BONE STRENGTH ACCRUAL ACROSS ADOLESCENT GROWTH AND THE INFLUENCES OF PHYSICAL ACTIVITY AND SEDENTARY TIME

by

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Abstract

With recent advances in imaging technologies, we are acquiring a better understanding of the complex hierarchy of bone and how bone adapts its geometry, microarchitecture and ultimately, its strength to withstand the loads imposed upon it during adolescent growth. Thus, in this thesis, I examine the influence of physical activity (PA), sedentary time, maturity and sex on estimated bone strength and its determinants[1] (i.e., microarchitecture, geometry and density) across adolescence.

This thesis is based on the UBC Healthy Bones III Study (HBSIII), a mixed longitudinal cohort of healthy girls and boys age 8-12 years at study entry. We assessed bone strength, geometry and density at the tibial shaft using peripheral quantitative computed tomography (pQCT) and bone strength, microarchitecture, geometry and density at the distal tibia and radius using high-resolution pQCT (HR-pQCT). We assessed PA and sedentary time using accelerometry.

Four studies comprise this thesis. First, I investigated cross-sectional associations between sedentary time and bone strength and its determinants at the distal tibia by HR-pQCT. I found no associations between sedentary time and bone parameters.

Second, I examined maturity- and sex-related adaptations of bone geometry and strength at the tibial shaft using pQCT. I found that larger bone area in boys provided them a greater bone strength advantage compared with girls across adolescence.

Third, I examined maturity- and sex-related adaptations of bone strength and its determinants by HR-pQCT at the distal tibia and radius. I found greater bone strength in boys across adolescence was underpinned by greater trabecular bone volume and total bone area.

Fourth, I examined prospective associations between PA, sedentary time and bone strength and its determinants at the distal tibia and radius using HR-pQCT. I observed greater bone strength and trabecular bone volume in participants engaging in more PA and lower total bone area in participants engaging in more sedentary time.

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[1]I use bone strength and *its determinants* to refer to *bone microarchitecture, geometry and density* throughout this dissertation.

Collectively, these studies enhance our understanding of how bone is gained during adolescence and add a unique perspective to the benefits of PA for bone strength and its determinants.

Lay Summary

Bone strength is the bottom line in fracture prevention. However, the intricacies of how bone strength is gained during adolescence are not completely understood. Thus, I used advanced medical imaging tools to study how bone strength is gained across 12 years of adolescent growth in 393 participants from the UBC Healthy Bones III Study. I also examined the influence of maturity, physical activity and sedentary behaviour on bone strength accrual in boys and girls.

The studies that make up this thesis make several novel contributions to the pediatric bone research field. First, they represent the longest studies of bone growth during adolescence using three-dimensional imaging techniques and advanced statistical modelling approaches. Second, they challenge a pre-existing paradigm regarding differences in how bone is accrued between boys and girls. Finally, they highlight adolescence as a critical 'window' for bone health and underscore the importance of physical activity for bone strength accrual.

Preface

This dissertation is an original intellectual product of the author, Leigh Gabel. Chapters 3-6 of this dissertation are versions of stand-alone manuscripts in the peer-reviewed academic literature. The first three chapters are published (Chapters 3-5) and the fourth (Chapter 6) has been submitted for publication and is currently undergoing peer-review. As first author, I led each of these chapters. I provide details of my contributions and those of my collaborators for each publication below.

This dissertation is based on the University of British Columbia (UBC) Healthy Bones III Study (HBSIII). The HBSIII was conceived of and designed by Professor Heather McKay (University of British Columbia) and received ethics approval from the UBC Behavioural Research Ethics Board (H15-01194, H07-02013, H2-70537). I began doctoral studies during the last year of the HBSIII data collection (2012), and I conducted and analyzed all pQCT assessments/scans during the 4-week data collection period. I subsequently became proficient in HR-pQCT scan acquisition and analysis and took a lead role overseeing all aspects of HBSIII HR-pQCT imaging data. I independently reviewed all HR-pQCT scans (over 2000 scans from 5 years of data collection) to quantify motion artifacts, conducted all standard HR-pQCT analyses and applied a customized segmentation algorithm to assess cortical porosity and thickness, including visually inspecting all segmentations to ensure correct differentiation between the cortex and trabeculae. Further, I led statistical analysis of the longitudinal HBSIII data using advanced multilevel modeling techniques. I conducted all of the statistical data analysis in this thesis. Throughout this dissertation, I use 'I' to refer to my individual contributions and 'we' to refer to contributions of the research team.

Chapter 1: A version of this material was published as Gabel L and Macdonald HM. Exercise and the female skeleton. In: Gordon CM and LeBoff MS (Eds.) The female athlete triad: A clinical guide. Springer, New York; 2015: 39-69. As lead author of this book chapter, I was responsible for the literature review and drafting the manuscript. Dr. Macdonald defined the chapter outline and provided detailed feedback and edits on all versions of the chapter.

Chapter 3: A version of this material was published as Gabel L, McKay HA, Nettlefold L, Race D, and Macdonald HM. Bone architecture and strength in the growing skeleton: the role of sedentary time. Medicine and Science in Sports and Exercise, 2015, 47(2): 363-72. As lead

author I was responsible for defining the research question, conducting the statistical analyses and drafting the manuscript. HR-pQCT data were collected in 2009 by Dr. Danmei Liu (Centre for Hip Health and Mobility) and accelerometry data were cleaned and processed by Douglas Race (HBSIII study coordinator). Dr. Lindsay Nettlefold and Douglas Race provided guidance related to accelerometry and feedback on near final drafts of the manuscript. Drs. Heather McKay and Macdonald provided detailed feedback and edits on all versions of the manuscript.

Chapter 4: A version of this material was published as Gabel L, Nettlefold L, Brasher PM, Moore SA, Ahamed Y, Macdonald HM, and McKay HA. Reexamining the surfaces of bone in boys and girls during adolescent growth: a 12-year mixed longitudinal pQCT study. Journal of Bone and Mineral Research. 2015, 30(12): 2158-67. As lead author I was responsible for data collection (in 2012), conducting the statistical analyses and drafting the manuscript. I defined the research question in collaboration with Drs. McKay and Macdonald, whom also helped draft the manuscript and provided detailed feedback on all versions. Dr. Brasher provided statistical guidance and Dr. Nettlefold assisted with statistical analyses and manuscript reviews. Sarah Moore assisted with the estimation of maturity using age at peak height velocity. Drs. Macdonald and Ahamed assisted with data collection between 2001 and 2008.

Chapter 5: A version of this material was published as Gabel L, Macdonald HM, and McKay HA. Sex differences and growth-related adaptations in bone microarchitecture, geometry, density and strength from childhood to early adulthood: a mixed longitudinal HR-pQCT study. Journal of Bone and Mineral Research. 2017, 32(2): 250-63. As lead author I developed the research question, analyzed the HR-pQCT scans, conducted the statistical analyses and drafted the manuscript. Drs. McKay and Macdonald provided detailed feedback on all versions of the manuscript.

Chapter 6: A version of this material was published ahead of print as Gabel L, Nettlefold L, Macdonald HM, and McKay HA. Physical activity, sedentary time and bone strength during adolescence: a mixed-longitudinal HR-pQCT study. Journal of Bone and Mineral Research. E-pub ahead of print, DOI: 10.1002/jbmr.3115. As lead author I developed the research question, analyzed the HR-pQCT scans, conducted the statistical analyses and drafted the manuscript. Drs. McKay and Macdonald provided detailed feedback on all versions of the manuscript. Dr. Nettlefold provided guidance regarding accelerometry analyses and feedback on the manuscript.

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List of Abbreviations

ABBREVIATION	TERMS
2D/3D	Two-/Three-dimensional
aBMD	Areal bone mineral density by dual energy X-ray absorptiometry
AIC	Akaike information criterion
APHV	Age at peak height velocity
BC	British Columbia
BIC	Bayesian information criterion
BMC	Bone mineral content
BMD	(volumetric) Bone mineral density
BSI	Bone strength index
BV/TV	(trabecular) Bone volume fraction
CHMS	Canadian Health Measures Survey
CI	Confidence interval
CIHR	Canadian Institutes of Health Research
cpm	Counts per minute
CSA	Cross-sectional area
CSMI	Cross-sectional moment of inertia
Ct.Ar	Cortical bone area
Ct.BMD	Cortical bone mineral density
Ct.Po	Cortical porosity
Ct.Th	Cortical thickness
DXA	Dual energy X-ray absorptiometry
FEA	Finite element analysis
FFQ	Food frequency questionnaire
F.Load	Failure load
GH	Growth hormone
HBSIII	Healthy Bones III Study
HHQ	Health history questionnaire
HR-pQCT	High-resolution peripheral quantitative computed tomography

ABBREVIATION	TERMS
IBDS	Iowa Bone Development Study
ICC	Intraclass correlation coefficient
IGF-1	Insulin-like growth factor-1
IU	International units
LL	Log likelihood
LSC	Least significant change
μSv	microSieverts
MCSA	Muscle cross-sectional area
Me.Ar	Medullary area
MES	Minimal effective strain
MES_m	Minimal effective strain for modeling
MES_r	Minimal effective strain for remodeling
METs	Metabolic equivalents
MRI	Magnetic resonance imaging
MVPA	Moderate-to-vigorous physical activity
NHANES	National Health and Nutrition Examination Survey
PA	Physical activity
PAQ-A	Physical Activity Questionnaire for Adolescents
PAQ-C	Physical Activity Questionnaire for Children
PBMAS	Pediatric Bone Mineral Accrual Study
PHV	Peak height velocity
pQCT	Peripheral quantitative computed tomography
PVE	Partial volume effect
RCT	Randomized controlled trial
ROI	Region of interest
SSI_p	Polar strength-strain index
Tb.Ar	Trabecular area
Tb.BMD	Trabecular bone mineral density
Tb.N	Trabecular number
Tb.Th	Trabecular thickness

ABBREVIATION	TERMS
Tt.Ar	Total bone area
Tt.BMD	Total bone mineral density
UBC	University of British Columbia
U.Stress	Ultimate stress
Z	Section modulus

Acknowledgements

I would like to express my sincerest thanks to my supervisors, Drs. Heather McKay and Heather Macdonald. I am fortunate to have spent the past five years learning from you – it has been a truly fulfilling journey. Thank you both for challenging my thinking, encouraging me to ask questions and for pushing me out of my comfort zone. To Heather McKay, I admire your dedication and enthusiasm for research, your leadership, vision and writing finesse. I am grateful for your mentorship. To Heather Macdonald, I cannot speak highly enough about my experience under your tutelage; your encouragement, guidance, attention to detail and spot-on feedback have been instrumental to my growth as a researcher. You set high expectations, but provided me the independence and flexibility to develop in my own time and to cultivate my style and voice. I also thank you for encouraging me to pursue research abroad, conference and teaching opportunities – these experiences and the connections I made as a result are invaluable. I am equally appreciative of my committee members. To Dr. Lindsay Nettlefold for your Stata and accelerometry expertise, morning chats, feedback, support and friendship. To Louise Mâsse for discussions about study design and analyses. I could not have asked for a more supportive and talented committee. Your expertise, editing and thought-provoking discussions were critical to this dissertation.

I feel privileged to have been a part of the UBC Healthy Bones III Study team. I am indebted to the numerous researchers, staff, investigators and participants of the UBC Healthy Bones III Study. This dissertation would not have been possible without your years of involvement, hard work and dedication. In particular, I would like to thank Douglas Race, study coordinator extraordinaire, for your knowledge on everything Healthy Bones III Study-related and for your assistance with accelerometry processing and database management. To Sarah Moore for recruiting the final Healthy Bones III Study cohort, your work on the maturity offset piece and support throughout my studies. To Danmei Liu for your pQCT and HR-pQCT expertise, availability and guidance. To Mikko Määttä for your assistance with auto-segmentation and finite element analyses. Thank you also to the Canadian Institutes of Heath Research for your generous support of the Healthy Bones III Study.

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Fellowship. To Prof. Jo Salmon and her team at Deakin University in Melbourne, thank you for inviting me into your lab and for being gracious hosts during my research fellowship. To Penny Brasher for providing statistical guidance during the early part of my studies. To Lesa Hoffman for making complex statistical modelling accessible to non-statisticians. To my CHHM family, thank you for a supportive and fun work environment. In particular, thank you to Christa Hoy (and Lindsay) for lunch time conversations where I often thought out loud and sought your opinions. To Anna Chudyk, Amanda Frazer and Christine Voss, my office mates over the years, for your enthusiasm and friendship.

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Chapter 1: Introduction, Literature Review, Rationale, Objectives & Hypotheses

1.1 Introduction

Bone strength[2] is irrefutably the most important parameter of skeletal health and is underpinned by bone's material properties, quantity, dimensions (size and material distribution) and microarchitecture.[1,2] This tenet has guided a paradigm shift away from assessing only two-dimensional (2D) measures of bone mass (measured with dual energy X-ray absorptiometry, DXA) to three-dimensional (3D) measures of bone geometry and microarchitecture. As imaging devices such as peripheral quantitative computed tomography (pQCT) and high-resolution pQCT (HR-pQCT) become more commonly used, we acquire a better understanding of the hierarchical structure of bone. As important, we also gain insight as to how complex bone structures adapt in response to physical activity (PA)[3].

Despite recent advances in bone imaging, the intricacies of how bone is gained in childhood and lost in later life are still not completely understood. For example, it is unclear whether bone is gained or resorbed at the endocortical surface of the diaphysis during adolescent growth. We also do not fully understand how maturity-related adaptations differ between boys and girls. Adaptations specific to bone microarchitecture during growth are also unclear as few studies have used HR-pQCT to examine maturity- and sex-related differences during childhood and adolescence. Of these, all were cross-sectional or had a short follow-up period[4-6] and scanned different regions of the distal radius and/or tibia. Gaps in knowledge are due in part to inadequate methods used to control for the substantial variation in maturational status among adolescents and the reliance on cross-sectional compared with prospective data. A thorough understanding of bone strength accrual is crucial to appreciate the influence of PA on bone adaptation during growth.

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[2] I use bone strength to refer to estimated bone strength in studies that used non-invasive imaging.

[3] PA defined as any bodily movements expending energy.[3]

The ability of bone to adapt to mechanical loads resulting from weight-bearing PA was first described more than a century ago.[7,8] In recent decades, we amassed a substantial body of evidence to support an integral role for PA and weight-bearing PA, specifically, for developing and maintaining a healthy skeleton. In particular, the critical period of adolescence, when more than one quarter of adult bone mass is accrued,[9] and childhood represent windows during which the skeletal benefits of weight-bearing PA may be enhanced.[10-15] In turn, optimal PA during the growing years may prevent adult bone health problems, such as osteoporosis and fragility fractures.

Although we do not know the precise PA prescription (e.g., frequency, intensity, duration, type) for optimal bone strength accrual, evidence from animal studies, school-based interventions and observational studies suggest that "a little goes a long way". Specifically, short bouts of high-impact PA implemented over relatively short timeframes may be sufficient to enhance bone mass and strength accrual during adolescence.[15] Conversely, we know less about bone microarchitecture adaptations to weight-bearing PA, although they also influence bone strength during childhood and adolescent growth and development.

Despite numerous health benefits associated with PA, today's youth spend roughly 60% of their waking hours being sedentary (defined as ≤ 1.5 metabolic equivalents (METs).[16] A focus on the consequences of 'not loading' a healthy growing skeleton is relatively new. No studies to date have investigated the relationship between sedentary time and bone strength (estimated using pQCT or HR-pQCT) in children and youth. Thus, it is unclear how the potentially deleterious impact of sedentary time interacts with the osteogenic effect of PA in healthy, ambulatory children and adolescents.

Therefore, the primary aim of my thesis is to determine the influence of PA and sedentary time on bone strength accrual and its determinants during childhood and adolescence. I extend previous studies that focused primarily on accrual of bone mass and areal bone mineral density (aBMD; by DXA) as outcomes. My secondary aim is to examine the maturity- and sex-related adaptations in bone strength accrual and its determinants during adolescent growth. To achieve these aims, I employ two novel bone imaging techniques, pQCT and HR-pQCT, and measure PA and sedentary time objectively using accelerometry. In addition, I use general linear mixed models to account for the longitudinal nature of my data, which permits investigation of inter- and intra-individual variation across time. My thesis is divided into four research chapters

(Chapters 3-6). In Chapter 3, I describe cross-sectional associations between sedentary time and bone strength and its determinants at the distal tibia using HR-pQCT. In Chapter 4, I examine longitudinal maturity- and sex-related adaptations in bone strength and geometry from childhood to early adulthood at the tibia midshaft using pQCT. In Chapter 5, I investigate longitudinal maturity- and sex-related adaptations in bone strength and its determinants at the distal tibia and radius from childhood to early adulthood using HR-pQCT. Finally, in Chapter 6, I examine the prospective associations between PA, sedentary time and bone strength at the distal tibia and radius during adolescence using HR-pQCT.

1.2 Literature review

In this section, and in six parts, I provide an overview of pertinent literature that informs this thesis: basic bone biology, bone biomechanics, bone imaging in children and adolescents, bone development during childhood and adolescence and factors that influence bone development, with a specific focus on PA and sedentary time.

Bone is a complex and dynamic tissue that serves to provide structural support and withstand loads imposed on it by external and internal forces.[17] In addition, bones serve as levers for locomotion, a reservoir for calcium, protector of internal organs, a site for hematopoiesis (formation of blood cells) and attachment sites for muscles, ligaments and tendons.[17] Bones must serve all of these functions while remaining lightweight for locomotion and adapting to substantial changes in morphology imposed by growth.[18]

1.2.1 Bone biology and bone growth

In this section, I briefly describe basic bone biology related to human long bones, the focus of my thesis. I briefly review mechanisms that influence bone development and maintenance.

1.2.1.1 Bone tissue: composition and organization

Bone is a composite material of minerals, collagen, water, non-collagenous proteins and lipids.[18] The mineral component makes up approximately 70% of bone by weight and consists of calcium and phosphate arranged in crystals of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). Hydroxyapatite provides stiffness, mechanical resistance and a source of minerals (e.g., calcium and phosphate).[18] The non-mineralized component makes up 20-25% of bone by weight and is primarily type 1 collagen and non-collagenous proteins (98%). Collagen is a connective protein that binds hydroxyapatite, providing elasticity and the ability to resist tension.[18] The remainder of bone tissue is comprised of bone cells (osteoblasts, osteoclasts and osteocytes). Together, the mineral and collagen matrix produce a connective tissue with high stiffness and strength[18] that allows bone to withstand stresses in bending, compression and torsion.

There are two types of bone tissue: woven and lamellar bone.[19] Woven, or immature, bone is characterized by an irregular pattern of collagen fibre orientation. Woven bone comprises all bone at birth and can be found at ligament and tendon insertions in healthy adult skeletons.[17] Woven bone can be temporarily found during fracture healing as it is formed faster than lamellar bone.[20] Most woven bone is resorbed and replaced by lamellar bone by about four years of age.[21] In contrast, lamellar bone, or mature bone, is characterized by a consistent arrangement of collagen fibres along lines of force. Lamellar bone is the building block of both cortical and trabecular bone, such that the structural subunits, lamellae, are oriented parallel to trabeculae in trabecular bone (also termed cancellous bone) and arranged in osteons in cortical bone (Figure 1.1).[17] Osteons are the major structural units of cortical bone and are cylindrical arrangements of cortical bone (lamellae) around a Haversian canal, channeling a blood vessel for nutrition. Osteons are usually aligned along the long axis of bones and are connected by Volkmann's canals running at right angles.[19]

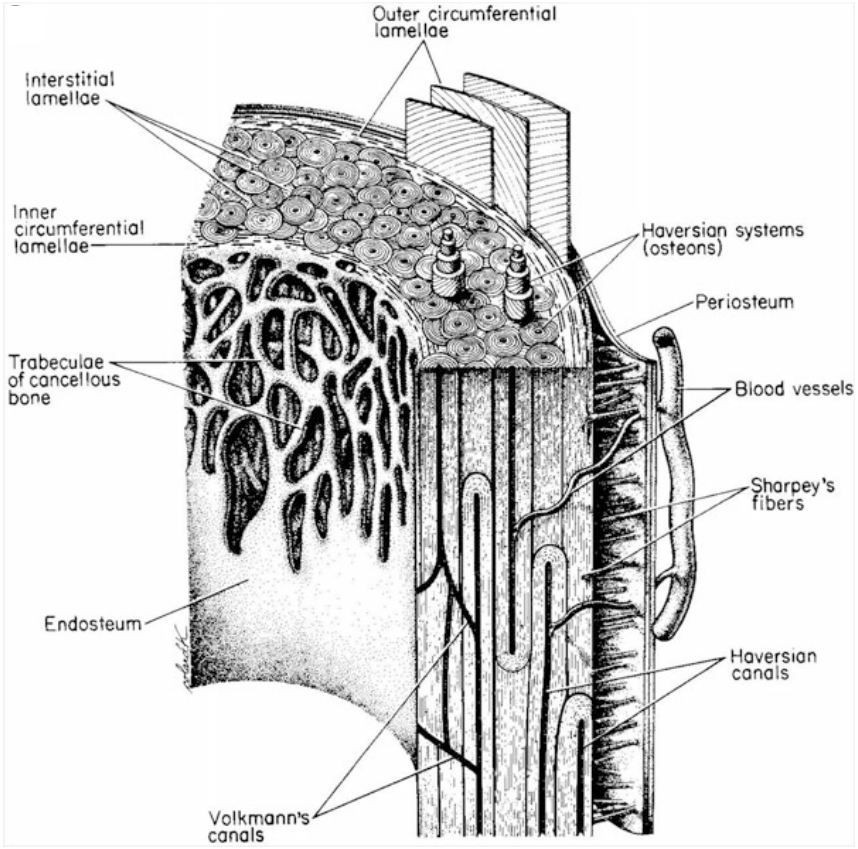


Figure 1.1 Diagram of structural elements of long bones, illustrating cortical bone, comprised of osteons surrounding blood vessels, and trabecular bone. Reprinted from Martin et al.,[22] with permission from Springer New York.

Although cortical and trabecular bone are made of the same material, their respective bone matrices are arranged differently, and thus serve unique structural and functional roles.[21] Cortical bone provides structure, protects organs and has a porosity of 5-30% of bone volume. Calcium comprises upwards of 80-90% of cortical bone volume.[21,23] Cortical bone forms the diaphyses of long bones and is also located in the thin shells of metaphyses (Figure 1.2). Trabecular bone, on the other hand, is more porous than cortical bone (30-90%) and calcium comprises only 15-25% of bone volume.[21,23] Trabecular bone is found in the epiphyses and metaphyses of long bones and in vertebral bodies. Trabecular bone's 3D lattice geometry enables transfer of loads through bending moments.[21,24] Trabecular bone is arranged so that bone marrow, blood vessels and connective tissue are in contact with bone. Given its large surface area and proximity to bone marrow, trabecular bone is suited for metabolic activities associated with bone turnover. Trabecular bone is typically 'younger' than cortical bone as it is more metabolically active and remodeled frequently.[25] The human skeleton is comprised of

predominantly cortical bone (~80%); however, the relative contribution of cortical and trabecular bone to total bone volume varies between and within skeletal sites. For example, the femoral head is 50% cortical bone, while the radial diaphysis is 95% cortical bone.[26]

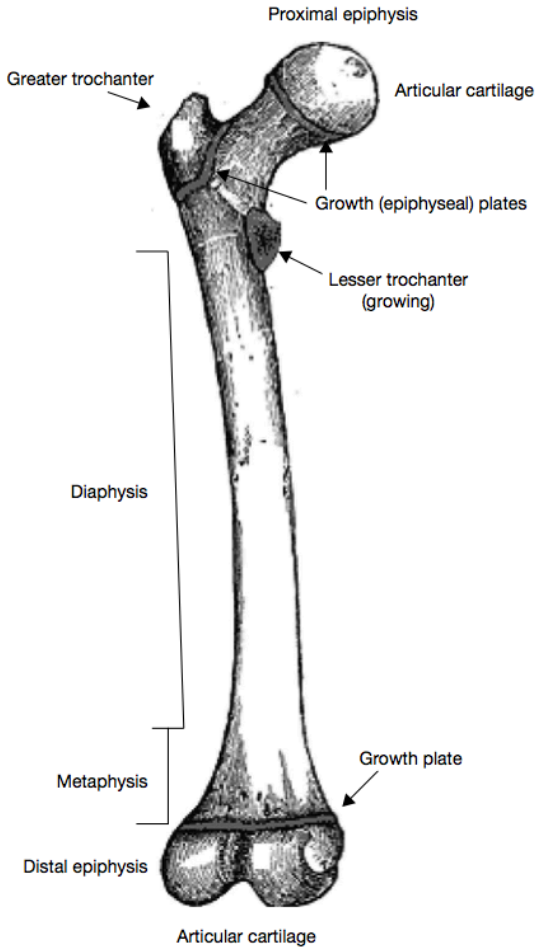


Figure 1.2 Anterior view of the right human femur with basic anatomy, including diaphysis, epiphysis and metaphysis. Modified from the online edition of the 20th US edition of Gray's *Anatomy of the Human Body*, and originally published in 1918 and reprinted from Kontulainan et al.,[27] with permission from Karger.

Three cell types found within cortical and trabecular bone regulate bone turnover: osteoblasts, osteoclasts and osteocytes. Osteoblasts, bone-forming cells, lay down extracellular matrix and regulate bone mineralization. Osteoblasts secrete an initial collagen matrix (osteoid, unmineralized protein), which forms the basic framework of bone tissue, and subsequently mineralize the collagen.[18] Osteoclasts, bone-resorbing cells, resorb fully mineralized bone. Osteocytes, the most abundant cell in bone, are derived from former osteoblasts that become

embedded within the bone matrix. Osteocytes communicate with other bone cells, detect mechanical loading and coordinate modeling and remodeling activity (described in further detail in Section 1.2.1.2).[18,28]

1.2.1.2 Bone modeling and remodeling

Bone modeling and remodeling allow bone to adapt its size, shape and distribution of microarchitecture throughout the lifespan.[29] These processes optimize bone strength and minimize bone mass by adding bone where it is needed and removing bone from where it is not.[30] Bone modeling involves independent actions of osteoblasts and osteoclasts in response to physiological or mechanical influences, resulting in gradual adaptation of the skeleton to biomechanical forces.[26] Bone modeling increases bone length and size; thus, it predominates during growth and is reduced at skeletal maturity. Modeling enlarges the diaphysis during growth, such that osteoblasts deposit bone onto periosteal surfaces and osteoclasts remove bone from endocortical surfaces.[17] As bone length increases, the wider metaphyses are modelled to match the thinner cross-section of the diaphysis; a process referred to as metaphyseal inwaisting.[31] These processes reshape long bones, preventing the cortex from becoming excessively heavy and thick and positioning the diaphyseal cortex farther from the centre of the bone.[27]

In contrast to modeling, bone remodeling couples the action of osteoblasts and osteoclasts (the basic multicellular unit) to preserve bone strength and mineral homeostasis.[26] During remodeling, osteoclasts resorb pockets of old or damaged bone and osteoblasts subsequently fill the pockets with collagen matrix and mineralize the collagen to form new bone. Remodeling occurs throughout the lifespan in response to mechanical loading and to replace dead and damaged bone tissue.[17] Bone resorption takes 2 to 4 weeks, while formation takes 4 to 6 months to complete; thus, one full remodeling cycle takes approximately 5-7 months.[26,32] Modeling and remodeling are integral for strengthening the growing skeleton and I discuss both in greater detail in section 1.2.2.2.2.

1.2.1.3 Long bone geometry

The human skeleton contains many different types of bones: long, short, flat, irregular and sesamoid. In this chapter, I focus primarily on long bones, which, during growth, are comprised of the diaphysis, epiphysis and metaphysis (Figure 1.2). The diaphysis is a cylindrical shaft in the middle of the long bone; the outer portion contains cortical bone that encloses the medullary cavity. Cortical bone has two surfaces: the periosteum or periosteal surface, which is the outer surface facing the soft tissue, and endosteum or endocortical surface, which is the inner surface adjacent to bone marrow. Both are active sites of bone modeling and remodeling, lined with osteoblasts and osteocytes. The periosteum contributes to appositional bone growth through modeling-related increases in bone diameter during development.[21] Cortical bone diameter is thinner towards the metaphyses and epiphyses, where the medullary cavity is replaced by trabecular bone. The metaphysis is a transitory region located between the diaphysis and epiphysis, and is comprised of trabecular and cortical bone. A layer of cartilage known as the growth plate separates the metaphysis and epiphysis (Figure 1.2).[21] The flared shape of long bone ends distributes joint forces and reduce stress transmitted from trabecular bone in the metaphysis to cortical bone in the diaphysis.[33]

1.2.1.4 Longitudinal growth of long bones

Skeletal growth (in length and width) occurs between birth and maturity in preparation for a lifetime of loading, and is controlled by systemic, local and mechanical factors.[34] Skeletal growth in length occurs at the growth plates in epiphyseal and metaphyseal regions, where cartilage proliferates.[26] Endochondral ossification is the complex process whereby cartilaginous tissue is replaced by bone (Figure 1.3).[35] The epiphyseal end of the growth plate is predominantly cartilaginous tissue composed of chondrocytes, cartilage producing cells. Approximately 80% of cartilage is resorbed during growth. The remaining cartilage provides a scaffold for osteoblasts to deposit bone matrix, forming primary trabeculae, a mixture of cartilage and bone tissue.[34] During growth, metaphyseal trabeculae thicken and are eventually integrated into the metaphyseal cortex (a process termed trabecular coalescence) and later into the diaphyseal cortex.[34] Metaphyseal trabeculae, located at the centre of long bones, are

completely resorbed leaving the diaphysis empty of trabeculae. As a result, the age of bone tissue at a certain distance from the growth plate is directly related to the rate of longitudinal growth. Bone tissue at a given distance from the growth plate will be relatively younger when the rate of longitudinal growth increases, such as during the pubertal growth spurt.[36] Of note, the contribution of distal and proximal growth plate activity to overall long bone growth varies between bone sites during different periods of growth.[37,38] For example, 57% of longitudinal bone growth occurs at the proximal metaphysis of the tibia between 10 and 15 years of age and 43% occurs at the distal metaphysis.[39] After growth plate closure, bone age parallels chronological age.[36]

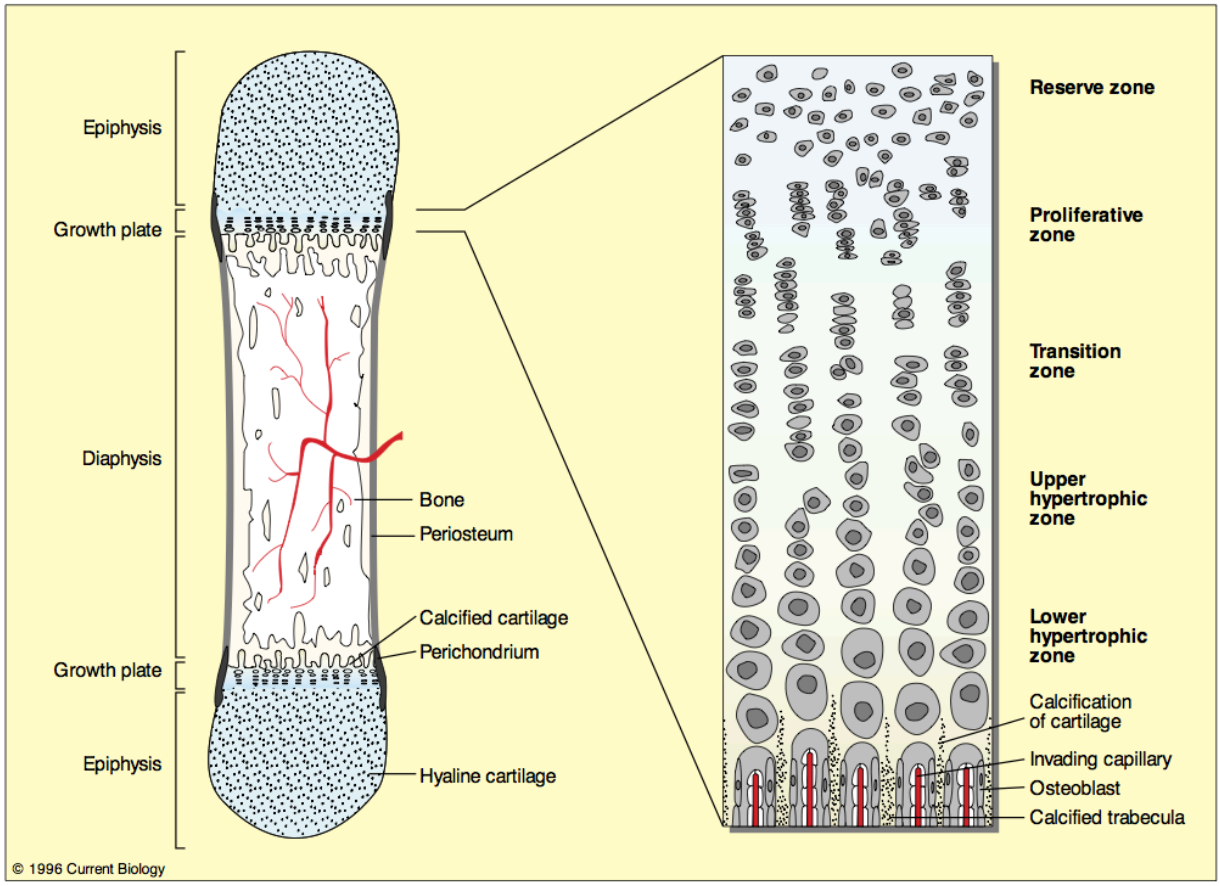


Figure 1.3. The schematic on the left is of a long bone during embryonic development, including the growth plate. The image on the right shows proliferation of chondrocytes at the growth plate. Reprinted from Wallis,[40] with permission from Elsevier.

1.2.2 Bone biomechanics

In this section, I discuss bone's mechanical properties and mechanisms underlying bone's adaptation to mechanical loading.

1.2.2.1 Material and mechanical properties of bone

The hierarchical geometry of bone ranges from smaller than the nanoscale to greater than the millimeter scale, with all levels contributing to bone's mechanical behaviour and function (Figure 1.4).[25] Bone *macro*architecture includes cortical and trabecular bone properties greater than the millimeter scale (i.e., cross-sectional area).[41] Bone *micro*architecture refers to individual trabeculae, Haversian systems and osteons, ranging from 10 to 500 micrometers (e.g., cortical thickness and porosity, trabecular number and thickness).[25] Organization of bone on an even smaller scale includes the sub-microarchitecture (1-10 μm; e.g., individual lamella), nanostructure (a few hundred nanometers to 1 μm; e.g., collagen fibres) and sub-nanostructure (less than a few hundred nanometers; e.g., bone mineral crystals).[25] In this thesis, I focus on bone's organization at the macro- and microarchitecture levels as these are within the measurement capacity of the imaging tools I used for my research.

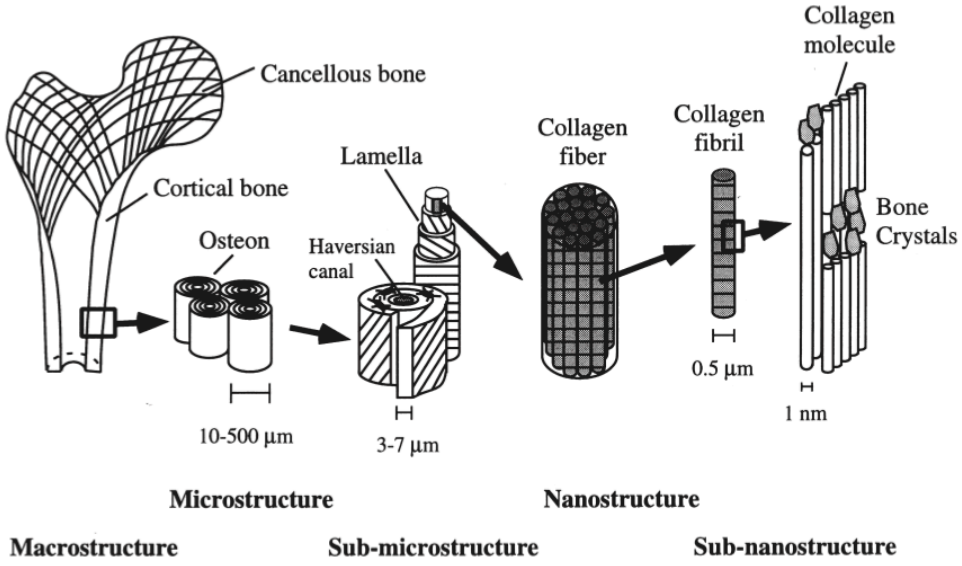


Figure 1.4 The hierarchical organization of bone, reprinted from Rho et al.,[25] with permission from Elsevier.

Bone's material behaviour reflects its intrinsic material properties and is independent of geometry (size).[42] Material properties include *stress, strain, Young's modulus, ultimate stress and toughness*. Structural behaviour, on the other hand, reflects both material and geometric properties. Material properties of bone are typically determined by mechanical tests (destructive testing to determine force required to cause failure) on uniform specimens of intact bone (i.e., part of the bone machined using a saw), whereas structural behaviour is determined by mechanical tests of whole bone specimens (i.e., whole sections of intact bones) where bone geometry is preserved.[2]

In this section, I provide a brief overview of bone's material behaviour, although the concepts I discuss apply to many materials other than bone. *Stress* is a measure of the intensity of force applied to a material, and is defined as force applied per unit area (N/m^2).[43] When normalized to cross-sectional area, direct comparison can be made between specimens of different size and loads of different magnitudes.[42] *Strain*, on the other hand, is a measure of material deformation and is calculated as relative change in bone dimensions (change in length / initial length; unit-less, but is often expressed as microstrain because it is small in magnitude in bone).[44] Stresses and strains are categorized as normal or shear. Normal stresses act perpendicular to a given plane and the strains either pull apart and elongate the bone (tensile) or compact and shorten the bone (compression). Shear stresses and strains, on the other hand, act parallel to the plane and define the angular change during deformation.[42] Bones experience both normal and shear stresses and strains during normal function.[42] Bones are typically loaded in one of four ways (compression, tension, bending and torsion) or in combination (i.e., long bones are often loaded in bending, but also in compression and torsion).[18]

As illustrated in Figure 1.5, the stress-strain curve reflects bone's material behaviour and describes the amount of stress required to produce a unit of strain.[42] Material properties of *stiffness*, *ultimate stress* and *Young's modulus* are derived from the stress-strain curve.

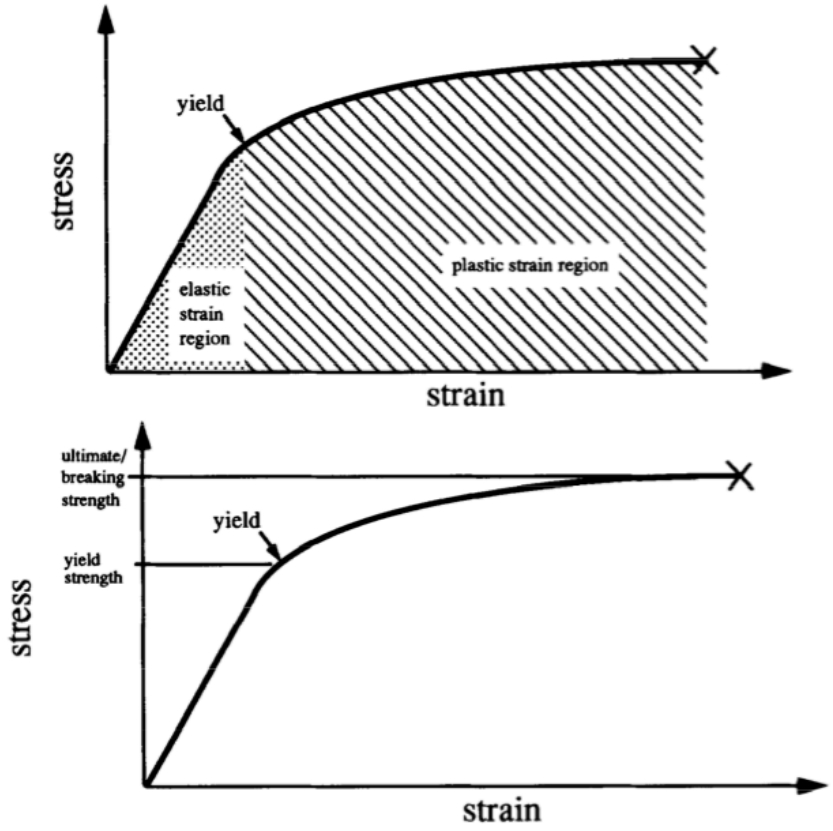


Figure 1.5. The top image is a stress-strain curve divided into elastic and plastic regions. The bottom image displays the measurement of strength from the stress-strain curve. X marks the stress and strains where failure occurs. Reprinted from Turner and Burr,[45] with permission from Elsevier.

Young's modulus of elasticity is the amount of force necessary to deform a structure. It is calculated as the slope (stress divided by strain) of the linear segment of the stress-strain curve.[43] The stiffer the material (more force needed to cause deformation), the steeper the slope of the stress-strain curve. *Ultimate stress* is the maximum stress a bone can sustain without failing, while *toughness* is the amount of energy a bone can absorb prior to fracture (derived from area under the stress-strain curve).[45] In the elastic portion of the stress-strain curve, a section of the curve prior to a yield point, stress and strain are linearly related. Any strain in the elastic region is only temporary and bone will return to its original shape once a load is removed. The plastic region of the stress-strain curve begins beyond the yield point where the slope decreases. Strain along this section of the curve is permanent and occurs until a fracture point or point of failure is reached.[45] With these mechanical properties in mind, most bones respond by growing large and thick enough to stay within the elastic region of the stress-strain curve.[24]

Long bones of the appendicular skeleton primarily experience stresses and strains at the diaphysis in bending and torsion. Cortical bone experiences the highest loads and deformations.[46] In contrast, at metaphyseal sites where trabecular bone predominates, the greatest stress is experienced in compression and bending.[46]

1.2.2.1.1 Material properties of cortical bone

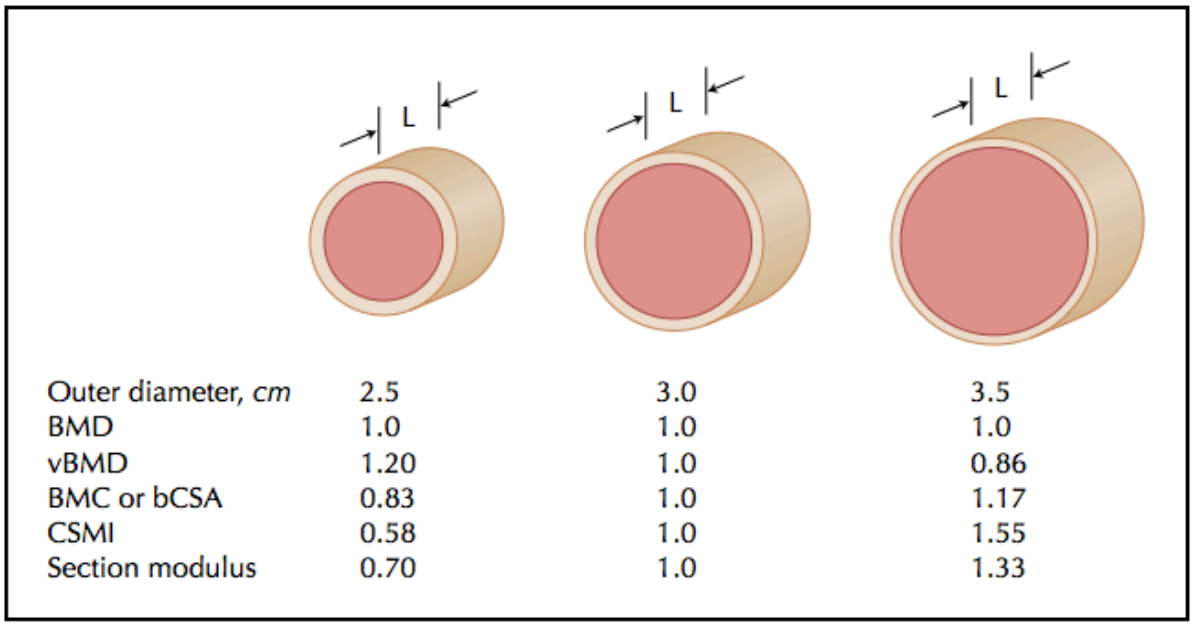
Material properties of cortical bone depend greatly on degree of matrix mineralization and porosity.[47,48] Cortical bone is anisotropic, such that its elastic properties and strength are dependent upon orientation of bone with respect to the applied load.[23] Cortical bone is stronger and stiffer longitudinally versus transversally and is stronger in compression than in tension.[23] It is a viscoelastic (time-dependent) material, such that its mechanical properties are dependent on strain duration and strain rate.[23] The contribution of cortical bone to whole bone strength is also site-specific. For example, cortical bone's contribution to long bone strength is much greater at the cortical-rich diaphysis compared with the metaphyses, where a combination of cortical and trabecular bone contribute to whole bone strength.[23]

1.2.2.1.2 Material properties of trabecular bone

Trabecular bone is largely responsible for bone's energy absorbing capacity.[49] As with cortical bone, trabecular bone is anisotropic (material properties are dependent upon the orientation); in contrast, trabecular bone is more porous and highly heterogeneous throughout the body, resulting in varied mechanical properties.[49] The heterogeneity of trabecular bone stems from differences in volume fraction, arrangement of individual trabeculae (i.e., microarchitecture) and tissue properties.[50] Trabecular bone strength and elastic modulus are site-specific, varying with the changing function of bone with respect to location and type of stress. For example, when tested in different directions, there are up to ten-fold differences in the elastic modulus of trabecular bone at the same anatomical site.[51] Mechanical properties of trabecular bone are influenced by changes in the thickness, number, separation and connectivity of trabeculae.[52]

1.2.2.1.3 Bone strength in bending and compression

Long bone diaphyses experience loading in tension, compression, bending or torsion; alone or in combination.[46] In theory, a hollow tube provides the greatest strength with least mass in response to torsional or bending loads.[43] Bending strength is proportional to the square of the material's distance from the cross-section's center of mass[53] and is influenced by bone's cross-sectional moment of inertia (CSMI), which quantifies the distribution of material around the bending moment.[42] This principle is illustrated in Figure 1.6, where the bone with the greater outer circumference and larger hollow center is significantly stronger and stiffer than the bone with a smaller outer diameter and hollow centre. Thus, bone added to the periosteal surface contributes more to bending strength than that removed from the endocortical surface.[53] Therefore, the most efficient structure is one where mass is placed furthest from the neutral axis.[18]



Outer diameter, *cm*	2.5	3.0	3.5
BMD	1.0	1.0	1.0
vBMD	1.20	1.0	0.86
BMC or bCSA	0.83	1.0	1.17
CSMI	0.58	1.0	1.55
Section modulus	0.70	1.0	1.33

Figure 1.6. Scale drawings of three cylindrical cross-sections with different outer diameters, fixed length (L), but equal areal bone mineral density (BMD). Corresponding values of volumetric BMD (vBMD), bone mineral content (BMC) or cross-sectional area (bCSA), cross-sectional moment of inertia (CSMI) and section modulus. Reprinted from Beck,[54] with permission.

Metaphyseal sites are primarily loaded in compression, thus resistance to bending is not an appropriate index of strength.[55] In addition, strength indices derived from cortical geometry,

such as CSMI, require an accurate measure of the cortical compartment. This may prove challenging in children and at distal sites where the cortical shell is thin. Bone strength index (BSI) is a noninvasive estimate of bone strength in compression that incorporates both bone material properties and its distribution. BSI is the product of total cross-sectional area (CSA) and the square of total BMD. It predicts up to 85% of the variance in failure load at the distal tibia (4% site).[55] Thus, bone strength in compression can be increased by adding bone on the periosteal surface and with increased trabecular BMD. Compressive bone strength can also be estimated using high-resolution images and finite element analysis (FEA). I discuss FEA in greater detail in section 1.2.3.3.1.

1.2.2.2 Bone's response to mechanical stimuli

Above, I presented that bone is a complex and dynamic tissue. Bone's primary role is to provide structural support and withstand loads imposed by external and internal forces (e.g., gravitational and muscular forces).[24] Increased bone strength can be achieved in a number of ways – through increased BMD, changes in bone geometry and/or distribution of microarchitecture. The skeleton is continually exposed to a loading environment and bone is deposited and resorbed to achieve an optimum balance between bone strength and mass.[18] In this section, I consider bone's response to mechanical stimuli in the context of mechanical problems, how bone perceives applied forces, the osteogenic response to applied loads and factors that influence bone's response to mechanical stimuli.

1.2.2.2.1 Mechanotransduction

As early as the 19th century, Julius Wolff described how bone architecture adapts to mechanical loads applied to it, remodeling over time to better resist similar strains.[8] Bone responds to mechanical loading through mechanotransduction, a process whereby a biophysical force is converted into a cellular response.[56] Briefly, osteocytes sense mechanical strain and initiate a signaling cascade to effector cells (osteoblasts and osteoclasts).[56] The skeleton responds to mechanical strains through modeling and remodeling and adjusts bone mass and geometry to match the demands of the mechanical environment.[18,57]

First, in mechanocoupling (Figure 1.7), a mechanical force applied to bone produces fluid shear stresses along the cell membrane that are detected by osteocytes.[58] Deformation of bone creates pressure gradients within osteocyte canaliculi, triggering interstitial fluid flow and communication at gap junctions.[18] Second, biochemical coupling transduces fluid shear stress into a biochemical signal through various biomechanical pathways, including cyclooxygenase (COX) and nitric oxide synthase (NOS).[59] Third, the signal is transmitted from the sensor cell (osteocyte) to effector cell (osteoblasts or osteoclasts) and finally, the effector cell responds to the signals.[59] Once strain is transduced, osteogenic cells initiate one of four possible outcomes: 1) no response, 2) osteoblasts add new bone, 3) osteoclasts resorb bone, or 4) both osteoblasts and osteoclasts are recruited in coordination.[18]

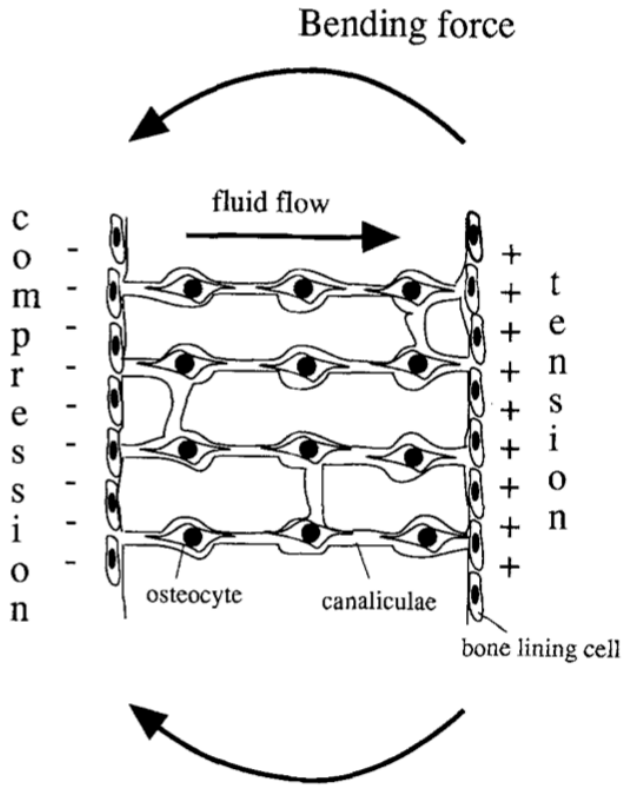


Figure 1.7. Illustration of mechanocoupling. Bending forces cause deformation of osteocytes and create pressure gradients that drive fluid through canaliculae, from regions of compression to tension. The fluid flow generates shear stress on cell membranes. Reprinted from Duncan et al,[56] with permission from Springer.

1.2.2.2.2 Mechanostat theory

Frost's mechanostat theory proposes that bone's mechanical competence is a function of mechanosensory negative feedback loops that sense load-induced strains and respond by adapting bone mass, geometry and strength to maintain bone strain at an optimal level.[60] Osteocytes sense strain and send out signals to initiate bone modeling and remodeling that increase bone strength.[60] Frost describes setpoints, minimum effective strain (MES) thresholds, whereby loads above and below such setpoints stimulate or attenuate bone mineral deposition or resorption (Figure 1.8).[60] When strains exceed the modeling threshold (MESm, ~2000 microstrain), bone modeling is activated, increasing bone strength. Within the remodeling threshold (MESr, ~50-200 microstrain), the amount of bone resorbed and accrued tends to be balanced. Below the remodeling threshold (MESr), (disuse or trivial loading zone), the theory contends that more bone is resorbed than accrued.[61,62] Finally, at the upper end of the spectrum, strains beyond the pathological MES theoretically cause accumulating fatigue damage.[63] Others proposed that non-mechanical factors such as nutrition and hormones alter the MES thresholds.[57] I discuss the influences of non-mechanical factors on bone adaptation in section 1.2.5.

During growth, mechanical loads associated with increased bone length and muscle forces increase bone tissue strain above MESm. After skeletal maturity, peak bone tissue strains are reduced, and remodeling enables bone conservation (MESr). In aging adults, PA and muscle strength decreases. Consequently, mechanical loads imposed on bones diminish, and based on Frost's theory, strains downshift below the remodeling threshold region into the disuse zone.[62]

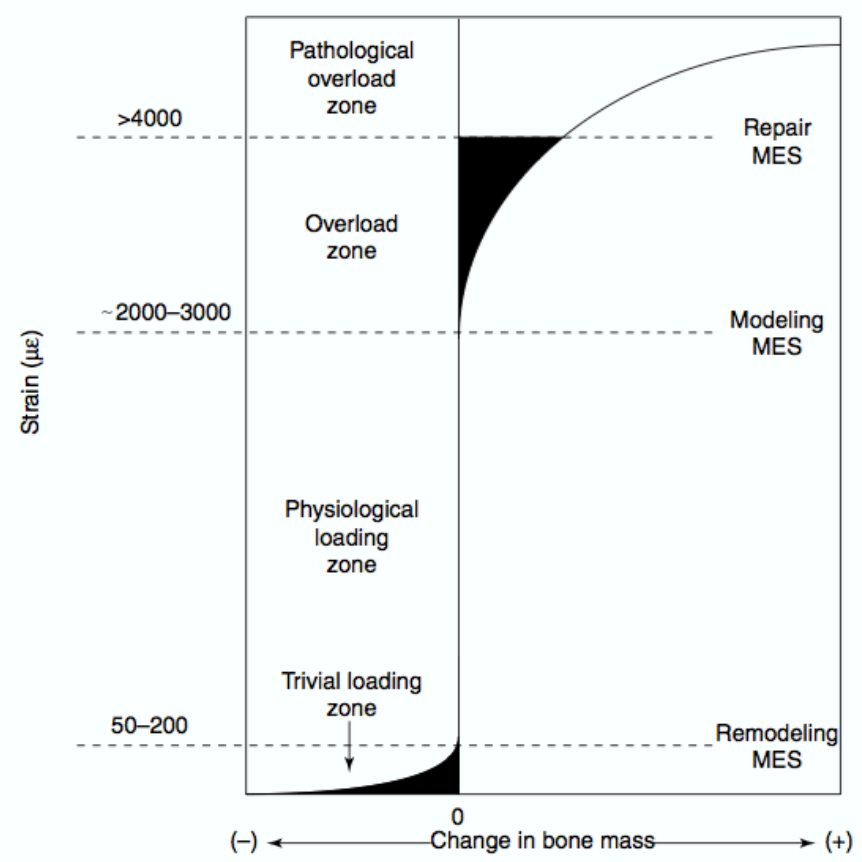


Figure 1.8. Illustration of the mechanostat theory and influence of mechanical strain on bone modeling and remodeling. Theoretically, bone remodeling occurs in the upper limit of the trivial loading zone (or disuse zone) and in the physiological loading zone; bone modeling occurs in the overload zone; and microdamage repair occurs in the pathological overload zone. Based on Forwood and Turner[64] and reprinted from Bachrach et al,[65] with permission from Elsevier.

Frost's mechanostat theory significantly advanced our understanding of bone's response to mechanical loading; however, more recent work highlights several inaccuracies.[66] Namely, mechanostat theory cannot account for why bone resorption does not predominate at non-weight bearing sites due to disuse. Cellular accommodation theory attempts to reconcile inconsistencies in mechanostat theory using mathematical principles that assume: 1) bone cells adapt their set point in response to a change in loading environment and 2) set points vary from site to site based on the local strain environment.[66] Therefore, the set point will be higher in weight bearing bones than non-weight bearing bones. Animal studies support the cellular accommodation theory of mechanoadaptation, whereby bone's response to loading resembled adaptation predicted by cellular accommodation theory, but not adaptation predicted by mechanostat theory.[67]

Specifically, bone adaptation in adult rats was proportional to the initial peak load magnitude and bone desensitized to loading after the initial weeks of loading.[67]

1.2.2.2.3 The functional model of bone development

Muscular contractions impose the largest voluntary loads on the body.[62] In contrast, body weight incurs relatively small static loads on bones, which are amplified by muscular contractions.[57] During longitudinal growth, increases in bone length and muscle forces result in greater bone deformation.[57] In their functional model of bone development, an extension of mechanostat theory, Rauch and Schoenau proposed that a negative feedback loop between tissue strain and bone strength is central to bone's regulation (Figure 1.9). This model also proposes that physiologic loads from muscle forces trigger a cascade of events that allow bones to maintain functional structural integrity and strength.[57]

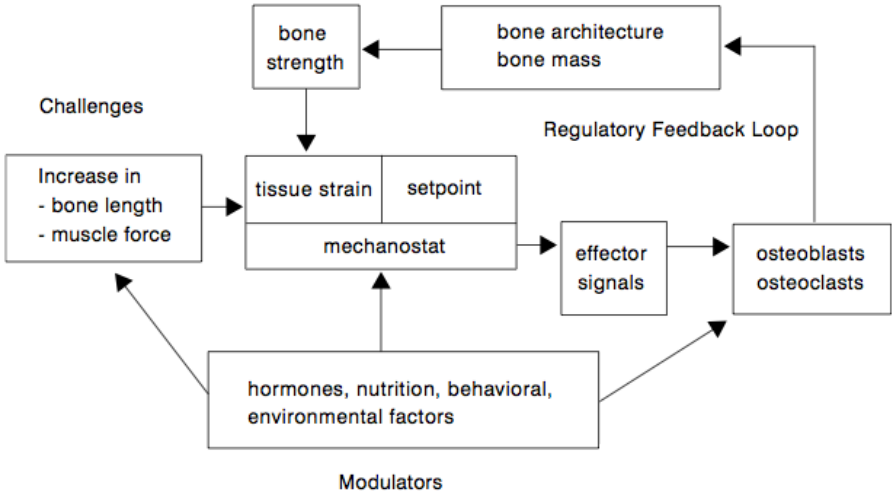


Figure 1.9. The functional model of bone development based on mechanostat theory. A feedback loop between bone deformation and bone strength is the central component of regulation of bone development and adaptation. During growth, this homeostatic system must continually adapt to external challenges (increases in bone length and muscle force) to keep tissue strain close to a preset value. Factors shown in the bottom box modulate the regulatory system. Reprinted from Schoenau[68] and adapted from Rauch and Schoenau,[57] with permission from Nature Publishing Group.

During growth, bone adapts its strength in response to mechanical stimuli through several mechanisms: 1) periosteal apposition to increase bone CSA; 2) periosteal apposition in

conjunction with reduced endocortical resorption to increase cortical thickness; and/or 3) modified cortical and trabecular microarchitecture (i.e., increased trabecular thickness or number or decreased cortical porosity) to increase tissue density.[11,69] In the following section, I focus on the role of mechanical loading and resultant adaptations in bone strength, geometry and microarchitecture during growth.

1.2.2.2.4 Experimental evidence for bone adaptation to mechanical stimuli

Experimental evidence from animal models advanced our understanding of how bone adapts to mechanical loads.[70-76] Based on this evidence, Charles Turner proposed three fundamental 'rules' that predict bone structural adaptations to mechanical stimuli.[77] First, dynamic loading drives bone adaptation, such that the stimulus for bone adaptation increases with load magnitude or frequency. Second, only short bouts of mechanical loading are necessary to elicit an osteogenic response. There is a ceiling effect for bone tissue stimulation (loading frequency or duration), beyond which bone adaptation is subject to diminishing returns. Third, bone cells become accustomed to routine strain; structural change is driven by abnormal strains ('strain distribution theory'[78]). These 'rules' provide insight into how different intensities and modalities of exercise predict human bone adaptation.

We cannot directly translate results from animal studies to studies of children and youth; however, animal models shed light on adaptation in bone's microarchitecture that underpin increased bone strength during growth. For example, pubertal rats (6 weeks old) subjected to 8 weeks of exercise (freefall jumps from 45 cm; strains were similar in magnitude to those observed in human athletes such as triple jumpers) had significantly greater trabecular bone volume fraction (BV/TV) and trabecular thickness (Tb.Th), but not trabecular number (Tb.N; all by micro-CT) at the proximal ulna compared with the control group (no freefall jumps).[79] In the same study, geometric adaptation to loading at the proximal ulna included thicker cortices (Ct.Th) as a result of enhanced endocortical contraction (by pQCT). Compared with controls, exercised rats had significantly greater cortical area (Ct.Ar), periosteal and endocortical circumference at the ulnar shaft and greater trabecular BMD (Tb.BMD) at the distal ulna. The ulnar shaft and distal site were not assessed using micro-CT. Collectively, adaptations in geometry and microarchitecture contributed to 36% greater mechanical strength (ultimate force

and energy to failure) in exercised rats compared with controls.[79] Similar bone microarchitecture adaptations were observed in young rats after 10 weeks of treadmill running.[80] As Figure 1.10 illustrates, exercised rats had greater BV/TV, Tb.Th and Tb.N at the distal femur compared with controls. At the femoral shaft, exercised rats had greater Ct.Ar, Ct.Th and maximum load, compared with controls. In line with the adaptations I discussed in section 1.2.2.1.3, these findings confirm that in response to loading, microarchitecture adaptations in trabecular bone predominate at metaphyseal sites whereas changes in cortical bone predominate at diaphyseal sites.

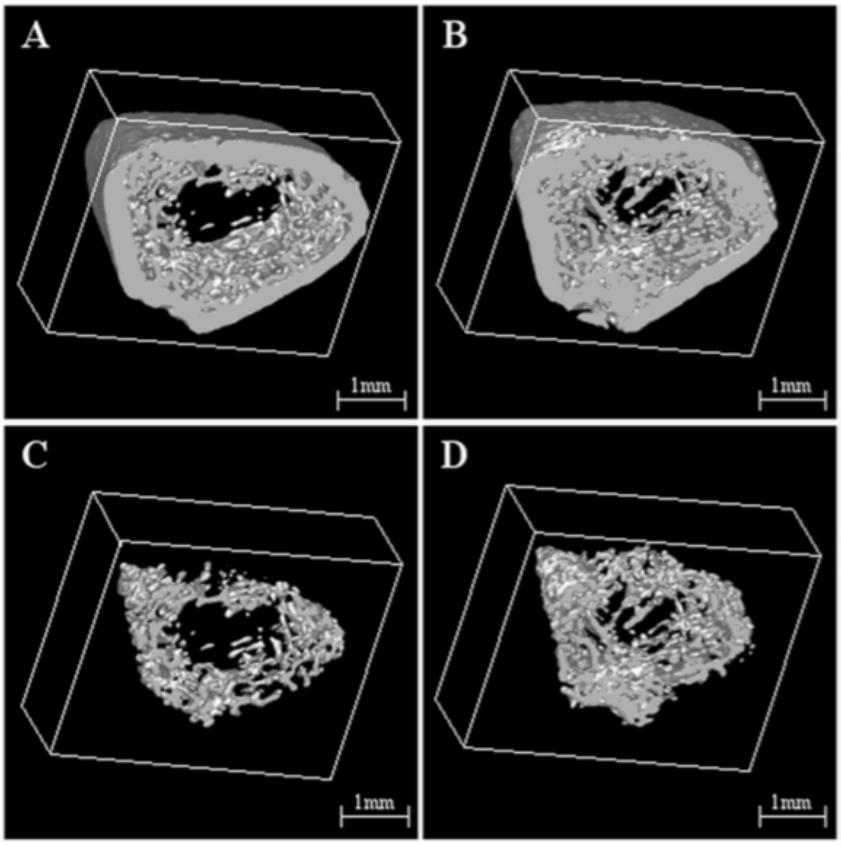


Figure 1.10. Three-dimensional images from micro-computed tomography (micro-CT) of exercise-related adaptations in bone microarchitecture at the distal femoral diaphysis in rats. Sedentary controls in A and C, exercised rats in B and D; cortical compartment in the top images, trabecular compartment in the bottom images. Reprinted from Joo et al.,[80] with permission from Elsevier.

Animal studies also provide insight into the prolonged effects of exercise training on the skeleton. For example, rats subjected to axial loads on the right forearm for 7 weeks (approximately the same relative timespan as a human childhood) had significant gains in bone

mass (by DXA), Ct.Ar and estimated bone strength (minimum second moment of area; by pQCT) compared with the left forearm.[81] However, only bone geometry and strength gains persisted after 92 weeks of detraining.[81]

The mature skeleton also adapts its strength in response to mechanical loading; however, the adaptive mechanisms may differ to that of growing bone and may be site-specific. For example, in a turkey loading model, young turkeys (1-year olds) experienced significant structural benefits at the ulnar shaft (30% gain in Ct.Ar by microradiography) following an 8-week loading program, while older turkeys (3-years old) reaped no such benefits (-3% change in Ct.Ar).[82] Conversely, a 14-week running program in a rat model elicited similar gains in bone breaking loads (N, compression testing) at the proximal femoral neck in both young and adult rats (30% and 28%, respectively). Gains in bone strength in young rats were attributed to significant gains in total area (Tt.Ar by pQCT; 25%) and no gains in total BMD (Tt.BMD by pQCT), whereas gains in bone strength in adult rats were attributed to significant increases in Tt.BMD (23%) with no increases in Tt.Ar.[83] Collectively, evidence from animal models confirms that the growing skeleton has greater capacity to adapt to mechanical loads at the diaphysis, compared with the mature skeleton. The growing skeleton preferentially adapts to loading at the metaphysis through enhanced bone geometry, whereas the mature skeleton adapts through gains in density. I discuss human bone adaptation to mechanical loading in section 1.2.6.

1.2.3 Measuring bone in children and adolescents

As I described in section 1.2.2.1, direct measurements of whole bone strength can only be acquired through mechanical testing using animal models. However, a number of imaging tools are commonly used to estimate bone's ability to resist fracture. A number of factors influence choice of imaging tool, including the study aim, site of skeletal assessment and cost. In this section, I discuss three imaging modalities in detail, including strengths and limitations of each: dual energy X-ray absorptiometry (DXA), peripheral quantitative computed tomography (pQCT) and high-resolution pQCT (HR-pQCT).

1.2.3.1 DXA

DXA was introduced nearly 30 years ago and is the current clinical gold standard for assessing bone health. Advantages of DXA include its wide availability, relatively low radiation exposure, low cost, short scan time and ability to scan clinically relevant sites such as the lumbar spine and proximal femur in addition to the whole body.[84] The effective dose equivalent for a whole body scan is low; approximately 1.4 to 13 μSv (comparable to effective daily background radiation dose ~ 4 μSv/day) depending on the scan.[85] In addition, availability of pediatric normative values make DXA an attractive tool for clinical and research settings.

DXA quantifies bone mass through attenuation of photons of two different energies, based on known density of different tissues.[84] Attenuation of each pixel of the X-ray beam is measured as it projects from an X-ray source above the participant to one or more X-ray detectors beneath the table. Beam attenuation is greater in mineralized tissues compared with soft tissues; bone is more dense than soft tissue due to heavier calcium and phosphate composition.[86] Figure 1.11 illustrates how photon attenuation is measured within each pixel of a DXA image. DXA measures the mass of hydroxyapatite (in g/cm^2) along a straight path from the X-ray source to the detector.[53] X-ray beam attenuation within pixels located in a given region (i.e., whole body, femoral neck) above a certain threshold (set by the manufacturer) are averaged to provide areal BMD (aBMD, g/cm^2). Bone area (BA, cm^2) is the total area of all pixels that exceeded the bone threshold. Bone mineral content (BMC, g) is the product of aBMD and BA.

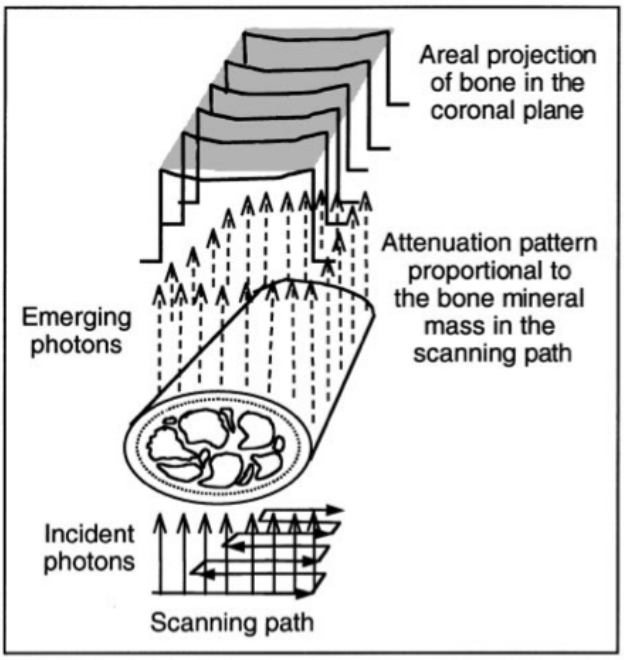


Figure 1.11. Illustration of densitometry. Photons are attenuated during transmission, producing an attenuation profile proportional to the mass of mineralized bone in the scanning path. Reprinted from Seeman,[87] with permission from Endocrine Society.

Despite widespread use, DXA is not without limitations. First, because DXA derives its output by summing the mineral mass between the X-ray source and detector, it cannot determine 3D cross-sectional geometry, distribution (thickness) of mass[86] or true volumetric density (g/cm^3).[88] Given its planar, two dimensional (2D) nature, DXA cannot account for bone depth; thus, measures of bone mass are strongly influenced by body size (e.g., larger bones have greater aBMD compared with smaller bones because of differences in size).[84] For example, aBMD is systematically underestimated in shorter people. Further, catch-up growth results in higher values of aBMD even though actual volumetric BMD has not changed.[88] This issue is problematic when comparing bone mass between children of different sizes or within the same children longitudinally. Strategies to overcome DXA's limitations include adjusting BMC for bone area, height and age, body weight or muscle mass.[88] Method of adjustment largely depends on the nature of available reference data.[89]

Second, DXA cannot differentiate between cortical and trabecular bone compartments. These parameters must be considered as only quantifying bone mass does not adequately describe bone's mechanical competence. Further, because bone responds to loading by adding

new bone tissue where mechanical demands are greatest, small increases in bone mass can substantially improve bone strength. This was highlighted in several animal studies whereby minimal exercise-induced increases in DXA-derived BMC and aBMD (< 10%) were accompanied by substantial increases (> 60%) in bone strength (by micro-CT).[90,91] The importance of assessing bone geometry is illustrated in Figure 1.6; marked variation in bone bending strength (section modulus) occurs despite bones having the same measured aBMD.[54] Finally, body composition influences DXA outcomes. DXA assumes homogenous distribution of fat around bone;[92] however, soft tissue thickness affects beam magnification, such that non-uniform distribution of fat around bone may lead to inaccurate assessment of BMC and aBMD.[93]

1.2.3.1.1 Hip structural analysis

Application of hip structural analysis (HSA) to proximal femur DXA scans is a strategy commonly used to counter DXA's 2D limitations. Based on mechanical engineering principles, HSA derives measurements of bone geometry, such as CSMI, and indices of bone strength, such as section modulus (a common measure of bone's resistance to bending at the femoral neck), from bone mineral data in the image plane.[94] As DXA only projects mineral in the cross-section, excluding soft tissue and voids, a bone thickness profile can be derived by dividing each pixel's mineral mass (g/cm^2) by the average mineral density of fully mineralized bone (1.05 g/cm^3).[53] The thickness profile represents the bone cross-section as if it were compressed into solid cortical bone.[53] The thickness profile collapses information regarding the distribution of mass along the X-ray paths, but preserves the distributional information in the image plane. Using HSA, mineral thickness profiles and cross-sections are extracted at three locations of the proximal femur: the narrow neck, the intertrochanteric region and across the shaft, from which CSMI and section modulus can be derived. Limitations of this approach include: 1) the assumption that the femoral neck and shaft are circular; 2) the assumption that tissue mineral density is constant; and 3) the proportion of cortical and trabecular bone is constant within the cross-section, which is often not the case.[84] As children's bones are less mineralized compared with adult bones, estimates of cross-sectional geometry are often underestimated in pediatric

HSA studies.[86] Further, scan dimensions are altered by participant/patient positioning, which makes it difficult to differentiate between actual dimensional changes and positioning errors.[53]

1.2.3.2 pQCT

Quantitative computed tomography (QCT) differs from DXA in that it directly measures bone cross-sectional geometry and volumetric BMD (g/cm^3) for a given region of interest (ROI). Central QCT systems are used clinically to image vertebral and femoral geometry and density, but are rarely used in pediatric research due to a higher dose of ionizing radiation (150-300 μSv; ~ one tenth to one fifth of total annual effective background radiation dose) compared with DXA.[41] In contrast, peripheral QCT (pQCT) is primarily a research tool used to assess bone geometry and BMD at distal and shaft sites of the tibia and radius. Like DXA, pQCT is common in pediatric research due to its short scan time (~3 min per scan) and minimal effective radiation dose (< 0.1 μSv for a complete scout view and pQCT scan).[95] pQCT is reliable, with in vivo reproducibility at tibial shaft sites ranging from 0.4-1.9% (coefficient of variation (CV)).[96] Reference data are available for children and young adults for cortical and trabecular BMD, CSA and cortical thickness.[97,98] The Centre for Hip Health and Mobility where I conducted my studies houses the XCT 3000 (Scanco Medical, Basserdorf, Switzerland). Thus, I focus my discussions on this model and on acquisition and analysis of bone images at the tibia shaft, specifically.

As with DXA, pQCT quantifies bone by evaluating attenuation of ionizing radiation (from an X-ray beam) through an object, from source to detector. The central pQCT gantry diameter is 300 mm. An X-ray tube produces a narrow beam with a focal spot size of 250 x 250 μm operating at 60 kV (Figure 1.12).[95] The gantry rotates in 12° steps for 15 translations to obtain a single image with a 2.5 mm slice thickness.[95] There is a minimal amount of scatter radiation from pQCT as the beam is tightly collimated (<1 μSv). One pQCT scan results in less than 1/1000 of the recommended yearly radiation exposure (1 mSv), and the effective dose is significantly less than that of a standard chest X-ray (100 μSv).[95] Despite these advantages, given its maximum imaging resolution of 0.2 mm, a limitation of pQCT is that it cannot accurately assess bone microarchitecture or separate cortical and trabecular bone in regions with a thin cortex, such as the distal radius.[99]

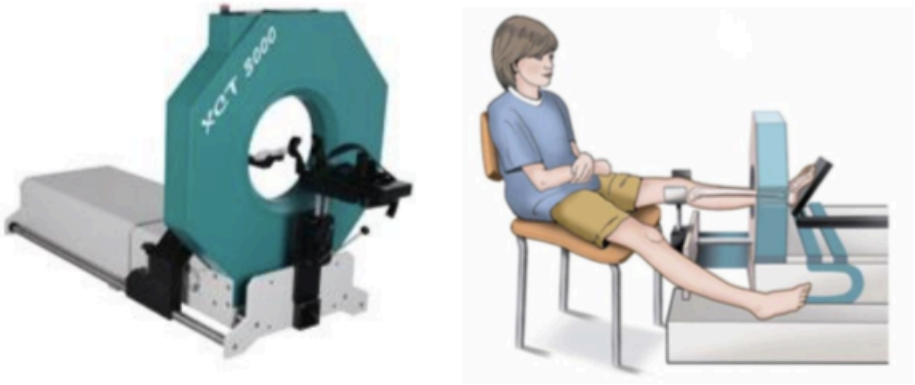


Figure 1.12. Image of peripheral quantitative computed tomography system (pQCT), model XCT 3000 (Stratec Medizintechnick GmbH). An illustration of leg positioning for pQCT tibia scans (by Vicky Earle, Medical Illustrator).

1.2.3.2.1 Image acquisition and analysis

Protocols for pQCT image acquisition are not standardized; thus, there exists considerable variability in reported scan protocols. The user can alter various parameters, including image resolution and scan speed. Radiation exposure varies with those parameters such that higher resolution scans increase radiation exposure. pQCT pixel sizes range from 0.2 mm (higher resolution) to 0.6 mm (lower resolution). Shorter scan speeds minimize radiation dose and movement artifacts, yet may sacrifice resolution. Use of a lower resolution pixel size increases the possibility of partial volume effects (PVE). The PVE refers to presence of tissues of varying densities (i.e., soft tissue and bone) within the same pixel (Figure 1.13),[100] which could underestimate BMD. Thus, minimizing radiation exposure and PVE should be carefully considered when choosing an acquisition protocol. Common protocol for pQCT image acquisition in pediatric studies is a 0.4 mm pixel size and a 30 mm/s scan speed.[101-103]

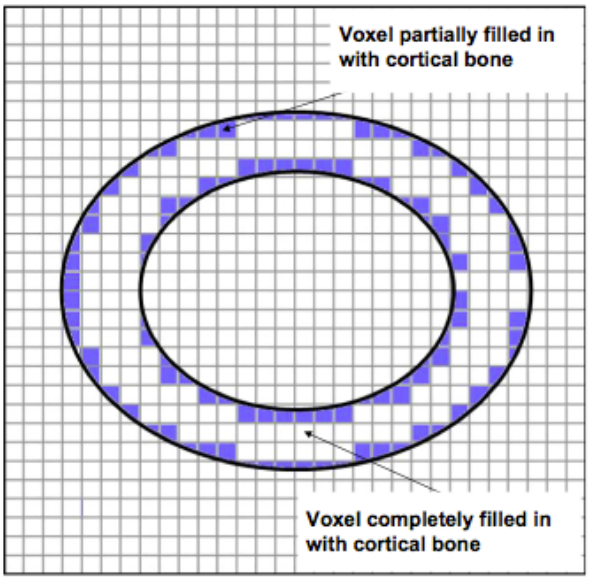


Figure 1.13. Illustration of the partial volume effect (PVE), whereby pixels at bone edges (blue pixels) contain both bone and soft tissue densities, resulting in a lower density for the blue pixels. Smaller bones have more pixels close to the bone edge and may be more affected by PVE. Reprinted from Zemel et al.,[100] with permission from Elsevier.

Reference line placement is also an issue with pediatric pQCT scans, as the measurement site continually migrates as bone grows. Thus, our research group chose to assess a site that is the same relative position from a fixed bony landmark and is reproducible during growth. Specifically, to assess cortical bone at the tibial midshaft we scan a site 50% of the distance proximal to the distal tibial endplate. We also assess muscle cross-sectional area (MCSA) at this site. This differs slightly from the manufacturer's recommendation that MCSA be measured at the 66% site as this is where the muscle belly is largest, on average.[104] However, MCSA at the two sites is strongly correlated ($r = 0.95$, $n = 20$ girls and boys aged 9-11 years; unpublished data from the Healthy Bones Study (HBS)). Further, acquiring MCSA at the 50% site reduces the number of scans needed in pediatric studies.

As with pQCT image acquisition, image analysis protocols are not standardized. Options include default settings and user-defined thresholds and modes. Prior to image analysis, the user defines a ROI, either automatically or manually. First, pQCT software separates bone from soft tissue by removing pixels below a user-defined threshold, leaving an outer edge of bone. Remaining (bone-filled) pixels are used to calculate total bone outcomes (Contour Mode; Tt.Ar and Tt.BMD). Second, the pQCT software removes all pixels in the ROI with an attenuation

coefficient below the defined threshold for cortical bone (Separation Mode; Ct.Ar, Ct.Th and cortical BMD (Ct.BMD)). At shaft sites, the pQCT software provides an estimate of bone strength (SSI) in bending and torsion. SSI is calculated as the integrated product of section modulus and Ct.BMD.[105] The ratio of Ct.BMD to normal physiological density (1200 mg/cm^3) provides an estimate of the modulus of elasticity. SSI can be determined with respect to the polar (z) axis (SSI_p, measures moment in torsion) or the bending (x,y) axes ($SSI_{x/y}$, measures moment in bending).[105] SSI_p explained over 90% of the variance in long bone torsional mechanical properties in adult cadavers.[106] Bone strength in compression is estimated at distal sites using BSI (described in Section 1.2.2.1.3).[55]

1.2.3.3 HR-pQCT

As with pQCT, high-resolution pQCT (HR-pQCT) uses an X-ray and detector that rotate around the lower leg or forearm (Figure 1.14). The X-ray tube has an 0.08 mm focal point, spanning a 12.6 cm field of view. The system acquires 110 parallel CT slices, stacked to form a 3D image. In contrast to pQCT, the imaging resolution of HR-pQCT ranges from 82 μm in first-generation scanners, to 61 μm in second-generation scanners.[107] In this thesis I focus on the first generation scanner (XtremeCT, Scanco Medical). Resolution of HR-pQCT permits accurate assessment of trabecular microarchitecture, such as trabecular number and thickness.[108] Adult trabecular thickness ranges from 100-300 μm,[41] whereas in children trabecular thickness ranges from ~60-100 μm.[109] Manufacturer's standard settings include an effective energy of 60 kVp, X-ray tube current of 900 μA and integration time of 100 ms. The < 3 μSv radiation exposure is equivalent to 0.2% of total annual background radiation in Canada; radiation scatter from a standard scan is very low (0.75 μSv).[110]

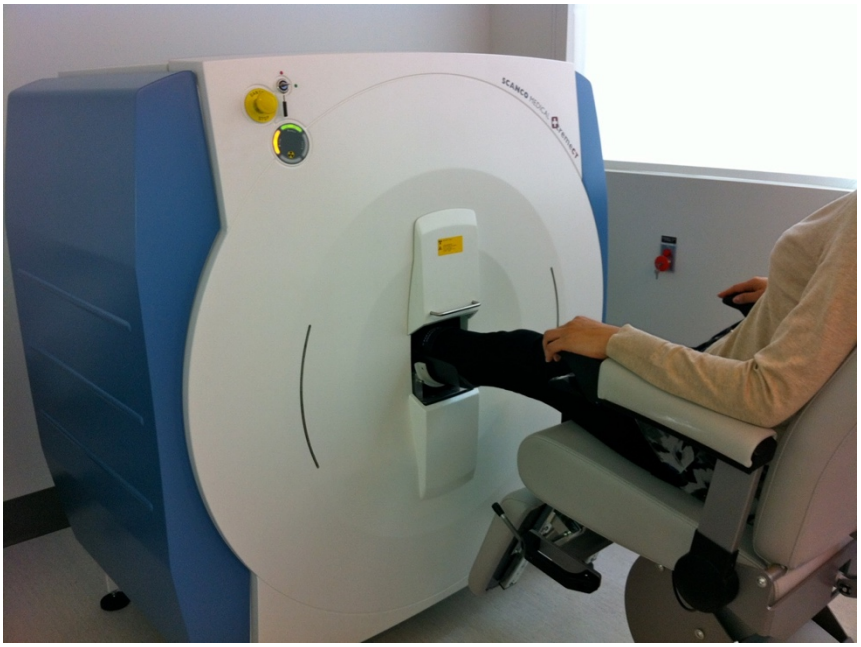


Figure 1.14. Image of high-resolution peripheral quantitative computed tomography (HR-pQCT) XtremeCT system (Scanco Medical) and leg positioning for tibia scan.

1.2.3.3.1 Image acquisition and analysis

To acquire HR-pQCT images, the skeletal site of interest (tibia or radius) is first immobilized in a carbon fiber cast and placed inside the scanner's gantry. A 2D anterior-posterior scout view scan is performed to identify the region of interest. Manufacturer's standard protocol uses a ROI of 9.5 mm and 22.5 mm proximal to the radial inclination tuberosity and tibial end plate, respectively. However, as with pediatric studies that use pQCT, a relative ROI is preferable over an absolute ROI for several reasons. First, in growing bone, a fixed site (e.g., 22.5 mm from a bony landmark) is a 'moving target' over time, one that is relatively more distal as the participant grows. A relative site (e.g., 8% of bone length from a fixed bony landmark) on the other hand, enables the operator to scan the same 'relative' region across growth.[111] Second, cortical and trabecular compartments differ markedly from distal to proximal sites along a long bone. Thus, relative positioning enables comparisons between participants of different sizes and with shorter versus longer limbs lengths. For example, a fixed ROI at 22.5 mm proximal to the tibial end plate is equivalent to a 5% site on a tall participant with a tibia length of 430 mm and a 7% site on a shorter participant with a tibia length of 340 mm. Comparisons between 5% and 7%

sites is confounded by a greater proportion of cortical compared with trabecular bone at the more proximal site. Finally, in children we must avoid irradiating the growth plate whenever possible. The 8% site of the distal tibia and 7% site of the distal radius includes both cortical and trabecular bone but excludes the growth plate in most children.[110,111]

Once the reference line is placed at the tibial end plate or distal medial edge of the radius, 110 tissue slices are scanned proximal to the 8% or 7% measurement site, respectively. In total, an approximate 9.02 mm region of the tibia or radius is scanned in less than 3 min. HR-pQCT outcomes from a standard morphological analysis include Tt.Ar (mm^2), Tt.BMD (mg HA/cm^3), Tb.BMD (mg HA/cm^3), BV/TV, Tb.N (1/mm), Tb.Th (mm) Tb.Sp (mm), Ct.BMD (mg HA/cm^3) and Ct.Th (mm). Of the trabecular measures, Tb.BMD and Tb.N are measured directly, while BV/TV, Tb.Th and Tb.Sp are derived from Tb.BMD and Tb.N[112] HR-pQCT demonstrates good agreement (R^2 = 0.59-.96) with micro-CT–derived (~20 μm voxel size) standard outcomes from cadaveric bone specimens.[113] In vivo estimates of reproducibility are less than 1% (root mean squared CV) for density parameters and between 0.7% (BV/TV) - 4.4% (Tb.Sp) for microarchitecture parameters at the distal tibia and radius.[114]

Standard HR-pQCT analysis frequently mistakes thin or porous cortical bone as trabecular bone and vice versa. Therefore, automated segmentation algorithms can be applied to HR-pQCT images using customized software to more accurately separate cortical and trabecular compartments (Figure 1.15).[115] Outcomes from automated segmentation algorithms include: Tt.Ar (mm^2), Ct.Ar (mm^2), cortical porosity (Ct.Po, %), Ct.BMD (mg HA/cm^3) and Ct.Th (mm).[115,116] In vivo estimates of reproducibility (root mean squared CV) for cortical parameters range from 0.6% for Ct.BMD to 13% for Ct.Po.[117]

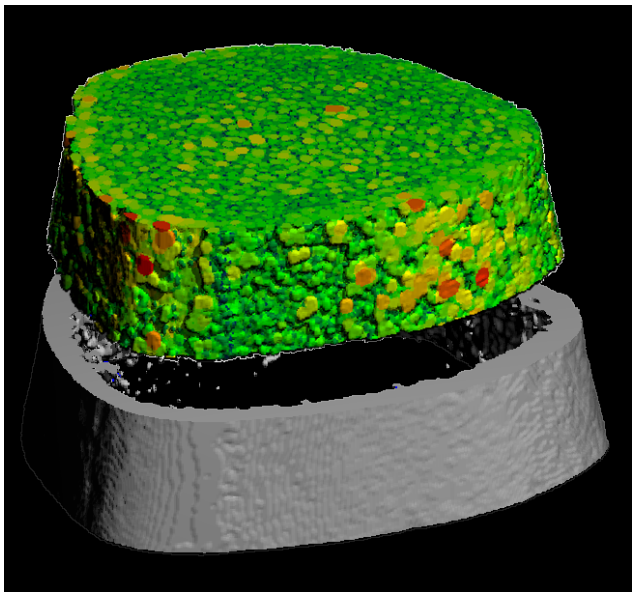


Figure 1.15. Illustration of trabecular (top image, green) and cortical (bottom image, grey) regions from a segmented high-resolution peripheral quantitative computed tomography scan.

Resolution of HR-pQCT is sufficient to obtain finite element analysis (FEA)-derived estimates of compressive bone strength. FEA is a computationally demanding numerical approach that converts 3D image data into FEA meshes voxel by voxel.[118] Conceptually, FEA breaks down a complex structure (i.e., bone) into smaller simpler elements. Computer-generated FEA models simulate applied loads, typically uniaxial compressive forces, onto the smaller elements. FEA outcomes include failure load (force that causes bone to fail; F.Load, N) and stiffness (reaction force using the FEA model at 1% strain divided by the average bone CSA from standard analyses; N/mm).[118] Stiffness is used to estimate ultimate stress (highest stress the bone can withstand per unit area without failing; U.Stress, MPa). F.Load derived from FEA is used to calculate load-to-strength ratio at the distal radius (ratio of estimated fall load applied to the outstretched hand after a fall from standing height; ϕ), an estimate of forearm fracture risk.[119,120]

FEA-derived U.Stress demonstrates good agreement ($R^2 > 0.94$) with experimentally-determined strength from destructive loading in human adult cadaver forearms, suggesting U.Stress is a good surrogate of bone strength (Figure 1.16).[118] Further, strong correlations between bone stiffness measures of the tibia and radius by HR-pQCT (and FEA) and stiffness of the lumbar vertebrae and proximal femur using central QCT (r = 0.69-70), suggest the mechanical competence of the distal radius and distal tibia reflect that of central, clinically

relevant sites.[121] However, no studies have investigated the relationship between FEA models and experimentally-determined bone strength in pediatric cadaver bone.

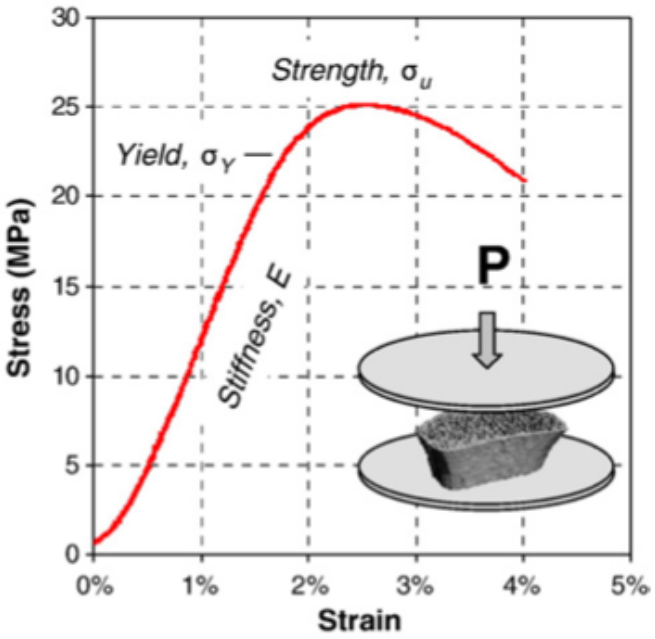


Figure 1.16 Illustration of stress-strain curve of destructive loading of cadaveric distal radii to determine linear and elastic failure regions. P = platen force. Reprinted from MacNeil et al.,[118] with permission from Elsevier.

HR-pQCT is limited to scanning the peripheral skeleton. However, it is an attractive imaging tool based on its high-resolution and short scan time (~2.8 min). Nevertheless, HR-pQCT acquisition and analysis protocols are not yet standardized, which limits comparisons across studies. Normative data for HR-pQCT are available in older adolescents (16-19 years)[122] and adults,[123] but not in children or younger adolescents. Finally, movement during high-resolution imaging increases the likelihood of motion artifacts (streaks or discontinuities on the scan), which may require that scans be repeated or excluded from analysis.[124]

1.2.4 Maturity- and sex-related differences in bone strength and its determinants

In the following sections, I outline several methods to assess maturity in children and adolescents. I also briefly review hormones that influence skeletal development and maturity- and sex-related differences in skeletal development.

1.2.4.1 Assessing maturity

Growth and maturation refer to a range of developmental processes (i.e., physical, sexual, cognitive). For the purpose of this thesis, I consider growth as somatic; that is, increases in body size or mass. I refer to maturation as the tempo and timing of biological (physical and sexual) changes associated with somatic growth.[125] As there is no constant relationship between maturity and time,[126] chronological age is not equivalent to stage of maturation.

1.2.4.1.1 Sexual maturation

Maturity in children and adolescents is commonly assessed as per the method of Tanner, which is based on development of secondary sex characteristics; breast and pubic hair development in girls and pubic hair and genital development in boys.[127] Tanner divided the continuous process of maturation into five discrete stages: Tanner stage 1 represents pre-puberty, Tanner 2 and 3 early-puberty, Tanner 4 peri-maturity and Tanner 5 post-puberty or reproductive maturity. A physician or nurse may perform the maturity assessment in clinical or research settings; however, if such personnel are unavailable, participants may self-assess using photographs or line drawings depicting stages of breast and genital or pubic hair development. Physician (or nurse)-determined Tanner stages correlate well with testosterone and estrogen levels in boys and girls.[128] However, when self-assessed, younger, less mature participants tend to overestimate their development, while more mature children are prone to underestimate development.[126] Further, the practicality of Tanner stages may be confounded by body composition; obese girls tend to overestimate their Tanner breast stage as adipose tissue can be mistaken for breast development.[129] Nevertheless, maturity assessment using the method of Tanner is attractive for clinical and research purposes since it is cost effective and only requires a one-time measurement. However, maturation is continuous and, thus discrete stages do not account for the large variation between two children of the same Tanner stage. In addition, there is sexual dimorphism in timing and tempo of maturity, such that girls mature at an earlier chronological age and Tanner stage compared with boys, on average.[126,130] For example, boys tend to reach peak height velocity (PHV) after entering pubic hair stage 4, while girls tend to

reach PHV after entering into breast or pubic hair stage 3.[130,131] Thus, boys and girls are not comparable at the same chronological age or stage of secondary sex development.

In girls, age at menarche is a common maturity indicator in cross-sectional and longitudinal studies.[125] Menarche (first menstrual period) is a relatively late event in sexual maturation, occurring at approximately Tanner breast stage 4.[132] Menarcheal status is typically self-reported by asking participants if they have experienced menarche. If yes, the participant is queried for a more precise date (month and/or year). Most girls can remember within a month of when their first 'period' occurred.[126] For boys, however, there is no such clear maturational event with timing that aligns with menarcheal status.

1.2.4.1.2 Skeletal maturation

Skeletal age, or bone age, is determined through radiography of the hand/wrist and is typically performed by trained clinicians based on one of three methods: Greulich-Pyle, Tanner-Whitehouse and Fels.[126] These methods use the left hand and wrist to estimate skeletal age; however, they differ with respect to bones assessed, scoring method and reference sample.[126] Skeletal age assesses fusion of the epiphyseal plate and is based on the premise that greater bone development and less cartilage will be observed in a more mature individual compared with a less mature individual.[125] While these techniques are used frequently in clinical settings, their broad use is limited by the ionizing radiation associated with radiography.

1.2.4.1.3 Somatic maturation

Maturation can also be assessed based on somatic changes in growth trajectories. Age at PHV (APHV), the age when maximum velocity in stature is attained, is a common indicator of somatic maturation and often termed 'biological age'.[126] As a continuous measure, APHV overcomes limitations of Tanner staging, as boys and girls can be aligned on a common maturational landmark. APHV occurs at approximately 11.7 years in girls and 13.4 years in boys,[133] with a range of approximately 4 years for each sex. APHV typically occurs when 90% of final adult stature is achieved,[134] which is 7-9 months prior to peak in bone mineral accrual velocity (Figure 1.17)[133] and 5-7 months before peak in femoral neck strength velocity.[135] In

addition, APHV is an important relative marker of function in boys and girls. Estimated velocities of many performance tasks in both boys and girls reach a peak at the same time as maximal growth in height.[136-138] Further, APHV approximates peak skeletal age velocity.[139,140]

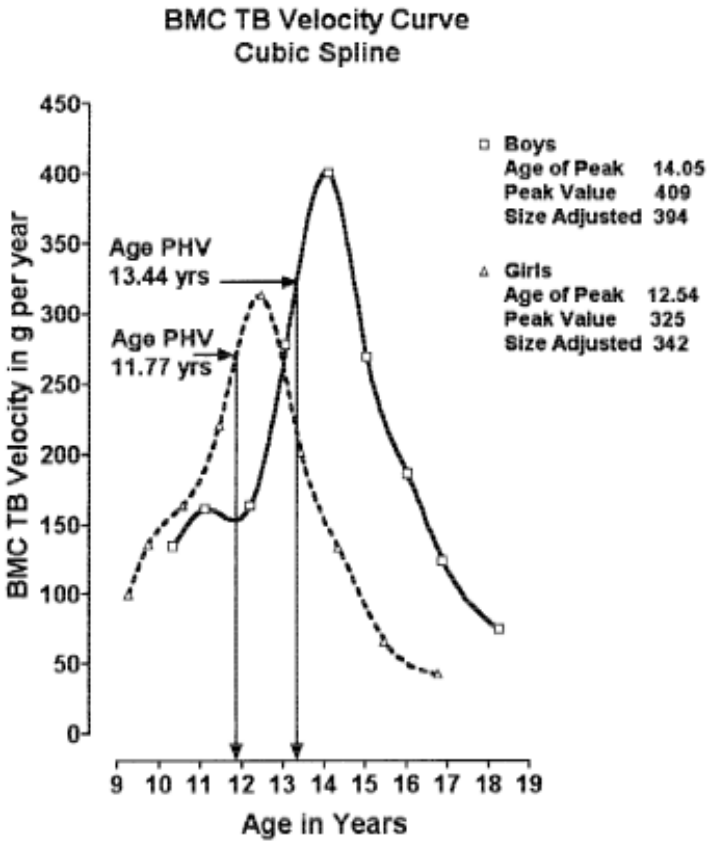


Figure 1.17. Total body bone mineral content (BMC TB) accrual velocity and ages at peak BMC and peak height velocity (PHV) for girls (dotted line) and boys (solid line) aligned on chronological age. The lag period between age at PHV and peak BMC is approximately 7-9 months. Reprinted from Bailey et al.,[133] with permission from John Wiley and Sons.

Direct assessment of APHV requires frequent (typically annual), serial assessments of height for at least two years surrounding peak growth; from these data growth trajectories can be mapped. However, in cross-sectional or short-term prospective studies, prediction equations can be used to estimate maturity offset (years from APHV).[141,142] Prediction equations use one-time measurements of anthropometric variables, including combinations of height, sitting height, leg length and age to estimate maturity offset. The Mirwald prediction equations are sex-specific and use height, sitting height, leg length, chronological age and their interactions to predict years

from APHV.[141] The Mirwald equation was cross-validated in longitudinal datasets (one Canadian sample and one Flemish sample) and predicted 88% and 89% of the variation in APHV in girls and boys, respectively.[141] However, accuracy of maturity offset prediction differs based on timing of maturation, such that the equation underestimates actual APHV in late-maturing boys and girls and overestimates APHV in early-maturing boys and girls.[143]

Our research group recently redeveloped the Mirwald equation to address concerns regarding its accuracy.[142] Specifically, longitudinal data (multiple observations per child) were analyzed using cross-sectional techniques, thereby ignoring within-person variation. This underestimated standard errors and *p*-values, and included spurious variables in the prediction model.[142] The re-developed equation includes fewer predictor variables and is as accurate as its predecessor. For example, the girls' equation includes age and height as predictors and explained 91% of the variance in APHV, while the re-developed boys' equation includes age and sitting height and explained 90% of the variance in APHV.[142] As sitting height may not be assessed in all growth studies, an alternate equation was developed for boys. This model used height instead of sitting height and demonstrated comparable accuracy ($R^2 = 0.90$). External validation of the model in two cohorts of children and adolescents demonstrated that 90% of predictions were within ± 1 year of actual APHV.[142] However, both Mirwald and Moore equations were developed and validated in white children only and may be inappropriate for use in ethnically diverse samples. Further, neither equation was robust enough to assess maturity prior to initiation of the growth spurt.

1.2.4.2 Maturity- and sex-related differences in bone development

In this section, I review development of bone strength and its determinants through adolescent growth. I focus on studies that used pQCT to assess maturity- and sex-related adaptations at the diaphysis and studies that used HR-pQCT to assess maturity- and sex-related adaptations at the metaphysis. Finally, I briefly discuss several important determinants of bone development throughout growth. I acknowledge that DXA studies were key to advance our understanding of bone adaptations to PA, and direct the reader to several excellent reviews of DXA-based studies.[10,144,145] In this section, I briefly describe findings from one prospective DXA study; I focus, whenever possible, on studies that employed 3D imaging tools.

One of the most widely cited studies, the University of Saskatchewan Pediatric Bone and Mineral Accrual Study (PBMAS), followed approximately 200 healthy children (age 8 to 15 years at study entry) through early adulthood. Researchers aligned children on APHV to control for maturation. Bone mass accrual peaked approximately 1 year after PHV, while approximately 35% of total body and lumbar spine BMC and more than 27% of femoral neck BMC was accrued during the 4-years around PHV.[9,133,134] Bone accrued in this short period represents more than will eventually be lost across 50 years of adulthood.[146] Sex differences in timing and magnitude of bone accrual were observed, such that total body BMC accrual occurred 1.4 years earlier in girls and was smaller in magnitude compared with boys.[133] Further, boys had significantly greater total body, femoral neck and lumbar spine BMC at all biological ages (-3 to +4 years from APHV).[147] Less is known about sex differences in bone strength and its determinants during growth. A better understanding could provide insight into the higher incidence of low-energy fractures in boys compared with girls during the adolescent growth spurt.[148]

Despite recent advances in high-resolution imaging technologies, only three studies (one from our group) used HR-pQCT to examine maturity- and sex-related adaptations in bone geometry and microarchitecture during adolescent growth.[4-6] Only two of these studies (one from our group) used FEA to estimate bone strength.[4,5] I briefly summarize these studies in Table 1.1 and refer to each by study name throughout this section.

Table 1.1. Overview of studies that used HR-pQCT to examine sex and maturity-related adaptations in bone strength and its determinants during adolescent growth.

Cohort	**Mayo Clinic**[5]	**Australian**[6]	**HBSIII**[4]
Study design	Cross-sectional	Cross-sectional	2 years at radius, 3 years at tibia; 1-year between measures
N	N = 127	N = 129	N = 398
Sex	66 girls / 61 boys	60 girls / 69 boys	212 girls / 186 boys
Ethnicity	96% white, 1% Asian, 3% other	100% white	47% white, 46% Asian, 7% other
Age	6-21 years	5-18 years	9-22 years
Maturity	14% bone age 6-8 years 26% bone age 9-11 years 23% bone age 12-14 years 23% bone age 15-17 years 14% bone age 18-21 years	51% Tanner 1 12% Tanner 2 9% Tanner 3 13% Tanner 4 15% Tanner 5	13% Tanner 1 24% Tanner 2/3 32% Tanner 4 31% Tanner 5
Site scanned	Radius (1 mm proximal to the epiphyseal growth plate of radius)	Radius (4% site); Tibia (7% site)	Radius (7% site); Tibia (8% site)
Bone outcomes	Standard analysis: BV/TV, Tb.Th, Tb.Sp, Tb.N Gaussian filter and threshold: Ct.BMD, Ct.Th, periosteal and endosteal circumference, Ct.Po index Finite element analysis: F.Load, Fall force, load-to-strength ratio, % load cortical bone	Standard analysis: Tt.BMD, BV/TV, Tb.Th, Tb.Sp, Tb.N, Tt.Ar, Ct.Ar, Ct.Th, Ct.BMD	Standard analysis: Tt.BMD, BV/TV, Tb.Th, Tb.Sp, Tb.N Automated segmentation: Ct.Po, Ct.Th, Ct.BMD, Ct.Ar, Tb.Ar, Tt.Ar Finite element analysis: U.Stress, F.Load, load-to-strength ratio

1.2.4.2.1 Bone strength

As discussed in section 1.2.2.2, changes in geometry, BMD and microarchitecture influence gains in bone strength during growth. Of the three cohorts assessed using HR-pQCT, all demonstrated substantial increases in bone strength throughout adolescent growth. Figure 1.18 illustrates that girls and boys in the HBSIII cohort had approximately 100% and 200% greater compressive bone strength (F.Load), respectively, at the distal radius during post-puberty compared with pre-puberty.[4] There were smaller differences (approximately 35% in girls and 50% in boys) at the distal tibia.[4] In concert with increased bone strength, load-to-strength ratio

(an indicator of distal radius fracture risk) decreased by half from pre- to post-puberty in girls and boys.[4,5] Similar maturity-related increases in bone bending strength (SSI_p or section modulus using pQCT) were observed at shaft sites. Specifically, there were gains of approximately 100% in girls and 200% in boys at the radius from pre- to post-maturity[149] and 24% in girls and 44% in boys at the tibia from early to post-puberty.[150] Maturity-related gains in bone strength appear larger at the radius compared with the tibia; however, this is an artifact of expressing change as a percentage. Absolute bone strength and gains in absolute bone strength were smaller at the radius than the tibia (likely due to the non-weight-bearing nature of the site). Despite this, the radius demonstrated greater percentage gains across growth due to smaller baseline (pre-puberty) values compared with the tibia.

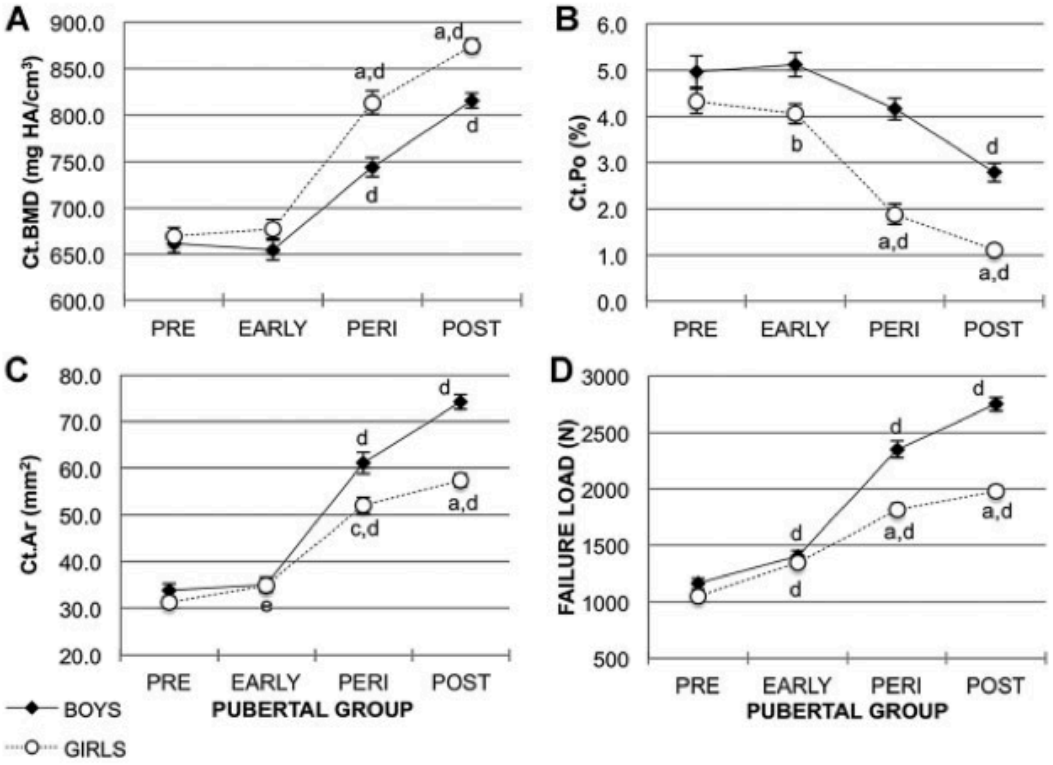


Figure 1.18. Illustrations of sex differences in high-resolution peripheral quantitative computed tomography (HR-pQCT) parameters at the distal radius by pubertal group based on the method of Tanner staging: A) cortical density (Ct.BMD), B) cortical porosity (Ct.Po), C) cortical area (Ct.Ar) and D) failure load. a, $p < 0.001$; b, $p < 0.01$; c, $p < 0.05$: significant difference between girls and boys within the same puberty group. d, $p < 0.001$; e, $p < 0.01$; significant difference between puberty group and the PRE group within sex. Reprinted from Nishiyama et al.,[4] with permission from John Wiley and Sons.

Sex differences in magnitude of bone strength accrual result in consistently stronger bones in boys compared with girls throughout adolescent growth at the weight-bearing tibia and from mid-puberty onwards at the radius. In the HBSIII cohort, F.Load was 16-25% greater in boys at the tibial metaphysis from pre- (Tanner 1) to post- (Tanner 5) puberty.[4] SSI_p (by pQCT) was 6% greater at the diaphysis in pre- and early-pubertal boys compared with girls.[151] At the radial metaphysis, F.Load was 27-39% greater in boys compared with girls from peri-(Tanner 4) to post-puberty in the HBSIII cohort (Figure 1.18),[4] and significantly greater in boys compared with girls from 12-14 years (bone-age) onwards in the Mayo Clinic cohort.[5] Similarly, SSI_p (by pQCT) was 18-32% greater in boys compared with girls at all pubertal stages at the radial diaphysis, except for a non-significant 14% difference at Tanner stage 4.[149] However, these comparisons are limited by their cross-sectional design and by use of Tanner staging to assess maturation. Prospective studies that use pQCT and HR-pQCT are needed to confirm the trajectory of bone strength accrual during adolescence and to illustrate how this may differ between sexes and skeletal sites.

1.2.4.2.2 Bone geometry

As described in section 1.2.2.1.3, bone CSA is a key determinant of overall bone strength, as bone's resistance to bending is proportional to its CSA to the third power.[152] Bone's resistance to compression is also proportional to CSA.[55] Boys' greater Tt.Ar confers them a strength advantage throughout growth compared with girls.[4-6,151] Substantial increases in bone strength during adolescent growth are underpinned by increases in Tt.Ar from pre- to peri-puberty; Tt.Ar plateaus thereafter.[4-6] For example, in the Australian cohort, Tt.Ar at the distal tibia was 35% and 55% greater in peri-pubertal girls and boys, respectively, compared with same sex pre-pubertal children.[6] The difference in distal radius Tt.Ar was 70% greater in peri- compared with pre-pubertal children.[6] Slightly smaller gains in distal tibia (10-20%) and radius (20-40%) Tt.Ar were observed in the HBSIII cohort from pre- (mean age 11 years) to peri- (mean age 16 years) puberty.[4]

Diaphyseal sites also demonstrated gains in Tt.Ar through periosteal expansion (Figure 1.19).[153,154] However, there is some discrepancy regarding sex-specific adaptation at the endocortical surface. Early cross-sectional radiographic studies of the second metacarpal

concluded that boys and girls experienced periosteal expansion and endocortical contraction at the diaphysis of the second metacarpal during growth. However, girls experienced more endocortical contraction compared with boys.[155-157] These findings are supported by a 2-year longitudinal study of pubertal girls (age 10-13 years at baseline) where narrowing of the tibial shaft (60% site) marrow cavity was observed in girls after menarche.[158] However, these results are discordant with reports of increased marrow cavity area (Tt.Ar – Ct.Ar) in both boys and girls throughout puberty (Figure 1.19).[153,154] Mechanistically, an increase in the CSA of bone and marrow would enhance bone strength by placing the neutral axis farther from the centre of mass. Contradictory findings may be due to differences in study design, imaging modality and/or method used to control for maturity. Importantly, none of the aforementioned studies examined changes at the endocortical surface in girls and boys relative to biological age.

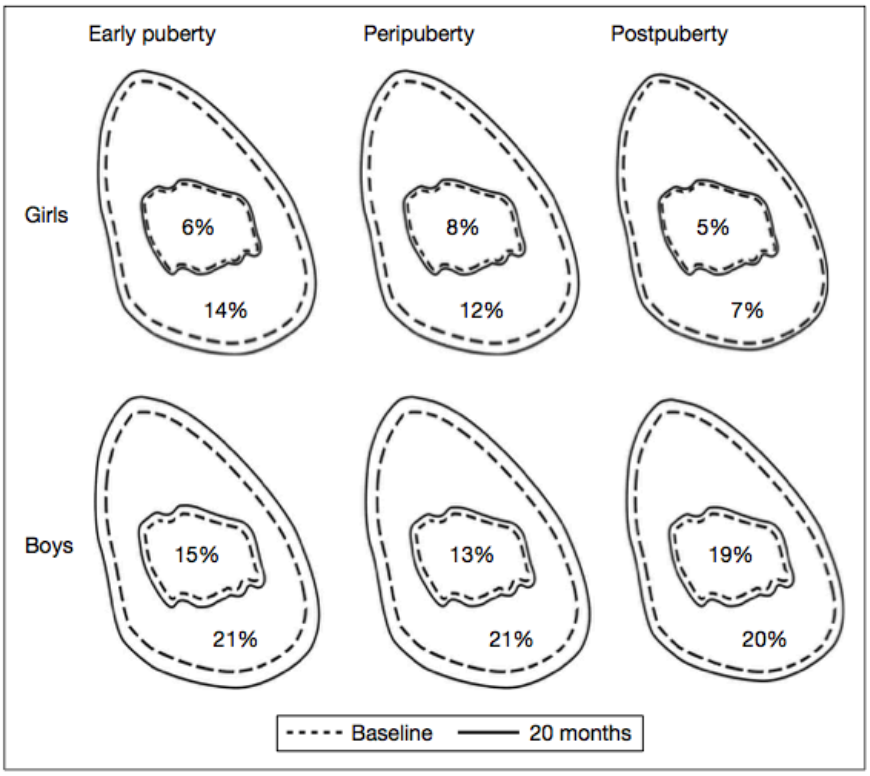


Figure 1.19. Illustration of bone growth over 20 months at the tibia midshaft in early-, peri- and post-pubertal boys and girls using peripheral quantitative computed tomography (pQCT). Numbers show the mean increase (%) in cortical and marrow cavity areas. Adapted from Kontulainen et al.,[153] and reprinted from Daly et al.,[159] with permission from Karger.

1.2.4.2.3 Bone density

Measures of Ct.BMD using 3D imaging techniques typically reflect mass of mineral per unit volume of the cortical compartment, including intracortical pores.[160] Bone modeling and remodeling activity is inversely related to BMD. During periods of active modeling or remodeling, more young bone matrix with lower mineral density is present compared with older, denser bone matrix.[160] Ct.BMD increases approximately 15-30% throughout adolescence at distal sites.[4] However, mid-puberty tends to be characterized by a transient decrease or plateau in Ct.BMD, followed by considerable increases in Ct.BMD throughout later maturity (i.e., 4% increase from pre-to early-puberty and 16% increase from early- to post-puberty in girls at the distal tibia) (Figure 1.18).[4,5] Similar maturity-related gains in Ct.BMD were observed at diaphyseal sites (by pQCT) in pubertal girls, such that greater consolidation of cortical bone (approximately 10% increase in Ct.BMD) occurred following menarche.[158,161] However, no transient decreases in Ct.BMD were observed in studies of the tibial shaft[158,161,162] or distal radius (by pQCT).[31] Nonetheless, the limited resolution of pQCT and subsequent PVE may preclude accurate assessment of Ct.BMD in bone with relatively thin cortices (< 2.5 mm) such as in children at the tibia and at all ages at the distal radius.

Consistent sex differences in Ct.BMD were observed at distal and shaft sites in later adolescence; girls demonstrated 2-10% greater Ct.BMD compared with boys from peri-puberty onwards.[4,5,162] Sexual dimorphism in Ct.BMD may arise in response to increased calcium demands during rapid adolescent growth,[163] and likely reflects boys' greater magnitude of growth and prolonged growth period compared with girls.[164] Future prospective studies should confirm these findings by aligning girls and boys on biological age.

1.2.4.2.4 Cortical microarchitecture

Growth-related increases in Ct.BMD throughout adolescence are underpinned by thickened cortices and decreased Ct.Po. Specifically, at the distal tibia and radius, Ct.Th increased by 50-70% from pre- to post-maturity in each of the three pediatric studies that use HR-pQCT.[4-6] Ct.Po decreased by 20-50% across the same period in the HBSIII cohort.[4] Further, transient decreases in Ct.BMD during mid-puberty were mirrored by transient decreases

in Ct.Th at the radius in boys and girls in Mayo Clinic and Australian cohorts [5,6] and increases in Ct.Po at the tibia and radius in HBSIII boys.[4] These maturity-related deficits at the cortex (i.e., a thin cortical shell and increased porosity during accelerated growth) may contribute to the heightened risk of fracture during the pubertal growth spurt, when growth outpaces consolidation of cortical bone.[36] Prospective studies are needed to confirm this hypothesis.

Sex differences in Ct.BMD are underpinned by sexual dimorphism in Ct.Po, such that boys have 25-175% greater Ct.Po compared with girls from early-puberty (Figure 1.18).[4] The timing of sex differences in Ct.Th is less clear. For example, in our HBSIII cohort, Ct.Th at the distal radius and tibia was 12-16% greater in boys compared with girls, but only during post-puberty.[4] In the Mayo Clinic cohort, boys demonstrated thicker cortices at the distal radius earlier in adolescence (early- and mid-puberty), but not in late- or post-puberty.[5] Finally, in the Australian cohort, girls demonstrated an advantage in Ct.Th compared with boys at both sites during peri-puberty. However, at post-puberty, cortices were thicker in boys compared with girls.[6] Discrepancies between studies likely reflect differences in methods used to segment the cortex, regions of interest and measures of maturity. Collectively, these findings suggest that greater Ct.BMD in girls compared with boys during peri- and post-puberty is largely a function of lower Ct.Po and is related to the increased intracortical bone turnover that boys experience as a result of greater magnitude of longitudinal growth.[163] Studies that align participants on biological age and incorporate automated segmentation algorithms are needed to clarify sexual dimorphism of the cortical shell.

1.2.4.2.5 Trabecular microarchitecture

As discussed in section 1.2.2.1.2, trabecular bone volume fraction (BV/TV; synonymous with Tb.BMD) is a function of the number, thickness and separation of trabeculae. Increases in BV/TV throughout growth may function to more efficiently transfer compressive loads from joint surfaces and increase bone's mechanical competence.[165] Histomorphometric study of the iliac crest (age 2-23 years; 33 females, 25 males) suggested that Tb.N varied little with age, while increased Tb.Th contributed to gains in BV/TV during growth.[166] This may occur due to remodeling with a positive balance, such that osteoblasts add more bone than was previously resorbed during each remodeling cycle (or through modeling, where new bone is added without

prior resorption), resulting in a gradual increase in BV/TV.[167] However, recent studies that used HR-pQCT to examine maturity-related changes in BV/TV were inconsistent. Figure 1.20 illustrates that BV/TV did not change significantly in girls at the distal radius or tibia throughout adolescent growth in all three pediatric cohorts mentioned previously.[4-6] In contrast, BV/TV at both sites were approximately 20% greater in peri- compared with pre-pubertal boys in the Mayo Clinic and Australian cohorts.[5,6] In the HBSIII cohort, boys' BV/TV did not differ between pre- to post-puberty.[4] Despite inconsistencies across these cohorts in growth-related adaptations in BV/TV, Tb.N did not vary with maturation in girls or boys at either bone site.[4-6] Therefore, as observed in the histomorphometric study, growth-related adaptations in BV/TV observed in boys were underpinned by 10-30% gains in Tb.Th across maturity,[4-6] potentially in response to increased serum testosterone.[5] It is difficult to explain why girls' trabecular parameters did not differ with stage of maturation. One hypothesis is that trabecular bone volume and microarchitecture are programmed early in life in girls.[5] Thus, prospective studies that span a longer period prior to adolescent growth might clarify maturity-related changes in trabecular microarchitecture.

Consistently greater BV/TV at the distal radius in boys (approximately 5-20%) compared with girls from peri-puberty onwards (Figure 1.20) was underpinned by adaptations in Tb.Th.[4-6] However, discrepancy exists regarding sexual dimorphism in trabecular microarchitecture at the tibia. In the Australian cohort, greater BV/TV in boys during peri- and post-puberty was related to significantly greater Tb.Th.[6] In contrast, boys' greater BV/TV at the tibia in the HBSIII cohort was a function of a more substantial network of trabeculae, as indicated by significantly greater Tb.N, but not Tb.Th, in boys compared with girls.[4] Additional study is warranted to clarify sex-related differences in trabecular microarchitecture throughout adolescent growth and how such differences influence bone strength.

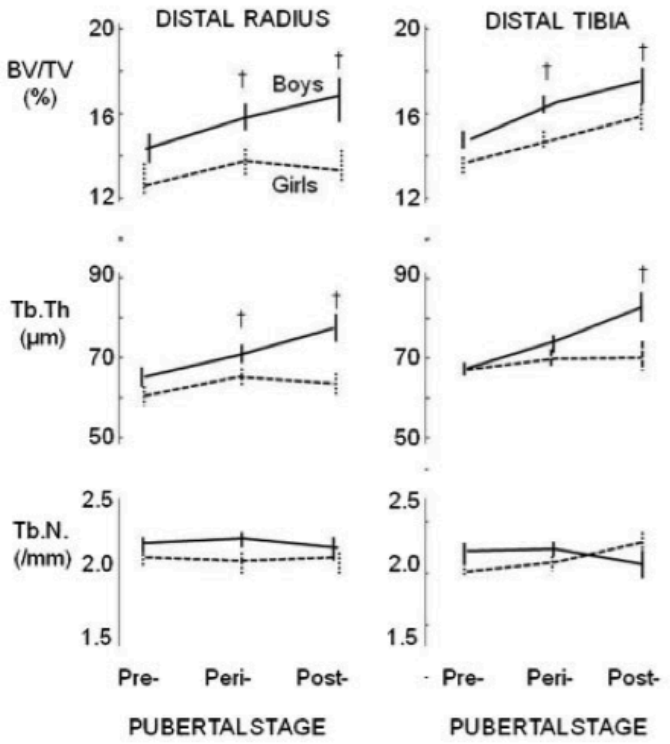


Figure 1.20. Illustration of sex differences in trabecular microarchitecture at the distal radius and tibia using high-resolution peripheral quantitative computed tomography (pQCT) across pubertal groups based on Tanner staging. ‡p<0.05 for sex difference. Reprinted from Wang et al.,[6] with permission from John Wiley and Sons.

1.2.5 Factors that influence of bone strength during growth

In this section, I briefly review intrinsic (i.e., genetics, hormones, ethnicity and muscle) and extrinsic (i.e., calcium, vitamin D, PA and sedentary time) factors that influence bone strength and its determinants in children and adolescents. I discuss the influence of PA and sedentary time on bone development in section 1.2.6 as they are primary variables of interest in this thesis.

1.2.5.1 Genetics

Total population variance for a given trait is explained by genetic and environmental factors and measurement error. Heritability is defined as the proportion of total population variance attributed to genetic factors.[168] Many genes regulate bone strength and its

determinants, such as those encoding receptors for steroid and calciotrophic hormones, local regulators of bone metabolism including growth factors and cytokines, bone matrix proteins and transporting factors.[169,170] Those implicated in bone remodeling include, but are not limited to, vitamin D, estrogen, calcitonin and parathyroid hormone receptors.[170]

Classic assessment of heritability is based on twin models, under the assumption that monozygotic and dizygotic twins experience similar environmental factors that may influence a trait. If monozygotic twins are more similar to one another than dizygotic twins, the twin model assumes this must be attributed to shared genes.[168] Monozygotic twins share 100% of their genes, whereas dizygotic twins share 50%. Thus, the correlation for a given trait for a monozygotic twin should be double that of the dizygotic twin if that trait is 100% genetically-determined.[169] However, the twin model cannot prove that genetic factors are the sole cause of any correlation, as there may be non-genetic explanations for a stronger relationship between monozygotic twins (i.e., gene-environment interactions related to lifestyle or preferential loading of limbs).[168]

Heritability estimates for BMC and aBMD (by DXA) at the lumbar spine and proximal femur range from 40-60% in family studies and 70-80% in twin studies. [169,171,172] However, much of this may be explained by body size, as heritability estimates for stature range from 60-80%.[169] Further, adjusting for body size attenuates heritability estimates of aBMD in twin and familial studies.[173]

Evidence from heritability studies of bone geometry and strength (by pQCT) suggest the influence of genetics may vary across skeletal sites. For example, heritability estimates of CSA at the distal radius within several Hutterite colonies in the United States ranged from 27% at the 4% site to 75% at the 20% site, after adjustment for age, sex, height and weight.[174] Site-specific differences in heritability may be partly explained by greater measurement error at the 4% site compared with the 20% site.[174] Heritability of compressive bone strength (BSI) in elderly female twins was greater at the distal radius (83%) compared with the distal tibia (61%).[175] Unlike the tibia, the distal radius is not subjected to compressive loads from body weight. Thus, the complex interaction between genetic and environmental influences is likely site-specific, such that the weight-bearing tibia may be more sensitive to environmental factors compared with the non-weight bearing radius.

1.2.5.2 Hormones

Significant alterations in the hormonal environment drive dramatic increases in linear growth and bone strength during maturation. For example, growth hormone (GH) and insulin-like growth factor (IGF-1) regulate longitudinal bone growth and influence bone modeling by stimulating osteoblasts and chondrocytes.[176] GH deficiency during childhood significantly reduces (50%) longitudinal bone growth resulting in smaller bone size and less bone mass accrual. [176] GH secretion peaks in concert with PHV and decreases thereafter (Figure 1.21), while IGF-1 peaks slightly later.[177] GH and IGF-1 continue to influence bone remodeling following cessation of linear growth.[176]

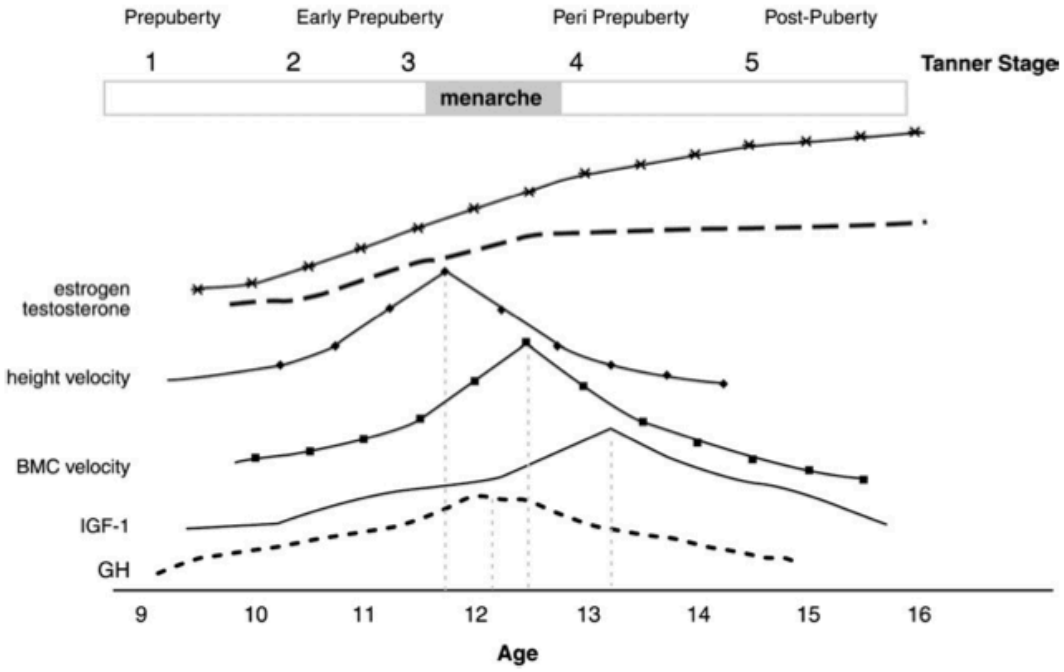


Figure 1.21. Illustration of peaks for sex steroids, height and BMC velocity, growth hormone and IGF-1 amplitude in relation to age and pubertal stage in girls. Reprinted from MacKelvie et al.,[144] with permission from BMJ Publishing Group Ltd.

Sex steroids, estrogen and testosterone, influence bone regulation throughout growth.[178] Estrogen's influence on bone development is biphasic in girls and boys, such that low concentrations during early-puberty stimulate skeletal growth through increased secretion of GH and IGF-1, while elevated concentrations during late-puberty limit growth by stimulating growth

plate closure.[179] Testosterone, on the other hand, directly encourages bone formation in girls and boys by inhibiting osteoblast apoptosis, promoting osteoblast formation at the growth plate[180] and stimulating GH and IGF-1.[178] In addition, testosterone indirectly affects bone formation through its anabolic effect on muscle mass which increases bending moments.[178]

As I discussed in section 1.2.4.2.2, growth-related adaptations in bone geometry enhance bone strength. Historically, estrogen was believed to inhibit periosteal apposition, such that sexual dimorphism in bone size (larger bones in men compared with women) was due to greater testosterone and less estrogen exposure in men compared with women.[178] However, case studies of boys with aromatase-deficiency (estrogen insensitivity) highlighted the critical influence of estrogen on normal skeletal growth in boys.[178,181] For example, a 16-year old boy had a bone age of just 12 years, despite normal levels of testosterone and full pubertal development. Three years of estrogen treatment increased his radius total CSA by 46%, Ct.Th by 12% (by pQCT) and increased bone age to 17 years.[181] Thus, estrogen and testosterone are essential for normal periosteal expansion. Males' greater bone size is attributed to greater periosteal apposition during adolescent growth, due to extended pubertal growth and later epiphyseal fusion, compared with girls.[178,179,182]

Calcitropic hormones (parathyroid hormone, vitamin D and calcitonin) also influence bone modeling and remodeling and regulate serum calcium. Parathyroid hormone regulates calcium homeostasis by interacting with bone, kidney and intestine.[183] Parathyroid hormone increases serum calcium by stimulating bone resorption and enhancing calcium absorption from the kidneys and intestine. Parathyroid hormone responds in accordance to serum calcium concentrations, such that large increases in serum calcium suppress parathyroid hormone secretion. Drops in serum calcium increase parathyroid hormone secretion.[183] Parathyroid hormone exerts anabolic and catabolic influences on bone, depending on its release pattern (intermittent or continuous) and subsequent stimulation of growth factors and cytokines.[183] In contrast, calcitonin influences calcium homeostasis by reducing serum calcium through inhibiting bone resorption and attenuating renal calcium resorption.[183] I discuss the influence of calcium and vitamin D on bone strength accrual in detail in section 1.2.5.4.

1.2.5.3 Ethnicity

Race is traditionally used to define biologic (genetic) differences in a person's appearance whereas ethnicity is commonly used to describe sociological and cultural factors such as nationality, ancestry and language. There is no consensus within the bone research literature as to which term is most appropriate to describe biological differences in bone development. Thus, I use the ethnicity to refer to biological and environmental factors that contribute to differences in bone strength and its determinants between people of different ancestries (i.e., white versus Asian). I use 'white' to describe those of European descent, 'Asian' to describe those of Asian descent, 'black' to describe those of African descent and 'other' to describe those of mixed ethnicity.

Evidence suggests that fracture incidence is lower among Asian children and adults compared with white children and adults.[184,185] However, we know little about how ethnic differences in bone strength and microarchitecture contribute to ethnic differences in fracture incidence. For example, in the multi-ethnic HBSIII cohort, Tt.Ar (by HR-pQCT) at the distal radius was smaller in Asian males compared with their same age white peers, independent of muscle mass and limb length.[186] However, Asian males and females had thicker and denser cortices compared with their white peers, while Asian males also had less porous cortices, contributing to similar estimates of bone strength (F.Load and load to strength ratio) between Asian and white participants. These data suggest that despite smaller bone geometry in Asian youth, bone adapts other parameters to maintain bone strength. With the exception of 11% greater trabecular separation in Asian females, there were no significant differences in trabecular microarchitecture between Asian and white adolescents and young adults.[186] Although data at weight-bearing sites are limited, one previous study reported similar bone outcomes between Asian and white boys and girls at the distal tibia, but smaller Ct.Ar and greater Ct.BMD at the tibial shaft (by pQCT) in Asian girls and smaller Ct.Ar in Asian boys compared with their white peers.[151] Genetics likely drives these ethnic-specific phenotypes; however, ethnic differences in timing of maturation may also explain the smaller bone geometry of Asian children who tend to mature earlier than their white peers.[187] Differences in modifiable lifestyle factors such as lower calcium intake and lower participation in PA in Asian compared with white children may also contribute to ethnic differences in bone accrual and geometry.[188]

Ethnic differences in bone strength and its determinants are also apparent in black compared with white children. These differences may contribute to a lower fracture incidence in blacks (half that of their white peers).[184] For example, after adjusting for tibia length and leg muscle MCSA, early- and peri-pubertal black children had 2-8% greater SSI_p, Ct.BMD and Tt.Ar (by pQCT at the tibia shaft) compared with white children.[189] This study and others[190,191] reported greater bone strength at the tibial diaphysis and metaphysis during childhood and adolescence in blacks compared with their white peers and suggest that these differences are already present in the early stages of puberty. Ethnic differences in markers of bone turnover reflect greater bone strength in black children who have greater levels of bone formation (osteocalcin and bone-specific alkaline phosphatase) and lower bone resorption (N-terminal telopeptide) markers, despite lower indices of modifiable factors such as vitamin D, dietary calcium and PA, compared with white children.[189] Studies that specifically investigate the influence of lifestyle and environmental factors on bone strength accrual among different ethnic groups are warranted.

1.2.5.4 Calcium and vitamin D

The dietary nutrients, calcium and vitamin D, impact development and maintenance of skeletal health. Calcium is the most abundant mineral in the human body and is stored primarily (99%) in bones and teeth. Calcium supports structural integrity of the skeleton and regulates metabolic function.[192] Vitamin D stimulates bone matrix formation and regulates calcium metabolism and absorption in concert with parathyroid hormone, such that intestinal calcium absorption doubles in the presence of adequate vitamin D.[192] Both nutrients are critical for skeletal health; inadequate intake or absorption of calcium and vitamin D during growth can result in rickets.[192] Current North American dietary standards recommend 1000 to 1300 mg/day of calcium and 600 IU daily of vitamin D for children and adolescents.[193]

Although calcium is the main building block of bone, whether or not calcium supplements are effective for bone accrual is equivocal. For example, a meta-analysis of 21 randomized controlled trials (RCTs) concluded that among children with normal baseline dietary calcium, supplemental calcium had little impact on total body BMC (by DXA). However, in children with low baseline intakes of calcium, a regimen of supplemental calcium significantly

increased total body and lumbar spine BMC.[194] In one of the longest trials of calcium supplementation to date, 3-years of calcium supplementation resulted in significantly greater increases in aBMD at the radius and lumbar spine in pre-pubertal, but not pubertal twins, compared with non-supplemented twin controls.[195] Thus, there may be a window of opportunity during pre-puberty when bone more positively responds to supplemental calcium.

We know less about the effects of calcium supplementation on bone strength and geometry. Few trials used pQCT to evaluate the bone strength response to supplemental calcium during growth. To my knowledge, no studies examined calcium supplementation independent of other lifestyle or dietary interventions (i.e., PA or vitamin D). A trial of pre-pubertal children (mean age 10 years) combined calcium supplementation and PA in an RCT across 12 months. Importantly, children in the study consumed recommended daily intakes for calcium at baseline.[196] Following daily calcium supplements (500 mg), Tb.BMD at the distal tibia (by pQCT) increased 5% more in non-gymnasts (defined as low-PA group) compared with non-supplemented non-gymnast controls, whereas no differences were observed in gymnasts (high-PA group).[196] No differences in bone density, geometry or strength were observed between calcium supplemented and non-supplemented groups at the distal radius or at the radial or tibial shaft for either non-gymnasts or gymnasts. Thus, supplemental calcium may interact with weight-bearing PA in pre-pubertal children, such that supplementation may not benefit those already engaging in high-impact PA. The bones of these athletes may have already adapted their density to high mechanical demands imposed through gymnastics.[196] No study has examined the interaction between calcium supplementation and PA in the adolescent skeleton.

As adequate vitamin D is necessary for optimal calcium absorption, several trials examined effectiveness of combined supplemental calcium and vitamin D on bone mineral (trabecular BMC and BMD) and strength accrual. In one trial, after 6-months of supplemental calcium (800 mg/day) and vitamin D (400 IU/day), peri-pubertal female identical twins (9-13 years) demonstrated 5% greater gains in Tb.BMD and Tb.Ar at the distal tibia and radius (by pQCT) and 6% greater increases in Ct.Ar at the tibial shaft compared with the non-supplemented control twin group; however, the latter did not translate into greater gains in SSI_p at the tibial shaft.[197] Similarly, a 12-month calcium (800 mg/day) and vitamin D (400 IU/day) supplementation trial in early and peri-pubertal girls (age 12 years) girls found a significant intervention effect for trabecular BMC and BMD at the distal tibia (by pQCT; no other sites were

evaluated).[198] Thus, combined supplementation of calcium and vitamin D may enhance trabecular volume at metaphyses and bone geometry at diaphysis. It is unclear how these gains influence bone strength or whether gains persist once supplementation ceases.

Vitamin D stimulates bone matrix formation, calcium and phosphate absorption in the small intestine, reabsorption of renal calcium and mobilization of calcium in bones.[199] Upwards of 90% of vitamin D is derived from the photo conversion of 7-dehydrocholesterol in the skin by solar UVB radiation, hence its termed the 'sunshine' vitamin.[200] Daily synthesis of 400 IU vitamin D in children and adolescents is possible through casual exposure of the face and hands to sunshine at midday in all latitudes during part of the year, but not in higher latitudes for the entire year.[201] Thus, factors such as living in northern or southern latitudes with low UVB production in winter months and clothing that covers most of the skin surface area can contribute to low vitamin D levels. Low serum vitamin D is prevalent in children and adolescents throughout the world.[202] The most recent estimates from the Canadian Health Measures Survey (CHMS; a representative sample of 5600 Canadians from 15 sites around the country) suggested that 11-29% of Canadian children and adolescents have serum vitamin D below recommended levels (< 50 nmol/L). [203]

While vitamin D deficiency increases risk for conditions such as rickets, supplements for children with adequate serum vitamin D may not enhance their bone mineral accrual. In a recent Cochrane systematic review of 6 placebo-controlled RCTs, which varied in geographical location and included higher latitude countries, supplemental vitamin D did not increase whole body BMC or aBMD of the forearm, hip and spine (by DXA).[204] However, when children who were deficient in serum vitamin D at baseline (< 35 nmol/l, with > 50 nmol/L considered adequate) received supplemental vitamin D, whole body BMC and lumbar spine aBMD increased significantly compared with a placebo group.[202,204] In contrast, a recent RCT supplemented vitamin D deficient post-menarcheal girls (< 37.5 nmol/liter) and found no effect on bone density, geometry or strength (by pQCT) or muscle force or power (by jumping mechanography) after 1-year.[205] Future trials using 3D imaging techniques would help to clarify the maturity-specific influence of supplemental vitamin D on bone strength.

1.2.5.5 Muscle force

In section 1.2.2.2.2, I highlighted the influence of muscular forces on bone strength development and maintenance. Muscle contractions impose the greatest mechanical challenge on bone (stresses several fold greater than body weight alone) and drive bone adaptation.[57,60] The functional model of bone development contends that bone continually adapts to mechanical loads induced by muscular strain by adjusting bone strength and its determinants (up or down) to maintain strains within safe limits.[57,60] Given the strong influence of muscle on bone development, growth-related changes in bone parameters should be considered in the context of the functional muscle-bone unit.

If the central tenet of the functional model of bone development is true, muscle development should precede bone development. This was observed in the 14-year University of Saskatchewan PBMAS. For example, peak total body muscle mass accrual (surrogate of muscle force; by DXA) preceded peak BMC accrual (by DXA) by approximately 6 months in girls and 4 months in boys.[206] In a subsequent analysis, peak total body muscle mass velocity occurred 2 to 4 months ahead of peak bone CSA and estimated bone strength (section modulus) velocity at the narrow neck and femoral shaft (by HSA; Figure 1.22)[207] Together these findings suggest that enhanced muscle mass promotes bone adaptation. In contrast, in a 7-year longitudinal study of Finnish girls, MCSA (surrogate of muscle force; by pQCT) peaked prior to BMC and BMD at the tibia shaft, but lagged behind total and cortical bone CSA (all bone measures by pQCT).[161] Thus, the muscle-bone relationship might not function uniformly across skeletal sites. In section 1.2.7.2.2, I provide further evidence from longitudinal pediatric bone studies that suggests muscle forces mediate the PA-bone relationship.[208,209]

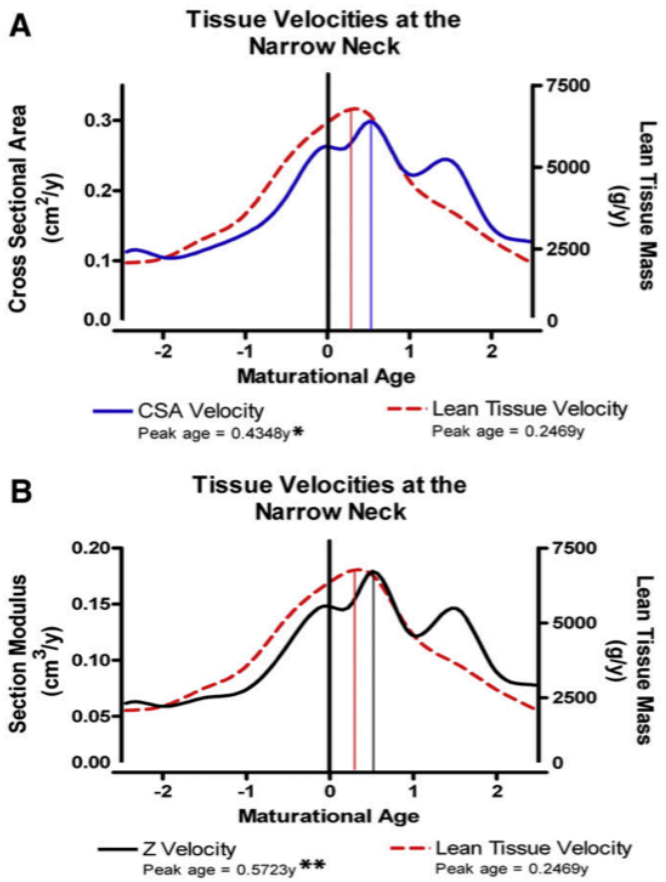


Figure 1.22. Illustration of tissue velocity curves for muscle mass, A) cross-sectional area (CSA) and B) section modulus (Z) at the femoral shaft aligned by maturational age (years from age at peak height velocity). The solid vertical line represents the maturational age when peak tissue velocities occurred. *Indicates significant difference between age of peak muscle velocity and peak CSA velocity. **Indicates a significant difference between age of peak muscle velocity and peak Z velocity. Reprinted from Jackowski et al.,[207] with permission from Elsevier.

Direct assessment of muscle force is not possible using non-invasive techniques. However, dynamometry and mechanography provide reliable estimates of muscle force. Hand-held dynamometers are an easy and reliable approach to measure maximal isometric grip force,[210] which is strongly associated (r = 0.80-0.90) with CSA and bone strength (BSI) at the distal radius (by pQCT). [211] In the lower limbs, jumping mechanography is commonly used to assess peak muscle force (N) and peak muscle power (W) during single- and two-legged jumps, respectively. However, to my knowledge no study has examined associations between lower limb muscle force or power (by mechanography) and bone strength in healthy children or adolescents (by pQCT or HR-pQCT). Given the high cost of the force platform, mechanography may not be feasible for field-based measures. Vertical jump height, on the other hand, is a simple

test used to estimate peak muscle power in conjunction with validated prediction equations.[212] Estimated muscle power was significantly associated (r = 0.54-0.78) with bone strength at the distal (4%; BSI by pQCT) and midshaft site (66%; SSI_p by pQCT) of the tibia in adolescents (mean age 17 years).[213] Thus, grip strength and vertical jump are easy and cost-effective tools to assess muscle force and power in the laboratory or in the field.

When functional measures of muscle force are not available, muscle mass (g) and MCSA (mm^2) are frequently used as surrogates. Muscle mass is derived from DXA whole body scans based on attenuation of X-rays through muscle tissue that is assumed to be of fixed density[214] Measures of muscle mass were highly correlated (r = 0.77) with leg muscle power in adolescent girls.[215] As with bone analyses using pQCT (described in Section 1.2.3.2.1), MCSA is derived using density thresholds that separate muscle from bone and fat. MCSA is highly correlated with estimated muscle power (using vertical jump height and prediction equation; r = 0.70) and bone strength at the distal tibia (r = 0.56-0.66; BSI by pQCT) and tibial shaft (r = 0.68; SSI_p by pQCT) in adolescents.[213]

1.2.6 Physical activity and sedentary time

In this section, I describe how PA and sedentary time are assessed and summarize the current literature regarding the influence of PA and sedentary time on bone strength and its determinants in children and adolescents.

1.2.6.1 Measurement of physical activity

PA is defined as any bodily movements expending energy.[3] Current Canadian PA guidelines recommend that children and adolescents (5 to 17 years) engage in 60 min/day of moderate-to-vigorous PA (MVPA) to achieve health benefits, while adults (18 to 64 years) should achieve 150 min of MVPA every week.[216] Guidelines for children and adolescents were based on a systematic review of the health benefits of PA in children and adolescents (including the influence of PA on aBMD by DXA).[217] Guidelines recommend that youth engage in muscle and bone-strengthening activities that use major muscle groups at least 3 days/week.[216]

Various tools are used to assess PA in children and youth. Measurement techniques include subjective administered or self-report questionnaires and direct monitoring devices, such as pedometers or accelerometers. Questionnaires are often the tool of choice as they are cost-effective, easy to administer and are have low participant burden.[218] However, self-report questionnaires are subject to recall bias. Thus, while they provide behavioural information regarding PA (setting and type of PA), they do not adequately capture PA intensity and duration. In contrast, objective tools (e.g., accelerometers) measure PA intensity, frequency and duration and can be time-stamped for time-of-day analyses. Further, ground reaction forces were strongly correlated with raw acceleration output in adults ($r = 0.85$)[219] and in children and adolescents (healthy children and those with osteogenesis imperfecta type 1, age 6-21 years; $r = 0.96$).[220] This suggests accelerometers are an appropriate tool with which to estimate mechanical loads associated with weight-bearing PA. However, high cost (i.e., $200-400 per unit), low wear compliance and inability to capture certain types of activity (i.e., swimming and biking) may limit use of accelerometry. Thus, investigators should consider the study aims and feasibility when choosing a PA measurement tool.

1.2.6.1.1 Self-report questionnaires to assess physical activity

Self-report questionnaires most often rely on a participant's ability to recall or report their PA. Participants tend to overestimate their PA in self-report questionnaires, compared with direct measures.[218] Structured activities may be easy to recall, but unstructured activities that make up most of daily PA are difficult to quantify. A recent systematic review concluded that no currently available PA questionnaire for children and adolescents (61 reviewed) were of both acceptable validity and reliability (based on an intraclass correlation coefficient (ICC) of > 0.70).[221]

The Physical Activity Questionnaires for Children (PAQ-C; 8-14 year olds or grades 4-8) and Adolescents (PAQ-A; 14-18 year olds or high school students) were designed for the Saskatchewan PBMAS and are widely used to estimate MVPA.[222,223] In both questionnaires, children/youth recall their participation in activities during the past 7 days. The PAQ-C is a nine-item questionnaire with the first question providing a checklist of common sport or leisure PA; the remaining questions are segmented by time-of-day (e.g., at lunch, after school) or day-of-the-week (e.g., last weekend). Items 1-9 are scored on a 1 (low PA) – 5 (high PA) scale; the

summary score is the average sum of nine questions.[222] Our research group modified the PAQ-C for HBSIII to include an estimate of dose (added time spent per activity session (item 1), involvement in extracurricular activities and number of nights of organized sport PA per week)[188] and perception of PA involvement on each day of the week (5-point scale ranging from none to very often). The PAQ-C demonstrated good test-retest reliability in 9-15 year old boys (r = 0.75) and girls (r = 0.82) using the summary score across seasons (r = 0.80 for average of two or three responses in fall, winter and spring).[222] However, the PAQ-C summary score demonstrated weak (r = 0.25; mean age 11 years)[224] to moderate (r = 0.39; participants in grades 4-8)[225] agreement with MVPA and activity counts respectively, by accelerometry. The PAQ-A is almost identical to the PAQ-C, with the exception that it does not include a question regarding PA during morning recess. The PAQ-A summary score was moderately correlated with accelerometry-derived MVPA (r = 0.49) in 14-year olds.[226] Despite limitations of self-reported PA, questionnaire-based assessment remains prevalent due to its ease of administration and ability to provide contextual information.

1.2.6.1.2 Accelerometry to assess physical activity

Accelerometers are non-invasive devices that record frequency, duration and intensity of everyday activities. Validation studies in children and adolescents demonstrate high reproducibility, validity and feasibility.[227] Thus, accelerometry is the preferred method to assess PA in children and adolescents.[228,229]

Accelerometers are small (approximately the size of a matchbox), light (< 30g) and robust devices typically attached to a band worn around the hip, although new models can be worn on the wrist. Some devices (triaxial accelerometers) measure motion in three planes: vertical, horizontal and perpendicular. For my thesis, I focus on ActiGraph GT1M accelerometer, as this model was available at the Centre for Hip Health and Mobility. The GT1M is worn at the hip and assesses motions in the vertical plane only. The sensors inside accelerometers detect acceleration of the body and produce an analog voltage proportional to the magnitude of acceleration. The analog signal is digitized (sample rate of 30 Hz) and filtered (bandwidth of 0.25 to 2.5 Hz; excludes non-human movement) to produce values known as 'counts'.[230] Counts are summed over user-specified intervals known as ‘epochs’ and stored in the unit’s memory.

Accelerometers assess PA in short measurement intervals (e.g., every 3 sec) over long periods of time (e.g., months). Early accelerometer models had a limited memory; thus, an epoch of 1-min was common. Newer devices have greater storage capacity and permit shorter epochs (e.g., 1-sec, 3-sec, 15-sec), which more accurately assess the intermittent nature of children's PA.[231] The potential for misclassification of PA increases in concert with epoch length. High-intensity PA may be underestimated when averaged over longer epochs, such that short bouts of high-intensity PA may be combined with bouts of low-intensity PA within the same epoch. To minimize misclassification, as short an epoch length as possible should be used. Given the importance of high-intensity PA for bone adaptation, a short epoch is particularly relevant for examining the relationship between PA and bone parameters. Further, accelerometry data can be easily re-integrated into longer epochs during post-acquisition data analysis, as needed.[228]

The number of days and h/day an accelerometer must be worn to accurately assess habitual PA is an important consideration. In children and adults, three to five days of monitoring are recommended to achieve reasonable reliability (r = 0.70-0.80).[232,233] Further, a minimum of 10 h/day of wear time is recommended to define a valid day, as this length minimized the effects of varying day length on PA outcomes in a study of over 6000, 11-year olds.[232]

Following the monitoring period, accelerometer data must first be screened for non-wear time. Much debate has concerned periods of non-wear time versus true sedentary time (e.g., reading, watching TV). This classification is crucial to accurately assess PA and sedentary time. Non-wear time can be defined visually during data analysis. This is conducted in combination with a PA diary filled out by the participant or parent that notes times when the accelerometer was worn (on-off times). However, this process is time consuming, subjective and inaccurate. Alternatively, some assumed that a certain number of minutes (e.g., 60-min) of consecutive '0' counts represents non-wear time. Given the sensitive nature of accelerometers, even small motions create values greater than zero.[234] A non-wear criteria defined as 20-min or longer of motionless data was recommended for youth, as 17.5 min, on average was the longest bout of motionless data over 7 days of monitoring in 115 youth (8-13 years).[235] Other commonly used non-wear criteria in youth ranged from 10-180 min of consecutive 0 counts, with the option of allowing for 1 to 2 min of counts between 0 and 100 during that period.[236] Although consensus has not been reached, a criteria of 60-min of consecutive 0 counts without interruptions was

recently suggested for use in children and youth, based on the most realistic number of non-wear periods per day observed in a more robust sample of 1000, 9-13 year olds.[237] The 60-min criteria resulted in a maximum of 4 non-wear periods per day, compared with an unrealistically high number of non-wear periods for the 20-min criteria (maximum of 10).[237] However, in future, non-wear criteria must be validated against direct measures such as video or direct observation.

Raw accelerometer counts are unit-less. Thus, they must be converted into a value that has meaning for the user. Accelerometer calibration studies used energy expenditure (indirect calorimetry, METs) or direct observation to develop count thresholds, known as cut points. Cut points are age- (and often study-) specific and correspond with PA intensity.[238] Thus, PA estimates are not comparable across studies that used different cut points. For example, five commonly used PA cut points for youth ranged from approximately ≥ 2200 cpm to ≥ 3600 cpm for moderate PA, to ≥ 4000 to ≥ 8200 cpm for vigorous PA. Accurate classification of MVPA against energy expenditure (using indirect calorimetry) was poor for 3 of the 5 cut points (those with higher cut points). This was based on low sensitivity (high false-negative rate) of MVPA that was misclassified as low-intensity PA.[239] Both Freedson[240] and Evenson[238]cut points demonstrated excellent classification accuracy for MVPA (receiver operating characteristic – area under the curve = 0.90) and fair accuracy for light PA (receiver operating characteristic – area under the curve: 0.69-0.70, respectively).[239] However, Evenson cut points are recommended because the MVPA cut point performed equally well among children and youth at all ages and all levels of PA intensity demonstrated acceptable classification accuracy.[239]

Expressing PA as min/day is a convenient way to convey recommendations to the public and practitioners and to assess compliance with PA recommendations. However, this is not without bias as expression of PA is influenced by duration of accelerometer wear time. Several approaches eliminate wear-time biases and facilitate comparisons between participants who had different wear times. For example, wear time can be accounted for: 1) by expressing PA relative to wear time,[234] 2) in regression-based analyses by including wear time as a predictor alongside PA, or 3) by using a residuals approach, which obtains the residuals from regressing the PA variable of interest on wear time.[241]

While accelerometers effectively capture frequency, duration and intensity of PA and eliminate reporting bias, they have limitations. Accelerometers cannot account for increases in

energy expenditure associated with walking up an incline or carrying a load.[242] Further, they do not accurately measure activities that occur mainly in the horizontal plane (e.g., skating). Finally, most accelerometers are not waterproof so cannot measure energy expenditure associated with swimming or other water sports.

1.2.6.2 Measurement of sedentary time

Sedentary time is activity defined by a low energy expenditure < 1.5 METs in a seated or reclined posture.[243] Parental or self-reported TV viewing, computer use, video games, phone use and reading are traditionally used to assess sedentary time.[244] However, more recently accelerometers have been used to objectively assess sedentary time in children and adolescents.

1.2.6.2.1 Self-report questionnaires to assess sedentary time

Unlike objective methods, self-report measures of sedentary time provide type and context to the behaviour.[244] However, few self-report measures of sedentary time demonstrated acceptable reliability and validity in children and adolescents (based on intraclass correlation coefficient > 0.70).[245] While many questionnaires demonstrated acceptable reliability (similar test-retest scores), most demonstrated poor construct validity compared with objective measures.[245] For example, self-reported sedentary/screen time in 6-11 year olds (n = 878) in the CHMS was weakly correlated (r = 0.17) with accelerometry-derived sedentary time measured over 4 to 7 days.[16] The low construct validity may be partially due to a mismatch between constructs addressed by questionnaires and comparison measures; questionnaires ask about specific leisure-time behaviours (e.g., time spent watching TV or playing video games), while accelerometers typically assess sedentary time over the entire day.[245]

1.2.6.2.2 Accelerometry to assess sedentary time

Accelerometery-derived sedentary time is more reliable and valid compared with self-reported sedentary time.[244] A cut point of < 100 cpm is commonly used to determine total sedentary time.[238,239] However, accelerometers cannot differentiate between sitting and standing

with minimal movement (standing by definition is not sedentary time). As mentioned previously, accelerometers do not provide context for a sedentary activity. Considering strengths and limitations of approaches to measure sedentary time, a combination of self-report questionnaires and objective monitors may best describe children's sedentary behaviours.

1.2.6.3 Sex- and age-related differences in physical activity and sedentary time

Boys are more active than girls during childhood and adolescence and PA declines from childhood into adulthood.[246,247] Two large nationwide surveys conducted in Canada and the United States examined prevalence of PA and sedentary time in children and youth. From 2007 to 2009, CHMS collected PA and sedentary time data in over 1600 children and adolescents at 15 measurement sites across Canada using parent-reports and accelerometry (Actical).[248] From 2003 to 2006, National Health and Nutrition Examination Survey (NHANES) collected accelerometer (ActiGraph) data from almost 1800 children and adolescents in the United States.[249] In both nationwide surveys, boys accumulated more MVPA compared with girls throughout childhood and adolescence.[248,249] MVPA decreased with age in both sexes to a similar extent.[248,249] From CHMS, children (6-10 years) engaged in approximately 1 h/day of MVPA (69 and 58 min/day for boys and girls, respectively), while adolescent boys and girls (11-14 years) accumulated less MVPA (59 and 47 min/day, respectively).[248] Compared with CHMS, NHANES reported slightly greater values for MVPA during childhood (6-11 years; 95 and 75 min/day for boys and girls, respectively), but lower values of MVPA during adolescence (12-15 years; 45 and 25 min/day respectively).[249] Based on estimates of MVPA, only 22% of Canadian boys and 24% of American boys (age 6-19 years) met MVPA recommendations of 60 min/day (using a 5 of 7 days criterion).[248,249] Just 11% of Canadian girls and 15% of American girls (age 6-19 years) achieved the recommended 60 min/day of MVPA (using a 5 of 7 days criterion).[248,249]

Both studies also assessed sedentary time using accelerometry. Those in the Canadian cohort (6-19 years) engaged in 8.6 h/day of sedentary time, on average. Sedentary time increased with age from approximately 7 h/day during childhood to 9 h/day during late adolescence.[248] Sedentary time was slightly lower in the American cohort (7.2 h/day; 6-19 years), on average, but increased by approximately 2 h/day (6 h/day to 8 h/day) from childhood to late

adolescence.[250] Sedentary time did not differ between Canadian boys and girls between 6 and 14 years of age, but was higher in girls thereafter (by 30 min/day).[248] In contrast, American girls were significantly more sedentary, compared with boys, throughout childhood and adolescence (by approximately 12 min/day).[250] Of note, the Canadian study did not control for differences in accelerometer wear time between participants, while the American study did. Thus, differences in wear time may confound comparisons of PA and sedentary time between studies. However, CHMS data confirm that boys are more active than girls across adolescence, MVPA decreases across adolescence in both sexes and sedentary time increases in both sexes from childhood to early adulthood.

1.2.7 Influence of physical activity and sedentary time on bone strength development

In this section, I briefly review current literature regarding how PA and sedentary time influence bone strength and its determinants during adolescent growth. I first highlight intervention studies, followed by observational studies in athletic and non-athletic cohorts and finish by presenting the link between PA during childhood and adolescence and adult bone outcomes.

The positive influence of PA on bone development is summarized in several excellent reviews.[12-15,251] As discussed in section 1.2.2.2.3, bone can adapt its strength in response to mechanical stimuli during growth through several mechanisms: 1) periosteal apposition can increase bone CSA; 2) periosteal apposition and reduced endocortical resorption can increase Ct.Th; 3) modifications to cortical and trabecular microarchitecture (i.e., increased Tb.Th or Tb.N or decreased Ct.Po) can increase tissue density.[11,69] Specifically, there is strong evidence to suggest that pre- and early-puberty may provide a 'window of opportunity' during which the skeleton is particularly responsive to loads associated with weight-bearing PA.[144,145] In contrast, we know less about the mechanisms underpinning bone's adaptation to PA in later adolescence.[10,15,252-255] This may be due, in part, to the use of imaging systems such as DXA, which cannot capture subtle adaptations in bone strength and its determinants and to the complex and extremely variable nature of adolescent growth.

1.2.7.1 Intervention studies of physical activity

Targeted bone-loading interventions were traditionally implemented in elementary schools as schools reach large numbers of children from diverse backgrounds. Effective PA interventions incorporated dynamic, high-impact activities that were of short duration, elicited 'unusual' strains and were separated by rest periods, thus mirroring the principles derived from the animal literature.[44] Length of PA intervention across studies ranged from 28 weeks to 2 years.[15] Most used DXA (11 of 14 RCTs) to monitor exercise-related gains in bone mass.[10,15] Importantly, children assigned to exercise intervention groups gained significantly more bone mass (1-6%) at the spine and hip compared with children in control groups, on average.[10]

One of the longest school-based RCTs conducted to date is the UBC HBS. Children (aged 9-11 years) who attended schools randomized to the exercise group participated in 10-12 min of high-impact jumping activities, 3 times per week for 7-months each year for two school years.[256,257] After the first school year, both groups demonstrated gains in BMC, but girls and boys who attended intervention schools demonstrated significantly greater gains in BMC and aBMD at the femoral neck and lumbar spine compared with children attending control schools. However, in girls, the intervention effect was only apparent in those who were early pubertal (Tanner stage 2 or 3) at baseline.[258,259] After two school years, significantly greater gains in femoral neck (5%) and lumbar spine (4%) BMC were observed in intervention girls[256] and in femoral neck (4%) BMC in intervention boys.[257] The HBS and similar interventions[254,260-262] highlighted that a simple exercise program, which requires very little time in the school day may enhance bone mass accrual.

Animal models demonstrated that the skeleton adapts to mechanical loading by adding bone to the periosteal surface of long bone shaft sites where strains are the greatest.[44,263] Although small in magnitude, these subtle structural adaptations confer dramatic increases in experimentally measured bone strength.[90,91] As mentioned previously, DXA cannot capture these bone adaptations to PA. Only 11 intervention trials conducted in the last decade used imaging tools (e.g., HSA, pQCT or MRI) able to assess exercise-induced adaptations in bone geometry and strength.[15] I review key findings from these studies below.

In the HBS, the greater gain in femoral neck BMC in early pubertal girls in the intervention group was associated with a 4% greater increase in femoral neck bone strength

(section modulus, HSA) compared with controls.[69] This strength gain was attributed to greater increase in CSA and reduced endocortical expansion, leading to a thicker cortex in the intervention group. In contrast, intervention-related gains in femoral neck bone strength were only observed in boys after the second year of the trial.[257] The apparent sex difference in the timing of structural adaptations to the HBS intervention may be related to maturity status. At baseline, 60% of girls were early-pubertal, whereas most boys were pre-pubertal. A later adaptation in bone strength in boys may be a result of advanced maturity (77% advanced to early- or peri-puberty) over the second year of the study and/or the prolonged intervention. These findings suggest that early-puberty may be a window of opportunity for femoral neck bone strength adaptations. As there were no differences in strength gains at the total proximal femur between groups during pre-puberty, a more intense exercise intervention may be necessary to confer beneficial structural adaptations at this larger site.

The influence of maturity status on bone structure adaptations to PA may also vary with skeletal site. To illustrate, Action Schools! BC involved short bouts of classroom-based exercise (including ~3 min/day of jumping) during a 16-month intervention period. Girls attending intervention schools that reported high compliance (≥ 80%) demonstrated 5% greater gains in femoral neck bone strength (section modulus, HSA) compared with girls attending control schools.[264] Conversely, an intervention effect was not observed at the distal or shaft site of the tibia (by pQCT) in girls, most of whom were early-pubertal at baseline.[265] Despite significantly greater gains in lumbar spine and total body BMC in boys attending intervention schools, there were no structural differences at follow-up compared with boys (pre- and early-pubertal boys pooled) attending control schools.[264] However, there was a significant group by maturity interaction, such that pre-pubertal boys at baseline in the intervention group demonstrated a 4% greater gain in distal tibia bone strength (BSI) compared with controls. The group by maturity interaction suggested the appendicular skeleton may be more responsive to loading during pre-puberty.[265] Alternatively, maturity-specific findings may be explained by lower participation in self-reported leisure time weight-bearing PA in pre-pubertal (6 h/week) compared with early-pubertal boys (8 h/week).[265] Thus, consistent with the cellular accommodation theory of mechanoadaptation, PA interventions may be less beneficial to those already engaging in high levels of weight-bearing PA. A more frequent and intense intervention may also be necessary to elicit an osteogenic response in the more active, mature group.

Not all intervention studies observed differences in bone outcomes between intervention and control groups. For example, pre-pubertal girls who completed seven months of a drop-jumping program using their non-dominant leg (3 times/week) did not demonstrate greater gains in bone strength at the mid-femur (by MRI) compared with girls in the control group.[266] As the jumps performed were unidirectional, of low magnitude (14-28 cm), over a short time period (28 weeks) and in a small sample (n = 13 in each of control, low- and high-impact groups), a more robust sample size with greater dynamic loads may be necessary to detect an osteogenic response.

School-based intervention trials are complex and challenging. Success depends largely upon participant and teacher compliance in intervention schools, activities conducted within control schools during the study period and activity levels of all participants at baseline – all are beyond a researcher's control. These and other factors (e.g., type, intensity, frequency and duration of the intervention, imaging tools used, scan acquisition and analysis procedure) represent significant heterogeneity across school-based intervention trials.[15] Nevertheless, the bone response to skeletal loading appears to be sex- and maturity-specific. Convincing evidence supports the role of high-impact exercise for augmenting bone mass (but less so structure and strength) in pre- and early-pubertal children. Further work is needed to better understand bone structural and microarchitectural adaptations to loads associated with weight-bearing PA and the optimal dose necessary to elicit meaningful bone health benefits.

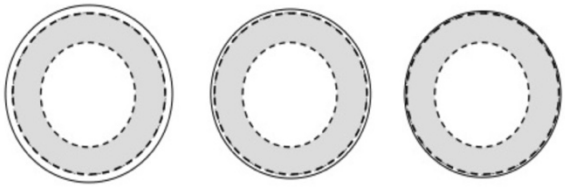
1.2.7.2 Observational studies of physical activity

Observational studies of habitual PA and athlete groups subjected to different loading conditions, represent a large body of evidence supporting a positive association between PA and bone development during growth. These studies traditionally relied on DXA to image bone; all demonstrated significant bone mass benefits in children who participated in weight-bearing sports such as gymnastics, tennis and running compared with non-athlete groups.[267-270] Similarly, leisure-time PA was a significant predictor of bone mass accrual in girls and boys across maturity.[133,271,272] With increased accessibility to 3D imaging tools, researchers are now gaining insight into bone structural advantages associated with weight-bearing PA. Thus, in this section I focus on observational studies that employed 3D imaging tools.

1.2.7.2.1 Athletic populations

Racquet Sports: Athletes in racquet sports such as tennis and badminton provide a unique model for investigating bone adaptation to loading. Within-subject comparisons of the playing versus non-playing arms controls for confounding factors such as genetics, hormones and diet. The seminal cross-sectional DXA study by Kannus and colleagues[267] paved the way for studies that used more sophisticated imaging tools. They reported side-to-side differences in BMC in the playing versus non-playing arms of female racquet sport players were significantly greater compared with controls (9-16% vs. 3-5%). However, of greater interest, players who initiated training prior to menarche had side-to-side differences nearly twice that of players who initiated training after menarche. This finding raised the possibility that skeletal benefits of weight-bearing PA are maximized during pre-menarcheal years.[267]

The bone mass advantage in the playing arm of female racquet sport athletes was also associated with significant bone strength benefits. To illustrate, bone strength (polar second moment of area) at distal and shaft sites of the humerus (by MRI) in young female tennis players was 11-23% greater in the playing arm compared with the non-playing arm.[273] Similarly, side-to-side differences in BMC, Ct.Ar, Tt.Ar and strength (BSI; all by pQCT) at distal and shaft sites of the radius and humerus were 8-22% greater in female racquet sport athletes compared with controls.[274] As in the Kannus et al. study, side-to-side differences in bone geometry and strength were double in magnitude in women who began racquet sport training prior to menarche compared with women who began training after menarche (Figure 1.23).[274] Finally, in the only prospective study of racquet sport athletes conducted to date, 12-month changes in bone geometry (Tt.Ar and Ct.Ar by MRI) were significantly greater among pre- and peri-pubertal female competitive tennis players compared with post-pubertal players.[275] These findings provide further support for a 'window of opportunity' during pre- and early-puberty when the skeleton is most responsive to mechanical stimuli.



	Side-to-side difference (%)		
	Young Starters	Old Starters	Controls
Total CSA	12.3	5.3	3.4
Cortical CSA	20.0	9.2	3.1
Cortical BMD	-0.8	-0.7	-0.9
Bone Strength (BSI)	26.5	10.2	4.0

Figure 1.23. Average side-to-side differences in humeral midshaft total bone cross-sectional area (CSA), cortical CSA, cortical bone mineral density (BMD) and bone strength index (BSI) between the playing and nonplaying arm in female racquet sport athletes as measured with peripheral quantitative computed tomography (pQCT). The solid line represents the playing arm (or dominant arm in controls) and the dotted line represents the nonplaying arm. Adapted from Macdonald et al.,[14] with permission from Future Medicine, Ltd.

In racquet sport athletes, adaptations in bone geometry at shaft and distal sites (Tt.Ar and Ct.Ar) during pre- and early-puberty were attributed to bone accrual on the outer bone surface (periosteal expansion) as opposed to endocortical expansion or contraction.[273-275] In contrast, training initiated after puberty was associated with greater bone contraction or decreased expansion on the endocortical surface, conferring little benefit to bone bending strength.[273] Similar sport-related gains in bone geometry and strength were observed in the playing-arms of young adult men, all of whom began training during childhood.[276,277]

Gymnastics: Artistic gymnastics imposes an extremely high mechanical stimulus on the skeleton (ground reaction forces greater than 10 times body weight).[278] Thus, gymnasts represent a unique population within whom to examine the effects of intense loading on bone. However, no longitudinal studies of gymnasts utilized pQCT or HR-pQCT to examine bone strength and geometry.

A 4-year DXA follow-up study of recreational gymnasts (aged 4-9 years at baseline) demonstrated 3% greater total body BMC, 7% greater femoral neck BMC[268] and 3-6% greater CSA at all three femoral neck sites compared with their non-gymnast peers.[279] Gymnasts also

showed 6-7% greater estimated bone strength (section modulus by HSA) at the narrow neck and intertrochanter compared with their non-gymnast peers.[279] Conversely, ex-recreational gymnasts (most of whom ceased participation between first and second measurement) did not demonstrate a bone advantage compared with non-gymnasts.[268,279] This suggests that continued participation is required to maintain benefits associated with recreational gymnastics during pre-puberty.

Gymnasts consistently demonstrated greater bone strength (by pQCT) in the upper and lower limbs compared with their non-gymnast peers.[103,165,279-281] As with racquet sport athletes, this bone strength advantage at shaft sites was due in part to enhanced bone geometry that conferred strength to long bones. For example, pre-pubertal elite gymnasts (aged 5-11 years) demonstrated 9% greater estimated bone strength (SSI_p by pQCT) at the radial shaft compared with non-gymnast controls. This advantage was likely driven by reported 5-7% greater Tt.Ar and Ct.Ar in the gymnasts.[165] Similar advantages were reported in 6-11 year old non-elite gymnasts (<16 h/week gymnastics; Figure 1.24)[103] and in 4-9 year old recreational current and ex-gymnasts (at least 45 min/week of gymnastics).[280] The distal site also demonstrated greater bone strength; however, adaptations were due to increased bone density. To illustrate, recreational gymnasts and ex-gymnasts had similar Tt.Ar. However, the 6-8% greater Tt.BMD in recreational gymnasts contributed to 22-25% greater bone strength (BSI) at the distal radius compared with non-gymnast controls.[280] Collectively, these findings suggest that participating in gymnastics at a recreational level confers bone health benefits during pre-puberty.

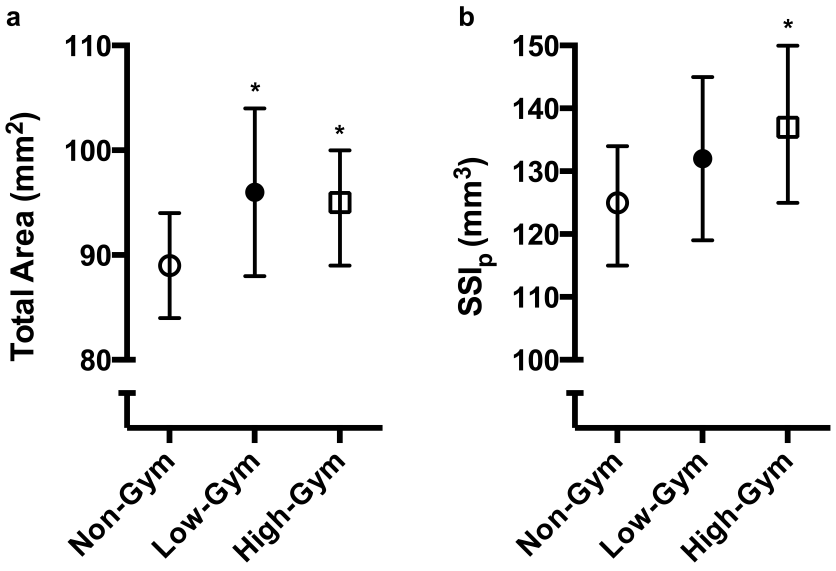


Figure 1.24. Illustration of a) bone geometry (total bone area) and b) estimated bone strength (polar strength-strain index, SSI_p) at the proximal radius (66% site) measured with peripheral quantitative computed tomography (pQCT) in pre-pubertal girls. Non-gymnasts (Non-Gym), low-training volume gymnasts (Low-Gym) and high-training volume gymnasts (High-Gym). *Indicates significantly different from Non-Gym. Bars represent 95% confidence intervals. Adapted from Burt et al.,[103] with permission from Springer.

Bone geometry and strength benefits associated with gymnastics training may persist into late adolescence. For example, post-menarcheal girls (n = 16, mean age 17 years) who participated in gymnastics during early-puberty (> 5 h/week for at least 2 years) but stopped training 1-year post-menarche, on average, had 19% greater Tb.BMD and 25-26% greater Ct.Ar and Tt.Ar. This conferred 34% greater estimated bone strength (BSI) at the distal radius compared with non-gymnasts.[281] Similarly, Tt.Ar and Ct.Ar were 22-33% greater in ex-gymnasts at the radial shaft. The larger bone area was associated with 46% greater bone strength (SSI_p) compared with non-gymnasts.[281] Longitudinal studies are needed to confirm these findings.

Other Sports: Studies in adolescents and young adults that used HR-pQCT support a benefit of high-impact PA on bone strength and microarchitecture. For example, late adolescent and young adult female athletes (age 14-21 years; at least 20 miles of running or 4 h/week of aerobic weight bearing exercise) demonstrated greater Tb.Ar, Tt.Ar and F.Load at the distal tibia compared with non-athletes.[282,283] Similarly, late adolescent and young adult male and female athletes who participated in high-impact sports (skiers and soccer players) demonstrated

significantly greater distal tibia Tb.BMD and F.Load compared with swimmers.[284] Further, female skiers and soccer players had greater Ct.Th and lower trabecular separation (Tb.Sp) compared with swimmers, while male soccer players had greater Tb.N compared with swimmers.[284] Thus, athletes who participated in high-impact sports had greater metaphyseal bone strength conferred by adaptations in trabecular microarchitecture and/or bone geometry.

1.2.7.2.2 Habitual physical activity

Leisure-time PA: Many children do not engage in structured PA such as gymnastics or racquet sports. Therefore, it is important to consider and better understand the influence of general, leisure-time PA on bone parameters. Observational studies, both cross-sectional and longitudinal, consistently demonstrated that more active children and adolescents accrued more bone mass and strength compared with their less active peers.[133,208,209] For example, vigorous PA (> 6 METs; using accelerometry) predicted 3-7% of femoral neck strength (by HSA) after adjusting for age, weight and height in pre-[285,286] and early-pubertal boys and girls.[286] Similarly, 9-13 year old girls in the highest PA quintile (via self-report questionnaire) demonstrated 7-9% greater estimated bone strength (SSI_p and BSI) and 3-4% greater periosteal circumference at the tibial diaphysis and metaphysis (by pQCT) compared with peers in the lowest PA quintile.[102] Finally, pre-pubertal girls who participated in high-impact PA (by questionnaire) demonstrated 3% greater CSA, 7% greater estimated bone strength (CSMI) and 6% greater Ct.Th at the tibial shaft (by pQCT) compared with girls who engaged in low-impact PA. However, no differences were observed in peri-pubertal girls.[287] While accepting known limitations of cross-sectional studies, these findings suggest a positive influence of weight-bearing PA on bone strength during pre- and early-puberty in boys and girls. The strength of associations are less clear in later adolescence, as few studies examined this age group. In those that did, results were equivocal. For example, self-reported weight-bearing PA was not associated with SSI_p at the tibial shaft in 11-14 year old peri-pubertal girls.[101] There was also no relationship between self-reported PA and radius strength (breaking bending resistance index by DXA; n = 1116 girls, Tanner 1-5).[288] In contrast, in adolescents and young adults (15-20 years old), impact PA (by questionnaire, impact > walking) was significantly positively associated with Tt.BMD, Tb.BMD and Tb.N at the distal tibia in girls and Tt.Ar and bone strength

(minimum and maximum moment of inertia) at the distal tibia in boys.[109] Differences in PA-bone associations are not surprising given the variation in methods between studies (i.e., imaging modalities, imaging of weight bearing and non-weight bearing sites, population and method used to assess PA). Higher quality prospective or intervention studies that use objective measures of PA are needed to clarify the relationship between habitual PA and bone strength in later adolescence.

Prospective studies such as the University of Saskatchewan PBMAS examined the influence of PA on normal bone accrual, while controlling for maturation using APHV. In their seminal study, Bailey and colleagues demonstrated that boys and girls in the highest quartile of PA (via self-report questionnaire) gained 7-18% more BMC at the femoral neck, lumbar spine and total body over 7 years compared with boys and girls in the lower quartile of PA.[133] In a subsequent analysis of the PBMAS cohort, self-reported PA positively predicted bone CSA and estimated bone strength (section modulus, HSA) at the femoral neck across maturity (Figure 1.25).[208] PA no longer predicted bone CSA or section modulus once muscle mass (surrogate of muscle force) was included in the multilevel model. This suggested a mediating role of muscle forces in the relationship between PA and bone geometry.[208] Similar findings were demonstrated in the Iowa Bone Development Study (IBDS). This ongoing longitudinal study of bone health during childhood, adolescence and young adulthood showed that MVPA (by accelerometry) positively predicted bone CSA and strength (section modulus by HSA) from age 5-11 years.[209] However, PA did not predict bone outcomes in girls when muscle mass was included in the multilevel model, adding more support for the mediating role of muscle force in the PA and bone health relationship. Despite the known influence of muscle forces on bone adaptation and development,[57,60] few observational studies examined the PA-bone relationship in the context of muscle.[15] Thus, the influence of muscle on associations between PA and bone parameters should be considered in studies of children and youth, in future.

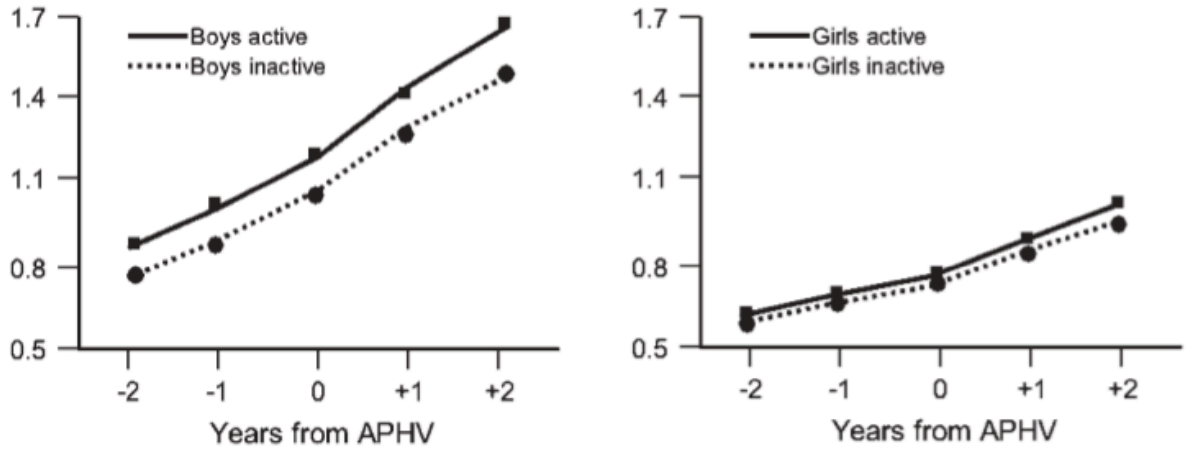


Figure 1.25. Illustration of growth curves for section modulus (Z) by hip structural analysis (HSA) of the femoral neck region in the longitudinal subset comparing 17 active girls or boys with 17 inactive girls or boys in relation to biological age, years from age at peak height velocity (APHV). Reprinted from Forwood et al.,[208] with permission from Elsevier.

In a subsequent analysis of the IBDS cohort, boys and girls who engaged in the highest trajectory of MVPA (by group-based trajectory modelling; no adjustment for muscle mass) throughout growth had significantly greater estimated bone strength at the distal (BSI) and 38% tibial site (polar moment of inertia by pQCT) at age 17 years compared with peers in the lowest MVPA trajectory.[289] Whether MVPA positively influences bone microarchitecture similarly is not yet known. That the association between PA and estimated bone strength did not vary with maturity in either longitudinal study[208,289] contrasts intervention studies that demonstrated greater benefits during pre- and early-puberty.[257,265] Such discrepancies highlight the complexity of bone adaptation to loading during growth and suggest that maturity-specific responses to PA may only be observed with more intense PA (i.e., jumping activities performed in RCTs).

1.2.7.3 Long-term effects of physical activity in childhood and adolescence

Benefits of PA on bone accrual are irrefutable. However, we do not have a clear understanding of whether PA-related gains in bone parameters are maintained into adulthood and associated with reduced fracture risk later in life. As discussed in section 1.2.2.2.4, elegantly designed studies that used animal models support lifelong benefits of exercise during growth on bone geometry, strength and fracture resistance.[81] A similar prospective study has not yet been

conducted in humans due to prohibitive methodological challenges. However, a recent cross-sectional study of former major league baseball pitchers and catchers demonstrated lifelong benefits of PA participation during youth (participants started throwing at mean age 6 years, ceased habitual throwing at mean age 31 years).[290] Side-to-side differences between throwing and non-throwing arms at the humeral midshaft were observed after 20 years of detraining for Ct.Th (by pQCT) and after 40 years of detraining for cortical BMC and Ct.Ar.[290] Tt.Ar and estimated bone strength (polar moment of inertia) were 0.56 mm^2 and 0.62 mm^4 greater, respectively, in the throwing compared with non-throwing arm after more than 50 years of detraining.[290] Compared with currently active professional baseball players, these values represent a 56% and 34% throwing-derived benefit in Tt.Ar and estimated bone strength, respectively, 50 years post-training.[290] Detraining and aging in later life, are characterized by a decline in bone strength and its determinants. Thus, support for preservation of bone geometry and strength across the lifespan in baseball pitchers and catchers suggests that PA during childhood and youth may have enduring benefits, despite reduced PA in adulthood.[290]

Prospective observational studies[291-293] and studies of athletes[294,295] demonstrated that PA during childhood and adolescence predicted bone parameters in young adulthood. Specifically, in the Penn State Young Women's Health Study (YWHS) and the University of Saskatchewan PBMAS, individuals who were most active during childhood and early adolescence maintained bone mass and strength advantages over their less active peers in later adolescence and young adulthood. Pre-menarcheal girls in the most active tertile (self-report questionnaire; mean age 12 years) at baseline in the YWHS had 10-11% greater estimated femoral neck strength (HSA) at age 17 compared with less active girls.[291] Similarly, when participants in the PBMAS cohort were followed up 9-11 years after baseline, young women and men 23-30 years of age who were most active as adolescents were still more active as adults compared with their peers.[292] Further, women who were in the upper quartile for PA during adolescence had 9-10% greater total hip and femoral neck BMC, 10-12% greater Ct.Ar and BMC at the tibia diaphysis and 3% greater trabecular content at the distal tibia in adulthood compared with their inactive peers (after adjusting for adult height, muscle area and adult PA). Men who were most active during adolescence demonstrated 10% greater Tt.Ar and 13% greater estimated bone strength (SSI_p) at the tibial diaphysis compared with their inactive peers.[293] However, bone parameters of women and men who reported average PA levels during

adolescence did not differ from inactive or active peers.[293] Thus, higher levels of PA during adolescence may be required to retain long-term benefits.

Skeletal benefits from gymnastics participation during childhood may also persist into later adolescence and young adulthood. For example, women aged 18-36 years who participated in high-level gymnastics during childhood and adolescence had 13-32% greater Ct.Ar and Tt.Ar and 16-25% greater BMC at radial and humeral shafts (pQCT) compared with same-aged women with no previous history of gymnastics participation.[294] Greater bone geometry in former gymnasts contributed to 36-38% greater estimated bone strength (SSI_p) at shaft sites of the radius and humerus (by pQCT).[294] Former gymnasts also had 15-18% greater MCSA at radial and humeral shafts compared with non-gymnasts.[294] Benefits from gymnastics were also observed at shaft sites of the tibia and femur and at the distal tibia, but were smaller in magnitude than those observed in the upper limbs.[294] Similarly, retired elite female gymnasts (10 years post-retirement, aged 22-30 years) had 10-50% greater estimated bone strength (BSI and SSI_p by pQCT) at distal and shaft sites of the radius and tibia compared with non-gymnast controls.[295] Bone strength adaptations in former gymnasts were associated with 15-28% greater Tt.Ar and BMC (total, cortical and trabecular) at the radius and 9-15% greater BMC (total, cortical and trabecular) and Tb.BMD at the tibia compared with non-gymnasts. Thus, very high-impact PA during adolescence may confer long-term benefits for bone geometry and strength.

Although these retrospective studies suggest that PA during childhood and adolescence may enhance bone strength later in life, a lifelong commitment to weight-bearing PA is recommended. However, we may never know whether such benefits in bone geometry and strength translate to reduced fracture risk later in life given the challenges of conducting such a study (i.e., long-term follow-up and associated costs).

1.2.7.4 Observational studies of sedentary time

Today's youth spend the majority of their waking hours in sedentary activities, yet few studies investigated the relationship between sedentary time and bone health (mass, geometry, or strength) in children and adolescents.[296-301] Too much sedentary time may negatively impact bone health by disrupting the balance between bone resorption and formation.[302] In an extreme example, prolonged sedentary time in a bed rest study of healthy adults increased bone

resorption rates without changes in bone formation rates.[303] In healthy children and youth, however, bone formation predominates. Thus, it is unclear how sedentary time interacts with the osteogenic effects of PA during growth and development. A recent systematic review suggested there was insufficient evidence to support an association between sedentary time (by accelerometry) and bone parameters (predominantly DXA-based studies).[304]

Studies that examined sedentary time-bone relationships primarily relied on DXA to assess BMC or aBMD. With exception of recent reports,[296,300,301] previous studies also relied on self-reported screen time to quantify sedentary time.[297-299,305] In brief, these studies suggested an inverse association between whole body BMC and internet use for non-academic purposes in adolescent boys;[297] a negative association between TV viewing and proximal femur aBMD in pre-pubertal girls;[306] and a negative association between TV viewing during childhood and adolescence (age 5, 8, 10, 14 and 17 years) and whole body BMC at age 20 years,[305] adjusting for PA. In contrast, three studies reported no association between self-reported sedentary time and whole body BMC or aBMD either with[298] or without[307,308] adjusting for PA. Results were equally mixed in the three studies that examined accelerometry-derived sedentary time. For example, sedentary time was positively associated with lumbar spine and proximal femur aBMD in adolescents and young adults, independent of MVPA.[296] Based on these limited findings, authors speculated that extended periods of sedentary time between bouts of PA might be required for optimal adaptation of bone to mechanical loading. Similarly, a 2-year follow-up study of 10-14 year old girls and boys found that increased sedentary time, when substituted for time spent in light PA, was positively associated with whole body BMC and aBMD.[301] In contrast, in peri-pubertal boys (age 11-13 years), a 5% increase in sedentary time over 12 months was negatively related to change in femoral neck aBMD (adjusted for vigorous PA).[300] Discrepancies clearly exist in the literature, and thus, well-designed prospective studies (appropriately powered for different rates and timing of maturity) are needed to clarify the bone strength-sedentary time relationship (measured objectively).

To supplement the paucity of information regarding the influence of sedentary time on bone microarchitecture, I briefly describe the Women International Space Simulation for Exploration (WISE) bed rest study, which provided an extreme example of unloading in twenty-four women aged 25-40 years. After 60 days of bed rest, BV/TV (by HR-pQCT) decreased by 0.1-0.3%, Tb.N decreased by 1-2% and Tb.Sp increased by 1-2% at both the distal tibia and

radius. At the distal tibia, Ct.Th also decreased by 1%.[309] Trabecular microarchitecture deficits persisted at one-year following cessation of bed rest, [309] suggesting that sustained periods of unloading may have long-term consequences for trabecular bone.

1.2.8 Summary of the literature

Bone strength is a function of bone geometry, density and microarchitecture, which all continually adapt to variable mechanical loads during growth. Maturity- and sex-related adaptations in bone strength and its determinants during childhood and adolescence are unclear. This is in part due to the large sample size needed to appropriately assess change across maturation given the substantial variation in its magnitude and timing. It is also due to few prospective studies that used 3D imaging to characterize bone. Although time and labour intensive, it is through longitudinal studies that we will better understand nuanced adaptations of bone over time. However, few such studies have been conducted during adolescent growth.

Therefore, in this thesis I overcome recognized gaps in the literature. That is, I examine bone strength and its determinants prospectively using pQCT and HR-pQCT. I also align boys and girls on biological age, APHV, and extend the scant literature that assessed the role of sedentary time on bone during growth using accelerometry. Further, I employ advanced statistical modelling approaches to maximize value of the HBSIII longitudinal dataset and to adequately distinguish within-person from between-person differences across adolescent growth. Collectively, these novel components represent the first time that maturity- and sex-related adaptations in bone strength and its determinants were examined prospectively across 12-years of adolescent growth, with participants aligned on biological age to control for maturational differences. Finally, it is also the first prospective study to investigate the influence of both PA and sedentary time on bone strength using HR-pQCT.

1.3 Rationale, objectives and hypotheses

In this section, I outline the rationale and specific aims and hypotheses for each of the four studies that comprise this dissertation.

1.3.1 Bone strength and microarchitecture in the growing skeleton: the role of sedentary time

Rationale: The benefits of PA for bone strength and parameters that underpin bone strength during childhood and adolescence are well established.[15] However, we know little about the potentially deleterious effects of sedentary time on bone during these key periods of growth.

Objectives:

1. To examine associations between self-reported screen time and bone strength and its determinants (bone parameters), independent of self-reported PA.
2. To examine associations between objectively measured volume[4] and patterns of sedentary time and bone parameters, independent of objectively-measured MVPA.
3. To assess the contribution of muscle force and modulator variables (i.e., maturity, ethnicity, dietary calcium and PA) to bone parameters.

Hypotheses:

1. Self-reported screen time will be negatively associated with bone parameters, independent of PA.
2. Objectively measured volume and patterns of sedentary time will be negatively associated with bone parameters, independent of MVPA.
3. Maturity and MCSA (surrogate of muscle force) will be the primary explanatory variables of tibial bone parameters.

Contribution: This cross-sectional study[310] is the first to examine the relationship between sedentary time and bone strength and its determinants using 3D bone imaging (HR-pQCT), which permits evaluation of Ct.Po and estimates of bone strength. Further, this study objectively measures the *volume* and *patterns* of sedentary time accumulation in addition to self-reported screen time. Current PA guidelines recommend limiting sedentary time for optimal health in children and youth;[311] however, there is a relatively limited body of evidence regarding how

[4] Volume refers to total duration of sedentary time (min/day)

unloading the skeleton may be detrimental to bone parameters in healthy children and adolescents. Thus, these findings may inform future public health recommendations regarding sedentary time of children and youth.

1.3.2 Re-examining the surfaces of bone in boys and girls during adolescent growth: a 12-year mixed longitudinal pQCT study

Rationale: A plethora of research supports childhood and adolescence as critical periods for bone mineral accrual.[9,312] However, the intricacies of how bone is gained during childhood is not completely understood. In the 1970s, a landmark study by Garn and colleagues examined surface-specific differences in bone growth and development. Specifically, this cross-sectional study examined radiographs of the second metacarpal and concluded that boys and girls experience periosteal expansion and endocortical contraction during adolescent growth. However, boys exhibit more periosteal expansion while girls exhibit more endocortical contraction.[155-157] The current study adopts a 12-year mixed longitudinal design and examines the tibial midshaft of boys and girls who are aligned on biological age (years from APHV) to revisit Stanley Garn's theory related to sex differences in periosteal expansion and endocortical contraction. Findings extend those from our previous study that used pQCT to assess bone development across 20 months.[153]

Objectives:

1. To compare rates of bone expansion and/or contraction at the periosteal and endocortical surfaces of the tibial midshaft between boys and girls pre- and post-APHV.
2. To compare rates of Ct.BMD and bone strength (SSI_p) accrual at the tibial midshaft between boys and girls pre-and post-APHV.

Hypotheses:

1. Both boys and girls will demonstrate expansion at the periosteal and endocortical surface. Boys will demonstrate a greater magnitude of change at both surfaces pre- and post-APHV.

2. Boys will demonstrate greater bone strength, but lower Ct.BMD compared with girls pre- and post-APHV.

Contribution: This 12-year study of adolescent bone growth using 3D imaging techniques (pQCT) is the longest to date.[313] Longitudinal studies are difficult to conduct, time consuming and relatively rare. Thus, the few existing studies that examined changes on bone surfaces during growth were cross-sectional or short term prospective. My study is uniquely able to account for the tremendous variability that accompanies bone adaptation throughout adolescence; I control for the potentially profound influence of maturity by aligning boys and girls on a common maturational landmark, APHV. Findings may challenge commonly held notions regarding sex differences in how bone is gained at the mid-tibia during growth and may improve our understanding of factors that influence fracture risk during adolescence.

1.3.3 Sex differences and growth-related adaptations in bone microarchitecture, geometry, density and strength: a mixed longitudinal HR-pQCT study

Rationale: Sex differences in adult bone strength and fracture risk are well-documented. However, we know less about adaptations in bone microarchitecture, geometry and density that accompany gains in bone strength during growth. Only three studies used HR-pQCT to evaluate sex differences in bone strength and its determinants during adolescence. Two of these were cross-sectional and one had a short follow-up period. Prospective studies of longer duration are key to evaluate the nuances of bone development over time and to further our understanding of factors that might contribute to the elevated fracture risk during adolescence, and ultimately, in later life.

Objectives:

1. To describe growth-related adaptations in bone strength and its determinants (parameters) at the distal tibia and radius in boys and girls.
2. To compare differences in growth-related adaptations in bone parameters between boys and girls.

Hypotheses:

1. Boys and girls will demonstrate increases in bone parameters throughout adolescence, with the exception that Ct.Po and load-to-strength ratio will decline during adolescence.
2. Boys will demonstrate greater bone strength, geometry and cortical porosity, but lower Ct.BMD throughout adolescence compared with girls.

Contribution: This longitudinal study of boys and girls across adolescent growth using HR-pQCT to evaluate bone, is the longest to date.[314] Uniquely, I use advanced mixed modelling approaches and align boys and girls on a common maturational landmark (APHV) to more clearly characterize changes in 3D aspects of bone microarchitecture, geometry, density and strength that accompany adolescent growth. This study provides new insight into sex differences in bone parameters and factors that may contribute to greater skeletal fragility during adolescence and, ultimately later in life.

1.3.4 Physical activity, sedentary time and bone strength from childhood to early adulthood: a mixed longitudinal HR-pQCT study

Rationale: Bone strength and its determinants continually adapt to increased mechanical loads during growth and PA is essential for optimal bone strength accrual. However, given the relatively recent evolution of bone imaging technologies, less is known about how bone microarchitecture adapts to PA and whether sedentary time independently influences bone parameters. A recent systematic review suggested there is insufficient evidence to ascertain an association between sedentary time and bone health in children and youth, independent of PA.[304] Thus, it remains unclear how the potentially deleterious impacts of sedentary time interact with the positive effects of PA to influence skeletal growth and development in healthy, children and youth. Prospective studies are poised to clarify adaptations in bone microarchitecture associated with independent effects of PA and sedentary time during growth.

Objectives:

1. To evaluate prospective associations between PA, sedentary time and growth-related adaptations in bone parameters at the distal tibia and radius in boys and girls across adolescence.

Hypotheses:

1. PA will positively predict adaptations in bone parameters. Sedentary time will be negatively associated with bone parameters, independent of PA.

Contribution: Previous cross-sectional studies evaluated the association between PA, sedentary time and bone parameters. These early studies used DXA to image bone and subjective measures to assess PA and sedentary time. I extend this body of literature in three distinct ways: 1) I use longitudinal data acquired across 4-years at the tibia and 3-years at the radius in boys and girls across adolescent growth; 2) I evaluate bone using more advanced 3D imaging techniques; 3) I assess PA and sedentary time objectively (using accelerometry). This is the first prospective study to examine how trabecular and cortical bone microarchitecture adapts to PA and sedentary time during adolescence. Findings will further clarify the consequences of positive health behaviours such as PA versus negative health behaviours such as sedentary time on bone parameters during adolescent growth. Outcomes might inform PA and sedentary time public health guidelines for youth, in future.

Chapter 2: Methods

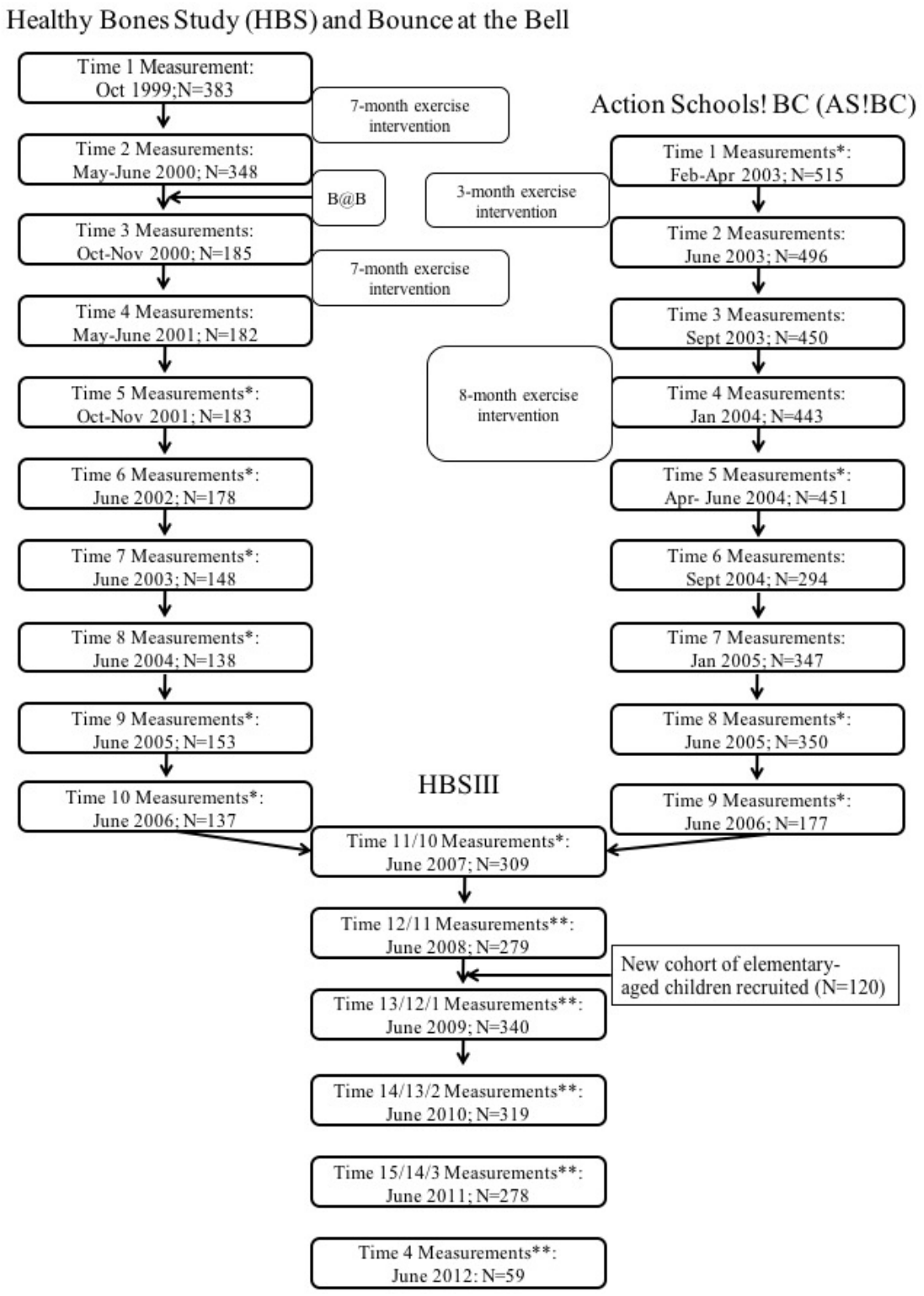
In this chapter, I present the research methods used to address my research aims. I first provide an overview of the study cohort in section 2.1 and then present specific methods used in section 2.2.

2.1 Healthy Bones Study overview

Participants were healthy girls (n=556) and boys (n=515) aged 8 to 23 years who participated in the mixed longitudinal University of British Columbia Healthy Bones III Study (HBSIII; Figure 1). The HBSIII cohort includes participants from three school-based studies: the Healthy Bones Study (HBS; Healthy Bones and Bounce at the Bell), which began in 1999; the Action Schools! BC (AS!BC) project, which began in 2003; and the most recent cohort, recruited in 2009. The three cohorts are collectively referred to as HBSIII and I describe this cohort in detail below.

2.1.1 Healthy Bones Study and Bounce at the Bell

HBS was a cluster randomized controlled school-based intervention that investigated effects of a 20-month exercise intervention on bone mass, as measured by DXA. We recruited participants (n = 383) in the fall of 1999 from grade 4, 5 and 6 classes in 14 schools in Richmond, BC, described in detail elsewhere.[257-259] We implemented the intervention over two academic years (2, 7-month intervention periods); the intervention consisted of brief (10-12 min) high-impact, weight-bearing exercise twice per week during physical education class and once per week in the classroom or outside. Participants in intervention and control schools took part in 40 min of physical education, twice per week, as mandated by the school board. We invited participants from intervention and control schools to attend annual assessments in the spring of each year following the intervention (until 2011). I describe the substantial efforts taken to retain participants for up to 12 years in section 2.1.4.



B@B, Bounce at the Bell participants added (N=51); * Indicates pQCT assessment; ** indicates pQCT and HR-pQCT assessment

Figure 2.1. Overview of the University of British Columbia Healthy Bones Study III (HBSIII).

The HBS companion study, Bounce at the Bell, investigated the effect of frequent bouts of jumping exercises on bone mass over 8-months (intervention period not indicated in Figure 1.1). We recruited participants (n = 51) in the fall of 2000 from grades 4 and 5 classes in 3 schools in Richmond, BC.[315] Participants performed 10 counter-movement jumps, 3 times/day (morning, noon and home bell; ~ 3 min/day of jumping) in addition to twice weekly physical education class. We invited participants to attend annual assessments each spring following the intervention (until 2011).

2.1.2 Actions Schools! BC

The Actions Schools! BC (AS!BC) trial was a 16-month cluster randomized controlled school-based intervention that evaluated the effectiveness of the AS!BC model for increasing bone mass and strength (as measured with DXA and pQCT). The AS!BC model is an active school model designed to promote PA in elementary schools. The model helps schools develop individualized action plans to promote healthy living based on evidence and best practice. The model is flexible and based on principles of health promotion.[316] We recruited participants (n = 515) in early 2003 from grade 4 and 5 classes at 10 elementary schools in Vancouver and Richmond, BC. [265] In phase one (3-months prior to summer holiday), we oriented participants to the program. In phase two (resumption of school in fall 2003), participants at intervention schools completed an 8-month active intervention.

As with the HBS cohort, we invited AS!BC participants to attend annual follow-up measurements each spring through 2011. We merged the HBS, Bounce at the Bell and AS!BC cohorts in 2006 because they employed nearly identical protocols and because participation in the exercise intervention was not associated with sustained benefits at the tibial shaft.[317] However, we excluded observations from children actively participating in the AS!BC intervention (n = 451, spring 2004) because we previously demonstrated a positive effect of a PA intervention on bone accrual.[258,259]

2.1.3 New cohort

In 2008, our research group acquired a first generation HR-pQCT and incorporated this into the HBSIII measurement protocol to assess bone microarchitecture. Since the youngest children from the HBS and AS!BC cohorts were in grade 10 (approximately 15 years old) by the time of first HR-pQCT assessment, we recruited a younger cohort (pre- and early-pubertal children) in order to examine changes in bone microarchitecture earlier and through the period of adolescent growth. We recruited the new cohort of younger participants (n=120; mean age 10.5 ± 0.6 years) in 2009 from grade 4 and 5 classes in 5 schools in Vancouver and Richmond, BC. We invited participants to attend annual assessments from spring 2009 through 2012.

In this thesis, I include bone data from HBSIII annual measurements conducted between spring 2001 and 2012. We obtained written informed consent from the parents or legal guardians, written assent from participants younger than 18 years of age and informed consent from participants 18 years of age and older. The University of British Columbia's Clinical Research Ethics Board approved all procedures (H15-01194, H07-02013, H2-70537).

2.1.4 Recruitment and retention

We employed similar recruitment methods for HBS and AS!BC studies. In brief, principals volunteered their schools to participate after the recruitment team made presentations to school principals at district meetings. Next, the recruitment team presented the project to grade 4, 5, and 6 teachers in volunteer schools. Finally, the recruitment team presented the study to grade 4, 5 and 6 students whose teachers had volunteered. We gave information letters and consent forms to classroom teachers for students to take home to parents (Appendix A). We obtained consent and assent for the follow-up studies for HBS participants in 2001, 2003, 2006 and 2009 and for AS!BC participants in 2004, 2006, 2007 and 2009.

We used similar recruitment methods to recruit the new cohort of grade 4 and 5 students. We distributed information letters and consent forms (Appendix A) in the classroom and obtained consent and assent in 2009.

We used several incentives over the 12-year study period to retain participants, including distributing items at assessments (i.e., snacks, stickers, pencils, socks, Frisbees, $20). We mailed

detailed individual and group (data de-identified, collapsed and reported by age and sex) results to each participant (Appendix B) in advance of the next years' data collection. Results included a picture of their whole body skeleton from DXA along with a note reminding them of upcoming data collection.

2.1.5 Data collection overview

For HBSIII participants attending elementary schools, the research coordinator contacted their teachers and arranged for participants from each class to be picked up at the school door to travel to the lab in groups of 5 (plus a research assistant as chaperone). The driver and research assistant accompanied participants during their trip from the elementary schools to the measurement site. The research coordinator contacted HBSIII study participants who attended secondary schools by telephone. Whenever possible, we booked group measurement sessions (up to 6 participants/session) for students attending the same secondary school (participants attended 41 different secondary schools across the study period), and we transported participants to the Bone Health Research Lab at Vancouver General Hospital (VGH) (and in 2012 to the Centre for Hip Health and Mobility at VGH) by minivan. For participants who already graduated secondary school, the research coordinator contacted participants individually to schedule assessment.

At the lab, participants rotated through 6 stations: anthropometry (5 min), questionnaires (30 min), mechanography (15 min), DXA (20 min), HR-pQCT (20 min) and pQCT (10 min). All members of the research team attended a full-day training session conducted by the research coordinator to learn measurement protocols prior to data collection. All research team members were trained to correctly conduct anthropometry and administer questionnaires. Team members practiced measurements during the training session to maintain quality assurance and were also trained on the ethics of data collection. Trained technicians/measurers conducted bone imaging and mechanography procedures.

2.2 Heathy Bones III Study protocol

In this section I discuss the specific measurements conducted for HBSIII. I personally acquired and analyzed all pQCT scans in 2012 and analyzed all HR-pQCT scans.

2.2.1 Anthropometry

Anthropometry included height (cm), sitting height (cm), body mass (kg), tibial and ulnar length (mm). We measured height and sitting height to the nearest 0.1 cm with a wall-mounted digital stadiometer (Seca Model 242, Hanover, MD) using stretch stature techniques. We assessed height with the participant's head positioned in the Frankfort plane, heels flat on the floor and shoes off and applied gentle traction to the participant's mastoid process.[318] We assessed body mass to the nearest 0.1 kg using an electronic scale (Seca Model 840, Hanover, MD). Participants removed any heavy clothing and shoes prior to stepping onto the scale. We assessed limb length to the nearest mm as the distance from the distal edge of the medial malleolus to the tibial plateau for the tibia and the distance from the olecranon to the ulnar styloid process for the ulna. Trained research assistants took all measurements in duplicate, unless differences were > 0.4 cm or 0.2 kg when they obtained a third measure. We used the mean of two values or the median of three for all analyses. In our laboratory, reproducibility (CV%) is < 0.3% for measures of stature and < 3.5% for tibia length.

2.2.2 Health history questionnaire

Parents completed a health history questionnaire for their child at baseline (HBS: 1999, Bounce at the Bell: 2000, AS!BC: 2003, new cohort: 2009) and participants completed a shorter version at subsequent annual visits (Appendix C). We excluded participants with diseases known to affect bone metabolism (e.g, osteogenesis imperfecta, fetal alcohol syndrome, Type 1 diabetes) or participants taking medication known to influence bone metabolism. We determined each participant's ethnicity based on their parents' and/or grandparents' place of birth as reported on the health history questionnaire at baseline. Parents were asked to classify their own, and their child's ethnicity. We classified participants as "Asian" if both parents or three of four

grandparents were born in Hong Kong, China, Japan, Taiwan, Philippines, Korea or India; "white" if both parents or three of four grandparents were born in North America or Europe; and "other" if the participant had parents of other or mixed ethnicities. We also considered parental self-report of ethnicity to ensure correct classification of each participant.

2.2.3 Maturity

2.2.3.1 Sexual maturation

We assessed maturity using the method of Tanner: self-reported pubic hair stage in boys and self-reported breast and pubic hair stage in girls;[127] however, we only used breast stage in girls as it showed better alignment with timing of menarche.[132] We gave participants a set of line drawings that depicted the 5 stages of sexual development and asked participants to select the drawing most similar to his/her own physical appearance. A brief description of the visual appearance at each stage accompanied drawings (Appendix C). Participants completed the questionnaire in private after receiving instructions from a research assistant and returned the questionnaire in a sealed envelope once completed. Participants who had reached maturity (Tanner stage 5) based on a previous year's data collection were not required to complete the questionnaire. I considered participants who were in Tanner stage 1 as pre-pubertal, Tanner stage 2 and 3 as early-/peri-pubertal and Tanner stage 4 and 5 as late-/post-pubertal.[4] We also assessed maturity in female participants using self-reported menarcheal status. A research assistant asked female participants if they had experienced their first menstrual period. If "yes", they were asked to recall the approximate date. Participants who reported reaching menarche at a previous year's data collection were not asked this question in subsequent years.

2.2.3.2 Age at peak height velocity

To control for well-known maturational differences between adolescent boys and girls of the same chronological age, we calculated age at peak height velocity (APHV; years) as an estimate of biological maturity. I provide a detailed description of this process in Appendix D. In brief, we fit an interpolating cubic spline to each participant's height velocity data.[142] The

magnitude of PHV was identified as growth per year (cm/year) that occurred at APHV. We used APHV to calculate a biological maturity offset (in years) by subtracting the APHV from chronological age at time of measurement. Thus, we generated a continuous measure of biological maturity offset (e.g., -1 year is equivalent to 1 year prior to attainment of APHV; +1 to one year after APHV). Due to missing and mistimed (e.g., 3 to 12 months between height measurements) measurements surrounding APHV, we were able to identify APHV for 235 of 1071 participants (112 boys, 123 girls).

2.2.3.3 Maturity offset equation

As we were unable to calculate APHV for all participants, I also estimated maturity offset (years from APHV) using a recalibrated version of the Mirwald prediction equation.[141,142] The recalibrated equation is a simplified version of the Mirwald equation that uses age and height for girls and age and sitting height for boys.[142] In the calibration sample from the Saskatchewan PBMAS (79 boys and 72 girls; 7.5-17.5 years), predicted APHV explained approximately 90% of variance in actual APHV.[142] Of note, the published equations by Moore et al., were based on data from white participants only. However, our research group also developed equations for Asian boys and girls (unpublished data). Therefore, I used the published equations[142] to predict maturity offset in white participants and in participants of other/mixed ethnicities. I used ethnic-specific equations to predict maturity offset in Asian participants.

Maturity offset estimation equations (age in years and height and sitting height in cm):

1. White/other boys: (-8.128741 + (0.0070346 x age x sitting height)
2. Asian boys: (-8.128741 + 0.7482624) + (0.0070346 x age x sitting height)
3. White/other girls: -7.709133 + (0.0042232 x age x height)
4. Asian girls: (-7.709133 + 0.7303442) + (0.0042232 x age x height)

For all participants I used anthropometry data from the measurement occasion closest to a reported average APHV (approximately 11.6 years in girls and 13.5 years in boys) to estimate maturity offset.[142]

2.2.4 Dietary calcium intake

All participants completed a validated food frequency questionnaire to estimate dietary intake of calcium (mg/day; Appendix C). Validity of the FFQ was assessed against a 1-day food recall (r = 0.98) and reliability was assessed on two occasions separated by 3 months (r = 0.76).[319] Participants reported how often they consumed 20 calcium-rich foods items (times per week, times per month) and how much they consumed each time (number of servings as per serving size described in the food frequency questionnaire).

2.2.5 Peak muscle power

We used the Leonardo Mechanograph Ground Reaction Force Plate (GRFP; Novotec, Germany) to assess peak leg muscle power from 2008 onwards, the mechanics of which are described in detail elsewhere. [320] Briefly, the GRFP is divided into two sections, which allows for simultaneous measurement of forces (vertical component only) applied to the right and left legs separately. The sample rate is set to 800 Hz (800 measurements/s for each force sensor). We used the manufacturer's software (Leonardo Mechanography v4.3) to detect, store and calculate mechanography outcomes. The software uses force and time data to calculate velocity of the movement (m/s), power (Watts, W) and jump height (m).

All participants performed a single two-legged countermovement vertical jump on the GRFP with their hands held static at their waist and their feet hip width apart. The research assistant explained the jumping protocol to all participants in a standardized manner. We asked participants to perform the countermovement jump after hearing a tone (from the computer). The research assistant instructed each participant to initiate a downwards movement and then immediately jump up as high as possible using both legs. We instructed participants to land with both feet on the platform (with each foot on the appropriate side of the middle line) and to remain still until after hearing a second tone from the computer signaling the end of the trial. Each participant performed one practice jump and three trial jumps. We used peak power during lift off phase (kW) from the jump associated with the maximum height for analysis.

2.2.6 Self-reported screen time and physical activity

We estimated screen time using a self-report questionnaire that inquired about h/day spent watching television and/or playing video or computer games during the previous week (Appendix C). There were five response options ranging from "none at all, or less than 1 hour per day" to "more than 4 hours per day". In addition to examining these data using the 5 response groups, I collapsed these responses into two groups (< 2 h/day and ≥ 2 h/day) in order to examine whether meeting current sedentary time guidelines for youth, which recommend "limiting recreational screen time to no more than 2 hours per day",[321] is associated with bone outcomes.

We assessed self-reported PA time over the previous week using the previously validated self-report Physical Activity Questionnaire for Children (PAQ-C) in elementary school participants and Physical Activity Questionnaire for Adolescents (PAQ-A) in participants in high school or older (Appendix C).[222,223] We calculated a general PA score as an average of the PAQ items in a continuous range between 1 (low activity) and 5 (high activity). Based on participants' estimates of time spent in common sports and activities in Item 1, we also estimated time spent in MVPA (min/day) and time spent in activities designated as loaded (impactPA in h/week; impact > walking).[109]

2.2.7 Objectively measured sedentary time and physical activity

In 2008, our research group acquired accelerometers to estimate PA and sedentary time (ActiGraph GT1M; Pensacola, FL). The GT1M is a small, uniaxial accelerometer that detects vertical accelerations of 0.05 – 2.00 g. The signal is band filtered to the frequency range of 0.25-2.50 Hz to exclude non-human movement. We attached each accelerometer to an elastic belt and instructed participants to wear the belt around the waist with the accelerometer positioned at the iliac crest. We asked participants to wear the device during all waking hours for seven consecutive days, except during water-based activities (e.g., swimming and showering). Participants received a log sheet to record accelerometer on and off times each day. We set the accelerometers to record in 15-sec epochs and analyzed all data using KineSoft software (v3.3.75; KineSoft, Loughborough, UK).

We included participants who recorded at least 10 h/day of data on three or more days[232] and defined non-wear time as 60-min of consecutive zero counts. As non-wear criteria are inconsistent within the literature and significantly alter accelerometer output, I determined my own non-wear criteria using HBSIII data based on suggestions from Mâsse and colleagues to examine the number of wearing interruptions observed in the data, as a high number of interruptions (e.g., 10 per day) are unlikely.[322] I examined several different non-wear criteria ranging from 10- to 60-min of consecutive zeros with or without a 2-min interruption. The 60-min non-wear criteria without interruptions resulted in an average of 1-2 wearing interruptions per day, compared with an average of 7 interruptions per day using the 10-min criteria without interruptions. Further, the longer criteria allowed me to include more participants (more valid wear days), while demonstrating strong rank order correlations with the other criterion (10-, 20-, 30-min without interruptions and 60-min with interruptions; r = 0.92-0.99). I presented these findings at the 2013 ICAMPAM conference in Amherst, Massachusetts (Gabel et al., Relationships between physical activity and adiposity: Does accelerometer non-wear criteria matter? *ICAMPAM Poster Presentation June 2013*).

I used a cut point of < 100 cpm to classify sedentary time[239] and the Evenson cut points[238,239] to determine intensities of PA: light ≥ 100 cpm, moderate ≥ 2296 cpm and vigorous ≥ 4012 cpm. Thus, I defined MVPA using an accelerometer cut point ≥ 2296 cpm.

2.2.8 Bone imaging

2.2.8.1 pQCT

Acquisition: We used pQCT to assess bone structure and strength at the tibial midshaft. One of 8 trained operators acquired pQCT scans over the 12-year period. Each operator conducted inter- and intra-rater reliability training to ensure consistency across technicians (e.g., scanning five participants with repositioning). We acquired a 2.3 mm slice at the midshaft (ROI: 50% site; proximal to the distal tibial endplate) of the left tibia using the XCT-2000 (Norland/Stratec Medizintechnic GmbH, Pforzheim, Germany) from 2001-2007 and the XCT-3000 (Norland/Stratec Medizintechnic GmbH, Pforzheim, Germany) from 2008-2012. We previously reported excellent agreement between XCT-2000 and XCT-3000 (root mean squared

coefficient of variation, 0.6-1.5% for tibial midshaft bone parameters).[323] We used a scan speed of 30 mm/sec and a resolution (pixel size) of 0.5 mm in participants recruited prior to 2003 and a resolution of 0.4 mm thereafter. Previous work confirmed no significant differences in Tt.Ar or Tt.BMD between 0.4 mm and 0.5 mm resolution pQCT scans at the distal radius.[324] We acquired a 30 mm planar scout view over the joint line to define the anatomic reference line, located at the distal aspect of the distal cartilage of the tibia (Figure 2.2). We used the same anatomical reference line to assess the same relative site each year. The effective dose equivalent (risk of exposure from a single tissue in terms of whole body exposure risk) for pQCT is negligible at 0.22 μSv (~ 5% of daily effective background radiation dose).

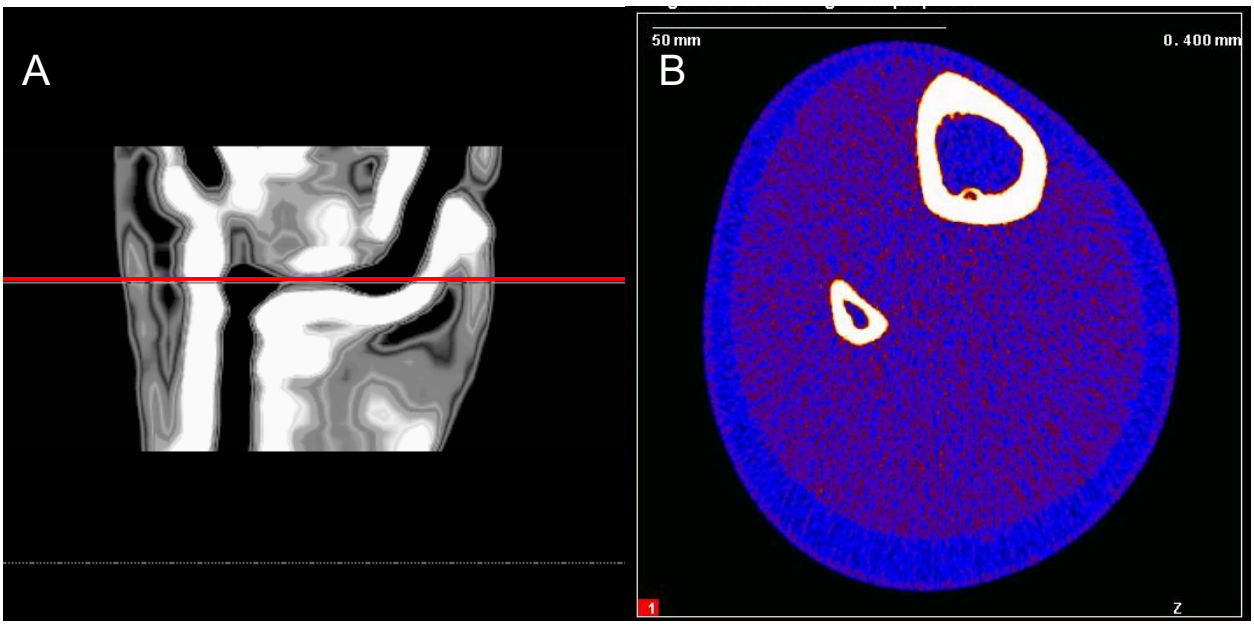


Figure 2.2. A) the anatomical reference line defining the distal aspect of the distal cartilage of the tibia and B) a peripheral quantitative computed tomography scan of the tibial midshaft. Bone is indicated in white, muscle in red/purple and subcutaneous fat in blue.

Prior to imaging, the pQCT technician screened participants to rule out pregnancy, ascertain prior exposure to ionizing radiation and to determine each participant's fracture history. If participants reported a prior fracture of the limb of interest in the past six months we scanned the contralateral side. Additionally, to maintain consistency with previous assessments, we scanned the contralateral side if it had been scanned in a previous year's assessment due to fracture of the limb of interest.

The pQCT technician briefly explained how pQCT worked and asked participants to extend their left leg into the pQCT gantry, resting on the supported platform (Figure 1.12). The operator secured the limb firmly with Velcro straps to minimize movement during the scan. The technician conducted a second scan if movement occurred during the first scan. Each scan took approximately 3 min to complete. I acquired and analyzed all scans in 2012 (n=59).

Analysis: We analyzed all scans using Stratec software version 6.0 as per our standard protocol.[151,153] An automatic ROI was generated after placing the cursor at the center of the tibia marrow cavity. The algorithm uses modes and thresholds set by the operator to determine numerous bone variables. I list modes, thresholds and outcome variables used in this thesis in Table 2.1. As discussed in section 1.2.3.2.1, pQCT protocols are not standardized; thus, I used modes and thresholds similar to those used in previous studies by our group and based on the manufacturer's recommendations.[151,153]

Table 2.1. Analysis modes, thresholds and outcome variables for pQCT measurements at the tibial midshaft (50% site).

Variable	**Analysis Mode (Threshold, mg/cm^3)**
Total bone cross-sectional area (Tt.Ar, mm^2)	Contour mode 1 (711 mg/cm^3)
Cortical Area (Ct.Ar, mm^2)	Peel mode 2 (540 mg/cm^3)
Cortical bone mineral density (Ct.BMD, mg/cm^3)	Separation mode 1 (711 mg/cm^3)
Polar strength-strain index (SSI_p, mm^3)	Contour mode 1 (711 mg/cm^3) Peel mode 2 (540 mg/cm^3) Separation mode 2 (480 mg/cm^3)
Muscle cross-sectional area (MCSA, mm^2)	Contour mode 1 (-100 mg/cm^3) Peel mode 2 (40 mg/cm^3) Separation mode 2 (711 mg/cm^3)

We determined in-vivo precision with repositioning at the 50% site using the XCT-2000 in 14 participants (12-27 years); the CV was less than 2% for all bone variables. [151] We maintained quality assurance by scanning a pQCT anthropomorphic phantom daily during measurement periods.

2.2.8.2 HR-pQCT

Acquisition: We assessed bone strength, microarchitecture, BMD and geometry at the non-dominant tibia and radius using HR-pQCT (XtremeCT; Scanco Medical AG, Brüttisellen, Switzerland.), unless the participant sustained a previous fracture of the tibia or radius, in which case we scanned the opposite limb.[4] We identified the preferred leg for kicking (i.e., "which leg would you use to kick a soccer ball") as the dominant tibia. We used a standard ROI to assess the same relative site from year to year. The ROI included both cortical and trabecular bone and excluded the growth plate in most children.[110]

Prior to each scan the HR-pQCT technician immobilized the limb in a carbon fibre cast shaped for the leg or forearm (Figure 2.3 A-B). The technician placed the limb into the gantry and adjusted the chair to ensure the participant was as comfortable as possible (Figure 2.3 C-D). The technician explained the importance of being still during the scans and dimmed the lights to create a relaxing environment.

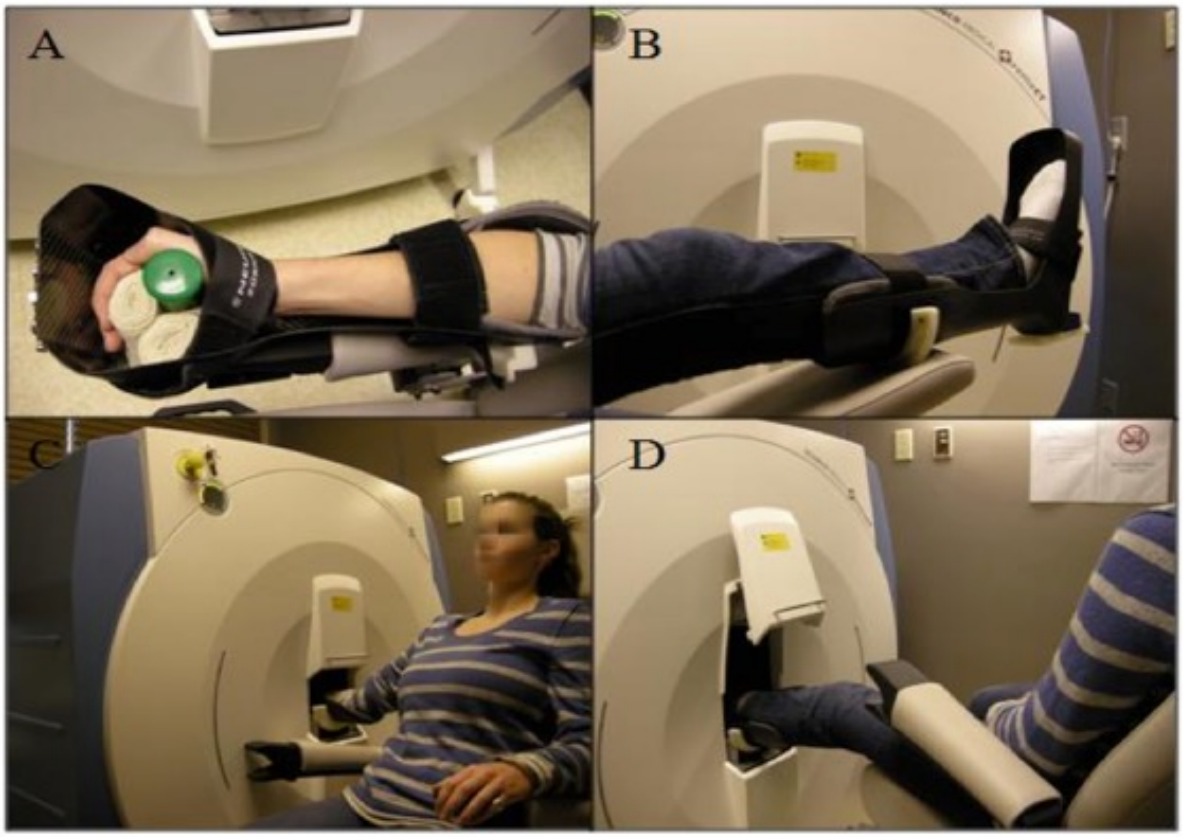


Figure 2.3. Set-up for high-resolution peripheral quantitative computed tomography (HR-pQCT) radius (A,C) and tibia (B,D) scans.

The technician first performed a 2D scout view over the joint line to identify the ROI. The technician placed a reference line at the distal tibia end plate (Figure 2.4) or medial edge of the distal radius (Figure 2.5) and defined the ROI as proximal to the reference line and equivalent to 8% of the total tibia length (Figure 2.4) or 7% of total ulnar length (Figure 2.5). We scanned all participants using the manufacturer's standard protocol of 60 kVp effective energy, 900 μA, matrix size of 1536 × 1536, 100 ms integration time and 82 μm nominal isotropic resolution. We acquired 110 slices (approximately 9.02 mm) scanned proximally toward the 8% or 7% site of the tibia and radius, respectively. The effective dose equivalent for the tibia scan is < 3 μSv per measurement (~ three-quarters of daily effective background radiation dose), with a measurement time of 2.8 min. A second scan was acquired if there were significant motion artifacts (> grade 3; Figure 2.6) on the first.[124] We conducted daily quality control procedures to assess density fluctuation and weekly procedures to standardize geometry using a calibration phantom provided by the manufacturer.

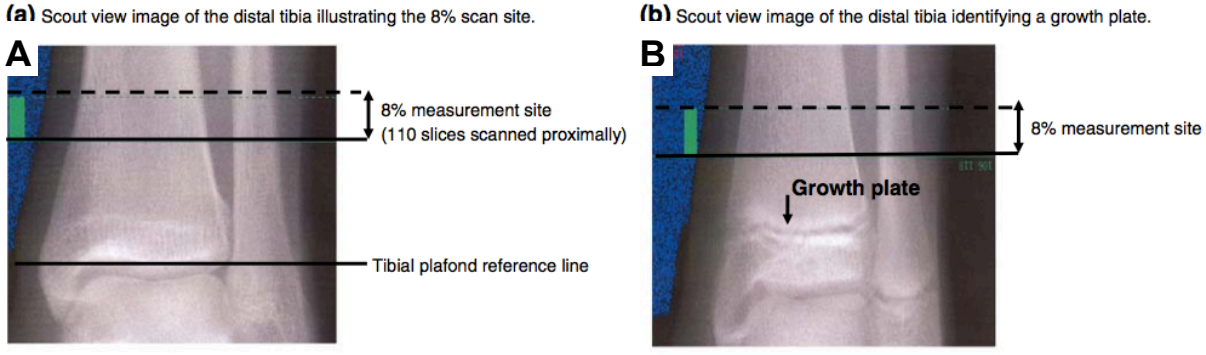


Figure 2.4. High-resolution peripheral quantitative computed tomography at the distal tibia. A) scout view image illustrating 8% scan site; B) scout view illustrating position of tibial growth plate. Reprinted from Burrows et al.,[110] with permission from Springer.

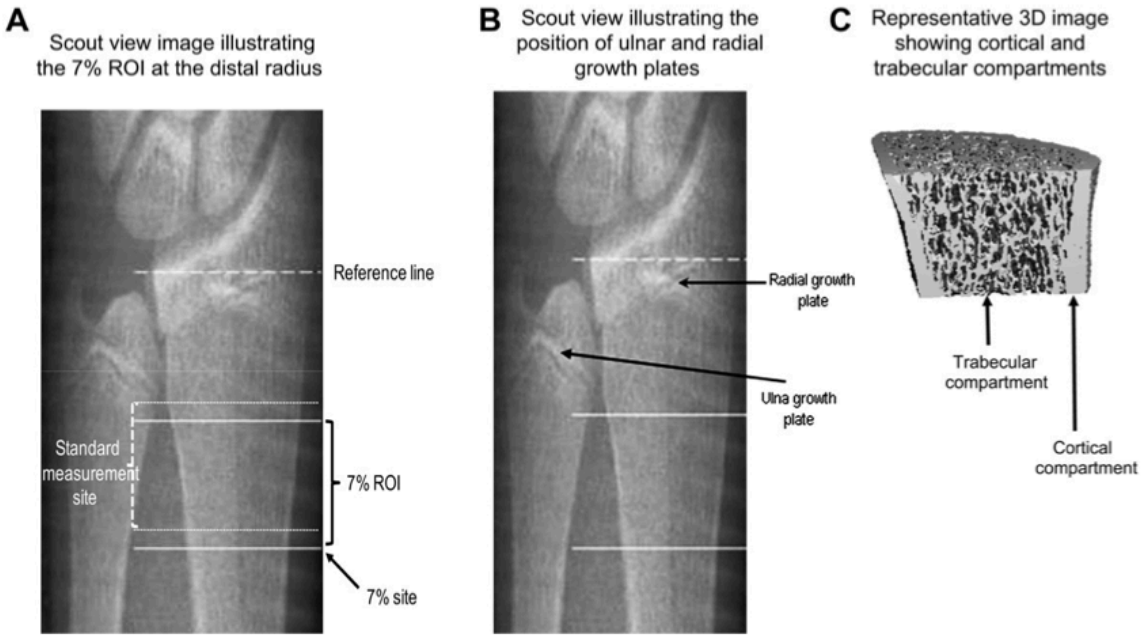


Figure 2.5. High-resolution peripheral quantitative computed tomography at the distal radius. A) scout view image illustrating 7% scan site; B) scout view illustrating position of ulnar and radial growth plates; C) representative three-dimensional image showing cortical and trabecular compartments. Reprinted from Burrows et al.,[111] with permission from Elsevier.

Analysis: I assessed all HR-pQCT images for motion artifacts using a grading scale from 1 (no motion) to 5 (medium-large streaks/discontinuities) (Figure 2.6).[124] I excluded scans with motion artifacts > 3.[124] Following motion grading, we performed three separate analyses to assess bone microarchitecture, geometry, BMD and estimate bone strength.

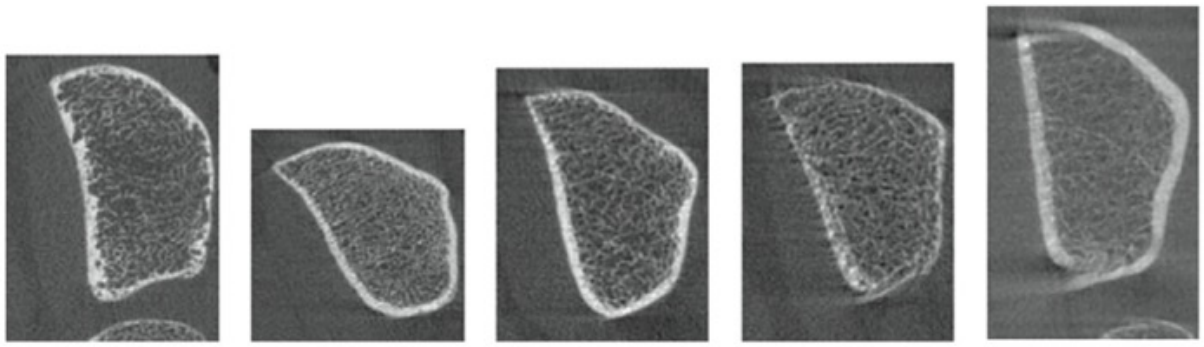


Figure 2.6. Distal radius scans illustrating motion artifact grading, ranging from 1 (no motion) on the left to 5 (large discontinuities) on the right. Reprinted from Pauchard et al.,[124] with permission from Elsevier.

Standard Protocol: The manufacturer's (Scanco) standard protocol separates cortical from trabecular bone using a semi-automated threshold based algorithm equivalent to 1/3 the apparent density of cortical bone.[325] This step requires hand drawn contours of the periosteal surface of the bone. I contoured all 110 slices for each scan and analyzed all HR-pQCT scans. I excluded the first three and last three slices from the analysis as per the manufacturer's protocol; thus, final values are based on 104 slices. The following parameters are directly measured from the standard analysis: Tt.BMD (mgHA/cm^3), Tb.BMD (mgHA/cm^3) and Tb.N (1/mm). Tb.N, the mean number of trabeculae per mm, is a truly 3D measure, and is calculated as the inverse of the mean spacing between the mid-axes of trabeculae. The following variables are derived from the standard analysis: BV/TV, Tb.Th (mm) and Tb.Sp (mm). BV/TV is calculated as:

$$\text{BV/TV} = \frac{\text{Tb.BMD}\ \left(\frac{\text{mgHA}}{\text{cm}^3}\right)}{1200\ \left(\frac{\text{mgHA}}{\text{cm}}\right)} \qquad \text{(Equation 1)}$$

It is not possible to directly measure Tb.Th because the HR-pQCT voxel size approximates the average thickness of individual trabeculae; thus, trabeculae would not be resolved at their actual thickness due to partial volume effects. Therefore, standard histomorphometry techniques are used to measure trabecular thickness as:[112]

$$\text{Tb.Th (mm)} = \frac{\text{BV/TV}}{\text{Tb.N}\left(\frac{1}{\text{mm}}\right)} \qquad \text{(Equation 2)}$$

Trabecular separation is also derived from the same measures as:

$$\text{Tb.Sp (mm)} = \frac{1-\text{BV/TV}}{\text{Tb.N}} \qquad \text{(Equation 3)}$$

Standard evaluation parameters of Tb.N, BV/TV and Tb.Sp are highly correlated with micro-CT measures of human cadaver bone at the distal tibia, r = 0.64-0.91[121] and radius, r = 0.59-0.96 in adults.[118] However, validity in the growing skeleton is currently unknown. Reproducibility in our lab is 0.2% (Tb.BMD) to 1.2% (Tb.Th) for all HR-pQCT acquired standard analysis measures at the tibia and radius (University of British Columbia Bone Health Research Group, unpublished data).

Automated Segmentation: The standard manufacturer's protocol performs well for quantifying trabecular microstructure; however, its utility is limited by its inability to accurately classify cortical bone. The standard threshold-based algorithm frequently mistakes thin or porous

cortical bone as trabecular bone and thick trabeculae for cortex.[112] A sophisticated dual-threshold automated-segmentation algorithm was developed to more accurately separate the cortical from trabecular compartment.[115] The automated-segmentation is a two-step protocol that first extracts the periosteal surface of the cortical shell (~170 mgHA/cm^3 threshold) and secondly extracts the endocortical surface of the cortical shell (~540 mgHA/cm^3) (Figure 2.5). Where the manufacturer's standard protocol would often 'clip' the endocortical surface of the bone by mistaking thin cortex for trabeculae or including thick trabeculae in the cortical shell, the automated segmentation algorithm more accurately identifies the endocortical surface.[115]

I applied the auto-segmentation algorithm and visually inspected all segmentations to ensure correct differentiation between cortex and trabeculae. The following variables are measured with the automated segmentation algorithm: Tt.Ar (total compartment CSA; mm^2), Ct.Po (as the number of void voxels within the cortex; %), Ct.BMD (apparent density of the cortex including all pore space; mgHA/cm^3), and Ct.Th (directly measured after removing intracortical pores; mm). Auto-segmentation parameters of Ct.Th and Ct.Po are highly correlated with micro-CT parameters in bone cadavers ($r = 0.80$ and $r = 0.98$, respectively).[116] The manufacturer now provides the automated segmentation algorithm as part of their standard HR-pQCT analysis software. As with the standard analysis, the validity of this algorithm for use in children and adolescents has yet to be confirmed.

Finite Element Analysis: Lastly, we applied a validated FEA to HR-pQCT images to estimate bone strength.[118] Post-doctoral fellow, Dr. Mikko Määttä, conducted the FEA, in consultation with me. We generated FE meshes from 3D HR-pQCT images using the voxel conversion approach.[326,327] We simulated uniaxial compression on each tibia section up to 1% strain using a single homogenous tissue modulus of 6829 MPa and a Poission's ratio of 0.3.[114] We used a custom FE solver (FAIM, Version 4.0, Numerics88Solutions, Calgary, Canada) on a desktop workstation (Mac Pro, OSX, Version 10.5.6, Apple Inc., Cupertino, CA, USA; 2 × 2.8 GHz Quad-Core Intel Xenon) to solve the FE models. FE outcomes were failure load (F.Load, N) and ultimate stress (U.Stress, MPa). We also calculated load-to-strength ratio of estimated fall load applied to the outstretched hand after a fall from standing height (simulation model that includes participant's height):[119,328]

$$\text{Fall Load (N)} = \frac{670\sqrt{2*9.81*\frac{height}{2}}}{10} \qquad \text{(Equation 4)}$$

670 is the damping coefficient (Ns/m), height = participant height (cm) and 9.81 = gravitational constant (m/s^2).

$$\text{Load-to-strength ratio } (\phi) = \frac{\text{Fall Load (N)}}{\text{Failure Load (N)}} \quad \text{(Equation 5)}$$

2.2.9 Statistical analysis

I this section I provide an overview of the statistical analyses performed in this thesis. I performed all analyses in Stata Version 12.1 (StataCorp, College Station, TX, USA). I visually inspected all data using histograms for continuous variables and dotplots for categorical variables. For cross-sectional analyses, I examined scatterplots to assess relationships between descriptive and predictor variables against bone parameters. For longitudinal analyses, I examined scatter plots of bone parameters against maturity offset for each participant. I used these plots to identify potential measurement errors or outliers. For each statistical model (cross-sectional and longitudinal), I examined model adequacy using histograms of residuals, residual vs. fit plots and residual vs. covariate plots.

In Chapter 3, I used multivariable linear regression to investigate cross-sectional associations between sedentary time and bone parameters during adolescence. In Chapters 4 and 5, I used general linear mixed models to evaluate maturity- and sex-related differences in bone parameters at the tibial midshaft and distal tibia and radius, respectively, across adolescence. In Chapter 6, I used general linear mixed models to examine longitudinal associations between PA, sedentary time and bone parameters across adolescence. In section 1.2.9.1, I provide an overview of the general linear mixed models used in Chapters 4-6. I provide a detailed description of the statistical analysis specific to each research aim within each research chapter.

2.2.9.1 General linear mixed models

Our longitudinal data are comprised of repeated measures of bone parameters that are unique to that individual and are related to each other. Thus, I used general linear mixed models, also known as multilevel models or random coefficients models, to allow each individual to have his or her own slope and intercept, just as in a summary measures approach. However, unlike the

summary measures approach, mixed models imposes conditions on the distribution of the coefficients.[329] Using mixed models allows for any pattern of repeated measures and estimates the coefficient for an individual as a weighted average of the average for the sample and the individual's ordinary least squares estimate. This means that the slope and intercept values calculated from the population data are used to pull or 'shrink', towards the grand mean, the slope and intercept values of those participants who had fewer data points and/or shorter time ranges between measurements.[329] By allowing for variation in both intercepts and slopes within individuals, growth velocities may vary between individuals. I used maturity offset centered at 0 as the time indicator. I provide an equation for a mixed model below:

Random linear maturity model, including the fixed effects of sex and ethnicity predicting the intercept and sex predicting the linear slope

$$\textit{Level 1: } y_{ti} = \beta_{0i} + \beta_{1i}MO_{ti} + \varepsilon_{ti}$$

$$\textit{Level 2: Intercept: } \beta_{0i} = \gamma_{00} + \gamma_{01}Boys_i + \gamma_{02}Ethnicity_i + \mu_{0i}$$

$$\textit{Linear time: } \beta_{1i} = \gamma_{10} + \gamma_{11}Boys_i + \mu_{1i}$$

$$\textit{Composite: } y_{ti} = [\gamma_{00} + \gamma_{10}MO_{ti} + \gamma_{01}Boys_i + \gamma_{11}MO_{ti}*Boys_i + \gamma_{02}Ethnicity_i] + [\mu_{0i} + \mu_{1i}MO_{ti} + \varepsilon_{ti}]$$

MO is maturity offset (centered at 0, APHV); Boys = 0, girl; 1, boy

Ethnicity= 0, Asian; 1, white; 2, other

where y_{ti} is the bone parameter on measurement occasion t in the i^{th} individual,

$(\mu_{0i}, \mu_{1i}) \sim N(0,\Sigma)$ is the vector of random effects for the i^{th} individual and

$\varepsilon_{ij} \sim N(0,\sigma^2)$ is the within-subject residual error.

Thus, the intercepts γ_{00}, $\gamma_{01}Boys_i$ and $\gamma_{02}Ethnicity_i$ represent the mean value of the bone parameter and the fixed effect of sex and ethnicity on the mean intercept of the bone parameter when maturity offset is zero, respectively, while μ_{0i} is the person-specific deviation from the mean intercept, assumed to follow a normal distribution with a mean of zero and variance of σ^2. The slopes γ_{10} and $\gamma_{11}Boys$ represent the fixed linear effect of maturity and the fixed effect of sex on linear maturity at APHV, respectively, while μ_{1i} is the person-specific deviation from the fixed linear effect of time. The slope for a given individual is $\gamma_{10} + \gamma_{11}Boys + \mu_{1i}$, such that it will be higher or lower than the overall slope, $\gamma_{10} + \gamma_{11}Boys$, by and amount μ_{1i}. I specified the covariance structure as unstructured to allow the random intercepts and slopes to covary.

Chapter 3: Bone Architecture and Strength in the Growing Skeleton: The Role of Sedentary Time

SYNOPSIS: We know little about the potentially deleterious effects of sedentary time on bone strength and its determinants during the key period of adolescent growth. In this chapter, I explore associations between sedentary time and bone strength and its determinants in children, adolescents and young adults. I present this chapter in its published format with minor modifications.[5]

3.1 Introduction

Today's youth spend close to sixty percent of their waking hours in sedentary activities.[248] The benefits of PA for bone health during childhood and adolescence are well established;[10,13] however, we know very little about the potentially deleterious effects of sedentary time on bone during these key periods of growth and development. Too much time spent sedentary is thought to negatively impact bone health by disrupting the balance between bone resorption and formation;[302] yet few studies have evaluated this phenomenon in healthy, ambulatory populations. Extreme examples of prolonged sedentary time, such as experienced during bed rest, highlight increases in rates of bone resorption without changes in rates of bone formation.[303] In healthy growing individuals, however, where bone formation predominates, it is unclear how sedentary time interacts with the osteogenic effects of PA.

A focus upon the potential consequences of *'not loading'* a healthy growing skeleton is relatively new and few studies have investigated the relationship between sedentary time and bone health (mass, structure, or strength) in children and adolescents, with contradictory findings.[297-299,330] During growth, the primary mechanical challenges driving bone adaptation are increases in bone length (a longer lever arm) and muscle force. These mechanical stimuli are modulated by hormones, nutrition and PA.[57] Thus, the landscape is complex and the

[5] A version of this chapter is published: Gabel L, McKay HA, Nettlefold L, Race D, Macdonald HM. Bone architecture and strength in the growing skeleton: the role of sedentary time. Med Sci Sports Exerc 2015;47(2):363–72.

independent influence and the interplay between factors warrants further investigation. Despite an abundance of cross-sectional and longitudinal studies that used DXA to assess BMC and aBMD during adolescent growth,[10,133,297-299] the discussion regarding the contribution of different key factors that influence bone strength, geometry, BMD and microarchitecture is relatively new.

Previous studies that investigated associations between sedentary time and bone health used 2D imaging technology (DXA) to assess BMC or aBMD.[297-299,330] As a planar instrument, DXA is unable to capture aspects of bone microarchitecture (cortical and trabecular mircroarchitecture) and bone geometry that contribute to bone strength.[41] While DXA studies considerably advanced our understanding of how bone adapts to PA,[10] the importance of bone strength for describing skeletal health has guided a paradigm shift away from 2D measures of bone mass to 3D measures of bone microarchitecture, geometry and BMD. With technological advances such as HR-pQCT (82 μm voxel size), we are now able to evaluate parameters previously only measured with invasive bone biopsies, such as trabecular and cortical bone microarchitecture,[110] including customized algorithms to assess cortical porosity.[115,116] We are also able to apply validated finite element analysis (FEA) techniques to HR-pQCT images to estimate bone strength[118] of the distal radius and distal tibia.

With the exception of the most recent report,[330] previous DXA studies of the sedentary behaviour-bone health relationship were also limited by their reliance on self-reported measures to quantify sedentary behavior[297-299] – techniques that are highly susceptible to recall bias.[229] Moreover, self-report questionnaires often assess only TV viewing or screen time, representing just one of many important sedentary behaviours. In contrast, accelerometers objectively measure total *volume* of sedentary time as well as *patterns* that reflect how sedentary time is accumulated (e.g., in short or long bouts). Recent literature indicates patterns of sedentary time negatively influence cardiometabolic health in adults independent of total sedentary time and MVPA;[241] however, it is unknown whether such patterns of sedentary time influence the growing skeleton.

To address the limitations of previous work, I used HR-pQCT to examine: i) whether self-reported screen time is associated with bone strength and its determinants independent of PA; ii) whether objectively measured volume and patterns of sedentary time is associated with bone strength and its determinants independent of MVPA and, iii) the contribution of muscle

force (using MCSA as a surrogate) and modulator variables (maturity, ethnicity, MVPA) to bone parameters. I hypothesized that screen time and sedentary time would be detrimentally associated with bone parameters. My secondary hypothesis was that MCSA would be the primary explanatory variable of tibial bone parameters, after adjusting for maturity status.

3.2 Methods

I provide a detailed description of study design and methods for data collection in Chapter 2 and a brief overview in the following sections.

3.2.1 Study design

Participants in this cross-sectional analysis were evaluated as part of the mixed longitudinal HBSIII study. For the current study, I included healthy children, adolescents and young adult boys (n = 159) and girls (n = 181) aged 9 to 20 years who were measured in 2009 (largest and youngest cross-sectional sample of HBSIII with HR-pQCT data). We considered participants white (n = 156) if both parents or 3 of 4 grandparents were born in North America or Europe, and Asian (n = 153) if both parents or 3 of 4 grandparents were born in Asia (i.e., Hong Kong, China, India, Philippines, Vietnam, Korea, or Taiwan). Thirty-one participants were classified as other (mixed ethnicity or other ethnic origins).

I excluded six participants who did not have an HR-pQCT scan due to concerns regarding exposure to radiation during recent clinical examinations, one participant who did not complete the maturity assessment, two participants with type 1 diabetes and three participants with diseases known to affect bone metabolism (Crohn's disease, fetal alcohol syndrome, osteogenesis imperfecta). No participants were taking medication known to affect bone metabolism. Thus, I included 154 boys and 174 girls in my analyses examining self-reported screen time. In analyses examining objectively measured sedentary time, I excluded 122 participants who did not meet accelerometry inclusion criteria (10 h/day on three or more days); thus, I included 89 boys and 117 girls.

3.2.2 Anthropometry, maturity and dietary calcium

We assessed height, body mass and tibia length using standard protocols. We assessed maturity using the method of Tanner (self-reported pubic hair stage) in boys[127] and in girls using self-reported menarcheal status and self-reported Tanner breast stage. All participants completed a validated food frequency questionnaire to estimate dietary intake of calcium (mg/day).[319]

We used pQCT (Norland/Stratec XCT 3000, Strate Medizintechnic GmbH, Pforzheim, Germany) to measure MCSA (mm^2) at the 50% site of the left tibia. This site primarily captures the soleus and gastrocnemius. We analyzed MCSA at this site using Contour mode 1 (-100 mg/cm^3), Peel mode 2 (40 mg/cm^3) and Cort mode 1 (710 mg/cm^3), as in our previous work.[151] One trained technician performed all measurements and analyses. A cone phantom was scanned daily during measurement to maintain quality assurance.

3.2.3 Sedentary time and physical activity

We estimated screen time using a self-report questionnaire and impact loading PA (impactPA, min/week) over the previous week in the full cohort using the modified PAQ-C or PAQ-A.[222,223]

We estimated objectively measured volume and patterns of sedentary time and MVPA using accelerometers with a 15-sec epoch. To control for differences in accelerometer wear time between participants, I estimated volume of sedentary time as a percentage of total accelerometer wear time (sedentary time (min/day)/average wear time (min/day)). I represented patterns of sedentary time as frequency of breaks in sedentary time (breaks/hr), calculated as average daily breaks[6] in sedentary time divided by hours of sedentary time, whereby more frequent breaks or interruptions in sedentary time is considered beneficial.[241]

[6] I defined a break in sedentary time as any time the accelerometer count rose above 100 cpm.

3.2.4 Bone microarchitecture, geometry, BMD and strength

We assessed bone microarchitecture, geometry, BMD and strength at the non-dominant tibia (8% site) using HR-pQCT (XtremeCT; Scanco Medical AG, Brüttisellen, Switzerland). If participants had a prior tibia fracture, we scanned the contralateral limb (n = 5). I evaluated all HR-pQCT images for motion artifacts. As motion artifacts were negligible (< 3 on scale from 1 to 5; Figure 2.6), I retained all HR-pQCT scans for analysis. I used system software and the manufacturer threshold-based algorithms to segment and evaluate all images,[325] and an automatic segmentation algorithm to segment the cortical and trabecular regions.[115] I report standard morphologic measures using standard manufacturer analysis including: Tt.BMD (mgHA/cm^3), BV/TV, Tb.N (1/mm) and Tb.Th (mm).

I used a validated automated segmentation algorithm[115,116] to determine Tt.Ar (mm^2) and the following cortical bone parameters: Ct.BMD (mg HA/cm^3), Ct.Po (%; number of void voxels within the cortex), and Ct.Th (mm; mean cortical volume divided by the outer bone surface). Finally, we applied a validated FEA[118] to HR-pQCT images to estimate F.Load (N).

3.2.5 Statistical analysis

I performed separate analyses for boys and girls due to known sex differences in bone accrual and in the tempo and timing of growth and maturation.[133,147] I compared descriptive and bone variables between the full cohort and the subsample with accelerometry data and between boys and girls using unpaired Student's t-tests for continuous variables and Chi-squared tests for categorical variables.

I fit univariate multivariable regression models to examine associations between sedentary time and bone parameters. Dependent variables included: trabecular bone microarchitecture (BV/TV, Tb.N, Tb.Th), cortical bone microarchitecture (Ct.Po, Ct.Th), geometry (Tt.Ar), density (Ct.BMD, Tt.BMD) and bone strength (F.Load). To assess my objectives, I developed four regression models. In Model 1, I evaluated associations between recreational self-reported screen time and bone parameters, controlling for MCSA (an estimate of muscle force), tibia length (an estimate of moment arm), maturity, ethnicity (Asian, white, other), dietary calcium and impactPA, as in previous work from our group.[151] I chose

menarcheal status as the maturity indicator for girls due to the strong relationship between age at menarche and the timing of peak bone mass accrual.[331] I subsequently developed two regression models using accelerometry-derived variables. In Model 2, I evaluated associations between objectively measured volume of sedentary time and bone parameters, controlling for MCSA, tibia length, maturity, ethnicity, dietary calcium and accelerometry-derived MVPA. In Model 3, I replaced objectively measured volume of sedentary time with patterns of sedentary time (i.e., breaks/h of sedentary time). In Model 4, I evaluated the contribution of MCSA, tibia length, maturity, ethnicity, dietary calcium and MVPA (by accelerometry) to bone parameters. I calculated the additional variance explained by each variable in the model (ΔR^2) by holding all other variables constant. I used histograms, quartile-quartile and scatter plots of residuals to assess normality, linearity and homoscedascity.

3.3 Results

3.3.1 Descriptive characteristics

I provide descriptive characteristics for participants in Table 3.1 and bone outcomes in Table 3.2. Descriptive characteristics, MVPA, sedentary time, and bone parameters were not significantly different between the full cohort and the subsample with accelerometry data. Boys were significantly older, taller, heavier, had longer tibias, greater MCSA, and engaged in significantly more self-reported impactPA and accelerometery-derived MVPA compared with girls ($p < 0.05$ for all). Self-reported screen time and accelerometry-derived sedentary time did not differ between boys and girls. Boys had greater BV/TV, Tb.N, Ct.Po, Ct.Th, Tt.Ar and F.Load compared with girls ($p < 0.05$ for all).

Self-reported screen time was positively correlated with accelerometry-derived volume of sedentary time (boys: r = 0.24, girls: r = 0.36, $p < 0.001$ for both) and negatively related to breaks in sedentary time (boys: r = -0.23, girls: r = -0.33, $p < 0.001$ for both). Accelerometry-derived volume of sedentary time was negatively correlated with breaks in sedentary time (boys: r = -0.93, girls: r = -0.96, $p < 0.001$ for both) and MVPA (boys: r = -0.50, girls: r = -0.54, $p < 0.001$ for both). Self-reported ImpactPA was positively correlated with accelerometry-derived MVPA (boys: r = 0.47, girls: r = 0.21, $p < 0.05$ for both).

Table 3.1. Descriptive characteristics and estimates of sedentary time for boys and girls in the full cohort and in the subsample with accelerometry data. Values are mean (SD) unless otherwise indicated.

	Boys			Girls		
	Full Cohort (n=154)	**Subsample (n=89)**	***P* value***	**Full Cohort (n=174)**	**Subsample (n=117)**	***P* value***
Age (years)	15.6 (3.3)	15.1 (3.3)	0.24	14.6 (3.9)	14.1 (3.9)	0.21
Tanner Stage (# 1/ 2-3 /4-5)	19/19/116	13/14/62	0.62	30/62/82	25/46/46	0.40
Menarcheal status (# pre/post)				72/102	55/62	0.34
Ethnicity (# Asian/white/other)	68/72/14	37/44/8	0.92	82/75/17	60/47/10	0.77
Height (cm)	167.8 (15.6)	166.4 (16.2)	0.51	154.4 (11.8)	152.5 (11.3)	0.17
Weight (kg)	61.7 (19.0)	58.2 (16.6)	0.14	50.0 (14.9)	47.6 (14.1)	0.25
Dietary calcium intake (mg/day)	1135 (762)	1158 (742)	0.82	1017 (631)	1039 (604)	0.77
Tibia length (mm)	406 (40)	404 (42)	0.68	369 (31)	365 (29)	0.31
MCSA (mm^2)	4668 (1129)	4466 (1042)	0.17	3862 (971)	3764 (960)	0.40
Self-reported variables						
Screen time (h/day)	2.9 (1.3)	2.8 (1.3)	0.99	2.6 (1.2)	2.6 (1.2)	0.98
Screen time guidelines (# <2h/≥2h)	70/84	42/47	0.79	86/88	59/58	0.87
Impact PA (min/week)	363 (324)	321 (304)	0.32	246 (269)	245 (269)	0.99
Accelerometer-derived variables						
Total wear time (min/day)		844.7 (70.3)			823.3 (65.2)	
Sedentary time (% of wear time)		68.5 (10.2)			69.5 (8.7)	
Breaks in sedentary time (# breaks/h sedentary time)		31.0 (11.9)			32.6 (10.8)	
MVPA (min/day)		57.8 (23.8)			41.3 (17.5)	

*Difference between the full cohort and the subsample with accelerometry data tested using unpaired Student's t-tests for continuous variables and Chi-squared tests for categorical variables

MCSA = muscle cross-sectional area; PA = physical activity; MVPA = moderate to vigorous physical activity.

Table 3.2. Bone parameters at the distal tibia assessed using high-resolution peripheral quantitative computerized tomography (HR-pQCT). Values are mean (SD).

	Boys			Girls		
	Full Cohort (n=154)	**Subsample (n=89)**	***P* value***	**Full Cohort (n=174)**	**Subsample (n=117)**	***P* value***
F.Load (N)	6387 (1824)	6053 (1790)	0.17	4878 (1375)	4715 (1322)	0.32
BV/TV	0.167 (0.026)	0.164 (0.025)	0.50	0.155 (0.025)	0.154 (0.025)	0.92
Tb.N (1/mm)	1.93 (0.29)	1.92 (0.27)	0.84	1.84 (0.25)	1.84 (0.27)	0.94
Tb.Th (mm)	0.088 (0.014)	0.087 (0.014)	0.61	0.085 (0.014)	0.085 (0.014)	0.94
Ct.Po (%)	5.8 (2.3)	5.8 (2.4)	0.97	4.4 (2.3)	4.6 (2.5)	0.40
Ct.Th (mm)	1.25 (0.36)	1.17 (0.35)	0.11	1.03 (0.31)	1.01 (0.31)	0.48
Ct.BMD (mg HA/cm^3)	781.7 (83.4)	768.4 (84.1)	0.23	786.7 (111.7)	773.0 (111.9)	0.30
Tt.BMD (mg HA/cm^3)	302.1 (59.0)	292.0 (58.7)	0.20	280.4 (61.9)	275.9 (62.9)	0.55
Tt.Ar (mm^2)	747.5 (135.3)	735.1 (136.2)	0.49	624.6 (94.1)	616.6 (86.6)	0.47

*Difference between the full cohort and the subsample with accelerometry data tested using unpaired Student's t-tests for continuous variables and Chi-squared tests for categorical variables.

F.Load = failure load; BV/TV = trabecular bone volume fraction; Tb.N = trabecular number; Tb.Th = trabecular thickness; Ct.Po = cortical porosity; Ct.Th = cortical thickness; Ct.BMD = cortical bone mineral density; Tt.BMD = total bone mineral density; Tt.Ar = total area.

Table 3.3. Unstandardized beta coefficients and model variances for multivariable regression analyses of bone parameters in boys. Beta coefficients ± standard error. Values in bold are significant at $p < 0.05$.

		Adj. R^2	MCSA (10^3)	Tibia Length (10^2)	Maturity[c]	Ethnicity[d]	Dietary Calcium (10^3)	Physical Activity (10^2)	Sedentary Time (10^2)
Model 1[a]	**BV/TV**	0.17	**0.007 ± 0.003**	**-0.018 ± 0.008**	**0.019 ± 0.009**	-0.002 ± 0.005	**0.006 ± 0.003**	0.074 ± 0.038	-0.076 ± 0.156
	Tb.N	0.24	**0.15 ± 0.03**	0.02 ± 0.08	**-0.40 ± 0.10**	0.01 ± 0.05	**0.07 ± 0.03**	0.58 ± 0.40	-0.44 ± 1.64
	Tb.Th	0.28	**-0.003 ± 0.001**	**-0.010 ± 0.004**	**0.028 ± 0.004**	-0.002 ± 0.002	0.0003 ± 0.001	0.007 ± 0.018	-0.003 ± 0.077
	Ct.Po	0.08	**0.10 ± 0.2**	1.0 ± 0.7	**-2.5 ± 0.9**	0.4 ± 0.4	0.2 ± 0.3	1.6 ± 3.6	-4.0 ± 14.8
	Ct.Th	0.53	**0.06 ± 0.03**	0.03 ± 0.08	**0.41 ± 0.09**	-0.07 ± 0.05	**0.07 ± 0.03**	**1.11 ± 0.39**	1.46 ± 1.63
	Ct.BMD	0.57	**13.9 ± 5.8**	-13.9 ± 17.7	**122.6 ± 21.1**	-14.0 ± 10.5	0.97 ± 6.69	94.1 ± 87.5	87.4 ± 362.2
	Tt.BMD	0.40	**12.0 ± 4.8**	**-29.4 ± 14.8**	**78.0 ± 17.6**	-9.9 ± 8.8	**12.8 ± 5.6***	**151.2 ± 73.0**	37.8 ± 302.3
	Tt.Ar	0.45	**49.1 ± 10.5**	**175.1 ± 32.2**	-57.2 ± 38.4	11.9 ± 19.2	-15.8 ± 12.1	285.9 ± 159.1	15.8 ± 65.8
	F.Load	0.69	**607 ± 106**	**889 ± 326**	**1331 ± 388**	-191 ± 194	132 ± 123	**6338 ± 1160**	3565 ± 6662
Model 2[b]	**BV/TV**	0.13	0.006 ± 0.004	-0.018 ± 0.010	0.022 ± 0.013	0.002 ± 0.006	0.007 ± 0.004	0.009 ± 0.014	-0.002 ± 0.047
	Tb.N	0.09	0.09 ± 0.05	0.06 ± 0.11	-0.24 ± 0.14	0.01 ± 0.06	0.05 ± 0.04	-0.08 ± 0.15	-0.55 ± 0.50
	Tb.Th	0.33	-0.001± 0.002	**-0.013 ± 0.005**	**0.023 ± 0.006**	0.001 ± 0.002	0.001 ± 0.002	0.010 ± 0.007	0.029 ± 0.023
	Ct.Po	0.13	0.06 ± 0.4	1.7 ± 1.0	**-3.9 ± 1.2**	0.2 ± 065	0.5 ± 0.4	**2.8 ± 1.3**	5.9 ± 4.4
	Ct.Th	0.50	0.05 ± 0.04	-0.01 ± 0.10	**0.42 ± 0.14**	-0.08 ± 0.06	0.08 ± 0.004	-0.02 ± 0.15	-0.08 ± 0.49
	Ct.BMD	0.60	10.1 ± 1.0	-27.4 ± 22.8	**165.0 ± 29.8**	-8.1 ± 13.8	-8.3 ± 8.6	**-67.3 ± 32.4**	-194.8 ± 105.6
	Tt.BMD	0.39	7.9 ± 8.3	-33.1 ± 19.7	**92.7 ± 25.7**	-4.5 ± 11.5	11.9 ± 7.4	-14.3 ± 28.0	-42.6 ± 91.3
	Tt.Ar	0.52	**67.7 ± 17.0**	**168.8 ± 40.5**	**-105.4 ± 52.7**	6.2 ± 23.5	-3.6 ± 15.2	**123.3 ± 57.4**	71.1 ± 187.2
	F.Load	0.68	**708 ± 184**	716 ± 437	**1200 ± 569**	-145 ± 254	192 ± 165	935 ± 620	-512 ± 2050

BV/TV=Trabecular bone volume fraction; Tb.N=Trabecular number (1/mm), Tb.Th=Trabecular thickness (mm); Ct.Po=Cortical porosity (%); Ct.BMD=Cortical bone mineral density (mg HA/cm^3); Tt.BMD=Total bone mineral density (mg HA/cm^3); Tt.Ar=Total area (mm^2); F.Load=Failure Load (N); MCSA=Muscle cross-sectional area (mm^2).

[a] model included MCSA, tibia length, maturity, ethnicity, dietary calcium, self-reported impactPA, self-reported screen time (n=154)

[b] model included MCSA, tibia length, maturity, ethnicity, dietary calcium, accelerometry-derived MVPA and sedentary time (n=89)

[c] Late-/post-pubertal (Tanner 4-5) in reference to pre-pubertal boys (Tanner 1). Coefficients for peri-pubertal (Tanner 2-3) vs pre-pubertal boys not significant and not shown.

[d] White in reference to Asian boys. Coefficients for Other vs. Asian boys not significant and not shown.

Table 3.4. Beta coefficients and model variances for multivariable regression analyses of bone parameters in girls. Beta coefficients ± standard error. Values in bold are significant at $p < 0.05$.

		Adj. R^2	MCSA (10^3)	Tibia Length (10^2)	Maturity	Ethnicity[c]	Dietary Calcium (10^3)	Physical Activity (10^2)	Sedentary Time (10^2)
Model 1[a]	**BV/TV**	0.24	**0.009 ± 0.002**	**-0.021 ± 0.001**	**0.017 ± 0.005**	0.005 ± 0.004	**0.008 ± 0.003**	0.051 ± 0.039	-0.121 ± 0.152
	Tb.N	0.21	**0.05 ± 0.02**	-0.05 ± 0.07	**-0.14 ± 0.05**	**0.12 ± 0.04**	**0.08 ± 0.03**	**0.94 ± 0.40**	-0.15 ± 1.54
	Tb.Th	0.29	-0.002 ± 0.001	**-0.010 ± 0.004**	**0.016 ± 0.003**	-0.003 ± 0.002	0.001 ± 0.002	-0.018 ± 0.021	-0.056 ± 0.083
	Ct.Po	0.45	**0.5 ± 0.2**	0.8 ± 0.5	**-3.6 ± 0.4**	0.2 ± 0.3	0.4 ± 0.2	-0.5 ± 3.0	-14.3 ± 11.6
	Ct.Th	0.67	**0.06 ± 0.02**	0.10 ± 0.06	**0.39 ± 0.04**	-0.03 ± 0.03	0.02 ± 0.02	0.16 ± 0.32	0.26 ± 1.23
	Ct.BMD	0.86	3.3 ± 4.6	23.2 ± 13.7	**192.5 ± 9.9**	1.5 ± 7.3	0.8 ± 5.5	-27.8 ± 74.9	432.9 ± 289.2
	Tt.BMD	0.61	**13.1 ± 4.2**	-21.0 ± 12.6	**89.9 ± 9.1**	2.6 ± 6.7	9.8 ± 5.0	50.0 ± 68.8	10.7 ± 265.7
	Tt.Ar	0.31	**39.8 ± 8.5**	**129.8 ± 25.5**	**-60.1 ± 18.4**	21.5 ± 13.6	-1.2 ± 10.2	205.1 ± 139.7	-52.6 ± 539.3
	F.Load	0.71	**526 ± 81**	**851 ± 241**	**1195 ± 174**	82 ± 129	146 ± 97	2636 ± 1318	1375 ± 5089
Model 2[b]	**BV/TV**	0.22	**0.010 ± 0.003**	-0.015 ± 0.009	0.011 ± 0.007	0.002 ± 0.005	**0.008 ± 0.004**	0.026 ± 0.015	0.007 ± 0.043
	Tb.N	0.18	**0.09 ± 0.03**	-0.12 ± 0.10	-0.09 ± 0.08	**0.13 ± 0.05**	**0.10 ± 0.04**	-0.20 ± 0.17	-0.74 ± 0.47
	Tb.Th	0.32	0.002 ± 0.002	-0.003 ± 0.005	**0.011 ± 0.004**	**-0.005 ± 0.003**	0.001 ± 0.002	**0.024± 0.008**	0.034 ± 0.023
	Ct.Po	0.46	**0.8 ± 0.3**	0.3 ± 0.8	**-3.7 ± 0.6**	-0.2 ± 0.4	0.2 ± 0.3	0.002 ± 1.3	-4.6 ± 3.5
	Ct.Th	0.67	**0.09 ± 0.02**	0.03± 0.07	**0.35 ± 0.06**	-0.02 ± 0.04	0.01 ± 0.03	0.24 ± 0.12	0.07 ± 0.34
	Ct.BMD	0.83	6.5 ± 6.4	10.7 ± 19.3	**181.5 ± 14.9**	6.6 ± 10.1	0.1 ± 7.5	16.2 ± 31.7	94.9 ± 89.2
	Tt.BMD	0.60	**17.1 ± 5.5**	-22.2 ± 16.7	**79.7 ± 12.8**	2.3 ± 8.7	9.8 ± 6.5	**54.3 ± 27.3**	40.4 ± 76.9
	Tt.Ar	0.36	**46.7 ± 9.6**	**131.2 ± 29.2**	**-94.7 ± 22.5**	16.2 ± 15.3	3.2 ± 11.4	-34.7 ± 47.9	97.1 ± 134.7
	F.Load	0.74	**642 ± 93**	**843 ± 284**	**809 ± 218**	27 ± 148	200 ± 111	**939 ± 465**	1550 ± 1308

BV/TV=Trabecular bone volume fraction; Tb.N=Trabecular number (1/mm), Tb.Th=Trabecular thickness (mm); Ct.Po=Cortical porosity (%); Ct.BMD=Cortical bone mineral density (mg HA/cm^3); Tt.BMD=Total bone mineral density (mg HA/cm^3); Tt.Ar=Total area (mm^2); F.Load=Failure Load (N); MCSA=Muscle cross-sectional area (mm^2).

[a] model included MCSA, tibia length, maturity, ethnicity, dietary calcium, self-reported impactPA, self-reported screen time (n=174)

[b] model included MCSA, tibia length, maturity, ethnicity, dietary calcium, accelerometry-derived MVPA and sedentary time (n=117)

[c] White in reference to Asian girls. Coefficients for Other vs. Asian girls not significant and not shown.

Table 3.5. Beta coefficients and model variances for multivariable regression analyses of bone parameters in boys and girls (Model 3). Beta coefficients ± standard error.

		Adj. R^2	MCSA (10^3)	Tibia Length (10^2)	Maturity[a]	Ethnicity[b]	Dietary Calcium (10^3)	Physical Activity (10^2)	Sedentary Breaks (10^2)
Boys	**BV/TV**	0.13	0.006 ± 0.004	-0.017 ± 0.010	0.024 ± 0.013	0.002 ± 0.006	0.006 ± 0.004	0.008 ± 0.012	0.010 ± 0.035
	Tb.N	0.17	0.09 ± 0.05	0.06 ± 0.11	-0.27 ± 0.14	0.01 ± 0.06	0.04 ± 0.04	-0.01 ± 0.12	0.32 ± 0.38
	Tb.Th	0.33	-0.001 ± 0.021	**-0.012 ± 0.005**	**0.025 ± 0.006**	0.001 ± 0.003	0.001 ± 0.002	0.006 ± 0.006	-0.012 ± 0.017
	Ct.Po	0.11	0.01 ± 0.4	1.8 ± 1.0	**-3.3 ± 1.2**	0.1 ± 0.6	0.5 ± 0.4	1.9 ± 1.1	-2.0 ± 3.3
	Ct.Th	0.50	0.05 ± 0.04	0.01 ± 0.11	**0.41 ± 0.13**	-0.07 ± 0.06	0.08 ± 0.04	-0.06 ± 0.12	-0.01 ± 0.37
	Ct.BMD	0.59	11.9 ± 9.7	-29.5 ± 23.2	**149.2 ± 29.4**	-5.6 ± 13.4	-7.9 ± 8.8	-38.0 ± 27.2	74.2 ± 80.7
	Tt.BMD	0.39	8.2 ± 8.3	-33.4 ± 19.7	**90.2 ± 25.1**	-4.1 ± 11.4	11.8 ± 7.5	-8.4 ± 23.2	21.5 ± 68.7
	Tt.Ar	0.52	**67.2 ± 17.0**	**169.4 ± 40.5**	-101.0 ± 51.3	5.4 ± 23.4	-3.6 ± 15.4	**113.3 ± 47.5**	-34.4 ± 140.8
	F.Load	0.68	**704 ± 183**	720 ± 436	**1224 ± 553**	-145 ± 252	186 ± 166	977 ± 512	570 ± 1517
Girls	**BV/TV**	0.22	**0.010 ± 0.003**	-0.015 ± 0.009	0.011 ± 0.007	0.002 ± 0.005	0.009 ± 0.004	0.026 ± 0.013	-0.010 ± 0.030
	Tb.N	0.17	**0.08 ± 0.03**	-0.11 ± 0.10	-0.13 ± 0.08	**0.13 ± 0.05**	**0.09 ± 0.04**	-0.09 ± 0.15	0.28 ± 0.33
	Tb.Th	0.32	0.002 ± 0.002	-0.003 ± 0.005	**0.012 ± 0.004**	**-0.005 ± 0.003**	0.001 ± 0.002	**0.019 ± 0.007***	-0.016 ± 0.016
	Ct.Po	0.45	**0.8 ± 0.3**	0.4 ± 0.8	**-3.8 ± 0.6**	-0.2 ± 0.4	0.1 ± 0.2	0.5 ± 1.1	2.8 ± 2.5
	Ct.Th	0.67	**0.09 ± 0.02**	0.02 ± 0.07	**0.36 ± 0.05**	-0.02 ± 0.04	0.01 ± 0.03	**0.23 ± 0.11**	-0.02 ± 0.24
	Ct.BMD	0.83	6.8 ± 6.4	9.4 ± 19.3	**184.3 ± 14.3**	6.1 ± 10.1	0.3 ± 7.6	5.0 ± 27.6	-53.4 ± 62.8
	Tt.BMD	0.60	**17.2 ± 5.5**	-22.8 ± 16.6	**80.5 ± 12.3**	2.2 ± 8.7	9.8 ± 6.4	**50.1 ± 23.7**	-26.1 ± 54.0
	Tt.Ar	0.36	**465.8 ± 9.6**	**129.9 ± 29.1**	**-95.2 ± 21.5**	16.5 ± 15.2	3.5 ± 11.4	-41.2 ± 41.5	-84.6 ± 94.6
	F.Load	0.74	**645 ± 93**	**821 ± 283**	**837 ± 209**	23 ± 148	204 ± 111	782 ± 404	-1030 ± 920

BV/TV=Trabecular bone volume fraction; Tb.N=Trabecular number (1/mm), Tb.Th=Trabecular thickness (mm); Ct.Po=Cortical porosity (%); Ct.BMD=Cortical bone mineral density (mg HA/cm^3); Tt.BMD=Total bone mineral density (mg HA/cm^3); Tt.Ar=Total area (mm^2); F.Load=Failure Load (N); MCSA=Muscle cross-sectional area (mm^2).

Model included MCSA, tibia length, maturity, ethnicity, dietary calcium, accelerometry-derived MVPA and breaks in sedentary time

[a] Late-/post-pubertal (Tanner 4-5) in reference to pre-pubertal boys (Tanner 1). Coefficients for peri-pubertal (Tanner 2-3) vs pre-pubertal boys not significant and not shown. Post-menarcheal in reference to pre-menarcheal girls.

[b] White in reference to Asian participants. Coefficients for Other vs. Asian participants not significant and not shown

3.3.2 Screen time and bone parameters

Self-reported screen time did not predict bone parameters in boys or girls, adjusting for MCSA, tibia length, maturity, ethnicity, dietary calcium and impactPA (Model 1, Table 3.3 and Table 3.4). Similarly, bone parameters were not associated with meeting or not meeting screen time guidelines.

3.3.3 Objectively measured sedentary time and bone parameters

Volume of sedentary time did not predict bone parameters in boys or girls, adjusting for MCSA, tibia length, maturity, ethnicity, dietary calcium and MVPA (Model 2, Figure 3.1 and Table 3.3 and Table 3.4). Likewise, breaks in sedentary time did not predict bone parameters in boys or girls (Model 3, see Table 3.5).

3.3.4 Factors that influence bone parameters

Figure 3.2 displays the additional contribution of each variable in Model 4 (MCSA, tibia length, maturity, ethnicity, dietary calcium and MVPA) to bone parameters. With the exception of BV/TV in boys ($p = 0.053$), I observed a strong relationship between maturity and bone strength and its determinants in boys and girls, explaining an additional 3-35% of the variance in multivariable models. MCSA accounted for an additional 4-14% of the variance in Tb.N, Tt.Ar and F.Load in boys and girls and in BV/TV, Ct.Po, Ct.Th and Tt.BMD in girls. Tibia length was a significant predictor of Tt.Ar in boys and girls, Tb.Th in boys only and F.Load in girls only, explaining an additional 2-11% of the variance in multivariable models. White girls had significantly greater Tb.N compared with Asian girls; ethnicity accounted for an additional 5% of the variance in Tb.N in girls. Dietary calcium explained an additional 4% of the variance in BV/TV and Tb.N in girls. MVPA explained an additional 2-4% of the variance in Tt.Ar and F.Load in boys and BV/TV, Tb.Th, Ct.Th and Tt.BMD in girls. MVPA was also a weak, but non-significant predictor of F.Load ($p = 0.10$) in girls.

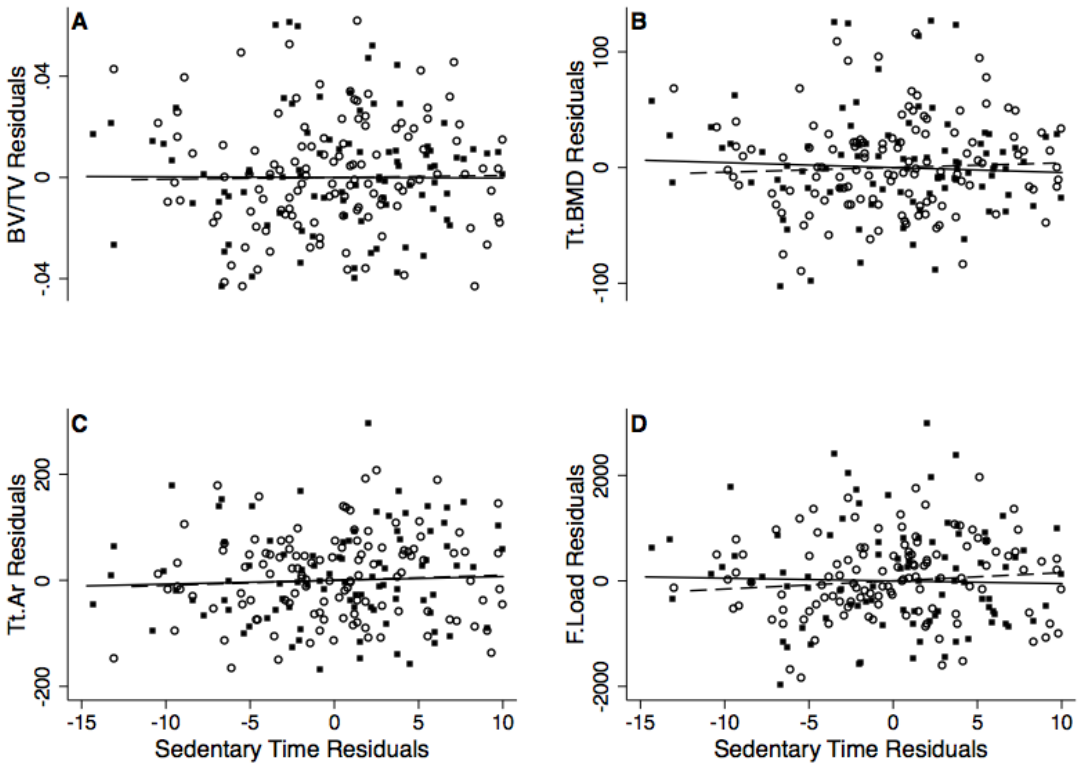


Figure 3.1. Scatterplots of sedentary time (as a % of wear time) regression residuals and bone architecture, BMD and strength regression residuals. Boys are represented by black squares and solid lines; girls are represented by open circles and dashed lines. (A) trabecular bone volume fraction (BV/TV), (B) total bone mineral density (Tt.BMD, mg HA/cm^3), (C) total area (Tt.Ar, mm^2), (D) failure load (F.Load, N).

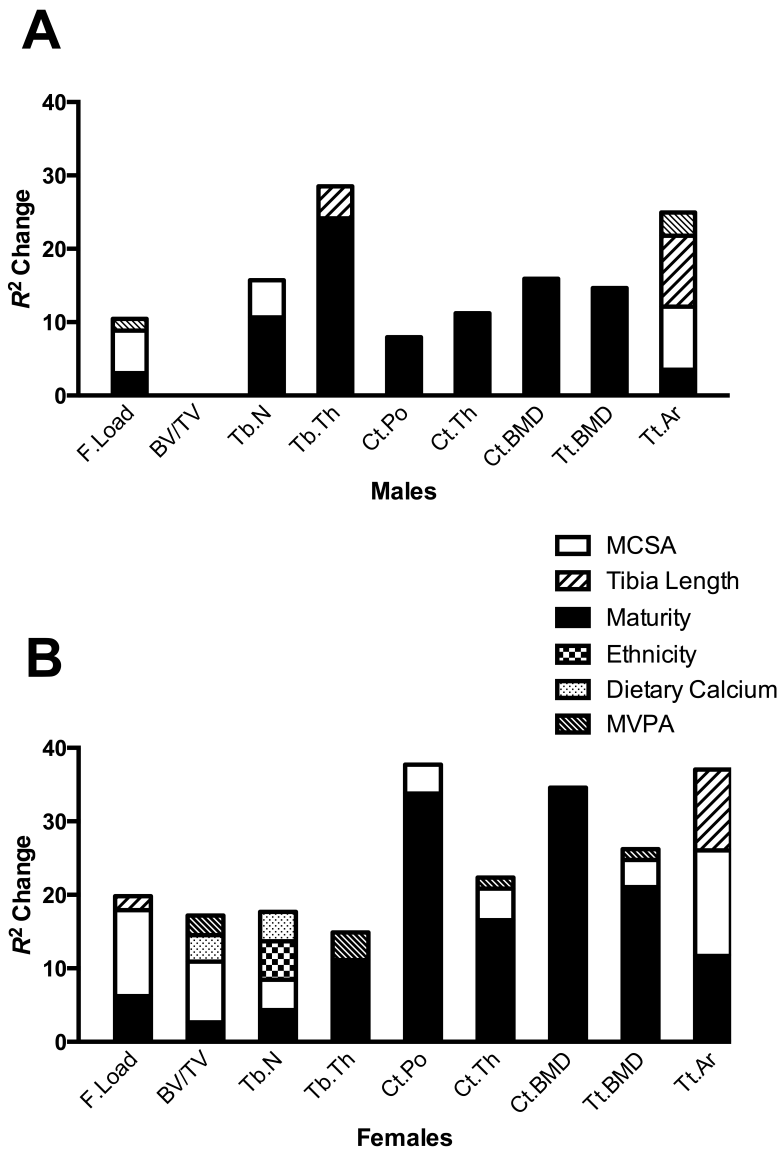


Figure 3.2. Contribution of muscle cross-sectional area (MCSA), tibia length, maturity, ethnicity, dietary calcium and accelerometry-derived moderate-to-vigorous physical activity (MVPA) to the prediction of bone architecture, BMD and bone strength in Model 4 in A) boys and B) girls (n = 206). For example, the solid black bar represents the additional variance in bone outcomes explained by maturity when MCSA, tibia length, ethnicity, dietary calcium and MVPA are held constant. F.Load = failure load; BV/TV = trabecular bone volume fraction; Tb.N = trabecular number; Tb.Th = trabecular thickness; Ct.Po = cortical porosity; Ct.Th = cortical thickness; Ct.BMD = cortical bone mineral density; Tt.BMD = total bone mineral density; Tt.Ar = total area.

3.4 Discussion

This study makes several unique contributions to the body of literature that evaluates the association between sedentary time and bone health in children and youth. First, I built upon current literature[297-299,330] with the use of a novel imaging tool, HR-pQCT, and evaluated Ct.Po and estimated bone strength using advanced analysis techniques. Second, I used accelerometers to objectively measure volume and patterns of sedentary time in addition to self-reported screen time. In contrast to my hypotheses, sedentary time, whether by self-report or accelerometry, did not predict bone bone parameters, in either boys or girls.

I did not observe an association between self-reported screen time or meeting screen time guidelines and bone parameters. This observation confirms findings from a recent DXA-based study[297] that found no relationship between whole body BMC and screen time in adolescent girls after adjusting for maturity, height and lean mass. On the other hand, Gracio-Marco and colleagues observed a negative relationship in 12-17 year old boys between whole body BMC and Internet use for non-study purposes after adjusting for lean mass and MVPA, but not for TV viewing or computer/video game use.[297] It is unclear why such behaviour was only related to whole body BMC in boys and how Internet use for non-study purposes would differentially influence bone mass compared with other sedentary activities. My results also contrast those of Chastin and colleagues who identified a negative association between TV watching and BMC at the proximal femur in 8-22 year old boys and girls and lumbar spine BMC in girls, after adjusting for MVPA.[330] In addition, proximal femur BMC was negatively related to total self-reported screen time, but not computer time, in girls.[330] However, these results must be interpreted with caution as authors failed to account for body size or maturation in their analyses, both primary determinants of BMC.[147]

It is important to highlight that high engagement in sedentary activities does not preclude participation in PA. The challenge for sedentary behaviour researchers is discerning the independent contribution of each (sedentary time and PA) to selected outcomes, as they share a significant amount of variance. Therefore, it is important to adjust for PA to determine the unique contribution of sedentary time. For example, Vicente-Rodriguez and colleagues observed significantly higher odds for having low total body BMC (< 10^{th} percentile for z-score) in 13-19 year old boys who spent > 3 h/day watching TV; however, these results did not persist after

adjusting for PA.[298] As intervention studies demonstrate that very short bouts of weight-bearing PA elicit an osteogenic response,[10,13] the potentially deleterious effect of large amounts of sedentary time on bone health may theoretically be counteracted through small bouts of weight-bearing PA.

As with screen time, I did not discern a negative association between objectively measured volume of sedentary time and bone parameters in healthy children and youth. Similarly, patterns of sedentary time were not associated with any bone parameters. Although this is the first study to examine the relationship between accelerometry-derived sedentary time and bone outcomes by HR-pQCT, my findings are in contrast to the only DXA-based study that assessed sedentary time with accelerometry[330] as well as studies that assessed other health measures in youth and adults.[332,333] In particular, Chastin et al., identified a positive association between accelerometry-derived sedentary time and BMC at the spine and proximal femur in boys and girls (aged 8-22 years) after adjusting for MVPA,[330] suggesting that sedentary time might be beneficial for skeletal health. Nonetheless, these results must be interpreted with caution since body size and maturity were not accounted for -- two variables of considerable importance in such a broad cohort. Failure to adjust for these important determinants may confound the sedentary time-BMC relationship. With respect to other health measures, a relatively recent body of literature reported independent effects of objectively-measured sedentary time (after adjustment for MVPA) on several chronic disease risk factors including adiposity and insulin sensitivity in youth.[332,333] Further, the examination of specific patterns of sedentary time has piqued research interest, as studies in adults demonstrated prolonged bouts of sedentary time were negatively associated with cardiometabolic health, independent of total sedentary time and MVPA.[241] Nevertheless, as the potential mechanisms driving the detrimental relationship between a sedentary lifestyle and cardiometabolic health are much different than those for bone health,[302] it is not entirely surprising that my results are discordant with those conducted in other areas of health research.

To reiterate, although participants in the current study spent most of their waking hours (69% of the day) in sedentary pursuits, they still experienced significant mechanical loading as indicated by daily MVPA comparable to other Canadian youth (58 vs. 53 min/day in adolescent boys and 41 vs. 39 min/day in adolescent girls).[248] Further, adaptation of bone to PA is site-specific and varies based on strain type, magnitude and location along the length of the bone.[257]

Thus, our measurement site may have influenced our ability to detect an effect of sedentary time on bone outcomes. Specifically, we assessed a weight-bearing site (the distal tibia) that experiences mechanical loads as a consequence of normal ambulation. Therefore, this site may be less sensitive to all but very high volumes of sedentary time or prolonged periods of non-weight bearing such as during bed rest.[303]

During growth, muscle forces and increasing bone length present the most significant mechanical challenges for bone.[57] To maintain structural integrity, bone must adapt both its mineral properties and architecture. Thus, it is not surprising that both MCSA, a surrogate of muscle force, and tibia length, an estimate of moment arm, predicted bone geometry and BMD in boys and girls. The distal end of the tibia, in particular, experiences high axial compressive forces through most of stance primarily due to internal muscle forces.[334] Accordingly, it appears that some elements of bone microarchitecture adapt to these internal mechanical forces.

Maturity-related changes in bone microarchitecture, geometry and BMD are well documented in the literature,[4,150] and explained the majority of the variance in bone parameters in the present study. Although not the focus of my study, I included ethnicity as a covariate in my models based on known differences in bone architecture between Asian and white adolescents.[151,186] I noted apparent ethnic differences in trabecular microarchitecture in girls, such that Asian girls had a smaller number of trabeculae. Similar trends were previously observed at the distal radius in this cohort[186] and were also demonstrated in adult women.[335] Finally, my study provides additional support that modulators of the functional model of bone development, such as dietary calcium and MVPA, are weak but significant predictors of bone parameters during childhood, adolescence and young adulthood.[13,109]

My study reaffirms a strong role for maturity and surrogates of muscle force as predictors of bone strength. These findings are consistent with our earlier work[150,151] and the functional model of bone development, which proposes that both muscle forces and longitudinal growth are the primary drivers of bone adaptation.[57] Although MVPA was only a statistically significant predictor of bone strength in boys, I observed the same trend in girls. We observed similar sex-specific findings previously,[109] and speculate that the strength of the relationship between MVPA and bone strength in girls, or lack thereof, may simply reflect their relatively low engagement in MVPA.

I acknowledge several limitations of my study. First, based on the cross-sectional design, I cannot infer any causal relationships between sedentary time and bone parameters. Second, I cannot extend my results to the general population due to our convenience sampling methods. Third, I recognize the limitations associated with self-assessment of maturity status. I attempted to overcome this limitation by using menarcheal status in girls; however, more accurate measures of maturity (i.e., APHV) should be considered in future prospective studies. Fourth, limited sample size precluded stratification of analyses by maturity group. Given the notion of potential 'windows of opportunity' for bone development,[144] examination of maturity-specific responses is warranted in future work. Finally, uniaxial accelerometers were not designed specifically for measuring ground reaction forces, nor can they distinguish between different postures, such as sitting, standing, and lying down. Ground reaction forces are strongly associated with accelerometry output,[219] and thus, accelerometers are proposed as an appropriate tool for estimating mechanical loads associated with weight-bearing PA. However, I acknowledge that some PA may have been misclassified and I may have included some standing time in my estimates of sedentary time.

3.5 Conclusions

I provide new evidence that self-reported screen time and objectively measured sedentary time (volume and patterns) are not associated with tibial bone parameters in a sample of children, adolescents and young adults. Longitudinal studies are needed to clarify whether there is an independent influence of sedentary time on bone strength and its determinants across longer periods of growth and development and if this relationship is moderated by maturity status. The field of pediatric bone health would also benefit from studies that evaluate bone parameters at more clinically relevant sites such as the distal radius (using HR-pQCT) or at the spine and hip using other imaging modalities. Based on previous work highlighting the benefits of PA for bone health,[10,13] strategies designed to increase PA may be more beneficial for bone health during youth and young adulthood than strategies aimed at reducing sedentary time; however, targeted RCTs are needed to confirm this. That said, approaches that counter the alarming increase in sedentary time during childhood and adolescence[248] might concurrently increase PA, thereby justifying a multi-pronged approach.

Chapter 4: Re-examining the Surfaces of Bone in Boys and Girls During Adolescent Growth: A 12-year Mixed Longitudinal pQCT Study

SYNOPSIS: The specifics of how bone is gained during childhood and adolescence are not completely understood. In this chapter, I evaluate the accrual of bone on the periosteal and endocortical surfaces of the tibia and compares rates of accrual between boys and girls across 12 years. I present this chapter in its published format with minor modifications.[7]

4.1 Introduction

Childhood and adolescence are critical periods for bone mineral accrual.[133,312,336,337] However, the intricacies of how bone is gained (in childhood) and lost (in later life) are still not completely understood. In the 1960s and 1970s, Garn and colleagues examined surface-specific changes that accompany bone growth and development.[155-157] They conducted cross-sectional radiographic studies of the second metacarpal and concluded that both boys and girls exhibit endocortical (innermost cortical surface) contraction and periosteal (outer cortical surface) expansion within the diaphysis of the second metacarpal during adolescent growth, but that girls experienced more endocortical contraction compared with boys and that boys experienced more periosteal expansion compared with girls.[155-157] Others proposed that this sexual dimorphism contributes to increased bone fragility in women compared with men in older adulthood.[152,338]

The pioneering studies of Garn and colleagues advanced our understanding of sex differences in bone development. However, this early work was limited by the use of planar radiographs to evaluate bone surfaces and caution must be applied when generalizing Garn's theory to all skeletal sites, as his radiographic studies focused on the non-weight bearing metacarpals. The advent of pQCT permits scrutiny of the commonly held tenets regarding bone apposition and resorption on surfaces of growing bone. In a 20-month longitudinal study of boys and girls (10-13 years at baseline) using pQCT, we did not observe endocortical bone contraction

[7] A version of this chapter is published: Gabel L, Nettlefold L, Brasher PM, Moore SA, Ahamed Y, Macdonald HM, et al. Reexamining the surfaces of bone in boys and girls during adolescent growth: a 12-year mixed longitudinal pQCT study. J Bone Miner Res 2015;30(12):2158–67.

or expansion in girls (assessed as the area of the medullary canal (Me.Ar)) at the tibial midshaft.[153] Similarly, boys did not demonstrate endocortical contraction, but compared with girls, boys displayed significant endocortical expansion.[153] In contrast, a 2-year longitudinal study of girls (10-13 years at baseline) reported both periosteal expansion and endocortical contraction at the tibia shaft after menarche.[158] The contradictory findings of endocortical contraction in the studies by Garn et al.[155-157] and Wang et al. [158] compared with the absence of endocortical contraction in the study by Kontulainen et al.[153] have not been explored in a longitudinal study that spans a longer period of adolescent growth.

Garn and colleagues also did not control for maturational status in their cross-sectional studies. Failure to consider the tremendous variation in maturational status of children at the same chronological age can dramatically affect outcomes of cross-sectional and intervention studies.[125] APHV is most commonly used as an indicator of somatic maturity in longitudinal studies of childhood and adolescent growth[125,137] and is highly correlated with sexual maturation.[137,339] APHV refers to the age when maximum linear growth in height occurs and generally occurs in boys and girls at a maturational time point when approximately 90% of adult stature has been achieved.[134] Serial measurements surrounding maximal height velocity are required to determine APHV.

Therefore, I aimed to advance the classic studies of Garn and colleagues by evaluating the sex- and surface-specific pattern of bone accrual on the periosteal and endocortical surfaces of the weight bearing mid-tibia in boys and girls, aligned on maturity offset. My objectives were to: 1) compare rates of bone expansion and/or contraction at the periosteal and endocortical surfaces of the tibia and 2) compare rates of cortical bone density and bone strength accrual at the tibia, between boys and girls pre- and post-APHV. This current study extends our previous 20-month pQCT study to 12 years to further evaluate these bone surface-specific events.

4.2 Methods

I provide a detailed description of study design and methods for data collection in Chapter 2 and a brief overview in the following sections.

4.2.1 Study design

Participants were drawn from a cohort of healthy girls (n = 556) and boys (n = 515) aged 8 to 12 years at baseline who comprised the University of British Columbia HBSIII (Figure 4.1). In the present analysis, I included bone data from annual measurements conducted between May 2001 (first year of pQCT measurements) and June 2012. Of the full cohort (n = 1071) there were a median of 3 annual measurements (interquartile range: 2 to 7). I excluded observations from children actively participating in an intervention (n = 451, spring 2004)[265] as we previously demonstrated a positive effect of a PA intervention on bone accrual.[258,259] However, I included all additional follow-up measurements (2005-2011) regardless of group assignment, as participation in an exercise intervention was not associated with sustained benefits at the tibial shaft.[317] Specifically, I explored the influence of group assignment on bone parameters from 2005-2011; since group assignment was not a statistically significant predictor of bone parameters (Appendix E, Table E.1), the data for exercise and control groups were pooled. For the purpose of this analysis, I refer to data obtained at the first pQCT measurement as baseline.

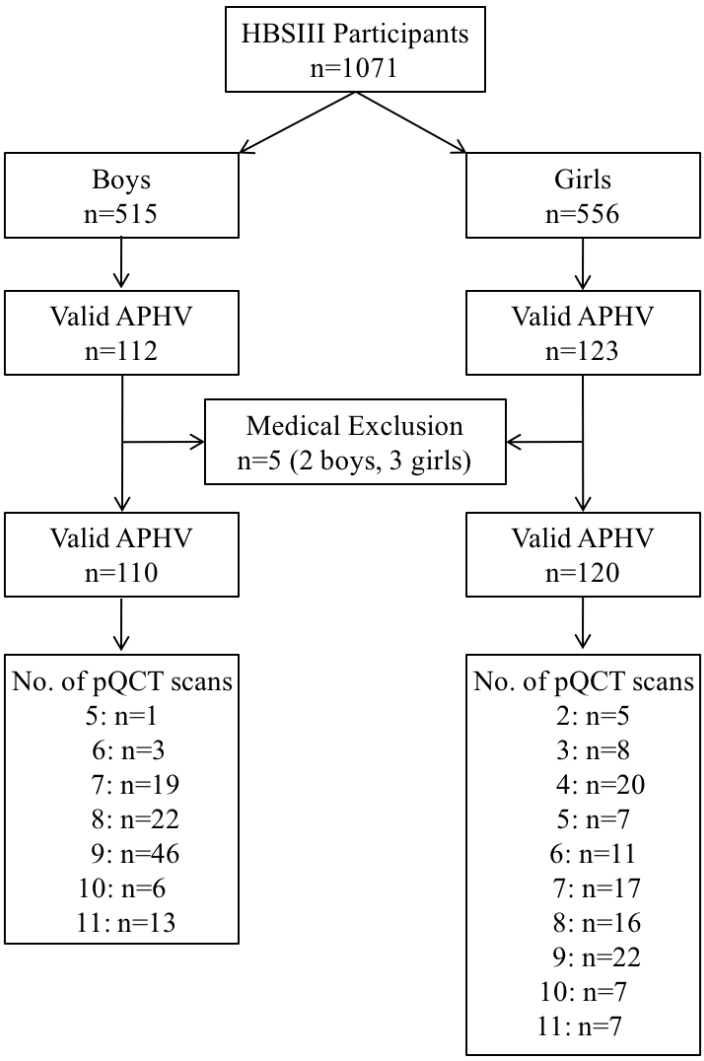


Figure 4.1. Number of participants recruited and the number of valid peripheral quantitative computed tomography (pQCT) follow-up scans for boys and girls

4.2.2 Anthropometry and age at peak height velocity

We assessed standing and sitting height, body mass and tibia length using standard methods. To control for well-known maturational differences between adolescent boys and girls of the same chronological age, we calculated years from APHV as an estimate of biological maturity. I provide a detailed description of this process in Appendix D. Due to missing and mistimed measurements surrounding APHV, we were able to identify APHV for 235 of the 1071 participants (112 boys, 123 girls). I used APHV to calculate a biological maturity offset (in years) by subtracting the APHV from chronological age at time of measurement. Thus, I

generated a continuous measure of biological age (e.g., -1 year is equivalent to 1 year prior to attainment of APHV; +1 to one year after APHV).

4.2.3 Healthy history and dietary calcium

I identified medical exclusions and ethnicity using a health history questionnaire. Based on questionnaire responses, I identified five participants who had conditions that prevented their participation in regular PA and/or reported medical conditions known to influence bone metabolism (osteogenesis imperfecta, fetal alcohol syndrome, type 1 diabetes, leukemia, congenital heart defect). I excluded these participants from my analysis. Thus, I included 230 healthy participants (110 boys, 120 girls) in my analysis. We assessed dietary calcium intake (mg/day) using a validated food-frequency questionnaire.[319] We assessed leisure-time PA using the modified PAQ-C or PAQ-A[222,223] and calculated a general PA score as the average of the PAQ items in a range between 1 (low activity) and 5 (high activity), MVPA (min/day) and loaded PA (impact > walking; h/week).

4.2.4 Bone geometry, density and strength

We assessed bone geometry, density and strength at the left tibia (50% site) using pQCT (Norland/Stratec, Medizintechnic GmbH). I acquired and analyzed all pQCT scans in 2012. My outcome variables were Tt.Ar (mm^2) to assess change on the periosteal surface; Ct.Ar (mm^2) to evaluate change in cross-sectional area between the periosteum and endocortical surfaces; the ratio of Ct.Ar to Tt.Ar (Ct.Ar/Tt.Ar) to assess change in cortical thickness; medullary canal area (Me.Ar, mm^2) to assess change on the endocortical (inner) surface, calculated as Ct.Ar subtracted from Tt.Ar; Ct.BMD (mg/cm^3) and SSI_p (mm^3), an estimate of torsional bone strength.

We analyzed all scans using Stratec software version 6.0. I analyzed all scans in 2012. I included all participants with at least one pQCT scan. I excluded all scans with motion artifacts (n = 9 across 12-years; Appendix E, Figure E.1).

4.2.5 Data cleaning

Prior to modeling data, I examined scatter plots for bone area, density and strength against maturity offset for each participant. I specifically looked for negative changes in anthropometry and/or bone parameters – known to occur due to slight differences in positioning, limb length measures or other measurement error. I identified potential measurement errors using the following protocol. A negative change in height during childhood and adolescence would likely represent measurement error, as would a negative change in Tt.Ar at the tibia midshaft. Thus, I considered a negative change in Tt.Ar as an indicator of measurement error for bone measures. I allowed for a 0.7% buffer based on in vivo precision estimates from our lab.[151,153] When Tt.Ar was > 0.7% lower than the previous years recorded value, I flagged this measurement time point for further inspection. I first visually confirmed all 'flagged' Tt.Ar data points. I then applied a linear interpolation between the data point prior to and after the flagged data point for all bone parameters for that measurement time point (n = 49/1756 total measures). For example, if Tt.Ar was flagged, linear interpolation was applied to measures of Tt.Ar, Ct.Ar, Me.Ar, Ct.BMD and SSI_p at that measurement period. If a data point was flagged at the last measurement period (n = 15), I used the previous year's value. For density measures, I allowed a 2-year buffer around APHV within which negative changes were accepted, as density measures may transiently decrease during maturation.[6] Of note, I re-ran all models without interpolation or last observation carried forward and results were nearly identical, with the exception of minor changes to model coefficients (Appendix E, Tabel E.2). Lastly, in 2009 a scanner malfunction with the XCT-3000 required use of a different XCT-3000 for all HBSIII scans. Phantom scans indicated a systematic underestimation of cortical bone density measures by the replacement XCT-3000 (no other bone measures were influenced); thus, I interpolated all Ct.BMD measures for 2009 (n = 158 scans). I then manually examined scatter plots of all bone measures against maturity offset for each participant to verify linear interpolation.

4.2.6 Statistical analysis

I used general linear mixed models to compare the annual rate of change in the bone parameters between girls and boys pre- and post-APHV. Maturity offset was centered at 0, a

common maturational landmark used in pediatric studies.[125] For each bone variable, I fit a piecewise linear model with a breakpoint at APHV, i.e., maturity offset = 0. I included sex, ethnicity, a linear spline for maturity offset (MO_1, MO_2) and the interaction of sex and maturity offset as fixed effects in the model. I included a random intercept and random slopes, allowing each individual's profile to vary about the average curve.

The mixed model is:

Conditional piecewise model (random linear piecewise maturity model)

Level 1: $y_{ti} = \beta_{0i} + \beta_{1i}MO_{1ti} + \beta_{2i}MO_{2ti} + \varepsilon_{ti}$

Level 2: Intercept: $\beta_{0i} = \gamma_{00} + \gamma_{01}Boys_i + \gamma_{02}Ethnicity_i + \mu_{0i}$

Linear time pre-APHV: $\beta_{1i} = \gamma_{10} + \gamma_{11}Boys_i + \mu_{1i}$

Linear time post-APHV: $\beta_{2i} = \gamma_{20} + \gamma_{21}Boys_i + \mu_{2i}$

Composite: $y_{ti} = [\gamma_{00} + \gamma_{10}MO_{1ti} + \gamma_{01}Boys_i + \gamma_{11}MO_{1ti}*Boys_i + \gamma_{20}MO_{2ti} + \gamma_{21}MO_{2ti}*Boys_i + \gamma_{02}Ethnicity_i] + [\mu_{0i} + \mu_{1i}MO_{1ti} + \mu_{2i}MO_{2ti} + \varepsilon_{ti}]$

Boys = 0, girl; 1, boy

MO_1 = MO, preAPHV; 0, postAPHV

MO_2 = 0, preAPHV; MO, postAPHV

Ethnicity = 0, Asian; 1, white; 2, other

where y_{ti} *is the bone parameter on measurement occasion t in the* i^{th} *individual,*

$(\mu_{0i}, \mu_{1i}, \mu_{2i}) \sim N(0,\Sigma)$ *is the vector of random effects for the* i^{th} *individual and*

$\varepsilon_{ij} \sim N(0,\sigma^2)$ *is the within-subject residual error.*

The average curves for an Asian participant, for example, are:

Girls: $y_i = \gamma_{00} + \gamma_{10}MO_{1i} + \gamma_{20}MO_{2i}$

Boys: $y_i = (\gamma_{00} + \gamma_{01}Boys_i) + (\gamma_{10} + \gamma_{11}Boys_i)*MO_{1i} + (\gamma_{20} + \gamma_{21}Boys_i)*MO_{2i}$

Thus, γ_{00} represents the mean value of the bone parameter when maturity offset is zero and μ_{0i} the person-specific deviation from the mean intercept; γ_{10} represents the fixed linear effect of maturity pre-APHV and μ_{1i} the person-specific deviation from the fixed linear effect of maturity; γ_{20} the fixed linear effect of maturity at post-APHV and μ_{2i} the person-specific deviation from the linear effect of maturity. $\gamma_{01}Boys$ represents the fixed effect of sex on the mean intercept of the bone parameter; $\gamma_{11}Boys$ the fixed effect of sex on the linear slope pre-

APHV; and $\gamma_{21}Boys$ the fixed effect of sex on the linear slope post-APHV. $\gamma_{02}Ethnicity$ represents the fixed effect of ethnicity on the mean intercept of the bone parameter. Thus, the intercepts γ_{00} and ($\gamma_{00} + \gamma_{01}Boys_i$) represent the average value of the bone parameter when maturity offset is zero for Asian girls and boys, respectively. Similarly, the slopes $\gamma_{10}MO_{1i}$ and ($\gamma_{10} + \gamma_{11}Boys_i$)*$MO_{1i}$ represent the average annual rates of change pre-APHV for girls and boys, respectively. $\gamma_{20}MO_{2i}$ and ($\gamma_{20} + \gamma_{21}Boys_i$)*$MO_{2i}$ represent the average rates of change post-APHV for girls and boys, respectively. I checked model adequacy graphically using plots of residuals.[340] Diagnostic checking of the fitted models revealed some serial correlation in the residuals; however, attempting to incorporate a serial correlation component into the model led to problems with model convergence, an issue identified by others.[340] Models that included serial correlation and only a random intercept yielded similar results to the random coefficients only model. I summarized between-sex differences as rate ratios and nonlinear combinations of the model's coefficients and growth velocities. I considered p-values < 0.05 statistically significant.

4.3 Results

4.3.1 Descriptive characteristics

I provide participant characteristics and baseline bone parameters in Table 4.1. The proportion of girls and boys included in the present analysis and ethnic diversity was similar to that in the larger HBSIII cohort (52% girls, 47% white). Baseline height was also similar between participants included in the current analyses (n = 230) and those we excluded if we were unable to calculate APHV (n = 829). Excluded participants were 0.1 years older and weighed 1.6 kg more at baseline, on average, compared with those participants included in the analyses.

Table 4.1. Characteristics of boys and girls at first pQCT measurement. Data are reported as mean (standard deviation) unless otherwise indicated.

	Boys (n=110)	**Girls (n=120)**
Age (years)	11.0 (1.2)	10.9 (1.0)
# 9/10/11/12/13/14 (yrs)	20/42/25/20/0/3	22/58/15/25/0/0
#Asian/ white /other	45/56/9	56/52/12
APHV (years)[a]	13.1 (1.2)	11.5 (0.8)
Height (cm)	146.3 (10.1)	145.5 (9.7)
Weight (kg)	40.1 (10.3)	39.1 (10.6)
Sitting height (cm)	76.6 (4.9)	76.5 (5.1)
Tibial length (mm)	338.8 (28.2)	337.0 (26.5)
Physical activity score[b]	3.1 (0.6)	2.9 (0.5)
MVPA (min/day)	110.5 (68.5)	83.1 (55.7)
Load time (h/wk)	7.4 (5.2)	5.3 (4.4)
Dietary calcium (mg/day)	956 (538)	880 (426)
Tt.Ar (mm^2)	329.4 (68.9)	311.2 (62.0)
Ct.Ar (mm^2)	211.3 (44.6)	204.6 (42.2)
Ct.Ar/Tt.Ar	0.64 (0.05)	0.66 (0.05)
Me.Ar (mm^2)	118.1 (31.9)	106.6 (29.2)
Ct.BMD (mg/cm^3)	1039.0 (33.9)	1060.9 (33.9)
SSI_p (mm^3)	1132.7 (361.2)	1060.7 (312.0)

[a]APHV not determined at baseline visit.
[b]Average of PAQ items ranging from 1 (low activity) to 5 (high activity).
APHV = age at peak height velocity; MVPA = moderate-to-vigorous physical activity; pQCT bone parameters: Tt.Ar = Total area; Ct.Ar = Cortical area; Ct.Ar/Tt.Ar = ratio of cortical to total area; Me.Ar = Medullary canal area; Ct.BMD = Cortical bone mineral density; SSI_p = polar strength-strain index.

4.3.2 Comparisons of bone parameters between boys and girls at APHV

I provide mean values at APHV for all bone parameters for boys and girls in Table 4.2. For all bone variables (except Ct.Ar/Tt.Ar and Ct.BMD) boys' mean values at APHV were 24-36% greater than girls'. Boys had 2 and 3% lower values for Ct.Ar/Tt.Ar and Ct.BMD, respectively, at APHV compared with girls. These differences are depicted in the individual growth curves and lowess curves aligned on maturity offset (Figure 4.2) and in a schematic representation of differences in bone parameters in relation to maturity offset (Figure 4.3). At APHV, white participants and participants of other ethnicity had significantly greater Tt.Ar (Ratio: 1.11; 95% CI: [1.06, 1.16], 1.15; [1.07, 1.23]), Ct.Ar (1.13; [1.08, 1.18], 1.15; [1.07,

1.23]), Me.Ar (1.07; [1.00, 1.14], 1.15; [1.03, 1.27]) and SSI_p (1.13; [1.06, 1.19], 1.17; [1.06, 1.23]), respectively, compared with Asian participants. White participants had significantly greater Ct.Ar/Tt.Ar (1.02; [1.00, 1.04]) and lower Ct.BMD (0.99, [0.98. 1.00]) compared with Asian participants at APHV (Figure 4.4).

Table 4.2. Estimates of model intercepts. Intercepts represent the average value of the bone parameter at APHV (maturity offset = 0). Numbers in brackets are the standard error of the parameter estimate or the 95% confidence interval for the ratio.

	Boys	**Girls**	**Ratio**	***p*-value**
Tt.Ar (mm^2)	413.4 (7.2)	326.6 (6.8)	1.27 (1.21 to 1.32)	<0.001
Ct.Ar (mm^2)	267.9 (4.7)	215.6 (4.5)	1.24 (1.18 to 1.30)	<0.001
Ct.Ar/Tt.Ar	0.647 (0.006)	0.662 (0.006)	0.98 (0.96 to 1.00)	0.027
Me.Ar (mm^2)	145.5 (3.7)	111.0 (3.5)	1.31 (1.22 to 1.40)	<0.001
Ct.BMD (mg/cm^3)	1042.9 (3.6)	1080.7 (3.5)	0.97 (0.96 to 0.97)	<0.001
SSI_p (mm^3)	1585.8 (36.6)	1165.5 (35.0)	1.36 (1.28 to 1.45)	<0.001

Tt.Ar, total area; Ct.Ar, cortical area; Me.Ar, medullary canal area; Ct.BMD, cortical bone mineral density; SSI_p, polar strength-strain index.
For this purpose, intercepts presented refer to Asian participants.

4.3.3 Comparison of annual accrual rates for bone parameters between boys and girls pre-and post-APHV

I aligned participants on maturity offset and provide between-sex comparisons for annual accrual rates pre- and post-APHV for each bone parameter (Table 4.3). Boys and girls demonstrated periosteal bone formation (represented by an increase in Tt.Ar) and net bone loss at the endocortical surface (represented by a net increase in Me.Ar) over the measurement period. Boys demonstrated significantly greater annual accrual rates compared with girls for Tt.Ar and Me.Ar pre-APHV, and significantly lower annual accrual rates for Ct.Ar/Tt.Ar and Ct.BMD pre-APHV compared with girls. There were no significant between-sex differences in annual accrual rates pre-APHV for Ct.Ar and SSI_p. Post-APHV, there were no significant between-sex differences in annual accrual rates for Ct.Ar/Tt.Ar; however, boys demonstrated significantly greater annual accrual rates for all other bone parameters compared with girls (Figure 4.2).

Table 4.3. Estimates of fixed effects slopes and comparison between boys and girls. Slopes represent annual rates of accrual pre- and post-age at peak height velocity (APHV), adjusted for maturity offset and ethnicity. Numbers in brackets are the standard error of the parameter estimate or the 95% confidence interval for the ratio.

	Slope Pre-APHV				Slope Post-APHV			
	Boys	**Girls**	**Ratio**	***p*-value**	**Boys**	**Girls**	**Ratio**	***p*-value**
Tt.Ar (mm^2/yr)	55.4 (2.0)	47.0 (2.7)	1.18 (1.02 to 1.34)	0.011	19.2 (0.6)	10.2 (0.6)	1.89 (1.64 to 2.13)	<0.001
Ct.Ar (mm^2/yr)	38.3 (1.6)	35.0 (2.2)	1.10 (0.94 to 1.26)	0.211	15.2 (0.5)	8.7 (0.5)	1.75 (1.50 to 1.99)	<0.001
Ct.Ar/Tt.Ar	0.007 (0.001)	0.013 (0.002)	0.56 (0.29 to 0.83)	0.021	0.005 (0.0005)	0.005 (0.0005)	1.01 (0.71 to 1.31)	0.928
Me.Ar (mm^2/yr)	15.2 (0.6)	11.4 (0.9)	1.34 (1.11 to 1.57)	<0.001	4.1 (0.2)	1.6 (0.2)	2.63 (1.80 to 3.45)	<0.001
Ct.BMD (mg/cm^3/yr)	-1.0 (0.9)	16.4 (1.6)	-0.07 (-0.17 to 0.04)	<0.001	18.2 (0.5)	14.8 (0.6)	1.23 (1.11 to 1.35)	<0.001
SSI_p (mm^3/yr)	271.6 (10.0)	237.4 (14.6)	1.14 (0.98 to 1.30)	0.054	164.0 (4.8)	84.2 (5.0)	1.95 (1.69 to 2.20)	<0.001

Tt.Ar, total area; Ct.Ar, cortical area; Me.Ar, medullary canal area; Ct.BMD, cortical bone mineral density; SSI_p, polar strength-strain index. Maturity offset is years from APHV.

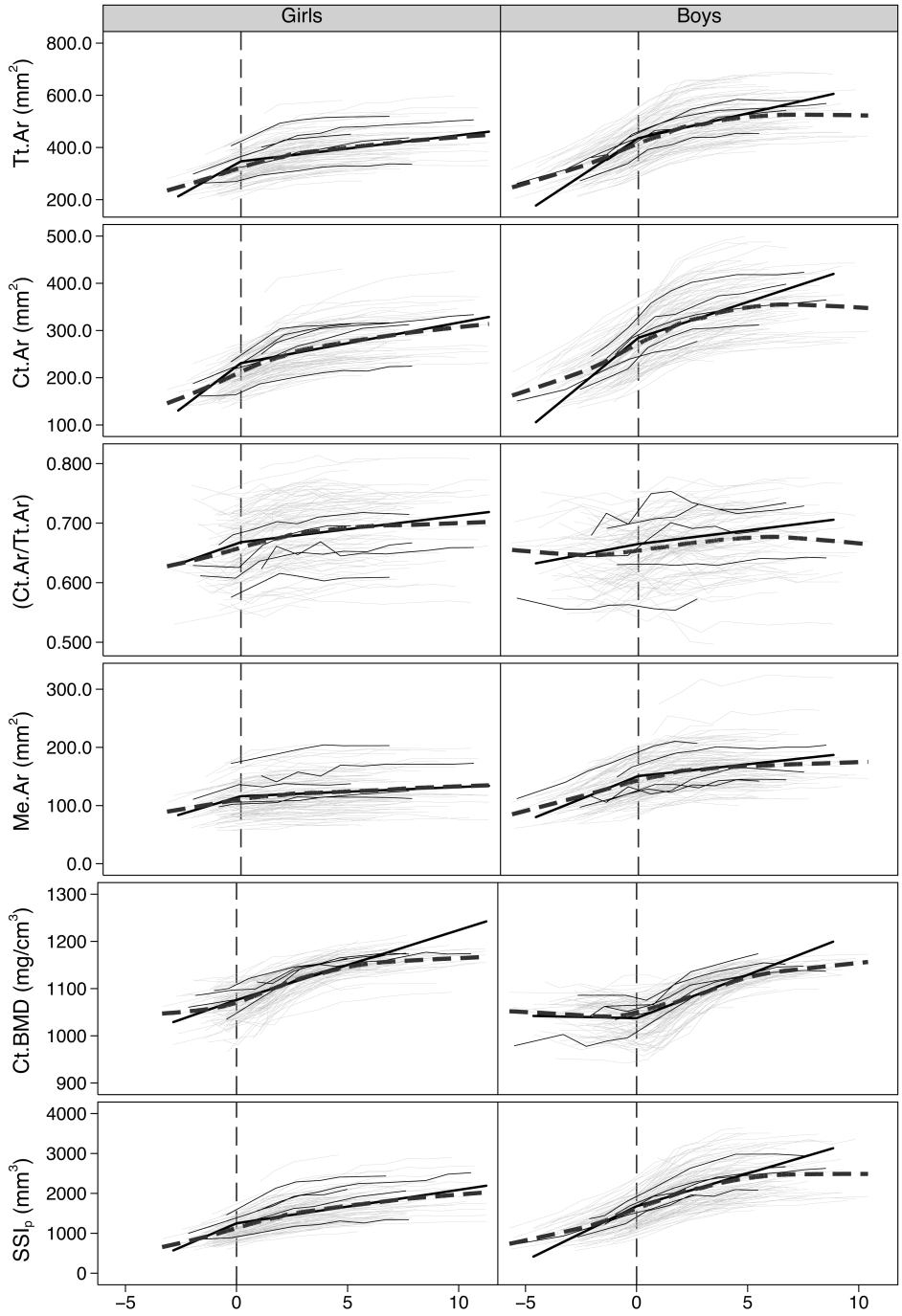


Figure 4.2. Individual growth curves (thin, light gray lines), individual growth curves of five randomly selected girls and boys (thin, black lines), a lowess-smoothing curve (thick, dark gray dashed line) and the polynomial mixed model curves (thick, black line) of total area (Tt.Ar), cortical area (Ct.Ar), ratio of cortical to total area (Ct.Ar/Tt.Ar), medullary are (Me.Ar), cortical bone mineral density (Ct.BMD) and polar strength-strain index (SSI_p), plotted against maturity offset. The vertical line indicates maturity offset (years from age at peak height velocity) of 0.

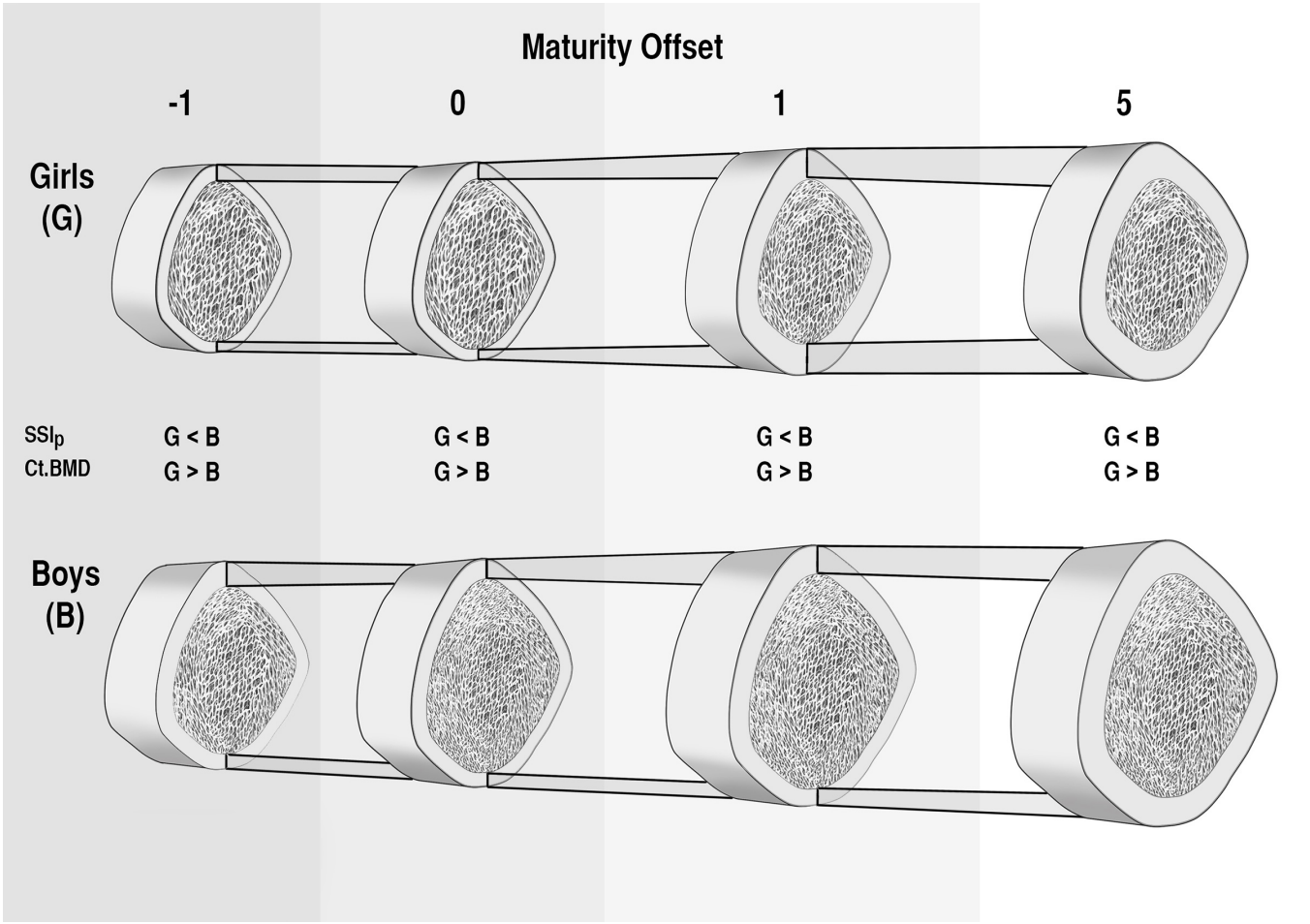


Figure 4.3. A schematic representation of differences in total area (Tt.Ar), cortical area (Ct.Ar) and medullary area (Me.Ar) in boys and girls in relation to maturity offset (years from age at peak height velocity). I present maturity offset at -1, 0, 1 and 5 years. Significant differences between girls and boys are shown for polar strength-strain index (SSI_p), where boys' values exceed girls' at all time points, and Ct.BMD, where girls' values exceed boys' at all time points. (Diagram not to exact scale).

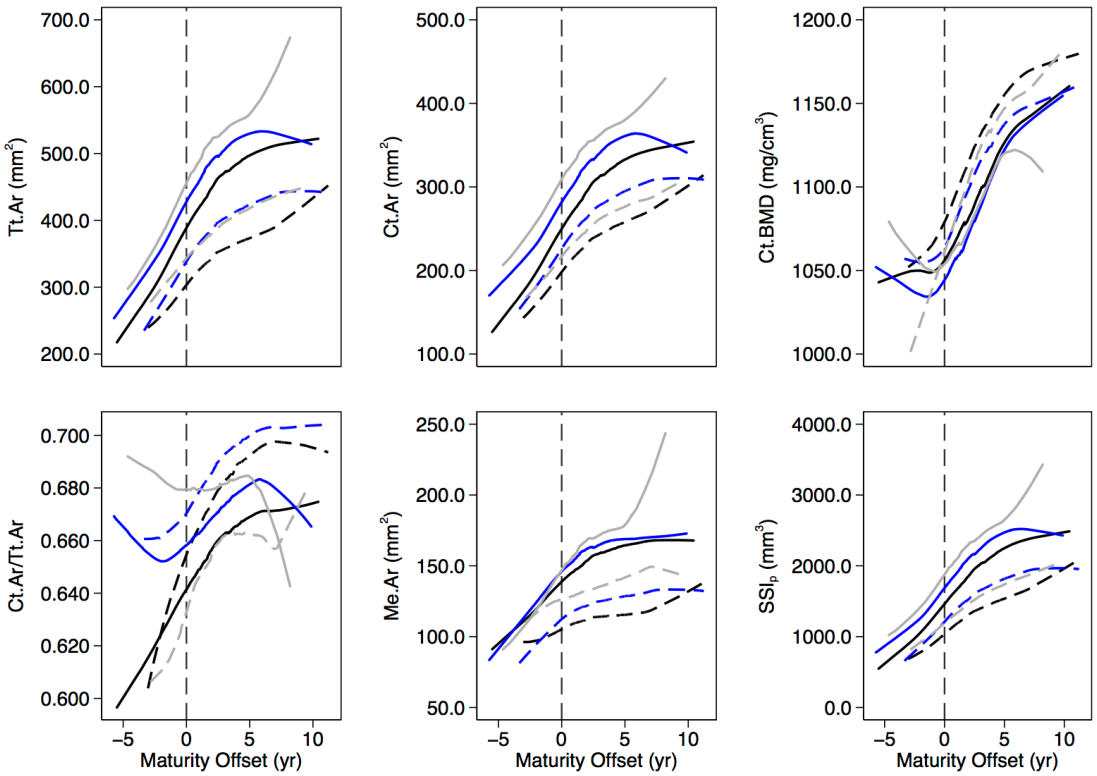


Figure 4.4. Curves of predicted total area (Tt.Ar), cortical area (Ct.Ar), ratio of cortical to total area (Ct.Ar/Tt.Ar), medullary area (Me.Ar), cortical bone mineral density (Ct.BMD) and polar strength-strain index (SSI_p), plotted against maturity offset for boys (solid lines) and girls (dashed lines), Asian (black line), white (blue line) and other (grey line) participants. The vertical line indicates maturity offset (years from age at peak height velocity) of 0.

4.4 Discussion

I revisit Garn's classic studies[155-157] and more recent reports[152,338] that suggest bone strength during adolescence is accrued primarily through periosteal expansion in boys compared with endocortical contraction in girls. In this analysis, I extend Garn's methods (cross-sectional study of planar radiographs of the second metacarpal) by aligning boys and girls on a common maturational landmark (APHV). I then modeled 3D aspects of tibial bone geometry, density and strength (acquired using pQCT) across 12-years. Should Garn's general findings persist in my study, boys would accrue more bone on the periosteal surface (contributing to increased Tt.Ar)

and girls would accrue more bone on the endocortical surface (contributing to a narrower medullary cavity (reduction in Me.Ar)).

Although both boys and girls accrued substantial amounts of bone on the periosteal surface (as estimated by changes in Tt.Ar), boys' instantaneous accrual rates were approximately 56% greater compared with girls' at APHV (age 13.1 years in boys and 11.5 years in girls). These findings support those of Garn who reported boys had 48% greater periosteal expansion at the second metacarpal compared with girls from childhood to late adolescence (8-22 years of age).[155-157] The current results also extend our earlier findings across 20-months where we reported 8% greater periosteal expansion in boys compared with girls during early- and peri-puberty and 14% greater periosteal expansion in boys post-puberty at the tibial midshaft.[153] The distribution of bone away from the neutral axis confers a considerable strength advantage to boys at long bone shafts such as the mid-tibia.[87]

I used Me.Ar to estimate changes at bone's endocortical surface. I observed expansion of the medullary canal for both boys and girls across maturity. However, annual accrual rates in boys were more than double (105% greater) that of girls at APHV. Contrary to Garn's findings that bone formation at the endocortical surface was enhanced in girls during puberty,[155-157] our 12-year longitudinal data demonstrated a small net loss of bone at the endocortical surface in girls. This was represented by a small net increase in Me.Ar (11 mm^2/year pre-APHV and 2 mm^2/year post-APHV, on average). My findings also differ from Wang et al. who analyzed data from a 2-year longitudinal study and observed an increase in Me.Ar in girls until menarche and a slight decrease thereafter.[158] However, fewer than 10 participants were assessed at 24-months post menarche; thus, interpretations must be made with caution.[158] My findings across a 12-year period are consistent with our previous work, where we reported a 5-8% increase in Me.Ar over 20-months in early- to post-pubertal girls.[153] Thus, our data do not support increased endocortical bone formation during adolescence as noted by Garn. On the contrary, I noted endocortical bone expansion predominates in both boys and girls; however, girls' bone was preserved to a greater extent (less expansion) compared with boys.

There are several plausible explanations for the differences between Garn's early findings and the present study. First, bone formation and resorption differ considerably by anatomical region.[341,342] In earlier studies, we reported that Ct.BMD also varied across sectors within the same bone cross-section.[162,343] As the metacarpal is non-weight bearing compared with the

weight-bearing tibia, the substantially different forces experienced at each site may contribute to site-specific differences between the two studies. To enhance bone strength in response to greater loads associated with bending and torsion, the tibia preferentially adapts by distributing bone further away from the neutral axis (through an increase in Me.Ar in addition to increases in Tt.Ar). This could be achieved with either less endocortical apposition or more endocortical resorption compared with the non-weight-bearing metacarpal.[87] Second, there are significant limitations associated with estimating bone cross-sectional geometry using 2D radiographs.[344] Third, despite relatively large sample sizes, Garn used cross-sectional data for his analyses. The limitations (e.g., selection/attrition, cohort differences) of age-heterogeneous cross-sectional samples for evaluating rates of change are reviewed in detail elsewhere.[345] Longitudinal data are better able to represent the tremendous growth-related variability among children and also permit separation of age-related mean trends from estimates of associations between age-related variables. Finally, Garn et al. did not control for substantial maturational differences between boys and girls of the same chronological age, which may have influenced their findings.

I used the ratio of Ct.Ar to Tt.Ar (Ct.Ar/Tt.Ar) as a surrogate of cortical thickness to examine changes across time as we did in our previous 20-month study.[153] I observed relative thickening of the cortex (increase in Ct.Ar/Tt.Ar) and thus a reciprocal decrease in Me.Ar relative to total bone size in both boys and girls across the study period. The cortex contributed similarly to overall bone size in boys compared with girls at APHV. Consistent with our previous study over a 20-month period,[153] boys' greater overall growth rate (Tt.Ar and Ct.Ar) post-APHV resulted in no observed differences in Ct.Ar/Tt.Ar between boys and girls during this time.

I report significantly lower Ct.BMD for boys compared with girls in the current study as per previous reports at the tibial midshaft[151,153,162] and the proximal radius[346] assessed using pQCT, and at the distal tibia and radius assessed using HR-pQCT.[4] More dense cortices in girls might reflect lower rates of intracortical remodeling associated with a shorter period and smaller magnitude of growth during adolescence in girls, compared with boys.[164] Although I noted sex differences in Ct.BMD accrual appeared prior to APHV, I was unable to clearly discern if these differences were present at earlier maturational stages. Girls in my study were relatively close to APHV at baseline and mean curves appeared to converge several years prior to APHV (Figure 4.2). Given the greater contribution of geometry to bone strength compared with density at long

bone shafts,[87,347] girls' more dense bones would only partially compensate for their smaller bone size, on average, compared with boys. In adults, larger bone size rather than differences in density or amount of bone within the periosteal envelope is thought to account for men's greater bone strength compared with women.[338]

Although bone size, shape and density contribute to adult bone strength,[41] the relative contribution of these components to bone strength in children is largely unknown. On average, boys had significantly greater estimated bone strength (represented by SSI_p) and greater annual accrual rates compared with girls. In boys, the surge of testosterone during puberty is largely responsible for greater magnitude and prolonged duration of the pubertal growth spurt, which results in greater gains in periosteal and longitudinal bone formation across puberty in boys compared with girls.[348] As bone's ability to resist bending forces is directly proportional to the distribution of mass about the neutral bending axis, an incremental increase in the external diameter of a long bone (increased bone size) improves bones' resistance to bending and torsional loading and substantially increases bone's strength.[43,347] Thus, in the current study, boys' larger bones (greater Tt.Ar), enhanced rates of change in bone parameters and prolonged duration of longitudinal growth, compared with girls, contributed to boys' greater estimated bone strength (SSI_p). Should this advantage persist similar to the continuity of bone area from young to middle adulthood as reported by Garn and colleagues,[349] boys and men would be conferred a bone strength advantage throughout life.

Although there are known ethnic differences in the timing and tempo of maturation[350] (Asian participants in our cohort attained APHV approximately 7 months prior to white participants), I attempted to account for this in my analysis by aligning on APHV. White participants had 1% lower Ct.BMD and 2-13% greater values for all other bone parameters compared with their Asian peers at APHV. These findings are consistent with our previous reports demonstrating larger Ct.Ar (at the tibial midshaft by pQCT) in pre- and early-pubertal white boys and girls[151] and lower Ct.BMD (at the distal tibia by HR-pQCT) in mid to late-pubertal white boys and girls compared with their Asian peers.[186] I cannot discount the possibility that ethnic differences in bone parameters may have been present prior to the onset of puberty and influenced by a shorter duration of growth (earlier onset of maturation) in Asian participants. I note that although our sample reflects the ethnic diversity of Metro Vancouver,

where visible minority groups represent 47% of the population (compared with 19% in the rest of Canada),[351] it may not be representative of populations outside of this area.

The purpose of this analysis was to compare rates of change pre-APHV and post-APHV. Thus, I fit a piecewise linear regression model; I did not attempt to model bone parameter growth curves. I highlight several strengths of my study. Most importantly, longitudinal data are difficult, time consuming and costly to collect and are therefore relatively rare. The few previous studies that reported changes on bone surfaces during growth utilized cross-sectional data[155-157] or longitudinal data across 2-years[158] and were therefore unable to capture the tremendous variability that accompanies growth of tissues throughout adolescence. Our 12-year longitudinal data enabled us to identify APHV for participants and in turn, align boys and girls on the same maturational landmark. Additionally, we examined 3D changes in bone geometry and strength at a weight-bearing site, the tibial midshaft, using an advanced imaging technique (pQCT).

I acknowledge several limitations of my study. First, as in any repeated measures study of growing bone it is not possible to reassess the same bone cross-section over time. Long bone growth is both complex and disproportionate; at the tibia, 57% of longitudinal growth occurs at the proximal metaphysis and 43% occurs at the distal metaphysis.[39] Therefore, we used a standard anatomical landmark to identify the same relative site along the length of the tibia at each measurement in every child. Second, based on differences in maturational timing between boys and girls at the same chronological age, many of the girls in my study approached APHV at baseline. Thus, I was unable to compare boys and girls across several years prior to APHV and may have underestimated annual rates of change for girls prior to APHV. Third, these results are specific to adaptations at the tibial shaft and do not necessarily represent other skeletal regions. Although I did not discern net endocortical contraction in girls at the tibia, it may occur at other skeletal sites or even at different sites along the length of the tibia. Fourth, the resolution of pQCT (0.4 mm pixel size) may have limited our ability to detect small changes in cortical bone area and subsequently small changes on the endocortical surface. Finally, I acknowledge that our convenience sampling methods limit the external validity of our results.

4.5 Conclusions

In summary, the pioneering studies of Garn and colleagues established an important premise that prompted researchers to more closely examine sex differences on the surfaces of growing long bones. Boys' larger bones confer a greater bone strength advantage during adolescence. However, it would be of interest to better understand whether benefits persist into adulthood and older age. Although evidence from animal studies[81]and retrospective studies of athletes[290,294,295] support that enhanced long bone strength accrued during the younger years persists, the implications of this on preventing osteoporosis and fragility fractures in later life, remains largely unknown.

Chapter 5: Sex Differences and Growth-Related Adaptations in Bone Microarchitecture, Geometry, Density and Strength from Childhood to Early Adulthood: A Mixed Longitudinal HR-pQCT Study

SYNOPSIS: The specifics of how bone microarchitecture and strength are accrued during childhood and adolescence are not completely understood. In this chapter, I evaluate growth-related adaptations of bone at the distal tibia and radius and differences in bone accrual and adaptation between boys and girls. I present this chapter in its published format with minor modifications, including additional detail to statistical analyses.[8]

5.1 Introduction

Bone strength, the 'bottom line' of fracture prevention, is influenced by many factors, including bone size, bone mineral density and the organization of bone microarchitecture.[352] However, the intricacies of bone microarchitecture adaptations as they relate to increased bone strength and fracture risk during growth are not well understood.

To date, few studies used HR-pQCT to evaluate sex differences in bone strength and its determinants. Of these, most were cross-sectional or had only a short follow-up period.[4-6,353] However, these early studies were crucial as they began to shed light on changes in bone parameters that occur during puberty. Two cross-sectional studies of children and adolescents aged 5-21 years observed transiently thinner and less dense cortices at the radius in boys compared with girls [5] and at both radius and tibia[6] during mid-puberty. In our previous study, over a 2-3 year follow-up period, we noted significantly more porous cortices in boys compared with girls as early as Tanner Stage 2 and 3.[4] We do not know whether these microarchitectural differences during puberty and deficits at the cortex, specifically, explain the high incidence of fractures in boys during periods of rapid pubertal growth.[148,354] Boys may be at a disadvantage during adolescent growth due to higher rates of bone turnover, longer duration of linear growth

[8] A version of this chapter is published: Gabel L, Macdonald HM, McKay HA. Sex differences and growth-related adaptations in bone microarchitecture, geometry, density and strength from childhood to early adulthood: a mixed longitudinal HR-pQCT study. J Bone Miner Res 2017; 32(2): 250-63.

and greater PHV compared with girls.[163,355] Evaluating bone strength and the parameters that underpin bone strength accrual prospectively over a longer time frame will further our understanding of factors that might contribute to elevated fracture risk during adolescence.

To assess sex differences in bone accrual during growth, it is essential to account for the influence of maturational status, which varies considerably between children of the same chronological age.[125] Self-reported stage of maturation as per the method of Tanner is commonly used in cross-sectional and short-term prospective pediatric studies; however, boys and girls cannot be aligned on a similar maturational time point using this method.[130,131] In contrast, I[313] and others[133,147] compared bone accrual between boys and girls aligned on a common maturational landmark, APHV. Commonly used as an indicator of somatic maturity in longitudinal studies,[125,137] APHV refers to the age when maximum linear growth in height occurs and generally occurs in boys and girls at a maturational time point when approximately 90% of adult stature has been achieved.[134] APHV can be determined either directly in longitudinal studies with adequate serial measures acquired around the period of maximal height velocity, or predicted in cross-sectional or short-term prospective studies using validated equations that incorporate common anthropometric measures such as height and sitting height.[141,142] APHV has not previously been used in pediatric bone studies that utilized HR-pQCT, to control for what could potentially be substantial differences in growth between and within sexes.

Thus, my objectives were to: 1) describe growth related adaptations in bone strength and its determinants at the distal tibia and radius in boys and girls, and 2) compare differences in growth related adaptations in bone strength and its determinants between boys and girls. I hypothesized that boys would demonstrate greater bone strength, geometry and Ct.Po, but lower Ct.BMD throughout growth compared with girls.

5.2 Methods

I provide a detailed description of study design and methods for data collection in Chapter 2 and a brief overview in the following sections.

5.2.1 Study design

Participants were drawn from a cohort of healthy girls (n = 556) and boys (n = 515) aged 8 to 12 years at study entry who comprised the University of British Columbia HBSIII (Table 5.1). In the present analysis, I included bone data from annual measurements conducted between May 2008 (first year of HR-pQCT measurements) and June 2012. In the cohort with HR-pQCT data (n = 399) we acquired a median of 4 annual measurements at the distal tibia (interquartile range: 3 to 4) and a median of 3 annual measurements at the distal radius (interquartile range: 2 to 3). For the purpose of this analysis, I refer to data obtained at the first HR-pQCT measurement as baseline.

Table 5.1. Overview of study participants that comprise the Healthy Bones Study III cohort.

Cohort	**N (sex; ethnicity)**	**Years of data collection (HR-pQCT assessment)**	**Study Objective**
Healthy Bones Study and Bounce at the Bell[257-259,315]	N=436 (50% boys; 45% Asian, 44% white, 11% other)	1999-2011 (2008-2011)	Examine effect of a 20-month cluster-randomized school-based exercise intervention on bone mass
Action Schools! BC[265]	N=515 (50% boys; 55% Asian, 32% white, 13% other)	2003-2011 (2008-2011)	Examine effect of a 16-month cluster-randomized school-based physical activity intervention on bone mass and strength
New Cohort[4]	N=120 (33% boys; 47% Asian, 44% white, 9% other)	2009-2012 (2009-2012)	Prospective cohort to evaluate changes in bone microarchitecture and strength during the growing years

5.2.2 Anthropometry and age at peak height velocity

We assessed standing and sitting height, body mass and tibia length using standard methods. We estimated years from APHV as an estimate of biological maturity, as described in Appendix D. Due to missing and mistimed measurements surrounding APHV, we were only able to identify APHV using the cubic spline method in 198 participants (50% of cohort). For the remaining participants, I used the Moore equation[142] and anthropometric data from the measurement occasion closest to reported APHV (approximately 11.6 years in girls and 13.5

years in boys) to estimate APHV. Thus, I used the measurement occasion when girls were closest to 11.6 years (range 9.5 to 13.1 years) and boys were closest to 13.0 years (10.8 to 14.3 years), on average, to estimate APHV. For all participants, I used APHV to calculate a continuous measure of biological maturity offset (in years) by subtracting APHV from chronological age at time of measurement (e.g., -1 year is equivalent to 1 year prior to attainment of APHV; +1 to one year after APHV).

5.2.3 Health history and ethnicity

I determined health history and ethnicity using a questionnaire, completed by parents at baseline and by participants at subsequent annual visits. Based on questionnaire responses, I identified six participants who had conditions that prevented their participation in regular PA and/or reported medical conditions known to influence bone metabolism (osteogenesis imperfecta, fetal alcohol syndrome, type 1 diabetes, leukemia, congenital heart defect). With these exclusions, I used data from 393 healthy participants (184 boys, 209 girls) for analysis.

5.2.4 Bone microarchitecture, geometry, density and strength

We assessed bone strength and its determinants at the non-dominant distal tibia (8% site) and distal radius (7% site) using HR-pQCT (XtremeCT; Scanco Medical AG, Brüttisellen, Switzerland; Figure 5.1). I evaluated all HR-pQCT scans for motion artifacts and analyzed all scans as per manufacturer's standard protocol.[113,356] I excluded 1 tibia scan and 33 radius scans (3%) due to motion artifact > 3 (on a scale from 1 to 5);[124] exclusions were fewer compared with other studies of the radius that used HR-pQCT in adolescent populations.[5,357] I used a semi-automated segmentation method to trace the periosteal surface of the tibia and radius and a threshold-based algorithm to separate the cortical and trabecular bone. I report standard morphological measures including: Tt.BMD (mg/cm^3), Tb.N (1/mm), Tb.Th (mm), Tb.Sp (mm) and BV/TV. Additionally I used an automated segmentation algorithm to separate trabecular and cortical bone[115,116] to determine: Tt.Ar (mm^2), Ct.BMD (mg/cm^3), Ct.Po (%) and Ct.Th (mm). Finally, we applied a validated FE analysis to HR-pQCT images to estimate bone strength. FEA outcomes were F.Load (N) and U.Stress (MPa). We also calculated load-to-strength ratio (distal

radius only) as the a ratio of estimated fall load applied to the outstretched hand after a fall from standing height.[119]

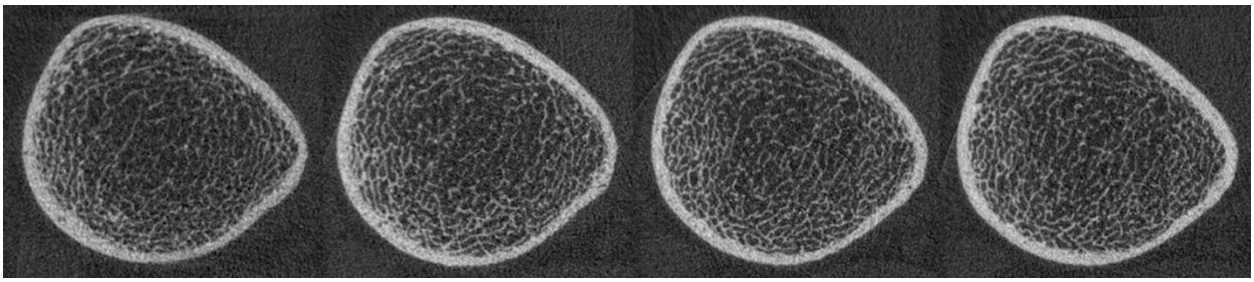


Figure 5.1. Representative high-resolution peripheral quantitative computed tomography images at the distal tibia from a single participant across 4 years acquired at 11- (far left), 12-, 13- and 14- (far right) years of age. Images not to scale.

5.2.5 Statistical analysis

I considered *p*-values < 0.05 statistically significant. Prior to modeling my data, I first examined scatter plots generated for bone parameters against maturity offset for each participant. I fit general linear mixed models to compare annual rate of change in bone parameters between girls and boys. I centered maturity offset 0, a maturational landmark used previously in pediatric studies.[125] All models were estimated using maximum likelihood estimation, as there was only a 0.3% downward bias in the random intercept variance compared with restricted maximum likelihood estimation (assessed using Ct.Ar at the distal tibia as the outcome).

I used the following process to determine the best fitting model for all variables. First, I conducted an empty means random intercept model to determine the amount of variance attributed to between-person and within-person differences. Next, I assessed a fixed linear time, random intercept model, with maturity offset (years from APHV) as the time variable, followed by a random linear time model. I followed these models with fixed and random quadratic and cubic time models. I used Wald test p-values to determine significance of individual fixed effects and log likelihood (-2*log likelihood (LL)) statistics to determine significance of random effects variances and covariances between nested models given the difference in model degrees of freedom. I determined the best fitting unconditional growth model by the largest reduction in the deviance test (-2LL) and the parsimony of the model (Akaike and Bayesian information criterion (AIC and BIC) values). I examined change in pseudo R^2 with addition of each fixed polynomial

time variable (as computed from the square of the correlation between the observed bone variable and the outcomes predicted by the fixed effects) to assess the potential for overfitting of the model. If a negligible change in pseudo R^2 (< 1%) suggested the presence of overfitting, I used the previous model. I then examined the effect of sex and ethnicity on the intercept and maturity effects.

5.2.5.1 Model building

I provide a detailed example of model building using Ct.Ar at the distal tibia as the outcome. I began with an empty means, random intercept model that indicated 92% of the variance in Ct.Ar was due to between persons (i.e., individual mean differences across age), while the remaining 8% was within persons (i.e., time-specific deviations about one's mean value). Fixed linear effect of maturity offset and its random variance across participants were each significant ($-2\Delta LL(\sim 2) = 262.6$, $p < 0.001$), indicating a significant increase in Ct.Ar with maturity, on average, and individual differences therein. Next, to improve model fit with respect to non-linear shape of the growth curves, I examined polynomial models to describe the overall pattern of individual differences in change in Ct.Ar across maturity, centered at maturity offset of 0 (APHV). A fixed quadratic effect of maturity was significant ($p < 0.001$), indicating a deceleration in Ct.Ar velocity over time, on average. However, a model including a random effect for quadratic maturity did not converge. Next, inclusion of a cubic effect of maturity significantly improved model fit ($p < 0.001$), but change in pseudo R^2 was negligible (0.2%), suggesting overfitting of the model, so I selected a fixed quadratic, random linear time model as the best fitting unconditional growth model.

Equation 1: Unconditional growth model (fixed quadratic, random linear maturity model)

$$\textit{Level 1: } y_{ti} = \beta_{0i} + \beta_{1i}MO_{ti} + \beta_{2i}MO_t^2 + \varepsilon_{ti}$$

$$\textit{Level 2:Intercept: } \beta_{0i} = \gamma_{00} + \mu_{0i}$$

$$\textit{Linear time: } \beta_{1i} = \gamma_{10} + \mu_{1i}$$

$$\textit{Quadratic time: } \beta_{2i} = \gamma_{20}$$

$$\textit{Composite: } y_{ti} = [\gamma_{00} + \gamma_{10}MO_{ti} + \gamma_{20}MO_{ti}^2] + [\mu_{0i} + \mu_{1i}MO_{ti} + \varepsilon_{ti}]$$

MO is maturity offset (centered at 0, APHV),

where y_{ti} is the bone parameter on measurement occasion t in the i^{th} individual,

$(\mu_{0i}, \mu_{1i}) \sim N(0,\Sigma)$ is the vector of random effects for the i^{th} individual and

$\varepsilon_{ij} \sim N(0,\sigma^2)$ is the within-subject residual error.

Thus, Equation 1 represents the best-fitting unconditional growth model, where γ_{00} represents the mean value of the bone parameter when maturity offset is 0 and μ_{0i} the person-specific deviation from the mean intercept; γ_{10} represents the fixed linear effect when maturity offset is 0 (APHV) and μ_{1i} the person-specific deviation from the fixed linear effect of time; and γ_{20} the fixed quadratic effect when maturity offset is 0 (APHV). This model included a fixed intercept for the expected Ct.Ar at maturity offset of 0 of 89.5 mm^2, with a 95% random intercept confidence interval of 46.3 to 132.8 mm^2. The significant instantaneous fixed linear effect at maturity offset of 0 of 8.8 mm^2 per year had a 95% random linear age slope confidence interval of 1.5 to 16.0mm^2 per year, indicating that a continuous increase in Ct.Ar was predicted for all participants at maturity offset of 0. Finally, the significant fixed quadratic effect of maturity of -0.5 mm^2 indicated the linear effect of maturity became less positive by 0.5 mm^2 per $year^2$. The fixed effects of linear and quadratic maturity accounted for approximately 40% of the total variance in Ct.Ar (i.e., pseudo R^2 as computed from the square of the correlation between observed Ct.Ar and the outcomes predicted by the fixed effects). I applied this process of model fitting to all other bone outcome variables to determine the best fitting model.

I then examined the effect of participants' sex as a time-invariant predictor (53% girls, 47% boys; Equation 2). Sex significantly moderated the intercept at maturity offset of 0 and the linear maturity effects. Specifically, compared with girls, boys were predicted to have significantly greater Ct.Ar at maturity offset of 0 by 25.9 mm^2, and significantly more positive linear change by 1.3 mm^2 per year. Sex accounted for an additional 23% of the total variance in Ct.Ar, including 35% of the random intercept variance and 10% of the random linear maturity slope variance. Ethnicity significantly moderated the intercept at maturity offset of 0 and accounted for an additional 1% of the total variance in Ct.Ar, including 4% of the random intercept variance. The full model including sex and ethnicity accounted for 64% of the total variance in Ct.Ar. Thus, the final model used to predict bone outcomes was a random linear,

fixed quadratic maturity model, that included fixed effects of sex and ethnicity predicting the intercept, and sex predicting the linear and quadratic maturity slopes (Equation 2).

Equation 2: Random linear, fixed quadratic maturity model, including fixed effect of sex and ethnicity predicting intercept, and sex predicting linear and quadratic maturity slope

$$\textit{Level 1: } y_{ti} = \beta_{0i} + \beta_{1i}MO_{ti} + \beta_{2i}MO_t^2 + \varepsilon_{ti}$$

$$\textit{Level 2: Intercept: } \beta_{0i} = \gamma_{00} + \gamma_{01}Boys_i + \gamma_{02}Ethnicity_i + \mu_{0i}$$

$$\textit{Linear time: } \beta_{1i} = \gamma_{10} + \gamma_{11}Boys_i + \mu_{1i}$$

$$\textit{Quadratic time: } \beta_{2i} = \gamma_{20} + \gamma_{21}Boys_i$$

$$\textit{Composite: } y_{ti} = [\gamma_{00} + \gamma_{10}MO_{ti} + \gamma_{20}MO_{ti}^2 + \gamma_{01}Boys_i + \gamma_{11}MO_{ti}*Boys_i + \gamma_{21}MO_{ti}^2*Boys_i + \gamma_{02}Ethnicity_i] + [\mu_{0i} + \mu_{1i}MO_{ti} + \varepsilon_{ti}]$$

MO is maturity offset (centered at 0, APHV); Boys = 0, girl; 1, boy

Ethnicity= 0, Asian; 1, white; 2, other

where y_{ti} is the bone parameter on measurement occasion t in the i^{th} individual,

$(\mu_{0i}, \mu_{1i}) \sim N(0,\Sigma)$ is the vector of random effects for the i^{th} individual and

$\varepsilon_{ij} \sim N(0,\sigma^2)$ is the within-subject residual error.

The intercepts γ_{00}, $\gamma_{01}Boys_i$ *and* $\gamma_{02}Ethnicity_i$ represent the mean value of the bone parameter and the fixed effect of sex and ethnicity on the mean intercept of the bone parameter when maturity offset is zero, respectively, while μ_{0i} is the person-specific deviation from the mean intercept. The slopes γ_{10} and $\gamma_{11}Boys$ represent the fixed linear effect of maturity and the fixed effect of sex on linear maturity at maturity offset of 0, respectively, while μ_{1i} is the person-specific deviation from the fixed linear effect of time. The slopes γ_{20} and $\gamma_{21}Boys$ represent the fixed quadratic effect of maturity and the fixed effect of sex on quadratic maturity, respectively.

I checked model adequacy graphically using plots of residuals.[340] Diagnostic checking of fitted models revealed some serial correlation in the residuals; however, attempting to incorporate a serial correlation component into the model led to problems with model convergence, an issue identified by others.[340] Models that included serial correlation and only a random intercept yielded similar results to the random coefficients only model. I calculated

adjusted means and estimated sex differences in bone parameters at each maturity offset using the margins command in Stata and a Bonferroni adjustment to account for multiple comparisons. Accordingly, the level of statistical significance was set to $p < 0.0042$ ($p < 0.05$ divided by 12) for sex differences.

5.3 Results

5.3.1 Descriptive characteristics

I provide participant characteristics and bone parameters at first HR-pQCT measurement in Table 5.2. There were 1240 total observations at the distal tibia and 915 total observations at the distal radius (Table 5.3). Between-person differences in bone parameters accounted for 79% (for Ct.Po) to 96% (for BV/TV) of the variance in bone parameters, while the remaining 4-21% was attributed to within-person differences. I deliberately did not compare parameters between boys and girls at baseline as their chronological age and maturity varied significantly. Alternatively, I compared differences in all parameters between boys and girls at the same approximate maturity offset (years from APHV). I provide predicted model parameters and growth curves at the tibia (Table 5.4, Figure 5.2) and at the radius (Table 5.5, Figure 5.3). I compare sex differences in bone parameters at each maturity offset across 12 years (Figure 5.4, Figure 5.5 and Table 5.6 and Table 5.7). Due to few measurements at maturity offsets before 2 years prior to APHV in girls (n = 3) and after 9 years post-APHV in boys (n = 6), I limited the range of between-sex comparisons from 2 years prior to APHV to 9 years post-APHV.

Table 5.2. Characteristics of boys and girls at first HR-pQCT measurement.

	Boys (n=184)			Girls (n=209)		
	Mean (SD)	**Min**	**Max**	**Mean (SD)**	**Min**	**Max**
Age (yrs)	15.1 (2.6)	9.5	19.8	14.5 (3.4)	9.5	20.3
No. Asian/ white /other	82/83/19	-	-	97/92/20	-	-
No. Tanner 1/2/3/4/5	20/14/11/63/75	-	-	31/37/33/58/50	-	-
Maturity offset (yrs)	2.1 (3.0)	-4.1	8.2	3.1 (3.5)	-2.6	9.3
Height (cm)	167.1 (14.3)	129.7	192.2	155.5 (11.6)	130.0	181.6
Weight (kg)	60.8 (17.3)	27.8	127.3	50.1 (14.1)	22.2	95.9
Sitting height (cm)	88.0 (7.3)	67.2	102.4	83.1 (6.5)	68.6	95.0
Tibial length (mm)	403 (37)	306	482	370 (30)	300	444
Ulnar length (mm)	274 (28)	204	333	246 (21)	196	293
Distal Tibia						
Tb.N (1/mm)	1.90 (0.27)	1.18	2.41	1.81 (0.26)	1.10	2.55
Tb.Th (mm)	0.088 (0.014)	0.055	0.122	0.086 (0.014)	0.056	0.127
Tb.Sp (mm)	0.450 (0.076)	0.326	0.742	0.477 (0.076)	0.320	0.788
BV/TV	0.165 (0.025)	0.108	0.235	0.154 (0.025)	0.093	0.236
Ct.Th (mm)	1.20 (0.37)	0.55	2.35	1.03 (0.31)	0.42	2.01
Ct.Po (%)	5.6 (2.4)	1.6	17.0	3.8 (2.1)	0.7	10.4
Ct.BMD (mg/cm^3)	748.7 (88.5)	577.5	925.3	773.8 (112.6)	552.9	938.1
Tt.BMD (mg/cm^3)	294.4 (59.6)	167.8	441.6	281.7 (60.3)	165.4	466.9
Ct.Ar (mm^2)	119.0 (36.4)	49.4	231.4	92.8 (27.3)	38.2	162.7
Tt.Ar (mm^2)	749.9 (133.2)	454.6	1127.8	624.9 (90.5)	426.9	943.3
F.Load (N)	6212.5 (1683.0)	2185	10700	4912.8 (1317.5)	2434	8425
U.Stress (MPa)	34.3 (9.9)	10.8	55.9	31.7 (9.7)	13.2	60.3
Distal Radius[a]						
Tb.N (1/mm)	1.98 (0.26)	1.28	2.56	1.97 (0.26)	1.37	2.48
Tb.Th (mm)	0.080 (0.015)	0.050	0.136	0.072 (0.010)	0.053	0.120
Tb.Sp (mm)	0.433 (0.070)	0.316	0.689	0.446 (0.073)	0.315	0.673
BV/TV	0.158 (0.030)	0.082	0.246	0.141 (0.026)	0.075	0.237
Ct.Th (mm)	1.02 (0.32)	0.40	1.81	0.87 (0.28)	0.41	1.62
Ct.Po (%)	3.4 (2.1)	0.4	11.2	2.4 (1.9)	0.1	9.8
Ct.BMD (mg/cm^3)	729.9 (109.7)	485.5	934.6	735.3 (138.8)	446.3	966.6
Tt.BMD (mg/cm^3)	326.3 (81.8)	155.7	558.9	305.4 (77.9)	167.1	498.9
Ct.Ar (mm^2)	60.7 (21.3)	24.1	104.4	45.1 (16.7)	18.7	92.5
Tt.Ar (mm^2)	262.7 (59.6)	142.8	431.1	206.3 (36.8)	128.7	317.8
F.Load (N)	2295.3 (851.6)	628.5	4576.0	1588.1 (571.1)	477.0	3103.0
U.Stress (MPa)	34.9 (13.5)	4.7	67.4	29.5 (11.4)	6.1	59.9
Load-to-strength Ratio	1.39 (0.62)	0.63	3.80	1.89 (0.73)	0.90	5.11

[a]Sample size for radius parameters: boys (n = 166), girls (n = 185)

Maturity offset is estimated as years from age at peak height velocity (APHV)

SD, standard deviation; Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation; BV/TV, trabecular bone volume to total volume fraction; Ct.Th, cortical thickness; Ct.Po, cortical porosity, Ct.BMD, cortical bone mineral density; Tt.BMD, total bone mineral density; Ct.Ar, cortical area; Tt.Ar, total area; F.Load, failure load; U.Stress, ultimate stress.

Table 5.3 Number of HR-pQCT measurements by sex, site and maturity offset.

	Tibia		Radius	
Maturity offset	**Boys**	**Girls**	**Boys**	**Girls**
-4	3	-	3	-
-3	22	3	22	3
-2	36	19	35	18
-1	37	55	34	54
0	31	69	26	69
1	31	67	15	66
2	59	33	19	26
3	65	21	44	11
4	79	39	52	10
5	76	61	58	33
6	68	57	50	44
7	43	63	38	42
8	28	64	25	40
9	12	38	12	35
10	5	34	5	33
11	1	19	1	19
12	-	2	-	2
Total	596	644	439	505

Table 5.4. Estimates of model intercepts for the effects of maturity, sex and ethnicity as predictors of bone parameters at the distal tibia at age at peak height velocity. Numbers in brackets are the standard error of the parameter estimate. Bold values are $p<0.05$.

	Intercept (γ_{00})	**Maturity (γ_{10})**	**Maturity^2 (γ_{20})**	**Maturity^3 (γ_{30})**	**Boys (γ_{01})**	**Boys by Maturity (γ_{11})**	**Boys by Maturity^2 (γ_{21})**	**Boys by Maturity^3 (γ_{31})**	**Ethnicity (γ_{02})**	
									white	**other**
Tb.N (1/mm)	**1.78 (0.02)**	**-0.01 (0.003)**	-	-	**0.07 (0.04)**	**0.01 (0.004)**	-	-	**0.12 (0.03)**	0.05 (0.04)
Tb.Th (mm)	**0.082 (0.001)**	**0.0026 (0.0003)**	**-0.0002 (0.00002)**	-	**0.004 (0.001)**	**-0.002 (0.0004)**	**-0.0001 (0.00004)**	-	**-0.004 (0.001)**	-0.002 (0.002)
Tb.Sp (mm)	**0.489 (0.007)**	0.001 (0.001)	-	-	**-0.003 (0.001)**	**-0.003 (0.001)**	-	-	**-0.034 (0.008)**	-0.013 (0.013)
BV/TV	**0.147 (0.002)**	**0.002 (0.0003)**	**-0.0001 (0.00003)**	-	**0.010 (0.003)**	**0.002 (0.001)**	0.0001 (0.0001)	-	0.005 (0.003)	0.003 (0.004)
Ct.Th (mm)	**0.84 (0.02)**	**0.07 (0.01)**	0.001 (0.001)	**-0.004 (0.001)**	**0.12 (0.003)**	**0.02 (0.01)**	**0.01 (0.002)**	**-0.001 (0.0001)**	-0.01 (0.02)	**0.10 (0.02)**
Ct.Po (%)	**4.9 (0.2)**	**-0.3 (0.1)**	**-0.1 (0.01)**	**0.01 (0.001)**	**2.2 (0.3)**	-0.1 (0.1)	**-0.1 (0.02)**	**0.01 (0.002)**	0.3 (0.2)	-0.1 (0.3)
Ct.BMD (mg/cm^3)	**679.9 (4.6)**	**25.7 (1.4)**	**2.3 (0.3)**	**-0.3 (0.02)**	**-11.8 (6.0)**	**-3.9 (1.8)**	**2.7 (0.4)**	**-0.3 (0.04)**	-4.1 (3.9)	**16.2 (4.6)**
Tt.BMD (mg/cm^3)	**245.7 (4.5)**	**8.1 (0.9)**	**1.2 (0.2)**	**-0.1 (0.01)**	6.1 (5.5)	**4.0 (1.3)**	**1.0 (0.3)**	**-0.1 (0.02)**	1.1 (4.9)	15.8 (8.3)
Ct.Ar (mm^2)	**74.3 (1.9)**	**8.3 (0.4)**	**-0.5 (0.04)**	-	**25.6 (2.2)**	**1.4 (0.6)**	0.1 (0.1)	-	3.5 (2.2)	**10.7 (3.7)**
Tt.Ar (mm^2)	**575.9 (10.3)**	**39.4 (1.8)**	**-6.7 (0.5)**	**0.3 (0.03)**	**162.3 (12.1)**	**-7.6 (2.5)**	**-2.5 (0.6)**	**0.3 (0.1)**	**66.4 (11.8)**	27.8 (19.7)
F.Load (N)	**3938.6 (90.2)**	**485.6 (15.8)**	**-27.8 (1.4)**	-	**1381.8 (104.8)**	**56.0 (22.7)**	-1.7(2.3)	-	**346.8 (105.7)**	**484.2 (177.0)**
U.Stress (MPa)	**26.8 (0.7)**	**1.9 (0.2)**	0.004 (0.039)	**-0.01 (0.003)**	1.7 (0.9)	0.2 (0.2)	**0.2 (0.1)**	**-0.02 (0.004)**	-0.4 (0.8)	**2.6 (1.3)**

Maturity is estimated as years from age at peak height velocity (APHV).

Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation; BV/TV, trabecular bone volume to total volume fraction; Ct.Th, cortical thickness; Ct.Po, cortical porosity; Ct.BMD, cortical bone mineral density; Tt.BMD, total bone mineral density; Ct.Ar, cortical area; Tt.Ar, total area; F.Load, failure load; U.Stress, ultimate stress.

Table 5.5. Estimates of model intercepts for the effects of maturity, sex and ethnicity as predictors of bone parameters at the distal radius at age at peak height velocity. Numbers in brackets are the standard error of the parameter estimate. Bold values are $p<0.05$.

	Intercept (γ_{00})	**Maturity (γ_{10})**	**Maturity^2 (γ_{20})**	**Maturity^3 (γ_{30})**	**Boys (γ_{01})**	**Boys by Maturity (γ_{11})**	**Boys by Maturity^2 (γ_{21})**	**Boys by Maturity^3 (γ_{31})**	**Ethnicity (γ_{02})**	
									white	**other**
Tb.N (1/mm)	**2.00 (0.02)**	**-0.05 (0.01)**	**0.004 (0.001)**	-	0.03 (0.03)	**0.02 (0.01)**	-0.002 (0.001)	-	0.04 (0.02)	0.01 (0.04)
Tb.Th (mm)	**0.070 (0.001)**	**0.002 (0.0003)**	**-0.0001 (0.00003)**	-	**0.005 (0.001)**	**0.001 (0.0004)**	0.000001 (0.0001)	-	-0.0001 (0.0012)	-0.002 (0.002)
Tb.Sp (mm)	**0.437 (0.006)**	**0.013 (0.002)**	**-0.001 (0.0002)**	-	-0.011 (0.007)	**-0.007 (0.002)**	0.001 (0.0003)	-	**-0.013 (0.006)**	0.001 (0.011)
BV/TV	**0.139 (0.003)**	-0.0001 (0.0004)	-	-	**0.010 (0.003)**	**0.003 (0.001)**	-	-	0.004 (0.003)	-0.002 (0.005)
Ct.Th (mm)	**0.63 (0.01)**	**0.03 (0.01)**	**0.02 (0.002)**	**-0.001 (0.0001)**	**0.05 (0.02)**	**0.04 (0.01)**	**0.01 (0.002)**	**-0.001 (0.0002)**	**-0.07 (0.02)**	-0.02 (0.03)
Ct.Po (%)	**3.8 (0.2)**	**-0.2 (0.04)**	**-0.1 (0.01)**	**0.01 (0.001)**	**1.1 (0.2)**	**-0.1 (0.03)**	-	-	**0.3 (0.1)**	0.1 (0.2)
Ct.BMD (mg/cm^3)	**599.2 (5.2)**	**14.4 (2.0)**	**7.9 (0.6)**	**-0.6 (0.04)**	1.4 (7.4)	**5.1 (2.5)**	-0.3 (0.7)	-0.1 (0.1)	**-20.9 (4.8)**	-7.7 (8.0)
Tt.BMD (mg/cm^3)	**244.7 (4.5)**	**4.2 (1.3)**	**4.1 (0.4)**	**-0.3 (0.03)**	8.4 (5.8)	**8.5 (1.7)**	0.3 (0.5)	-0.1 (0.4)	**-16.6 (5.1)**	-10.0 (8.6)
Ct.Ar (mm^2)	**30.5 (0.8)**	**3.2 (0.2)**	**0.5 (0.08)**	**-0.1 (0.01)**	**8.2 (1.1)**	**2.0 (0.3)**	**0.4 (0.1)**	**-0.1 (0.01)**	-0.1 (0.9)	0.7 (1.5)
Tt.Ar (mm^2)	**185.0 (3.9)**	**21.3 (1.1)**	**-3.9 (0.3)**	**0.2 (0.02)**	**58.2 (5.1)**	**-4.5 (1.4)**	0.5 (0.64)	-0.02 (0.04)	**40.1 (4.3)**	**21.3 (7.2)**
F.Load (N)	**1124.7 (34.4)**	**185.8 (8.4)**	**-9.5 (0.9)**	-	**567.3 (40.6)**	**28.7 (11.5)**	1.8 (1.5)	-	**193.0 (41.1)**	113.8 (68.6)
U.Stress (MPa)	**22.1 (0.8)**	**1.4 (0.3)**	**0.3 (0.1)**	**-0.03 (0.01)**	**2.5 (1.1)**	**1.0 (0.3)**	0.1 (0.1)	**-0.02 (0.01)**	**-1.8 (0.9)**	-0.8 (1.5)

	Intercept (γ_{00})	**Maturity (γ_{10})**	**Maturity^2 (γ_{20})**	**Maturity^3 (γ_{30})**	**Boys (γ_{01})**	**Boys by Maturity (γ_{11})**	**Boys by Maturity^2 (γ_{21})**	**Boys by Maturity^3 (γ_{31})**	**Ethnicity (γ_{02})**	
									white	**other**
Load-to-Strength Ratio	**2.4 (0.04)**	**-0.3 (0.01)**	**0.02 (0.001)**	**-**	**-0.6 (0.1)**	**0.1 (0.02)**	**-0.004 (0.002)**	**-**	**-0.1 (0.03)**	-0.1 (0.1)

Maturity is estimated as years from age at peak height velocity (APHV).
Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation; BV/TV, trabecular bone volume to total volume fraction; Ct.Th, cortical thickness; Ct.Po, cortical porosity; Ct.BMD, cortical bone mineral density; Tt.BMD, total bone mineral density; Ct.Ar, cortical area; Tt.Ar, total area; F.Load, failure load; U.Stress, ultimate stress.

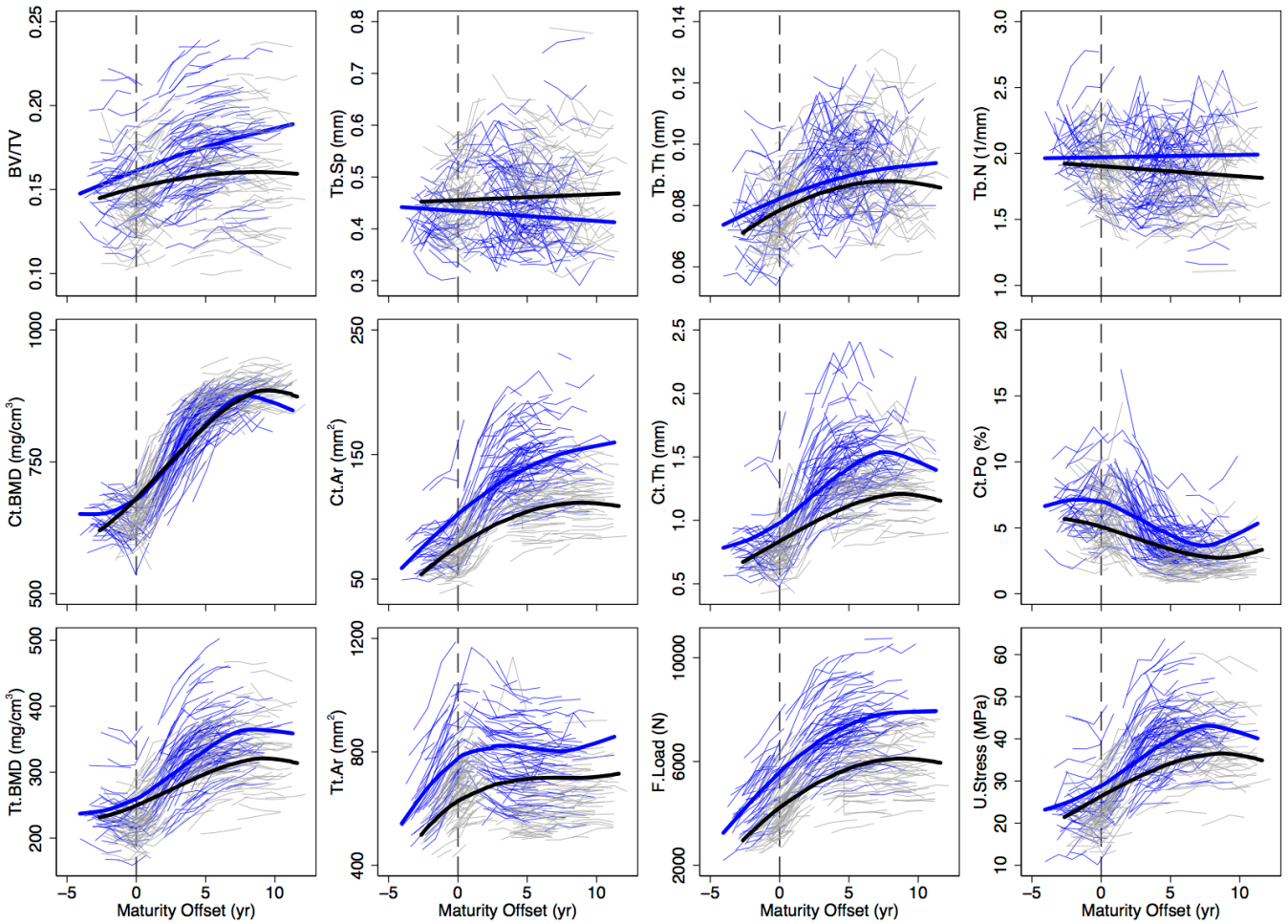


Figure 5.2. Distal tibia individual growth curves for boys (thin, blue lines) and girls (thin, grey lines) and the polynomial mixed model growth curves for boys (thick, blue line) and girls (thick, black line) for trabecular bone volume fraction (BV/TV), separation (Tb.Sp), thickness (Tb.Th) and number (Tb.N), cortical BMD (Ct.BMD), area (Ct.Ar), thickness (Ct.Th) and porosity (Ct.Po), and total BMD (Tt.BMD), area (Tt.Ar), failure load (F.Load), and ultimate stress (U.Stress). The vertical line indicates maturity offset (years from age at peak height velocity) of 0.

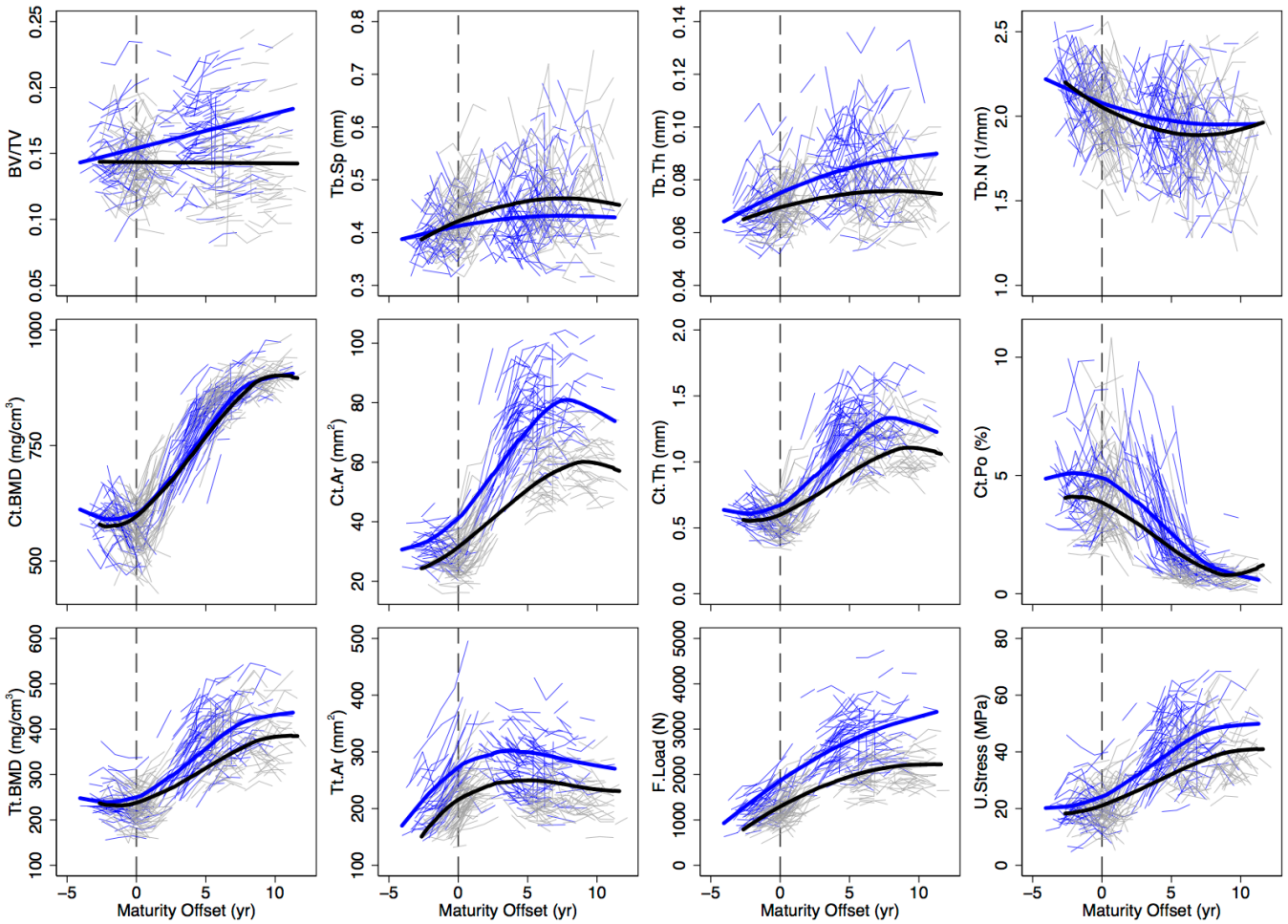


Figure 5.3. Distal radius individual growth curves for boys (thin, blue lines) and girls (thin, grey lines) and the polynomial mixed model growth curves for boys (thick, blue line) and girls (thick, black line) for trabecular bone volume fraction (BV/TV), separation (Tb.Sp), thickness (Tb.Th) and number (Tb.N), cortical BMD (Ct.BMD), area (Ct.Ar), thickness (Ct.Th) and porosity (Ct.Po), and total BMD (Tt.BMD), area (Tt.Ar), failure load (F.Load), and ultimate stress (U.Stress). The vertical line indicates maturity offset (years from age at peak height velocity) of 0.

5.3.2 General growth patterns at the distal tibia and radius

Based on growth curves for HR-pQCT outcomes (Figures 5.2 and 5.3), boys and girls demonstrated net gains in Tb.Th, Ct.Th, Ct.Ar, Tt.Ar, Ct.BMD, Tt.BMD, F.Load and U.Stress and net losses in Ct.Po across 12 years of adolescent growth at both the distal tibia and radius. Trajectories for most parameters at both sites indicated increases during adolescence in boys and girls. However, curves for Ct.BMD, Tt.BMD and Ct.Th at the distal radius suggest transient decreases around APHV (approximately 13.1 years in boys and 11.5 years in girls). Conversely, curves for Ct.Po suggest a transient increase around APHV at both sites in boys and at the radius in girls, prior to declining after APHV.

The magnitude of change in bone parameters during adolescence was similar between boys and girls at both sites (percent change provided in Tables 5.6 and 5.7). However, trabecular microarchitecture parameters demonstrated some site- and sex-specific variation with maturation. At the distal tibia, Tb.N remained relatively unchanged across adolescence (1% and -5% change in boys and girls, respectively; between maturity offset of -2 to +9). However, at the radius, Tb.N decreased in boys (-9%) and girls (-13%) between maturity offset -2 to +9. Similarly, Tb.Sp demonstrated little change at the distal tibia (-5% and 3% change in boys and girls, respectively), but increased at the distal radius (7% and 17% in boys and girls, respectively). Boys demonstrated approximately 20% increase in BV/TV from maturity offset -2 to +9 at both sites. Girls' BV/TV increased by 10% at the distal tibia and remained relatively stable at the distal radius.

5.3.3 Comparisons of model estimates of bone parameters between boys and girls

5.3.3.1 Tibia

At the distal tibia, boys, compared with girls, demonstrated significantly greater Ct.Th, Ct.Po, Ct.Ar, Tt.Ar, and F.Load at equivalent maturational time points across growth (Figure 5.4). Values were significantly greater for boys from 1 year prior to APHV onwards for BV/TV; 1 year post-APHV and beyond for Tb.N and U.Stress; and beyond 1 year post-APHV for Tt.BMD. Boys demonstrated significantly greater Tb.Th compared with girls prior to APHV and

no differences thereafter. Tb.Sp was significantly lower in boys compared with girls at all maturity offsets post-APHV. Ct.BMD was similar between boys and girls at all maturity offsets except at 9 years post-APHV when girls' values were greater than boys'.

5.3.3.2 Radius

At the distal radius, boys demonstrated significantly greater Ct.Ar, Tt.Ar and F.Load across 12 years of adolescent growth compared with girls (Figure 5.4). Load-to-strength ratio was significantly lower in boys compared with girls at all maturity offsets (Figure 5.5). Bone parameters were significantly greater in boys from 1 year prior to APHV onward for Tb.Th; from APHV onward for BV/TV and Ct.Th; and greater post-APHV for U.Stress and Tt.BMD. Tb.N was significantly greater in boys from 3-5 years post-APHV. Tb.Sp was significantly lower in boys compared with girls from 2 to 8 years post-APHV. Ct.Po was greater in boys compared with girls at all maturity offsets except at 9 years post-APHV, when boys' Ct.Po was the same as girls'. There were no significant sex differences in Ct.BMD at any maturity offset.

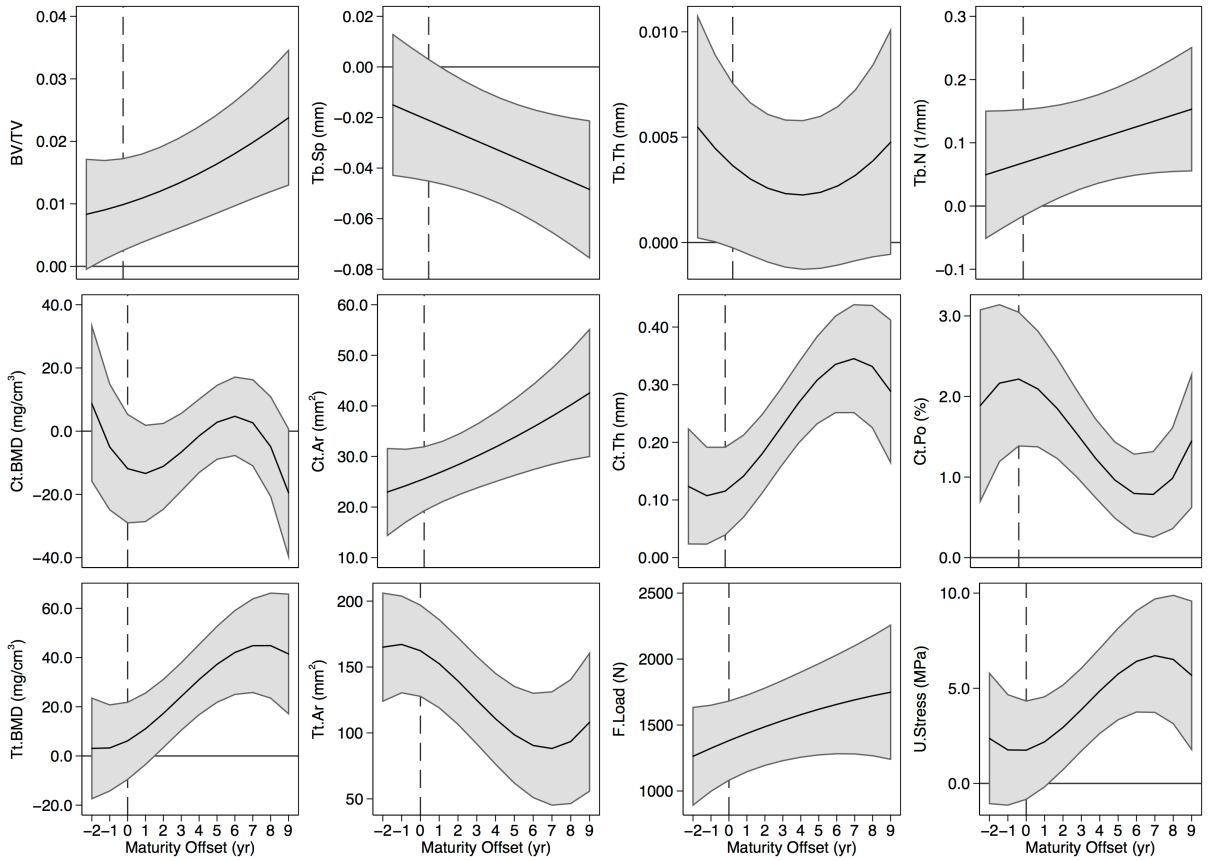


Figure 5.4. Sex differences in distal tibia trabecular bone volume fraction (BV/TV), separation (Tb.Sp), thickness (Tb.Th) and number (Tb.N), cortical BMD (Ct.BMD), area (Ct.Ar), thickness (Ct.Th) and porosity (Ct.Po), and total BMD (Tt.BMD), area (Tt.Ar), failure load (F.Load), and ultimate stress (U.Stress) across maturity. The solid black line represents the mean predicted sex difference (boys - girls) accompanied by a shaded 95% confidence interval, correcting for multiple comparisons using a Bonferroni adjustment. Estimates above 0 indicate significantly greater values in boys, while estimates below zero indicate significantly greater values in girls. Confidence intervals that cross 0 indicate non-significant sex differences. The vertical line indicates maturity offset (years from age at peak height velocity) of 0.

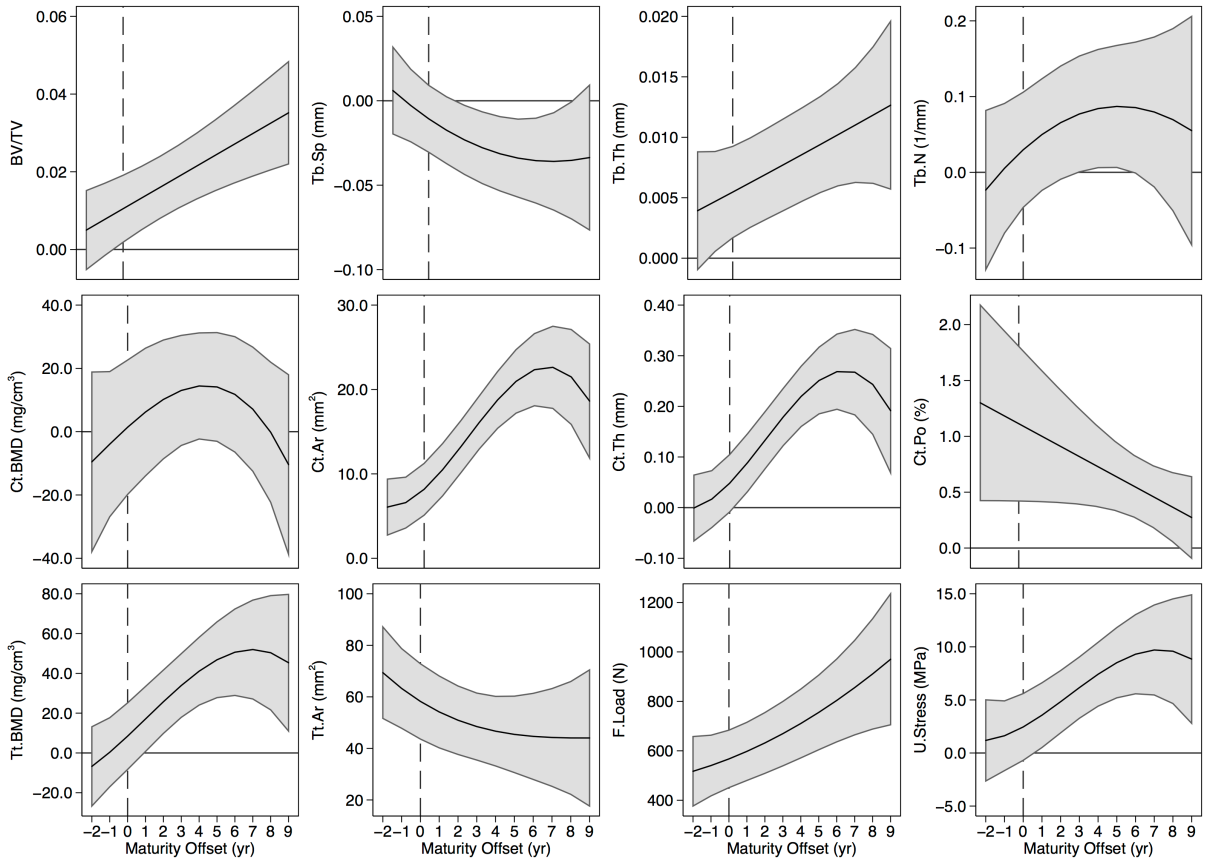


Figure 5.5. Sex differences in distal radius trabecular bone volume fraction (BV/TV), separation (Tb.Sp), thickness (Tb.Th) and number (Tb.N), cortical BMD (Ct.BMD), area (Ct.Ar), thickness (Ct.Th) and porosity (Ct.Po), and total BMD (Tt.BMD), area (Tt.Ar), failure load (F.Load), and ultimate stress (U.Stress) across maturity. The solid black line represents the mean predicted sex difference (boys - girls) accompanied by a shaded 95% confidence interval, correcting for multiple comparisons using a Bonferroni adjustment. Estimates above 0 indicate significantly greater values in boys, while estimates below zero indicate significantly greater values in girls. Confidence intervals that cross 0 indicate non significant sex differences. The vertical line indicates maturity offset (years from age at peak height velocity) of 0.

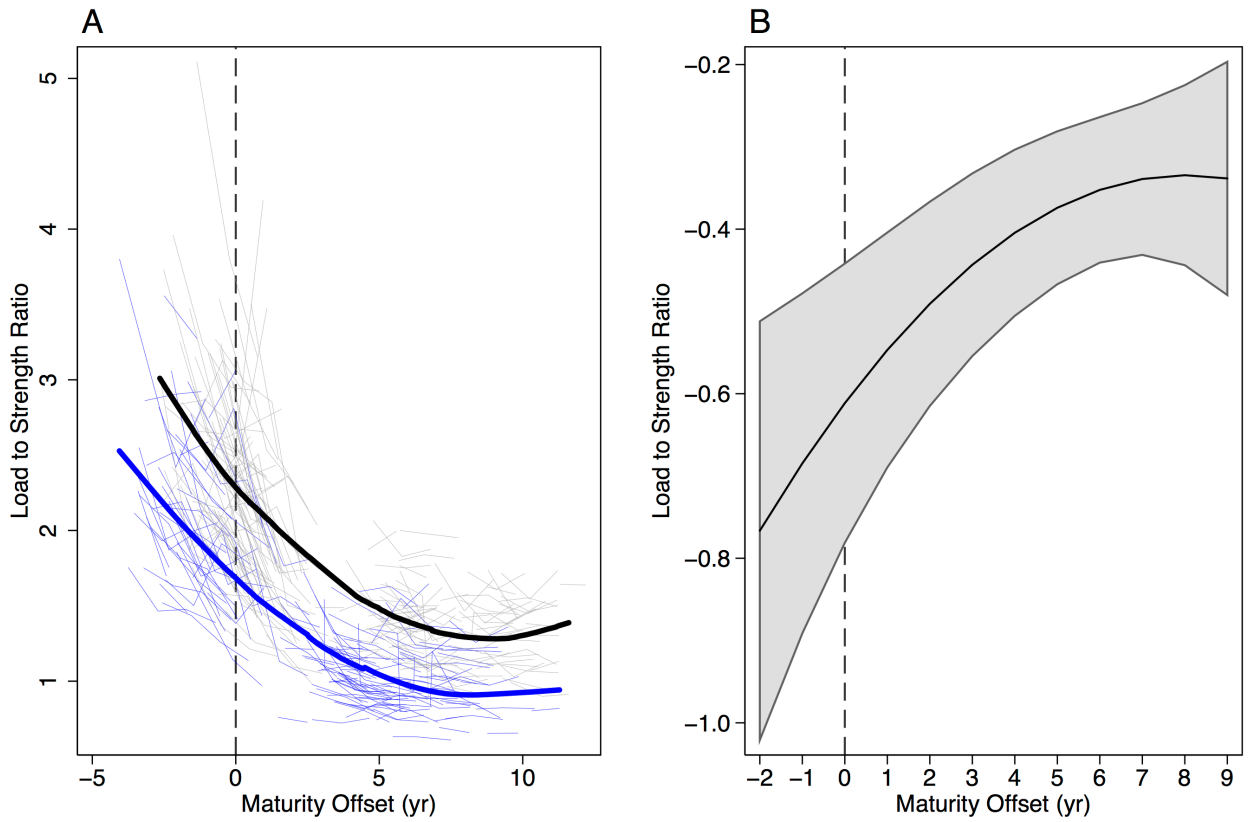


Figure 5.6. Load to strength ratio at the distal radius. (A) displays individual data and predicted growth curves for boys (thin black lines and thick black line) and girls (thin grey lines and thick blue line). (B) displays predicted sex differences (boys-girls) across maturity with 95% confidence intervals, correcting for multiple comparisons using a Bonferroni adjustment. Estimates above 0 indicate significantly greater values in boys, while estimates below zero indicate significantly greater values in girls. Confidence intervals that cross 0 indicate non significant sex differences.

Table 5.6. Adjusted means for bone parameters at the distal tibia at each maturity offset in boys (B) and girls (G). Maturity offset is years from age at peak height velocity. Data are presented as mean (standard error). Percent change is calculated over 12 years (from a maturity offset of -2 to a maturity offset of +9).

		Maturity offset												
		-2	**-1**	**0**	**+1**	**+2**	**+3**	**+4**	**+5**	**+6**	**+7**	**+8**	**+9**	**%Δ**
Tb.N (1/mm)	B	1.91 (0.02)	1.91 (0.02)	1.91 (0.02)	1.91 (0.02)[a]	1.91 (0.02)[a]	1.91 (0.02)[a]	1.92 (0.02)[a]	1.92 (0.02)[a]	1.92 (0.02)[a]	1.92 (0.02)[a]	1.92 (0.02)[a]	1.93 (0.03)[a]	1.0
	G	1.86 (0.02)	1.85 (0.02)	1.84 (0.02)	1.83 (0.02)	1.83 (0.02)	1.82 (0.02)	1.81 (0.02)	1.80 (0.02)	1.80 (0.02)	1.79 (0.02)	1.78 (0.02)	1.77 (0.02)	-4.5
Tb.Th (mm)	B	0.080 (0.001)[a]	0.082 (0.001)[a]	0.084 (0.001)	0.086 (0.001)	0.088 (0.001)	0.089 (0.001)	0.091 (0.001)	0.092 (0.001)	0.093 (0.001)	0.093 (0.001)	0.094 (0.001)	0.095 (0.001)	18.1
	G	0.075 (0.001)	0.078 (0.001)	0.081 (0.001)	0.083 (0.001)	0.085 (0.001)	0.087 (0.001)	0.088 (0.001)	0.089 (0.001)	0.090 (0.001)	0.090 (0.001)	0.090 (0.001)	0.090 (0.001)	20.4
Tb.Sp (mm)	B	0.455 (0.007)	0.453 (0.006)	0.451 (0.006)	0.449 (0.006)	0.447 (0.005)	0.445 (0.005)	0.444 (0.005)	0.442 (0.005)	0.440 (0.006)	0.438 (0.006)	0.436 (0.007)	0.434 (0.007)	-4.6
	G	0.473 (0.007)	0.471 (0.006)	0.472 (0.006)	0.473 (0.006)[a]	0.475 (0.005)[a]	0.476 (0.005)[a]	0.477 (0.005)[a]	0.478 (0.005)[a]	0.479 (0.005)[a]	0.480 (0.005)[a]	0.481 (0.006)[a]	0.483 (0.006)[a]	2.6
BV/TV	B	0.152 (0.002)	0.156 (0.002)[a]	0.159 (0.002)[a]	0.162 (0.002)[a]	0.165 (0.002)[a]	0.168 (0.002)[a]	0.171 (0.002)[a]	0.173 (0.002)[a]	0.176 (0.002)[a]	0.178 (0.002)[a]	0.180 (0.003)[a]	0.182 (0.003)[a]	19.5
	G	0.144 (0.002)	0.147 (0.002)	0.149 (0.002)	0.151 (0.002)	0.153 (0.002)	0.155 (0.002)	0.156 (0.002)	0.157 (0.002)	0.158 (0.002)	0.158 (0.002)	0.158 (0.002)	0.158 (0.002)	9.9
Ct.Th (mm)	B	0.85 (0.02)[a]	0.89 (0.02)[a]	0.96 (0.02)[a]	1.06 (0.02)[a]	1.16 (0.02)[a]	1.27 (0.02)[a]	1.37 (0.02)[a]	1.46 (0.02)[a]	1.53 (0.02)[a]	1.57 (0.02)[a]	1.57 (0.03)[a]	1.53 (0.03)[a]	79.3
	G	0.73 (0.03)	0.79 (0.02)	0.85 (0.02)	0.91 (0.02)	0.98 (0.02)	1.04 (0.02)	1.10 (0.02)	1.16 (0.02)	1.20 (0.02)	1.23 (0.02)	1.24 (0.02)	1.24 (0.03)	70.1
Ct.Po (%)	B	7.2 (0.3)[a]	7.4 (0.2)[a]	7.2 (0.2)[a]	6.8 (0.2)[a]	6.1 (0.1)[a]	5.4 (0.1)[a]	4.7 (0.1)[a]	4.0 (0.1)[a]	3.5 (0.1)[a]	3.2 (0.1)[a]	3.3 (0.2)[a]	3.9 (0.2)[a]	-46.6
	G	5.3 (0.3)	5.2 (0.2)	5.0 (0.2)	4.7 (0.2)	4.3 (0.2)	3.9 (0.1)	3.4 (0.1)	3.0 (0.1)	2.7 (0.1)	2.5 (0.1)	2.4 (0.2)	2.4 (0.2)	-54.9
Ct.BMD (mg/cm^3)	B	648.3 (5.8)	651.5 (4.9)	667.8 (4.2)	694.0 (3.7)	727.0 (3.3)	763.4 (3.0)	800.2 (2.9)	834.2 (3.0)	862.1 (3.2)	880.7 (3.5)	886.9 (4.3)	877.5 (5.9)	35.4
	G	639.6 (6.3)	656.5 (4.9)	679.6 (4.2)	707.4 (3.8)	738.1 (3.4)	770.1 (3.1)	801.7 (2.8)	831.4 (2.8)	854.4 (2.9)	878.0 (3.2)	891.8 (3.5)	896.9 (3.9)[a]	40.2
Tt.BMD (mg/cm^3)	B	240.2 (5.0)	244.2 (4.3)	253.9 (3.9)	267.9 (3.6)	284.8 (3.4)[a]	303.3 (3.4)[a]	321.8 (3.6)[a]	339.1 (4.0)[a]	353.6 (4.4)[a]	364.0 (4.8)[a]	368.8 (5.5)[a]	366.7 (6.4)[a]	52.6
	G	237.2 (5.1)	240.9 (4.3)	247.7 (3.9)	256.9 (3.6)	267.6 (3.4)	279.1 (3.3)	290.8 (3.4)	301.8 (3.7)	311.5 (4.1)	319.1 (3.5)	323.9 (5.1)	325.2 (5.6)	37.1
Ct.Ar (mm^2)	B	81.2 (2.1)	92.3 (1.8)	102.5 (1.6)	111.8 (1.5)	120.2 (1.5)	127.7 (1.6)	134.3 (1.7)	140.0 (1.9)	144.8 (2.2)	148.7 (2.5)	151.7 (2.9)	153.9 (3.3)	89.4

		Maturity offset												
		-2	**-1**	**0**	**+1**	**+2**	**+3**	**+4**	**+5**	**+6**	**+7**	**+8**	**+9**	**%Δ**
	G	58.3 (2.1)[a]	68.1 (1.8)[a]	76.9 (1.6)[a]	84.8 (1.5)[a]	91.6 (1.5)[a]	97.4 (1.5)[a]	102.2 (1.7)[a]	106.1 (1.8)[a]	108.9 (2.0)[a]	110.7 (2.2)[a]	111.5 (2.5)[a]	111.3 (2.8)[a]	91.0
Tt.Ar (mm^2)	B	665.5 (10.0)[a]	729.5 (9.1)[a]	771.2 (8.5)[a]	794.3 (8.3)[a]	802.9 (8.2)[a]	800.7 (8.4)[a]	791.7 (8.8)[a]	779.7 (9.4)[a]	768.6 (10.1)[a]	762.3 (10.9)[a]	764.6 (12.0)[a]	779.4 (13.6)[a]	17.1
	G	500.4 (10.2)	562.4 (9.0)	608.8 (8.5)	641.8 (8.2)	663.5 (8.1)	675.9 (8.0)	681.1 (8.2)	681.2 (8.7)	678.1 (9.4)	674.1 (10.3)	671.1 (11.2)	671.2 (12.1)	34.1
F.Load (N)	B	4378.8 (91.0)[a]	4982.0 (80.2)[a]	5526.1 (74.4)[a]	6011.2 (72.6)[a]	6437.2 (73.9)[a]	6804.1 (77.2)[a]	7111.9 (81.9)[a]	7360.6 (88.1)[a]	7550.3 (95.8)[a]	7680.8 (105.7[a]	7752.3 (118.1)[a]	7764.7 (133.7)[a]	77.3
	G	3115.7 (91.7)	3657.9 (80.6)	4144.3 (73.7)	4575.1 (70.6)	4950.1 (70.7)	5269.5 (73.1)	5533.1 (77.1)	5741.1 (82.4)	5893.4 (88.9)	5989.9 (96.5)	6030.8 (105.7)	6015.9 (116.6)	93.1
	B	25.5 (0.8)	26.7 (0.7)	28.6 (0.6)	31.0 (0.6)	33.6 (0.5)[a]	36.3 (0.5)[a]	38.9 (0.6)[a]	41.1 (0.6)[a]	42.9 (0.7)[a]	43.9 (0.8)[a]	44.0 (0.9)[a]	43.1 (1.0)[a]	69.1
U.Stress (MPa)	G	25.5 (0.8)	25.0 (0.7)	26.9 (0.6)	28.8 (0.6)	30.7 (0.6)	32.4 (0.5)	34.0 (0.5)	35.4 (0.6)	36.5 (0.6)	37.2 (0.7)	37.5 (0.8)	37.4 (0.9)	61.8

[a]Significant difference between boys and girls with Bonferroni adjustment, $p < 0.0042$.

Table 5.7. Adjusted means for bone parameters at the distal radius at each maturity offset in boys (B) and girls (G). Maturity offset is years from age at peak height velocity. Data are presented as mean (standard error). Percent change is calculated over 12 years (from a maturity offset of -2 to a maturity offset of +9).

		Maturity offset												
		-2	**-1**	**0**	**+1**	**+2**	**+3**	**+4**	**+5**	**+6**	**+7**	**+8**	**+9**	**%Δ**
Tb.N (1/mm)	B	2.12 (0.03)	2.08 (0.02)	2.05 (0.02)	2.02 (0.02)	2.00 (0.02)	1.97 (0.02)[a]	1.96 (0.02)[a]	1.94 (0.02)[a]	1.93 (0.02)	1.93 (0.03)	1.93 (0.03)	1.93 (0.04)	-9.0
	G	2.14 (0.03)	2.08 (0.02)	2.02 (0.02)	1.97 (0.02)	1.93 (0.002)	1.90 (0.02)	1.87 (0.02)	1.86 (0.02)	1.85 (0.02)	1.85 (0.02)	1.86 (0.02)	1.87 (0.02)	-12.6
Tb.Th (mm)	B	0.070 (0.001)	0.073 (0.001)[a]	0.075 (0.001[a]	0.078 (0.001[a]	0.080 (0.001)[a]	0.082 (0.001)[a]	0.083 (0.001)[a]	0.085 (0.001)[a]	0.086 (0.001)[a]	0.087 (0.001)[a]	0.088 (0.002)[a]	0.088 (0.002[a]	26.4
	G	0.066 (0.001)	0.068 (0.001)	0.070 (0.001)	0.071 (0.001)	0.073 (0.001)	0.074 (0.001)	0.075 (0.001)	0.075 (0.001)	0.076 (0.001)	0.076 (0.001)	0.076 (0.001)	0.076 (0.001)	14.8
Tb.Sp (mm)	B	0.408 (0.006)	0.415 (0.005)	0.420 (0.005)	0.425 (0.005)	0.429 (0.005)	0.433 (0.005)	0.436 (0.005)	0.438 (0.006)	0.439 (0.006)	0.439 (0.007)	0.439 (0.010)	0.438 (0.012)	7.3
	G	0.402 (0.007)	0.417 (0.005)	0.431 (0.005)	0.443 (0.005)	0.453 (0.005)[a]	0.461 (0.005)[a]	0.467 (0.005)[a]	0.472 (0.006)[a]	0.474 (0.006)[a]	0.475 (0.007)[a]	0.474 (0.007)[a]	0.472 (0.009)	17.3
BV/TV	B	0.146 (0.003)	0.149 (0.002)	0.152 (0.002)[a]	0.154 (0.002[a]	0.157 (0.002)[a]	0.159 (0.002)[a]	0.162 (0.002)[a]	0.165 (0.002)[a]	0.167 (0.003)[a]	0.170 (0.003)[a]	0.173 (0.003)[a]	0.175 (0.003)[a]	20.0
	G	0.141 (0.002)	0.141 (0.002)	0.141 (0.002)	0.141 (0.002)	0.141 (0.002)	0.141 (0.002)	0.141 (0.002)	0.141 (0.002)	0.141 (0.002)	0.140 (0.002)	0.140 (0.003)	0.140 (0.003)	-0.7
Ct.Th (mm)	B	0.61 (0.02)	0.60 (0.01)	0.65 (0.01)	0.73 (0.01)[a]	0.84 (0.01)[a]	0.97 (0.01)[a]	1.09 (0.01)[a]	1.21 (0.02)[a]	1.31 (0.02)[a]	1.38 (0.02)[a]	1.40 (0.03)[a]	1.36 (0.03)[a]	129.3
	G	0.61 (0.02)	0.59 (0.01)	0.60 (0.01)	0.64 (0.01)	0.71 (0.01)	0.79 (0.01)	0.87 (0.01)	0.96 (0.02)	1.04 (0.02)	1.11 (0.02)	1.15 (0.02)	1.17 (0.03)	89.1
Ct.Po (%)	B	5.1 (0.3)[a]	5.2 (0.2)[a]	5.1 (0.2)[a]	4.7 (0.2)[a]	4.2 (0.1)[a]	3.6 (0.1)[a]	3.0 (0.1)[a]	2.3 (0.1)[a]	1.8 (0.1)[a]	1.3 (0.1)[a]	0.9 (0.1)[a]	0.8 (0.1)	-85.4
	G	3.8 (0.2)	4.0 (0.2)	4.0 (0.2)	3.7 (0.2)	3.3 (0.1)	2.8 (0.1)	2.3 (0.1)	1.7 (0.1)	1.2 (0.1)	0.8 (0.1)	0.5 (0.1)	0.5 (0.1)	-87.4
Ct.BMD (mg/cm^3)	B	587.1 (6.5)	579.1 (5.9)	590.3 (5.7)	616.9 (5.2)	654.8 (4.7)	700.2 (4.2)	749.1 (4.1)	797.4 (4.2)	841.4 (4.5)	876.9 (4.9)	900.1 (5.9)	907.0 (8.5)	54.5
	G	596.7 (7.5)	583.0 (5.3)	588.9 (4.8)	610.7 (4.7)	644.6 (4.6)	687.2 (4.4)	734.6 (4.2)	783.3 (4.2)	829.6 (4.5)	869.8 (4.7)	900.3 (4.9)	917.4 (5.1)	53.8
Tt.BMD (mg/cm^3)	B	239.6 (4.9)	236.7 (4.5)	244.7 (4.3)	261.3 (4.1)[a]	284.3 (3.9)[a]	311.4 (4.0)[a]	340.5 (4.3)[a]	369.3 (4.8)[a]	395.5 (5.5)[a]	416.9 (6.3)[a]	431.3 (7.4)[a]	436.5 (9.3)[a]	82.2
	G	246.4 (5.0)	236.4 (4.0)	236.2 (3.9)	244.2 (3.9)	258.6 (3.9)	277.5 (3.9)	299.4 (4.1)	322.4 (4.6)	344.9 (5.2)	365.0 (6.0)	381.0 (6.8)	391.1 (7.6)	58.8
Ct.Ar (mm^2)	B	32.4 (0.8)[a]	34.4 (0.8)[a]	38.7 (0.8)[a]	44.7 (0.8)[a]	51.7 (0.8)[a]	59.2 (0.8)[a]	66.6 (0.8)[a]	73.2 (1.0)[a]	78.5 (1.1)[a]	81.8 (1.2)[a]	82.6 (1.5)[a]	80.2 (1.8)[a]	147.3

		Maturity offset												
		-2	**-1**	**0**	**+1**	**+2**	**+3**	**+4**	**+5**	**+6**	**+7**	**+8**	**+9**	**%Δ**
	G	26.4 (0.9)	27.8 (0.7)	30.5 (0.7)	34.2 (0.8)	38.5 (0.8)	43.1 (0.8)	47.8 (0.8)	52.2 (0.9)	56.1 (1.0)	59.2 (1.2)	61.1 (1.3)	61.6 (1.5)	133.5
Tt.Ar (mm^2)	B	214.9 (4.4)[a]	243.2 (4.0)[a]	263.6 (3.8)[a]	277.1 (3.5)[a]	284.7 (3.3)[a]	287.4 (3.2)[a]	286.3 (3.4)[a]	282.4 (3.8)[a]	276.7 (4.2)[a]	270.3 (4.8)[a]	264.2 (5.7)[a]	259.3 (7.2)[a]	20.7
	G	145.5 (4.4)	179.9 (3.6)	205.4 (3.4)	223.0 (3.4)	233.8 (3.3)	239.0 (3.2)	240.0 (3.3)	237.0 (3.6)	232.1 (4.0)	226.1 (4.6)	220.1 (5.1)	215.3 (5.7)	48.0
F.Load (N)	B	1331.2 (34.4)[a]	1568.9 (30.6)[a]	1791.0 (29.4)[a]	1997.7 (29.8)[a]	2189.0 (31.0)[a]	2364.8 (32.6)[a]	2525.1 (34.8)[a]	2670.0 (37.9)[a]	2799.4 (42.8)[a]	2913.4 (49.8)[a]	3011.9 (59.4)[a]	3095.0 (71.6)[a]	132.5
	G	814.1 (34.8)	1028.4 (29.8)	1223.7 (27.8)	1400.0 (28.1)	1557.2 (29.6)	1695.4 (31.7)	1814.6 (33.9)	1914.7 (36.6)	1995.8 (39.9)	2057.8 (44.3)	2100.8 (50.4)	2124.8 (58.5)	161.0
	B	21.2 (0.9)	21.8 (0.9)	23.6 (0.8)	26.5 (0.8)[a]	30.0 (0.7)[a]	33.9 (0.7)[a]	37.9 (0.8)[a]	41.8 (0.8)[a]	45.2 (0.9)[a]	47.9 (1.1)[a]	49.7 (1.3)[a]	50.2 (1.7)[a]	137.0
U.Stress (MPa)	G	20.0 (1.0)	20.2 (0.8)	21.2 (0.7)	22.9 (0.7)	25.1 (0.7)	27.7 (0.7)	30.5 (0.7)	33.3 (0.8)	35.9 (0.9)	38.2 (1.0)	40.1 (1.1)	41.3 (1.3)	106.8
Load-to-Strength Ratio	B	2.10 (0.06)	1.88 (0.05)	1.69 (0.04)	1.51 (0.04)	1.36 (0.03)	1.23 (0.03)	1.13 (0.02)	1.05 (0.02)	0.99 (0.02)	0.96 (0.02)	0.95 (0.03)	0.96 (0.04)	-54.8
	G	2.87 (0.06)[a]	2.57 (0.05)[a]	2.30 (0.04)[a]	2.06 (0.03)[a]	1.85 (0.03)[a]	1.68 (0.03)[a]	1.53 (0.03)[a]	1.42 (0.02)[a]	1.34 (0.02)[a]	1.30 (0.02)[a]	1.28 (0.02)[a]	1.30 (0.03)[a]	-54.5

[a]Significant difference between boys and girls with Bonferroni adjustment, $p < 0.0042$.

5.4 Discussion

In this study, I use longitudinal data and mixed modeling approaches to demonstrate sex differences in bone strength and its determinants at the distal tibia and radius across adolescence and into young adulthood. Longitudinal data capture nuanced adaptations of bone over time and overcome many limitations of cross-sectional data. Despite this, few long term prospective studies have been conducted, most likely due to the time and labour intensive nature of this type of investigation. I align boys and girls on a common maturational landmark (maturity offset; years from APHV) to more clearly characterize changes in 3D aspects of bone quality across adolescence. My study highlights similarities in magnitude of bone accrual at the distal tibia compared with the distal radius, and sex differences therein, despite substantially different loading environments. Further, I report significant sex differences in bone microarchitecture, geometry and strength at both sites across growth. I observed substantially more porous cortices in boys in the years around peak linear growth, which may contribute to greater skeletal fragility in boys during adolescence.

5.4.1 Trabecular microarchitecture

Trabecular bone density may increase by way of gains in material density or trabecular number, or through thickening of trabeculae. My finding of increased Tb.Th throughout growth is consistent with previous work.[4,166] That is, increases in the amount of trabecular bone (expressed as BV/TV) during growth are underpinned by thickening of trabeculae, with little to no change in trabecular number or separation. Thickening of trabeculae during growth has been attributed to remodeling with a positive balance, such that more bone is added during each remodeling cycle than was previously resorbed.[166,167] Although a slow process, in theory, trabeculae thicken with each subsequent remodeling cycle.

I also observed site-and sex-specific differences in trabecular volume across adolescence. Boys in my study and others'[4-6] demonstrated consistent increases in trabecular bone volume throughout growth at both distal sites. However, BV/TV at the distal radius did not change significantly in girls. Thus, given similar Tb.N and Tb.Sp, the observed sex difference in BV/TV at the radius is driven by thicker trabeculae in boys. The mechanism underlying the sex-

specificity of BV/TV is not entirely clear. Although we did not assess hormonal markers in our study, Kirmani and colleagues assessed growth and reproductive hormonal markers in their cross-sectional study of bone architecture at the distal radius in 127 participants aged 6-21 years. They noted a significant relationship between BV/TV and testosterone in boys, but no such relationship with any hormones in girls. The authors speculated that perhaps girls' trabecular bone volume at the radius is programmed early in life.[5] Finally, I observed comparable Tb.Th in boys and girls at the distal tibia, suggesting similar adaptations to the greater mechanical loads experienced at this weight-bearing site.

5.4.2 Cortical microarchitecture, bone geometry and estimated bone strength

I noted consistently larger bone size in boys compared with girls at the distal tibia and radius. Similar findings were reported in previous studies that assessed bone using pQCT and HR-pQCT and that accounted for differences in maturational status using the method of Tanner or APHV.[4,6,153,313,358] Even small differences in bone size confer substantial increases in resistance to bone compressive and bending forces.[29] Thus, it is not surprising that boys demonstrated consistently greater bone strength compared with girls across adolescence and into young adulthood at both skeletal sites. At the distal radius, greater bone strength in boys also contributed to a lower load-to-strength ratio, an indicator of fracture risk,[119] compared with girls.

Adaptations at the cortex, specifically changes in porosity during peak growth, also contribute to bone strength. The exponential relationship between porosity and strength dictates that small decreases in porosity may result in large gains in bone strength.[359] I found that adolescent growth is characterized by significant decreases in Ct.Po and concurrent increases in Ct.Th. Importantly, my data and that of others[4,5] show a transient period of increased porosity at the cortex during periods of rapid growth at both skeletal sites. Moreover, I confirm that boys demonstrate greater porosity compared with girls at both bone sites, a finding we previously noted over a shorter time period.[4] This is likely explained by boys' longer period of adolescent growth and greater linear growth velocity.[164,355]

My data also suggest a transient decrease in in Ct.Th and Ct.BMD around peak growth at the distal radius; also observed by others.[5,6] I did not observe thickening of the distal radius

cortex until 1 year post-APHV (approximately 12.5 years and 14.1 years in girls and boys, respectively). This is consistent with the cross-sectional study by Rauch and colleagues, whereby Ct.Th at the distal radius (by pQCT) did not increase until after Tanner Stage 4, or approximately 13.0 years and 15.0 years of age in girls and boys, respectively.[31] The authors contended that distal radius Ct.Th remained relatively stable until late puberty, as endocortical apposition cannot keep pace with rapid periosteal resorption that dominates the process of metaphyseal inwaisting during periods of rapid longitudinal growth.[31] The lag in Ct.Th I observed at the radius may contribute to increased forearm fracture risk. However, this finding contrasts growth patterns at the distal tibia in the current study and in my previous study at the tibial shaft (Chapter 4),[313] where I observed thickening of the cortex throughout growth. Site-specific differences in growth-related adaptations may contribute to substantially different forces experienced at the non weight-bearing radius compared with the weight-bearing tibia.

A heightened period of bone fragility during the pubertal growth spurt is thought a direct result of increased calcium demands, resulting in higher rates of intracortical bone turnover and increased porosity due to incomplete consolidation of bone.[163] Alternatively, trabecular coalescence may be incomplete.[33] Longitudinal growth produces new trabeculae at the growth plate, which eventually coalesce into the cortical shell. That is, the further bone is from the growth plate, the greater number of loading cycles it experiences.[33] Newly formed bone requires time (and probably mechanical stimulus) to coalesce – luxuries not available during rapid adolescent growth. The underlying mechanism for the sex-difference in porosity aligns well with the theory by Tanck,[33] whereby more rapid growth in boys results in more immature bone at metaphyses compared with girls. In my study, Ct.Po was the only bone parameter deficit in boys compared with girls in the years around peak linear growth. Thus, while I cannot rule out other determinants, my findings support the hypothesis that increased Ct.Po during peak growth may contribute to higher incidence of forearm fractures rates in boys during this time.[163]

It is interesting that sex differences in Ct.Po did not manifest in estimates of bone strength and fracture risk at the distal radius. Specifically, boys, in comparison with girls, demonstrated greater estimated bone strength and lower fracture risk despite greater Ct.Po. This finding is consistent with a recent cohort study in which adult forearm fracture cases exhibited significantly more porous cortices at the distal radius (by HR-pQCT) compared with non-fracture controls, despite similar estimates of bone strength.[360] There are several possible explanations

for this discrepancy. First, greater Ct.Po in boys may be somewhat compensated for by their substantially larger bones compared with girls. Second, Ct.Po may be localized to specific regions of the cortex that contribute less to mechanical competence. Porosity varies significantly across regions of the cortex,[361] and porosity on the endocortical surface experiences lower mechanical stress compared with porosity localized to the periosteal surface.[359] Thus, regional analyses within the same bony compartment may help clarify the porosity-strength relationship during growth, in future.[361] Third, the resolution of HR-pQCT may be insufficient to accurately assess porosity at the distal end of the radius where the cortical shell is quite thin; thereby, systemically underestimating Ct.Po,[362] an explanation offered by Vilayphiou and colleagues.[363] Finally, it is possible that FEA estimates of bone strength cannot capture changes in Ct.Po that contribute to transient increases in bone fragility. Forearm fractures are a consequence of compressive and bending forces.[2] Therefore, FEA-estimates of bone strength need to incorporate stress associated with bending in addition to compressive loads.[4]

The transient decreases in Ct.BMD and Tt.BMD observed at the distal radius surrounding peak growth may reflect increased porosity during this time. Despite significant sex differences in porosity and contrary to our previous studies,[4,353] I did not observe denser cortices in girls compared with boys at the distal tibia or radius (with the exception of greater Ct.BMD in girls 9 years post-APHV). Although similar Ct.BMD despite greater Ct.Po among boys suggests that boys may compensate for larger cortical pores with greater cortical material mineral density, I am unable to confirm this using currently available imaging methods. The discrepancy of sex differences in Ct.BMD may be partially explained by methodological differences in maturity assessment, as we relied on self-reported stage of sexual maturation for our previous studies.[127] While feasible for use in cross-sectional and short-term prospective studies, comparisons between sexes at the same Tanner stage are confounded by differences in the timing of growth relative to development of secondary sex characteristics. Specifically, the majority of girls attain PHV by Tanner stage 3, whereas most boys do not reach PHV until Tanner stage 4.[130,131]

In our previous study,[4] when we compared girls and boys at the same maturational stage as per the method of Tanner, we noted significantly greater Ct.BMD in girls compared with boys at peri- (Tanner stage 4) and post-puberty (Tanner stage 5) at both skeletal sites. However, girls would have been more mature than boys at these Tanner Stages, on average, based on APHV. If I account for this by comparing values for girls at Tanner Stage 4 with boys in Tanner Stage 5,

Ct.BMD values are virtually identical at the distal tibia (813 ± 100.8 vs. 815.5 ± 68.6) and radius (835.3 ± 52.1 vs. 821.7 ± 42.7). This premise held true when I visually examined cross-sectional HR-pQCT values at the distal radius as per Kirmani et al[5] and at the radius and tibia as per Wang and colleagues.[6] If one supports this contention, it also explains sex differences reported at the distal radius using pQCT.[358] Thus, the notion girls have greater Ct.BMD at distal sites in later maturity may be an artifact of the method used to control for maturity.

My study has several limitations. First, as in any repeated measures study of growing bone, it is not possible to reassess the exact same bone cross section over time. Long bone growth is both complex and disproportionate; at the tibia, 57% of longitudinal growth occurs at the proximal metaphysis and 43% occurs at the distal metaphysis.[39] Therefore, we used a standard anatomical landmark to identify the same relative site along the length of the tibia and radius at each measurement in every child. Second, based on differences in maturational timing between boys and girls at the same chronological age, many of the girls in my study were post-APHV at baseline. Thus, I was only able to compare boys and girls up to 2 years prior to APHV. Third, I note that our ethnically diverse sample limits the generalizability of my findings outside the Metro Vancouver area, where visible minority groups represent 47% of the population.[351] While I did not specifically aim to examine ethnic differences in bone accrual in the present study, I recognized the need to control for known differences in the timing and tempo of maturation between ethnic groups. For example, Asian participants in our cohort attained APHV approximately 7 months prior to their white peers. I accounted for this by aligning participants on APHV. Fourth, I was unable to align bone data to fracture occurrence. Although we explored the association between bone microarchitecture and forearm fractures in a separate cohort,[357] prospective studies are warranted to clarify the influence of bone microarchitecture on fracture risk. Finally, the minimal change in Tb.N and Tb.Sp observed across growth (1-17%) was in some cases comparable with relatively high least significant change values (LSC % ~15-20%) reported previously.[364] Thus, observed decreases in Tb.N across growth at the distal radius may represent a measurement artifact in cases where resolution of HR-pQCT was unable to capture thin trabeculae.

5.5 Conclusions

My study was uniquely positioned to examine sex differences in growth-related adaptations in bone strength and its determinants across adolescent growth, with boys and girls aligned on a common measure of somatic maturity. I noted boys' superior bone size and strength compared with girls' across maturity. Contrary to previous HR-pQCT studies that compared boys and girls according to self-reported stage of sexual maturation, I did not observe sex differences in Ct.BMD during peak growth. I suggest that compared with girls, boys' substantially more porous cortices throughout growth may partially explain their greater skeletal fragility during the pubertal growth spurt. This hypothesis would benefit from prospective studies comparing microarchitectural parameters between boys and maturity-matched female peers who have sustained a fracture.

Chapter 6: Physical Activity, Sedentary Time and Bone Strength from Childhood to Early Adulthood: A Mixed Longitudinal HR-pQCT Study[9]

SYNOPSIS: In this chapter, I evaluate the influence of PA and sedentary time on growth-related adaptations in bone strength and its determinants at the distal tibia and radius across adolescence. I present this chapter its submitted format with minor modifications.

6.1 Introduction

PA and weight-bearing exercise are essential to develop and maintain a healthy skeleton.[15] In particular, there is strong evidence to suggest that the critical period of pre- and early-puberty may provide a 'window of opportunity' when skeletal benefits of weight-bearing PA can be optimized.[144] In contrast, we know less about the mechanisms underpinning bone's adaptation to PA in later adolescence.[10] This may be due, in part, to reliance on imaging systems such as DXA, as they may be unable to capture subtle adaptations in bone strength and its determinants (i.e., geometry, density and microarchitecture).

In recent years, 3D imaging tools such as pQCT provided new evidence for PA as an important driver of bone strength and its determinants during growth. For example, a cross-sectional study demonstrated significant positive associations between vigorous PA (by accelerometry) and cortical geometry, density and estimated strength at the mid-tibia (strength strain index, SSI; by pQCT) in adolescent boys and girls (n = 1748).[365] Total daily steps (by pedometer) were associated with bone strength (BSI; by pQCT) at the distal tibia and femur in 8 to 13-year old girls (n = 349).[366] In addition, earlier in my thesis (Chapter 3), I demonstrated a positive association at the distal tibia between accelerometry-derived MVPA and estimated bone strength (F.Load assessed with HR-pQCT and FE analysis) and geometry in boys and trabecular and cortical microarchitecture in girls (n = 209).[310] In the only longitudinal study of PA that used pQCT to image bone, the Iowa Bone Development Study demonstrated boys and girls (n =

[9] A version of this chapter was published ahead of print: Gabel L, Macdonald HM, Nettlefold L, McKay HA. Physical activity, sedentary time and bone strength from childhood to early adulthood: a mixed longitudinal HR-pQCT study. Journal of Bone and Mineral Research; Epub ahead of print, DOI: 10.1002/jbmr.3115.

346) who engaged in high levels of MVPA (by accelerometry) throughout growth had significantly greater estimated bone strength at the distal and 33% site of the tibia (BSI and polar moment of inertia) at age 17 compared with those in the lowest MVPA trajectory (based on three MVPA trajectories by group-based trajectory modelling).[289]

Despite numerous health benefits associated with PA, today's youth spend roughly 60% of their waking hours in sedentary activities.[248] Whether sedentary time independently influences health outcomes or simply displaces other forms of PA, is currently debatable.[367] A focus upon the consequences of 'not loading' a healthy growing skeleton is relatively new and few studies (4 DXA studies[286,296,300,301] and 1 HR-pQCT study[310]) investigated the relationship between objectively-measured sedentary time and bone in adolescents, with contradictory findings.[304] A recent systematic review suggested there was insufficient evidence to support an association between sedentary time and bone health, independent of PA.[304] Thus, whether the potentially deleterious influence of sedentary time interacts with the osteogenic effect of PA in healthy, ambulatory children and adolescents remains unclear. Prospective studies would clarify the structural and microarchitectural adaptations associated with sedentary time during growth. They would also ascertain whether sedentary time influences bone development independent of PA.

Thus, my primary aim was to prospectively evaluate associations between PA and growth-related adaptations in bone strength and its determinants at the distal tibia and radius in boys and girls. My secondary aim was to prospectively evaluate associations between sedentary time and growth-related adaptations in bone strength and its determinants at the distal tibia and radius in boys and girls, independent of PA. I hypothesized that PA would positively predict adaptations in bone strength and its determinants, while sedentary time would be negatively related to bone strength and its determinants, independent of PA.

6.2 Methods

I provide a detailed description of study design and methods for data collection in Chapter 2 and a brief overview in the following sections.

6.2.1 Study design

Participants were drawn from a cohort of healthy girls (n = 556) and boys (n = 515) aged 8 to 12 years at study entry who comprised the University of British Columbia HBSIII cohort. My analyses in this study included bone data from annual measurements conducted between May 2008 (first year of HR-pQCT measurements) and June 2012. In the cohort with HR-pQCT and accelerometry data (n = 308; Figure 6.1) we acquired a median of 3 annual measurements at the distal tibia (interquartile range: 2 to 4) and a median of 2 annual measurements at the distal radius (interquartile range: 1 to 3). Average duration between measures was 1.0 years at the tibia and radius. For the purpose of this study, I refer to data obtained at the first HR-pQCT measurement as baseline.

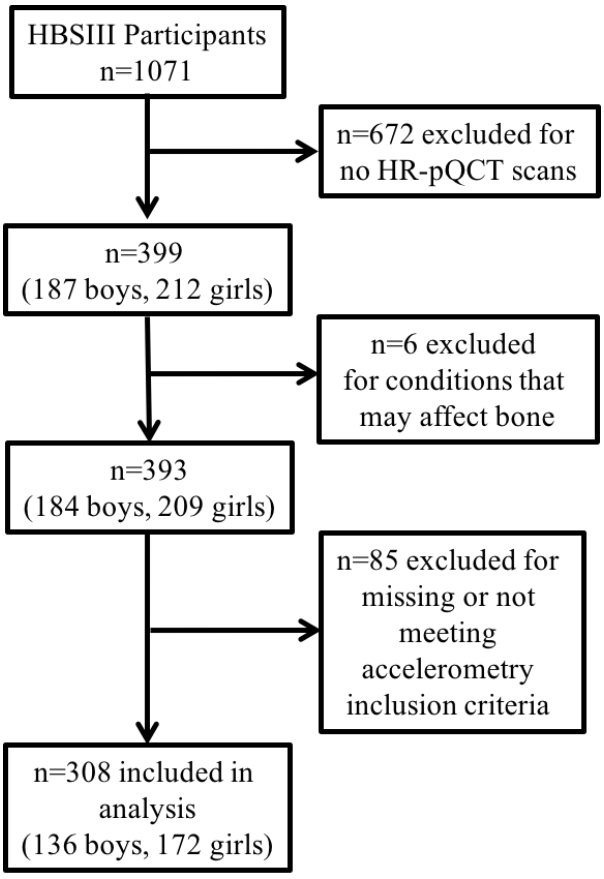


Figure 6.1. Participant inclusion diagram.

6.2.2 Anthropometry and age at peak height velocity

We assessed standing and sitting height, body mass and limb lengths using standard methods. We estimated years from APHV as an estimate of biological maturity offset, as described in Appendix D. Due to missing and mistimed measurements surrounding APHV, we were only able to identify APHV using the cubic spline method in 198 participants (50% of cohort). For the remaining participants, I used the Moore equation[142] and anthropometric data from the measurement occasion closest to the expected APHV (approximately 11.6 years in girls and 13.5 years in boys) to estimate APHV. Thus, I used the measurement occasion when girls were closest to 11.6 years (range 9.5 to 13.1 years) and boys were closest to 13.0 years (10.8 to 14.3 years), on average, to estimate APHV. For all participants I used APHV to calculate a continuous measure of biological maturity offset (in years) by subtracting APHV from chronological age at time of measurement (e.g., -1 year is equivalent to 1 year prior to attainment of APHV; +1 to one year after APHV).

6.2.3 Health history, ethnicity and dietary calcium

We determined health history and ethnicity using a questionnaire, completed by parents at baseline and by participants at subsequent annual visits. Based on questionnaire responses, I identified six participants who had conditions that prevented their participation in regular PA and/or reported medical conditions known to influence bone metabolism (osteogenesis imperfecta, fetal alcohol syndrome, type 1 diabetes, leukemia, congenital heart defect). Following these exclusions, my sample included HR-pQCT data from 393 healthy participants (184 boys, 209 girls). All participants completed a validated food frequency questionnaire to estimate dietary intake of calcium (mg/day).[319]

6.2.4 Physical activity and sedentary time

I estimated objectively measured volume and patterns of sedentary time and MVPA using accelerometers (ActiGraph GT1M; Pensacola, FL) with a 15-sec epoch. Based on accelerometry

wear time criteria (10 h/day on at least 3 days), we excluded 85 participants who did not meet inclusion criteria.

I used a cut point of < 100 cpm to classify sedentary time[239] and the Evenson cut point of ≥ 2296 cpm to classify MVPA.[238,239] I used the residuals approach to control for differences in accelerometer wear time between participants.[368]

6.2.5 Peak muscle power

Muscular contractions impose the largest physiological loads on the skeleton.[68] Thus, we used the Leonardo Mechanograph Ground Reaction Force Plate (GRFP; Novotec, Germany) to assess peak leg muscle power (Watts). I used peak leg muscle power to characterize overall functional muscle power, as we did not assess upper limb muscle power in the current study. Bohannon and colleagues observed a strong correlation between lower leg and upper limb grip strength in adults (r = 0.77-.81), suggesting that lower leg strength may be an acceptable surrogate for grip strength.[369]

6.2.6 Bone microarchitecture and strength

We assessed bone strength and its determinants at the non-dominant distal tibia (8% site) and distal radius (7% site) using HR-pQCT (XtremeCT; Scanco Medical AG, Brüttisellen, Switzerland). I evaluated all HR-pQCT scans for motion artifacts and analyzed all scans as per manufacturer's standard protocol.[113,356] I excluded 1 tibia scan and 33 radius scans (3%) due to motion artifact > 3 (on a scale from 1 to 5).[124] I report standard morphological measures including: Tt.BMD (mg/cm^3), Tb.N (1/mm), Tb.Th (mm), Tb.Sp (mm) and BV/TV. I used an automated segmentation algorithm to separate trabecular and cortical bone[115,116] to determine: Tt.Ar (mm^2), Ct.BMD (mg/cm^3), Ct.Po (%), and Ct.Th (mm). Finally, we applied a validated FEA to HR-pQCT images to estimate bone strength. FEA outcomes were F.Load (N) and load-to-strength ratio (distal radius only).[118] The short-term reproducibility in our lab is < 3.8% for all HR-pQCT standard analysis-derived parameters, 1.2% (Ct.BMD) to 17.3% (Ct.Po) for automated segmentation measures and 4.0% for FE-based F.Load (University of British Columbia Bone Health Research Group, unpublished data).

6.2.7 Statistical analysis

I considered *p*-values < 0.05 statistically significant. Prior to modeling my data, I first examined scatter plots generated for bone strength and its determinants against maturity offset for each participant. I fit general linear mixed models (also called random coefficients regression models or multilevel models) to evaluate the influence of PA and sedentary time across maturity. As in my previous analysis I centered maturity offset at 0, and I used the following process to determine the best fitting model for all bone parameters.[314]

First, I fit an empty means random intercept model to determine the amount of variance in bone parameters attributed to between- and within-person differences. Next, I fit a fixed linear time random intercept model, with maturity offset as the time variable, followed by a random linear time model (allowing each participant his or her own slope for the effect of maturity). I followed these models with fixed and random quadratic and cubic time models. I used Wald test p-values to determine significance of individual fixed effects and maximum likelihood log likelihood (-2*log likelihood (LL)) statistics to determine significance of random effects variances and covariances between nested models given the difference in model degrees of freedom. I examined reduction in the deviance test (-2ΔLL) and model parsimony (Akaike and Bayesian information criterion (AIC and BIC) values) to determine the best fitting unconditional growth model. To assess potential for overfitting the model, I examined change in pseudo R^2 with addition of each fixed polynomial time variable (as computed from the square of the correlation between the observed bone variable and the outcomes predicted by the fixed effects) and used the previous model if negligible change in pseudo R^2 (< 1%). I estimated all models using maximum likelihood estimation, as there was only a 0.3% downward bias in the random intercept variance compared with restricted maximum likelihood estimation. I then examined the effect of sex and ethnicity (Asian/white/other) on the intercept and maturity effects.

Once I determined the best-fitting growth model, which included the fixed effects of sex and ethnicity, I developed a series of models to address my primary and secondary objectives – the longitudinal relationship between PA, sedentary time and bone parameters. Model 1 included MVPA or sedentary time as fixed effects. Model 2 also included lower leg muscle power, limb length (surrogate of lever arm) and dietary calcium as fixed effects. I fit an additional model for

sedentary time (Model 3) that examined the influence of sedentary time independent of MVPA (min/day).

I used the following approach to model effects of time-varying fixed effects and covariates (i.e., MVPA, sedentary time, muscle power, limb length, dietary calcium). First, I examined an empty means random intercept model for each covariate to determine the amount of variance attributed to between- and within-person differences. Next, I examined the extent to which each covariate demonstrated individual change over time using a fixed linear time (maturity offset) random intercept model, followed by a random linear time model, then a fixed quadratic time model. As longitudinal data are made of up repeated measures of attributes that change over time, it is important to examine the inter-individual variation (i.e., differences between people) along with the intra-individual variation (i.e., differences over time within the same person).[370] Therefore, I person-mean centered all covariates, such that the deviation from the individual's mean value across years (i.e., $MVPA_{ti} - \overline{MVPA}_i$; where $MVPA_{ti}$ is MVPA on measurement occasion *t* in the i^{th} individual and $\overline{MVPA}_i$ is that individual's mean MVPA across years) represented the within-person (level 1) effect. The individual's mean value across years represented the between-person (level 2) effect.[371] I retained both within- and between-persons fixed effects of muscle power, limb length and dietary calcium in the models if either the within- or between-persons effect was significantly association with the bone parameter in question. I provide the specific covariates retained for each bone parameter in Table 6.1. I added interaction terms to Model 1 to examine potential moderation of the effects of MVPA and sedentary time at each level by sex. However, as the interaction terms did not significantly improve model fit (based on -2ΔLL and AIC and BIC values) I removed them from the model. I also examined potential moderation of the effects of MVPA and sedentary time by maturity and included interaction terms where model fit was significantly improved based on a reduction in the deviance test (-2ΔLL) and model parsimony (AIC and BIC) values. I provide an example of a mixed model below:

Random linear, fixed quadratic maturity model, including fixed effect of sex, ethnicity, MVPA, muscle power, limb length and dietary calcium predicting intercept, and sex predicting linear and quadratic maturity slope

Level 1: $y_{ti} = \beta_{0i} + \beta_{1i}MO_{ti} + \beta_{2i}MO_{ti}^2 + \beta_{3i}(MVPA_{ti} - \overline{MVPA}_i) + \beta_{4i}(Musclepower_{ti} - \overline{Musclepower}_i) + \beta_{5i}(Limblength_{ti} - \overline{Limblength}_i) + \beta_{6i}(Calcium_{ti} - \overline{Calcium}_i) + \varepsilon_{ti}$

Level 2: Intercept: $\beta_{0i} = \gamma_{00} + \gamma_{01}Boys_i + \gamma_{02}Ethnicity_i + \gamma_{03}(\overline{MVPA}_i) + \gamma_{04}(\overline{Musclepower}_i) + \gamma_{05}(\overline{Limblength}_i) + \gamma_{06}(\overline{Calcium}_i) + \mu_{0i}$

Linear time: $\beta_{1i} = \gamma_{10} + \gamma_{11}Boys_i + \mu_{1i}$

Quadratic time: $\beta_{2i} = \gamma_{20} + \gamma_{21}Boys_i$

Within-person MVPA: $\beta_{3i} = \gamma_{30}$

Within-person Muscle power: $\beta_{4i} = \gamma_{40}$

Within-person Limb length: $\beta_{5i} = \gamma_{50}$

Within-person Dietary calcium: $\beta_{6i} = \gamma_{60}$

Composite: $y_{ti} = [\gamma_{00} + \gamma_{01}Boys_i + \gamma_{02}Ethnicity_i + \gamma_{03}(\overline{MVPA}_i) + \gamma_{04}(\overline{Musclepower}_i) + \gamma_{05}(\overline{Limblength}_i) + \gamma_{06}(\overline{Calcium}_i) + \gamma_{10}MO_{ti} + \gamma_{20}MO_{ti}^2 + \gamma_{11}MO_{ti}*Boys_i + \gamma_{21}MO_{ti}^2*Boys_i + \gamma_{30}(MVPA_{ti} - \overline{MVPA}_i) + \gamma_{40}(Musclepower_{ti} - \overline{Musclepower}_i) + \gamma_{50}(Limblength_{ti} - \overline{Limblength}_i) + \gamma_{60}(Calcium_{ti} - \overline{Calcium}_i)] + [\mu_{0i} + \mu_{1i}MO_{ti} + \varepsilon_{ti}]$

MO is maturity offset (centered at 0, APHV); Boys = 0, girl; 1, boy
Ethnicity= 0, Asian; 1, white; 2, other
where y_{ti} *is the bone parameter on measurement occasion t in the* i^{th} *individual,*
$(\mu_{0i}, \mu_{1i}) \sim N(0,\Sigma)$ *is the vector of random effects for the* i^{th} *individual and*
$\varepsilon_{ij} \sim N(0,\sigma^2)$ *is the within-subject residual error.*

Thus, the intercepts γ_{00}, $\gamma_{01}Boys_i$, $\gamma_{02}Ethnicity_i$, $\gamma_{03}(\overline{MVPA}_i)$, $+\gamma_{04}(\overline{Musclepower}_i)$, $\gamma_{05}(\overline{Limblength}_i)$, $\gamma_{06}(\overline{Calcium}_i)$, $\gamma_{30}(MVPA_{ti} - \overline{MVPA}_i)$, $\gamma_{40}(Musclepower_{ti} - \overline{Musclepower}_i)$, $\gamma_{50}(Limblength_{ti} - \overline{Limblength}_i)$ and $\gamma_{60}(Calcium_{ti} - \overline{Calcium}_i)$ represent the mean value of the bone parameter and the fixed effect of sex, ethnicity, and between- and within-person MVPA, muscle power, limb length and dietary calcium on the mean intercept of the bone parameter when maturity offset is zero, while μ_{0i} is the person-specific deviation from the mean intercept. The slopes γ_{10} and $\gamma_{11}Boys$ represent the fixed linear effect of maturity and the fixed effect of sex on linear maturity offset of 0, respectively, while μ_{1i} is the person-specific deviation from the

fixed linear effect of time. The slopes γ_{20} and $\gamma_{21}Boys$ represent the fixed quadratic effect of maturity and the fixed effect of sex on quadratic maturity, respectively. I checked model adequacy graphically using plots of residuals.[340] Diagnostic checking of fitted models revealed some serial correlation in the residuals; however, attempting to incorporate a serial correlation component into the model led to problems with model convergence, an issue identified by others.[340] Models that included serial correlation and only a random intercept yielded similar results to the random coefficients only model. For models including a maturity by MVPA (or sedentary time) interaction term, I calculated the marginal effect at each maturity offset using the margins command in Stata and a Bonferroni adjustment to account for multiple comparisons. Due to few measurements at maturity offsets before 2 years prior to APHV in girls (n = 3) and after 9 years post-APHV in boys (n = 3), I limited my range of marginal effects from 2 years prior to APHV to 9 years post-APHV. Accordingly, the level of statistical significance was set to $p < 0.0042$ ($p < 0.05$ divided by 12 maturity offsets) for maturity by MVPA or sedentary time interaction effects.

I rescaled results by the interquartile range (IQR) for MVPA and sedentary time (adjusted for wear time) in order to account for the substantially different volume of each type of activity (i.e., substantially lower volume of MVPA compared with sedentary time). Thus, the reported coefficients represent associations with bone parameters for every IQR increment in MVPA or sedentary time (i.e., difference in bone parameter between an individual at the 75th percentile (upper quartile) for MVPA or sedentary time compared with an individual at the 25th percentile (lower quartile) for MVPA or sedentary time). The upper quartile of MVPA approximated current MVPA recommendations[216] of 60 min/day (61 min/day), while the lower quartile was < 31 min/day. The upper quartile for sedentary time approximated 11 h/day, while the lower quartile approximated 9 h/day.

Table 6.1. Covariates used in mixed effects models, not including sex, ethnicity, MVPA and sedentary time. Time-varying covariates were retained if $p < 0.05$. Interactions terms were retained if they significantly improved model fit based on a reduction in the deviance test (-2ΔLL) and model parsimony (AIC and BIC) values.

Bone Parameter	Maturity	Time-varying Covariates	Interaction terms
Distal Tibia			
BV/TV	MO, MO^2	Muscle power, limb length, dietary calcium	
Tb.Th (mm)	MO, MO^2	Limb length	
Ct.Th (mm)	MO, MO^2, MO^3	Muscle power, limb length, dietary calcium	MO, MO^2, MO^3 by SED and MVPA[a]
Ct.Po (%)	MO, MO^2, MO^3	Muscle power, limb length	
Ct.BMD (mg/cm^3)	MO, MO^2, MO^3	Muscle power	MO, MO^2, MO^3 by SED and MVPA[a]
Tt.Ar (mm^2)	MO, MO^2, MO^3	Muscle power, limb length	MO, MO^2, MO^3 by MVPA and SED
F.Load (N)	MO, MO^2	Muscle power, dietary calcium	
Distal Radius			
BV/TV	MO	Muscle power, limb length, dietary calcium	
Tb.Th (mm)	MO, MO^2	Muscle power, limb length, dietary calcium	
Ct.Th (mm)	MO, MO^2, MO^3	Muscle power, limb length	
Ct.Po (%)	MO, MO^2, MO^3	Muscle power, limb length	
Ct.BMD (mg/cm^3)	MO, MO^2, MO^3	Muscle power, limb length, dietary calcium	
Tt.Ar (mm^2)	MO, MO^2, MO^3	Muscle power, limb length	MO, MO^2, MO^3 by SED
F.Load (N)	MO, MO^2	Muscle power, limb length, dietary calcium	
Load-to-strength Ratio	MO, MO^2	Muscle power, limb length, dietary calcium	MO, MO^2 by MVPA

BV/TV, trabecular bone volume fraction; Tb.Th, trabecular thickness; Ct.Th, cortical thickness; Ct.Po, cortical porosity; Ct.BMD, cortical bone mineral density; Tt.Ar, total area; F.Load, failure load.
MO, maturity offset (years from age at peak height velocity); MVPA, moderate to vigorous physical activity, SED, sedentary time.
[a]MVPA interaction in Model 3 only

6.3 Results

6.3.1 Descriptive characteristics

A total of 308 participants (795 observations) and 259 participants (588 observations) met the inclusion criteria of valid HR-pQCT and accelerometry data at the distal tibia and distal radius, respectively. I provide participant characteristics, including MVPA and sedentary time data at first HR-pQCT measurement in Table 6.2 and bone parameters at first HR-pQCT measurement in Table 6.3. Between-person differences in bone parameters accounted for 85% (for Tb.Th) to 96% (for BV/TV) of the variance in bone parameters, while the remaining 4-15% was attributed to within-person differences.

Table 6.2. Characteristics of boys and girls at first HR-pQCT measurement.

	Boys (n=136)			**Girls (n=172)**		
	Mean (SD)	**Min**	**Max**	**Mean (SD)**	**Min**	**Max**
Age (yrs)	14.9 (2.9)	9.5	21.6	14.4 (3.5)	9.5	21.4
No. Asian/ white /other	64/60/12	-	-	84/72/16	-	-
No. Tanner 1/2/3/4/5	16/12/10/44/53	-	-	26/36/27/41/42	-	-
Maturity offset (yrs)	1.8 (3)	-4.1	9.3	2.9 (3.6)	-2.6	10.6
Height (cm)	165.6 (14.4)	129.7	192.2	154.9 (11.4)	130.0	181.6
Weight (kg)	58.4 (15.9)	27.8	108.6	49.6 (13.9)	22.2	87.5
Sitting height (cm)	87.1 (7.4)	67.2	99.6	82.7 (6.3)	68.6	95.0
Tibial length (mm)	400 (37)	306	482	370 (30)	300	444
Ulnar length (mm)[a]	271 (28)	211	325	245 (20)	196	286
Leg muscle power (kW)	2.7 (1)	1.0	5.4	1.9 (0.6)	0.8	3.6
Dietary calcium (mg)	1155 (726)	95	3253	938 (602)	73	2803
Accelerometry Variables						
MVPA (min/day)	59.0 (26.4)	14.7	142.8	40.9 (17.6)	4.8	104.1
Sedentary time (min/day)	584.8 (105.2)	347.4	804.0	593.4 (101.2)	324.6	868.1
Accelerometer wear time (min/day)	839.8 (69.7)	662.0	990.5	835.1 (74.2)	655.9	1073.0

[a]259 participants with data at the distal radius 142 girls, 117 boys
Values of moderate-to-vigorous physical activity, MVPA, and sedentary time are unadjusted for wear time.

Table 6.3. Bone parameters for boys and girls at first HR-pQCT measurement.

	Boys (n=136)			**Girls (n=172)**		
	Mean (SD)	**Min**	**Max**	**Mean (SD)**	**Min**	**Max**
Distal Tibia						
BV/TV	0.163 (0.025)	0.108	0.235	0.152 (0.025)	0.093	0.236
Tb.Th (mm)	0.087 (0.014)	0.057	0.120	0.085 (0.014)	0.056	0.127
Ct.Th (mm)	1.15 (0.36)	0.56	2.15	1.02 (0.3)	0.42	2.01
Ct.Po (%)	5.6 (2.4)	1.6	17	3.9 (2.2)	0.8	10.4
Ct.BMD (mg/cm^3)	738.9 (86.1)	594.7	889.0	768.7 (112.6)	562.9	935.5
Tt.Ar (mm^2)	745.3 (136.1)	454.6	1127.8	620 (87.1)	426.9	900.7
F.Load (N)	6007.1 (1638.8)	2185.0	10440.0	4821.3 (1227.2)	2434.0	7788.0
U.Stress (MPa)	33.2 (9.8)	10.8	55.8	31.4 (9.6)	13.2	60.3
Distal Radius[a]						
BV/TV	0.156 (0.028)	0.082	0.224	0.142 (0.027)	0.075	0.237
Tb.Th (mm)	0.08 (0.016)	0.051	0.136	0.072 (0.01)	0.053	0.120
Ct.Th (mm)	0.96 (0.32)	0.38	1.76	0.85 (0.29)	0.41	1.62
Ct.Po (%)	3.5 (2.1)	0.2	10.0	2.6 (2.1)	0.1	9.8
Ct.BMD (mg/cm^3)	710.5 (113.2)	478.2	978.0	722.4 (140.9)	446.3	966.9
Tt.Ar (mm^2)	259.3 (58.8)	142.8	421.1	201.6 (35.1)	128.7	317.8
F.Load (N)	2156.2 (828.8)	628.5	4739.0	1540.7 (555.3)	477.0	3103.0
Load-to-strength Ratio	1.48 (0.64)	0.61	3.8	1.93 (0.72)	0.90	5.11

BV/TV, trabecular bone volume fraction; Tb.Th, trabecular thickness; Ct.Th, cortical thickness; Ct.Po, cortical porosity; Ct.BMD, cortical bone mineral density; Tt.Ar, total area; F.Load, failure load.

[a] 259 participants with data at the distal radius 142 girls, 117 boys

Twenty-four percent of our cohort (43% of boys, 9% of girls) met recommendations of 60 min/day of MVPA. MVPA declined by roughly 31% across adolescence, from approximately 60 min/day in boys and 43 min/day in girls at 2 years prior to APHV (age 11.1 years in boys and 9.5 years in girls) to 49 min/day in boys and 32 min/day in girls 9 years post-APHV (age 22.1 years in boys and 20.5 years in girls). On average, MVPA was significantly greater for boys compared with girls across growth (~16 min/day difference, $p < 0.001$). Sedentary time was not significantly different between boys and girls ($p = 0.60$) and increased by roughly 30% across adolescence, from approximately 8.1 h/day at 2 years prior to APHV to 10.6 h/day at 9 years post-APHV.

6.3.2 Influence of physical activity and sedentary time on bone parameters

6.3.2.1 Moderate to vigorous physical activity

I provide results of mixed effects models fit to examine the longitudinal relationship between MVPA and bone parameters across growth in Table 6.4. At the *distal tibia*, between-person differences in MVPA positively predicted BV/TV, Ct.Po, Tt.Ar and F.Load in Model 1 (adjusted for sex, ethnicity and maturity) and Model 2 (additionally adjusted for muscle power, limb length and dietary calcium). Participants in the upper quartile of MVPA (~60 min/day) had approximately 4% greater BV/TV across growth compared with their peers in the lowest quartile of MVPA (~< 30 min/day; Figure 6.2). I observed a significant interaction between MVPA and maturity for Ct.Po, Tt.Ar and F.Load, whereby participants in the upper quartile of MVPA had approximately 12-14% greater Ct.Po between APHV and 3 years post-APHV; 4-6% greater Tt.Ar between 1 year prior to APHV to 2 years post-APHV; and 6-7% greater F.Load between 1 year prior to APHV and 8 years post-APHV compared with their peers in the lowest quartile of MVPA (Figure 6.4). Within-person change in MVPA did not significantly predict bone parameters at the distal tibia.

At the *distal radius*, between-person differences in MVPA positively predicted BV/TV and F.Load and negatively predicted load-to-strength-ratio in both models. Participants in the upper quartile of MVPA (~60 min/day) had approximately 5% greater BV/TV and 8% greater F.Load compared with their peers in the lowest quartile of MVPA (~< 30 min/day; Figure 6.3)

across growth. I observed a significant interaction between MVPA and maturity for load-to-strength ratio, whereby participants in the upper quartile of MVPA had approximately 7-12% lower load-to-strength ratio between 2 years prior to APHV and 2 years post-APHV compared with their peers in the lower quartile of MVPA (Figure 6.4). Within-person change in MVPA did not significantly predict bone parameters in either model at the distal radius.

Table 6.4. Longitudinal associations of between-person moderate-to-vigorous physical activity (MVPA; per IQR, 30 min) with bone parameters at the distal tibia and radius. Coefficients (95% CI) represent the difference in bone parameter between an individual in the upper quartile for MVPA compared with an individual in the lower quartile MVPA at maturity offset (years from age at peak height velocity) of 0.

Bone Parameter	Model	Tibia	Radius
BV/TV	1	0.006 (0.002 to 0.011)**	0.008 (0.003 to 0.013)**
	2	0.006 (0.002 to 0.010)**	0.007 (0.002 to 0.012)**
Tb.Th (mm)	1	0.001 (-0.001 to 0.003)	0.002 (-0.000 to 0.004)
	2	0.001 (-0.001 to 0.003)	0.002 (-0.001 to 0.004)
Ct.Th (mm)	1	0.02 (-0.02 to 0.07)	0.01 (-0.02 to 0.05)
	2	0.03 (-0.02 to 0.07)	0.02 (-0.01 to 0.06)
Ct.Po (%)	1	0.8 (0.3 to 1.3)**	0.1 (-0.1 to 0.3)
	2	0.8 (0.3 to 1.3)**	0.1 (-0.1 to 0.3)
Ct.BMD (mg/cm^3)	1	-7.2 (-17.0 to 2.5)	-3.5 (-17.1 to 10.2)
	2	-6.1 (-15.8 to 3.6)	-0.3 (-13.4 to 12.9)
Tt.Ar (mm^2)	1	38.6 (17.7 to 59.6)***	6.8 (-1.0 to 14.6)
	2	38.9 (18.1 to 59.8)***	4.5 (-2.5 to 11.6)
F.Load (N)	1	329.3 (150.4 to 508.2)***	116.4 (49.7 to 183.1)**
	2	324.6 (168.3 to 481.0)***	117.3 (57.4 to 177.2)***
Load-to-strength	1	-	-0.20 (-0.31 to -0.10)***
ratio	2	-	-0.19 (-0.29 to -0.09)***

BV/TV, trabecular bone volume fraction; Tb.Th, trabecular thickness; Ct.Th, cortical thickness; Ct.Po, cortical porosity; Ct.BMD, cortical bone mineral density; Tt.Ar, total area; F.Load, failure load. Model 1 adjusted for sex, ethnicity and maturity. Model 2 additionally adjusted for muscle power, limb length and dietary calcium. $^{**}p < 0.01$, $^{***}p < 0.001$.

6.3.2.2 Sedentary time

I present results of the mixed effects models used to examine the longitudinal relationship between sedentary time and bone parameters across growth at the *distal tibia* in Table 6.5. Between-person differences in sedentary time negatively predicted Tt.Ar and F.Load and positively predicted Tb.Th, Ct.Th and Ct.BMD in Model 1 (adjusted for sex, ethnicity and maturity) and Model 2 (additionally adjusted for muscle power, limb length and dietary).

Associations persisted after additional adjustment for MVPA (Model 3), with the exception of F.Load, which was no longer negatively associated with sedentary time ($p = 0.15$). Participants in the upper quartile of sedentary time had 10% greater Tb.Th across growth compared with their peers in the lowest quartile of sedentary time (Figure 6.2). I observed significant interactions between sedentary time and maturity for Ct.Th, Ct.Po, Ct.BMD and Tt.Ar, whereby participants in the upper quartile of sedentary time had 9-12% greater Ct.Th from APHV to 3 years post-APHV; 24% greater Ct.Po at 2 years prior to APHV and 18-24% lower Ct.Po at 3 and 4 years post-APHV; 3-5% greater Ct.BMD from APHV to 4 years post-APHV; and 9-14% lower Tt.Ar from 1 year prior to APHV and onwards compared with their peers in the lowest quartile of sedentary time (Figure 6.5). Sedentary time models adjusted for MVPA (Model 3), demonstrated that MVPA was also positively associated with Tb.Th (B: 0.005; 0.004 to 0.011, $p < 0.001$) and demonstrated a positive interaction with maturity from 1 to 6 years post-APHV for Ct.Th (B*:* 0.09 to 0.010; $p = 0.027$ to 0.003) and 2 to 4 years post-APHV for Ct.BMD (B: 15.6 to 16.3; $p =$ 0.035 to 0.011). Within-person change in sedentary time was not related to bone parameters at the distal tibia.

At the *distal radius*, between-person differences in sedentary time positively predicted Ct.BMD in Model 1, but were no longer significant in Model 2 (adjusted for muscle power, limb length and dietary calcium; Table 6.5). F.Load became negatively associated with sedentary time in Model 2, but was no longer significantly associated with sedentary time after additional adjustment for MVPA (Model 3). I observed a significant interaction between sedentary time and maturity for Tt.Ar, whereby participants in the upper quartile for sedentary time had 9-13% lower Tt.Ar from 3 to 9 years post-APHV compared with their peers in the lower quartile for sedentary time (Figure 6.5). Within-person change in sedentary time was not related to bone parameters at the distal radius.

Table 6.5. Longitudinal associations of between-person sedentary time (per IQR, 106 min) with bone parameters at the distal tibia and radius. Coefficients (95% CI) represent the difference in bone parameter between an individual in the upper quartile for sedentary time compared with an individual in the lower quartile for sedentary time at maturity offset (years from age at peak height velocity) of 0.

Bone Parameter	Model	Tibia	Radius
BV/TV	1	-0.005 (-0.010 to 0.000)	-0.005 (-0.011 to 0.001)
	2	-0.005 (-0.010 to 0.001)	-0.003 (-0.010 to 0.003)
	3	0.002 (-0.005 to 0.009)	0.003 (-0.005 to 0.011)
Tb.Th (mm)	1	0.003 (0.001 to 0.006)**	0.000 (-0.002 to 0.003)
	2	0.003 (0.001 to 0.006)**	0.000 (-0.002 to 0.003)
	3	0.008 (0.004 to 0.011)***	0.003 (-0.001 to 0.006)
Ct.Th (mm)	1	0.07 (0.02 to 0.12)**	0.03 (-0.01 to 0.07)
	2	0.07 (0.01 to 0.12)*	-0.01 (-0.05 to 0.03)
	3	0.10 (0.04 to 0.17)**	0.02 (-0.03 to 0.07)
Ct.Po (%)	1	-0.3 (-0.8 to 0.3)	-0.1 (-0.4 to 0.2)
	2	-0.4 (-1.0 to 0.1)	-0.1 (-0.4 to 0.2)
	3	0.1 (-0.6 to 0.9)	0.1 (-0.4 to 0.3)
Ct.BMD (mg/cm^3)	1	22.0 (11.2 to 32.7)***	22.3 (6.8 to 37.9)*
	2	19.8 (8.5 to 31.1)***	8.4 (-7.5 to 24.4)
	3	26.7 (11.8 to 41.5)***	15.2 (-3.3 to 33.7)
Tt.Ar (mm^2)	1	-48.3 (-70.7 to -25.8)***	-7.9 (-18.8 to 3.1)
	2	-72.0 (-95.7 to -48.3)***	-5.8 (-16.7 to 5.0)
	3	-75.1 (-105.6 to -44.5)***	-8.5 (-21.5 to 4.5)
F.Load (N)	1	-231.2 (-425.3 to -37.1)*	-19.1 (-97.1 to 59.0)
	2	-388.8 (-570.5 to -207.1)***	-84.2 (-162.4 to -5.9)*
	3	-171.8 (-406.1 to 62.4)	15.1 (-81.8 to 112.0)
Load-to-strength ratio	1	-	0.06 (-0.01 to 0.13)
	2	-	0.03 (-0.04 to 0.10)
	3	-	-0.01 (-0.10 to 0.08)

BV/TV, trabecular bone volume fraction; Tb.Th, trabecular thickness; Ct.Th, cortical thickness; Ct.Po, cortical porosity; Ct.BMD, cortical bone mineral density; Tt.Ar, total area; F.Load, failure load. Model 1 adjusted for sex, ethnicity and maturity. Model 2 additionally adjusted for muscle power, limb length and dietary calcium. Model 3 additionally adjusted for moderate-to-vigorous physical activity, MVPA in min/day. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

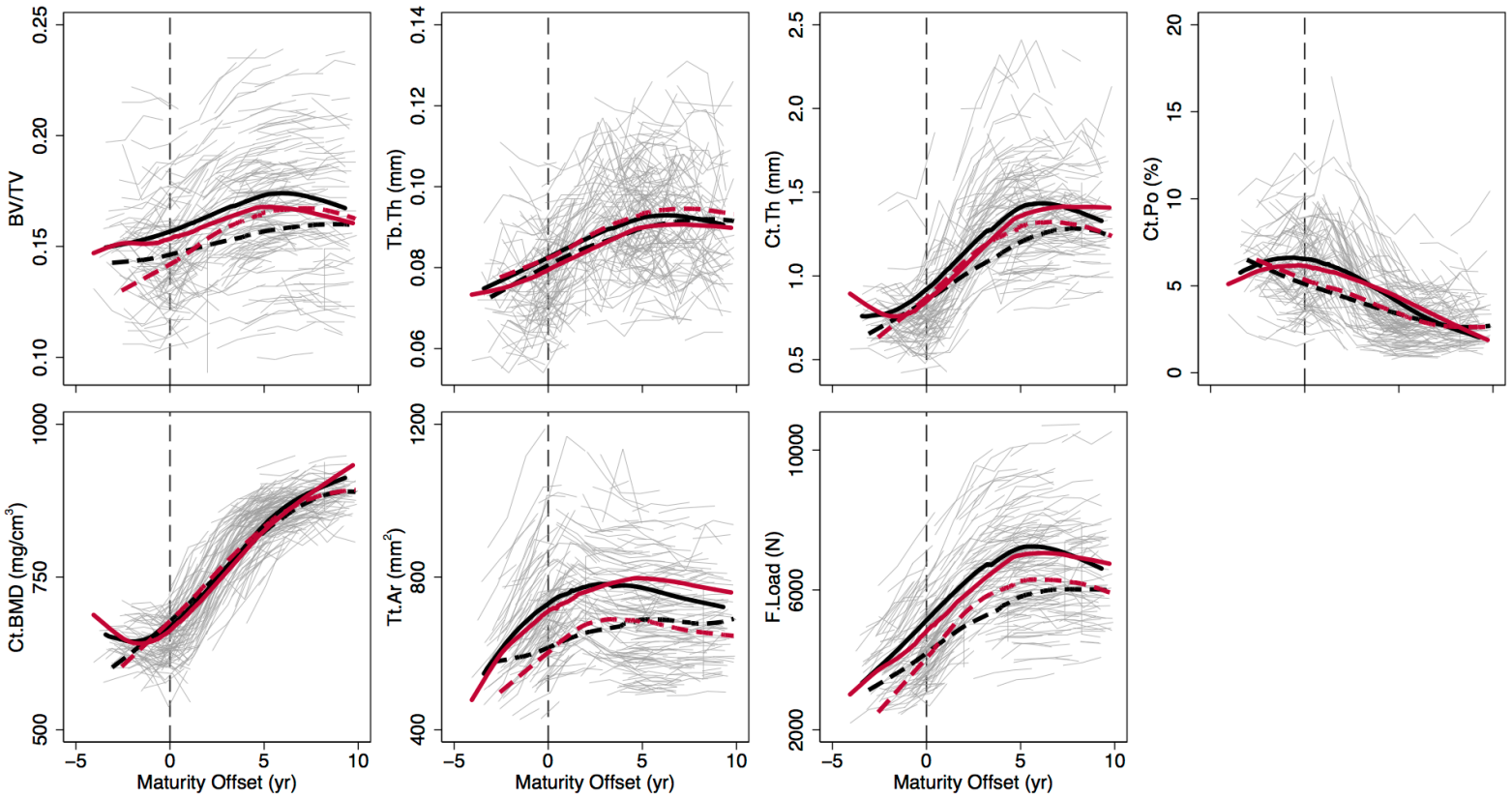


Figure 6.2. Distal tibia individual growth curves (thin, light grey lines) and estimated growth curves from the polynomial mixed model for participants in the upper (~60 min/day; red solid line) and lower quartile of MVPA (~< 30 min/day, black dashed line), and the upper quartile (~11 h/day; blue dashed line) and lower quartile of sedentary time (~< 9 h/day; red solid line) for trabecular bone volume fraction (BV/TV), and thickness (Tb.Th), cortical BMD (Ct.BMD), thickness (Ct.Th) and porosity (Ct.Po), total area (Tt.Ar), and failure load (F.Load). The vertical line indicates maturity offset (years from age at peak height velocity) of 0. Mixed model growth curves are adjusted for maturity, sex, ethnicity, lower limb muscle power, limb length and calcium. Growth curves for sedentary models are additionally adjusted for MVPA.

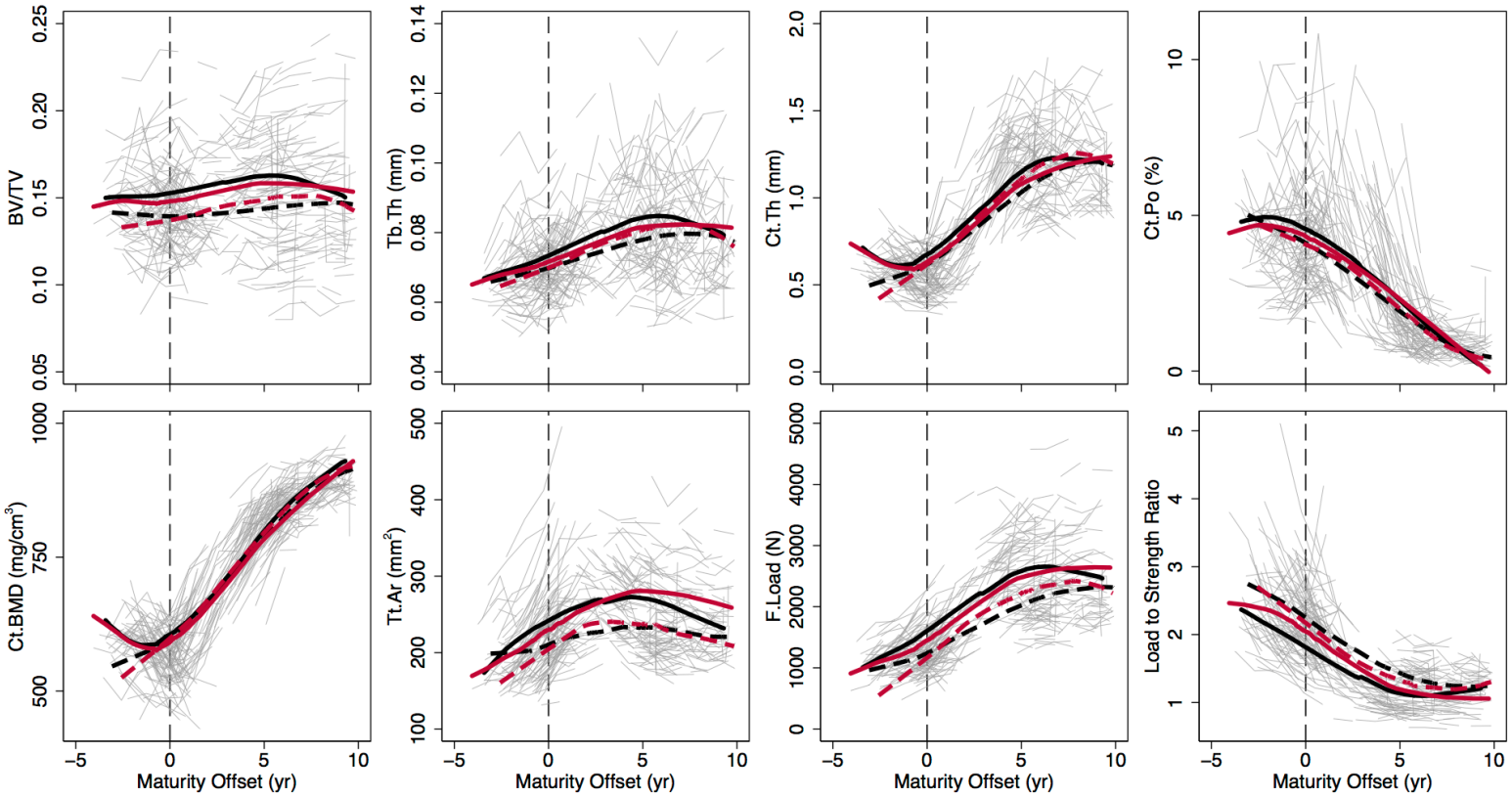


Figure 6.3. Distal radius individual growth curves (thin, light gray lines) and estimated growth curves from the polynomial mixed model for participants in the upper (~60 min/day; black solid line) and lower quartile of MVPA (~<30 min/day, black dashed line), and the upper quartile (~11 h/day; red dashed line) and lower quartile of sedentary time (~<9 h/day; red solid line) for trabecular bone volume fraction (BV/TV), and thickness (Tb.Th), cortical BMD (Ct.BMD), thickness (Ct.Th) and porosity (Ct.Po), and total area (Tt.Ar), failure load (F.Load) and load-to-strength ratio. The vertical line indicates maturity offset (years from age at peak height velocity) of 0. Mixed model growth curves are adjusted for maturity, sex, ethnicity, lower limb muscle power, limb length and calcium. Growth curves for sedentary models are additionally adjusted for MVPA.

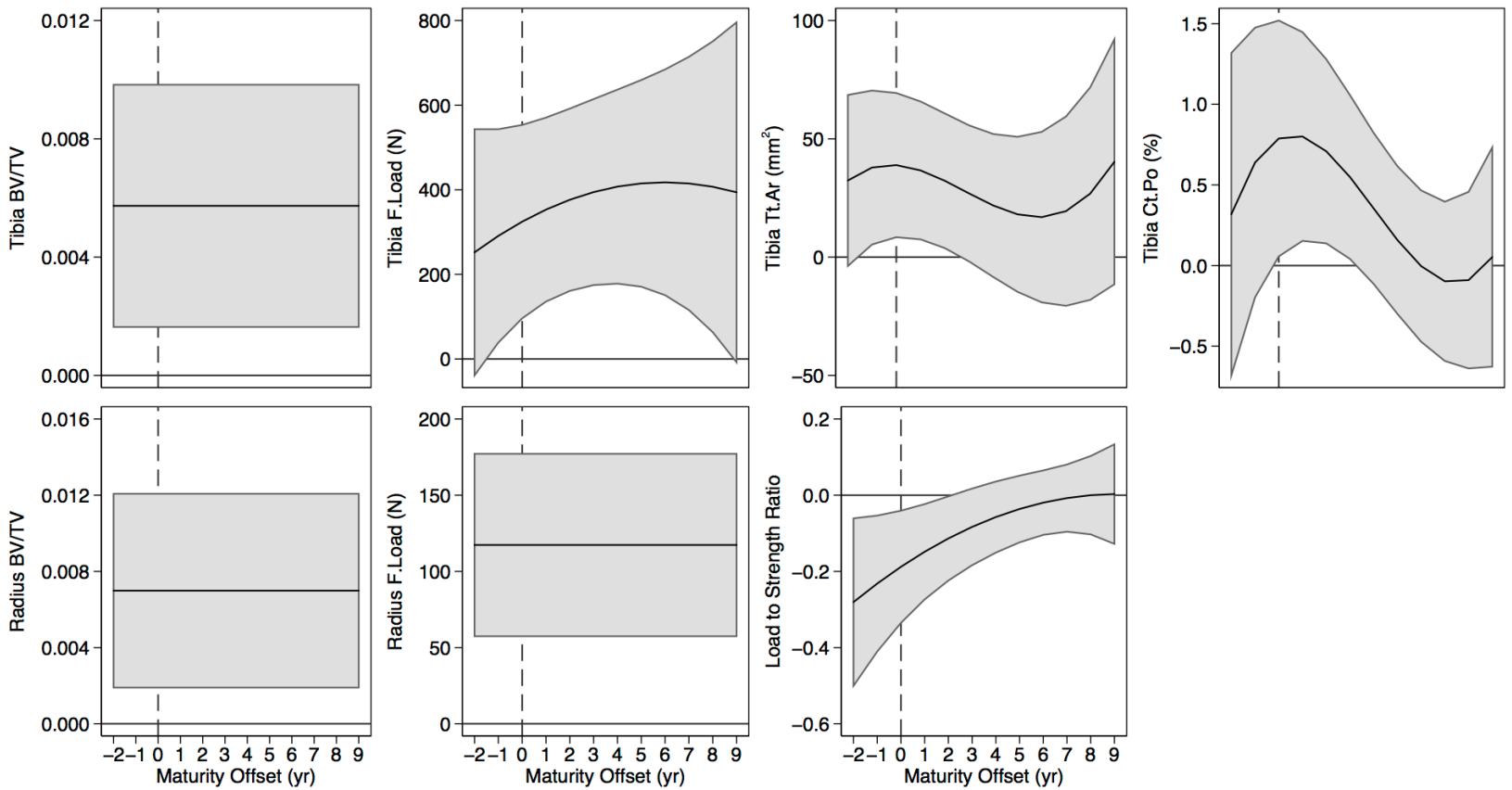


Figure 6.4. Interaction of MVPA and maturity with bone parameters across growth at the distal tibia and radius. The solid black line represents the coefficient of MVPA accompanied by a shaded 95% confidence interval, correcting for multiple comparisons using a Bonferroni adjustment. Estimates above 0 indicate significant positive relationship with MVPA, while estimates below 0 indicate significant negative relationship with MVPA. Confidence intervals that cross 0 indicate non-significant relationship. The vertical line indicates maturity offset (years from age at peak height velocity) of 0. Bone volume fraction (BV/TV), failure load (F.Load), total area (Tt.Ar), and cortical porosity (Ct.Po).

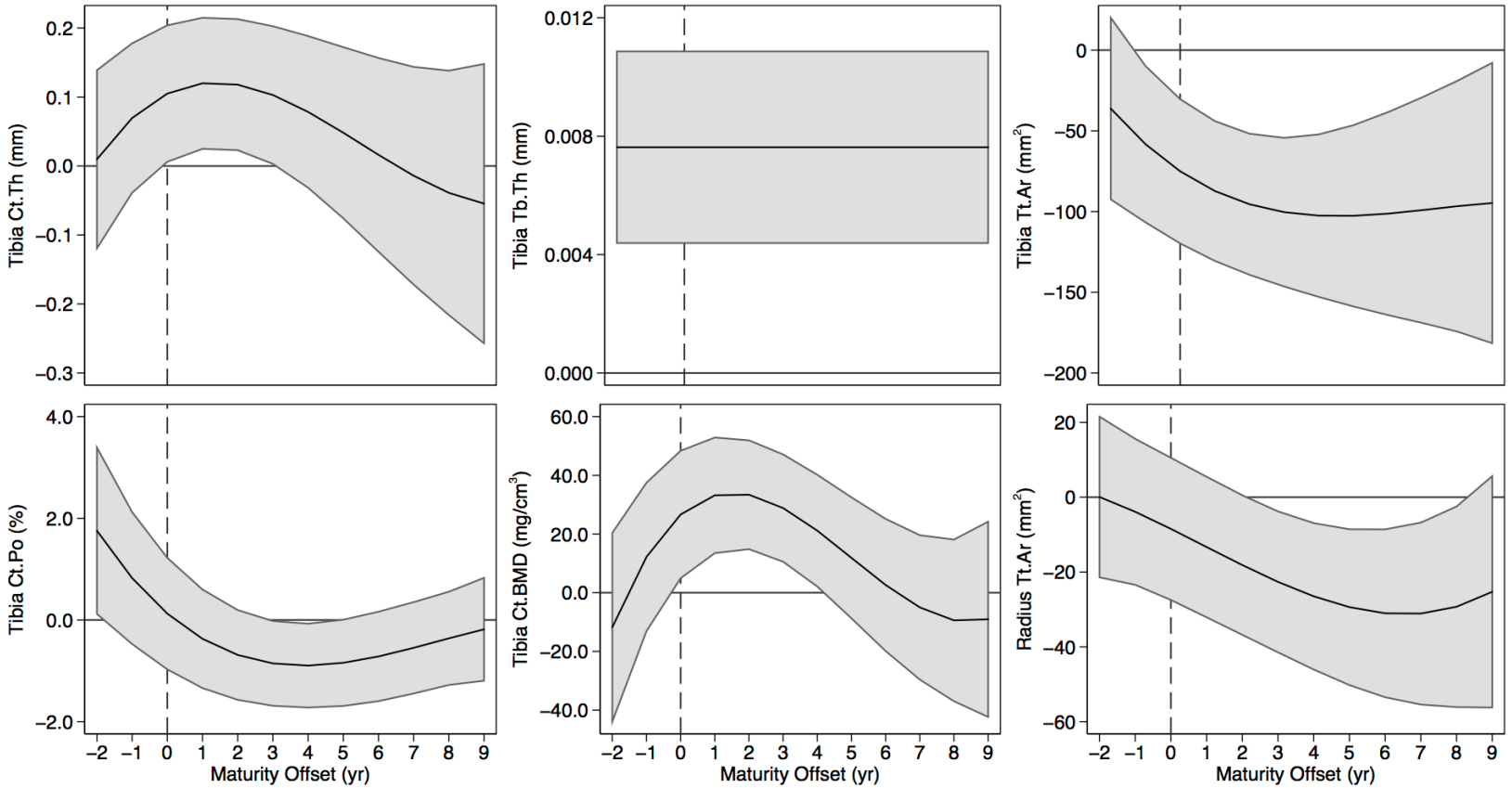


Figure 6.5. Interaction of sedentary time and maturity with bone parameters across growth at the distal tibia and radius. The solid black line represents the coefficient of sedentary time accompanied by a shaded 95% confidence interval, correcting for multiple comparisons using a Bonferroni adjustment. Estimates above 0 indicate significant positive relationship with sedentary time, while estimates below 0 indicate significant negative relationship with sedentary time. The vertical line indicates maturity offset (years from age at peak height velocity) of 0. Cortical thickness (Ct.Th), trabecular thickness, (Tb.Th), total area (Tt.Ar), cortical porosity (Ct.Po), and cortical BMD (Ct.BMD).

6.4 Discussion

The HBSIII study is the longest prospective study conducted to date that spans the adolescent growth spurt and used HR-pQCT to examine the tibia and radius. Key elements of my study include aligning girls and boys on a common maturational landmark and using an objective measure of MVPA and sedentary time. I provide unique insight into consequences of elevated and reduced loading of the skeleton during adolescent growth. My results suggest that adolescents who participate in more intense PA (MVPA) have greater trabecular bone tissue volume at both skeletal sites and greater bone area at the tibia, which contribute to superior bone strength. Conversely, sedentary adolescents have smaller bones (Tt.Ar), on average at both skeletal sites, but greater Tb.Th, Ct.Th and Ct.BMD at the tibia. My findings also highlight the maturity-specific nature of bone's response to elevated and reduced loading and suggest that early and mid-puberty may be particularly important years to target these behaviours.

6.4.1 Physical activity and bone strength

The osteogenic effects of PA are irrefutable.[44] I extend the relatively few longitudinal studies that used HR-pQCT to characterize growing bone. In doing so, I also extend evidence that supports MVPA as a predictor of bone strength at both the tibia and radius across growth in boys and girls. My findings are consistent with a recent longitudinal study that used pQCT to assess bone[289]and previous cross-sectional studies that demonstrated a significant association between objectively-measured PA (via accelerometer or pedometer) and pQCT-[365,366] and HR-pQCT-[310] estimated bone strength. In my recent analysis of the HBSIII cohort, bone strength (F.Load by HR-pQCT) increased by approximately 47-65% in boys and 59-91% in girls at the distal tibia and radius in the 4 years around peak growth (Chapter 5).[314] Considering the strong influence of maturation and other biological determinants of bone strength (i.e., genetic factors account for approximately 83% of distal radius and 61% of tibial bone strength by pQCT),[175] I view the significant relationship between MVPA and bone strength across growth in girls and boys as meaningful. From a population health perspective, the significant associations I observed for MVPA suggest that meeting current PA recommendations may benefit adolescents' bone strength.

To my knowledge, this is the first study to show significant positive associations between habitual MVPA and bone strength at the distal radius. Greater bone strength in adolescents who engaged in more MVPA also contributed to a lower load-to-strength ratio, indicating a lower risk of forearm fracture. The weight-bearing tibia is more sensitive to environmental factors (i.e., mechanical loading due to locomotion) compared with the radius.[175] Further, counts from accelerometers worn at the hip were strongly associated with ground reaction forces,[219] and accurately quantified energy expenditure of upper limb activities.[372] Although counterintuitive at first glance, a similar influence of PA at both skeletal sites may be due to participants predominantly engaging in higher intensity activities that demand concordant upper and lower body movements (e.g., walking, running) and that stimulate movement and muscular contraction in both upper and lower limbs.

Metaphyseal sites are primarily loaded in axial compression, whereby bone's ability to resist loading (bone strength) is roughly proportional to the product of total bone CSA and square of bone density.[55] Thus, adding bone mass to the periosteal surface during growth substantially increases compressive bone strength. My finding that MVPA positively predicted Tt.Ar at the distal tibia in early and mid-puberty is consistent with previous studies in children and adolescents that demonstrated a positive relationship between PA (via accelerometery or self-report) and CSA (by DXA and HSA at the narrow neck, intertrochanter and proximal femur shaft,[285] periosteal circumference at the tibial shaft (by pQCT)[102] and Tt.Ar at the distal tibia (by HR pQCT).[109,310] Given that Tt.Ar at the distal tibia tends to plateau approximately 3 years after APHV (age 16.1 years in boys and 14.5 years in girls; Chapter 5),[314] the period of accelerated growth during early and mid-puberty represents the previously reported 'window of opportunity' to enhance bone geometry through the osteogenic effect of PA.[44]

To my knowledge, ours is also the first longitudinal study to examine adaptation of trabecular microarchitecture to PA during adolescence. My findings suggest that PA-related adaptations in trabecular bone volume likely contribute to greater bone strength in more active youth. The positive association I observed between MVPA and BV/TV at both skeletal sites across growth is consistent with previous cross-sectional studies, where I observed a positive association between MVPA and BV/TV[310] and self-reported loaded PA with Tb.BMD in adolescent girls at the distal tibia.[109] Our results also concur with cross-sectional studies that used high-resolution MRI and pQCT to assess bone in athletic populations and observed a

beneficial effect of weight-bearing PA on Tb.BMD.[165,281,295,373] The adaptation of trabecular tissue volume to mechanical loading may more efficiently transfer compressive loads from the joint surfaces and increase bone's mechanical competence.[165] That I did not observe an association of PA with Tb.Th is consistent with a cross-sectional study in collegiate gymnasts that found no difference in Tb.Th (assessed using high-resolution MRI) between gymnasts and controls.[373] However, my findings contrast animal studies that reported significant gains in Tb.Th in response to mechanical loading.[79,80] There is a need for intervention studies to clarify trabecular bone adaptation to PA during adolescence.

6.4.2 Physical activity and cortical bone

Until recently, assessment of cortical microarchitecture at metaphyseal sites was not possible, as low resolution imaging devices precluded quantification of thickness and porosity at sites with thin cortices, such as the distal radius. Thus, the influence of habitual PA on Ct.BMD and microarchitecture remained unclear. In the current study, I did not observe a relationship between MVPA and Ct.BMD in either sex during growth. These findings are consistent with our previous cross-sectional HR-pQCT studies where we did not observe an association between PA (self-reported[109]and accelerometry-derived MVPA[310]) and Ct.BMD at the distal tibia. Similarly, cross-sectional studies of young athletes who participated in weight-bearing sports showed little adaptation of Ct.BMD to loading at metaphyseal and diaphyseal sites.[165,280-282] However, studies of the tibial shaft (by pQCT) reported both positive[287] and negative [365] associations between PA (by self-report or accelerometry) and Ct.BMD in girls and boys, respectively. Discrepancies across studies may be due to differences in the imaging modality and/or the scan site (i.e., metaphyseal vs. diaphyseal site). Given that the metaphyseal cortex is rapidly modeled during periods of accelerated growth,[36] it is possible that greater mechanical loads applied during this period may delay consolidation of cortical bone due to incomplete trabecular coalescence or increased intracortical remodeling.

Few studies examined associations between PA and cortical microarchitecture at metaphyseal sites during adolescence. I found no relationship between Ct.Th and MVPA, which is consistent with our previous cross-sectional HR-pQCT study where we reported no association between weight-bearing PA (self-reported) and Ct.Th at the distal tibia in adolescents.[109] The

influence of PA on Ct.Th may be maturity- and site-specific, whereby pre-pubertal children and diaphyseal sites may be particularly responsive to adaptation to weight-bearing PA. For example, at diaphyseal sites, PA was positively associated with Ct.Th (by pQCT) in pre-pubertal children,[165,287,374] but not in a cohort of pre-, peri- and late-pubertal girls.[102] The influence of PA on metaphyseal cortical porosity is equally uncertain. My findings of greater Ct.Po at the tibia in participants who engaged in higher levels of MVPA during mid-puberty contrast my previous cross-sectional study where this positive association was not evident.[310] Ackerman and colleagues observed significantly greater Ct.Po at the tibia, but not the radius, in adolescent and young adult female athletes compared with non-athletic controls.[282] Authors speculated greater modelling/remodeling activity in athletes might drive greater porosity at the tibia. However, they cautioned that absolute values of porosity were low. Discrepancies regarding the influence of PA on Ct.Po may be partially due to the inability of HR-pQCT to resolve small pores, especially in growing children and adolescents.[4] Thus, future studies that use higher-resolution technology (i.e., XtremeCT II, 61 μm imaging resolution) may serve to clarify cortical microarchitectural adaptations to loading.

6.4.3 Sedentary time and bone parameters

Mechanistically, excessive sedentary time may negatively affect bone health by disrupting bone formation-resorption balance, as occurs in immobilized individuals.[303] However, it is unclear how sedentary time may affect healthy, ambulatory children and adolescents, and interact with or counter the osteogenic effects of PA. As in my previous cross-sectional study of the distal tibia in adolescents (Chapter 3),[310] I did not discern an association of bone strength with sedentary time independent of MVPA during adolescence. An apparent paradox exists whereby our cohort spent most of their waking hours (~70% of the day) in sedentary pursuits; however, this did not preclude their participation in PA. For example, although deemed sedentary, participants experienced mechanical loading and engaged in daily MVPA at levels comparable with other Canadian youth (59 min/day vs. 53 min/day in adolescent boys and 41 min/day vs. 39 min/day in adolescent girls).[248] Thus, the potentially detrimental effect of sedentariness on bone strength may be offset by the overriding osteogenic effect of MVPA.

MVPA did not attenuate the detrimental relationship between sedentary time and bone geometry. That is, sedentary adolescents had lower Tt.Ar at both skeletal sites, independent of MVPA during mid- to late-puberty. These results contrasted findings from my previous cross-sectional study where sedentary time was not associated with Tt.Ar.[310] It appears that a high volume of sedentary time in and of itself, beyond displacing PA, may be detrimental to periosteal expansion. Although the precise mechanism is unclear, periosteal expansion (as estimated by change in Tt.Ar) may be attenuated by reduced mechanical stimuli associated with sedentariness. Experimental dose-response studies are needed to advance our understanding of mechanisms by which sedentary behaviours negatively influence periosteal expansion.

The positive association between sedentary time and trabecular and cortical bone microarchitecture and density at the distal tibia during mid-puberty was unexpected and challenged my hypothesis. It may be that bone tissue is distributed and mineralized differently in adolescents who have smaller bones (due to lower levels of mechanical loading) as a means to maximize bone strength. Alternatively, high levels of PA may provide an osteogenic stimulus, while a high volume of sedentary time may promote bone consolidation. A similar positive association between sedentary time and BMC (by DXA) was observed at the spine and proximal femur in adolescents and young adults, independent of MVPA.[330] Our finding might be explained by experimental studies that demonstrated the importance of recovery periods between loading bouts for optimal biomechanical adaptation and to restore mechano-sensitivity of bone cells.[91,375] That sedentary time and MVPA both positively predicted Tb.Th, Ct.Th and Ct.BMD at the distal tibia was also unexpected. The correlation between MVPA and sedentary time was r = -0.59, which did not prompt concerns of collinearity (Variance Inflation Factor of 1.5). This scenario suggests that not all sedentary time is detrimental and a high active adolescent may benefit from also being highly sedentary (an 'active couch potato'),[376] as a means to 'recover' and enhance bone microarchitecture and BMD.

The sedentary time – bone relationship was maturity-specific, such that sedentary individuals had thicker and denser cortices during mid- to late-puberty (post-APHV). The metaphyseal cortex is rapidly modeled during early puberty; thus, bone turnover is elevated in both active and sedentary individuals. Beyond APHV, when longitudinal growth and bone modeling are reduced, differential adaptation of bone tissue to mechanical loading (i.e., active vs. sedentary adolescents) may be more apparent. For example, whereas intracortical remodeling

and periosteal apposition are heightened in active adolescents due to greater mechanical loading, cortical bone consolidation may be greater in sedentary adolescents due to lower intracortical remodeling and periosteal apposition associated with reduced mechanical stimuli. Regardless of differences in bone tissue distribution, the larger bones of active adolescents provide them a bone strength advantage at the distal tibia throughout adolescence. Future investigations into the pattern of sedentary time accumulation (i.e., long bouts of extended sedentary time vs. short bouts of sedentary time interspersed with PA) may help clarify the mechanism by which sedentary time influences bone microarchitecture.

I did not observe an association of within-person differences in MVPA or sedentary time with bone parameters throughout growth. Thus, habitual PA may be a stronger predictor of bone strength and its determinants in general populations of youth compared with individual changes in these behaviours from one year to the next. This may be a function of the lengthy bone modeling process, which requires 4 to 6 months to complete.[26] Therefore, significant time is required for changes in the loading environment to be discernable in the skeleton. On the other hand, the relationship between within-person differences in PA, sedentary time and bone parameters may have been tempered by only two to three years of follow-up data. Longer prospective studies would clarify the influence of inter- and intra-individual differences in PA and sedentary time on bone parameters.

I acknowledge several limitations of my study. First, as in any repeated measures study of growing bone, it is not possible to measure exactly the same bone cross section over time. Long bone length increases through endochondral ossification at both the proximal and distal growth plates and increases differentially at proximal and distal ends at different time points. [39] Therefore, we used a standard anatomical landmark to identify the same relative site along the length of the tibia and radius at each measurement in each participant. Second, we did not assess endocrine markers that represent activation of the hypothalamic-gonadal-pituitary axis or hand-wrist x-rays of skeletal maturity. Instead, I aligned participants on a common maturational landmark, maturity offset (years from APHV), as a means to control for the significant variation in maturation of children of the same chronological age. Third, my analyses were limited to peripheral sites, and may not represent relationships between bone parameters, MVPA and sedentary time at clinically relevant central sites. However, distal tibia parameters (by HR-pQCT) also reflected mechanical competence of the central skeleton (i.e., proximal femur and

lumbar spine).[121] Thus, increases in tibial bone strength due to PA may also indicate gains in strength at clinically relevant central sites. Fourth, high variability of Ct.Po may obscure the PA/sedentary time-bone relationship. Finally, uniaxial accelerometers do not directly measure ground reaction forces, nor can they accurately capture certain activities (i.e., bicycling or carrying loads) or distinguish between different sedentary postures, such as sitting, standing and lying down. Thus, it is possible that some activities were under-represented in my measures of PA and standing time may have been included in estimates of sedentary time.

6.5 Conclusions

Despite these limitations, my study was uniquely positioned to examine the influence of PA and sedentary time on growth-related adaptations in bone strength and parameters that underpin bone strength across adolescence. My study adds a unique perspective to evidence regarding the positive benefits of PA on bone strength and its determinants through childhood into adolescence. This relationship appears to be driven primarily by greater trabecular volume at distal sites, in concert with greater bone area at the tibia. I also provide new evidence that sedentary time may be detrimental to bone geometry, independent of PA. However, sedentary time may also be beneficial to some bone parameters in the presence of high levels of PA. Further investigation into patterns of PA and sedentary time accumulation and specific dose-response studies may shed light on the optimal dose of loading and recovery to enhance bone strength and its determinants during growth. Future studies should also examine these behaviours over a longer time frame to establish whether the benefits of habitual PA during childhood and adolescence persist into mid- and late-adulthood and how they influence fracture risk. As the beneficial role of PA for bone is well-established,[15] interventions that address the significant declines in MVPA and concurrent increases in sedentary time during adolescence are warranted.

Chapter 7: Integrated Discussion

The overall aim of my thesis was to examine the influence of PA and sedentary time on bone strength accrual across adolescent growth. To this end, I conducted four studies. In this chapter, I review and integrate key findings from each study and highlight how they contribute to the literature. I then discuss unique aspects and implications of my findings to the field of pediatric bone research. Finally, I discuss methodological challenges of longitudinal studies, the public health implications of my findings and my thoughts on future directions for this field.

7.1 Overview of findings

7.1.1 Bone strength and microarchitecture in the growing skeleton: the role of sedentary time

Summary (primary objective – self-reported screen time):

a) Self-reported screen time was not associated with bone parameters at the distal tibia, independent of self-reported impact PA, maturity (Tanner stage for boys and menarcheal status for girls), ethnicity, tibia length, MCSA and dietary calcium.

Summary (secondary objective – objective measured sedentary time):

a) Objectively-measured sedentary time (volume and patterns) was not associated with bone parameters at the distal tibia, independent of MVPA maturity (Tanner stage for boys and menarcheal status for girls), ethnicity, limb length, MCSA and dietary calcium.

Summary (tertiary objective – muscle force and modulator variables):

a) Maturity (by Tanner stage in boys and menarcheal status in girls) predicted 3-35% of variance in bone parameters at the distal tibia. BV/TV in boys was the only parameter not significantly associated with maturity ($p = 0.053$).

b) MCSA predicted 4-14% of variance in Tb.N, Tt.Ar and F.Load in boys and girls and BV/TV, Ct.Po, Ct.Th and Tt.BMD in girls participants at the distal tibia.
c) Tibia length predicted 2-11% of variance in Tt.Ar in boys and girls, Tb.Th in boys and F.Load in girls at the distal tibia.
d) Dietary calcium predicted 4% of variance in BV/TV and Tb.N in girls at the distal tibia.
e) MVPA predicted 2-5% of variance in Tt.Ar and F.Load in boys and BV/TV, Tb.Th, Ct.Th and Tt.BMD in girls at the distal tibia.

Conclusions:

a) Self-reported screen time and objectively-measured sedentary time (volume and patterns) are not associated with distal tibia bone strength, geometry, BMD or microarchitecture in a sample of healthy children, adolescents and young adults.
b) Maturity, surrogates of muscle force and limb length are important predictors of bone strength and its determinants during adolescence.
c) MVPA is a weak, but important predictor of bone strength and its determinants during adolescence. The non-significant relationship between MVPA and bone strength in girls ($p = 0.10$) may reflect relatively low engagement in MVPA in girls compared with boys.

In Chapter 3, I conducted a cross-sectional study to determine associations between sedentary time and bone parameters in children, adolescents and young adults. My findings extended a relatively limited body of evidence regarding the role of sedentary time on bone parameters during childhood and adolescence. For example, two retrospective cohort studies examined associations between self-reported TV viewing during childhood and aBMD and BMC (by DXA) in young adulthood;[299,305] one short-term (2-year) prospective study examined associations between objectively-measured sedentary time (by accelerometry) and aBMD and BMC accrual (by DXA);[301] and no studies investigated the influence of sedentary time on bone parameters using 3D imaging.

Most of what we know about the growth and development of human bone comes from early DXA studies. There studies (including many from our lab) predominated from the mid-1990s to mid-2000s. As new technologies emerged, our research group acquired novel 3D

imaging tools such as pQCT and HR-pQCT to characterize bone geometry and microarchitecture. My additional contribution was to recognize the need to quantify the influence of sedentary time (as well as PA) on growing bone. Thus, my study is the first to assess associations between sedentary time and bone parameters using 3D imaging. My study is also the first to examine associations between patterns of sedentary time (number of breaks in bouts of sedentary time using accelerometry) and bone parameters. I found no association between sedentary time and bone strength and its determinants at the distal tibia in boys or girls. I used multivariable regression to examine bone strength's association with sedentary time, independent of PA, maturity, ethnicity, MCSA, limb length and dietary calcium, as these factors are all known to play a role in bone accrual.

I found it interesting that sedentary time was not a detriment to bone parameters in the HBSIII cohort. Several reasons might explain this. First, boys and girls participated in MVPA (min/day) comparable with other Canadian and American youth.[248,249] Thus, this level of PA may have been sufficient to counteract potential negative effects of prolonged sitting on weight-bearing bone parameters. Second, given the cross-sectional design of my study, I could not infer causality and I consider this first study as hypothesis generating and a strong base to guide future work. For example, focused studies with a 'stronger' research design (i.e., prospective studies, such as in Chapter 6) help clarify how reduced loading of the skeleton may influence bone health in children and youth. The functional model of bone development is a central tenet to describe bone accrual in pediatric research.[57,60] My study aligns with this position as it reaffirms a strong role for muscle force and maturity as predictors of bone strength.

Based on my findings and given the known limitations of cross-sectional studies (e.g., inability to separate mean differences across age groups from age-dependent associations),[345] it seems that public health initiatives that aim to increase PA may prove more beneficial for bone health in children and adolescents than those that aim to decrease sedentary time. However, targeted RCTs are needed to confirm this as none currently exist. Further, I focused on the weight-bearing tibia in this study. Thus, there is also a need to explore relationships between sedentary time and bone parameters at more clinically relevant sites, such as the distal radius.

7.1.2 Re-examining the surfaces of bone in boys and girls during adolescent growth

Summary (primary objective – periosteal and endocortical surfaces):

a) Boys and girls demonstrated periosteal and endocortical bone expansion (increase in medullary area) throughout adolescence at the tibial midshaft. Boys had 27% and 31% greater Tt.Ar and Me.Ar, respectively, at APHV compared with girls.
b) Boys demonstrated greater annual accrual rates at the periosteal surface (Tt.Ar) of the tibial midshaft compared with girls, pre- (18%) and post-APHV (89%).
c) Boys demonstrated greater endocortical expansion (Me.Ar) at the tibial midshaft compared with girls, pre- (34%) and post-APHV (163%).

Summary (secondary objective – bone strength and Ct.BMD):

a) Boys and girls demonstrated increases in bone strength (SSI_p) at the tibial midshaft throughout adolescence. Boys had 36% greater SSI_p at APHV compared with girls.
b) Boys and girls had similar annual accrual rates of SSI_p pre-APHV (272 and 237 mm^3/yr; $p = 0.054$).
c) Boys demonstrated 95% greater annual accrual rates of SSI_p post-APHV compared with girls.
d) Boys demonstrated decreases in Ct.BMD prior to APHV and increases in Ct.BMD post-APHV, whereas girls experienced increases in Ct.BMD pre- and post-APHV at the tibial midshaft. Girls had 3% greater Ct.BMD at APHV compared with boys.
e) Girls had greater annual accrual rates of Ct.BMD pre-APHV compared with boys (-1 and 16 mg/cm^3/yr in boys and girls, respectively).
f) Boys had 23% greater annual accrual rates of Ct.BMD post-APHV.

Conclusions:

a) Periosteal expansion is accelerated during adolescence at the tibial shaft, and is more evident in boys than girls.

b) Girls experience diminished endocortical expansion compared with boys.
c) More dense cortices in girls may reflect lower rates of intracortical remodeling associated with smaller magnitude of growth compared with boys.
d) Boys' larger bones (greater Tt.Ar), enhanced rates of change in bone parameters, and prolonged duration of longitudinal growth confer boys a bone strength advantage throughout adolescence.

In Chapter 4, I challenged a pre-existing paradigm within the pediatric bone literature that bone is accrued preferentially on the periosteal surface in boys and the endocortical surface in girls. Thus, I sought to clarify sex differences in bone expansion and contraction on bone surfaces at the tibial midshaft during adolescence.

My study is the first prospective study to align boys and girls on a common maturational landmark (APHV) to examine sex differences in bone development (using pQCT) during adolescent growth. This is key given the well known influence of maturation on bone accrual. This 12-year mixed longitudinal study is the longest study of adolescent bone growth conducted to date that used 3D bone imaging techniques.

In the 1970s, a pioneer in our field, Stanley Garn, conducted a landmark cross-sectional study using radiography (a planar technique) to image the second metacarpal. He reported boys gained more bone at the periosteal surface compared with girls, while girls gained more bone at the endocortical surface compared with boys during adolescence.[155-157] My prospective findings using more advanced imaging concur with Garn's reports of greater periosteal bone expansion in boys compared with girls. However, my findings challenge the notion of a sex difference in endocortical bone contraction. Importantly, I reported that endocortical bone expansion predominates in both boys and girls during adolescent growth. However, at the mid-tibia, girls' bone was preserved to a greater extent (less expansion) compared with boys. Greater endocortical expansion in boys, coupled with periosteal expansion, serves to distribute bone further away from the neutral axis, thereby enhancing bone strength in bending and torsion. This might explain the bone strength advantage conferred to boys and men throughout life, assuming this skeletal advantage persists from adolescence into late-adulthood.

Importantly, girls had more dense cortices compared with boys across adolescence. More dense bones may partially compensate for girls' smaller bone size, compared with boys, on

average. However, at long bone shafts, bone geometry influences bone strength to a greater extent than does density. More dense cortices in girls may reflect lower rates of intracortical remodeling due to smaller magnitude and shorter duration of longitudinal growth compared with boys.

I conclude that greater bone strength in boys at the weight-bearing tibia across adolescence appears to be driven by boys' larger bones and longer duration of longitudinal growth compared with girls. It seems important to evaluate whether similar sexual dimorphism in bone strength and bone accrual exists at other skeletal sites, such as the clinically relevant distal radius, as this has not yet been examined. We do not know whether or how these sex differences in bone accrual might influence fracture risk later in life. Longer term prospective trials should, in future, evaluate whether boys retain their bone strength advantage into adulthood and older age. However, given the challenges and costs associated with long term follow-up, a prospective study from adolescence through late adulthood is improbable. In the absence of these studies, mixed-cohort studies that span early- through late-adulthood would serve to clarify the strength advantage in boys and men and how this advantage influences sex differences in fracture risk.

7.1.3 Sex differences and growth-related adaptations in bone microarchitecture, geometry, density and strength

Summary (primary objective – growth-related adaptations):

a) Boys and girls demonstrated growth-related gains in Tb.Th, Ct.Th, Ct.Ar, Tt.Ar, Ct.BMD, Tt.BMD, F.Load and U.Stress across adolescent growth at the distal tibia and radius, declines in Ct.Po at both sites and declines in load-to-strength ratio at the radius.
b) Tb.N remained relatively constant across adolescent growth in boys and girls at the distal tibia, but decreased at the radius. Conversely, Tb.Sp increased at the distal radius but remained relatively constant at the tibia in boys and girls across adolescent growth.
c) BV/TV increased across growth in boys at both sites and in girls at the distal tibia, but remained relatively constant in girls at the radius.

d) Distal radius growth curves for Ct.BMD, Tt.BMD and Ct.Th suggested transient decreases around APHV in boys and girls.
e) Growth curves for Ct.Po suggested a transient increase around APHV at both sites in boys and at the radius in girls, prior to declining after APHV.

Summary (secondary objective – sex-related adaptations):

Across adolescence and compared with girls:

a) Boys demonstrated 28-63% greater F.Load at both skeletal sites.
b) Boys demonstrated 13-45% greater Ct.Ar and Tt.Ar at both skeletal sites.
c) Boys demonstrated 28-80% greater Ct.Po at both skeletal sites.
d) Load-to-strength ratio at the distal radius was 26-27% lower in boys, indicating lower risk of distal forearm fracture.
e) Boys had similar Ct.BMD at both skeletal sites.
f) BV/TV was 6-25% greater in boys from 1-year prior to APHV at the tibia and from APHV onwards at the radius.

Conclusions:

a) Boys' superior bone strength at the distal tibia and radius is underpinned by their consistently larger bones, thicker cortices and greater trabecular bone volume.
b) Boys' higher incidence of forearm fracture during adolescence may be a function of greater porosity compared with girls across adolescence.
c) Similar Ct.BMD between sexes despite greater Ct.Po in boys suggests boys may compensate for larger cortical pores with greater cortical material mineral density.
d) Increases in BV/TV during growth are underpinned by thickening of trabeculae.

In Chapter 5, I extended the pediatric bone literature by examining growth- and sex-related adaptations in bone strength and its determinants at the distal tibia and radius across adolescent growth. This study is important as fracture risk is ultimately related to bone strength.

It is only through precise and accurate 3D imaging systems that we can evaluate the strength of growing bone. Specifically, my study is the longest study to date to use HR-pQCT to assess bone parameters in boys and girls across adolescence. Importantly, based on the longitudinal nature of my data, I was able to align boys and girls on a common maturational landmark (maturity offset) to compare bone parameters between sexes at an equivalent maturational time point.

I found that Ct.BMD (by HR-pQCT) at the distal tibia and radius was similar in boys and girls. This finding challenged my hypothesis that girls would demonstrate denser cortices compared with boys. My hypothesis was driven by our previous study of adolescents (same cohort, but fewer years of follow-up and aligned on Tanner staging) where girls demonstrated greater Ct.BMD (by HR-pQCT) compared with boys in peri- and post-puberty.[4] Similar Ct.BMD in boys and girls also contrasted my findings at the tibial shaft (by pQCT; Chapter 4), where girls demonstrated greater Ct.BMD at APHV compared with boys.

These differences in findings at the cortex between boys and girls may be rooted in the skeletal site assessed (i.e., primarily compressive stresses at distal sites versus bending at shaft sites) and imaging modality used to assess bone (i.e., more accurate assessment of the cortical shell with HR-pQCT versus pQCT). Probably of most significance, I contend that reportedly more dense cortices at distal sites in girls compared with boys during later maturity may be an artifact of how researchers controlled for maturity. For example, our previous analysis aligned boys and girls using the method of Tanner and found significantly greater Ct.BMD in girls compared with boys;[4] whereas I did not observe sex differences in Ct.BMD when aligned on maturity offset. Comparisons of Ct.BMD between sexes aligned on the method of Tanner may be confounded by differences in timing of growth relative to secondary sex characteristics. That is, Tanner staging, although more convenient and popular, is not a perfect maturity match between boys and girls who may have different tempo and timing of indicators of sexual maturity. We do a better job of controlling for maturity by aligning participants on a reliable universal maturational indicator – linear growth in height. That said, the field would move forward if more studies align boys and girls on maturity offset (based on APHV). Further, studies that use similar segmentation methods to accurately separate cortical and trabecular bone are warranted to confirm my findings. Finally, as girls mature before boys, more girls than boys in my study were near or at APHV at study entry. Thus, future studies that follow girls and boys beginning at a younger age (i.e., pre-puberty) would clarify sex differences in Ct.BMD prior to APHV.

Of interest, Ct.Po was the only bone parameter that favored girls at the distal tibia and radius during peak adolescent growth. This biological disadvantage in boys may partially explain adolescent boys' higher incidence of forearm fractures compared with girls.[148] However, despite deficits in Ct.Po, boys had consistently greater bone strength and lower estimates of forearm fracture risk compared with girls across adolescent growth. Thus, compared with girls, boys' substantially larger bones likely compensated for any deficit in Ct.Po. In addition, although a tremendous advancement in bone imaging, resolution of HR-pQCT may be insufficient to accurately assess porosity in regions where the cortical shell is thin, such as in rapidly growing children and adolescents at the tibia and at all ages at the distal radius. In future, studies that employ the next generation of 3D imaging techniques with higher resolution (i.e., XtremeCT2) may help to clarify sex differences at the cortex.

These findings and my results in Chapter 4, suggest boys' greater bone size and strength at the distal tibia and radius will confer them an advantage across adolescent growth, compared with girls. Boys' more porous cortices, on average, may contribute to higher fracture incidence during adolescence; however, this needs to be evaluated in light of boys' increased risk taking behaviours.[377] Although a relatively daunting task (i.e., large number of participants to achieve adequate statistical power), long-term prospective studies of fracture risk are needed to clarify the influence of bone microarchitecture on fracture risk. An important next step is to recruit a large sample of boys and girls who have sustained a fracture and compare microarchitecture parameters between boys and maturity-matched female peers.

7.1.4 Physical activity, sedentary time and bone strength from childhood to early adulthood

Summary (primary objective – PA):

a) MVPA was greater in boys compared with girls (~16 min/day), but declined by approximately 31% (11 min/day) in both sexes across adolescence. Sex did not moderate the relationship between MVPA and bone parameters.
b) Between-person differences in MVPA positively predicted bone strength (F.Load) and BV/TV at the distal tibia and radius across adolescence. Participants in the upper quartile

of MVPA had 6-8% greater F.Load and 4-5% greater BV/TV compared with participants in the lowest quartile of MVPA.

c) Tt.Ar was 4-6% greater in participants in the upper quartile of MVPA from 1-year prior to APHV to 3-years post APHV compared with peers in the lowest quartile of MVPA.
d) Participants in the upper quartile of MVPA had 7-12% greater load-to-strength ratio between 2-years prior to APHV and 2-years post APHV compared with peers in the lowest quartile of MVPA.
e) Within-person change in MVPA did not significantly predict bone strength or its determinants at the distal tibia or radius.

Summary (secondary objective – sedentary time):

a) Sedentary time was similar between boys and girls and increased approximately 30% (2.5 h/day) across adolescence. Sex did not moderate the sedentary time – bone relationship.
b) Between-person differences in sedentary time were negatively associated with bone strength (F.Load), independent of sex, ethnicity, maturity, muscle power, limb length and dietary calcium, but not after additional adjustment for MVPA.
c) Compared with peers in the lowest quartile of sedentary time, participants in the upper quartile of sedentary time had: 1) 9-14% lower Tt.Ar at the distal tibia from 1-year prior to APHV onwards and 2) 9-13% lower Tt.Ar at the distal radius from 3 to 9 years post-APHV.
d) At the distal tibia, participants in the upper quartile of sedentary time had: 1) 10% greater Tb.Th across growth, 2) 9-12% greater Ct.Th from APHV to 3-years post-APHV, 3) 24% greater Ct.Po at 2-years prior and 18-24% lower Ct.Po at 3 and 4-years post-APHV and 4) 3-5% greater Ct.BMD from APHV to 4-years post-APHV.
e) MVPA was positively associated with Tb.Th, Ct.Th and Ct.BMD in sedentary time models that adjusted for MVPA.
f) Within-person change in sedentary time did not significantly predict bone strength (F.Load) or its determinants at the distal tibia or radius.

Conclusions:

a) Bone parameters demonstrate maturity-specific associations with MVPA and sedentary time across growth. Thus, early- and mid-puberty may be particularly important years to target these behaviours.
b) My findings support the importance of MVPA for bone strength accrual across adolescent growth.
c) The MVPA – bone strength relationship at distal sites appears to be driven by adaptations in trabecular bone volume.
d) MVPA attenuates the detrimental relationship between sedentary time and bone strength.
e) Sedentary time is detrimentally related to bone geometry, independent of MVPA.
f) Sedentary time may benefit some bone parameters. High levels of MVPA may provide an osteogenic stimulus, while a high volume of sedentary time may promote bone consolidation.

Given the fundamental relationship between mechanical loading and bone mass and strength accrual, in Chapter 6, I examined associations between PA, sedentary time and bone parameters across adolescence. These prospective data are novel as is the use of HR-pQCT to assess bone parameters over 3 to 4 years of longitudinal growth. Further, I objectively assessed PA and sedentary time (using accelerometry). This methodology and my focus on reduced loading of bone is unique within the pediatric bone health literature, as subjective methods (questionnaires) and a focus on elevated loading of the skeleton are still most common.

My findings reaffirm that MVPA is associated with bone strength accrual. Early and mid-puberty represent a 'window of opportunity' where bone strength accrual might be enhanced through engaging in higher intensity PA (i.e., MVPA). This study is important as it is the first to show a positive association between habitual MVPA and bone strength at the non-weight bearing distal radius. Although the mechanism at this non-weight bearing site is not as intuitive as activities that load the weight-bearing tibia, participation in common activities (e.g., running) stimulates muscular contraction in both upper and lower limbs. My study is also the first to prospectively examine the influence of elevated and reduced loading on trabecular and cortical

bone microarchitecture across adolescence. Importantly, positive adaptations in bone strength at the distal tibia and radius were driven by gains in trabecular bone volume.

A plethora of evidence demonstrated the benefits of weight-bearing PA for bone health. However, the problem that currently plagues Canadian children and youth is the propensity to sit in front of TV and computer screens.[248] Thus, perhaps the more relevant question is what happens when we reduce loading of the skeleton during adolescence. My findings suggest that children or youth who engage in high-intensity PA offset the potentially detrimental effect of sedentariness on bone strength. However, these activities did not attenuate the detrimental relationship between sedentary time and bone geometry. This contrasts the non-significant association between sedentary time and bone geometry I report in Chapter 3. This difference likely reflects the cross-sectional versus prospective study design and the more accurate assessment of maturity using APHV in Chapter 6. I observed positive associations between sedentary time and Tb.Th, Ct.Th and Ct.BMD at the distal tibia, which also contrasted my null-findings in Chapter 3.

My results present the profile of a highly active, highly sedentary person (so called 'active couch potato').[376] An example of this would be a child or youth who is involved in school sport, but who is sedentary outside of actively engaging in practice or playing games related to that sport. This kind of activity does not appear to be detrimental to bone and may in fact benefit some bone parameters by promoting bone consolidation.

Prospective studies that examine types, intensities and patterns of PA as well as sedentary time accumulation (i.e., bouted PA and sedentary time) are needed to help us understand the 'optimal dose' of PA and recovery to enhance strength of the adolescent skeleton. Further, given the intricacy and interrelatedness of factors that influence bone accrual, complex statistical approaches are needed. Compositional analysis techniques similar to those used in recent cardiometabolic health studies[378,379] may provide an integrated approach to evaluate the interrelatedness of sedentary time and PA (i.e., how sedentary time changes when MVPA increases or decreases, and vice versa).[378,379]

Null associations between within-person change in MVPA and sedentary time and bone parameters may reflects: 1) the lengthy bone modeling process and 2) the relatively short follow-up of my study (two to three years). Thus, prospective studies that follow the same participants

from pre- to post-puberty would clarify inter- and intra-individual differences in the influence of elevated and reduced loading on bone parameters.

My results clearly highlight the complexity of bone growth and development wherein the bone response to different kinds of loads is site-, bone compartment- and maturity-specific. Thus, there remains much room within this field to investigate these associations and responses across different kinds of activities and different stages of maturity. In closing, my study adds a unique perspective the PA-bone strength relationship. My findings suggest that adolescents who meet current PA guidelines may achieve bone strength benefits. However, the optimal PA dose for bone strength accrual and whether benefits of PA for adolescent bone health persist into adulthood, remain to be determined.

7.2 Challenges and future directions

In this section, I briefly describe challenges associated with examining bone strength, PA and sedentary time during adolescence. I include implications of my work and its application to future research.

7.2.1 The use of pQCT and HR-pQCT imaging systems in pediatric bone research

In sections 1.2.3.2 and 1.2.3.3, I describe how pQCT and HR-pQCT offer several advantages over DXA imaging. Specifically, pQCT and HR-pQCT permit us to examine 3D bone geometry and estimate bone strength. Of particular relevance to my thesis, HR-pQCT allows us to examine trabecular and cortical bone microarchitecture. However, efforts are needed to standardize protocols for scan acquisition (e.g., reference line placement and measurement site), scan analysis and establish long-term precision in prospective studies. Further, calibration within and between scanners is necessary for normative data to be of value (available for pQCT in youth[97] and HR-pQCT in late adolescents[122]).

Long-term precision is a key issue in longitudinal studies that use pQCT and HR-pQCT to examine bone parameters in children and adolescents. During growth, long bones lengthen through endochondral ossification at proximal and distal growth plates. However, the process is complex, as tempo and timing of longitudinal bone growth differs at the proximal compared with

distal end of long bones.[39] Thus, it is not possible to assess exactly the same site on the tibia and radius over time.

Several approaches are used to reproduce the same measurement site over time. Although researchers used a fixed landmark for adult (and some pediatric) studies, this represents a fundamental challenge when assessing growing bone. A fixed distance region of interest (ROI), represents a "moving target" in children and adolescents in that it shifts relative to timing and magnitude of longitudinal bone growth; of greatest concern during rapid adolescent growth. Therefore, our lab uses a percent distance ROI from a fixed anatomical region, as I described in section 1.2.3.2.1. Although the 'exact' same ROI will not be measured in its entirety across growth as bone migrates proximally, we identify the same relative ROI across years. I suggest that researchers consider the most appropriate ROI as it relates to their research question and study population. If the goal is to conduct longitudinal studies during growth or to compare between participants of different body sizes, I believe the relative ROI approach is most appropriate. However, I recognize this approach is not without limitations.[110,111] For example, manual assessment of limb length (to determine the relative ROI) may introduce measurement error not present using the fixed distance approach. Importantly, limb length imprecision should introduce random error (noise) rather than a systematic difference between individuals. Nevertheless, adequate training (with evaluation of intra- and inter-rater reliability) and having the same measurer/technician across all years of a study will reduce measurement error.

Long bones are complex, heterogeneous structures; thus, lower resolution of pQCT (0.2 – 0.5 mm pixel size) compared with HR-pQCT may limit our ability to detect small changes in the cortex and endocortical surface. Further, the number, size and shape of pores, vary along the length of bone and within the cross-section of the cortex. Accurate definition of periosteal and endocortical surfaces remains a challenge during HR-pQCT analysis. Partial volume effects may prevent accurate separation of cortical and trabecular bone in regions with thin cortices. This is especially true at the endocortical surface at metaphyseal sites, due to the gradual transition from cortical to trabecular bone. As I discussed in section 1.2.1.4, trabecular bone originates at the growth plate through endochondral ossification and subsequently thickens and coalesces into cortical bone at the endocortical surface.[380] During periods of rapid growth, 'corticalization' of trabeculae is delayed. Thus, HR-pQCT segmentation algorithms may misclassify incompletely

coalesced trabeculae as cortical bone, thereby overestimating thickness and porosity of the cortex.[6]

Two approaches are commonly used to segment cortical bone: 1) threshold or 2) density-based approach. A comprehensive review of limitations associated with each approach is beyond the scope of this thesis but are documented elsewhere.[362] In brief, the threshold-based approach (described in section 2.2.8.2) underestimates Ct.Po as it cannot accurately detect smaller pores. In contrast, the density-based approach overestimates Ct.Po and assumes homogenous mineralization throughout bone. Approaches used to segment the cortex need to be standardized in studies that use similar imaging technologies. The most accurate approach will likely be informed by newer generation HR-pQCT systems (imaging resolution 61 μm).

Clinically, we are concerned with where and how bones fail. Thus, the focus in older adults is at the proximal femur, lumbar spine and forearm. Bone strength at the hip and spine cannot be assessed in the growing skeleton using 3D imaging without considerable ionizing radiation. Although bone parameters assessed at the distal tibia (by HR-pQCT) were strongly correlated with mechanical competence of the central skeleton (i.e. proximal femur and lumbar spine) in adults,[121] we do not know whether the same is true in children and youth. Further, we do not know if estimates of bone strength by pQCT or HR-pQCT predict fracture in children and adolescents. In my study, fracture incidence was low (7 prospective fractures at all sites; i.e., metacarpal, scaphoid, radius, ulna, tibia) and we did not obtain details on fracture mechanism (i.e., low or high energy). Therefore, I was unable to align bone data based on fracture occurrence. Prospective studies that clarify short- and long-term implications of maturity- and sex-related differences in bone parameters on fracture risk are warranted.

7.2.2 Maturity

Longitudinal studies of bone strength during adolescence are rare because they present a substantial challenge compared with studies of the mature skeleton. To illustrate, as mentioned earlier in my thesis, maturation strongly influences bone accrual; however, maturity status varies between children of the same chronological age. It is therefore imperative that researchers account for maturity in all studies of bone conducted in children and adolescents. Nevertheless, precise assessment of maturity remains a challenge. For the study I describe in Chapter 3, I

accounted for maturity using the method of Tanner for boys and menarcheal status in girls. However, timing of maturation differs between sexes, and it was not possible to compare boys and girls at the 'exact' same stage of maturity. Age at menarche is a discrete maturational event in girls but there is no equivalent maturational marker in boys.

In Chapters 4-6, I had access to longitudinal data and aligned participants on a common somatic maturational landmark, APHV (maturity offset). As a continuous measure, maturity offset overcomes limitations of maturity assessments as per the method of Tanner and menarcheal status. Direct assessment of maturity offset in longitudinal studies requires serial measures of height surrounding APHV, so is not an option in cross-sectional, short-term prospective studies or any study without measures of height across the pubertal growth spurt. In cross-sectional or short-term prospective studies, validated equations may be used to predict maturity offset, but may not be as accurate as direct assessment.[141,142]

Although a reliable approach to identify a common maturation landmark, maturity offset also has limitations. APHV is a global measure of linear growth in height and does not account for timing, tempo and regional differences in appendicular and axial growth patterns. For example, peak growth in leg length precedes peak in sitting height or trunk growth. Further, truncal growth extends over a longer period and contributes proportionally more to overall height during the adolescent growth spurt compared with leg length. Importantly, these processes occur at a similar (and predictable) relative time in boys and girls. For example, peak leg velocity occurs approximately six months prior to APHV in boys and girls, while peak sitting height velocity occurs approximately two months post-APHV in boys and girls.[381,382] Thus, in both sexes, peak growth of the axial and appendicular skeleton occurs at roughly the same relative time. Further, estimated velocities of many performance tasks in boys and girls reach a peak at the same approximate time as maximal growth in height.[136-138] Thus, APHV is an important relative marker of function in boys and girls. In the few studies that examined APHV relative to skeletal age, APHV approximated that of peak skeletal age velocity.[139,140] Ideally researchers would assess endocrine markers that represent activation of the hypothalamic-gonadal-pituitary axis or hand-wrist x-rays of skeletal maturity; however, these assessments are not always possible in studies of healthy children. Therefore, despite some limitations, APHV is an acceptable and perhaps preferred approach to assess maturity in pediatric bone studies as a means to compare bone outcomes between sexes.

7.2.3 Assessment of physical activity and sedentary time

It is irrefutable that weight-bearing PA or mechanically loading the skeleton is critical to enhance bone strength. Thus, it is imperative to accurately quantify PA in order to examine PA-related adaptations in bone parameters. For my study, I assessed PA objectively using accelerometry. Accelerometry is reliable[232] and provides a measure of intensity and duration of PA. Accelerometers do not directly measure ground reaction forces; however, raw vertical accelerations from accelerometers were strongly associated with ground reaction forces (by mechanography) in adults ($r = 0.85$)[219] and in children and adolescents (healthy children and those with osteogenesis imperfecta type 1, age 6-21 years; $r = 0.96$).[220] Thus, I contend that accelerometry is an acceptable surrogate to represent mechanical loading experienced by human bone. Accelerometers are limited by their inability to accurately capture certain activities (e.g., bicycling or carrying loads) and low participant compliance, particularly in pediatric studies. Waist-worn accelerometers are cumbersome, uncomfortable and easy to forget to put on in the morning after they are removed at night.[229,383] I observed poor compliance in my studies; my sample size decreased by approximately 22% after I excluded participants with fewer than 3 days (of 7 days) and at least 10 h/day of wear time.

Wrist-worn accelerometers may improve accelerometer wear compliance in future studies. To illustrate, NHANES switched from waist-worn to wrist-worn accelerometers for the 2011-2012 measurement cycle. In doing so, compliance increased to 70-80% (at least 6 days of data with 10 h/day of wear) from 35% in the 2003-2004 cycle when waist-worn accelerometers were used. In addition, NHANES used waterproof wrist-worn accelerometers, which allowed participants to wear the accelerometer continuously (i.e., 24 h/day) during the measurement period. Monetary incentives, reminder phone calls and text messages may also improve accelerometer compliance.[229]

Popularity of wearable technology (e.g., Fitbit) may also represent an opportunity to enhance compliance in PA studies.[384] For example, compliance was 98% (24 h/day for 5 days) in a study of 7-10 year olds that used Polar Active wrist monitors. Polar Active monitors are waterproof devices with a device display and a tri-axial accelerometer (epoch length of 30-sec only). They can be purchased at a fraction of the cost of a traditional accelerometer ($99 vs.

$400).[385] However, criterion validity of PA in children and youth assessed with new generation wearable devices must first be ascertained.[386]

Objective measures of sedentary time are important to accurately assess the influence of reduced loading on bone adaptation. Sedentary time is typically defined as an energy expenditure ≤ 1.5 METs in a seated or reclined posture.[243] Accelerometers can estimate sedentary time, but cannot distinguish between different postures, such as sitting, standing and lying down. Thus, standing without movement is often misclassified as sedentary even though standing is a weight-bearing activity and considered light PA by definition. Use of postural measurement devices (e.g., ActivPAL) in studies that assess sedentary time may better differentiate between standing and sitting.

Finally, comparing PA and sedentary time across studies is confounded by different devices (e.g., Actical in CHMS versus ActiGraph in NHANES), cut points, epochs and non-wear definitions. These methodological differences could alter dose-response relationships and public health recommendations (e.g., duration and intensity of PA for optimal bone health). Consensus within the research community is needed to standardize accelerometry methods for children and youth. In the interim, researchers must be fully transparent regarding accelerometry methods and associated limitations.

7.3 Challenges with longitudinal study designs

Longitudinal data are invaluable as they capture nuances of bone growth and development across time. However, longitudinal studies present a number of methodological challenges. First, attrition can result in significant declines in number of participants across time. To illustrate, of 1071 total HBSIII participants at baseline, only 306 remained at study completion 12 years later. As I described in section 2.1.4, we used many incentives during HBSIII to retain participants. Loss to follow-up was likely compounded by the challenge of tracking/locating participants as they matured and left home to study or travel across the 12-year study. Second, measurement error may be introduced during longitudinal studies as research staff turns over. To reduce measurement errors in HBSIII, we conducted inter- and intra-rater reliability training every year with research assistants/technicians responsible for anthropometry and imaging. Third, changes in measurement tools (e.g., new technology) may introduce bias if

outcomes from the new device are not comparable with those from previous technology. In HBSIII, we acquired pQCT scans using XCT-2000 (gantry aperture of 140 mm) from 2003-2007. We acquired an XCT-3000 in 2008 (gantry aperture of 300 mm) to accommodate larger limbs of adolescents and young adults. To ensure comparable data between XCT-2000 and 3000, we assessed bone density, geometry and strength parameters in 28 adults at the tibial midshaft and confirmed excellent agreement (r = 0.90-0.99) between instruments.[323] Finally, longitudinal data are not independent measures (i.e., there is within-person correlation between measurements). Thus, I used linear mixed models in Chapters 4-6 to account for the correlated nature of my data. With mixed models, I used random intercepts and slopes to provide each participant his or her own trajectory across time.[370]

Finally, I acknowledge several other limitations of my studies. First, many girls in HBSIII were close to APHV at baseline assessment (using pQCT) and were post-pubertal at first HR-pQCT assessment (Table 6.3). Thus, I was limited to comparing boys and girls and maturity-related differences at 2-years before APHV (but not earlier). Prospective studies that target pre-pubertal children would clarify maturity- and sex-related adaptations in bone parameters prior to APHV. Second, volunteer sampling methods limit external validity of my results. Finally, our ethnically diverse sample of children from Metro Vancouver (where visible minority groups represent 47% of the population)[351] may limit generalizability of our findings outside this geographic region.

7.4 Public health implications

Taken together, my results suggest that early- and mid-puberty are critical windows that may provide a unique opportunity to optimize bone strength accrual with PA. As I demonstrated in Chapter 6 and others have observed globally,[387,388] adolescence is characterized by substantial declines in PA and increases in sedentary time. Given moderate tracking of PA from adolescence through adulthood,[389] the foundation for PA is set early in life. Thus, we must promote healthy movement behaviours during the formative childhood and adolescent years. Dose-response studies are needed to inform specific prescription (e.g., frequency, intensity and duration) of PA for optimal bone strength accrual, which may lead to modifications of current PA guidelines for musculoskeletal health.

Further study is also required to inform public health guidelines related to sedentary time and bone health. Canadian public health guidelines recommend children and youth limit screen time and prolonged periods of sitting, based on detrimental associations between screen time and cardiovascular and metabolic health outcomes [390]. Research into the sedentary behaviour – bone health relationship is in its infancy compared with other health outcomes and we have much to learn about how bone adapts to reduced loading while simultaneously interacting with the osteogenic effects of PA. What limited evidence exists regarding the influence of sedentary time on bone parameters in children and youth is equivocal; even within this dissertation, results are mixed. Thus, currently there is insufficient evidence to inform public health guidelines or recommend limiting sedentary time for bone health.

In the six decades since Jerry Morris and colleagues first described a lack of PA as a key risk factor for heart disease in bus drivers,[391] we have amassed a substantial body of evidence demonstrating the link between a lack of PA (inactivity) and morbidity and mortality in adults.[392,393] PA also benefits a plethora of health outcomes across the lifespan.[394,395] Today, the pediatric bone literature is replete with observational and intervention studies that demonstrated the beneficial role of PA for bone mass and strength.[15] Despite convincing evidence and numerous 'calls for action' to increase PA in children and youth, wide-scale adoption of behaviour change initiatives is largely lacking or unsuccessful.[396,397] Therefore, future studies should examine factors that support implementation of effective strategies to encourage children and youth to be more active. Strategies delivered at scale are needed to improve health at the population level. Future efforts should include multi-pronged approaches to enhance PA across home, school and community settings to counter the downward trajectory in this health behaviour.

7.5 Future research

In this dissertation I provide novel insight into how bone is gained during adolescence and the influence of movement behaviours on bone strength accrual during adolescence. My research also provides a foundation for future investigations, as it addresses several gaps in the literature. In this final section, I highlight specific research questions and studies that may

enhance our understanding of bone strength accrual during growth and the influence of movement behaviours on bone parameters in children and youth.

1. Using data from HBSIII, examine:
    a. The influence of objectively-measured patterns of PA and sedentary time accrual (e.g., frequency and duration of bouts) on bone parameters to better inform PA and sedentary behaviour guidelines.
    b. The interrelatedness of PA and sedentary time and how combinations of movement behaviours influence bone parameters using compositional analyses.
2. Are maturity- and sex-related adaptations at the distal tibia and radius consistent with adaptations at central skeletal sites?
3. Use second generation HR-pQCT (higher resolution) in adolescents to confirm sex differences in cortical bone parameters (i.e., Ct.Po and Ct.BMD), with boys and girls aligned on maturity offset.
4. Recruit a younger cohort of children (pre-pubertal) and use HR-pQCT to examine whether sex differences in bone parameters are apparent prior to puberty.
5. Re-consent the HBSIII cohort in 5-10 years and use HR-pQCT to examine:
    a. Whether sex differences in bone parameters persist into adulthood.
    b. Age-related adaptations in bone parameters from childhood through adulthood.
    c. The influence of bone strength accrual during adolescence on adult skeletal health.
    d. The influence of PA and sedentary time during adolescence on adult skeletal health.
6. Conduct a large prospective fracture trial in children and adolescents, with boys and girls aligned on maturity offset, to investigate:
    a. The influence of bone microarchitecture on fracture risk.
    b. The influence of sex-differences in bone parameters on fracture risk.
    c. Whether factors that influence fracture risk differ between boys and girls.
    d. The influence of PA and sedentary time on fracture risk.

7. Conduct a large randomized controlled trial in children and youth that uses HR-pQCT and aligns boys and girls on maturity offset to examine the dose-response relationship between PA (e.g., varying frequencies and durations) and bone parameters.
    a. Include accelerometers to accurately assess habitual PA.
    b. Include monitoring devices that measure posture to accurately differentiate between sitting time and standing time with no movement.
    c. Examine the relationship between meeting current PA guidelines and bone parameters.
8. Conduct a multi-component PA dissemination trial that includes high-impact exercises in school and at home, assesses effectiveness with HR-pQCT and accelerometry and aligns participants on maturity offset. Follow participants from elementary through high school.

Exploring these research questions in future will improve our understanding of factors that influence fracture risk during adolescence and provide insight into the influence of adolescent bone strength accrual for adult skeletal health. Findings have the potential to shape public health guidelines for PA and sedentary time during childhood and adolescence and inform effective interventions that improve skeletal health across the lifespan.

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Appendix A: Information to Participants, Consent and Assent Forms

Healthy Bones Study Follow-Up

Principal Investigator: Heather McKay PhD, [redacted]
Co-Investigators: Adam Baxter-Jones PhD, Melonie Burrows PhD, Karim Khan MD PhD, David Cooper
PhD Research Coordinator: Melonie Burrows PhD [redacted]

Information to Families:

The UBC Bone Health Research Group is continuing a study of students that looked at whether a program of physical activity, and specifically, jumping exercises benefited the bones of growing children. We also looked at the role of calcium and other nutrients on bone development. The ultimate aim of this study was to identify the role of physical activity and proper nutrition during the childhood years and the risk factors for the prevention of osteoporosis and bone fractures in later life. Results after 2 years of intervention clearly demonstrated an approximate 5% increase in bone strength in the 'jumping' schools compared with control or 'non-jumping' schools. However, we do not know if these advantages are maintained into maturity. Therefore, the fourth part of a study that began with grade 3 and 4 students during the 1997-98 school years aims to determine if the significant bone health benefits we observed in the exercising schools persist through into adulthood. We would like to continue to follow the Healthy Bones subjects for another three years as you proceed through adolescence and enter into adulthood. Principals and teachers of Healthy Bones subjects have been asked to make time available for you to participate, and all have agreed. If you have graduated we will make available a variety of times to accommodate to your work schedule.

We would like to continue to follow-up the students who were previously enrolled in this study. Students who choose to be involved in the follow-up study will have their bone status and growth and development measured one time in the spring of the next three years. The total time you will be away from school for measurements will be approximately 2.5 hours for each measurement session. You will be picked up from your school or from a school near your home in groups of 6 by mini-van and be transported to VGH by an experience driver. Detailed information about all measurements that will occur during these sessions is provided in the attached consent form. You may sign the consent form and return it to the Bone Healthy Research Group in the envelope provided.

The Healthy Bones Study will potentially offer new information aimed at the prevention of osteoporosis. If you agree to participate please sign the attached consent form and return it in the self addressed stamped envelope provided. Should you have any questions about this study please contact Melonie Burrows PhD [redacted] or Dr. Heather McKay [redacted]. Thank you for your interest in this study. We look forward to hearing from you.

Sincerely,

Heather McKay, Ph.D.

[redacted]

Healthy Bones Study Follow-Up Consent Form

Procedures:
Your child's continued participation in the project will involve one testing session each spring of the next three years at the Vancouver General Hospital Research Pavilion [redacted]). Total testing time per session will be approximately 3 hours, including transportation time.

1. **Anthropometry:** Measures of height, seated height, calf girth, hip girth, waist girth and weight will be taken.

2. **Questionnaires:** You will be asked to complete questionnaires that will assess your physical activity, calcium intake and updated health history. The health history is to determine if there are any reasons to exclude you from the research study and to identify any conditions or medications that may affect study outcomes. A trained study staff person will discuss the importance of these assessments with you. Following individual instruction, you will be asked to complete the physical maturity assessment forms. There is a space in our laboratory where you may do this in private, seal the results and return the envelope to us. Results remain confidential and data entry is by subject number only so that you cannot be identified. You do not need to answer any questions which make you feel uncomfortable.

3. **Musculoskeletal Fitness:** Musculoskeletal fitness (i.e. muscle strength and power) will be assessed by performing a standing long jump, a vertical jump assessment and a leg extension test. A standing long jump will require you to stand with both feet together, to bend your knees and jump as far as you can horizontally using your arms and legs. The vertical jump is the same but requires you to jump as high as you can vertically. The leg extension test requires you to sit on a chair and perform leg extensions, one leg at a time, as fast as you can over 60 seconds.

4. **Bone Densitometer:** Your whole body and hip bone status will be evaluated by a bone densitometer. This is a machine which takes a picture of your bones. This procedure is painless and routinely used in modern medical practice. It requires only that you lie still on the padded measurement table for about 15 minutes. Although the bone measurement is X-ray based, the total patient effective dose per session will be less than 10 millirem which is similar to the background radiation one would receive by taking a one-way flight from Vancouver to Halifax. To put this in perspective, the annual background radiation in Vancouver due to natural sources is around 150 millirem per year. The current permissible level for the general population is 500 millirem per year. These values can be used to compare the relative risk of less than 10 millirem exposure from the bone density procedure. All bone density measurements will be conducted by a trained operator. Less than 15 minutes is required for all the bone measurement procedures.

5. **pQCT:** Analysis of bone geometry of the lower leg will be performed using images generated by peripheral Quantitative Computerized Tomography (pQCT). The pQCT involves a minimal amount of radiation (0.1 microSV), that's 1 tenth the radiation of a normal chest x-ray, similar to the radiation exposure one would receive flying to Toronto from Vancouver. You will have to remain still with your leg extended into the device for three measures of the lower leg, one at 8% one at 50% and one at 66% of the length of the lower leg from the ankle. Additionally there will be one measure of the forearm at 8% of the length of the forearm from the wrist. A trained operator will conduct the scans which will take a total of 15 minutes.

6. **Accelerometers**: We will monitor your physical activity with an accelerometer. This is a motion sensor that is a 'smart' pedometer, working like the lights used in yards and carports. Like these lights, the motion sensor is always on, but is activated by movement. The motion sensor will give us an idea of the typical physical activity patterns of children in our studies. The motion sensor is safe, non-invasive and is only attached to the body by the belt around the waist. You will wear the accelerometer (on a belt around your waist) from the time you get up until the time you go to bed (approximately 12 hours) for 7 consecutive days. A research assistant will provide

clear instructions on how to wear the accelerometer. This will take no more than ten minutes in the Spring while you are at school. Accelerometers will be worn for 7 days. We will also ask you to complete a log while you are wearing your accelerometer. The student log takes approximately 10 minutes to fill out. After the seven days we will come to your school and pick up your accelerometer and completed log. This will take 5 minutes.

Possible Harms:

Although the bone densitometer scan is x-ray based, the total patient effective dose per session will be less than 10 millirem which is similar to the background radiation one would receive by taking a one-way flight from Vancouver to Halifax. To put this in perspective, the annual background radiation in Vancouver due to natural sources is around 150 millirem per year. The current permissible level for the general population is 500 millirem per year. The pQCTscan involves a minimal amount of radiation (0.1 microSV), that's 1 tenth the radiation of a normal chest x-ray, similar to the radiation exposure one would receive flying to Toronto from Vancouver. The wearing of the accelerometer around the waist by the use of a belt may cause some discomfort.

Benefits:

If you choose to participate in the Healthy Bones Follow-up Study, you will learn more about how physical activity can contribute to improved health. At the end of the study you will receive a summary of the results indicating the general findings of the study and your personal performance. It is our hope that through this program, you will achieve the many health benefits that accompany an active lifestyle. No one knows if you will receive any direct benefit from participating in the study. The investigators cannot guarantee that you will derive any benefit.

Rights and Welfare of the Individual:

You have the right to refuse participation in this study. It is understood that you are free to withdraw from any or all parts of the study at any time without penalty. Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the Investigator or his or her designate by representatives of the UBC Research Ethics Board for the purpose of monitoring the research. However, no records which identify you by name or initials will be allowed to leave the Investigators' offices. Files are kept in the Vancouver General Hospital, Bone Health Research Lab. The lab remains locked and only those directly involved in the study (namely, the Healthy Bones III Research Evaluation Team) will have access to your records and results. Your individual results will remain confidential as they will not be discussed with anyone outside the research team. Please be assured that you may ask questions at any time. We will be glad to discuss your results with you when they have become available and we welcome your comments and suggestions. Should you have any concerns about this study or wish further information please contact Dr. Heather McKay, [redacted] Melonie Burrows [redacted]. If you have any concerns about your rights or treatment as a research subject, you may contact the Research Subject Information Lines at the University of British Columbia at [redacted]

Compensation for Injury:

Signing this consent form in no way limits your legal rights against the sponsors, investigators or anyone else.

Healthy Bones Study Consent Form – Spring 2009

Please sign this consent form

I agree to participate in the Healthy Bones study as outlined previously (the anthropometry, questionnaires, musculoskeletal fitness, bone densitometry, pQCT and accelerometers).

I understand that at any time during the study I am free to withdraw without jeopardizing any medical management, employment or educational opportunities. I understand the contents of all pages of this form, the proposed procedures and possible risks. I have had the opportunity to ask questions and have received satisfactory answers to all inquiries regarding this program.

Checklist

- I have read and understood the subject information and consent form.
- I have had sufficient time to consider the information provided and to ask for advice if necessary.
- I have had the opportunity to ask questions and have had satisfactory responses to my questions.
- I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.
- I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.
- I understand that I am not waiving any of my legal rights as a result of signing this consent form.
- I understand that there is no guarantee that this study will provide any benefits to me (if applicable).
- I have read this form and I freely consent to participate in this study.
- I have been told that I will receive a dated and signed copy of this form.

Signatures

_____	_____	_____
Printed name of subject	Signature	Date
_____	_____	_____
Printed name of witness	Signature	Date
_____	_____	_____
Printed name of principal investigator	Signature	Date

Principal Investigator:
Heather McKay PhD

Co-Investigators
Darren Warburton PhD, Naylor PJ, Ryan Rhodes PhD

Research Coordinator
Melonie Burrows [redacted] extension [redacted]

Information to Families:

Thank you for your involvement in Action Schools! BC during the 2005-2006 school year. The program continues to be a great success and we hope that your child enjoyed being a part of the evaluation. Our ultimate goal is to "make healthy choices the easy choices" to enhance the health and well-being of all children. We are pleased to announce that your child's school will continue to be a part of Action Schools! BC during the 2006-2007 school year.

We would like to invite your child to participate in the Action Schools! BC evaluation once again for four years. The evaluation will take place in April-June 2007 to 2010 at the Bone Health Research Lab at VGH. Children will be transported in groups of 6 by mini-van to VGH by an experienced driver and chaperone. There we will assess bone health and administer questionnaires to assess physical activity, dietary intake and psychosocial well-being. In addition, your child's cardiovascular health will be assessed by means of a shuttle run test and blood pressure measurement. This session will require that your child be away from school for approximately 2 to 3 hours. Detailed information for all measurements is provided in the attached consent form.

In addition, your child may be asked to wear an accelerometer for five days. Accelerometers are 'motion sensors' that work using the same technology as the motion sensor lights for houses and carports. The purpose of the motion sensor is to get an idea of your child's physical activity patterns. The accelerometer is small and lightweight and is worn on a belt around the waist while they continue on with their normal daily activities.

At this time we would ask that you please consider your child's participation in the Action Schools! BC evaluation. We invite you to read, complete and sign the attached consent form. Please place it in the stamped, addressed envelope provided and return it in the mail.

We are excited to continue working with the students, teachers and parents in the schools involved in Action Schools! BC. Your child's continued involvement is vital to the success of this program. If you have any questions please contact Melonie Burrows at [redacted] or Dr. Heather McKay at [redacted]

Sincerely,

Dr. Heather McKay, Professor
UBC Dept of Orthopaedics

Healthy Bones Study Follow-Up Consent Form

Procedures:
Your child's continued participation in the project will involve one testing session each spring of the next three years at the Vancouver General Hospital Research Pavilion (828 West 10th Avenue, Vancouver). Total testing time per session will be approximately 3 hours, including transportation time.

1. **Anthropometry:** Measures of height, seated height, calf girth, hip girth, waist girth and weight will be taken.

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Benefits:

If you choose to participate in the Healthy Bones Follow-up Study, you will learn more about how physical activity can contribute to improved health. At the end of the study you will receive a summary of the results indicating the general findings of the study and your personal performance. It is our hope that through this program, you will achieve the many health benefits that accompany an active lifestyle. No one knows if you will receive any direct benefit from participating in the study. The investigators cannot guarantee that you will derive any benefit.

Rights and Welfare of the Individual:

You have the right to refuse participation in this study. It is understood that you are free to withdraw from any or all parts of the study at any time without penalty. Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the Investigator or his or her designate by representatives of the UBC Research Ethics Board for the purpose of monitoring the research. However, no records which identify you by name or initials will be allowed to leave the Investigators' offices. Files are kept in the Vancouver General Hospital, Bone Health Research Lab. The lab remains locked and only those directly involved in the study (namely, the Healthy Bones III Research Evaluation Team) will have access to your records and results. Your individual results will remain confidential as they will not be discussed with anyone outside the research team. Please be assured that you may ask questions at any time. We will be glad to discuss your results with you when they have become available and we welcome your comments and suggestions. Should you have any concerns about this study or wish further information please contact Dr. Heather McKay, [redacted] or Melonie Burrows [redacted]. If you have any concerns about your rights or treatment as a research subject, you may contact the Research Subject Information Lines at the University of British Columbia at [redacted].

Compensation for Injury:

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Healthy Bones Study Consent Form – Spring 2009

Please sign this consent form and return it

Parental/Guardian Consent:

I agree to have my child participate in the central components of the Healthy Bones III evaluation (height, weight, questionnaires, bone measurements, musculoskeletal Fitness, accelerometry, blood pressure) for the next three years and I authorize the Department of Orthopaedics, as agent of the University of British Columbia, to arrange transportation of my child to and from the Vancouver General Hospital, Bone Health Research Laboratory located at the Vancouver General Hospital - Research Pavilion - 5th Floor, 828 West 10th Avenue, Vancouver, British Columbia.

I understand that at any time during the Healthy Bones III evaluation we will be free to withdraw without jeopardizing any medical management, employment or educational opportunities. I understand the contents of all pages of this form, the proposed procedures and possible risks. I have had the opportunity to ask questions and have received satisfactory answers to all inquiries regarding this program.

Checklist

- I have read and understood the subject information and consent form.
- I have had sufficient time to consider the information provided and to ask for advice if necessary.
- I have had the opportunity to ask questions and have had satisfactory responses to our questions.
- I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.
- I understand that the participation in this study is voluntary and that our child is completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that they shall receive.
- I understand that we are not waiving any of our legal rights as a result of signing this consent form.
- I understand that there is no guarantee that this study will provide any benefits to our child (if applicable).
- I have read this form and freely consent for our child to participate in this study.
- I have been told that we will receive a dated and signed copy of this form.

The parent(s)/guardian(s)and the investigator are satisfied that the information contained in this consent form was explained to the child to the extent that he/she is able to understand it, that all questions have been answered, and that the child assents to participating in the research.

Signatures

_____	_____	_____
Printed name of parent/Guardian	Signature	Date
_____	_____	_____
Printed name of witness	Signature	Date
_____	_____	_____
Printed name of principal investigator	Signature	Date

Healthy Bones Study Assent Form – Spring 2009

Child's Assent:

Invitation

I am being invited to be part of a research study. It is up to me if I want to be in this study. No one will make me be part of the study. Even if I agree now to be part of the study, I can change my mind later. No one will be mad at me if I choose not to be part of this study.

Why Are We Doing This Study?

This study will help us learn more about how your exercise and what you eat helps your bones grow as you get older.

What Will Happen in This Study?

If I agree to be in this study, I will go to the Bone Health Research Group laboratory for one, three hour visit each year (3 visits over 3 years). Each time I go to the laboratory I will have my height and weight taken, and my leg, hip and waist size measured. I will answer questions about how much exercise I do, how much I eat and how my body is growing. I will do some jumps to see how far and how high I can jump, and some leg kicks (like kicking a ball) to see how strong my legs are. I will have some pictures of my bones taken to see how strong they are. These pictures will be of my hips, back bone, whole body, leg and arm. During my visit I will be given a small box attached to a belt to wear around my hips for 7 days to see how much exercise I do. After the 7 days I will give the box to my school office.

Who Is Doing This Study?

Dr Heather Mckay and other doctors from the University of British Columbia will be doing this study. They will answer any questions I have about the study. I can also call them [redacted] if I am having any problems or if there is an emergency and I cannot talk to my parents.

Can Anything Bad Happen to Me?

When I am wearing the belt and box I may feel itching from the belt rubbing on my skin – similar to what I may feel when I wear a belt with my pants. I should tell my parents/guardian if I feel itching from the belt.

Who Will Know I Am in the Study?

Only my school, doctors and people who are involved in the study will know I am in it. When the study is finished, the doctors will write a report about what was learned. This report will not say my name or that I was in the study. My parents and I do not have to tell anyone I am in the study if we don't want to."

When Do I Have To Decide?

I have as much time as I want to decide to be part of the study. I have also been asked to discuss my decision with my parents.

Signature:

If I put my name at the end of this form, it means that I agree to be in the study

_____	_____	_____
Printed name of child	Signature	Date

Healthy Bones Study III

Principal Investigator: Heather McKay PhD, [redacted]
Co-Investigators: Adam Baxter-Jones PhD, Melonie Burrows PhD, Karim Khan MD PhD, David Cooper PhD Research Coordinator: Melonie Burrows PhD [redacted]

Information to Families:

We would like to thank you for taking the time to read this information. We are inviting your child along with other classmates from their school to participate in a health related research project to answer questions about how growth and development progresses over time and how it is affected by different variables.

The UBC Bone Health Research Group has been conducting research projects such as this one in the Richmond and Vancouver School districts since 1999 where we have measured nearly 1000 students. Many of these students have been coming to our lab for annual measurement ever since. Our research aims to understand several of the important factors in healthy development as we pass from childhood through to adulthood. Primarily we look at the role of physical activity, nutrition, calcium and other nutrients on bone development. The ultimate aim of this study is to identify the role of physical activity and proper nutrition during the childhood years and the risk factors for the prevention of osteoporosis and bone fractures in later life. Secondarily we would like a clear consecutive view of what normal healthy development looks like biologically as adolescents pass from childhood into adulthood. We would like to invite your child to participant in the Healthy Bones research project for three years (spring 2009, spring 2010, and spring 2011) as they proceed through childhood into adolescence. Once consented, scheduling of your child is done in coordination with his/her teacher and principal to minimize disruption to school.

Students who choose to be involved in the follow-up study will have their bone status and growth and development measured one time in the spring of the next three years. The total time your child will be away from school for measurements will be approximately 3 hours for each measurement session. Your child will be picked up by an experienced driver and a chaperone from their school in groups of 5 by mini-van and be transported to VGH. Detailed information about all measurements that will occur during these sessions is provided in the attached consent form.

The Healthy Bones Study will potentially offer new information aimed at the prevention of osteoporosis. If you agree to participate please sign the attached consent form and return it to your child's teacher. Should you have any questions about this study please contact Melonie Burrows PhD [redacted] or Dr. Heather McKay [redacted]. Thank you for your interest in this study. We look forward to hearing from you.

Sincerely,
Heather McKay, Ph.D.

[redacted]

Healthy Bones Study Follow-Up Consent Form

Procedures:
Your child's continued participation in the project will involve one testing session each spring of the next three years at the Vancouver General Hospital Research Pavilion (828 West 10th Avenue, Vancouver). Total testing time per session will be approximately 3 hours, including transportation time.

1. **Anthropometry:** Measures of height, seated height, calf girth, hip girth, waist girth and weight will be taken.

2. **Questionnaires:** You will be asked to complete questionnaires that will assess your physical activity, calcium intake and updated health history. The health history is to determine if there are any reasons to exclude you from the research study and to identify any conditions or medications that may affect study outcomes. A trained study staff person will discuss the importance of these assessments with you. Following individual instruction, you will be asked to complete the physical maturity assessment forms. There is a space in our laboratory where you may do this in private, seal the results and return the envelope to us. Results remain confidential and data entry is by subject number only so that you cannot be identified. You do not need to answer any questions which make you feel uncomfortable.

3. **Musculoskeletal Fitness:** Musculoskeletal fitness (i.e. muscle strength and power) will be assessed by performing a standing long jump, a vertical jump assessment and a leg extension test. A standing long jump will require you to stand with both feet together, to bend your knees and jump as far as you can horizontally using your arms and legs. The vertical jump is the same but requires you to jump as high as you can vertically. The leg extension test requires you to sit on a chair and perform leg extensions, one leg at a time, as fast as you can over 60 seconds.

4. **Bone Densitometer:** Your whole body and hip bone status will be evaluated by a bone densitometer. This is a machine which takes a picture of your bones. This procedure is painless and routinely used in modern medical practice. It requires only that you lie still on the padded measurement table for about 15 minutes. Although the bone measurement is X-ray based, the total patient effective dose per session will be less than 10 millirem which is similar to the background radiation one would receive by taking a one-way flight from Vancouver to Halifax. To put this in perspective, the annual background radiation in Vancouver due to natural sources is around 150 millirem per year. The current permissible level for the general population is 500 millirem per year. These values can be used to compare the relative risk of less than 10 millirem exposure from the bone density procedure. All bone density measurements will be conducted by a trained operator. Less than 15 minutes is required for all the bone measurement procedures.

5. **pQCT:** Analysis of bone geometry of the lower leg will be performed using images generated by peripheral Quantitative Computerized Tomography (pQCT). The pQCT involves a minimal amount of radiation (0.1 microSV), that's 1 tenth the radiation of a normal chest x-ray, similar to the radiation exposure one would receive flying to Toronto from Vancouver. You will have to remain still with your leg extended into the device for three measures of the lower leg, one at 8% one at 50% and one at 66% of the length of the lower leg from the ankle. Additionally there will be one measure of the forearm at 8% of the length of the forearm from the wrist. A trained operator will conduct the scans which will take a total of 15 minutes.

6. **Accelerometers**: We will monitor your physical activity with an accelerometer. This is a motion sensor that is a 'smart' pedometer, working like the lights used in yards and carports. Like these lights, the motion sensor is always on, but is activated by movement. The motion sensor will give us an idea of the typical physical activity patterns of children in our studies. The motion sensor is safe, non-invasive and is only attached to the body by the belt around the waist. You will wear the accelerometer (on a belt around your waist) from the time you get up until the time you go to bed (approximately 12 hours) for 7 consecutive days. A research assistant will provide clear instructions on how to wear the accelerometer. This will take no more than ten minutes in the Spring while you are at school. Accelerometers will be worn for 7 days. We will also ask you to complete a log while you are wearing your accelerometer. The student log takes approximately 10 minutes to fill out. After the seven days we will come to your school and pick up your accelerometer and completed log. This will take 5 minutes.

Possible Harms:
Although the bone densitometer scan is x-ray based, the total patient effective dose per session will be less than 10 millirem which is similar to the background radiation one would receive by taking a one-way flight from Vancouver to Halifax. To put this in perspective,

the annual background radiation in Vancouver due to natural sources is around 150 millirem per year. The current permissible level for the general population is 500 millirem per year. The pQCTscan involves a minimal amount of radiation (0.1 microSV), that's 1 tenth the radiation of a normal chest x-ray, similar to the radiation exposure one would receive flying to Toronto from Vancouver. The wearing of the accelerometer around the waist by the use of a belt may cause some discomfort.

Benefits:

If you choose to participate in the Healthy Bones Follow-up Study, you will learn more about how physical activity can contribute to improved health. At the end of the study you will receive a summary of the results indicating the general findings of the study and your personal performance. It is our hope that through this program, you will achieve the many health benefits that accompany an active lifestyle. No one knows if you will receive any direct benefit from participating in the study. The investigators cannot guarantee that you will derive any benefit.

Rights and Welfare of the Individual:

You have the right to refuse participation in this study. It is understood that you are free to withdraw from any or all parts of the study at any time without penalty. Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the Investigator or his or her designate by representatives of the UBC Research Ethics Board for the purpose of monitoring the research. However, no records which identify you by name or initials will be allowed to leave the Investigators' offices. Files are kept in the Vancouver General Hospital, Bone Health Research Lab. The lab remains locked and only those directly involved in the study (namely, the Healthy Bones III Research Evaluation Team) will have access to your records and results. Your individual results will remain confidential as they will not be discussed with anyone outside the research team. Please be assured that you may ask questions at any time. We will be glad to discuss your results with you when they have become available and we welcome your comments and suggestions. Should you have any concerns about this study or wish further information please contact Dr. Heather McKay, [redacted] or Melonie Burrows, [redacted]. If you have any concerns about your rights or treatment as a research subject, you may contact the Research Subject Information Lines at the University of British Columbia at 604.822.8598.

Compensation for Injury:

Signing this consent form in no way limits your legal rights against the sponsors, investigators or anyone else.

Healthy Bones Study Consent Form – Spring 2009

Please sign this consent form and return it to your child's teacher

Parental/Guardian Consent:

I agree to have my child participate in the central components of the Healthy Bones III evaluation (height, weight, questionnaires, bone measurements, musculoskeletal Fitness, accelerometry, blood pressure) for the next three years and I authorize the Department of Orthopaedics, as agent of the University of British Columbia, to arrange transportation of my child to and from the Vancouver General Hospital, Bone Health Research Laboratory located at the Vancouver General Hospital - Research Pavilion - [redacted] Vancouver, British Columbia.

I understand that at any time during the Healthy Bones III evaluation we will be free to withdraw without jeopardizing any medical management, employment or educational opportunities. I understand the contents of all pages of this form, the proposed procedures and possible risks. I have had the opportunity to ask questions and have received satisfactory answers to all inquiries regarding this program.

Checklist

- I have read and understood the subject information and consent form.
- I have had sufficient time to consider the information provided and to ask for advice if necessary.
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- I understand that there is no guarantee that this study will provide any benefits to our child (if applicable).
- I have read this form and freely consent for our child to participate in this study.
- I have been told that we will receive a dated and signed copy of this form.

The parent(s)/guardian(s)and the investigator are satisfied that the information contained in this consent form was explained to the child to the extent that he/she is able to understand it, that all questions have been answered, and that the child assents to participating in the research.

Signatures

_____	_____	_____
Printed name of parent/Guardian	Signature	Date
_____	_____	_____
Printed name of witness	Signature	Date
_____	_____	_____
Printed name of principal investigator	Signature	Date

Healthy Bones Study Assent Form – Spring 2009

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Invitation

I am being invited to be part of a research study. It is up to me if I want to be in this study. No one will make me be part of the study. Even if I agree now to be part of the study, I can change my mind later. No one will be mad at me if I choose not to be part of this study.

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This study will help us learn more about how your exercise and what you eat helps your bones grow as you get older.

What Will Happen in This Study?

If I agree to be in this study, I will go to the Bone Health Research Group laboratory for one, three hour visit each year (3 visits over 3 years). Each time I go to the laboratory I will have my height and weight taken, and my leg, hip and waist size measured. I will answer questions about how much exercise I do, how much I eat and how my body is growing. I will do some jumps to see how far and how high I can jump, and some leg kicks (like kicking a ball) to see how strong my legs are. I will have some pictures of my bones taken to see how strong they are. These pictures will be of my hips, back bone, whole body, leg and arm. During my visit I will be given a small box attached to a belt to wear around my hips for 7 days to see how much exercise I do. After the 7 days I will give the box to my school office.

Who Is Doing This Study?

Dr Heather Mckay and other doctors from the University of British Columbia will be doing this study. They will answer any questions I have about the study. I can also call them at [redacted]), if I am having any problems or if there is an emergency and I cannot talk to my parents.

Can Anything Bad Happen to Me?

When I am wearing the belt and box I may feel itching from the belt rubbing on my skin – similar to what I may feel when I wear a belt with my pants. I should tell my parents/guardian if I feel itching from the belt.

Who Will Know I Am in the Study?

Only my school, doctors and people who are involved in the study will know I am in it. When the study is finished, the doctors will write a report about what was learned. This report will not say my name or that I was in the study. My parents and I do not have to tell anyone I am in the study if we don't want to."

When Do I Have To Decide?

I have as much time as I want to decide to be part of the study. I have also been asked to discuss my decision with my parents.

Signature:

If I put my name at the end of this form, it means that I agree to be in the study

_____	_____	_____
Printed name of child	Signature	Date

Appendix B: Results for Study Participants

Healthy Bones Study 2011 Reports

Results for:

Table 1

	Test Date	Age (yrs)	Height (cm)	Weight (kg)	Long Jump (cm)	Vertical Jump (cm)	Calcium (mg/day)	Physical Activity Score
Spring 2011	15/05/2011	21.1	179.2	71.0	153.0	52.1	1313	1.4
Spring 2010	05/05/2010	20.1	179.1	69.7	148.2	52.1	3946	1.3
Spring 2009	20/03/2009	19.0	179.1	70.6	146.7	39.4	2337	1.3
Spring 2008	27/03/2008	18.0	177.6	66.9	153.0	48.3	3253	1.9
Spring 2007	23/04/2007	17.1	176.8	64.1	141.0	40.6	1781	2.5

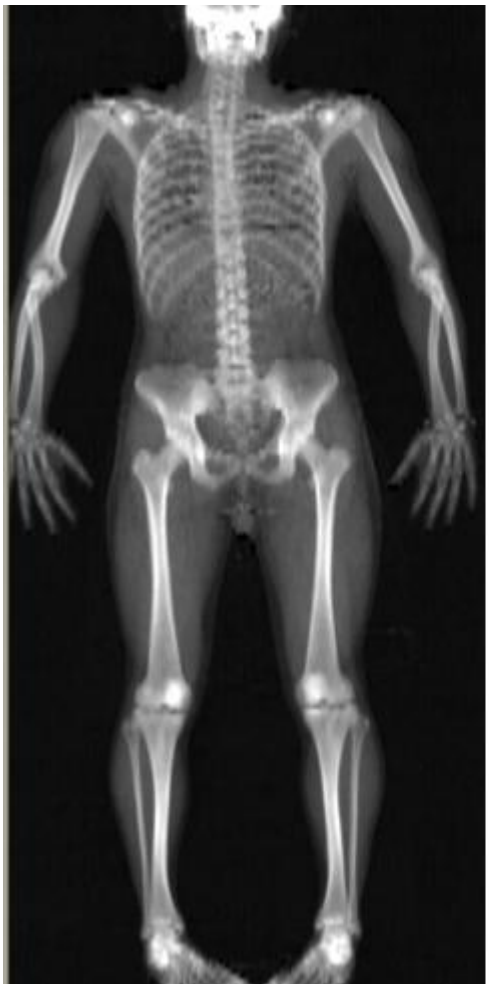
Table 2

Boys' Averages

	Spring 2011	Spring 2010	Spring 2009	Spring 2008	Spring 2007	
Number of Male Subjects	31	33	37	50	63	
Average Age	21.5	20.5	19.5	18.5	17.6	yrs
Average Height	176.6	176.1	176.7	176.3	176.3	cm
Average Weight	71.8	72.0	71.7	71.4	71.0	kg
Average Long Jump	193.8	195.9	193.6	195.4	200.4	cm
Average Vertical Jump	48.2	53.1	51.5	50.4	49.5	cm
Average Calcium	861.9	895.9	1063.3	1145.3	1107.9	mg/day
Average Physical Activity	1.8	1.9	1.9	2.0	2.1	

Thank you so much for coming in to see us again this year for measurement! It has been many years and we appreciate your commitment to the study. We hope that you come back to see us again next time! These are some of your results from your visit. In Table 1 you can see YOUR measurements: your age; height and weight; how long and high you jumped; how much calcium you ate; and your activity score. Table 2 shows the average measurements of your PEERS, who are approximately the same age and the same gender as you. You will see the numbers from this year and from the three previous years. Note that there is tremendous variation with the timing and magnitude of growth changes, so if you fall outside of the average this is perfectly normal! You may just be an early or late maturing person. Interestingly, girls will reach their maximum gains in height approximately two years before boys; this is the point in adolescence in which the rate of growth is the greatest.

As you probably know, it is important to get plenty of exercise and eat foods rich in calcium for bones to develop and become strong. The physical activity score above is out of 5 points. As you get older, your physical activity may decrease, but there are simple things that we can do to stay active. Think about walking to and from school or work, parking the car further away from the store, meeting with friends for a hike or a game of ultimate frisbee, or anything else that gets you to break a sweat! And, what about your diet? You should try to eat 1300mg of calcium every day for maximum benefit. You can get enough calcium by eating foods like yogurt, cheese, dark green leafy vegetables, canned fish with the bones in, or drinking milk or milk beverages. Being active and eating right won't just help your bones either, but will make you a healthier person all around!



Your Total Body Bone Mass (grams)	
Spring 2011	2890
Spring 2010	2892
Spring 2009	2736
Spring 2008	2625
Spring 2007	2503

Boys' Averages	
Spring 2011	2606
Spring 2010	2656
Spring 2009	2640
Spring 2008	2570
Spring 2007	2560

You would be amazed at how much your skeleton does for you! It protects important organs like your heart; it creates the structure of your body, lets you move in many different ways, and even makes blood cells for you. The picture above is your most recent DXA scan that was performed in our lab. Did you know that when you were born you had more than 300 bones in your body! As you grow up, some bones fuse together, and you will end up with 206 bones as an adult. Osteoporosis is the deterioration of bone tissue later in life. It is important to build bone now while you have the chance in order to decrease the likelihood of developing this disease in the future. You have a window of opportunity during growth to optimize the growth and development of your skeleton!

You will see your total body bone mass above, which is the number of grams of bone that you currently have in your body. The average represents your peers who are the same age and same gender. Like your height and weight, there is a lot of variation between individuals as the amount of bone mass is very closely related to your body size. As you get taller and bigger, your bones will adapt and become bigger and stronger too! We are so glad that you have chosen to be a part of the Healthy Bones Study. You are the most important part of our research team! If you (or your parents or guardians) have any questions about the study or your results, please don't hesitate to contact us!

Appendix C: Questionnaires

Health History Questionnaire – Winter 2009

Please take the time to answer the following questions about your child's health. This questionnaire is voluntary and you are free to leave any questions unanswered. Please be assured that all information will remain strictly confidential and will only be available to the researchers. If you have any questions regarding the contents of this questionnaire, please contact Melonie Burrows (604.875.4111 Extension 61104) or Dr. Heather McKay (604.875.5346) at the University of British Columbia. You can also email any questions to Melonie.burrows@hiphealth.ca. Please return this questionnaire to your child's teacher along with the consent form if you and your child choose to participate. Thank you for your participation in the Evaluation Component of Action Schools! BC.

PARENT(S) REGARDING YOU:

1.0 Where were you born?

Mother: _____ Father: _____

1.1 Where were **your parents** born?

Maternal Mother: _____ Maternal Father: _____

Paternal Mother: _____ Paternal Father: _____

1.2 How long have you lived in North America? Years: _____ Months:_____

1.3 Where did your family live before moving to North America? _____

1.4 How would you classify your family ethnically? (i.e., Caucasian-Canadian, Japanese-Canadian, etc.)

_____

1.5 If you wish to have your child's results sent home at the end of the study, please provide us with the following contact information.

Mailing Address:

_____

Phone Number_____ **Email**_____

REGARDING YOUR CHILD:

Child's name: _____ Age: _____

Birth Date: Day_____ Month_____Year_____

Child's birth weight_____(grams or lbs/ozs)

2.0 **Nutrition History:**

2.1 Who prepares your child's meals (i.e. mother, father, grandmother, nanny)?_____

2.2 Does your child drink milk every day?

_____YES: if **yes:**

On Average how many cups per day? _____

Has your child always drank milk every day (after being weaned from breast or bottle)?
yes _____ no _____

if **no**, at what age did she/he start drinking milk every day? _____ years old.

_____NO: if **no:**

Has your child ever drank one or more cups of milk per day (after being weaned from breast or bottle)?

_____ yes: at what age did she/he stop drinking milk every day? _____ years old.

How many cups did he/she drink until that age? _____ cups per day

_____ no : (never drank milk on a daily basis after being weaned)

2.3 Is your child on a special diet? _____ Yes _____ No

If **yes:** _____ vegetarian

_____ low sodium

_____ low cholesterol

_____ other

Please specify: _____

3.0 **Medical History and Status:**

3.1 Has your child ever been treated for any of the following conditions?

	Yes	No
food allergies	O	O
hypothyroidism	O	O
other allergies	O	O
hyperthyroidism	O	O
asthma	O	O

Any other conditions (please list)_____

_____

_____

3.2 Is your child currently taking any medications? _____ Yes _____ No

If **yes**, what medication(s) is your child taking? _____

_____

_____

What are these medication(s) for? _____

_____

_____

3.3 Has your family doctor ever said that your child has a heart condition and that he/she should only do physical activity recommended by a doctor? _____Yes _____No

3.4 Does your child complain of chest pain when they are doing physical activity?
_____Yes _____No

3.5 In the past month, has your child complained of chest pain when they were not doing any physical activity?
_____Yes _____No

3.6 Does your child have a bone or joint problem that could be made worse by a change in their physical activity? _____Yes _____No

3.7 Does your child lose their balance because of dizziness or do they ever lose consciousness?
_____Yes _____No

3.8 Do you know of any other reason why your child should not participate in physical activity?
_____Yes _____No

4.0 **<u>Bone History:</u>**

4.1 Has your child ever been hospitalized, confined to bed or had a limb immobilized (i.e., arm in a cast)?
_____ Yes _____ No

If **yes:** list condition, approximate date and time involved
(Example: wrist fracture summer, 1990 10 weeks)

Reason	**Date**	**Time Involved**

4.2 Is there a history of wrist, hip, or spine fractures in your family? _____ Yes _____ No

If **yes**: indicate who was affected

_____ mother _____ father
_____ maternal grandmother _____ paternal grandmother
_____ maternal grandfather _____ paternal grandfather

4.3 Is there a history of osteoporosis in your family? _____ Yes _____ No

If **yes:** indicate who was affected

_____ mother _____ father
_____ maternal grandmother _____ paternal grandmother
_____ maternal grandfather _____ paternal grandfather

4.4 Is there a history of any other bone disease in your family?
_____ Yes _____ No

If **yes:** please indicate the family member(s) affected

1. _____
2. _____

What is the name of the condition(s) affecting this family member?

1. _____
2. _____

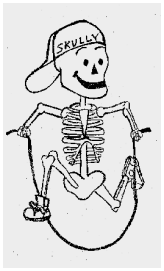
5.0 **<u>Physical Activity:</u>**

5.1 How would you rate the physical activity level of your child?
Physical activity is defined as vigorous activity that makes them sweat and/or breathe hard.

_____ Inactive
_____ Sometimes active
_____ Moderately active
_____ Often active
_____ Very active

THANK YOU FOR YOUR PARTICIPATION

Action Schools! BC



ID: _____
Date:_____
Checked by: _____

Spring 2012
Health History Questionnaire

Many of our Healthy Bones III participants are no longer attending secondary school. This creates significant variety in people's activity patterns. With this in mind we would like you to answer the following questions about what you are currently doing so that we can better understand your current level of activity.

1. Do you currently attend Secondary School? O Yes O No
2. Do you currently attend a College or University? O Yes O No

   If YES:
   a) Is your program part time or full time? _____

   b) What type of program are you in? _____

3. Do you currently work? O Yes O No

   If YES:
   a) How many hours a week do you usually work? _____

   b) What type of work do you do? _____

REMINDER ANY INFORMATION CONTAINED HEREIN WILL BE TREATED AS CONFIDENTIAL

4. Are you right handed or left handed? O Right O Left

5. Has your diet changed over the past year? O Yes O No

   If **yes:**
   _____ vegetarian
   _____ dairy-free
   _____ low sodium
   _____ low cholesterol
   _____other (Please specify: _____)

6. Do you drink milk every day? O Yes O No

   If **yes:** How many cups per day? _____

7. Do you currently take any medications? O Yes O No

   If **yes:** What medication(s) are you taking? _____

8. Did you break any bones during the past year (March 2011 to present)? O Yes O No

If **yes:** What bone(s), when (month), and for how long was the bone in a cast? (indicate R or L, and upper or lower arm/leg).
For example: right radius (or lower arm), July 2011, 6 weeks.

_____
_____
_____

How did you break the bone(s)? (check one)

a. ___ fell while running;
b. ___ fell while walking/standing;
c. ___ contact during sports* (i.e., to person, equipment, ground (includes snowboarding/ skateboarding); ***indicate sport (i.e., soccer)** _____
d. ___ fell from height (i.e., playground equipment, bike, tree, stairs);
e. ___ trauma in car/skidoo/boat accident;
f. _____ other (specify)

9. Were you sick for longer than one month during the past year? O Yes O No

If **yes**, what did you have? _____

10. Were you hospitalized during the past year? O Yes O No

If **yes**, for how long? _____

11. Has any member of your family been diagnosed with osteoporosis during the past year?
O Yes O No

If **yes**, who? _____

12. Has any member of your family been diagnosed with cardiovascular disease or stroke during the past year?
O Yes O No

If **yes**, who? _____

13. Do you drink cola? O Yes O No

If no go to question 14.

O Sometimes
O One to two cans per day
O Three cans or more per day

14. Do you drink coffee? O Yes O No

If no go to question 15.

- O One or two cups a day
- O Three or more cups a day
- O Sometimes
- O Never

15. Have you ever drunk some kind of alcoholic beverage? O Yes O No

If no go to question 16.

15.1 How often did you drink some kind of alcoholic beverage?

- O Daily or almost every day
- O Three or four times a week
- O Once or twice a week
- O Once or twice a month
- O Less than once a month
- O Never
- O Don't know
- O I've only tasted or had a sip from someone else's drink.

15.2 At what age did you start to drink alcohol? _____

16. Have you ever smoked tobacco products (cigarettes, cigars, or nicotine based products)? O Yes O No

16.1 Have you ever smoked for six months or more? O Yes O No

16.2 How long did you smoke? _____

16.3 Do you still smoke?

- O Yes, daily
- O Yes, occasionally
- O No, not at all

16.4 When you are/were smoking how many cigarettes do/did you usually smoke per day?

About _____ per day

16.5 At what age did you start to smoke daily? _____

17. Have you, or your immediate family(mother, father and / or siblings), ever had:

		WHO
17.1 Rheumatoid arthritis	O Yes O No	_____
17.2 Osteoporosis	O Yes O No	_____
17.3/17.4 An overactive/underactive thyroid Or parathyroid gland	O Yes O No	_____
17.5 Alcoholism	O Yes O No	_____
17.6 Chronic liver disease	O Yes O No	_____
17.7 Cancer	O Yes O No	_____
17.8 Stomach ulcers	O Yes O No	_____
17.9 Lactase deficiency (inability to digest milk)	O Yes O No	_____
17.10 Eating Disorders (anorexia nervosa or bulimia)	O Yes O No	_____

18. For the following medications, please circle whether you have taken them in the past, currently taking them, or have never taken the drug:

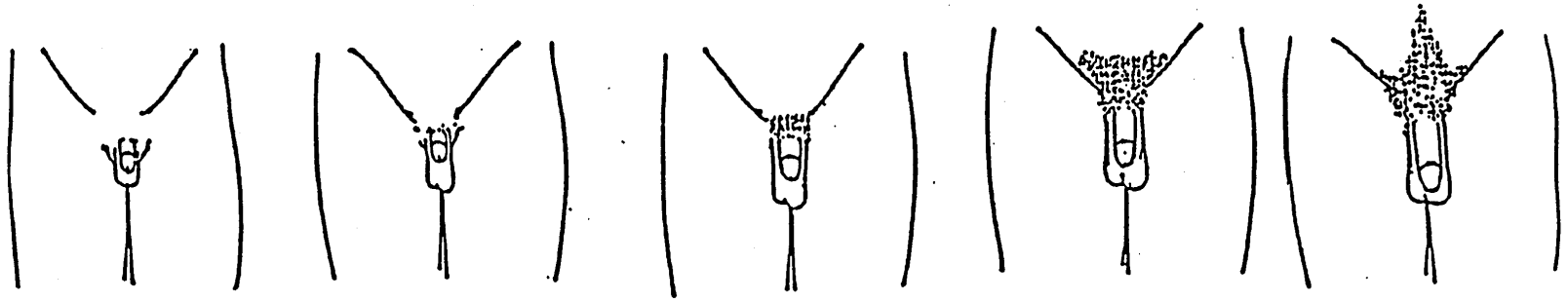
18.1 Cortisone or similar drug	O Past	O Currently	O Never
18.2 Anabolic steroids	O Past	O Currently	O Never
18.3 Thyroid hormone pills	O Past	O Currently	O Never
18.4 Asthma medication	O Past	O Currently	O Never
18.5 Medication for heartburn or indigestion (eg Tums, Rolaids, Maalox or Mylanta antacids)	O Past	O Currently	O Never
18.6 Lithium (for over one year) (a mood stabilizing medication)	O Past	O Currently	O Never
18.7 Anticonvulsant drugs	O Past	O Currently	O Never

ID #:_____
Checked by: _____

Healthy Bones and Action Schools! Study – Spring 2012

BOYS: After reading the descriptions under each drawing, please place a check mark above the drawing that looks closest to your stage of pubic hair development. Seal your response in the envelope provided. Thank you!

1 _____ **2 _____** **3 _____** **4 _____** **5 _____**



1	2	3	4	5
There is no pubic hair at all.	**There is a small amount of long, lightly coloured hair. This hair may be straight or a little curly.**	**There is hair that is darker, curlier and thinly spread out to cover a somewhat larger area than in stage 2.**	**The hair is thicker and more spread out, covering a larger area than in stage 3.**	**The hair now is widely spread and covering a large area, like that of an adult male.**

ID #:_____
Checked by: _____

Healthy Bones and Action Schools! Study – Spring 2012

Self-Assessment of Maturity Status: Boys

As you keep growing over the next few years, you will see changes in your body. These changes happen at different ages for different children, and you may already be seeing some changes, others may have already gone through some changes. Sometimes it is important to know how a person is growing without having a doctor examine them. It can be hard for a person to describe themselves in words, so doctors have drawings of stages that all children go through.

There are 5 drawings of pubic hair growth which are attached for you to look at. All you need to do is pick the drawing that looks like you do now. Put a check mark above the drawing that is closest to you stage of development for pubic hair. Put the sheet in the envelope and seal it so your answer will be kept private.

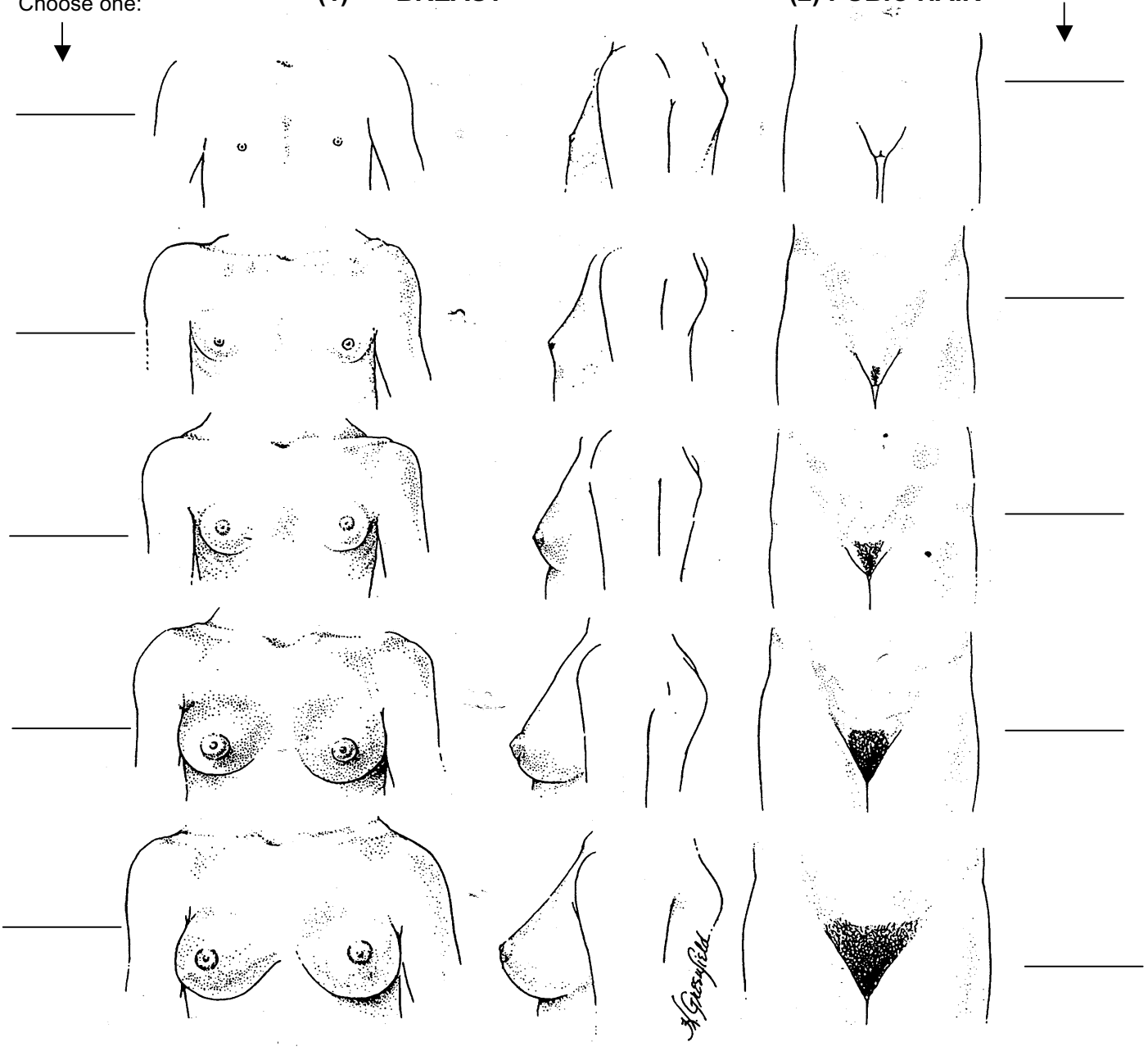
ID #:_____

Checked by: _____

Healthy Bones and Action Schools! Study– Spring 2012

Please put a check mark on the drawing that looks most like (1) your stage of breast development, and (2) your stage of pubic hair development. Seal your response in the envelope provided. Thank you!

Choose one: **(1) BREAST** **(2) PUBIC HAIR** Choose one:



Healthy Bones and Action Schools! Study– Spring 2012

Self Assessment of Maturity Status: Girls

As you keep growing over the next few years, you will see changes in your body. These changes happen at different ages for different children. You may already be seeing some changes, and some of your friends may have already gone through some changes. Sometimes it is important to know how a person is growing without having a doctor examine them. It can be hard for a person to describe themselves in words, so doctors have drawings of stages that all children go through. There are 5 drawings of breast growth, and 5 drawings of pubic hair growth on the next page. All you need to do is pick the drawings that look like you now. Put one check mark on the line at the drawing that is closest to your stage of development for breast growth, and one check mark at the drawing that is closest to your stage of pubic hair growth. Put the sheet in the envelope and seal it so that your answer will be kept private.

ID:
_____
Checked by:

Healthy Bones and Action Schools
Food Frequency Questionnaire Spring 2011

dd_____mm_____yy_____

INSTRUCTIONS

We would like to know about some of the foods you eat. For each food listed please fill in how often you usually eat a portion of the size stated.

If you eat the food:

- *every day* or *more than once a day*, fill in how many times you have it **Per Day**
- *less than once a day* but *more than once a week*, fill in the times **Per Week**
- *less than once a week*, but *more than once a month*, fill in the times **Per Month**
- *less often than once a month*, or *never eat it*, put a **'✔'** under **'Do Not Eat'**.

EXAMPLE: Janice has **a glass of orange juice** every morning, along with **two slices of toast**. She usually has **two sandwiches** at lunch, and eats **french fries** about 3 times per week. She almost never eats **cauliflower**.

Here is how Janice would fill in her answers:

		Per Day	Per Week	Per Month	Do Not Eat
Orange juice	1 cup (250 mL)	**1**	_____	_____	O
French fries	Regular serving	_____	**3**	_____	O
Cauliflower	½ cup (125 mL)	_____	_____	_____	✔
Bread or toast	1 slice	**6**	_____	_____	O

NOW IT'S YOUR TURN!
Make sure you only fill in **ONE ANSWER** for each different food

Go to the next page

Remember to give only ONE answer for each food!

		NUMBER OF TIMES I EAT THE FOOD			
		Per Day	**Per Week**	**Per Month**	**Do Not Eat (✔)**
Bread or toast	1 slice *or* 1 roll	_____	_____	_____	O
Muffin	1 large muffin	_____	_____	_____	O
Pizza	1 medium slice	_____	_____	_____	O
Cheeseburger or Veggie burger with cheese	1 burger	_____	_____	_____	O
Cheese: processed or hard cheese (plain or in sandwich)	1 slice	_____	_____	_____	O
Broccoli	½ cup (125 mL)	_____	_____	_____	O
Gai-lan (Chinese broccoli)	½ cup (125 mL)	_____	_____	_____	O
Bok- choi (Chinese cabbage)	½ cup (125 mL)	_____	_____	_____	O
Ice cream	1 large scoop	_____	_____	_____	O
Frozen yogurt	1 large scoop	_____	_____	_____	O
Fast-food milkshake	1 medium	_____	_____	_____	O
Cottage Cheese	½ cup (125 mL)	_____	_____	_____	O
Yogurt	1 small carton or bowl (174 mL)	_____	_____	_____	O
Canned salmon, sardines (with bones)	½ small can	_____	_____	_____	O
Soft drink	1 can or large glass	_____	_____	_____	O
Tofu	2 oz (60 g)	_____	_____	_____	O
Milk on cereal		_____	_____	_____	O
Orange juice	1 cup (250 mL)	_____	_____	_____	O
Milk: any type including chocolate	1 cup (250 mL)	_____	_____	_____	O
Macaroni & Cheese	1 cup (250 mL)	_____	_____	_____	O

Go on to more questions on the next few pages…

I usually drink (check ✔ ***one***):

O milk
O flavoured milk (chocolate, strawberry, etc.)
O soy milk
O rice milk

Are you allergic to any foods?

O No
O Yes

If you anwered 'Yes', what foods are you allergic to?

List food(s): _____

_____

In the questions below, we would like to know if you use any **VITAMIN and/or MINERAL supplements**. Tell us what supplements you use, the brand or name of each supplement, and how often you use it.

Please Note: This question is* ***not* *about any* medications *you may be taking.*

INSTRUCTIONS:

- If you use a supplement, write down the brand or name. If you forget the name, describe what you can about the supplement or its container (i.e. colour of box or bottle, is it chewable, etc.)
- Put a check mark (✔) in one of the circles below for how many times you use it.
- **NOTE**: Put **only *ONE*** check mark for each supplement.

TYPE OF SUPPLEMENT		**HOW MANY TIMES?**				
	Brand or Name	**Daily**	**More than 3 times per week**	**1 to 3 times per week**	**Less than once per week**	**Do Not Use**
Multivitamin	_____	O	O	O	O	O
Multivitamin/mineral	_____	O	O	O	O	O
Iron	_____	O	O	O	O	O
Vitamin C	_____	O	O	O	O	O
Calcium	_____	O	O	O	O	O
Other: _____	_____	O	O	O	O	O
Other: _____	_____	O	O	O	O	O

Fruit and Vegetable Food Frequency Questionnaire

INSTRUCTIONS: Think about what you ate **last week** and answer the questions below by filling in one circle with your pencil.

- **Please think about <u>all</u> the fruits and vegetables that you ate last week. Include those that were:**
  - Raw and cooked,
  - Eaten as snacks and at meals,
  - Eaten at home and away from home (restaurants, friend's house),
  - Eaten alone or mixed with other foods

1) Over the past week, how many times per week or day did you drink ***100% fruit juice*** such as orange, apple, grape or grapefruit juice? **Do *<u>not</u>* count fruit drinks** like Kool-Aid, lemonade, Hi-C, iced tea, cranberry juice drink and Tang.

O	O	O	O	O	O	O	O	O
Never	1-2x/week	3-4x/week	5-6x/week	1x/day	2x/day	3x/day	4x/day	5+times/day

2) Over the last week, how often did you eat **french fries** or **fried potatoes**?

O	O	O	O	O	O	O	O	O
Never	1-2x/week	3-4x/week	5-6x/week	1x/day	2x/day	3x/day	4x/day	5+times/day

3) Over the past week, how often did you eat **other white potatoes**? Count baked, boiled, and mashed potatoes, potato salad, and white potatoes that were ***<u>not</u>*** fried.

O	O	O	O	O	O	O	O	O
Never	1-2x/week	3-4x/week	5-6x/week	1x/day	2x/day	3x/day	4x/day	5+times/day

<u>Food in the MORNING</u>

For the next ***two*** questions, think about all the food you ate at your **morning meal** or **morning snacks** in the last week

4) On how many days did you eat **FRUIT** for your <u>morning</u> meal or <u>morning</u> snacks?
Count any kind of fruit: fresh, canned, and frozen. *Do ***<u>not</u>*** count juices.*

O	O	O	O	O
Never	1-2 days/week	3-4 days/week	5-6 days/week	Everyday

5) On how many days did you eat **VEGETABLES** for your morning meal or morning snacks?
Count salads, vegetables in mixtures (i.e. sandwiches, omelettes, casseroles, Chinese dishes, stew, stir-fry, soup, etc.), tomato pasta sauce and all other raw, cooked and canned vegetables.
Do not include white potatoes.

O	O	O	O	O
Never	1-2 days/week	3-4 days/week	5-6 days/week	Everyday

LUNCHTIME and AFTERNOON SNACKS

For the next ***two*** questions, think about all the food you ate at **lunchtime** or for **afternoon snacks** in the last week

6) On how many days did you eat **FRUIT** at lunchtime or for your afternoon snacks?
Count any kind of fruit: fresh, canned, and frozen. *Do **not** count juices.*

O	O	O	O	O
Never	1-2 days/week	3-4 days/week	5-6 days/week	Everyday

7) On how many days did you eat **VEGETABLES** at lunchtime or for you afternoon snacks?
Count salads, vegetables in mixtures (i.e. sandwiches, omelettes, casseroles, Chinese dishes, stew, stir-fry, soup, etc.), tomato pasta sauce, and all other raw, cooked and canned vegetables.
Do not include white potatoes.

O	O	O	O	O
Never	1-2 days/week	3-4 days/week	5-6 days/week	Everyday

SUPPERTIME and EVENING SNACKS

For the next ***two*** questions, think about all the food you ate at **suppertime** or for **evening snacks** in the last week

8) On how many days did you eat **FRUIT** at suppertime or for your evening snacks?
Count any kind of fruit: fresh, canned, and frozen. *Do **not** count juices.*

O	O	O	O	O
Never	1-2 days/week	3-4 days/week	5-6 days/week	Everyday

9) On how many days did you eat **VEGETABLES** at suppertime or for your evening snacks?
Count salads, vegetables in mixtures (i.e. sandwiches, omelettes, casseroles, Chinese dishes, stew, stir-fry, soup etc.), tomato pasta sauce, and all other raw, cooked and canned vegetables.
Do not include white potatoes.

O	O	O	O	O
Never	1-2 days/week	3-4 days/week	5-6 days/week	Everyday

Thank You!

ID: _____
Checked by: _____

Action Schools! BC Follow-up

Elementary Physical Activity Questionnaire Spring 2011

We would like to know about the **physical activity** you have done in the **last 7 days**. This includes sports or dance that make you sweat or make your legs feel tired, or games that make you huff and puff, like tag, skipping, running, and climbing.

REMEMBER:
- **There are no right or wrong answers – THIS IS NOT A TEST.**
- **Please answer all questions as honestly and accurately as you can – this is very important.**

This question is about **PHYSICAL ACTIVITY IN YOUR SPARE TIME**. Have you done any of the following activities in **PAST 7 DAYS**? For **HOW MANY MINUTES** did you do each activity?

INSTRUCTIONS:
- Tick (✔) ***ONE*** circle per row for how many times you did the activity in the **last 7 days.**
- Write down how long you did the activity for in the "**Minutes per Session**" column.

*NOTE***:** *When you are thinking of how long you did the activity for, remember recess is 15 min. and lunch is usually 30 min!*

1)	**HOW MANY TIMES PER WEEK?**					**Minutes per Session**
	No	**1-2**	**3-4**	**5-6**	**7 or more**	
Skipping	O	O	O	O	O	_____
Four Square	O	O	O	O	O	_____
Creative Playground	O	O	O	O	O	_____
Tag	O	O	O	O	O	_____
Walking for exercise	O	O	O	O	O	_____
Bicycling	O	O	O	O	O	_____
Jogging/running	O	O	O	O	O	_____
Swimming	O	O	O	O	O	_____
Baseball/Softball	O	O	O	O	O	_____
Dance	O	O	O	O	O	_____
Football	O	O	O	O	O	_____
Badminton	O	O	O	O	O	_____
Skateboarding/Scooter	O	O	O	O	O	_____
Soccer	O	O	O	O	O	_____
Ice Hockey/Ringette	O	O	O	O	O	_____
Ice Skating	O	O	O	O	O	_____
Street Hockey	O	O	O	O	O	_____
Floor Hockey	O	O	O	O	O	_____
Rollerblading	O	O	O	O	O	_____
Skiing/Snowboarding	O	O	O	O	O	_____
Cross-country Skiing	O	O	O	O	O	_____
Martial Arts	O	O	O	O	O	_____
Gymnastics	O	O	O	O	O	_____
Basketball	O	O	O	O	O	_____
Volleyball	O	O	O	O	O	_____
Other: _____	O	O	O	O	O	_____

Please read the following questions carefully before answering

2) In the **last 7 days**, during your **PHYSICAL EDUCATION (PE) CLASSES**, how often were you **very active** (playing hard, running, jumping and throwing)? *Check only one*.

O I don't do PE
O Hardly ever
O Sometimes
O Quite often
O Always

3) In the **last 7 days**, what did you do most of the time at **RECESS?** *Check only one*.

O Sat down (talking, reading, doing school work) .
O Stood around or walked around.
O Ran or played a little bit.
O Ran around and played quite a bit.
O Ran and played hard most of the time.

4) In the **last 7 days**, what did you normally do **AT LUNCH** (besides eating lunch)? *Check only one*.

O Sat down (talking, reading, doing school work)
O Stood around or walked around.
O Ran or played a little bit.
O Ran around and played quite a bit.
O Ran and played hard most of the time.

5) In the **last 7 days**, on how many days **RIGHT AFTER SCHOOL**, did you do sports, dance, or play games in which you were very active? *Check only one*.

O None.
O 1 time last week.
O 2 or 3 times last week.
O 4 times last week.
O 5 times last week.

6) In the **last 7 days**, on how many **EVENINGS** did you do sports, dance, or play games in which you were very active? *Check only one*.

O None.
O 1 time last week.
O 2 - 3 times last week.
O 4 - 5 times last week.
O 6 - 7 times last week.

7) How many times did you do sports, dance, or play games in which you were very active **LAST WEEKEND?** *Check only one*.

O None.
O 1 time.
O 2 - 3 times.
O 4 - 5 times.
O 6 or more times.

For the next two questions, read all 5 responses before deciding on the ONE answer that describes you.

8) Which **one** of the following five statements describes you best for the **last 7 days**? *Check only one.*

O **All or most of my free time** was spent doing things that involved **little physical effort** (e.g. watching TV, homework, playing computer games, Nintendo).

O **I sometimes (1-2 times last week) did physical things** in my free time (e.g. played sports, went running, swimming, bike riding, did aerobics).

O **I often (3-4 times last week) did physical things** in my free time.

O **I quite often (5-6 times last week) did physical things** in my free time.

O **I very often (7 or more times last week) did physical things** in my free time.

9) How many **hours per day** did you **watch television** or **play video games** (PlayStation, X-Box) or **computer games LAST WEEK**? (NOTE: Each show is usually a ½ hour). *Check only one.*

O None at all, or less than 1 hour per day.
O More than 1 hour but less than 2 hours per day.
O More than 2 hours but less than 3 hours per day.
O More than 3 hours but less than 4 hours per day.
O More than 4 hours per day.

10) Were you sick last week, or did anything **prevent** you from doing your **normal physical activities**?

O No
O Yes ➔ **If you answered 'Yes', what prevented you**?: _____

11) For each day of the week below, put a check (✔) in the circle that **best describes how often you did physical activity** (sports, games, dance, or other activities). This includes PE classes, lunch, recess, after school, evenings, and other spare time.

a) Check only ***ONE*** circle for each day

	HOW OFTEN DID YOU DO PHYSICAL ACTIVITY?				
	None	**Little Bit**	**Medium**	**Often**	**Very Often**
Monday	O	O	O	O	O
Tuesday	O	O	O	O	O
Wednesday	O	O	O	O	O
Thursday	O	O	O	O	O
Friday	O	O	O	O	O
Saturday	O	O	O	O	O
Sunday	O	O	O	O	O

b)	**Check (✔) the days you had PE classes at school *in the last week*:**	**Monday**	**Tuesday**	**Wednesday**	**Thursday**	**Friday**
		O	O	O	O	O

12) Do you participate in **organized SPORT outside of school** (i.e. soccer team, dance class, karate, etc.)?

O Yes
O No

13) Do you participate in other **organized ACTIVITIES outside of school** (i.e. music lessons, Chinese school, tutoring, Guides/Scouts, church group, musical theatre, drama, art lessons, volunteering, etc.)?

O Yes
O No

14) If you do participate in **organized sport** or **organized activities**, how many evenings during the week do you do these sports and/or activities?

Write the sport or activity on the line and then put a check (✔) under the correct number of evenings per week.

SPORT OR ACTIVITY	**EVENINGS PER WEEK**						
	1	2	3	4	5	6	7
_____	O	O	O	O	O	O	O
_____	O	O	O	O	O	O	O
_____	O	O	O	O	O	O	O
_____	O	O	O	O	O	O	O
_____	O	O	O	O	O	O	O
_____	O	O	O	O	O	O	O

15) Think about what other **EDUCATION**, **ENTERTAINMENT**, or **SOCIAL** activities you did **yesterday**. If you did the activity, check (✔) the circle and write down how many minutes you did it for.

	ACTIVITY	**I did this yesterday (✔)**	**How many minutes did you do the activity?**
a)	Computer/Internet	O	_____
b)	Video Games	O	_____
c)	Watching TV or movies	O	_____
d)	Reading (not for school)	O	_____
e)	Sitting and listening to music	O	_____
f)	Sitting and talking with friends (not on the phone)	O	_____
g)	Talking on the phone	O	_____
h)	Homework/Studying	O	_____
i)	Other: _____	O	_____
j)	Other: _____	O	_____

Thank You!

Healthy Bones III and Action Schools! Spring 2012
Physical Activity Questionnaire – High School Students

ID #: _____
Checked by: _____

Date (dd/mm/yy): _____

We would like to know about the physical activity you have done in the last 7 days. This includes sports or activities that make you sweat or make your legs feel tired, or games that make you huff and puff, like tag, skipping, running, and climbing.

Remember:

A. There are no right or wrong answers – this is not a test.
B. Please answer all questions as honestly and accurately as you can – this is very important.

---

1. PHYSICAL ACTIVITY IN YOUR **SPARE TIME (this <u>does not</u> include P.E classes)**.
Have you done any of the following activities in the **past 7 days**? If yes, how many times and for how long?

Tick only one circle per row	No	1-2	3-4	5-6	7 or more times	time per session
Skipping	O	O	O	O	O	_____
Four Square	O	O	O	O	O	_____
Creative Playground	O	O	O	O	O	_____
Tag	O	O	O	O	O	_____
Walking for exercise	O	O	O	O	O	_____
Bicycling	O	O	O	O	O	_____
Jogging or running	O	O	O	O	O	_____
Swimming	O	O	O	O	O	_____
Baseball, softball	O	O	O	O	O	_____
Dance	O	O	O	O	O	_____
Football	O	O	O	O	O	_____
Badminton	O	O	O	O	O	_____
Skateboarding/Scooter	O	O	O	O	O	_____
Soccer	O	O	O	O	O	_____
Street Hockey	O	O	O	O	O	_____
Volleyball	O	O	O	O	O	_____
Floor Hockey	O	O	O	O	O	_____
Basketball	O	O	O	O	O	_____
Ice skating	O	O	O	O	O	_____
Cross-country skiing	O	O	O	O	O	_____
Ice hockey/ringette	O	O	O	O	O	_____
Martial Arts	O	O	O	O	O	_____
Gymnastics	O	O	O	O	O	_____
Rollerblading	O	O	O	O	O	_____
Skiing/Snowboarding	O	O	O	O	O	_____
Other: _____	O	O	O	O	O	_____
Other: _____	O	O	O	O	O	_____

2. In the last 7 days, during your **PHYSICAL EDUCATION (PE) CLASSES**, how often were you very active (playing hard, running, jumping and throwing)? Check only one.

- O I don't do PE
- O Hardly ever
- O Sometimes
- O Quite often
- O Always

3. In the last 7 days, what did you normally do **AT LUNCH** (besides eating lunch)? Check only one.

- O Sat down (talking, reading, doing school work)
- O Stood around or walked around.
- O Ran or played a little bit.
- O Ran around and played quite a bit.
- O Ran and played hard most of the time.

4. In the last 7 days, on how many days **RIGHT AFTER SCHOOL**, did you do sports, dance, or play games in which you were very active? Check only one.

- O None.
- O 1 time last week.
- O 2 or 3 times last week.
- O 4 times last week.
- O 5 times last week.

5. In the last 7 days, on how many **EVENINGS** did you do sports, dance, or play games in which you were very active? Check only one.

- O None.
- O 1 time last week.
- O 2 - 3 times last week.
- O 4 - 5 times last week.
- O 6 - 7 times last week.

6. How many times did you do sports, dance, or play games in which you were very active **LAST WEEKEND?** Check only one.

- O None.
- O 1 time.
- O 2 - 3 times.
- O 4 - 5 times.
- O 6 or more times.

7. Which **ONE** of the following five statements describes you best for the last 7 days? Read all 5 before deciding on the one answer that describes you.

O All or most of my free time was spent doing things that involved **little physical effort** (e.g. watching TV, homework, playing computer games, Nintendo).

O **I sometimes (1-2 times last week) did physical things** in my free time (e.g. played sports went running, swimming, bike riding, did aerobics).

O **I often (3-4 times last week) did physical things** in my free time.

O **I quite often (5-6 times last week) did physical things** in my free time.

O **I very often (7 or more times last week) did physical things** in my free time.

8. How many hours per day did you watch television or play Nintendo last week? (each show is usually a half hour or 30 minutes). Check only one.

O I watched less than 1 hour or have no TV.
O I watched more than 1 hour but less than 2.
O I watched more than 2 hours but less than 3.
O I watched more than 3 hours but less than 4.
O I watched more than 4 hours.

9. Were you sick last week, or did anything prevent you from doing your normal physical activities?

O Yes
O No

If yes, what prevented you?_____

10. Mark how often you did physical activity (like playing sports, games, doing dance or any other physical activity) for each day last week (this includes P.E, lunch, recess, after school, evenings, spare time, etc).

	None	Little Bit	Medium	Often	Very Often
Monday	O	O	O	O	O
Tuesday	O	O	O	O	O
Wednesday	O	O	O	O	O
Thursday	O	O	O	O	O
Friday	O	O	O	O	O
Saturday	O	O	O	O	O
Sunday	O	O	O	O	O

11. Do you participate in **organized sport**, (school volleyball team, martial arts practices, swimming lessons) outside of school?

O Yes
O No

If yes, please list the **SPORTS** that you do beside the number that matches the number of times you do those sports during the week. For example, if you have swimming lessons on 2 nights of the week, check the circle beside "2" and write swimming lessons on the line. You can have more than one activity on a line.

O 1 activity: _____
O 2 activity: _____
O 3 activity: _____
O 4 activity: _____
O 5 activity: _____
O 6 activity: _____
O 7 activity: _____

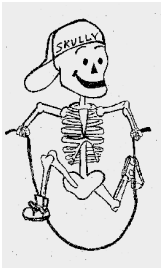
12. Do you participate in **organized activities** (music lessons, Chinese school, tutoring, girl guides, boy scouts) outside of school?

O Yes
O No

If yes, please list the **activities** that you do beside the number that matches the number of times you do those activities during the week. For example, if you have girl guides on 2 nights of the week, check the circle beside "2" and write girl guides on the line. You can have more than one activity on a line.

O 1 activity: _____
O 2 activity: _____
O 3 activity: _____
O 4 activity: _____
O 5 activity: _____
O 6 activity: _____
O 7 activity: _____

THANK YOU!



HEALTHY BONES III 7-DAY ACTIVITY LOG – Spring 2012

ID:

Directions:

1) Please wear the motion sensor under your clothing.

2) The motion sensor should fit snugly on the waist with the sensor positioned above the right hip. The belt should feel comfortable but not floppy.

3) The motion sensor should be worn from the time you wake up until the time you go to bed for at least 12 hours each day and should only be removed during that period if you go swimming or have a bath or a shower. It is not waterproof.

4) Please record the time when the motion sensor is first put on and when it is taken off daily on the log, which is on the reverse side of this form. Also record anything that affected your physical activity level on any given day, such as an illness, injury or unfavorable weather.

5) The motion sensor is like a smart 'pedometer' and it is very valuable. A Healthy Bones III researcher will collect them from the main office of your school.

Thank you very much for your help!

Monitor:	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday
Dates							
On Time AM							
Off Time PM							
Did weather change your routine?	No	No	No	No	No	No	No
	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Did illness change your routine?	No	No	No	No	No	No	No
	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Was the motion sensor removed during wear time?	No	No	No	No	No	No	No
	Yes	Yes	Yes	Yes	Yes	Yes	Yes
If yes, what time was it removed?	___:____ to ___:____	___:____ to ___:____	___:____ to ___:____	___:____ to ___:____	___:____ to ___:____	___:____ to ___:____	___:____ to ___:____
Why was the monitor removed?							
Any problems? Please explain.							

ID #: 3313
Date:_____
Checked by:_____

HBS III Spring 2012

Bone Imaging Information Sheet

DXA Technician (*initial*):_____

HR-pQCT Technician (*initial*):_____

pQCT Technician (*initial*):_____

Required medical information:

1. Do they have a history of bone fracture (check for non-dominant limb)? Yes No

2. Have they had any nuclear medicine tests within the last 10 days? Yes No

3. Are they at risk of being pregnant? Yes No

4. Have you had your first menstrual period?

If Yes;

4a. Do you know the date of your first menstrual period?

4b. What was the date of your last menstrual period?_____

5. Do you take oral contraceptives (i.e., Birth Control)? Yes No

If Yes;

5a. What brand name of oral contraceptives do you take?_____

*If the participant is male, cross out the above section

DXA	Scan Completed	Comments OR if the scan has been repeated provide details
Whole Body	Yes No	
Lumbar Spine	Yes No	
Proximal Femur	Yes No	

HR-pQCT (site)	Scan Site	Comments OR if the scan has been repeated provide details
Radius 7%	Left Right	
	Left Right	
Tibia 8%	Left Right	
	Left Right	

pQCT (site)	Comments OR if the scan has been repeated provide details
Left Tibia 50%	

Appendix D: Determination of Age at Peak Height Velocity

To control for well-known maturational differences between adolescent boys and girls of the same chronological age, we calculated age at peak height velocity (APHV) as an estimate of biological maturity. To ensure we measured participants during the anticipated period of maximal height gain, we set an age criteria. For boys' data to be used for APHV calculation, participants needed a height measurement before age 11.5 years and after age 16.5 years with a minimum of five measurements during this time period. Age criteria for boys approximated the average 10th and 90th percentiles from six well-known growth and development studies.[1-7] Girls for whom we calculated APHV needed a height measure before 11.0 years and after 13.0 years, with a minimum of four measurements during this time period. Age criteria for girls approximated the average 15th and 85th percentiles of the six aforementioned studies. We used a less conservative range for girls due to age of entry limitations in our cohort. Using the 15th and 85th percentiles instead of the 10th and 90th allowed us to include 81 additional girls in our study cohort.

Due to variation between studies, time between height measurements ranged from 3 to 12 months. Missing and mistimed visits resulted in measurement intervals in some cases of up to 30 months in boys and 36 months in girls. However, we minimized these gaps during the critical period of rapid growth (11.5-16.5 years in boys and 11.0-13.0 years in girls), as boys required 5, and girls required 4 measures for data to be included in analyses. We calculated multiple running annual height velocities (4 velocity calculations per height measurement) as growth during the time-interval divided by the time-interval (cm/year). From these we retained one calculated velocity, closest to the ideal measurement interval of 0.85 to 1.15 years.[8] We then fitted an interpolating cubic spline[9-13] on a regular grid (10 grid points/year) to each participant's height velocity data. The age associated with the maximum interpolated height velocity was identified as the provisional APHV. Peak height velocity was identified as growth per year (cm) that occurred at the provisional APHV.

We then visually inspected the height velocity vs. age curve for each participant. If age at the first or last velocity point was identified as APHV, the magnitude of PHV had to be $\geq$ 90th percentile relative to normative data (described below) for the APHV value to be accepted. We used data from six previously conducted growth and development studies[1-7] to calculate the 90th percentile of PHV for girls ($\geq$ 9.0 cm/year) and boys (10.5 cm/year). We modeled our approach after Little et al.[14] who accepted first or last velocity points as APHV only if the magnitude was

≥ 90th percentile of the magnitude identified by Buckler[15] and Anderson et al.[16] We fit the spline using all velocity data; however, we may have overestimated velocities based on intervals < 0.85 due to measurement error and seasonal variation or underestimated those based on intervals > 1.15.[14] We visually inspected each participant's velocity curve to ensure that calculations based on time intervals outside the 0.85-1.15 years did not impact determination of PHV. Finally, we used APHV to calculate a biological maturity offset (in years) by subtracting APHV from chronological age at the time of measurement. Thus, we generated a continuous measure of biological age.

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Appendix E: Additional Data for Chapter 4

Table E.1. Estimates of model intercepts and fixed effects slopes between boys and girls without interpolation for measurement error. Slopes represent annual rates of accrual pre- and post-age at peak height velocity (APHV), adjusted for maturity offset and ethnicity. Numbers in brackets are the standard error of the parameter estimate.

	Intercept at APHV			Slope Pre-APHV			Slope Post-APHV		
	Boys	**Girls**	***p*-value**	**Boys**	**Girls**	***p*-value**	**Boys**	**Girls**	***p*-value**
Tt.Ar (mm^2/yr)	408.8 (7.2)	321.7 (6.8)	<0.001	52.1 (1.6)	41.4 (2.3)	<0.001	19.7 (0.6)	11.1 (0.6)	<0.001
Ct.Ar (mm^2/yr)	265.1 (4.7)	212.7 (4.5)	<0.001	36.8 (1.4)	31.9 (1.9)	0.041	15.6 (0.5)	9.3 (0.5)	<0.001
Ct.Ar/Tt.Ar	0.648 (0.006)	0.663 (0.006)	0.025	0.007 (0.001)	0.013 (0.002)	0.012	0.005 (0.0004)	0.004 (0.0004)	0.738
Me.Ar (mm^2/yr)	144.2 (3.7)	109.6 (3.5)	<0.001	14.4 (0.5)	9.9 (0.8)	<0.001	4.2 (0.2)	1.9 (0.2)	<0.001
Ct.BMD (mg/cm^3/yr)	1042.3 (3.7)	1078.6 (3.5)	<0.001	-1.9 (0.8)	14.2 (1.6)	<0.001	17.8 (0.6)	14.9 (0.6)	<0.001
SSI_p (mm^3/yr)	1563.2 (34.9)	1140.9 (34.9)	<0.001	262.8 (9.2)	216.9 (13.4)	0.005	166.9 (4.7)	89.6 (4.8)	<0.001

Tt.Ar, total area; Ct.Ar, cortical area; Me.Ar, medullary canal area; Ct.BMD, cortical bone mineral density; SSI_p strength-strain index. Maturity offset is years from APHV.

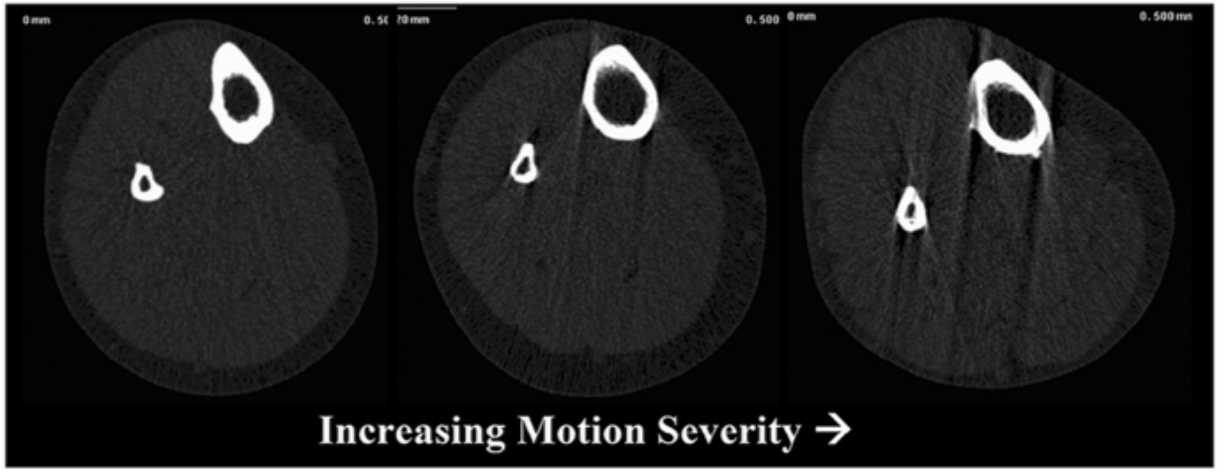

Figure E.1. Illustration of motion artifact from pQCT tibia scans. Scans with streaks in the cortical shell (far right image) are excluded from analysis. Reprinted from Chan et al.,[1] with permission from Elsevier.

Table E.2. Estimates of model intercepts between intervention and control group participants. Intercepts represent the average value of the bone parameter at age at peak height velocity (APHV; maturity offset = 0). Numbers in brackets are the standard error of the parameter estimate.

	Intervention	**Control**	***p*-value**
Tt.Ar (mm^2)	322.9 (7.5)	331.7 (8.2)	0.245
Ct.Ar (mm^2)	213.9 (4.9)	217.8 (5.4)	0.409
Ct.Ar/Tt.Ar	0.664 (0.006)	0.660 (0.007)	0.589
Me.Ar (mm^2)	109.4 (3.8)	113.3 (4.2)	0.306
Ct.BMD (mg/cm^3)	1082.6 (3.7)	1079.7 (4.0)	0.361
SSI_p (mm^3)	1142.8 (38.0)	1201.3 (41.2)	0.093

Tt.Ar, total area; Ct.Ar, cortical area; Me.Ar, medullary canal area; Ct.BMD, cortical bone mineral density; SSI_p strength-strain index. For this purpose, slopes presented refer to Asian girls at APHV. There were no group by sex interactions.

Table E.3. Baseline Pearson correlations of age, sex, ethnicity, maturity, and anthropometric variables with bone parameters at the tibial midshaft by peripheral quantitative computed tomography (n=230). Correlations with sex, Tanner stage and ethnicity are Spearman's rank order correlations.

	Tt.Ar	**Ct.Ar**	**Ct.Ar/Tt.Ar**	**Me.Ar**	**Ct.BMD**	**SSI_p**
Age	0.56***	0.57***	0.05	0.40***	-0.04	0.59***
Sex	0.14*	0.08	-0.14*	0.19**	-0.30***	0.12
Ethnicity	0.25***	0.30***	0.08	0.10	-0.20**	0.25***
Tanner Stage	0.44***	0.47***	0.12	0.25***	-0.01	0.47***
Maturity	0.42***	0.48***	0.16*	0.23***	0.14*	0.46***
Height	0.81***	0.82***	0.06	0.58***	-0.13	0.82***
Weight	0.80***	0.79***	0.00	0.60***	-0.07	0.79***
Sitting Height	0.78***	0.78***	0.04	0.56***	-0.05	0.79***
Tibia Length	0.79***	0.80***	0.05	0.57***	-0.20**	0.78***

Tt.Ar, total area (mm^2); Ct.Ar, cortical area (mm^2); Ct.Ar/Tt.Ar, ratio of cortical to total area; Me.Ar , medullary canal area (mm^2), Ct.BMD, cortical bone mineral density (mg/cm^3); SSI_p, strength-strain index (mm^3); Age (years); Sex (girls=0, boys=1); Ethnicity (Asian=0, white=1, other=2); Tanner stage (1,2,3,4,5); Maturity, years from age at peak height velocity; Height (cm); Weight (kg); Sitting height (cm), Tibia length (mm).*p<0.05, **p<0.01, ***p<0.001.

Reference

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Appendix F: Additional Data for Chapters 5 and 6

Table F.1. Baseline Pearson correlations of age, sex, ethnicity, maturity, anthropometric variables, muscle power, dietary calcium and accelerometry variables with bone parameters at the distal tibia by high-resolution peripheral quantitative computed tomography (n = 393). Correlations with sex, Tanner stage and ethnicity are Spearman's rank order correlations.

	BV/TV	**Tb.N**	**Tb.Th**	**Tb.Sp**	**Ct.Th**	**Ct.Po**	**Ct.BMD**	**Tt.BMD**	**Ct.Ar**	**Tt.Ar**	**F.Load**	**U.Stress**
Age	0.28***	-0.19***	0.43***	0.14**	0.70***	-0.42***	0.85***	0.66***	0.71***	0.27***	0.67***	0.66***
Sex	0.21***	0.17***	0.09	-0.19***	0.22***	0.40***	-0.14**	0.11*	0.37***	0.48***	0.39***	0.13*
Ethnicity	0.06	0.17***	-0.11*	-0.16**	0.04	0.01	0.05	0.05	0.08	0.14**	0.09	0.02
Tanner Stage	0.35***	-0.11*	0.43***	0.04	0.71***	-0.28***	0.68***	0.64***	0.75***	0.38***	0.74***	0.65***
Maturity	0.26***	-0.21***	0.44***	0.16**	0.68***	-0.52***	0.9***	0.68***	0.65***	0.13*	0.59***	0.67***
Height	0.29***	-0.06	0.33***	0.02	0.64***	-0.09	0.55***	0.50***	0.78***	0.63***	0.80***	0.55***
Weight	0.35***	0.14**	0.21***	-0.18***	0.65***	-0.10*	0.54***	0.53***	0.77***	0.63***	0.80***	0.53***
Sitting Height	0.35***	-0.06	0.39***	0.01	0.69***	-0.15**	0.63***	0.57***	0.8***	0.57***	0.81***	0.60***
Tibia Length	0.20***	-0.01	0.20***	-0.01	0.55***	0.00	0.41***	0.37***	0.69***	0.63***	0.71***	0.43***
Muscle Power	0.38***	0.07	0.31***	-0.12*	0.69***	-0.04	0.51***	0.54***	0.83***	0.64***	0.84**	0.57***
Dietary Calcium	0.11*	0.24***	-0.09	-0.23***	0.01	0.17***	-0.15**	0.01	0.05	0.08	0.06	-0.01
MVPA	0.18**	0.21***	0.01	-0.22***	0.03	0.29***	-0.18**	0.02	0.11	0.27***	0.19**	0.01
Sedentary Time	0.06	-0.33***	0.33***	0.29***	0.45***	-0.36***	0.57***	0.37***	0.44***	0.14*	0.40***	0.41***
Wear Time	0.01	-0.21***	0.19**	0.18**	0.28***	-0.22***	0.34***	0.20***	0.30***	0.17**	0.28***	0.23***

BV/TV, trabecular bone volume to total volume fraction; Tb.N, trabecular number (1/mm); Tb.Th, trabecular thickness (mm); Tb.Sp, trabecular separation (mm); Ct.Th, cortical thickness (mm); Ct.Po, cortical porosity (%); Ct.BMD, cortical bone mineral density (mg/cm^3); Tt.BMD, total bone mineral density (mg/cm^3); Ct.Ar, cortical area (mm^2); Tt.Ar, total area (mm^2); F.Load, failure load (N); U.Stress, ultimate stress (MPa); Age (years); Sex (girls=0, boys=1); Ethnicity (Asian=0, white=1, other=2); Tanner stage (1,2,3,4,5); Maturity, years from age at peak height velocity; Height (cm); Weight (kg); Sitting height (cm), Tibia length (mm), Muscle power (W), Dietary calcium (mg); MVPA (min/day); Sedentary time (min/day); Wear time (min/day). [a] n = 269 for accelerometry data. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table F.2. Baseline Pearson correlations of age, sex, ethnicity, maturity, anthropometric variables, muscle power, dietary calcium and accelerometry variables with bone parameters at the distal radius by high-resolution peripheral quantitative computed tomography (n = 351). Correlations with sex, Tanner stage and ethnicity are Spearman's rank order correlations.

	BV/TV	**Tb.N**	**Tb.Th**	**Tb.Sp**	**Ct.Th**	**Ct.Po**	**Ct.BMD**	**Tt.BMD**	**Ct.Ar**	**Tt.Ar**	**F.Load**	**U.Stress**	**L:S**
Age	0.10	-0.36***	0.36***	0.29***	0.77***	-0.57***	0.88***	0.72***	0.81***	0.48***	0.73***	0.67***	-0.78***
Sex	0.29***	0.03	0.30***	-0.09	0.24***	0.27***	-0.04	0.12*	0.39***	0.48***	0.43***	0.21***	-0.41***
Ethnicity	-0.01	0.09	-0.07	-0.07	-0.09	0.02	-0.03	-0.08	-0.01	0.20***	0.06	-0.07	-0.04
Tanner Stage	0.18***	-0.28***	0.44***	0.20***	0.70***	-0.51***	0.71***	0.67***	0.76***	0.57***	0.77***	0.65***	-0.76***
Maturity	0.06	-0.36***	0.31***	0.30***	0.76***	-0.64***	0.91***	0.73***	0.75***	0.36***	0.66***	0.66***	-0.71***
Height	0.17**	-0.27***	0.37***	0.18***	0.63***	-0.23***	0.56***	0.49***	0.78***	0.77***	0.8***	0.53***	-0.77***
Weight	0.19***	-0.13*	0.30***	0.07	0.65***	-0.30***	0.58***	0.54***	0.76***	0.63***	0.74***	0.54***	-0.70***
Sitting Height	0.19***	-0.29***	0.41***	0.20***	0.69***	-0.29***	0.63***	0.56***	0.83***	0.73***	0.83***	0.59***	-0.81***
Tibia Length	0.19***	-0.24***	0.37***	0.15**	0.63***	-0.18***	0.52***	0.48***	0.77***	0.73***	0.78***	0.54***	-0.75***
Muscle Power	0.30***	-0.18***	0.46***	0.09	0.69***	-0.21***	0.54***	0.55***	0.83***	0.72***	0.84***	0.60***	-0.75***
Dietary Calcium	0.19***	0.20***	0.06	-0.22***	-0.06	0.21***	-0.14**	-0.05	-0.02	0.09	0.05	-0.02	0.00
MVPA	0.20**	0.11	0.14*	-0.13	0.06	0.17*	-0.06	0.01	0.16*	0.33***	0.24***	0.07	-0.20**
Sedentary Time	-0.01	-0.38***	0.27***	0.32***	0.54***	-0.36***	0.56***	0.46***	0.56***	0.34***	0.52***	0.46***	-0.54***
Wear Time	-0.02	-0.23***	0.16*	0.20**	0.35***	-0.18**	0.31***	0.24***	0.41***	0.33***	0.39***	0.27***	-0.37***

BV/TV, trabecular bone volume to total volume fraction; Tb.N, trabecular number (1/mm); Tb.Th, trabecular thickness (mm); Tb.Sp, trabecular separation (mm); Ct.Th, cortical thickness (mm); Ct.Po, cortical porosity (%); Ct.BMD, cortical bone mineral density (mg/cm^3); Tt.BMD, total bone mineral density (mg/cm^3); Ct.Ar, cortical area (mm^2); Tt.Ar, total area (mm^2); F.Load, failure load (N); U.Stress, ultimate stress (MPa); Load:strength, load-to-strength ratio; Age (years); Sex (girls=0, boys=1); Ethnicity (Asian=0, white=1, other=2); Tanner stage (1,2,3,4,5); Maturity, years from age at peak height velocity; Height (cm); Weight (kg); Sitting height (cm), Tibia length (mm), Muscle power (W), Dietary calcium (mg); MVPA (min/day); Sedentary time (min/day); Wear time (min/day). [a]n = 216 for accelerometry data.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.