.95)	0.96 (1.25)	0.80 (1.16)	0.91 (1.03)	**1.84 (1.17)**[e]	**2.67 (1.07)**
R ultra distal BMC	0.00 (1.00)	−0.27 (0.52)	0.35 (0.86)	0.74 (1.18)	0.33 (1.07)	0.35 (0.80)	**1.13 (1.03)**[e]	**1.89 (0.90)**
R total BMC	0.00 (1.00)	−0.18 (0.69)	0.66 (0.93)	0.93 (1.24)	0.72 (1.14)	0.81 (0.98)	**1.72 (1.15)**[e]	**2.54 (1.04)**
H BMC	0.00 (1.00)	−0.11 (0.85)	0.85 (0.98)	1.01 (1.26)	0.96 (1.18)	1.10 (1.11)	**2.06 (1.20)**[e]	**2.89 (1.13)**
HN BMC	0.00 (1.00)	−0.22 (0.61)	0.55 (0.91)	0.87 (1.22)	0.57 (1.11)	0.64 (0.91)	**1.51 (1.11)**[e]	**2.32 (0.99)**
LS BMC	0.00 (1.00)	−0.23 (0.58)	0.50 (0.89)	0.84 (1.21)	0.51 (1.10)	0.57 (0.88)	**1.42 (1.10)**[e]	**2.21 (0.96)**

Data presented as mean (SD). Bold values denote significant differences in BA and BMC Z-scores comparing nonfracturing and fracturing white males.
BA = bone area; BMC = bone mineral content; TBLH = total body less head; R = radius; H = total hip; HN = hip neck; LA = lumbar spine.
[a] $p < 0.05$ for all BA and BMC *Z*-scores between nonfracturing black females and white females with and without fractures.
[b] $p < 0.01$ for all BA and BMC *Z*-scores between nonfracturing black females and black males with and without fractures except for the RA ultra distal BMC *Z*-score in black males with fractures.
[c] $p < 0.001$ for all BA and BMC *Z*-scores between nonfracturing black females and white males with and without fractures.
[d] $p < 0.05$.
[e] $p < 0.01$, significant differences within the same sex and age group.

significant difference in physical activity at age 10 or 15 years in black or white females or in black males between those who did or did not report a previous fracture.

Discussion

This is the first study to demonstrate heterogeneity in the pathogenesis of fractures in children of different ethnic groups. White males who reported a previous fracture were more physically active at ages 10 and 15 years and had greater BA and BMC at the same ages at all sites than their nonfracturing white peers. Thus increased physical activity seems to be the key contributory factor in the pathogenesis of fractures in white urban South African males. These differences were not present in white females or in black children; however, in white females, BMI and lean mass appeared to be contributory factors. The association with BMI in females has been reported in other studies,[17,18] but the differences in lean mass have not been reported previously. No anthropometric or bone mass and size factors were found to be associated with fractures in black children when performing comparisons within the same sex and ethnic groups. Comparing body and bone mass variables of the different ethnic and sex groups with those of nonfracturing black females had shown that white males at 10 and 15 years, white females, and black males at 15 years had a greater lean mass; and white males and females and black males at 15 years had higher BA and BMC at all sites.

Our findings support those of Clark and colleagues[19] in a high-income country setting, who described the relationship between physical activity, bone mass, and fracture risk in mainly white Caucasian children (3.1% were of nonwhite ethnicity) and showed that despite having a higher BMD, daily or more vigorous physical activity increased fracture risk. To the best of our knowledge, the current study is the first to investigate the relationship between physical activity, bone mass, and fracture risk in children of different ethnic origins.

There are both bone-dependent and bone-independent factors that contribute to fracture risk in childhood.[20] Many studies have investigated the influence of physical activity, socioeconomic status, exposure to sunlight, breastfeeding in early life, and maternal smoking during childhood on bone mass.[21–24] Flynn and colleagues[25] prospectively followed 8-year-old children for 8 years and concluded that there was an inverse relationship between bone mass at 8 years of age and upper limb fracture risk at 16 years of age, and that overweight or obesity at 8 years of age was also associated with an increased risk of fracture. In the Avon Longitudinal Study of Parents and

Table 6. Total Physical Activity Z-Scores at 10 and 15 Years in Children With and Without a History of Fractures at Age 15 Years Compared to Nonfracturing Black Females

	Physical activity Z-scores																	
	10 years								15 years									
	Without fractures				With fractures				Without fractures				With fractures					
	n	Total	Formal	Informal	*n*	Total	Formal	Informal	*n*	Total	Formal	Informal	*n*	Total	Formal	Informal		
Black females	136	0.0 (−0.6 to 0.4)	−0.5 (−0.6 to 0.3)	0.0 (−0.7 to 0.4)	16	0.2 (−0.4 to 0.4)	0.0 (−0.6 to 0.5)	0.0 (−0.7 to 0.4)	145	−0.3 (−0.6 to 0.1)	−0.4 (−0.4 to −0.1)	−0.4 (−0.6 to 0.1)	18	−0.2 (−0.6 to 0.3)	−0.4 (−0.4 to 0.1)	−0.3 (−0.6 to −0.1)		
White females	28	−0.8 (−1.3 to −0.5)	0.4 (−0.6 to 3.0)	−1.1 (−1.3 to −0.8)	20	−0.8 (−1.2 to −0.5)	0.8 (−0.0 to 2.1)	−1.1 (−1.3 to −0.8)	38	0.0 (−0.6 to 1.1)	0.8 (−0.1 to 2.5)	−0.5 (−0.6 to 0.0)	18	−0.3 (−0.5 to 1.0)	0.2 (−0.2 to 1.7)	−0.4 (−0.6 to 0.3)		
Black males	129	0.2 (−0.3 to 0.8)	−0.3 (−0.6 to 1.7)	0.1 (−0.3 to 0.6)	31	0.2 (−0.2 to 1.0)	0.1 (−0.6 to −1.1)	0.1 (−0.3 to 0.6)	140	0.1 (−0.4 to 0.9)	0.01 (−0.4 to 1.1)	−0.1 (−0.5 to 0.3)	32	0.2 (−0.2 to 0.9)	0.6 (−0.4 to 1.5)	−0.3 (−0.6 to 0.5)		
White males	23	−0.7 (−1.0 to 0.4)	**0.8 (−0.2 to 2.2)**[a]	−0.7 (−1.2 to −0.1)	20	−0.5 (−0.9 to −0.2)	**1.7 (0.5 to 3.8)**	−0.7 (−1.2 to −0.1)	24	**0.0 (−0.5 to 0.6)**[a]	**0.2 (−0.3 to 0.9)**[b]	−0.2 (−0.4 to 0.6)	22	**0.8 (0.0 to 2.1)**	**1.6 (0.6 to 3.8)**	0.0 (−0.4 to 0.4)		

Data presented as median (interquartile range). Each of the physical activity components of the black nonfracturing females were used as the control to create Z-scores for the other sex and ethnic groups. Bold values denote significant differences in physical activity Z-scores comparing nonfracturing and fracturing white males.

[a] $p < 0.05$.

[b] $p < 0.01$: significant differences within the same sex and age.

Children (ALSPAC) prospective study, an 89% increased risk of fracture per 1 SD decrease in size-adjusted BMC at age 9.9 years was found.[26] Despite the inverse relationship observed between fracture risk and bone mass, Clark and colleagues[26] were the first to suggest that the higher bone mass associated with increased physical activity in children does not necessarily compensate for the increased exposure to injuries and higher fracture risk.[19] Studies indicate that African American and Hispanic children have a significantly higher bone strength than white children, due to greater bone density at the distal trabecular bone regions and greater bone density and area at the cortical sites of the radius and tibia[27]; the authors suggest that the differences in fracture rates reported between different ethnic groups in adulthood may be traced back to these differences in bone strength in childhood. Several studies on the Bt20 cohort have reported greater BMD in black compared to white children at 9 and 10 years of age[7–9] and more recently Micklesfield and colleagues[11] have shown that South African black children have greater bone strength as measured by pQCT; however, the association between fracture risk and bone strength or geometry in South African children has to be further investigated. We postulate that structural differences in bone geometry may provide protection against fractures in black South Africans.

We have previously shown in the Bt20 cohort that there was a significant difference in the grading of trauma associated with fractures between white and black children, with white children fracturing at more severe levels of trauma than black children.[6] McVeigh and colleagues[10] have shown in the same cohort that white males with the highest physical activity scores have a greater BMD. Therefore, although more physically active white males may have higher BMD, this does not appear to protect them from fracturing.

We have also previously documented that black children are less physically active than white children,[10] but this does not seem to negatively impact their bone mass because black children have a greater hip bone mass, and black girls have a greater lumbar spine bone mass, than their white peers.[10] Despite South African black children being less active and consuming a diet lower in calcium (whites 703–711mg/day versus blacks 297–331mg/day) than white children,[28] we have now shown that they fracture less as well. The complexities of bone turnover and geometry have to be further investigated in the different ethnic groups to explain the possible pathogenesis of ethnic differences in fracture rates and bone mass among children.

A higher BMI at 10 and 15 years of age was the only difference between white females with and without a history of fractures. There were no significant differences in any bone mass or anthropometric measurements in black females with and without fractures. The Dortmund Nutritional and Anthropometric Longitudinal Designed (DONALD) study conducted in Germany on healthy children highlighted the inverse association between the accrual of bone mass and subcutaneous adipose tissue in prepubertal females and found that pubertal females with relatively high subcutaneous fat area (high ratio fat area/fat mass) were characterized by lower bone strength.[17] Our finding of increased adiposity being associated with greater fracture risk

in white girls is in keeping with previous studies conducted in white individuals. Goulding and colleagues[18] found that girls with fractured forearms are often overweight and those with recurrent fractures and high body weight have a substantially higher fracture risk than girls with a history of a single fracture.[29] We have yet to investigate the reasons why increased adiposity in white females is associated with increased fracture risk. No previous studies have compared body composition characteristics and fracture risks in black individuals and this is the first study to suggest that there does not appear to be an association between fat mass, or adiposity, and fracture risk in black girls or boys, despite an increasing (but similar) prevalence of overweight or obesity in both black and white females. At age 15 years, 24% of BF were overweight and 8% were obese; 22% of WF were overweight and 5% were obese; 8% of BM were overweight and 7.5% were obese; and 14% of WM were overweight and 4% were obese.

There are three potential limitations to this study: first, the small number of subjects in the white group, which was influenced by the original sampling method that represented the demographics of racial proportions in urban South Africa. Despite the small number of white children we were able to document statistically significant differences in bone size and area and physical activity between the white boys who did and did not fracture. Further, in white girls we were able to show an association between adiposity and fracture risk.

Second, we did not adjust for potential modifiers (socioeconomic, nutrition, and vitamin D status). Vidulich and colleagues[7] found no effect of nutritional factors on bone mass and stated that the site-specific ethnic differences in bone mass were not the result of poor nutrition or poorer households in South African black children but suggested rather that genetic factors might play a role. Poopedi and colleagues[30] found no significant relationship between vitamin D status and BMC in either black and white subjects in the same cohort; however, an inverse relationship between fat mass and 25-hydroxyvitamin D was found in both black and white children.[31] Despite black children having lower levels of 25-hydroxyvitamin D than white children, this does not appear to influence their bone health.[30]

Third, the lack of radiological confirmation of fractures might be considered a limitation of this study; the fracture rates for whites and blacks previously reported from the Bt20 cohort were similar to those reported in the current smaller subcohort and the previous findings were similar between the ethnic groups at all ages. Furthermore, fracture data that had been previously collected at year 13 and those collected in the current year-15 questionnaires confirmed the previous fracture rates. The use of skeletal diagrams also verified the site of fracture and were filled in with the help of the parent or caregiver for accuracy and completeness. Although there was no available radiological proof of fractures, both public and private health facilities in urban areas would have performed radiological assessments to confirm fractures, so self-reported data are likely to be reliable. Despite the lack of X-ray verification, there are no known systematic differences in the presentation of children from the different ethnic groups with fractures. All children less than 6 years old receive free health care at public hospitals and only a minimal fee for those older than 6 years is charged. Furthermore, primary and community health care clinics offer free services to all, thus individuals of a lower socioeconomic status can seek medical attention without any hindrance when warranted; it is therefore unlikely that fractures go undiagnosed. Unfortunately, there are no data available to confirm this.

In view of the more complete bone mass measurements for the black as opposed to the white children, there may be a potential ascertainment bias with regard to the bone mass findings; however, because the proportions of fractures in participants with fracture but no bone mass measurements are similar to those with bone mass measurements in both the black and white children, this bias is highly unlikely.

The present study did not explore ethnic differences in bone geometry and volumetric BMD measurements using peripheral quantitative computed tomography, or their relationship to fractures; it is possible that further insights into the reasons for the ethnic differences in fracture risk may come to light.

Conclusions

The pathogenesis of fractures in South African children appears to differ between ethnic groups. The white males with fractures were taller and had greater lean tissue mass than their nonfracturing peers. However, it appears that increased physical activity (formal sport), possibly leading to a greater opportunity for injury, is the contributory factor to the increased incidence of fractures despite greater bone mass and size. In white females with fractures, a higher BMI was found, in keeping with other studies. No such associations between physical activity or body composition and fractures were found in the black children. Further studies are necessary to investigate the relationship between bone geometry and fracture risk in children of different ethnic groups worldwide to determine whether genetic or other environmental factors are important contributory factors.

Disclosures

All authors state that they have no conflicts of interest.

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Authors' roles: KT was involved in the conception, acquisition of data, analysis, and interpretation of data and drafting of manuscript and approval of the final version of submitted manuscript. SAN, LKM, and JMP were involved in the design, critical revision, and approval of the final version of the submitted manuscript.

Appendix I: Authors' contributions to publications

Magan A, Nyati LH, Micklesfield LK, Norris SA and Pettifor JM (2017) Metacarpal growth through puberty and adolescence in a longitudinal South African cohort. Journal of Bone and Mineral Research 32(9):1926-1934. DOI: 10.1002/jbmr.3179

Authors' roles

Study design – JMP, SAN; Analysis of Radiographs – AM; Data analysis – AM and LN; Data interpretation – AM, LN, LKM, SAN, JMP; Integrity of data – AM and LN; Drafting and revising manuscript - AM, LKM, SAN, JMP.

Magan A, Micklesfield LK, Nyati LH, Norris SA and Pettifor JM. A longitudinal comparison of appendicular bone growth and strength through adolescence in a South African cohort using radiogrammetry and pQCT (2018) Osteoporosis international. DOI: 10.1007/s00198-018-4761-9

Authors' roles

Study design – JMP, LKM and SAN; Analysis of radiographs – AM; Data analysis: AM. Data interpretation – AM, LN, LKM, SAN and JMP; Integrity of data – AM and LN; Drafting and revising manuscript – AM, LKM, LN, SAN and JMP.

Magan A, Micklesfield LK, Norris SA, Thandrayen K, Munthali R and Pettifor JM. (2018) Metacarpal indices and their association with fracture in South African children and adolescents. Calcified Tissue International. DOI: 10.1007/s00223-018-0467-4

Authors' roles

Study design – JMP, SAN, LKM; Analysis of Radiographs – AM; Fracture data collection – KT; Integrity of data – AM and RJM; Data interpretation – AM, LKM, JMP, RJM; Drafting and revising manuscript – AM, LKM, SAN, KT, RJM, JMP.

Appendix J: PDF of Metacarpal growth during adolescence in a longitudinal South African cohort

ORIGINAL ARTICLE

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Metacarpal Growth During Adolescence in a Longitudinal South African Cohort

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ABSTRACT

To monitor the drift of the periosteal and endocortical surfaces during metacarpal growth longitudinally, radiogrammetry was carried out on hand-wrist X-rays of 572 children from the Birth to Twenty Bone Health Cohort annually from ages 9 to 21 years. This is the largest collection of longitudinal X-rays in African children. The second metacarpal bone length, bone width, and medullary width were measured using digital vernier calipers on a total of 4730 X-rays. Superimposition by Translation and Rotation (SITAR) was used to obtain age at peak metacarpal length velocity (PLV). Bone width and medullary width were modeled using SITAR against both chronological age and age from PLV. In black and white females, tempo and velocity of metacarpal length growth was synchronized. Black males, however, attained PLV 7 months later than white males ($p < 0.0001$). Compared to white males, black males had a longer second metacarpal ($p < 0.05$), and greater bone width size ($p < 0.02$), tempo ($p < 0.0009$), and velocity ($p < 0.0001$). Medullary width growth velocity in black participants peaked 2 years prior to attainment of PLV and exceeded that of their white peers ($p < 0.0001$) in whom it peaked 6 to 12 months post-PLV attainment. Black adolescents therefore had wider bones with relatively thinner cortices and wider medullary cavities than their white peers. Ethnic and sex differences also occurred in the timing of medullary width contraction that accompanied expansion in bone width and cortical thickness. In black males, medullary width contraction commenced approximately 3 years later than in black females, whereas in white males this occurred a year later than in white females. The ethnic and sex differences in bone acquisition reported in this study may differentially affect bone mass in later life. © 2017 American Society for Bone and Mineral Research.

KEY WORDS: RADIOGRAMMETRY; SECOND METACARPAL; BONE ACCRUAL; PUBERTY; ETHNICITY

Introduction

Bone mass accrual during childhood and adolescence is key to an individual's predisposition to skeletal fragility in later life.[1–3] Some 40% of bone mass is acquired during adolescence, making the pubertal phase an opportune time to influence bone mass accrual and future fracture risk.[2–4] Minimal differences in bone mass exist between the sexes in the prepubertal period.[5] Divergence arises during puberty when bone growth is influenced by sex hormones.[5,6] Estrogen limits periosteal bone expansion[7] but promotes endocortical contraction[8,9] while androgens stimulate periosteal bone apposition,[10] resulting in males having wider bones, greater cortical thickness, and wider medullary cavities than females.[11–14]

Radiogrammetry of the metacarpal bones can be used to monitor the movement of the periosteal and endocortical surfaces with growth,[15–20] thereby providing insight into the acquisition of bone architecture and the attainment of peak bone growth velocity. In US children, greater periosteal bone expansion has been reported in males compared to females.[6,11] Additionally, females experience bone apposition at the endocortical interface with subsequent medullary contraction and this occurs approximately 4 years earlier than in males.[6,11]

Metacarpal bone dimensions vary in different population groups.[6,21,22] Dequeker[22] reported greater metacarpal cortical area adjusted for bone width in US white and black adults compared to a European white and a Nigerian black population, whereas a metacarpal radiogrammetry study of 14-year-old South African (SA) children conducted over 40 years ago found that black children had narrower cortical thickness compared to white children.[21]

Most studies on metacarpal growth have been undertaken in developed countries,[6,11,23–25] and there is a paucity of longitudinal radiogrammetry data through adolescence.[6,24,25] The aim of this longitudinal study was to describe the growth in metacarpal dimensions in healthy black and white South Africans through puberty and adolescence into young adulthood. We hypothesized, first, that puberty has a differential sex effect on the periosteal and endocortical bone surfaces, and second, that black SA children have wider bones with thinner cortices compared to their white peers.

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Additional Supporting Information may be found in the online version of this article.
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Subjects and Methods

Participants and protocol

The participants of this Bone Health Cohort (BHC) study consisted of 563 children enrolled specifically from the large Birth to Twenty (Bt20) cohort to investigate factors relating to the acquisition of bone mass during puberty and adolescence. The Bt20 is a longitudinal birth cohort of 3273 singleton children born between April and June 1990 in the greater Johannesburg–Soweto metropolitan area, South Africa. The recruitment protocols for the cohort have been described previously by Richter and colleagues.[26] Ethnic classification was based on the race classification used in South Africa for demographic and restitution purposes. Ethnic classification was self-reported by the parents and only those children with both parents belonging to the same ethnic group were included in this study. Because of the small number of white children in the Bt20 cohort (being representative of the ethnic demographics in the region), an additional 120 white children born in the same period were recruited into the BHC at 9 years of age from schools in other areas. They were born during the cohort enrolment dates and their birth weight, socioeconomic status, maternal age, and education were similar to those of the initial white participants of the BHC.[27] Participation in the BHC was offered to the entire Bt20 cohort; however, our exclusion criteria included children suffering from chronic illnesses such as epilepsy and asthma because of possible effects of drug therapy on bone development and density. Informed assent from adolescent participants and consent from parents were obtained for inclusion in this study. Ethical clearance was obtained from the University of the Witwatersrand Committee for Research on Human Subjects. Annual measurements, taken at approximately the same time every year, included weight, height, and hand-wrist X-rays.

Anthropometric measurements

Weight and height of participants were measured while participants wore light clothing and no shoes. Weight was measured to the nearest 100 g using a digital scale (Dismed, Miami, FL, USA) and height to the nearest millimeter using a stadiometer (Holtain, Crymych, UK). Quality control annual training and monitoring ensured a coefficient of variation between measurers of less than 2%.

Puberty

All children were assessed by Tanner staging at each visit.[28] The ages of onset of puberty in this cohort have been published.[29] Because of the asynchrony between the tempo and velocity of growth relative to sexual maturity,[30] we used age at peak metacarpal length velocity (PLV) as an indicator of pubertal development. We found no differences between our unpublished data on age at peak height velocity (PHV) and age at PLV. In females, age at PHV corresponds to Tanner stage 3 and in males to Tanner stage 4.[31] The use of age at PLV allowed us to align females and males on a common biological maturity indicator.

Radiography

Posteroanterior hand-wrist radiographs of the left hand were taken annually from age 9 to 21 years by certified radiographers at the Charlotte Maxeke Academic Hospital in Johannesburg. The left hand was radiographed in all participants irrespective of hand dominance. The X-ray beam was focused on the distal aspect of the third metacarpal of the left hand. Radiographs were taken using cassettes with single-emulsion film under standard conditions of tube to film distance of 76 cm and exposure at 42 kV and 12 mA/s, and processed in an automatic developer in accordance with the optimal conditions described by Tanner.[32]

Radiogrammetry

Second metacarpal dimensions were measured on a total of 4730 hand-wrist X-rays by a single reader (AM). Measurements were carried out using a digital caliper calibrated to 0.01 mm.[11] The following parameters were measured in millimeters: total length of bone from proximal to distal end, and outer bone width (D) and inner medullary width (d) at the midshaft of the metacarpal. The cortical thickness was calculated as the difference between the bone width and medullary width (D – d). The geometric relationship between cortical thickness and medullary width was referred to as bone architecture.

Intraobserver reliability

Measurements of 30 randomly selected X-rays were repeated by the same researcher (AM) 1 month after initial measurement and again 16 months later to assess intraobserver reliability and reproducibility. The coefficients of variation for the radiogrammetry measures were as follows: (1) metacarpal length, 0.14%; (2) bone width, 0.80%; and (3) medullary width, 6.20%.

Interobserver reliability

The metacarpal measurements for the 10-year age group were evaluated against measurements performed on the same participants from this cohort in a previous study.[33] The difference between the results of the two studies was less than 3%.

Data analysis

Cross-sectional analyses

Participants were categorized into specific age groups for cross-sectional analyses. For example, individuals ≥ 9 and <10 years were categorized as age group 9.5 years. Data were analyzed cross-sectionally using Stata Version 14 (Stata Corporation, Inc., College Station, TX, USA) and SPSS Version 23 (IBM Corp., Armonk, NY, USA). No imputation of missing data was undertaken. Normality of the data was established. A one-way ANOVA was performed on data to determine between-group variation. Sex and ethnic group differences were ascertained using a Bonferroni multiple comparisons test (alpha level 0.05, degrees of freedom = 3). To determine ethnic and sex differences in bone measures with height as a covariate, an analysis of covariance (ANCOVA) with a Bonferroni multiple comparisons test (alpha level 0.05, degrees of freedom = 3) was performed. Mean annual differences for unadjusted bone measures were determined using a repeated measures ANOVA followed by a pairwise comparison of marginal linear predictions for unbalanced data. A value of $p < 0.05$ was considered to be statistically significant.

Longitudinal analyses

Superimposition by Translation and Rotation

Data were modeled using Superimposition by Translation and Rotation (SITAR) in R (Version 3.2.2; R Foundation for Statistical

Computing, Vienna, Austria; https://www.r-project.org/).[34] SITAR is a shape invariant model with a single fitted curve that summarizes individual growth patterns with three parameters: size, tempo, and velocity.[34] Individual curves are modeled and matched to the mean curve by shifting vertically (to represent differences in individual size), or horizontally for differences in tempo (timing) of growth. Individual differences in velocity cause a stretching or shrinking of the age scale, resulting in an increase or decrease of the slope.[34]

Joint models were fitted for metacarpal length, bone width, and medullary width data. These first combined the sexes by ethnic group, and second, the black and white participants by sex group. This allowed for group differences by sex or ethnicity to be tested. Distance curves for the bone dimensions of metacarpal length, bone width, and medullary width plotted against chronological age were produced. The SITAR model used was

$$E(y_{it}) = \alpha_i + h\left(\frac{t-\beta_i}{e^{-\gamma_i}}\right) \quad (1)$$

where y_{it} is the bone dimension of the ith participant at age t; α_i (size parameter), β_i (tempo parameter), and γ_i (velocity parameter) are random effects for participant i; $h(.)$ is a fitted cubic spline curve with degrees of freedom to minimize the Bayesian Information Criterion.[35]

To assess sex and ethnic differences in metacarpal growth standardized on a biological maturity indicator, bone measures were plotted against age from PLV. Age from PLV for each bone measure was calculated by subtracting chronological age at measurement from the age at PLV. Growth distance curves for bone width and medullary width were modeled against age from PLV.

Results

Participant recruitment and range in number of observations are presented in Fig. 1. Of the 683 participants recruited into the BHC, 111 participants elected to exit the study because of either time constraints or challenges with transportation. The 572 participants included in these analyses comprised 183 (32%) black females, 93 (16%) white females, 207 (36%) black males, and 89 (16%) white males. Attrition of participants and missing data waves during the study were due to the circular migration of individuals.[36] Participant characteristics at first measurement have been published.[37]

Metacarpal length

The individual curves for metacarpal length growth of female and male individuals of both ethnic groups are presented in Fig. 2*A* and *B*, respectively. PLV was attained at similar ages in black (11.7 ± 0.65 years) and white (12.0 ± 0.82 years) females (Fig. 2*A*; Table 1). White males attained PLV significantly earlier than their black peers (13.4 ± 0.64 years versus 14.0 ± 0.89 years; $p < 0.0001$) (Fig. 2*B*; Table 1). The tempo of the metacarpal growth spurt in black males lagged white males by 7 months (Fig. 2*B*; Table 1). In males, metacarpal length growth velocity was greater in white compared to black participants ($p < 0.002$). In both ethnic groups, the metacarpal growth spurt occurred significantly earlier in females with black females attaining PLV 2.6 years earlier than their male peers, and white females attaining PLV 1.5 years earlier than white males (Table 2). The timing of PLV was synchronized with that of PHV (our unpublished data). Significant ethnic differences were observed in metacarpal length in both sexes. Adolescent black children had on average 1 mm longer second metacarpals than white children ($p < 0.05$) (Table 1). These ethnic differences were accentuated after adjusting data for height (Supporting Tables 1 and 2). The second metacarpal of black and white adolescent males was longer than that of their female counterparts ($p < 0.0001$) (Supporting Fig. 1; Table 2).

Bone width

Black and white females had similar bone widths with no differences in tempo and velocity of growth (Fig. 3*A*; Table 1). Black males had significantly greater bone width size ($p < 0.02$), growth tempo ($p < 0.0009$), and velocity ($p < 0.0001$) than their white peers (Fig. 3*B*; Table 1). Bone width in both black and white adolescent males was approximately 1 mm greater than their respective female peers ($p < 0.0001$) (Table 2). Growth velocity in males also exceeded that of females in both ethnic groups ($p < 0.0001$) (Supporting Fig. 2; Table 2). Peak velocity in bone width was attained at approximately similar ages relative to age at PLV in both sexes and ethnic groups (Supporting Fig. 3). The increase in bone width was greatest during the pubertal growth spurt and continued until a later age in males compared to females (Fig. 3; Supporting Tables 1, 3, and 4).

Medullary width

Adolescent black individuals of both sexes had larger medullary cavities than their white peers ($p < 0.0001$) (Fig. 4*A*, *B*; Table 1). Peak growth velocity in medullary width in black participants occurred approximately 2 years prior to PLV, whereas in white participants, this occurred 6 to 12 months post–PLV (Supporting Fig. 5). Significant sex differences in medullary width size occurred in both ethnic groups, with males having greater medullary cavities than their female peers ($p < 0.0001$) (Supporting Fig. 4; Table 2). Growth velocity of medullary width was significantly greater in black ($p < 0.0001$) and white ($p < 0.03$) males relative to their female peers (Table 2). In both ethnic groups, when assessed against the age of PLV, the timing of peak medullary width growth was similar between females and males (Supporting Fig. 5).

In all participants, there was an initial increase in medullary width followed by a decrease (Supporting Tables 1, 3, and 4). Although annual changes in medullary width were not always significant, when the maximum medullary width attained (black and white females, 13.5 years; black males, 16.5 years; white males, 14.5 years) was compared to that at the last time of measure (20.5 years), the decrease was significant in both sexes and ethnic groups (Table 3).

Cortical thickness

In all participants, cortical thickness increased through adolescence (Supporting Tables 1, 3, and 4). Ethnic differences were evident, with white participants having thicker cortices than their black peers, although in females this difference was only until 16.5 years (Supporting Table 1). Significant annual increases in cortical thickness occurred for 2 years longer in males compared to their female peers (Supporting Tables 3 and 4). Sex differences with males having thicker cortices than their female peers were more marked in white compared to black participants (Supporting Table 1).

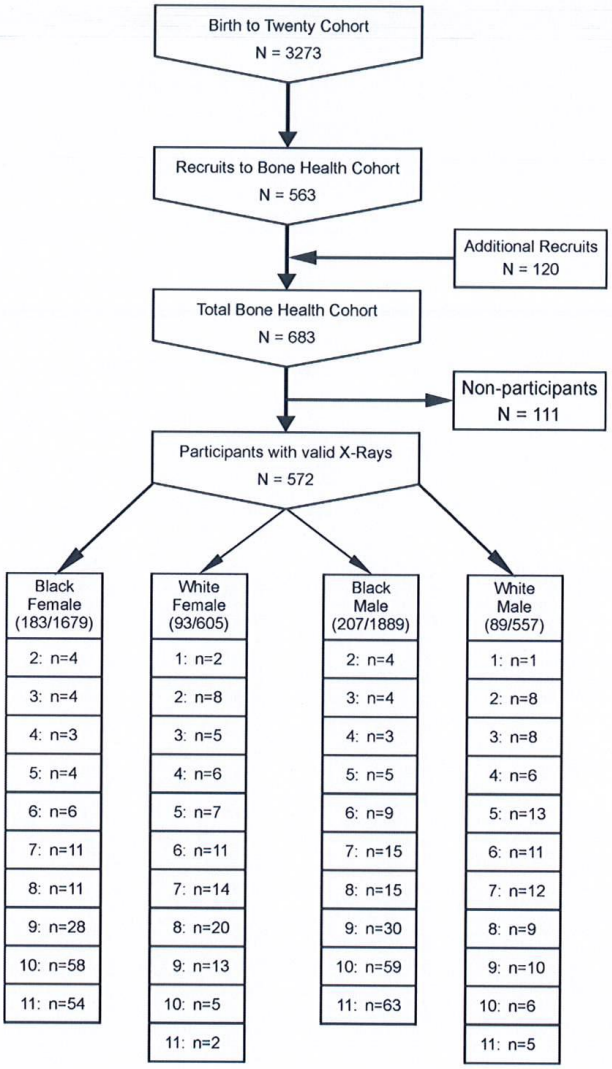

Fig. 1. Participant recruitment (N) and the number of participants (n) with their respective number of observations.

Discussion

This study presents unique longitudinal data on metacarpal bone development in an urban SA population spanning 12 years through puberty and adolescence and into young adulthood, a period during which significant physiological changes are experienced. The results of our study using radiogrammetry to measure metacarpal dimensions revealed distinctly different ethnic and sex patterns of bone growth compared to previous studies,[6,11] as well as ethnic differences in size and cross-sectional architecture of adolescent bone.

Ethnic differences in bone length were accentuated when data were adjusted for height with black individuals of both sexes having longer metacarpals than their white peers at all ages. A previous study of this cohort also reported longer forearm and leg lengths relative to stature in black children compared to their white counterparts at 9 years of age.[38] In US children, a similar pattern of ethnic differences in

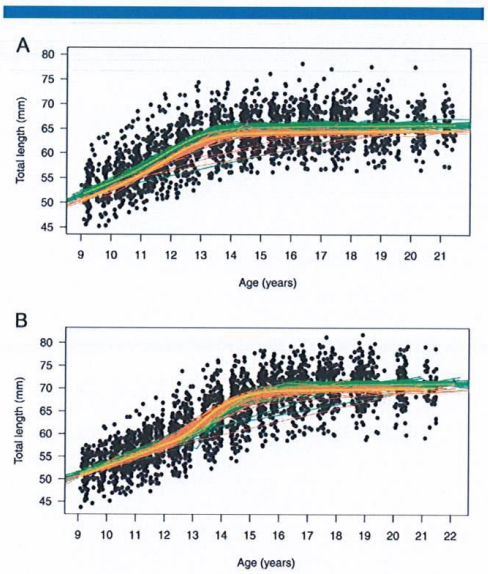

Fig. 2. Scatterplot of metacarpal length against chronological age, and individual curves adjusted for tempo and velocity, with the mean adjusted curve, for metacarpal length growth by ethnicity, in females (*A*) and males (*B*). Black participants, green color; white participants, orange color.

metacarpal lengths were reported in black and white children, although their data were not adjusted for height.[39] In contrast to SA black children, who are on average 5 to 6 cm shorter than their white peers (Nyati, unpublished data), prepubertal US black children had taller stature compared to their white peers.[40–42] Ethnic differences in the US were attributed to US black individuals being more efficient than their white peers in absorbing and preserving calcium, particularly during adolescence.[43,44] Calcium intake was not measured in our study; however, lower calcium intakes have been reported in black compared to white SA children.[45] Although low calcium intake may influence bone growth, it does not account for the longer metacarpals in black individuals in this study.

Ethnic differences in metacarpal width were reported in a SA study undertaken in 1971 in which 14-year-old urban black males had significantly narrower metacarpals than white males.[21] Our data do not support this finding. Comparing the results of the present study with those reported in 1971, the average metacarpal widths for our 14-year-old black and white males are 13.5% and 4.8% greater, respectively, while for black and white females they are 7% and 4.7% greater, respectively. These differences likely reflect secular changes in bone growth and the age of PHV in urban SA children, particularly in black individuals. Improved socioeconomic conditions and nutrition may have contributed to the secular trend in black children. The delay in age of PLV in black males has been suggested to be due to them being more sensitive to adverse environmental conditions.[46]

Table 1. Summary of SITAR Analyses of Ethnic Differences in the Size, Tempo, and Velocity of Metacarpal Length, Bone Width, and Medullary Width Growth During Adolescence as Measured Against Chronological Age

	Metacarpal length				Bone width				Medullary width	
	Females		Males		Females		Males		Females	Males
Participants/observations (*n*)	258/2099		275/2271		258/2099		275/2271		258/2099	275/2271
Degrees of freedom	5		5		5		6		8	5
Residual SD (mm)	0.51		0.66		0.10		0.11		0.19	0.25
Tempo-velocity correlation	−0.36		−0.47		−0.60		−0.01		−0.62	−0.01
Variance explained (%)	98.1		97.1		97.8		98.0		94.6	92.9
	Metacarpal length				Bone width				Medullary width	
	Females		Males		Females		Males		Females	Males
	Mean ± SE	*p*	Mean ± SE	*p*	Mean ± SE	*p*	Mean ± SE	*p*	Mean ± SE	*p*	Mean ± SE	*p*
Size (mm)	−1.04 ± 0.48	0.03	−1.10 ± 0.51	0.03	−0.13 ± 0.08	0.13	−0.24 ± 0.1	0.02	−0.40 ± 0.1	0.0001	−0.70 ± 0.12	0.0001
Tempo (years)	0.15 ± 0.13	0.27	−0.58 ± 0.13	0.0001	0.24 ± 0.19	0.23	−0.5 ± 0.15	0.0009	−0.72 ± 0.27	0.008	−1.74 ± 0.29	0.0001
Velocity (mm/year)	−0.01 ± 0.03	0.66	0.09 ± 0.03	0.002	0.02 ± 0.04	0.69	0.19 ± 0.05	0.0001	0.06 ± 0.05	0.18	0.03 ± 0.04	0.4

Data are for white relative to black females and males.

Table 2. Summary of SITAR Analyses of Sex Differences in the Size, Tempo, and Velocity of Metacarpal Length, Bone Width, and Medullary Width Growth During Adolescence as Measured Against Chronological Age

	Metacarpal length		Bone width		Medullary width	
	Black	White	Black	White	Black	White
Participants/observations (*n*)	390/3568	182/1162	370/3349	163/1022	370/3349	163/1022
Degrees of freedom	6	4	6	4	6	5
Residual SD (mm)	0.61	0.63	0.10	0.13	0.05	0.22
Tempo-velocity correlation	−0.28	−0.62	0.01	0.21	0.39	−0.01
Variance explained (%)	98.0	97.9	98.6	97.0	94.7	92.8

	Metacarpal length				Bone width				Medullary width			
	Black		White		Black		White		Black		White	
	Mean ± SE	*p*	Mean ± SE	*p*	Mean ± SE	*p*	Mean ± SE	*p*	Mean ± SE	*p*	Mean ± SE	*p*
Size (mm)	5.42 ± 0.38	0.0001	5.38 ± 0.53	0.0001	1.07 ± 0.07	0.0001	0.99 ± 0.10	0.0001	0.16 ± 0.02	0.0001	0.48 ± 0.16	0.0001
Tempo (years)	2.61 ± 0.10	0.0001	1.53 ± 0.17	0.0001	3.00 ± 0.11	0.0001	2.51 ± 0.20	0.0001	0.15 ± 0.01	0.0001	0.06 ± 0.02	0.0001
Velocity (mm/year)	0.13 ± 0.02	0.0001	0.24 ± 0.04	0.0001	0.26 ± 0.04	0.0001	0.32 ± 0.06	0.0001	0.22 ± 0.03	0.0001	0.12 ± 0.05	0.03

Data are for males relative to females in black and white participants.

Fig. 3. Scatterplot of bone width against chronological age, and individual curves adjusted for tempo and velocity, with the mean adjusted curve, for bone width growth by ethnicity, in female (*A*) and male (*B*) participants. Black participants, green color; white participants, orange color.

Fig. 4. Scatterplot of medullary width against chronological age, and individual curves adjusted for tempo and velocity, with the mean adjusted curve, for medullary width growth by ethnicity, in female (*A*) and male (*B*) participants. Black participants, green color; white participants, orange color.

Table 3. Differences in Bone Width, Cortical Thickness, and Medullary Width Between the Age at First Measurement and Age at Maximal Medullary Width, and Between the Age at Maximal Medullary Width and Age at Last Measurement

Group	Age range (years)[a]	Bone width (mm) (mean ± SE)	p	Medullary width (mm) (mean ± SE)	p	Cortical thickness (mm) (mean ± SE)	p
BF	9.5–13.5	1.09 ± 0.02	0.0001	0.30 ± 0.03	0.0001	0.79 ± 0.03	0.0001
	13.5–20.5	0.29 ± 0.02	0.0001	−0.23 ± 0.03	0.0001	0.52 ± 0.03	0.0001
WF	9.5–13.5	1.03 ± 0.05	0.0001	−0.10 ± 0.07	0.16	1.12 ± 0.08	0.0001
	13.5–20.5	0.35 ± 0.06	0.0001	−0.19 ± 0.08	0.02	0.54 ± 0.09	0.0001
BM	9.5–16.5	1.97 ± 0.03	0.0001	0.64 ± 0.04	0.0001	1.33 ± 0.04	0.0001
	16.5–20.5	0.23 ± 0.03	0.0001	−0.20 ± 0.04	0.0001	0.43 ± 0.04	0.0001
WM	9.5–14.5	1.69 ± 0.06	0.0001	0.38 ± 0.08	0.0001	1.31 ± 0.09	0.0001
	14.5–20.5	0.54 ± 0.07	0.0001	−0.38 ± 0.09	0.0001	0.91 ± 0.10	0.0001

BF = black female; WF = white female; BM = black male; WM = white male.

[a]Age at first measurement (9.5 years); age at maximal medullary width (BF = 13.5 years; WF = 13.5 years; BM = 16.5 years; WM = 14.5 years); age at last measurement (20.5 years).

The rate of bone growth was similar in both sexes prior to the pubertal growth spurt and consistent with those of other studies.[6,11,24] A divergence in bone length and width between females and males occurred during the pubertal growth spurt in response to the presumed differential expression of sex hormones. Although the sex differences in which males are taller and have longer and wider bones are believed to be the result of the later onset of puberty in males compared to females,[14,38] we also show significantly greater peak bone length and bone width velocity during puberty in males.

In keeping with the findings of Garn,[11] there were no significant sex differences in bone width prior to the onset of the pubertal growth spurt. However, during adolescence the degree of sexual dimorphism was similar in both ethnic groups, and these differences diminished when height was factored in. In contrast, Garn and colleagues[47] reported less sexual dimorphism in adults of largely African ancestry compared to European subjects when raw data were analyzed.

Our results showed that although sexual dimorphism emerged during the growth spurt, the pattern of bone growth in males was dissimilar to that of some previous metacarpal radiogrammetry studies.[6,11] In males, the expansion in bone width and cortical thickening during puberty has been attributed primarily to periosteal bone apposition,[11,13] with endocortical expansion continuing until 16 years in US white boys.[6] However, in our study, in white males, the endocortical area ceased to expand at an earlier age and was followed by contraction, concurring with the results of Martin and colleagues.[24] The relative delay in endocortical contraction in black males may be a consequence of their later entry into puberty and/or related to their different bone architecture.

Previous studies in females reported dimensional changes in cortical thickness due to endocortical apposition and this was attributed to the pubertal rise in estrogen.[11,24] Our results are consistent with these findings because greater endocortical contraction was observed in black and white adolescent females, and this occurred at similar ages to those previously reported.[6,11,24] Endocortical bone accrual is more closely associated with insulin-like growth factor-1 (IGF-1) in girls and with testosterone in boys.[48] It is therefore interesting that, in the present study, endocortical contraction commenced only a year later in white males compared to white females and conflicts with Garn's observations in Ohio whites.[11] Longitudinal hormonal assays through adolescence may reveal the endocrine influence on the endocortical surface. In a recent longitudinal pQCT study of the tibia it was proposed that in females, instead of increased endocortical bone deposition relative to males, there is less endocortical resorption.[49] The regulation of remodeling at the endocortical interface may therefore be region-specific[8,11] and/or genetically determined.[47] Given that in both sexes peak medullary width expansion in black participants occurred at least 2 years prior to attaining PLV compared to white participants, in whom it occurred at least 6 months after attainment of PLV, genetic influences at the endocortical surface may possibly override hormonal influences in bone development.

Distinct relationships of cortical thickness relative to medullary width and bone diameter were evident between black and white adolescents. Black adolescents had wider bones with thinner cortices and wider medullary cavities than white adolescents. These differences may be due to differences in calcium intake between the two ethnic groups.[45] A study comparing hypocalcemic and normocalcemic rural black SA children reported greater bone width and lower bone mineral density in the hypocalcemic group.[50] It was suggested that secondary hyperparathyroidism contributed to increased endocortical bone resorption and periosteal bone deposition.[50] The outward displacement of the cortex from the central axis seen in black participants may therefore be a compensatory mechanism enhancing the bone's resistance to torsional and bending forces.[51] Because the metacarpal is a non–weight bearing bone, the role of mechanical loading in determining bone size is unclear.[52] SA black children have smaller muscle cross-sectional area relative to bone size compared to white children and this may elicit a differential response to mechanical forces.[53]

The initiation and duration of the growth spurt and the attainment of PLV were synchronized between black and white females. In males, ethnic differences were observed whereby the black males' delayed entry into puberty allowed for greater prepubertal appendicular growth[38] and the attainment of PLV later than in white males is in keeping with the delayed skeletal maturity reported in the same cohort.[46] The lag in pubertal development in black males has been proposed to be a consequence of negative early life influences impacting growth.[46] An earlier onset of puberty is thought to be associated with an accelerated time through puberty;[46,54] however, in this study the duration of the growth spurt was similar in all groups regardless of the timing of the onset of the growth spurt.

The strengths of this study are its longitudinal nature spanning 12 years and its provision of information on a critical developmental period. Furthermore, it provides data based on ethnicity and sex and aligns these to biological maturity. SITAR has only been used to model stature[55] and skeletal maturity of short bones;[46] however, in this study we have shown its applicability in modeling appendicular bone growth. We acknowledge that errors may occur with repeated measures but because we expect repeat measures to vary with age we sought to minimize these errors by using standardized protocols for radiography and radiogrammetry. Although radiogrammetry was performed manually using calipers, precision of the method was validated by interobserver and intraobserver results. A digitized method may have been limiting in that it requires a minimum cortical thickness for recognition by an automated system, a challenge in pediatric populations.[56] Our study was standardized on the second metacarpal of the left hand irrespective of hand dominance. Previous radiogrammetry studies have shown greater bone width and cortical area of the right second metacarpal compared to the left regardless of hand dominance.[57,58] Participants who may have experienced a forearm fracture were not excluded because the fracture data on this cohort were obtained retrospectively. We do not, however, believe that this would have significantly impacted the metacarpal dimensions. A limitation of the study was the relatively smaller number of white participants.

In conclusion, in SA children distinct ethnic and sex differences in bone acquisition emerge during puberty. The resultant bone morphology and cross-sectional architecture at the end of skeletal maturity differs from previous studies in high-income countries. These dissimilarities in bone accrual during adolescence may differentially influence bone mass in later life.

Disclosures

All authors state that they have no conflicts of interest.

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Appendix K: PDF of A longitudinal comparison of appendicular bone growth and markers of bone strength in a South African cohort using radiogrammetry and pQCT

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ORIGINAL ARTICLE

CrossMark

A longitudinal comparison of appendicular bone growth and markers of strength through adolescence in a South African cohort using radiogrammetry and pQCT

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Abstract

Summary To compare growth patterns and strength of weight- and non-weight-bearing bones longitudinally. Irrespective of sex and ethnicity, metacarpal growth was similar to that of the non-weight-bearing radius but differed from that of the weight-bearing tibia. Weight- and non-weight-bearing bones have different growth and strength patterns.

Introduction Functional loading modulates bone size and strength.

Methods To compare growth patterns and strength of weight- and non-weight-bearing bones longitudinally, we performed manual radiogrammetry of the second metacarpal on hand-wrist radiographs and measured peripheral quantitative computed tomography images of the radius (65%) and tibia (38% and 65%), annually on 372 black and 152 white South African participants (ages 12–20 years). We aligned participants by age from peak metacarpal length velocity. We assessed bone width (BW, mm); cortical thickness (CT, mm); medullary width (MW, mm); stress-strain index (SSI, mm^3); and muscle cross-sectional area (MCSA, mm^2).

Results From 12 to 20 years, the associations between metacarpal measures (BW, CT and SSI) and MCSA at the radius (males $R^2 = 0.33$–0.45; females $R^2 = 0.12$–0.20) were stronger than the tibia (males $R^2 = 0.01$–0.11; females $R^2 = 0.007$–0.04). In all groups, radial BW, CT and MW accrual rates were similar to those of the metacarpal, except in white females who had lower radial CT (0.04 mm/year) and greater radial MW (0.06 mm/year) accrual. In all groups, except for CT in white males, tibial BW and CT accrual rates were greater than at the metacarpal. Tibial MW (0.29–0.35 mm/year) increased significantly relative to metacarpal MW (− 0.07 to 0.06 mm/year) in males only. In all groups, except white females, SSI increased in each bone.

Conclusion Irrespective of sex and ethnicity, metacarpal growth was similar to that of the non-weight-bearing radius but differed from that of the weight-bearing tibia. The local and systemic factors influencing site-specific differences require further investigation.

Keywords Functional loading · Growth patterns · Muscle · pQCT · Radiogrammetry · Stress-strain index

Introduction

Bone geometry and strength determine fracture risk [1]. Sex differences in metacarpal, radial and tibial bone size and strength have been documented in US adults [2] and children [3–5]. In South African (SA) children, in addition to sex differences, studies using radiogrammetry and peripheral quantitative computed tomography (pQCT) have shown distinct ethnic differences in bone diameter, the ratio of cortical thickness to medullary cavity diameter [6–8], and, in muscle cross-sectional area (MCSA) relative to bone size [6, 9].

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Bone size, mass and strength are modulated by mechanical loading due to body weight and muscle force [10–12]. Furthermore, muscle elicits a differential response to mechanical forces dependent on its cross-sectional area [6, 9]. The role of muscle in determining bone size and strength has been established at the radius [13, 14] and tibia [15], but its role at the metacarpal remains unclear [16].

Bone strength and resistance to fracture are dependent on the triad of its mass, geometry and the material properties of its matrix [17]. Historically, radiogrammetry was the means by which cortical measures from radiographs were translated into indices used as proxies for bone strength [3, 18]. Whilst radiogrammetry is a simple, accessible and inexpensive method which yields information about the amount of bone and its spatial organisation [3, 18]. pQCT, however, is a more advanced mode of assessing bone size and strength, and provides a 3-dimensional perspective of the quantity and matrix properties of both cortical and trabecular bone [19]. From the pQCT image, the stress-strain index (SSI) is derived using measures of bone geometry and volumetric bone density to provide a proxy for bone strength [20].

Whilst recent longitudinal studies from South Africa and Canada have reported on sex and ethnic differences in growth of the metacarpal bone (by radiogrammetry) [7], and the radius and tibia (by pQCT) [5, 8], to the best of our knowledge, the influence of muscle size on bone growth during adolescence has not been investigated longitudinally in African children. Our aim was to compare, in two ethnic groups, bone growth and strength patterns measured using two different technologies at weight- and non-weight-bearing appendicular sites, controlling for the influence of the associated musculature. We hypothesised firstly that, due to the metacarpal being a non-weight bearing bone, metacarpal bone measures and strength would correlate more closely with MCSA at the non-weight-bearing radius than at the weight-bearing tibia; therefore, radial MCSA could be used as a proxy for metacarpal MCSA. Secondly, irrespective of sex and ethnicity, the pattern of growth of the non-weight-bearing metacarpal and radial bones would be similar to each other and that metacarpal growth would differ from that of the weight-bearing tibia.

Materials and methods

Study participants and protocol

The participants in this study were black and white children from the Bone Health Cohort (BHC) which is a subgroup of the Birth to Twenty (Bt20) longitudinal birth cohort. The details of recruitment into both cohorts have previously been described [7, 21]. Briefly, the Bt20 cohort comprised 3273 singleton children born between April and June 1990 in the greater Johannesburg-Soweto metropolitan area, South Africa. At age 9 years, 563 of these children were included in the BHC to investigate bone development and its influencing factors during adolescence. Children were classified as black (African descent) or white (European descent) based on the race classification used in South Africa for demographic and restitution purposes. Ethnic classification was self-reported by the parents and only those children whose parents both belonged to the same ethnic group were included in this study. Previous genetic studies have shown little ethnic admixture between the groups in the Bt20 cohort [22]. Due to the small numbers of white participants originally enrolled into the Bt20 cohort, which represented the SA population demographics at that time, and, attrition of participants over time, an additional 120 9-year-old white children born during the same period were recruited into the BHC. Their mean birth weight, socioeconomic status and maternal age and education were commensurate with those of the original white participants of the BHC. Children with chronic illnesses such as epilepsy and asthma were excluded due to the possible negative effects of their medication on bone mass. Informed assent from adolescent participants and consent from parents were obtained for inclusion in this study. Ethics clearance was obtained from the University of the Witwatersrand Committee for Research on Human Subjects. At approximately the same time annually, participant height and weight were measured, and hand-wrist radiographs and peripheral quantitative computed tomography scans of the radius and tibia were obtained.

Anthropometric measurements

Weight and height of participants were measured whilst wearing light clothing and no shoes. Weight was measured to the nearest 100 g using a digital scale (Dismed, Miami, FL, USA) and height to the nearest millimetre using a stadiometer (Holtain, Crymych, UK). Quality control annual training and monitoring ensured a coefficient of variation between measurers of less than 2%.

Puberty

We used age at peak metacarpal length velocity (PLV) as an indicator of pubertal development [7]. The use of age at PLV allowed us to align black and white females and males on a common biological maturity indicator. Metacarpal bone length of participants aged between 9 and 21 years was modelled using Superimposition by Translation and Rotation (SITAR) in R (version 3.2.2, R Foundation for Statistical Computing, Vienna, Austria; https://www.r-project.org/) [23] to obtain age at PLV for each participant [7]. SITAR is a shape invariant model with a single fitted curve that summarises individual growth patterns with three parameters—size (amplitude), tempo and velocity [23]. Individual curves are modelled and matched to the mean curve by shifting vertically

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(to represent differences in individual size), or horizontally for differences in tempo (timing) of growth. Individual differences in velocity cause a stretching or shrinking of the age scale resulting in an increase or decrease of the slope [23].

Metacarpal, radial and tibial growth for each sex and ethnic group was standardised on PLV. Age from PLV for metacarpal, radial and tibial bone measures was calculated by subtracting the age at PLV from the chronological age at measurement. Age at PLV coincided with age at peak height velocity (unpublished data).

Radiography

Postero-anterior hand-wrist radiographs of the left hand were taken annually between 9 and 21 years of age by certified radiographers at the Charlotte Maxeke Academic Hospital in Johannesburg. The x-ray beam was focussed on the distal aspect of the third metacarpal of the left hand. Radiographs were taken using cassettes with single-emulsion film under standard conditions of tube to film distance of 76 cm and exposure at 42 kV and 12 mA/s, and processed in an automatic developer in line with the optimal conditions described by Tanner et al. [24].

Radiogrammetry

Radiogrammetry was performed on hand-wrist radiographs of 572 participants aged between 9 and 21 years to obtain age at PLV [7]. To compare growth patterns of the metacarpal bone to that of the radius and tibia, data was only used from 524 participants aged between 12 and 20 years to coincide with the availability of pQCT data. Second metacarpal dimensions were measured on a total of 4730 hand-wrist radiographs by a single reader (AM). Measurements were carried out using a digital calliper calibrated to 0.01 mm. The following parameters were measured in millimetres: total length of bone from proximal to distal end, and outer bone width and inner medullary width at the midshaft of the metacarpal. The cortical thickness was calculated as the difference between the bone and medullary widths. The SSI was calculated from the hand-wrist radiographs as described by Cointry and co-workers [25]. To assess intra-observer reliability and reproducibility, measurements of 30 randomly selected radiographs were repeated by the same researcher (AM) 1 month after initial measurement and again 16 months later. The coefficients of variation for the radiogrammetry measures were as follows: (i) metacarpal length, 0.14%; (ii) bone width, 0.80%; and (iii) medullary width, 6.20%.

Peripheral quantitative computed tomography imaging

Peripheral QCT imaging was carried out on the left forearm and lower leg annually on 524 participants of the BHC cohort between 12 and 20 years of age. At each visit, images were obtained from 2.3 mm thick diaphyseal slices taken at the tibia (38% and 65% sites) and the radius (65% site) (Stratec XCT-2000, Stratec Medical, Pforzheim, Germany). Forearm length was measured from the most distal end of the ulna styloid process to the tip of the olecranon process. The tibial length was measured from the distal end of the medial malleolus to the superior aspect of the medial tibial condyle. A 0.5-mm voxel size and a scan speed of 25 mm/s were used. Image processing and calculation of numerical values were performed using the manufacturer's software package (version 6; Stratec Medical, Pforzheim, Germany) at thresholds of 710 mg/cm^3 for cortical bone and 180 mg/cm^3 for total bone.

Analyses at the 38% tibial site and the 65% radial sites were done using the Cort mode 1 (threshold = 710 mg/cm^3) for total cross-sectional area (TotA, mm^2) and cortical bone area (CoA, mm^2). Bone measures were calculated as follows [6]: bone width = $2 \times \sqrt{(\text{TotA}/\pi)}$; medullary width = $2 \times \sqrt{(\text{TotA-CoA})/\pi}$; cortical thickness = bone width − medullary width. The SSI was determined from the pQCT bone measures at a threshold of 280 mg/cm^3 [8]. The individual density of each voxel was used in the calculation of the SSI to minimise the error due to the partial volume effect [26].

The 65% radial and tibial sites are associated with the largest muscle belly and were assessed for MCSA (cm^2). Muscle CSA, calculated as the area with a density between 40 and 180 mg/cm^3, was analysed using contour mode 3.

A scan of a phantom was performed daily for quality control. Throughout the study, all measurements and analyses were performed by two trained operators. The inter-operator variation was less than 1%. All pQCT scans were analysed by a single qualified individual. Images that had been distorted by movement during scanning or incorrect limb placement were excluded from this study.

Statistical analyses

Bivariate regression

All analyses were performed separately for each sex and ethnic group due to known differences in their bone geometry [7]. A bivariate regression was performed to determine the association between metacarpal bone width, thickness and SSI, and MCSA of the radius and tibia, and, between metacarpal SSI, and radial and tibial SSI. A Hausman test was performed on the data and the fixed effects model deemed to be appropriate for assessing the bone measures. The metacarpal outcome variables were log-transformed to approximately conform to normality. Fixed effects using the least squares dummy variable model (LSDV) removed the time-invariant characteristics of the data and assessed the net effect of the

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predictors on the outcome variable. The level of significance was set at $p < 0.05$.

Mixed-effects modelling

Analyses were conducted using Stata 14.0 (Stata Corporation Inc., College Station, TX, USA). Regression analyses showed that sex ($p < 0.0001$) and ethnicity ($p < 0.05$) were significantly associated with rates of bone growth; therefore, all analyses were conducted separately for each sex and ethnic group.

We used mixed-effects models to compare the growth patterns of bone width, cortical thickness, medullary width, and SSI as measured by radiogrammetry and pQCT, in the four groups when aligned by years from PLV. No imputation of missing data was undertaken as the mixed-effects model manages missing data by using information from other participants to obtain an overall curve. The regression model used was:

$$Y_{ij} = \beta_0 + \beta_1 \mathrm{Age}_{ij} + \beta_2 \mathrm{Age}^2_{ij} + \beta_3 \mathrm{Site}_j + \beta_4 \mathrm{MCSA}_j + \beta_5 \mathrm{Age}_{ij} \times \mathrm{Site}_j + \beta_6 \mathrm{Site}_j \times \mathrm{MCSA}_j + u_0 + u_1 \mathrm{Age}_{ij} + \varepsilon_{ij}$$

Y_{ij} is the bone variable of individual i measured at time j.

In the fixed effect of the model, β_0 and β_1 represent the average value of the bone variable for each individual and the overall slope of time (age from PLV), respectively. To adjust for the effect of bone site (metacarpal, radius and tibia) on the relationship between the bone variable and age, an interaction term for age and bone site (Age × Site) was included in the model. An additional interaction term Site × MCSA was included in the model to adjust for the effect of the associated musculature of each bone. Radial MCSA was used as a proxy for metacarpal MCSA. A quadratic function for age was included in the model as it improved model fit by lowering the Bayesian Information Criterion (BIC).

In the random effect of the model, u_0 is the individuals' residual distance from the intercept of the overall trajectory, u_1 is the residual difference for individuals' slopes around the overall effect of time (age from PLV), and, ε_{ij} is the residual of the individual points around the subject-specific trajectory. Random intercepts and slopes were included in all models as they improved model fit by lowering the BIC. The random effects allowed each individual's profile to vary around the average curve and for variation in the rate of growth between individuals. We used maximum log likelihood (−2log likelihood) to determine the significance of random effect variances and covariances between nested models. The level of statistical significance was $p < 0.05$.

Results

Participant recruitment, the number of participants in each sex and ethnic group, together with the total number of observations per group are shown in Supplementary Fig. 1. A total of 524 participants aged between 12 and 20 years had valid hand-wrist radiographs and radial and tibial pQCT scans. They comprised 174 (33%) black females (BF), 79 (15%) white females (WF), 198 (38%) black males (BM) and 73 (14%) white males (WM). The mean number of visits over the study period was 5 for black participants and 3.5 for white participants.

Associations of metacarpal bone measures with MCSA of radius and tibia

Metacarpal bone measures were associated with MCSA of the radius in all four groups (Table 1). The variance in metacarpal bone width and cortical thickness explained by radial MCSA was 34–45% in males ($R^2 = 0.34$–0.45) and 12–19% in females ($R^2 = 0.12$–0.19). There was no significant association between any of the metacarpal bone measures and tibial MCSA, except in black males (Table 1).

Associations of metacarpal SSI with MCSA and SSI of radius and tibia

The SSI of the metacarpal bone was significantly associated with MCSA of the radius in all four groups, although the variance explained in males was 36–44% ($R^2 = 0.36$–0.44) and only 15–20% in females ($R^2 = 0.15$–0.20) (Table 1; Supp. Fig. 2). Metacarpal SSI was associated with tibial MCSA in the black males only ($R^2 = 0.11$) (Table 1; Supp. Fig. 3).

Metacarpal SSI was significantly associated with radial and tibial SSI in all four groups (Table 1). The variance in metacarpal SSI explained by the radial SSI was 20–35% in females and 38–51% in males (females, $R^2 = 0.20$–0.35; males, $R^2 = 0.38$–0.51). Tibial SSI explained only 11–21% variance in metacarpal SSI in females but 27–30% in males (females, $R^2 = 0.11$–0.21; males, $R^2 = 0.27$–0.30).

Bone width

Metacarpal, radial and tibial bone width growth patterns are presented in Supplementary Fig. 4, and the average annual changes in bone width are shown in Table 2. Bone width over the period of study increased significantly in each of the three bones in all four groups, except for radial bone width in white females which did not increase significantly (Table 2). Metacarpal and radial bone width growth rates were similar to each other in both sex and ethnic groups. However, comparisons between the tibia

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Table 1 Fixed-effects regression estimates of metacarpal bone measures as the outcome and radial and tibial bone measures as predictors in black and white females and males

	Black female ($N = 174$)		White female ($N = 79$)	
	Coefficient [95% CI]	R^2	Coefficient [95% CI]	R^2
BW Met/MCSA radius	6.86E-05 [6.31E-05, 7.41E-0.5]*	0.19	8.36E-05 [6.89E-05, 9.83E-05]*	0.14
BW Met/MCSA tibia	1.71E-06 [− 1.05E-06, 4.47E-06]	0.007	5.25E-07 [− 4.74E-06, 5.79E-06]	0.03
CT Met/MCSA radius	2.23E-04 [2.01E-04, 2.44E-04]*	0.12	2.68E-04 [2.22E-04, 3.15E-04]*	0.13
CT Met/MCSA tibia	− 8.71E-06 [1.91E-05, 1.71E-06]	0.01	− 4.53E-06 [− 2.06E-05, 1.15E-05]	0.01
SSI Met/MCSA radius	2.92E-04 [2.66E-04, 3.18E-04]*	0.20	3.07E-04 [2.45E-04, 3.69E-04]*	0.15
SSI Met/MCSA tibia	5.52E-06 [− 7.27E-06, 1.83E-05]	0.009	1.07E-05 [− 1.06E-05, 3.20E-05]	0.04
SSI Met/SSI radius	1.97E-03 [1.77E-03, 2.16E-03]*	0.35	2.04E-03 [1.70E-03, 2.39E-03]*	0.20
SSI Met/SSI tibia	1.35E-04 [8.35E-05, 1.87E-04]*	0.21	2.32E-04 [1.12E-04, 3.52E-04]*	0.11
	Black male ($N = 198$)		White Male ($N = 73$)	
	Coefficient [95% CI]	R^2	Coefficient [95% CI]	R^2
BW Met/MCSA radius	7.68E-05 [7.39E-05, 7.96E-05]*	0.35	7.63E-05 [6.95E-05, 8.31E-05]*	0.33
BW Met/MCSA tibia	4.09E-05 [3.56E-05, 4.61E-05]*	0.09	8.92E-06 [7.81E-09, 1.78E-05]	0.06
CT Met/MCSA radius	1.83E-04 [1.73E-04, 1.93E-04]*	0.34	1.83E-04 [1.62E-04, 2.04E-04]*	0.45
CT Met/MCSA tibia	7.82E-05 [6.38E-05, 9.26E-05]*	0.06	9.04E-06 [− 1.43E-05, 3.24E-05]	0.01
SSI Met/MCSA radius	3.53E-04 [3.40E-04, 3.67E-04]*	0.44	3.31E-04 [2.98E-04, 3.64E-04]*	0.36
SSI Met/MCSA tibia	1.85E-04 [1.60E-04, 2.09E-04]*	0.11	3.95E-05 [− 2.56E-07, 7.92E-05]	0.02
SSI Met/SSI radius	3.29E-03 [3.14E-03, 3.44E-03]*	0.51	2.49E-03 [2.17E-03, 2.80E-03]*	0.38
SSI Met/SSI tibia	7.29E-04 [6.74E-04, 7.84E-04]*	0.30	7.41E-04 [6.04E-04, 8.79E-04]*	0.27

Log-transformed data are presented as β coefficients with 95% confidence intervals [CI] and coefficients of determination (R^2)

BW bone width (mm), *CT* cortical thickness (mm), *MCSA* muscle cross-sectional area (mm^2), *SSI* stress-strain index (mm^3), *Met* metacarpal

*$p < 0.0001$

and metacarpal bone revealed that tibial bone width growth rate among black females and white females (0.23 and 0.31 mm/year, respectively) was double that of the metacarpal (0.12 and 0.14 mm/year, respectively), whilst tibial bone width growth rate among black males and white males (0.68 and 0.82 mm/year, respectively) was 3–4 times greater than that of the metacarpal bone (0.22 and 0.28 mm/year, respectively).

Cortical thickness

The cortical thicknesses at the metacarpal, radial and tibial bone sites by years from peak metacarpal length velocity are shown in Supplementary Fig. 5. In all four groups, cortical thickness increased significantly over the period of study in the metacarpal, radius and tibia (Table 3). The average annual rate of growth in cortical thickness in the metacarpal and radius was similar to each other in all groups, except in white females in whom the radial cortical thickness growth rate was 0.04 mm/year less than that of the metacarpal ($p < 0.05$). In white males, the average annual cortical thickness growth rates of the three bones were similar. In the other three groups, the average annual increase in tibial cortical thickness growth rate was significantly greater than that of the metacarpal.

Medullary width

Metacarpal, radial and tibial medullary width growth patterns are presented in Supplementary Fig. 6, and the average annual changes in medullary width are shown in Table 4. Black and white females experienced a significant average decrease in metacarpal medullary width growth rate of − 0.03 and − 0.09 mm/year, respectively. The average annual change in metacarpal medullary width in white males was − 0.07 mm/year, while in black males, a significant increase in the metacarpal medullary width of 0.06 mm/year was noted. At the radius, medullary width decreased significantly in all four groups, while at the tibia, both black and white males experienced significant increases in medullary width of 0.17–0.20 mm/year. In females, the average annual change in tibial medullary width was not significant.

When comparing the metacarpal medullary width change to that of the radius, there were no significant differences in any of the groups, except in white females who experienced a greater average increase in radial

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Table 2 Results from mixed-effects growth models summarising differences in metacarpal, radial and tibial bone width growth

Outcome	Fixed effects					Random effects		
Bone width	YPLV	Bone	Intercept	Slope [CI]	Age × Site [CI]	Intercept	Slope	Residual
BF		Met	7.39	0.12 [0.11, 0.13]*				
	0.31 [0.25, 0.38]*	Rad	10.34	0.08 [0.04, 0.12]*	−0.004 [−0.03, 0.02]	0.72	0.0000	0.40
		Tib	21.14	0.23 [0.20, 0.26]*	0.10 [0.04, 0.15]*			
WF		Met	7.21	0.14 [0.12, 0.17]*				
	0.35 [0.23, 0.48]*	Rad	9.89	0.05 [−0.03, 0.12]	0.02 [−0.03, 0.07]	0.63	0.0000	0.40
		Tib	20.24	0.31 [0.25, 0.37]*	0.16 [0.06, 0.26]¥			
BM		Met	7.77	0.22 [0.20, 0.24]*				
	0.63 [0.55, 0.70]*	Rad	10.89	0.19 [0.15, 0.23]*	−0.01 [−0.06, 0.03]	0.87	0.001	0.71
		Tib	23.16	0.68 [0.64, 0.73]*	0.23 [0.17, 0.29]*			
WM		Met	7.35	0.28 [0.22, 0.33]*				
	0.94[0.77, 1.11]*	Rad	10.27	0.15[0.03, 0.27]¥	−0.02 [−0.12, 0.07]	1.04	0.005	0.65
		Tib	22.28	0.82 [0.73, 0.90]*	0.21 [0.10, 0.32]*			

Fixed effects = the intercept is the mean value for the outcome variable. Slopes represent annual rates of accrual as assessed by YPLV and are presented with 95% confidence intervals [CI]

Random effects = between-participant variation in intercepts and slopes with residual variances

Age × Site represents the slopes of the radius and tibia relative to the metacarpal bone

BF black female, *WF* white female, *BM* black male, *WM* white male, *YPLV* years from peak metacarpal length velocity, *Met* metacarpal, *Rad* radius, *Tib* tibia

Significant differences are given as $*p < 0.0001$, $**p < 0.001$, $¥p < 0.01$

medullary width of 0.06 mm/year relative to the metacarpal. Tibial medullary width increased significantly relative to the metacarpal in males, but not in females.

Stress-strain index

The SSI at the metacarpal, radial and tibial bone sites by years from peak metacarpal length velocity are shown in Supplementary Fig. 7. In all four groups, the SSI increased significantly in each bone, except for tibial SSI in white females in whom the average annual increase was not significant (Table 5). There was a greater average annual increase in radial than metacarpal SSI in black females and white males. Tibial SSI increased significantly compared to the metacarpal SSI in white females and black males (Table 5).

Discussion

In this longitudinal study, the growth patterns of weight- and non-weight-bearing appendicular bones, measured by radiogrammetry or pQCT, were assessed in black and white SA children through puberty and adolescence. We have shown that metacarpal bone width and cortical thickness are strongly associated with radial MCSA but not with tibial MCSA. Our findings are consistent with those of a pQCT study conducted in adults which showed a strong correlation between metacarpal bone mass and forearm muscle mass in both individuals with rheumatoid arthritis and healthy controls [27]. In the present study, the variance in metacarpal bone measures explained by radial and tibial MCSA was 1–45% in males, but only 0.7–20% in females. A sex difference has been previously reported in a study of young adults aged between 19 and 22 years in whom lean mass, measured by dual-energy x-ray absorptiometry, explained 34.5% of the variability in whole-body bone mineral density in males but only 18.3% of the variability in females [28]. This sex disparity which arises during puberty, is probably related to differences in muscle mass developing during this period [29]. It is of interest that peak muscle mass gain precedes peak bone mass gain by several months, as shown in a Canadian study in which peak muscle mass gain preceded peak bone mass gain by 0.36 years and 0.51 years in boys and girls, respectively [30], suggesting a role for muscle mass in determining peak bone mass.

We showed that there were no differences in the growth of bone width between the non-weight-bearing metacarpal and radial bones in all four groups when aligned on PLV; however, bone width growth of the weight-bearing tibia was significantly greater than that of the metacarpal bone, even after adjusting for the influence of the associated musculature. The greater increases in cross-sectional bone dimensions in the tibia may have been the result of mechanical loading [31–33] due to body weight and muscle contraction, the latter

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Table 3 Results from mixed-effects growth models summarising differences in metacarpal, radial and tibial cortical thickness growth

Outcome	Fixed effects					Random effects		
Cortical thickness	YPLV	Bone	Intercept	Slope [CI]	Age × Site [CI]	Intercept	Slope	Residual
BF		Met	2.88	0.14 [0.11, 0.17]*				
	0.28 [0.23,0.34]*	Rad	3.60	0.19 [0.16, 0.23]*	−0.01 [−0.03, 0.02]	0.24	0.0000	0.29
		Tib	7.53	0.22 [0.20, 0.25]*	0.11 [0.06, 0.16]*			
WF		Met	2.94	0.22 [0.17, 0.27]*				
	0.33 [0.23, 0.43]*	Rad	3.45	0.19 [0.14, 0.24]*	−0.04 [−0.08, −0.01]	0.13	0.0004	0.23
		Tib	8.00	0.32 [0.27, 0.37]*	0.10 [0.03, 0.18]¥			
BM		Met	2.72	0.16 [0.12, 0.19]*				
	0.27 [0.22,0.32]*	Rad	3.44	0.25 [0.22, 0.28]*	0.01 [−0.02, 0.05]	0.28	0.0000	0.37
		Tib	8.08	0.39 [0.36, 0.42]*	0.07 [0.02, 0.11]¥			
WM		Met	3.08	0.32 [0.23, 0.41]*				
	0.59 [0.48,0.70]*	Rad	3.19	0.29 [0.22, 0.35]*	−0.05 [−0.11, 0.01]	0.27	0.0004	0.30
		Tib	8.92	0.46 [0.40, 0.52]*	0.04 [−0.03, 0.11]			

Fixed effects = the intercept is the mean value for the outcome variable. Slopes represent annual rates of accrual as assessed by YPLV and are presented with 95% confidence intervals [CI]

Random effects = between-participant variation in intercepts and slopes with residual variances

Age × Site represents the slopes of the radius and tibia relative to the metacarpal bone

BF black female, *WF* white female, *BM* black male, *WM* white male, *YPLV* years from peak metacarpal length velocity, *Met* metacarpal, *Rad* radius, *Tib* tibia

Significant differences are given as $*p < 0.0001$, $**p < 0.001$, ¥$p < 0.01$, $p < 0.05$

creating the largest physiologic load on bone thus influencing bone strength [34].

In this study, cortical thickness of the three bones increased significantly during adolescence with a similar average annual rate in the metacarpal and radius in all groups, except in white females who had a lower rate of cortical thickness growth at the radius than the metacarpal. Tibial cortical thickness growth rate exceeded that of the metacarpal in all groups, except in white males. In an anthropological study of individuals aged between 1 and 30 years, Sumner et al. [31] found that cortical area as a function of bone length increased more rapidly in the weight-bearing femur than in the non-weight bearing humerus. The bone width and cortical thickness growth patterns that we observed in the weight-bearing tibia compared to the non-weight-bearing metacarpal are analogous to those reported in the femur and humerus by Sumner et al. [31].

Sexual dimorphism, irrespective of ethnicity, was apparent in the velocities of bone width and cortical thickness growth of the participants in our study, particularly in the tibia. Male participants experienced greater velocities which may be associated with higher rates of bone turnover [4, 35] and greater cortical porosity [36]. Whilst we did not analyse bone turnover or cortical porosity, it bears consideration, since the inverse relationship between cortical porosity and bone strength is exponential, with even a small increase in porosity resulting in a relatively large decrease in strength [37]. Changes in microstructure that accompany the greater growth rates in males may contribute to their higher fracture incidence [38] despite having superior strength, determined by SSI, than females [5].

In this study, the medullary width growth patterns differed between the three bones. In the non-weight-bearing bones there was an overall narrowing of the medullary cavity in all groups except for the metacarpal in black males in whom it expanded. At the tibia, the increase in medullary width was only significant in males. We have previously shown that there are ethnic differences in medullary width growth between black and white males in our cohort [7]. The contraction in metacarpal medullary width in our white males differs from cross-sectional studies of white children in the USA [39] but concurs with a more recent longitudinal study in European children [40], which suggests population differences in medullary cavity growth. The overall contraction of radial medullary width in both male and female participants in our study differs from that of a cross-sectional pQCT study at the 65% radius of white individuals between 6 and 40 years of age by Neu and co-workers [33]. In their study, from Tanner stage 3 onwards, there was no change in medullary width in females, whilst in males, it increased significantly which they attributed to modelling drift, that is, endocortical bone resorption in response to bone expansion as a result of periosteal bone deposition [33]. In contrast, in both sexes in our study, radial medullary width contraction was accompanied by bone width expansion indicating that bone deposition occurred at both the

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Table 4 Results from mixed-effects growth models summarising differences in metacarpal, radial and tibial medullary width change

Outcome	Fixed effects					Random effects		
Medullary width	YPLV	Bone	Intercept	Slope [CI]	Age × Site [CI]	Intercept	Slope	Residual
BF		Met	4.38	−0.03 [−0.06, −0.001]				
	0.03 [−0.05, 0.11]	Rad	6.56	−0.12 [−0.18, −0.06]*	0.004 [−0.03, 0.04]	0.80	0.0000	0.67
		Tib	13.61	0.01 [−0.02, 0.04]	0.005 [−0.07, 0.08]			
WF		Met	4.15	−0.09 [−0.14, −0.04]**				
	0.03 [−0.12, 0.18]	Rad	6.32	−0.15 [−0.26, −0.04]¥	0.06 [0.002, 0.12]	0.38	0.0000	0.56
		Tib	12.16	−0.02 [−0.08, 0.05]	0.12 [−0.001, 0.24]			
BM		Met	4.97	0.06 [0.02, 0.09]¥				
	0.33 [0.24, 0.43]*	Rad	7.41	−0.07 [−0.12, −0.01]	−0.03 [−0.09, 0.03]	1.30	0.0000	1.20
		Tib	15.10	0.29 [0.24, 0.34]*	0.17 [0.09, 0.25]*			
WM		Met	4.09	−0.07 [−0.16, 0.03]				
	0.26 [0.11, 0.42]*	Rad	6.94	−0.16 [−0.31, −0.02]	0.03 [−0.06, 0.12]	0.69	0.0000	0.63
		Tib	13.33	0.35 [0.27, 0.44]*	0.20 [0.09, 0.30]*			

Fixed effects = the intercept is the mean value for the outcome variable. Slopes represent annual rates of accrual as assessed by YPLV and are presented with 95% confidence intervals [CI]

Random effects = between-participant variation in intercepts and slopes with residual variances

Age × Site represents the slopes of the radius and tibia relative to the metacarpal bone

BF black female, *WF* white female, *BM* black male, *WM* white male, *YPLV* years from peak metacarpal length velocity, *Met* metacarpal, *Rad* radius, *Tib* tibia

Significant differences are given as $*p < 0.0001$, $**p < 0.001$, ¥$p < 0.01$, $p < 0.05$

Table 5 Results from mixed-effects growth models summarising differences in metacarpal, radial and tibial stress-strain index change

Outcome	Fixed Effects					Random Effects		
Stress-strain index	YPLV	Bone	Intercept	Slope [CI]	Age × Site [CI]	Intercept	Slope	Residual
BF		Met	392.00	38.59 [33.49, 43.69]*				
	48.39 [35.65, 61.14]*	Rad	115.00	14.08 [11.80, 16.36]*	7.87 [2.30, 13.45]¥	1.53E+04	0.0000	1.61E+04
		Tib	915.00	41.69 [29.00, 54.38]*	2.90 [−8.22, 14.03]			
WF		Met	357.00	34.08 [25.44, 42.71]*				
	54.62 [29.68, 79.65]*	Rad	61.97	13.61 [8.95, 18.27]*	5.29 [−4.28, 14.85]	1.17E+04	0.0000	1.55E+04
		Tib	852.00	19.17 [−4.44, 42.78]	23.49 [3.75, 43.22]			
BM		Met	324.00	66.86 [57.23, 76.49]*				
	99.79 [84.14, 115.00]*	Rad	144.00	23.35 [20.20, 26.49]*	5.07 [−5.34, 15.49]	2.64E+04	40.94	3.42E+04
		Tib	1230.00	104.00 [91.68, 116.00]*	23.78 [10.28, 37.27]¥			
WM		Met	263.00	95.95 [68.29, 124.00]*				
	121.00 [84.83, 158.00]*	Rad	73.47	22.65 [12.62, 32.68]*	22.65 [1.31, 44.00]	3.37E+04	0.0000	3.50E+04
		Tib	1000.00	114.00 [90.19, 138.00]*	16.12 [−8.66, 40.89]			

Fixed effects = the intercept is the mean value for the outcome variable. Slopes represent annual rates of accrual as assessed by YPLV and are presented with 95% confidence intervals [CI]

Random effects = between-participant variation in intercepts and slopes with residual variances

Age × Site represents the slopes of the radius and tibia relative to the metacarpal bone

BF black female, *WF* white female, *BM* black male, *WM* white male, *YPLV* years from peak metacarpal length velocity, *Met* metacarpal, *Rad* radius, *Tib* tibia

Significant differences are given as $*p < 0.0001$, $**p < 0.001$, ¥$p < 0.01$, $p < 0.05$

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periosteal and endocortical surfaces. While we adjusted for the influence of the associated musculature on bone growth in our study, we did not assess hormonal influences, which have been shown to differentially affect bone growth in the sexes [4]. Kirmani and co-workers [4] found that radial periosteal and endocortical bone accrual were driven by insulin-like growth factor-1 (IGF-1) in girls and by testosterone in boys. The greater expansion of tibial medullary width that we observed in male participants concurs with other longitudinal studies [5] and reflects the bone's compensatory mechanism to resist bending and torsional forces as the cortices move further from the bone's neutral axis. The site-specific differences at the endocortical surface that we observed suggest that in weight-bearing bones, there is modelling drift and that this functional adaptation to increase bone strength may possibly override hormonal influences.

The SSI, which is the product of the section modulus of the bone and the ratio of measured cortical density to physiologic bone density (1200 mg/cm^3), has been shown to be a strong proxy for bone strength and failure of loading [20]. We observed greater average annual changes in SSI of the metacarpal, radius and tibia in males than in females, which is in keeping with the greater increase in male bone width and cortical thickness over the period of measurement and consistent with mixed longitudinal pQCT and high-resolution pQCT studies of the radius and tibia in Canadian adolescents [5, 41]. Of interest, was the negligible change in tibial SSI in females despite an increase in bone width and cortical thickness through adolescence. Whilst a lower SSI might suggest that females may be more susceptible to fracture, childhood tibial fractures occur relatively infrequently. Up to the age of 12 years, tibial fracture incidence has been shown to be higher in British females compared to males [42]. Sex differences in proxies for tibial strength (section modulus and bone strength index), have previously been reported in a study of stress fracture in military recruits who averaged 19 years of age [43]. In male military recruits who had fractured, lower tibial strength indices were attributed to narrower bone widths, in contrast to female recruits who had fractured in whom lower tibial strength indices were a function of thinner cortices [43]. In our study, we did not differentiate between participants with and without a history of fracture and we are therefore unable to comment on the association between SSI and tibial fracture in our cohort.

The study has a number of limitations. Although we adjusted for differences in somatic maturity by using PLV, we assumed that 1 year of biological age is equivalent to 1 year of chronological age. This only accounted for differences in the timing (tempo) of growth and not for potential differences in the rate (velocity) of growth [8, 44]. Furthermore, the timing of peak growth velocity varies according to anatomical site [8, 44]. The effects of physical activity on bone are probably mediated via muscle [45] and although we did not consider physical activity in our analyses, we included MCSA to try to account for this. Our longitudinal data of bone growth of the metacarpal, radius and tibia in black and white children are unique; however, the number of white participants was relatively small which may have led to a type II error. This study only included radiographic and pQCT measurements from chronological age 12 years, by which time peak growth may have commenced or been completed in some individuals, particularly in females. Our data were obtained using two different technologies and at different diaphyseal regions; radiogrammetry was done at the midshaft of the metacarpal bones, and pQCT at the 65% radial and 38% and 65% tibial sites. The ratio of cortical to trabecular bone and the associated musculature of the three bones at these sites may differ. Although bone growth is region-specific, we do not believe that standardising measurements of all three bones at the diaphyseal midshaft would have changed the outcomes.

In conclusion, longitudinal analyses through adolescence showed that in both sex and ethnic groups, when aligned on a physiological maturity indicator, the growth patterns of the non-weight-bearing metacarpal bone and radius were overall similar to each other but differed from that of the weight-bearing tibia. The extent of sexual dimorphism in bone width growth patterns was similar in both ethnic groups, suggestive of common biological mechanisms which regulate functional adaptation, despite different genetic backgrounds.

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Compliance with ethical standards

Conflicts of interest None.

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Appendix L: PDF of Metacarpal indices and their association with fracture in South African children and adolescents

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Metacarpal Indices and Their Association with Fracture in South African Children and Adolescents

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Abstract
This prospective study assessed whether metacarpal indices predict fracture risk in children and adolescents. Radiogrammetry was performed at the second metacarpal midshaft on annual hand–wrist radiographs of 359 South African (SA) children aged 10–17 years. Bone length, bone width, and medullary width were measured, and the following proxies for bone strength calculated: metacarpal index (MCI), bone mineral density (BMD), section modulus (SM), stress–strain index (SSI), and slenderness index (SLI). Height and weight were measured annually. Self-reported physical activity (PA) and fracture history were obtained at ages 15 years (for the preceding 12 months) and 17 years, respectively. At 17 years, 82 (23%) participants (black, 16%; white, 42%; $p < 0.001$) reported a previous fracture. None of the bone measures or indices were associated with fracture in black participants. In white females, after adjusting for PA, a 1 standard deviation (SD) greater SLI doubled the fracture risk [odds ratio (OR) 2.08; 95% confidence interval (CI) 1.08, 3.98]. In white males, a 1 SD greater BMD was associated with a 2.62-fold increase in fracture risk (OR 3.62; 95% CI 1.22, 10.75), whilst a 1 SD greater SM (OR 2.29; 95% CI 1.07, 4.89) and SSI (OR 2.23; 95% CI 1.11, 4.47) were associated with a more than twofold increase in fracture risk, after height, and PA adjustment. No single index consistently predicted fracture across the four groups possibly due to ethnic and sex differences in bone geometry, muscle mass, and skeletal loading. Metacarpal radiogrammetry did not reliably predict fracture in SA children.

Keywords Radiogrammetry · Metacarpal indices · Bone strength · Fracture · Adolescents

Introduction

Childhood and adolescence is associated with a high incidence of fracture with at least 30% of children sustaining a fracture during this time [1–4]. Fractures in children occur predominantly during puberty, when there is dissociation between the ages of peak growth velocity and bone mineral accrual [5]. Compelling evidence exists for the antecedents of bone fragility in adulthood being established during the circumpubertal years [6–8]. Children who fracture are more inclined to fracture as adults [6, 9] and have evidence of reduced bone mineral density (BMD) and strength at previous fracture sites in adulthood [10].

The mechanical and functional properties of bone are a consequence of its morphological characteristics combined with tissue quality traits [11]. Biological constraints result in significant variation of these traits both within and across population groups [11]. Ethnic disparities in fracture incidence have been shown in US children [12]. In a South African (SA) study, a greater proportion of white (41%) than black (19%) children had fractured by age 15 years [3], despite white children having greater bone area and bone mineral content than their black peers after adjusting for differences in height and weight [3, 13]. The prevalence of fractures is also sex-specific, being greater in boys than girls [3, 4, 14]. Additionally, environmental factors such as physical activity (PA) and body mass index (BMI) independently contribute to variations in fracture incidence [13, 15].

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Metacarpal bone characteristics measured by radiogrammetry have been used to predict fracture risk in adults [16–18] and children [18–20]. There have been conflicting reports on the reliability of this method even though the strength of the association between wrist and forearm fractures and metacarpal measures obtained by radiogrammetry have been shown to be comparable to dual energy X-ray absorptiometry (DXA) [19]. The aim of this study was to determine the association between various metacarpal indices and fracture risk in a cohort of SA children and adolescents. We hypothesised that irrespective of ethnicity or sex, metacarpal bone dimensions and indices would predict fracture risk.

Materials and Methods

Participants and Protocol

The participants were members of the Bone Health Cohort (BHC) which is a sub-cohort of the Birth to Twenty Cohort (Bt20) whose participant recruitment protocols have previously been described [21]. Briefly, Bt20 is a longitudinal child health study of 3273 singleton children born over a 6-week period in 1990 in the greater Soweto-Johannesburg metropolitan area, South Africa. Children were classified as black (African descent) or white (European descent) based on the race classification used in South Africa for demographic and restitution purposes. Ethnic classification was self-reported by the parents and only those children whose parents both belonged to the same ethnic group were included in this study. Genetic studies have shown little ethnic admixture between the groups in the Bt20 cohort [22]. At age 9 years, 563 of these children were included in the BHC to investigate factors affecting the acquisition of peak bone mass during adolescence. An additional 120 white children born in the same period as the original cohort were enrolled into the BHC to compensate for the small number of white participants, which represented the population demographics of South Africa at that time. Birth weight, socioeconomic status, and maternal age and education of the additional white participants were similar to those of the existing white children of the BHC. All participants were healthy; those taking medication known to affect bone metabolism were excluded from the study.

Measurements included weight, height, and hand–wrist X-rays, and each participant's annual assessment was done at approximately the same time every year. The mean age and range at each annual assessment is presented in Supplementary Table 1.

Anthropometric Measurements

Weight and height of participants were measured whilst wearing light clothing and no shoes. Weight was measured to the nearest 100 g using a digital scale (Dismed, Miami, FL, USA) and height to the nearest millimetre using a stadiometer (Holtain, Crymych, UK). Quality control annual training and monitoring ensured a coefficient of variation between measurers of less than 2%. BMI was calculated as weight (kg)/height $(m)^2$.

Radiography

Postero-anterior hand–wrist radiographs of the left hand were taken annually between 9 and 21 years of age by certified radiographers at the Charlotte Maxeke Academic Hospital in Johannesburg. The left hand was radiographed in all participants irrespective of hand dominance. The X-ray beam was focussed on the distal aspect of the third metacarpal of the left hand. Radiographs were taken using cassettes with single-emulsion film under standard conditions of tube to film distance of 76 cm and exposure at 42 kV and 12 mA/s, and processed in an automatic developer in accordance with the optimal conditions described by Tanner [23].

Radiogrammetry

The dimensions of the second metacarpal were measured on annual radiographs from 359 participants between the ages of 10 and 17 years. Measurements were carried out using digital vernier callipers calibrated to 0.01 mm and the following parameters were obtained in millimetres: Total length of bone from proximal to distal end (L), and outer bone width (D) and medullary inner width (d) at the midshaft of the bone. The cortical thickness was calculated as the difference between the bone width and medullary width ($D - d$). Using these measurements, the following indices were calculated: MCI = $(D - d)/D$ [24], BMD = $\pi(D - d) \times [1 - ((D - d)/D)]$ [25], section modulus (SM) = $\pi[(D^4 - d^4)/32]/0.5D$ [26], slenderness index (SLI) = (bone length × body weight)/SM [27], stress–strain index (SSI) = SM × volumetric BMD [28]. A single researcher (AM) performed all measurements. Intraobserver reliability of metacarpal measurements were (i) metacarpal length 0.14%, (ii) bone width 0.80%, and (iii) medullary width 6.20% [21]. Interobserver reliability was evaluated by comparing the metacarpal measurements for the 10-year age group against measurements performed on the same participants from this cohort in a previous study. The difference between the results of the two studies was less than 3% [29].

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Fracture Questionnaire

Fracture history was obtained retrospectively at age 17 years. Participants completed a questionnaire with the assistance of their parent or caregiver. Information obtained included age at fracture, cause and site of fracture (with the aid of a skeletal diagram), by whom the fracture was diagnosed, whether radiographs were taken to confirm the fracture, and the treatment offered and performed. As our fracture data were collected retrospectively, the fractures could not be verified radiographically. Between 6 months and a year after completing the initial fracture questionnaire, 9% of the participants who were selected randomly, verified their fracture data telephonically. The grades of trauma causing fracture were classified as slight (grade 1), moderate (grade 2), and severe (grade 3) (Table 1) [3, 30]. Only those participants who had sustained fractures as a result of mild to moderate trauma were included in the analyses.

Physical Activity Levels

Self-reported PA was obtained at age 15 years for the preceding 12 months via an interviewer administered questionnaire which had been previously validated in a SA population [31]. Activities were categorized as formal (physical education, extra-mural school, and club sport) and informal (leisure time activities outside of the school or club environment). The duration of activity was recorded in minutes per week (min/week) and capped at 1680 min/week for informal activity. School and club sport were both capped at 1200 min/week.

Data Analysis

Cross-Sectional Data

Data for black and white participants were analysed separately because of differences between the two ethnic groups in anthropometry and bone mass measures obtained using radiogrammetry [21] and DXA [13]. Participants were categorized into yearly age groups for cross-sectional analyses. For example, age group 10 included participants aged between 10 and 10.9 years. Data were analyzed using STATA version 14 (Stata Corporation, Inc., College Station, TX, USA). A one-way ANOVA with a post-hoc Bonferroni multiple comparisons test (α level 0.05) was performed to determine sex and ethnic differences in fracture prevalence, and, in participant anthropometry, bone measures, bone indices, and PA levels. Participant height and BMI data were converted to Z-scores using the World Health Organisation Anthroplus software (http://www.who.int/growthref/tools/en). Black females were the largest group of non-fracturing participants and were, therefore, used as a reference group against which to compare the other groups' bone measures, bone indices, and PA scores after converting the variables to Z-scores based on black female data. The Z-scores were calculated by subtracting the reference mean from each individual score and dividing by the reference standard deviation (SD). Student t-tests were used to determine differences in Z-score bone measures between participants with and without fractures within the four ethnic and sex groups. Bivariate logistic regression analyses were used to determine the associations between anthropometric measures, bone measures, and bone indices and fracture history at 17 years separately for each sex and ethnic group. Multivariate logistic regression analyses, adjusting for height and formal PA, were also performed to examine the same associations at 17 years. Statistical significance was set at $p < 0.05$ for all analyses.

Longitudinal Data

Correlation analysis was performed on height at each year from 10 until 16 years with MCI, BMD, SM, and SSI to determine the influence of participant height on each index at different ages.

To determine the association between bone measures and indices prior to the age of peak metacarpal length velocity (PLV) [21] and a history of fracture at 17 years, logistic regression analysis was performed for each bone measure and index for the combined sex and ethnic groups of participants at 10 years of age to determine the odds ratios (ORs) for fracture at 17 years after adjusting for height.

Table 1 Grades of trauma causing fractures [3, 30]

Grades	Causes
Grade 1 (slight)	Falling to the ground from standing on the same level
	Falling from less than 0.5 m (falling from stools, chairs, and beds)
Grade 2 (moderate)	Falling from between 0.5 and 3 m
	Falling down stairs, from a bicycle, roller blades, skateboard, or swing
	Playground scuffles, sport injuries
Grade 3 (severe)	Falling from a height > 3 m (falls from windows or roofs)
	Motor vehicle or pedestrian accidents
	Injuries caused by heavy moving or falling objects (e.g., bricks or stones)

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A generalized estimating equations (GEE) model was used to analyze the correlated data of the bone measures and indices with the binary outcome of fracture. The combined data of participants from both ethnic and sex groups from ages 10 until 16 years were assessed against the cumulative fracture incidence between 10 and 17 years. A binomial family with a logit link was specified in the model. An autoregressive correlation structure was deemed the most appropriate to minimize loss of statistical power for the repeated measures. ORs were calculated for the bone indices and the binary outcome of fracture. Statistical significance was set at $p < 0.05$.

Results

Participant recruitment and number of observations are shown in Fig. 1. A total number of 359 participants, aged between 10 and 17 years, had valid hand–wrist X-rays and had completed the fracture and PA questionnaires. Attrition of participants and participants missing data collection waves during the study were due either to time constraints, challenges with transportation, or to the circular migration of individuals between their extended family in urban and rural areas.

Fracture Incidence

The mean age on completion of the fracture questionnaire was 17.69 ± 0.25 years. Eighty-two (23%) of these individuals had sustained one or more fractures during their lifetime. The percentage of white females who had fractured was more than triple that of black females (40 vs. 12%), whilst the percentage of white males who had fractured was more than double that of black males (44 vs. 20%). Within ethnic groups, there were no significant sex differences in fracture prevalence. The sites of fracture and grades of trauma are presented in Table 2. Five participants, who had sustained a fracture due to a grade 3 level of trauma, were excluded from further analyses.

Cross-Sectional Data

Cohort Characteristics at Age 17 Years

Characteristics of participants of the cohort at age 17 years are presented in Table 3. At 17 years of age, black participants were significantly shorter than their white peers. Black and white females were of similar weight; however, black males were lighter than white males. Black females and males had thinner cortices, greater medullary widths, and hence a lower MCI than their white peers. Black and white females had shorter and narrower bones with smaller medullary cavities than their respective male peers. Females, therefore, had lower BMD, SM, and SSI than males. Females had more slender bones than males as reflected by their higher SLI. At 15 years, black participants engaged in significantly less formal PA than their white peers.

Comparison of Fracture and Non-fracture Groups

Comparisons of anthropometry, bone measures and indices between the fractured and non-fractured groups at age 17 years are presented in Table 4. The only anthropometric difference between those who had and had not fractured was in white females, with those who had fractured having a significantly higher BMI Z-score than those who had not ($p = 0.04$). Metacarpal length, bone width, and cortical thickness did not differ between those who had and had not fractured in black females, black males, and white females. However, white males who had fractured had wider bones ($p = 0.03$) and greater medullary widths ($p = 0.02$) than those without fractures. There were no differences in any of the bone indices between the fracture and non-fracture groups in black participants. In white participants, females who had fractured had a greater SLI than their non-fractured peers ($p = 0.01$), while males who had fractured had lower MCI and SLI but higher BMD, SM, and SSI than their non-fracturing peers.

Associations Between Anthropometric Characteristics and History of Fractures at Age 17 Years

Bivariate logistic regression analyses showed that in white females a 1 SD greater BMI Z-score was associated with a 93% increase in fracture risk (OR 1.93; 95% confidence interval, CI 1.01, 3.71) (Supplementary Table 2). None of the anthropometric measures were associated with fracture risk in the other three groups (Supplementary Table 2).

Associations Between Bone Measures and History of Fractures at Age 17 Years

Bivariate logistic regression analyses showed that in white males, a 1 SD greater bone width was associated with a 96% increase in fracture risk (OR 1.96; 95% CI 1.03, 3.72) (Supplementary Table 2). When adjusted for height and PA (Fig. 2), the OR increased to 2.77 (95% CI 1.09, 7.09). In the same group, the odds for fracture increased 2.2-fold with a 1 SD greater medullary width (OR 2.22; 95% CI 1.05, 4.69) (Supplementary Table 2), however, the association was no longer significant when adjusted for height and PA (Fig. 2). In the other three groups, none of the bone measures were associated with fracture risk.

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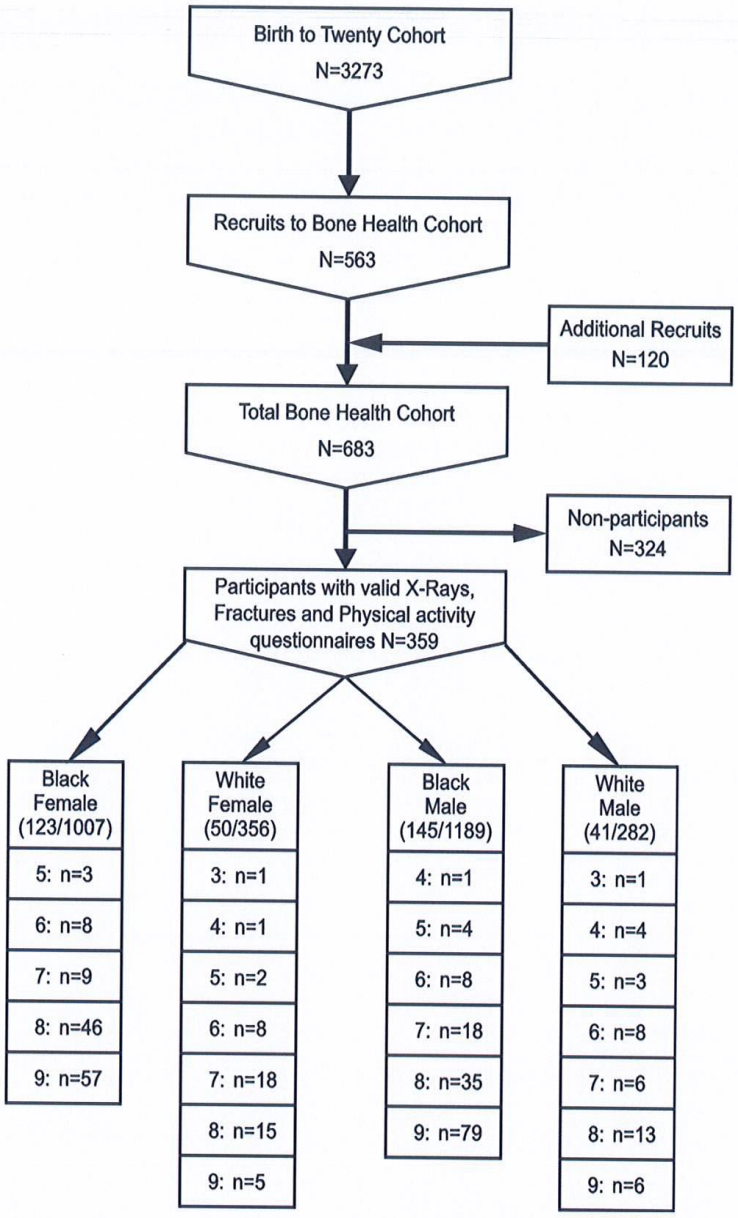

Fig. 1 Participant recruitment (*N*) and the number of participants (*n*) with their respective number of observations

Table 2 Cohort fractures by site and grade of trauma

Sites	Grade 1	Grade 2	Grade 3
Upper limb	10 (12%)	36 (44%)	0
Lower limb	6 (7%)	19 (23%)	4 (5%)
Other	0	6 (7%)	1 (1%)

Associations Between Bone Indices and History of Fractures at Age 17 Years

Bivariate logistic regression analyses showed that in white females a 1 SD greater SLI was associated with a twofold increase in odds for fracture (OR 2.00; 95% CI 1.11, 3.61) (Supplementary Table 2). After adjusting for PA, the OR did not change (OR 2.08; 95% CI 1.08, 3.98; Fig. 2). In contrast,

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Table 3 Cohort anthropometry, bone measures and bone indices at 17 years of age, and physical activity at 15 years of age

Variables	Black female ($n=123$)			White female ($n=50$)		
	Mean	SD		Mean	SD	
Height (cm)	158.20	6.13	ab	165.95	7.14	d
Weight (kg)	59.12	11.07		61.70	12.98	d
TL (mm)	65.38	3.70	b	65.56	3.85	d
BW (mm)	8.10	0.71	b	8.01	0.56	d
MW (mm)	3.98	0.87	ab	3.47	0.92	d
CT (mm)	4.12	0.53	a	4.54	0.59	d
MCI	0.51	0.08	ab	0.57	0.09	
BMD (g/mm^2)	6.21	0.61	b	5.97	0.71	d
SM (mm^3)	99.23	25.28	b	97.50	18.66	d
SSI (mm^3)	630.10	227.70	b	592.79	178.99	d
SLI (1/mm^2/kg)	40.69	10.39	b	42.84	11.91	d
FPA (min/week)	11.18	22.17	ab	44.32	58.45	
InFPA (min/week)	16.36	30.50		14.51	28.54	
Variables	Black male ($n=145$)			White male ($n=41$)		
	Mean	SD		Mean	SD	
Height (cm)	170.44	6.65	c	178.46	9.18	
Weight (kg)	59.45	9.13	c	72.01	12.61	
TL (mm)	70.53	3.87		70.94	3.74	
BW (mm)	9.14	0.79		9.16	0.83	
MW (mm)	4.83	1.07	c	4.20	0.94	
CT (mm)	4.31	0.68	c	4.96	0.59	
MCI	0.48	0.09	c	0.54	0.07	
BMD (g/mm^2)	6.95	0.64		6.99	0.76	
SM (mm^3)	138.89	32.43		146.04	36.44	
SSI (mm^3)	982.58	312.23		1045.41	353.15	
SLI (1/mm^2/kg)	31.41	7.32	c	36.78	10.26	
FPA (min/week)	26.21	31.60		33.42	34.67	
InFPA (min/week)	21.12	35.30		22.82	27.02	

a–d represent significant sex and ethnic differences: a—black female versus white female, b—black female versus black male, c—black male versus white male, d—white female versus white male ($p<0.05$). Data are presented as means with standard devisations (SD)

TL total length, *BW* bone width, *MW* medullary width, *CT* cortical thickness, *MCI* metacarpal index, *BMD* bone mineral density, *SM* section modulus, *SSI* stress–strain index, *SLI* slenderness index, *FPA* formal physical activity, *InFPA* informal physical activity

greater SLI in black females was associated with a reduced risk of fracture (OR 0.51; 95% CI 0.26, 1.00) (Supplementary Table 2), however, this was no longer significant when adjusted for PA (Fig. 2).

In white males, a 1 SD greater MCI was associated with a 55% decreased risk of fracture (OR 0.45; CI 0.20, 0.99) (Supplementary Table 2) which was no longer significant after adjusting for height and PA (Fig. 2). The bone indices BMD, SM, and SSI were associated with fracture risk in white males before and after adjusting for height and PA (Supplementary Table 2; Fig. 2). When adjusted for height and PA, a 1 SD greater BMD increased the risk of fracture threefold (OR 3.62; 95% CI 1.22, 10.75), and a 1 SD greater SM and SSI increased the odds of fracture by 129% (OR 2.29; 95% CI 1.07, 4.89) and 123% (OR 2.23; 95% CI 1.11, 4.47), respectively (Fig. 2).

Longitudinal Data

The bone indices BMD, SM, and SSI were moderately correlated with height at each year from age 10 until 16 years ($r=0.32$–0.55) (Supplementary Table 3). There was no consistent relationship between MCI and height across the age range (Supplementary Table 3).

When the association between bone indices prior to PLV and adolescent fracture history were assessed, logistic regression showed that for the combined groups of participants, none of the bone indices measured at age 10 years

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Table 4 Sex and ethnic differences in mean *Z*-scores with standard deviations (SD) for anthropometric measures, bone measures, metacarpal indices, and physical activity in 17-year old participants with and without a history of fracture

Variables	Black female			White female		
	No-fracture ($N=108$)	Fracture ($N=15$)	p	No-fracture ($N=30$)	Fracture ($N=20$)	p
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
BMI	0.51 (1.14)	0.23 (0.84)	ns	−0.08 (0.95)	0.57 (1.24)	0.04
Height	−0.53 (0.92)	−0.63 (0.95)	ns	0.46 (1.04)	0.41 (1.16)	ns
TL	0.00 (1.00)	−0.05 (1.24)	ns	−0.11 (0.97)	0.28 (1.19)	ns
BW	0.00 (1.00)	0.34 (1.22)	ns	0.00 (0.83)	−0.21 (0.78)	ns
MW	0.00 (1.00)	0.21 (1.31)	ns	−0.39 (1.01)	−0.88 (1.17)	ns
CT	0.01 (1.00)	0.11 (0.84)	ns	0.61 (0.91)	1.09 (1.28)	ns
MCI	0.04 (0.97)	−0.03 (1.05)	ns	0.55 (1.02)	1.09 (1.29)	ns
BMD	0.00 (1.00)	0.25 (0.96)	ns	−0.12 (1.12)	−0.72 (1.18)	ns
SM	0.00 (1.00)	0.34 (1.33)	ns	0.05 (0.81)	−0.14 (0.72)	ns
SSI	0.00 (1.00)	0.33 (1.39)	ns	−0.01 (0.86)	−0.31 (0.77)	ns
SLI	0.00 (1.00)	−0.54 (0.56)	ns	−0.18 (0.79)	0.60 (1.36)	0.01
FPA	0.02 (0.94)	−0.03 (0.52)	ns	1.59 (2.69)	1.04 (1.85)	ns
InFPA	0.00 (1.08)	−0.16 (0.62)	ns	−0.05 (1.13)	−0.11 (0.69)	ns
Variables	Black male			White male		
	No-fracture ($N=116$)	Fracture ($N=29$)	p	No-fracture ($N=23$)	Fracture ($N=18$)	p
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
BMI	−0.59 (1.11)	−0.71 (0.90)	ns	−0.04 (0.89)	0.52 (0.95)	ns
Height	−0.61 (0.89)	−0.64 (1.07)	ns	0.09 (1.22)	0.64 (1.17)	ns
TL	1.41 (1.05)	1.47 (1.19)	ns	1.44 (1.18)	1.67 (0.84)	ns
BW	1.54 (1.11)	1.60 (1.27)	ns	1.22 (1.12)	2.04 (1.18)	0.03
MW	1.01 (1.27)	1.15 (1.28)	ns	−0.07 (1.14)	0.74 (0.95)	0.02
CT	0.41 (1.20)	0.26 (1.48)	ns	1.66 (1.04)	1.46 (1.16)	ns
MCI	−0.40 (1.07)	−0.55 (1.15)	ns	0.70 (0.99)	0.10 (0.72)	0.04
BMD	1.24 (1.00)	1.23 (1.28)	ns	0.87 (1.31)	1.88 (0.91)	0.01
SM	1.67 (1.29)	1.74 (1.56)	ns	1.53 (1.27)	2.55 (1.63)	0.03
SSI	1.65 (1.38)	1.75 (1.71)	ns	1.43 (1.36)	2.64 (1.73)	0.02
SLI	−0.94 (0.66)	−0.91 (0.80)	ns	−0.33 (1.11)	−0.56 (0.73)	ns
FPA	0.57 (1.27)	0.85 (1.34)	ns	0.35 (0.92)	1.53 (1.61)	0.02
InFPA	0.11 (1.15)	0.30 (1.36)	ns	0.24 (1.12)	0.16 (0.67)	ns

Non-fracturing black females were used as a reference group to develop *Z*-scores for the bone measures and indices. WHO reference standards were used for anthropometry. *p*-values represent significant differences between fracture and non-fracture groups

ns no significant differences between fracture and non-fractured groups, *TL* total length, *BW* bone width, *MW* medullary width, *CT* cortical thickness, *MCI* metacarpal index, *BMD* bone mineral density, *SM* section modulus, *SSI* stress–strain index, *SLI* slenderness index, *FPA* formal physical activity, *InFPA* informal physical activity

significantly predicted fracture history at age 17 years (data not shown).

Longitudinal analyses of the bone measures and indices from ages 10 until 16 years and the odds of a positive fracture history by 17 years of age, before and after adjusting for height, using GEE for the combined groups are presented in Table 5. Increases in cortical thickness, MCI, and BMD were associated with decreases in fracture risk when unadjusted, but after adjusting for height, the relationships were reversed. When unadjusted, a 1 mm increase in cortical thickness between 10 and 16 years was associated with a 1% lower risk of fracture history at age 17 years ($p=0.03$). A unit increase in MCI between ages 10 and 16 years resulted in a 9% decrease risk of fracture risk by age 17 years ($p=0.02$), whilst a unit increase in BMD was associated with a 1% decreased risk of fracture by 17 years of age ($p=0.03$) using unadjusted data. After adjusting for height, there was a slight increase in fracture risk by age 17 years for a unit increase in CT ($p=0.01$), MCI ($p=0.02$), and BMD ($p=0.01$) between ages 10 and 16 years.

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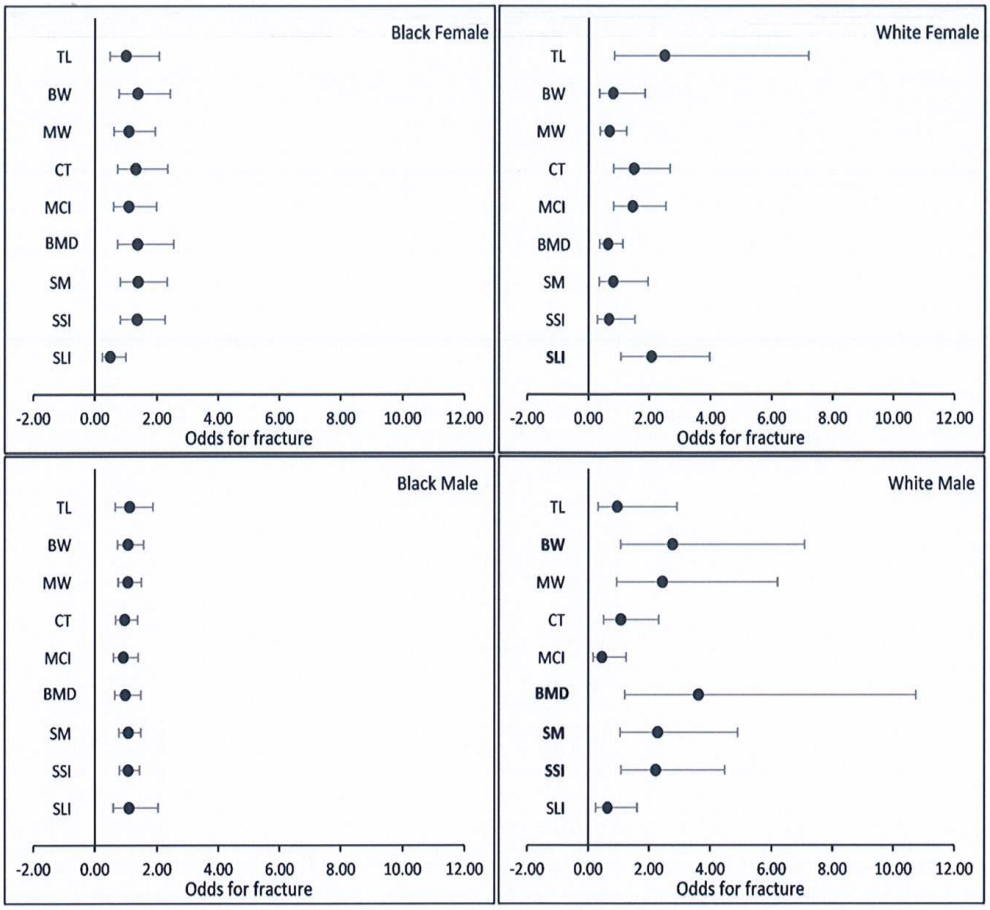

Fig. 2 Odds ratios for having a history of fracture, based on bone measures and indices at 17 years of age, after covarying for height and formal physical activity. Data are presented as odds ratios with 95% confidence intervals. *TL* total length, *BW* bone width, *MW* medullary width, *MCI* metacarpal index, *CT* cortical thickness, *BMD* bone mineral density, *SM* section modulus, *SSI* stress–strain index, *SLI* slenderness index. SLI covaried for formal physical activity only. Significant p-values < 0.05 are in bold

Discussion

Our study of SA children and adolescents from a longitudinal cohort comprising two ethnic groups assessed metacarpal bone measures and indices and their association with fracture risk during childhood and adolescence. We observed ethnic differences in fracture prevalence in the first 17 years of life, with substantially higher incidences in both white females and males relative to their black peers, consistent with results obtained at 10 and 15 years in the Bt20 cohort [13]. Similarly, ethnic differences in fracture incidence have previously been reported in SA adults in whom black individuals had a lower incidence of femoral neck fractures [32] even though their metacarpal BMD was lower than that of their white peers [33]. Whilst these studies taken together suggest that genetics may play a role in protecting against fracture, extrinsic factors which affect the attainment of peak bone mass may also contribute to the aetiology of skeletal fragility in late adulthood [6].

Fracture prevalence has been shown to be a function of bone geometry [34], thus geometric indices obtained by radiogrammetry such as the MCI and BMD, both of which reflect bone size and cortical thickness, have been the most widely used metacarpal indices to determine fracture risk [17, 19, 35]. Studies on both adults [16] and children [19] have shown a low MCI to be associated with a greater fracture risk. Even though we have previously shown that black children have thinner cortices relative to bone width [21], and hence a lower MCI than white children, we have also shown that they fractured less. In keeping with the above

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Table 5 GEE assessment of metacarpal bone measures and indices for the combined sex and ethnic groups from ages 10 until 16 years and odds ratios for a positive fracture history at age 17 years

Variables	Odds ratio	SE	95% CI	*p*
BW	1.004	4.75E−03	[0.99, 1.01]	0.44
Ht	1.00003	1.80E−04	[0.9997, 1.0004]	0.85
BW × Ht	1.000002	2.11E−05	[0.99996, 1.00004]	0.92
CT	0.99	3.93E−03	[0.98, 0.9989]	**0.03**
Ht	1.00001	9.07E−05	[0.9998, 1.0002]	0.92
CT × Ht	1.00006	2.29E−05	[1.00002, 1.0001]	**0.01**
MCI	0.91	3.81E−02	[0.84, 0.99]	**0.02**
Ht	0.99997	1.32E−04	[0.9997, 1.0002]	0.83
MCI × Ht	1.0006	2.51E−04	[1.0001, 1.001]	**0.02**
BMD	0.99	3.64E−03	[0.99, 0.999]	**0.03**
Ht	0.99998	9.50E−05	[0.9998, 1.0002]	0.84
BMD × Ht	1.00005	2.12E−05	[1.00, 1.0001]	**0.01**
SM	1.00006	1.68E−04	[0.9997, 1.0004]	0.71
Ht	1.0001	8.64E−05	[0.9999, 1.0003]	0.27
SM × Ht	1.00	7.99E−07	[0.999999, 1.000002]	0.92
SSI	1.00	2.06E−05	[0.99996, 1.00004]	0.98
Ht	1.0001	8.29E−05	[0.99996, 1.0003]	0.13
SSI × Ht	1.00	1.02E−07	[0.9999998, 1.00]	0.73

Data are presented as odds ratios with standard error of means (SE) and 95% confidence intervals (CI). Significant *p*-values < 0.05 are in bold

BW bone width, *Ht* height, *CT* cortical thickness, *MCI* metacarpal index, *BMD* bone mineral density, *SM* section modulus, *SSI* stress–strain index

findings, our white males at 17 years of age who had a history of fracture had greater bone widths and wider medullary cavities, reflective of a lower MCI, than their non-fracturing peers. They also had a 55% decrease in fracture risk associated with a 1 SD gain in MCI, but this was negated when height and PA were considered. The group of white males with a history of fractures participated in more formal PAs, thus any biomechanical advantage provided by the geometrical organization of their bones may have been overridden by greater force at the time of injury. The finding that white males who fractured were taller, played more formal sport, and had greater DXA-derived bone mass has been previously reported from this cohort [13]. We have also shown that white males who had fractured had a higher BMD than their non-fracturing peers. In contrast, in Europe, a lower BMD was reported in adolescent males who had fractured compared to those who had not [18]. Our results indicate that a higher BMD does not entirely compensate for higher risk-taking activities.

In our study, although there were no sex differences in fracture prevalence (possibly because of the small numbers), black males had a lower MCI than their female counterparts, whereas white males and females who had not fractured had a similar MCI. In contrast, 17–18-year old females in Europe with no history of fracture had a higher MCI than their male peers [18]. Collectively, in addition to sex differences, these results also reflect population differences in bone geometry. In both the fractured and non-fractured participants, we also observed sex differences in radiogrammetry-derived BMD, which reflects the greater bone widths and cortices in males compared to females. These sex differences are consistent with the greater DXA-derived BMD at 15 years in males compared to females in this cohort [13]. A shortcoming of both radiogrammetry- and DXA-derived BMD is that they represent bone size rather than volumetric BMD which may be obtained by more advanced high-resolution computed tomography. The latter technique may also give insight into factors such as cortical porosity that may contribute to fracture.

Long bone fracture is primarily the result of bending or torsional stresses [36]. When we assessed SM and SSI which are surrogate strength indices based on moments of inertia, these indices were associated with fracture only in white males. We found that a greater SM and SSI increased fracture risk significantly, even after adjusting for height and PA. Our white males who fractured had greater bone width and medullary width, and hence reduced cortical thickness, resulting in a higher SM and SSI compared to their non-fractured peers. This outward displacement of the cortex from the neutral axis of the bone should have conferred a greater resistance to bending and torsional forces, and a reduced propensity to fracture [37]. This incongruity may be attributed to the overriding influence of higher levels of PA participation.

Our study and others have shown that metacarpal indices are influenced by height [38] which is a major determinant of bone size and mass [39]. In our cohort, at 17 years of age, there were no differences in height or metacarpal length between participants who had fractured and those who had not. This finding differs from results obtained at 15 years of age where white males of this cohort who fractured were taller than their non-fracturing peers [13], and may be related to differences in age of pubertal development and peak height velocity. An additional influencing factor may have been our sample size which was 23% smaller than that at 15 years. Taller individuals are at a greater risk for fracture despite their longer and wider bones with relatively thinner cortices [40]. In a twin study, the taller twins had increased fracture risk due to increased porosity of the cortex, despite their bone geometry offering greater resistance to torsional and bending forces [40].

Since height influences metacarpal indices, we assessed the SLI, which is also a proxy for bone strength, for its association with a history of fracture. The SLI, is an index based on moments of inertia which factors in body size, that is, the product of weight and bone length [27]. We showed that

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the SLI was associated with a history of fracture in white females only. Whilst our white females with a history of fracture had a greater SLI and hence more slender bones than their non-fractured peers, we also showed that after adjusting for PA, a 1 SD increase in SLI doubled the risk of fracture. Since body weight is used in the calculation of the SLI, the greater weight in white females who fractured may account for the association between the SLI and fracture in this group only. At both 15 and 17 years, white females in this cohort who fractured had greater fat mass [13] which supports the findings of Goulding and co-workers in New Zealand [41].

Bone mass measures in 8-year old children obtained by DXA have been reported to predict upper limb fracture during puberty [42], but this was not found in our study. Metacarpal indices of the combined sex and ethnic groups evaluated at 10 years, which is prior to the age of PLV in these participants [21], were of no value in predicting adolescent fracture. This suggests that external risk factors may play a greater role than measures of bone mass in the association with fracture. Bone mass measures have been shown to track from childhood through adolescence [7]. In our longitudinal analyses, we have shown that an increase in bone width and/or cortical thickness, as reflected by the MCI, is associated with a 9% reduction of fracture history risk at age 17 years in SA children. However, it is unclear for how many years from the time of measurement that the index has predictive value and prospective studies would be needed to elucidate this. Radiogrammetry-derived BMD, when assessed longitudinally, was only weakly associated with decreased adolescent fracture. We acknowledge a shortcoming of this study in that the timing of the fractures was not documented as they occurred; these data may have provided better correlation with the indices longitudinally.

A strength of this study is that it presents longitudinal data on radiogrammetry-derived metacarpal indices and their associations with fracture history in SA children of two ethnic groups. To the best of our knowledge, there are no other such studies. We were limited by the overall small number of white individuals, and relatively few black participants with a history of fracture, which may have resulted in the loss of statistical power. In this study, we considered it essential to stratify the participants by sex and ethnicity due to known differences in their bone geometry. Another limitation is that radiogrammetry only provides quantitative rather than qualitative information. Our metacarpal measurements were at the mid-diaphyseal region where the composition of bone differs from metaphyseal sites which have a higher trabecular content and are the most prevalent sites of fracture [43]. Fractures were not verified radiographically, though there was consistency with fracture reports obtained at ages 10, 13, and 15 years [13]. Self-reported fracture history has been shown to be relatively accurate, particularly for upper limb and hip fractures [44]. In this study, the cause, site, and grade of fracture were not assessed separately due to the small participant numbers, nor were data adjusted for potential modifiers of skeletal health. Previous studies on this cohort have shown that despite ethnic disparities in nutrition, 25-hydroxy vitamin D levels and socio-economic status, black children had greater bone mass than their white peers at most sites [29, 45]. We also did not adjust for lean mass or the possible influence of bone's microstructural properties. We acknowledge that self-reported PA may be prone to recall bias, however, it is the most practical method to assess PA in a large longitudinal cohort.

In conclusion, metacarpal indices obtained at age 10 years, that is prior to the age of peak bone length velocity, did not predict fracture risk in SA adolescents. When the combined sex and ethnic groups were assessed longitudinally throughout adolescence, greater cortical thickness, MCI, and BMD were associated with a small decrease in fracture risk by age 17 years, but the opposite was seen when differences in height were adjusted for. At 17 years of age, the SLI was associated with fracture risk in white females, whereas greater bone width, BMD, SM, and SSI were associated with fracture risk in white males. There are ethnic and sex differences in the associations between radiogrammetry-derived metacarpal bone geometry measures and indices of moments of inertia with fracture in SA adolescents. We conclude that our study was unable to show that metacarpal radiogrammetry was reliably able to predict fracture in SA children, but this may possibly be due to sample size limitations.

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Compliance with Ethical Standards

Conflict of interest Ansuyah Magan, L.K. Micklesfield, S.A. Norris, K. Thandrayen, R.J. Munthali, and J.M. Pettifor declare no conflict of interest.

Human and Animal Rights and Informed Consent This study was approved by the University of the Witwatersrand Committee for Research on Human Subjects and was performed in accordance with the Ethical Standards as laid down in the 1964 Declaration of Helsinki and its later amendments. Informed assent from adolescent participants and consent from parents were obtained for inclusion in this study.

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