EFFECTS OF A SCHOOL-BASED PHYSICAL ACTIVITY INTERVENTION ON ADOLESCENT BONE STRENGTH, STRUCTURE AND DENSITY: THE HEALTH PROMOTING SECONDARY SCHOOLS (HPSS) BONE HEALTH STUDY

by

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Abstract

Physical activity (PA) benefits bone strength in children but little is known of the effects of PA on bone strength in adolescents. In this thesis, my primary aim was to determine the effect of a secondary school based PA intervention on bone strength, structure and density in adolescents.

This 8-month cluster, randomized-controlled, whole school-based intervention study had four intervention and five control schools. Participants were 210 Grade 10 students who were 15.3 years old, on average, at baseline. The Health Promoting Secondary Schools (HPSS) intervention was a choice-based model based on self-determination theory that aimed to increase PA, promote healthy eating and reduce screen time in adolescents. I used peripheral quantitative computed tomography (pQCT) to assess bone strength, structure and density at the distal and shaft sites of the tibia and radius. I assessed PA using a validated PA self-report questionnaire and I measured a sub-set of participants' PA objectively using accelerometry.

Part I is a systematic review and narrative synthesis of PA and pediatric bone literature. High-quality randomized-controlled trials (RCTs) with weight-bearing PA increased bone strength in children. Bone structure adaptations in response to PA were more common than adaptations in bone density (RCTs and observational studies). Only one RCT involved adolescents (average age 13.8 years) and studies often overlooked the influence of muscle on bone responses to PA.

In Part II, moderate-to-vigorous PA (MVPA), vigorous PA (VPA) and grip strength positively influenced bone strength in boys and girls after controlling for ethnicity, maturity, limb length and muscle mass. Sedentary time (SED) negated the positive influence of MVPA, but not VPA, on bone strength in girls.

In Part III, the HPSS intervention did not lead to significant gains in bone strength, structure or density in adolescents. The external factor of a province-wide teacher job action possibly hindered the execution of the HPSS intervention.

In summary, MVPA and VPA benefit bone strength in adolescents but further investigations are warranted to determine the effects of SED on bone strength. It remains to be determined the effects of a choice-based intervention on bone strength adaptations in adolescent boys and girls.

Preface

The Health Promoting Secondary Schools (HPSS) study was conceived and designed by Professor Joan Wharf Higgins and Associate Professor Patti-Jean Naylor (University of Victoria; UVic) in collaboration with Professor Heather McKay (University of British Columbia; UBC). The HPSS Bone Health Study (BHS) was primarily designed by Professor Heather McKay and Assistant Professor Heather Macdonald (UBC) while I provided input on aspects of study design, methods and targeted outcomes. Both studies received ethical approval from the UBC Behavioural Research Ethics Board (H10-01917) and UVic Ethics Board (10-168). My role was to coordinate the BHS from planning to execution. Thus, I organized and led data collection across three regions of British Columbia – Greater Vancouver, Interior and Vancouver Island, during both 6-week data collection periods. Prior to data collection, I trained and supervised research staff on all research protocols with the exception of bone imaging training. I acquired and analysed all of the dual-energy X-ray absorptiometry (DXA) scans, analysed all of the peripheral quantitative computed tomography (pQCT) scans and completed all anthropometry measurements. I entered and cleaned the data (except accelerometry data) and conducted all of the statistical data analysis for this thesis.

A version of Chapter 3 is published in the Journal of Bone and Mineral Research (Tan VPS, Macdonald HM, Kim SJ, Nettlefold L, Gabel L, Ashe M and McKay HA. The influence of physical activity on bone strength in children and adolescents: A systematic review and narrative synthesis. J Bone Miner Res, 2014, 29(10):2161-2181), the top peer-reviewed journal in our field. I was lead author on the review and collaborated with Professor McKay, Associate Professor Maureen Ashe and Dr. SoJung Kim (post-doctoral fellow) to design, conduct and write-up the systematic review. Professor McKay conceived the idea. I conducted the literature searches with guidance from information specialist Madeline Doyle-Waters. I extracted and compiled data from studies and conducted assessments of study quality with Dr.

Kim. I synthesized the data and wrote the initial draft of the systematic review. Assist. Prof Macdonald and Professor McKay provided detailed edits and feedback. Assoc. Professor Ashe, Dr. Lindsay Nettlefold and Leigh Gabel (doctoral candidate) also provided feedback on near final drafts of the systematic review.

I am currently preparing a version of Chapter 4 for submission, title: *Sedentary time negates the positive influence of moderate-to-vigorous physical activity but not vigorous physical activity on bone strength in adolescent girls*. I defined the research question, entered and cleaned the data and conducted the statistical analyses. Douglas Race (HPSS accelerometer coordinator) cleaned, uploaded and processed the accelerometer data. Professor McKay and Dr. Macdonald provided guidance on the focus of the paper and feedback on all versions of the manuscript.

For Chapter 5, I defined the research question, entered, cleaned and compiled all the data, with exception for accelerometry data where Douglas Race uploaded and processed the accelerometry data. I and conducted the statistical analyses. I wrote the draft for the chapter. Dr. Macdonald and Professor McKay provided feedback and guidance for every version of the chapter.

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List of Abbreviations

ABBREVIATION	TERMS
2-D/3-D	Two-/Three-dimensional
aBMD	Areal bone mineral density by dual energy X-ray absorptiometry
APHV	Age at peak height velocity
BC	British Columbia
BHS	Bone Health Study
BMAD	Bone mineral apparent density
BMC	Bone mineral content
BMD	Bone mineral density
BSI	Bone strength index
CCS	Canadian Cancer Society
CHHM	Centre for Hip Health and Mobility
CI	Confidence interval
CIHR	Canadian Institutes of Health Research
CON	Control
cpm	Counts per minute
CRD	Centre for Reviews and Dissemination
CSA	Cross-sectional area
Ct.Ar	Cortical bone area
Ct.Dn	Cortical bone mineral density
Ct.Th	Cortical thickness
DXA	Dual energy X-ray absorptiometry
FEA	Finite element analysis
FFQ	Food frequency questionnaire
G	Gravitational force or ground reaction force

ABBREVIATION	TERMS
GH	Growth hormone
HBS	Healthy Bones Study
HHQ	Health history questionnaire
HPSS	Health Promoting Secondary Schools
HR-pQCT	High resolution peripheral quantitative computed tomography
IGF-1	Insulin-like growth factor - 1
I_{max}	Maximum second moment of inertia
I_{min}	Minimum second moment of inertia
INT	Intervention
I_p	Polar second moments of area
IU	International units
LM	Lean body mass without bone
LPA	Light physical activity
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
MES_p	Minimal effective strain for repair
MPA	Moderate physical activity
MRI	Magnetic resonance imaging
MVPA	Moderate-to-vigorous physical activity
NHANES	National Health and Nutritional Examination Survey
OI	Osteogenic Index

ABBREVIATION	TERMS
PA	Physical activity
PAQ-A	Physical Activity Questionnaire for Adolescents
PAQ-C	Physical Activity Questionnaire for Children
PBMAS	Pediatric Bone Mineral Accrual Study
PBMCV	Peak bone mineral content velocity
PE	Physical education
PHV	Peak height velocity
pQCT	Peripheral quantitative computed tomography
PYPAQ	Past Year Physical Activity Questionnaire
PVE	Partial volume effect
RCT(s)	Randomized controlled trial(s)
ReaCT	Real Community Trial
SED	Sedentary time
SDT	Self-determination theory
SSI_p	Polar strength-strain index
Tb.Ar	Trabecular area
Tb.BV	Trabecular bone volume
Tb.Dn	Trabecular density
Tt.BMC	Total bone mineral content
Tt.Ar	Total bone area
Tt.Dn	Total bone mineral density
UBC	University of British Columbia
vBMD	Volumetric bone mineral density
VPA	Vigorous physical activity
Z	Section modulus

Acknowledgements

It all started with a long distance call from Canada to Malaysia that brought me to beautiful Vancouver for my doctoral studies. My thanks to Heather McKay for her leap of faith in taking me on as her doctoral student. Heather, you have inspired me in so many ways with your leadership and vision. Your guidance in research and writing are invaluable and your support in my endeavours are priceless. The other awesome Heather – Heather Macdonald, I am indebted to your many questions on 'whys', 'hows' and 'whats' that stimulated my thought process and challenged me to look at things differently. Heather and Heather, your contributions made my PhD journey richer. Thank you! My thanks also to Joan Wharf Higgins, for the HPSS study. It was a privilege to be part of such a unique study that gave respect and ownership to secondary school students to be decision-makers in their actions for health. To Lindsay Nettlefold, your friendship and enthusiasm in providing endless statistic discussions and for being my sifu in STATA, *gamsa hamida*! There are numerous others that have contributed to my learning in one way or another at CHHM and special thanks goes to Maureen Ashe for her mentorship during my systematic review paper and Danmei Liu for sharing her bone imaging expertise and her 'expert eye'.

To my HPSS Bone Health Study crew and posse, Sophie Kim So Jung and Paul Drexler, forgive me for being such a slave driver during our grueling data collection period. Your dedication, commitment and humour made it possible to obtain the quality data that we currently have. Christine Voss and Leigh Gabel, your timely appearance during our data collection period made us sane and provided the extra hands and smiles. Douglas Race, our research maestro, thank you for the many calls and your patience in handling the accelerometer data.

My doctoral experience in Vancouver was made extra special by the 'birds of a feather' friends that I will forever hold dear to my heart. Sarah Moore, you picked me up, literally, from the airport, and your encouragement and support at times when it seems to be the darkest, will always be cherished. The Salmonians -- Campbells, Gonzalezs, Mackenzies; Jungs and Ratus, your kindness, compassion and acceptance combined with water-gun fights, pumpkin smashin' and shared feasts taught me (and my family) that there is much to enjoy amidst our PhD 'sufferings'.

To my Universiti Sains Malaysia sponsors, your support and faith in me made this happen. My heartfelt thanks to the School of Health Sciences Dean, Prof. Ahmad Zakaria, and Exercise and Sports Science program chairman, Assoc. Prof. Mohamed Saat Ismail for giving me the chance to further my education. My dearest colleagues and cheerleaders Chen SC, Lim BH, Chen CK and Ooi FK, Ang BS that gave me sound advice and encouragement, *ribuan terima kasih*.

To my family both in Penang and Kota Bharu, I am sorry I missed out on so much and I am amazed at how you let me be worry-free during the years away. Your constant understanding and encouragement gave me peace to focus on what needs to be done. *Kam sia!* And last but not least, my deepest appreciation to my darling boys – Jayden, Lewis and SuperDad-Husband, Khuan. There were times when I wished I was alone so that I would be able to complete my studies without distractions and obligations. But without those smile-inducing, comforting 'distractions' and 'obligations', I would have been a very lonely soul. Thank you for sticking by me when I was a grumpy mommy or a distant, distracted wife. This thesis is possible because of you. I love you for being there with me. Thank you!

xxx

Dedication

Khuan, this is for you. Thanks for 'hanging out' with me…

Chapter 1: Introduction, Literature Review and Research Hypotheses

1.1 Introduction

Osteoporosis is a chronic condition that manifests in later adulthood increasing fracture risk and creating immense financial and societal burden. One in two women and one in three men over the age of 50 years will have an osteoporosis-related fracture in their lifetime (1). This leads to an up to three-fold increased risk of mortality, compared with healthy men and women without fractures (2). In 2001, the annual direct health care cost for osteoporotic hip fractures in Canada was an estimated $650 million and is projected to reach $2.4 billion by the year 2041 if trends are not reversed (3). More recently, the cost of osteoporosis for 2007/08 in Canada was $2.3 billion or 1.3% of total Canadian health care costs (4). Thus, the estimated cost to treat osteoporosis-related hip fractures alone by 2041 is tantamount to what it costs to treat osteoporosis and all related fractures in 2007/08. Also, by 2022, an estimated 18% of Canadian women and 15% of Canadian men over the age of 65 with a prior fragility fracture will have a major hip fracture (5). Approximately 40% of individuals (aged 50 years and above) with all types of fragility fractures required rehabilitative and home care services after discharge and up to 47% reported receiving informal care from friends and relatives (6). Overall, osteoporosis and related fractures also affected quality of life and those afflicted were less able to provide self-care, be mobile or to live without pain (7).

Clearly, there is a need to prevent osteoporosis and related fractures. Prevention can start early during the childhood and adolescent years (1,8). From longitudinal pediatric bone studies, we learned that the amount of bone mass accrued in the two years around puberty (26% of adult status) (9) is equivalent to the average amount of bone mass lost in men and women from age 20 to 90 years old (10). This provides a great window of opportunity for bone health optimization during adolescence.

Physical activity (PA) positively influences bone accrual in adolescents (11–13) as mechanical forces acting upon bone elicit differentiation of progenitor cells and/or proliferation of osteoblasts to form more bone (14). Sadly, the positive effects of weight-bearing PA on bone mass acquisition (11,12,15) and maintenance (16) are diminished by the reality that 93% of adolescents in Canada are not currently meeting the recommended guidelines for acquiring 60 minutes of moderate-to-vigorous PA (MVPA) daily (17). Worldwide, 80% of 13-15 year olds across 105 countries do not meet these guidelines (18). Taken together, this suggests that today's youth may be at increased risk of osteoporosis in later life.

Assessing PA, especially in children and youth has always posed a challenge. Studies that focus on PA and bone health rely mainly upon PA questionnaires as a data collection tool. Although many questionnaires have demonstrated validity (19,20), measures of type and duration of PA children and adolescents partake of, remains subjective.

Direct, objective measures of PA are now commonly used within the realm of child and youth PA research (21). Accelerometry-based activity monitors assess actual movements (most often based on vertical acceleration) (22). Validated thresholds are then used to classify raw accelerometer counts into PA intensity categories which can then be quantified with regards to duration and frequency (23). More recently, accelerometers have also been used to assess time spent being sedentary (24,25). However, very few child or adolescent bone health research studies used accelerometers to assess PA and even fewer objectively measured sedentary behaviour and reported its potential influence on bone health. Standardization and reproducibility of accelerometer-based PA outcomes was a concern in children and adolescents due to differences in energy expenditure if using adult-based thresholds to classify PA intensity (26). Also many studies did not report fully accelerometry protocols used and this hindered comparing results across studies (27). In light of this, more youth-based recent calibration studies (28), recommendations of standardized reporting in accelerometer studies (27), and newer models of

accelerometers (29) advanced the use of accelerometers to objectively measure PA in children and adolescents. Thus, the uses of accelerometry in pediatric bone studies are helpful to understand bone responses to PA in this population.

While a number of intervention studies targeted primarily children and bone mass (by dual energy x-ray absorptiometry (DXA)) as a primary outcome (30–34), we know relatively little about the effects of PA and sedentary time (SED) on adolescent bone structure and strength. For the purposes of my thesis I define bone structure as bone geometry and macroarchitecture such as cortical and trabecular area, cortical thickness, periosteal and endosteal circumference (35) and bone strength as the ability to resist fractures driven by bone's extrinsic (mass, structure) and intrinsic properties (degree of mineralization) (36). A detailed description of bone strength, structure and mass are discussed in section 1.2.3, where I discuss imaging tools used in assessing pediatric bone. A recent systematic review investigated the role of PA on bone strength and found only four publications from two randomized controlled trials (RCTs) in children and one RCT in adolescents (37). Four of the five studies were DXA-based and only one study (from our research group) used peripheral quantitative computed tomography (pQCT) to assess 10-year old children. All five studies implemented school-based interventions and all used subjective measures (questionnaires) to assess PA levels.

Schools may be the most effective setting to roll out PA interventions targeting children and adolescents (38). Health interventions conducted in schools can change adolescent health behaviour regardless of family environment (39,40). Those PA and bone health intervention studies conducted in the school setting that yielded significant results, reported high compliance (≥70%) (41,32,33). However, most PA intervention studies focused on elementary-age children; there are very few studies of adolescents (42). Skeletal changes observed in younger children in response to effective interventions cannot be applied to adolescents for a variety of reasons; 1) the influence of stage of maturity on bone

development (43), 2) the relevance of the PA intervention to adolescents and 3) differences in compliance to the intervention (44). Adolescents are not larger children—growth and development is occurring at an accelerated tempo across a wide range of time frames and youth are more autonomous and selective in the activities they choose to adopt (or not), compared with younger children.

The process of being physically active is closely coupled with the basic action of muscles – to generate force. Based on mechanostat theory, skeletal muscle generates forces that influence bone modeling and bone adapts to mechanical loading to be functionally competent (45). This is supported by longitudinal studies where peak lean mass accrual preceded peak bone mass (46–48) and bone strength accrual (49). In addition to biomechanical influences of muscle on bone development, muscle-bone interactions are mediated through various biochemical pathways (50). Thus, to examine how PA influences bone strength, structure and density, it is essential to consider the specific role that muscle plays in this adaptive process.

The primary aim of my thesis is to extend previous studies of elementary school, and the few secondary school, based PA interventions that focused primarily on bone mass (by DXA) as an outcome. To do so I will evaluate the effects of a unique choice-based PA intervention program on *adolescent bone strength, structure and density*. My secondary aim is to identify modifiable factors related to bone strength, structure and density in adolescents. Two other novel aspects of my thesis are that I used pQCT to assess bone variables and accelerometry to measure PA objectively, in combination with a validated PA questionnaire. My thesis is divided into three parts; Part I is a comprehensive systematic review of peer-reviewed, published RCTs and observational trials of PA and bone strength in children and adolescents. Part II examines the determinants of bone strength, structure and density in adolescents (including PA, muscle and sedentary time). Part III investigates the effect of a choice-based PA intervention (Health Promoting Secondary School (HPSS) study) on bone strength, structure and density in adolescents.

In Chapter 1, I review relevant literature on bone biology and biomechanics, imaging modalities used to assess bone strength and structure, maturity- and sex-related differences in bone, determinants of bone strength, structure and density in children and adolescents with a focus on the effects of PA and SED, measurement of PA and PA-related constructs (specifically muscle strength) on the growing skeleton. I then provide the rationale, specific objectives and hypotheses for the three main parts of this thesis. In Chapter 2, I describe the methods and protocols I used to assess my outcomes and steps taken to conduct the systematic review; the HPSS and Bone Health Study (BHS) design, protocols and primary outcomes, and the HPSS intervention program. In Chapters 3 to 5, I provide the results, discussion and conclusions for three different parts of my study. In the final integrative chapter (Chapter 6) I discuss the global outcomes of the thesis, propose recommendations for future research in pediatric bone, summarize the results and provide a conclusion related to the thesis as a whole.

1.2 Literature Review

In this section, I discuss the relevant literature that forms the basis for this dissertation in six parts: bone anatomy and physiology, bone biomechanics, bone imaging modalities, bone growth and development during adolescence, modulators of bone health and lastly, PA and bone health studies in adolescents.

1.2.1 Bone anatomy and physiology

In this section, I briefly describe basic bone anatomy related to long bones in the human body, as they are the focus of my study. I follow this with a brief overview of bone physiology that contributes to bone growth, development, repair and maintenance.

1.2.1.1 Whole bone composition and structure

Bone is composed of 65% mineral in the form of hydroxyapetite crystals, 25% organic material and 10% water. Ninety percent of the organic material consists of type-1collagen (cartilage) and the remaining 10% consists of non-collagenous proteins such as extracellular and cellular protein (51). The hard crystals and ductility of the flexible collagen matrix confer the stiffness of bone.

Bone features are distinct to their functions: birds have light and hollow bones for flight purposes while human ear bones are highly mineralized to transmit sounds for their acoustic role. There are numerous types of bones in the human body and the adage 'form follows function' adequately describes bone anatomy and its purpose. For example, the cranial bones are flat bones, comprised of a bony sponge-like network that absorbs and dissipates forces to protect the brain (52). Long bones, on the other hand, function to support our body mass and act as levers to enable movement (53). Long bone anatomy is uniquely shaped as being longer rather than wider, with articulate ends called epiphyses that are connected by a long shaft called the diaphysis (Figure 1.1) (54). The epiphyses are shaped to efficiently disperse compressive loads and contain growth plates that form trabeculae[1], also known as cancellous or spongy bone. As bone grows in length, trabecular bone is replaced by cortical or compact bone that forms the diaphysis. When linear growth ceases, the growth plate fuses and forms the epiphyseal line. A closer look at the formation of trabecular and cortical bone reveals highly organized structures that I discuss in more detail below.

[1] In this thesis, I use the term 'trabecular' and 'cortical' to refer to spongy or cancellous and compact bone tissue, respectively.

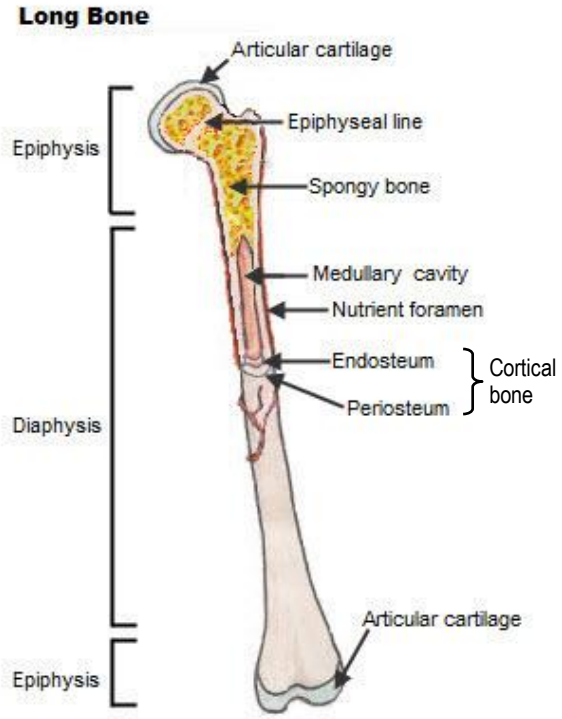


Figure 1.1. Long bone structure[2]. Adapted with permission from the National Institute of Health, through personal communication.

Formation of trabecular and cortical bone in long bones occurs via endochondral ossification where cartilage forms (from chondrocytes) prior to bone tissue deposition. The same chondrocytes form the growth plates in immature bones (55). After birth, primary ossification occurs within three weeks while secondary ossification can take up to a year to create complex Haversian systems (Figure 1.2), complete with osteons and vascular canals (51). The adult skeleton comprises 80% cortical and 20% trabecular bone (56).

[2] The term 'structure' is used interchangeably in this thesis with 'geometry' and 'architecture' to describe dimensions of bone (e.g., shape, size, area, thickness, spatial distribution)

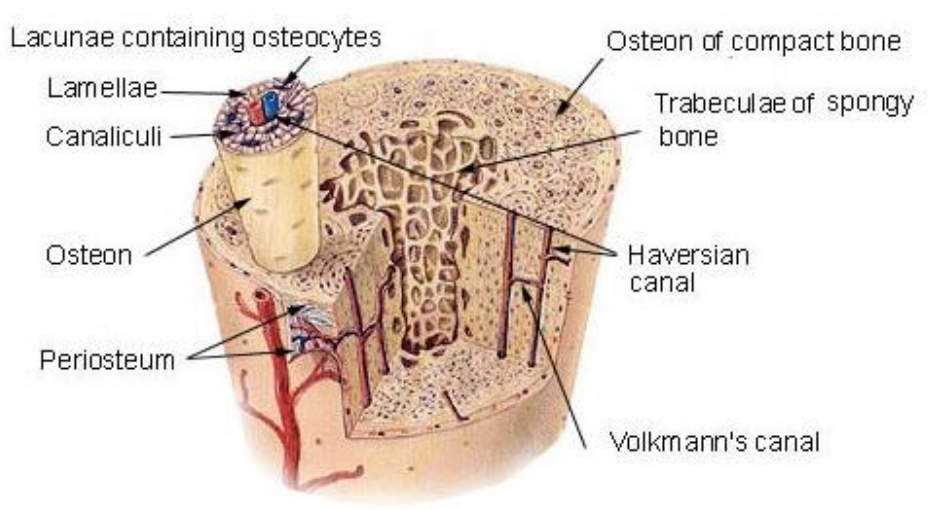


Figure 1.2. Structural elements in cortical (compact) and trabecular (spongy) bone. Osteons shaped from lamellar sheets surround blood vessel canals creating the Haversian system. Adapted with permission from the U.S. National Institute of Health.

Woven bone is the first bone matrix to be laid down before any trabecular or cortical bone is formed due to the rapid formation and quick mineralization of randomly placed type-1 collagen fibres. Therefore, woven bone is also created when injury occurs to bone tissue (55). As bone tissue repairs, a callus forms at the injury site, which provides stability during the healing process. Woven bone is later replaced by structured osteons. Half-osteons or hemi-osteons (with no vascular channels) form trabecular plates and rods with an average thickness of 200 μm (51). Although trabecular bone appears randomly constructed, it actually undergoes targeted modeling where the formation is highly organized and structured along the stress lines (51). The architectural construction of trabeculae support weight bearing and dissipate forces to the tougher cortical bone through the connected struts (Figure 1.3). The lattice-like network of trabecular bone provides a light yet strong and supportive structure.

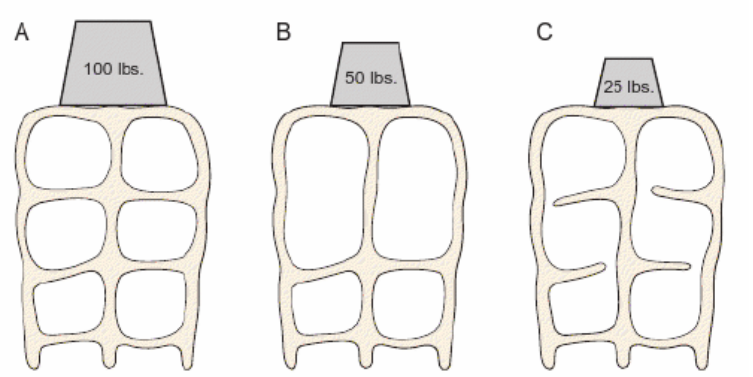


Figure 1.3. The importance of bone architecture, independent of the amount of material (i.e. mass). Trabecular bone in which the cross struts are disconnected (C) cannot support the same load as trabecular bone with connected cross struts (A), despite similar bone mass. A smaller bone mass that is buttressed properly (B) may be able to support more load than a greater mass in which the connectivity is compromised (C). Figures indicate maximum load. Reproduced from Burr and Akkus (51) with permission from Elsevier.

Cortical bone functions like a brick wall to defend the structural integrity of bone. It is made up of osteons (about 100-250 μm in diameter). The osteon is composed of a central canal (Haversian) channeling a blood vessel, nerves and lymphatics surrounded by concentric layers of lamellae. Each lamella is about 3-7 μm thick and is separated by an interlamellar layer (about 1μm thick) (51). Throughout cortical bone, Haversian and Volkmann (transverse links to Haversian) canals (Figure 1.2) provide a vast network of capillaries running through an otherwise dense and stiff tissue, contributing to 3-5% of the porosity in cortical bone. Interstitial bone is the remains of primary or secondary bone that fills the space between cylindrical osteons that are no longer remodelled (51). A cement line separates osteons from interstitial bone and serves as an important mechanical structure. The cement line controls fatigue and fracture processes by absorbing energy to stop fracture propagation and provides viscous dampening in cortical bone (51). Thus, cortical bone structure is tough not only due to the compact arrangement of osteons but also from built-in mechanisms that deter further fracture proliferation (57).

1.2.1.2 Physiology of bone growth, development and maintenance

Three basic bone cells – osteoclasts, osteoblasts and osteocytes – are involved in bone growth, development and maintenance. These cells act on four bone surfaces: periosteal (outer bone surface), endosteal (marrow bone surface), intracortical (inner canal of an osteon) and trabecular (51). Osteoclasts function to remove or resorb bone tissue (structural construction) and are derived from the hematopoietic monocyte macrophage lineage present in circulation and the bone marrow (58). Osteoclasts can resorb up to 200,000 μm^3 of bone per day (53), paving the way for new bone matrix. Without osteoclasts' resorption ability, bone formation would be disturbed. Dysregulation of osteoclasts can lead to excess or insufficient bone mass and compromised bone structure, two contradicting medical conditions known as osteopetrosis and osteoporosis, respectively (58). With osteopetrosis, bone is too stiff (brittle bones) due to high mineralization and is highly susceptible to fracture while osteoporosis, a condition of insufficient bone mass, leads to reduced bone strength, increasing fracture risks as well. The coordination of osteoclast activation depends on both mechanical forces and hormones. At the end of the resorption phase, osteoclasts undergo programmed cell death or apoptosis (58).

Osteoblasts are formed from mesenchymal progenitors, which also produce chondrocytes, myocytes and adipocytes. Osteoblast formation is regulated by several morphological transcription factors (58). Osteoblasts synthesize bone matrix for growth and repair by producing type-1 collagen and then deposit minerals to form new bone (58). Systemic, mechanical and local factors stimulate osteoblast activities. On average, osteoblasts lay down bone matrix or osteoid at a rate of 0.5-1.5 μm/day (53). During bone growth, osteoblasts can act independently on bone surfaces or be coupled with osteoclasts to form a bone multicellular unit to form and resorb bone, respectively. At the end of their lifecycle, osteoblasts are either embedded in bone matrix as osteocytes (5-20%) or transform into lining cells (15-30%) that cover the new bone surface. The remaining 50-80% of osteoblasts experience apoptosis (58).

Osteocytes formed from mature osteoblasts have a different morphology and role than their predecessors. They are the most abundant bone cells in skeleton, and are found in the bone matrix and on bone surfaces (58). Osteocytes form projections from their cell body, known as dendrites, which connect to other nearby osteocytes through the gap junction in canaliculi (microscopic canals in bone tissue) (58). These dendrites are thought to assist osteocytes in responding to mechanical and hormonal signals, and coordinate the function of osteoblasts and osteoclasts (59). When mechanical stimulus is low, osteocytes undergo apoptosis and this triggers osteoclast action to remove bone (58,60). Bone microdamage also triggers osteocytes to direct osteoclasts and osteoblasts to remove damaged bone and replace it with new bone (61). It is postulated that osteocyte viability is maintained by adequate mechanical stimulus (60).

1.2.1.2.1 Bone modeling and remodeling

Modeling and remodeling are the two main physiological mechanisms of bone growth and maintenance. I provide a general description of bone modeling and remodeling in Table 1.1.

Table 1.1. General overview of bone modeling and remodeling. Reproduced from Allen and Burr (62), with permission from Academic Press.

	Modeling	**Remodeling**
Goal	Shape bone, increase bone mass	Renew bone
Cells	Osteoblasts or osteoclasts and precursors	Osteoblasts, osteoclasts and precursors
Bone envelope	Periosteal, endosteal and trabecular	Periosteal, endosteal, trabecular and intracortical
Mechanisms	Activation-formation or activation-resorption	Activation- resorption - formation
Timing	Dominant in childhood but continues throughout life	Throughout life
Net effect on bone mass	Increase	Maintain or slight decrease

Bone modeling is prominent during growth and development in children and adolescents and functions to increase bone length and size and develop bone shape. *Formation modeling* and *resorption*

modeling signalled by local tissue strain consequently alter bone shape and structure on separate bone surfaces (62). During longitudinal bone growth, formation and resorption modeling occur on different bone surfaces. As illustrated in Figure 1.4, resorption modeling occurs on both periosteal and endosteal surfaces around the metaphysis (63). This causes 'bone drift' whereby bone mass is shifted away from the central bone axis to widen (and lengthen) bone (62). Modeling also occurs throughout our lifetime to reinforce cortical (through periosteal apposition) and trabecular (increased number and thickness) structure when load is applied on bone. I address bone loading more fully in section 1.2.2.

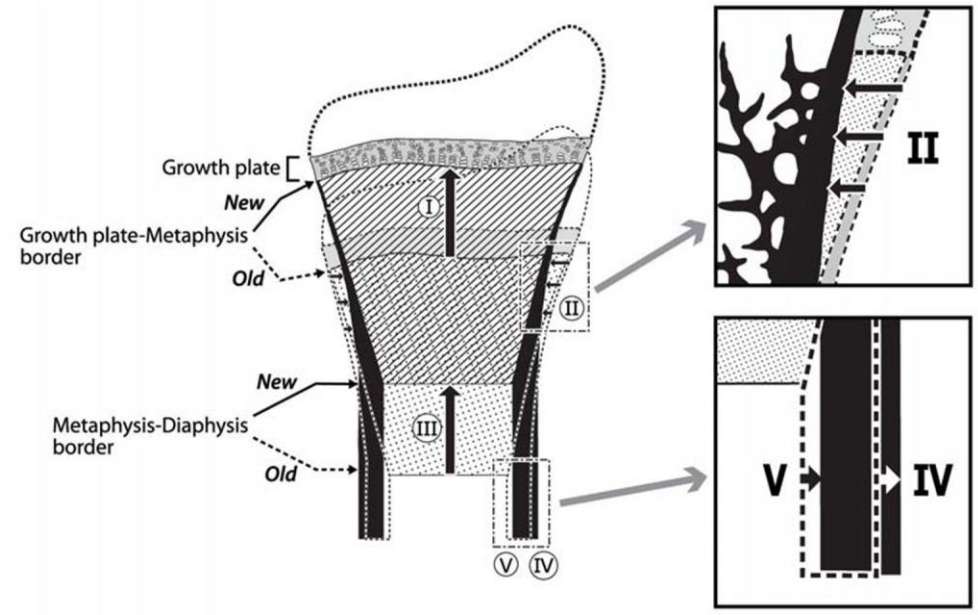


Figure 1.4. During dynamic growth, the growth plate contributes to linear growth (I) while resorption around the metaphyseal periosteum occurs to keep the bone shape (II). Resorption of trabeculae shifts the metaphysis-diaphysis border (III). Bone is added periosteally (IV) and resorbed endosteally (V) to increase bone width. Images reproduced from Rauch et al. (64), open access.

Bone remodeling is mainly carried out in 'pockets' by bone multicellular units in targeted areas that are activated by osteocyte apoptosis and microdamage (Figure 1.5) (62). Various stages of remodeling occur at any one time throughout the body but sequentially, the first stage involves recruitment of preosteoclasts to the damaged site through signals from osteocytes. Differentiation from preosteoclasts

into mature osteoclasts involves a multitude of signaling molecules, including macrophage colony stimulating factor (M-CSF), receptor activator for nuclear factor κB ligand (RANKL), tumor necrosis factor, interferon gamma, and inter-leukins (58). Next, mature osteoclasts create Howship lacunae, or resorption pits, to dissolve bone mineral and release collagen fragments. This process takes about four to six weeks. Then, osteoblasts are recruited to lay down new bone matrix at the resorption site during the reversal phase (62). Specific signaling pathways to recruit osteoblasts are still unconfirmed but we know several important signalling molecules regulate osteoblastic differentiation. These include bone morphogenetic proteins (BMPs), transforming growth factor (TGF)-β, Wnt glycolipoprotein, Indian Hedgehog protein, parathyroid hormone, insulin-like growth factor-1, fibroblast growth factors, and Notch (a transmembrane protein) (65). Bone matrix development and mineralization of bone by osteoblasts can take four to six months (66). After new bone is formed, some osteoblasts transform into lining cells or osteocytes while the rest undergo apoptosis (62).

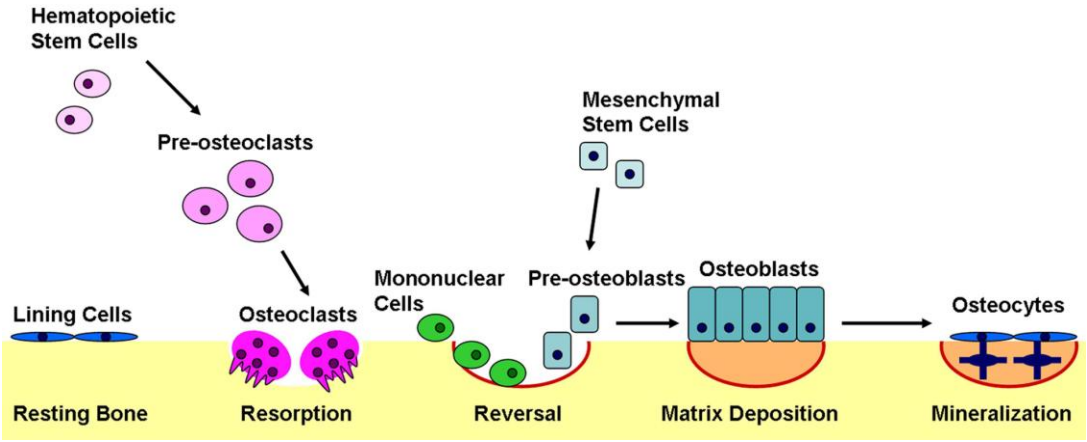


Figure 1.5. From the resting bone stage, bone remodeling is triggered by signals from osteocytes, which recruit preosteoclasts to start osteoclastogenesis where mature osteoclasts are formed to resorb bone. Next, preosteoblasts are recruited in the reversal stage to form mature osteoblasts. Osteoblasts then deposit bone matrix from the cement line (red line) to fill the lacuna. Finally, bone is mineralized by osteoblasts and subsequently osteoblasts undergo apoptosis, are entombed into osteocytes or transforms into lining cells. Reproduced from Kapinas and Delany 2011 (65), open access.

1.2.2 Bone biomechanics

In this section, I discuss the mechanical properties of bone, the theoretical basis of bone adaptation to mechanical stimuli and the animal and human studies that provided in-depth understanding of bone adaptation to mechanical loading.

1.2.2.1 Biomechanical properties of bone

Overall, human bones need to be stiff and strong yet light enough to allow movement of the body (67). Adequate bone strength is required to conduct daily activities without injury and to withstand larger forces sustained from low impact falls (i.e. falls from standing height or less). The ability of bone to absorb energy and avoid fracture depends on bones' material properties, mass and structure (67).

Mechanical testing of human cadaver bones defines several basic mechanical properties such as stiffness, ductility and toughness. Depending on the direction of applied loads, bones experience compressive, tensile, shear, bending and torsional forces (68,69). Results of mechanical tests on whole bone (including cortical and trabecular bone) are used to generate a load-deformation curve that defines the *extrinsic* properties of bone – that is the mechanical properties of whole bone structure (70). The two main regions below the curve are the *elastic* and *plastic* regions. Within the *elastic* region, an applied load to bone results in deformation; when the load is removed, the bone reverts back to its original form. The slope of the curve in the *elastic* region represents the extrinsic stiffness or rigidity of bone (68). When loads are applied past the *yield point*, within the *plastic* region, permanent deformation occurs. Consequently, bone will fracture under larger loads at the *failure point*. When controlled for bone size, the load-deformation curve converts to the stress-strain curve that describes bone's intrinsic properties.

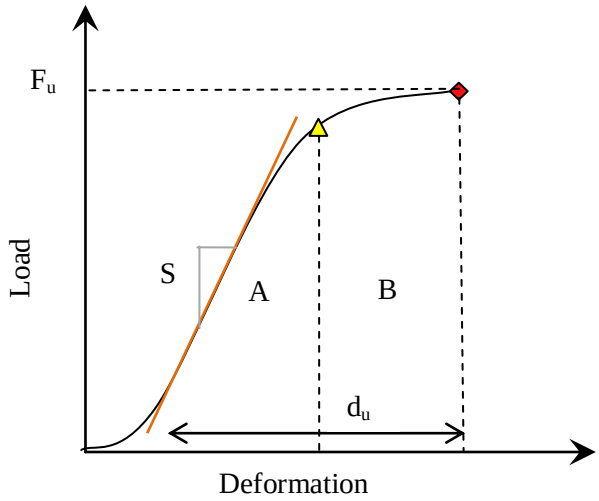


Figure 1.6. Load-deformation curve for whole bone properties. The slope (orange line) of the curve represents the extrinsic stiffness (S); the height of the curve denotes ultimate force (F_u); the yellow triangle is the yield point; the red diamond is the failure point; the area under the curve represents A, elastic region and B, plastic region with the combination of A+B equal to the work to failure; and the total deformation to fracture is ultimate deformation (d_u). Adapted from Turner (70) with permission from New York Academy of Sciences.

Intrinsic bone properties are represented by the stress-strain curve (Figure 1.7), which defines bone material properties at the tissue level. To understand these properties, I briefly present definitions for basic biomechanical terms. Stress at the tissue level, is defined as force per unit area (units: Pascal, (Pa) where 1 Pa = 1 N/m^2) (68). Strain at the tissue level, is a percentage change in length from the original dimensions. As strain is a relative deformation, there are no units. If a bone is stretched to 101% of its original length, the strain would be 0.01 or 1% of 10,000 microstrain (με) (68). With a change in bone length, bone width also changes and this length-width strain ratio is known as Poisson's ratio. Human cortical bone has a Poisson ratio of 0.28 to 0.45 which means a change of 0.28% to 0.45% in width is observed in the perpendicular (axial) direction of the 1% strain applied (71). Thus, bone tissue does not break instantaneously when loaded but deforms slightly due to bone's intrinsic material properties.

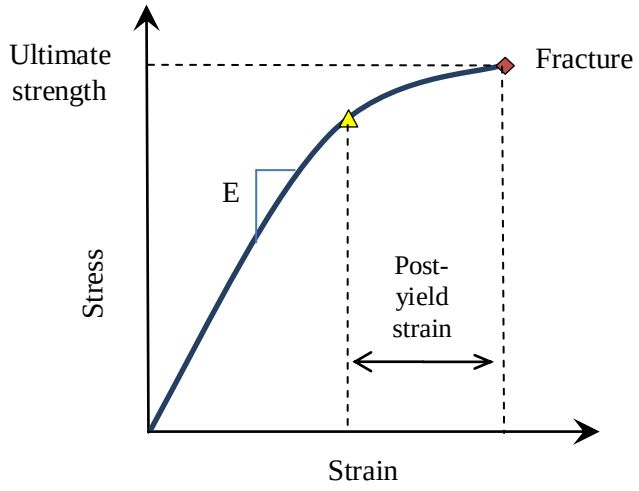


Figure 1.7. Stress-strain curve for bone tissue. The linear part of the curve represents the intrinsic stiffness (Young's modulus (E)). The height of the curve denotes ultimate strength. The yellow triangle is the yield point; the red diamond is the failure point. Strains above the yield point cause permanent damage to bone structure. Post-yield strain before fracture is equivalent to ductility (converse to brittleness). Adapted from Turner (70) with permission from New York Academy of Sciences.

The stress-strain curve describes bone's material properties with the linear slope representing bone's intrinsic stiffness, or Young's modulus (68). Young's modulus is similar across individuals, as the biomaterial used to construct bone is mainly hydroxyapatite and collagen. However, Young's modulus may differ depending on the direction of stress. Thus, bone tissue is known to be anisotropic (different properties in different directions) and anisotropy is attributed to the orientation of bone tissue. The direction osteons are laid down and formed directs the anisotropic properties and can be thought of as the 'grain' of a biomaterial such as the grain in wood. For example, the tensile strength of cortical femoral bone in the longitudinal direction is greater than in the transverse direction, 135 MPa and 53 MPa, respectively, while compressive strength (longitudinal) is even higher at 205 MPa (72). In elderly donors (cadaver femoral bones), cortical bone tensile strength was about 27% stronger compared with trabecular bone. Trabecular tensile and compressive strength was, on average, 85 MPa and 135 MPa, respectively (73). This aptly shows the impact that load direction and bone type (cortical or trabecular) have on the anisotropic characteristics of bone.

Bone strength is dependent on material (mineralization) and structural (size and shape) properties represented by the load-deformation and stress-strain curves. As strain increases past the yield point, permanent deformation occurs. The amount of energy needed to cause fracture is related to bone's ductility (inverse to brittleness). During linear growth in childhood and adolescence, bone is less mineralized and is therefore considered more ductile. Bone does not increase in size and density arbitrarily, as more energy would be required to move a bigger and heavier skeleton. Instead, bone formation is regulated by mechanical and non-mechanical influences as proposed in the mechanostat theory, which I discuss in detail below.

1.2.2.2 Mechanostat theory and mechanotransduction

More than a century ago, Julius Wolff proposed that mechanical stresses regulate bone architecture (74). More recently, Harold Frost (75) proposed the mechanostat theory which suggests that bone adaptation is regulated by several factors: mechanical (the dominant effector based on the Utah paradigm (45)) and non-mechanical, including a negative feedback mechanism that regulates bone physiology (76). According to the mechanostat theory, different strain thresholds, termed minimal effective strains (MES), dictate action of bone modeling (MES_m), remodeling (MES_r) or microdamage repair (MES_p) depending on the quantity of mechanical force received (Figure 1.8) (77). Mechanical forces exerted on bone are mainly through muscle contraction from PA and from ground reaction forces (63). Mechanical forces are transformed to biological pathways through the mechanotransduction process to regulate bone adaptation.

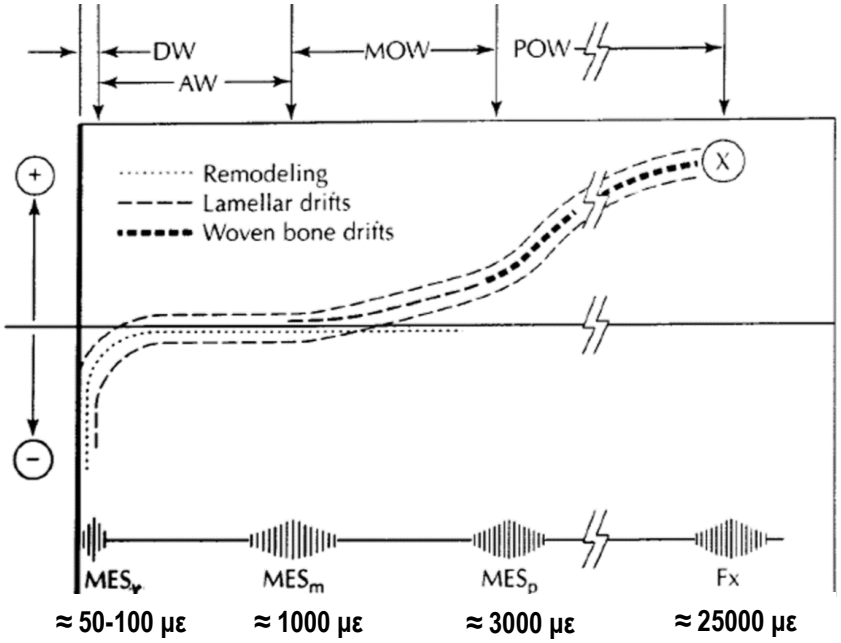


Figure 1.8. Illustration of the mechanostat theory in bone modeling and remodeling. Bone remodeling occurs in the upper limit of disuse window (DW); MES_m – minimal effective strain for bone modeling occurs between the adaptive window (AW) and the mild overload window (MOW); MES_p – minimal effective strain for microdamage repair is at the lower limit of the pathologic overload window (POW); Fx: bone fracture strain and με, microstrain. Adapted from Frost (77) with permission from A.R. Liss (etc).

There are four distinct phases in mechanotransduction, the process by which mechanical stimuli leads to bone formation or resorption. The first is *mechanocoupling* where the mechanical load perturbs the osteocytes and their dendrites (78). These perturbations result in fluid flow that produces a shear stress detected by mechanoreceptors located at cell membranes. Currently, there are three known groups of mechanoreceptors – mechanosensitive ion channels, cell adhesion/cytoskeletal molecules (e.g., connexin 43, β-integrin) and G-protein related molecules – that 'open' the channels to allow proteins, lipids and calcium to enter cells or be released into extracellular matrix (79). Movement of biochemical substances across membranes creates secondary biochemical signals that initiate the second phase known as *biochemical coupling*. Biochemical coupling occurs through various pathways, including the cyclooxygenase (COX)-prostaglandin E2 (PGE2), nitric oxide (NO) and Wnt-lipoprotein receptor-related

protein (LRP 5/6) pathways (80). The third phase of mechanotransduction involves *signal transmission* to bone cells from previous biochemical pathways. For example, the Wnt-LRP5/6 pathway in mature osteocytes promotes survival of β-catenin, which activates osteogenesis (58), while down regulating sclerostin (a negative bone formation regulator, expressed from the SOST gene) (81,82), which suppresses bone resorption. During the final phase, *effector cell response*, bone cells (osteoblasts, osteoclasts) directed by the biochemical signals form or resorb bone (83).

Although mechanical stimuli are the main drivers of bone adaptation, non-mechanical factors related to growth including genetics, nutrition and hormones also influence bone adaptation. I discuss these non-mechanical factors and their role in bone adaptation in section 1.2.6.

1.2.2.3 Principles governing bone adaptation to mechanical stimuli

Three basic principles govern bone adaptation to mechanical stimuli (84) illustrated by classic animal studies and supported by studies in humans. I review the key animal and human studies, below.

The first principle: bone adapts to dynamic loads and not static loads. In their classic experiment, Lanyon and Rubin experimented with functional loads and controlled external loading on turkey ulnas. They reported that static loads decreased bone formation and increased bone porosity while dynamic loads increased bone formation (85). Dynamic loads also effectively increased bone mineral content (BMC) by 134% through periosteal bone apposition; 36 cycles/day were as effective as 1800 cycles/day (86). When loaded, bone cells deform and exude fluids into interstitial space (87), causing fluid shear stress to initiate mechanocoupling (first step in mechanotransduction) (78). Intuitively, it makes sense that dynamic strain applied and removed in an on-off pattern, would elicit more fluid movement across cell membranes than a one-time static strain on bone cells. Hsieh and Turner showed that a strain frequency of 1 Hz, 5 Hz and 10 Hz on adult female rats increased ulnar bone formation rate in a dose-dependant manner (88).

Furthermore, bone is stimulated by strain magnitudes above strain thresholds. Rubin and colleagues used turkey ulnas to examine the dose-response of loading on bone cross-sectional area (CSA). They found a positive linear correlation between load magnitude and bone area whereby loads above 1000 με stimulated bone formation compared with the contralateral non-loaded ulnas (89). Turner and colleagues aimed to locate a threshold for bone formation in rats and found that a minimal load of 40N or a strain of 1050 με was required to induce bone formation and mineral apposition (90). The minimum strain threshold of 1000 με appears to be constant across several species but bone formation thresholds in humans are unknown.

The second principle: short periods of loading are more osteogenic compared with long periods of loading. Lengthy loading bouts are counterproductive for bone formation as bone cells lose their sensitivity to the mechanical stimulus (dependent on strain rate and magnitude) (91). Robling and colleagues showed this with their rat tibia loading model whereby 4 bouts/day of 90 bending cycles/bout resulted in greater gains in bone strength, structure and density compared with a single loading bout of 360 cycles (92–94). These findings support the theory that rest periods, anywhere from 14 seconds to 8 hours (92), are required to restore bone's sensitivity to mechanical loads. This is related to the fluid shifts in bone cells whereby continuous dynamic loading does not provide a refractory period sufficient enough to restore mechanical sensitivity (95).

The third principle: unique distribution of strains on bone elicits bone formation. Bone cells respond to unusual mechanical loading environments and are less responsive to routine or customary loading signals. Thus, in order to stimulate bone formation, mechanical loading needs to be 'abnormal' and 'error-driven'. Rubin and Lanyon demonstrated how rooster ulnas gain bone mass due to atypical direction of loading (loaded at 90 degrees – transverse direction from the natural wing-flapping strain distribution) (86). Since the peak strain magnitude (10,000-12,000 με), customary strain rate (30,000/sec

and 36,000/sec) and number of loading cycles (4-1800 cycles/day) were below hyperphysiological levels, the investigators attributed the bone changes observed through post-mortem histology and microradiography to the altered strain distribution (86). In a recent micro-CT study, Wallace and colleagues reported that young female mice exposed to diverse directional loading demonstrated greater trabecular volume fraction (1/Tb.BV) (12%), cortical area (Ct.Ar) (11%), cortical area fraction (1/Ct.Ar) (15%) and cortical thickness (Ct.Th) (12%) at the proximal humeri (100 μm distally) compared with mice exposed only to linear directional loads (96).

Based on these principles of bone adaptation, Turner and Robling proposed the osteogenic index (OI), which is a formula to quantify the osteogenic properties of an exercise protocol (97). The equation is derived from well-designed animal studies and takes into account intensity (ground reaction force) (98), number of loads/jumps and duration between exercise sessions (recovery sessions) (92) to generate a score for the particular exercise program (97). The OI equation is presented below,

$$\text{OI } (n\text{-session/day}) = \sum_{k=1}^{n} GRF \times \ln(N+1) \times \left(1 - e^{\frac{t}{\tau}}\right)^{-1}$$

where *GRF* is the peak ground reaction force or intensity, N is the number of loads per session, t is the time (hours) between sessions and τ a time constant of 6 hours. Although the OI provides a useful tool to quantify the osteogenic potential of an exercise regimen, it has, to my knowledge, not been validated in studies of children or adolescents. Studies using the OI in adults (99–102) are inconsistent. This is due to differences between studies in how the formula was used to calculate OI values (e.g., OI for a week vs. lifetime score) and the variability in bone outcomes (e.g., bone resorption vs. formation biomarkers).

A study by Lester and colleagues illustrates one application of the OI. They tested three different exercise programs in adult women (20.3±1.8 years) over an 8-week period: 1) resistance exercise (OI =

16.0), 2) running (OI = 20.6) and 3) combined running and resistance training (OI = 36.9). Trabecular volumetric bone mineral density (vBMD; by pQCT) increased 1.3% in the running and combined group (101). Erikson and colleagues conducted a proof-of-concept study (n=7 per group) and found that an 8-week jumping program in men (single or double jump sessions/day, equal number of jumps in both groups, resistance set at 80% of body mass) increased bone formation marker levels over time with significant changes in the twice/day group but bone marker levels were no different compared with controls (102). The investigators highlighted that although the OI might be useful, studies on the optimal OI score for bone modeling have not been conducted (102).

1.2.2.4 Differences in bone adaptation

Dynamic, high-magnitude loads that are of short duration and associated with abnormal strains are known to induce bone formation in animal models. However, the specific bone response varies with age and skeletal site. Further, bone adaptation to mechanical loads differs between cortical and trabecular bone. These factors come into play when results from studies are evaluated. I discuss several animal and human studies that contributed to our understanding of bone adaptation.

1.2.2.4.1 Age-specific bone adaptations

Results of several animal studies indicate that the growing skeleton has a greater capacity to adapt to mechanical loads compared with the mature skeleton. I highlight two classic animal and two recent human studies below. First, Turner and colleagues tested strain loads that ranged from 30 N to 64 N in young and old rats (103). Both young and old rats had periosteal bone apposition above 40 N but only increased 59% in the old rats compared with 100% gains in young rats. The investigators also reported that the minimal mechanical threshold for endosteal bone formation was 1050 με for young rats and 1700 με for old rats to achieve the same bone adaptation (103). Second, Jarvinen and colleagues exposed young

and adult rats to a 14-week running program. Femoral neck bone strength increased similarly in both groups of animals (104). In young rats, this was a result of increased CSA (25%) compared to vBMD (11%). In adult rats the reverse occurred; a 10% increase in CSA (not significantly different from controls) and a 23% increase in vBMD (104). This suggests that with aging, bone responds differently to mechanical loads (sensitivity, structural vs. density).

In human studies, increased bone strength in response to mechanical loading appears to be a result of changes in bone geometry rather than bone. A series of landmark contralateral comparison studies in racquet sport players (aged between early 20s to mid 50s) reported that bone size (by pQCT) was larger (9% to 32% more) on the playing arm compared with the non-playing arm due to sport-induced adaptations while side-to-side comparisons of vBMD were almost identical (-1% to 2%) (105,106). Similarly, young tennis players (girls, age 10 to 15 years) had stronger bones (by magnetic resonance imaging (MRI), polar second moment of area +11-23%) in their playing arm (humerus) versus their contralateral arm due to a greater Ct.Ar (also periosteal and endosteal circumferences). In contrast, there was no side-to-side difference in total humerus BMC (by DXA) (107). In older master athletes (athletes that train and compete above the age of 35 years; age 54.9±12.4 years in this study), there were no side-to-side differences in bone outcomes (BMC, vBMD, total bone area (Tt.Ar) by pQCT) between the dominant and non-dominant leg (108). However, there were sport-specific differences in Tt.Ar and BMC (but not vBMD) at all three bone sites, 4%, 14% and 66% of the tibia, between sprinters, hurdlers and triple jumpers. This indicates that bone adapts mainly by geometrical changes even in middle-aged athletes that are exposed to stringent exercise training and high impact forces.

To my knowledge, RCTs that looked at bone adaptations to PA across ages (youth to older adulthood) are missing in the literature. There are trials that either focused on children and adolescents (30,32,33) or adults (109–111) and each intervention program differed in frequency, intensity, type and

duration of PA. Overall, most PA intervention trials resulted in positive bone adaptations regardless of age. From a large cohort study, Tobias and colleagues (112) indicated that the level of impact forces required to positively affect bone adaptation may also be different between youth and adults. Thus, it appears that PA can benefit bone health across all ages but further investigation is required to establish the PA characteristics that optimize bone adaptations across different age groups.

1.2.2.4.2 Site and compartment-specific bone adaptations

Bone reacts to strains experienced and adapts where it is most needed. For example, rodent bone tends to bend in the medio-lateral direction when loaded mechanically. Thus, resultant bone formation was observed at medial and lateral sections more so than at anterior-posterior sections relative to the neutral axis (Figure 1.9) (79). Similar site-specific adaptations were observed in an RCT that investigated the adaptation of cortical bone surfaces in boys and girls after a 20-month intervention (113). Prepubertal boys who participated in jumping exercises (3 min/day of countermovement jumps) and classroom PA (additional 15 min 5 days/week on top of regular PE), displayed bone adaptations at the anterior, posterior and medial sections, but not the lateral side, of the tibial midshaft (Figure 1.10) (113).

In addition to local adaptations (anterior-posterior, medial-lateral), peripheral and axial sites also respond differently depending on the type of loading, timing of assessment and the bone compartment (cortical, trabecular) assessed. As it is, timing of bone mineral accrual was different depending on the bone site assessed. From a longitudinal study following 8 year olds until age 30 years (114), the femoral neck was the first to achieve peak bone mass accrual, 2 years after age of peak height velocity (APHV). This was followed by peak bone mass accrual at the lumbar spine (4 years after APHV) and total body peak BMC accrual at six years post APHV (114). An understanding of site-specific characteristics that change across time also informs on the PA-induced bone adaptations. In adults, aged 20 to 99 years, Macdonald and colleagues used high resolution pQCT (HR-pQCT) to assess differences in trabecular and

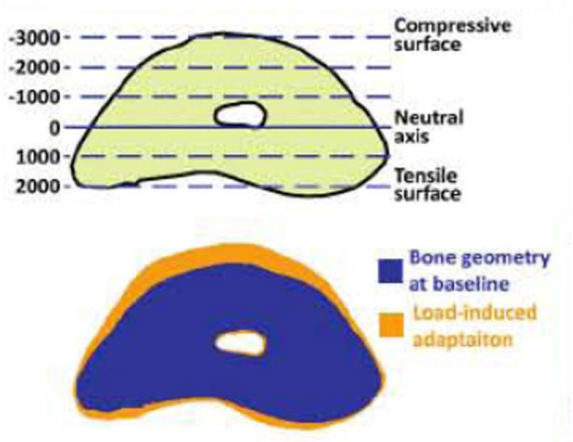


Figure 1.9. Bone adaptations to loading in a cross section sample of the ulnar midshaft of a rodent. The schematic shows the strain distributions (upper panel) and bone formed (lower panel) after ulnar loading. Mechanical loading of the right ulnar, 3 days/week for 16 weeks, resulted in apposition of new bone predominantly on the periosteal surface. New bone formed as a result of the experiment can be visualized (orange area) at the outer medio-lateral bone surface. Adapted from Robling et al. (79) with permission from Academic Press.

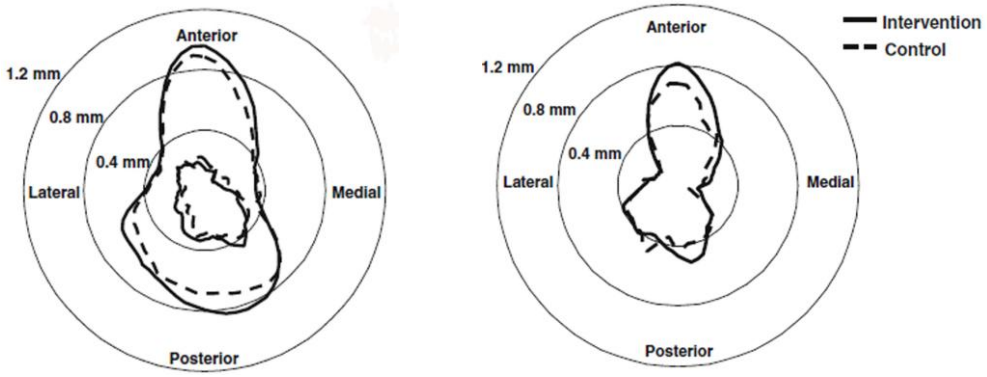


Figure 1.10. Schematic of the tibial midshaft that shows radial plots of periosteal and endosteal surface changes (left) and cortical thickness changes (right), measured by peripheral quantitative computed tomography. The space between the dotted and solid lines is the difference between pre-pubertal boys randomly assigned to intervention (20 months of 15 min/week extra classroom physical activity and bone loading program) or control groups. Adapted from Macdonald et al. (113), with permission from Springer-Verlag London.

cortical bone at the distal radius and tibia as age increases (115). They found that trabecular bone reduced in volume with advancing age depending on skeletal site. Distal tibia reductions in trabecular bone volume

were observed starting at young adulthood while loss of bone volume at the distal radius began in middle adulthood (115). Looking at bone compartments, the distal tibia experiences almost equal reduction in trabecular number and thickness in women and men (115). But for the distal radius, women have reduced trabecular number and men have thinner trabeculae as they age (115). This trend was also reported by Khosla and colleagues who also used HR-pQCT to assess similar bone sites (116). For the cortical bone compartment, again, the distal tibia seemed to deteriorate earlier (pre and peri-menopausal) compared with the distal radius (after menopause) in women (115,117). The changes that occur at different sites (local, peripheral, axial) and within different bone compartments (trabecular and cortical) need to be considered when trying to assess whether bone adaptation are truly from PA or natural progression due to age and sex differences.

1.2.3 Bone imaging

As bone strength is the 'bottom line' of fracture prevention (118), there is a need to understand how bone strength is estimated using different imaging modalities in order to better interpret bone outcomes. In this section, I present a general overview of DXA and pQCT, along with the strengths and limitations of each. I also briefly describe HR-pQCT, MRI and quantitative ultrasound (QUS) as the other imaging tools used in pediatric bone research.

1.2.3.1 Dual-energy X-ray absorptiometry (DXA)

With the development of DXA in 1987 (119), safety and image resolution of bone imaging improved. This provided a catalyst for pediatric bone health research. Briefly, DXA emits two high- and low-energy photons through the body and a detector picks up photons not attenuated by bone and soft tissue (119). DXA scan data is processed pixel-by-pixel depending upon the degree of beam attenuation,

to map out soft tissue regions adjacent to bone (119). The total projected bone area (cm^2) is a sum of the pixels within the bone edges. The reported value of areal bone mineral density (aBMD, g/cm^2) is defined as the integral mass of bone mineral per unit of this projected area (119). Bone mineral content (BMC, g) is obtained by multiplying aBMD by the area scanned (119).

For DXA, the effective dose – defined as the uniform whole body dose of radiation that would be equivalent to risk of carcinogenic and genetic effects - depends on scan time and size of the individual being scanned (119). Longer scan time means a longer exposure to the radiation energies. In a 15 year-old, the effective dose equivalent for a whole body scan using DXA (Discovery-A, Hologic Inc; scan time of 60 seconds) is 4.2 μSv (120). This dose is less than 0.2% of annual natural background radiation (121). Thus, DXA is a safe as well as a valid, reliable and precise tool to assess clinically relevant sites such as the hip, spine and forearm (122). It is also easy to use and low cost, making it highly desirable for both clinical and research settings (123). Finally, DXA is the current clinical gold standard to diagnose osteoporosis and monitor treatment in adults.

Despite the clinical advantages of DXA, there are limitations when assessing the growing skeleton and the first of which is DXA's planar technology. In general, this remains the primary weakness, as DXA is unable to assess volume. For example, Figure 1.11 depicts the overestimation of aBMD from DXA projections of large versus small specimens (124). There have been efforts made to develop algorithms (122,125–127) and to correct aBMD for size (126,128) in order to estimate bone strength from DXA images of the hip and spine. One approach was size-adjustments of vertebral segments measured by DXA to assess bone mineral apparent density (BMAD, g/cm^3) (126,128). Carter and colleagues used a cuboidal formula (126) while Kroger and colleagues used an elliptical or cylindrical formula (128) to obtain vertebral BMAD. However, there was no consensus as to the most acceptable way to account/correct for size. Other than accounting for size, the International Society for Clinical Densitometry (ISCD) cautioned

that aBMD results need be considered in conjunction with factors such as height, sex, age, pubertal stage, ethnicity and fracture history to avoid misinterpretation in children and adolescents (129).

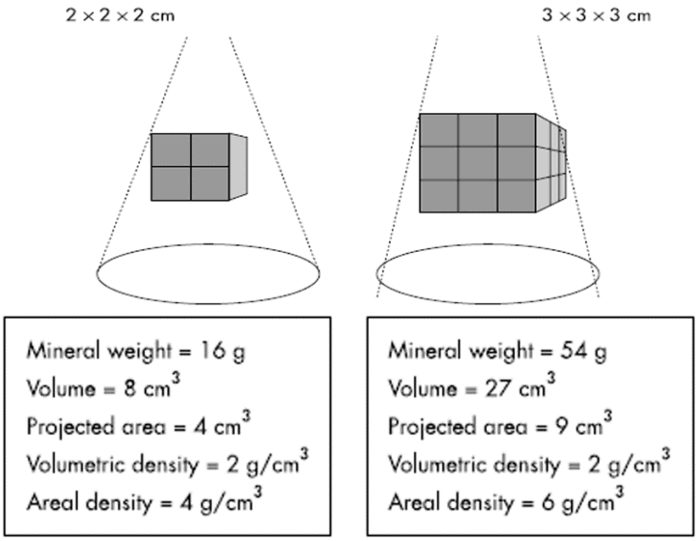


Figure 1.11. Effects of size on bone parameters assessed by dual-energy X-ray absorptiometry (DXA). Both samples have identical volumetric density but the areal density of the 3x3x3 specimen is greater because of the projected area captured by DXA. Adapted from Carter et al. (126), with permission from Mary Ann Liebert, Inc.

Hip structural analysis (HSA) (125) was developed to assess bone structure and estimate bone strength at the proximal femur. However, this approach is not without its own limitations. The ISCD 2014 position statement highlighted that it may not be possible to accurately identify bone markings required by hip assessment protocols (e.g., HSA) in the growing femur (129).

Other than the planar nature of DXA, several other DXA limitations needs to be noted. First, a limitation of DXA is its spatial resolution (about 0.5 mm) (130). Thus, DXA cannot discriminate between trabecular and cortical bone compartments (131). Second, DXA outcomes can also be affected by scanning position, movement and body composition (132). Inaccuracies occur as soft tissue absorbs and attenuates low energy beams from DXA. Thus, soft tissue thickness will affect assessment of bone mineral

(133). Also, the relative distance of bone from the detector due to increased soft tissue thickness leads to systematically higher BMC, aBMD and apparent bone area values (133). In spite of this, analysis and monitoring of body fat and bone mineral free lean mass by DXA are considered valid and reliable in children and adolescents (134,135). Lastly, outcomes across different DXA manufacturers (Lunar, Hologic and Norland) are not comparable due to the different systems used to generate (K-absorptiometry vs. high-low voltage switch), calibrate (different bone and fat mass sources) and detect (remove vs. correct beam hardening) X-rays in the imaging systems (133,136).

1.2.3.2 Peripheral quantitative computed tomography (pQCT)

In light of DXA's limitations, the use of 3-D imaging tools is becoming more widespread in pediatric bone research. To date, pQCT has been used most frequently in research, as it provides a means to obtain valid and reliable measures of bone structure and estimates of bone strength in the growing skeleton with low radiation exposure. In addition, unlike DXA, pQCT can separate trabecular and cortical bone compartments at peripheral sites (distal and shaft sites of the radius and tibia) (137). In this section, I provide an overview of pQCT technology, image acquisition and analysis and common estimates of bone strength obtained using pQCT.

Briefly, pQCT consists of an X-ray tube that produces a narrow beam with a focal point of 250 x 250 μm. pQCT operates at 60 kV (0.3A) and uses an aluminium and copper filter which minimizes radiation exposure and assists to obtain a good contrast image (138). The diameter of the central gantry opening is 300 mm (XCT 3000 model) (Figure 1.12). The gantry rotates in 12^{o} steps for a total of 15 translations and obtains a single image with a slice thickness of 2.5 mm (139). Attenuated images from pQCT are calibrated based on the density of water (60 mg of hydroxyapatite density) to obtain vBMD values (140). The effective radiation dose for a scout scan (to place the reference line) and a pQCT scan is

less than 0.1 µSv (139). As a system, pQCT is compact (1280 x 740 x 910 mm) and is easily portable (weight=90kg) (141).

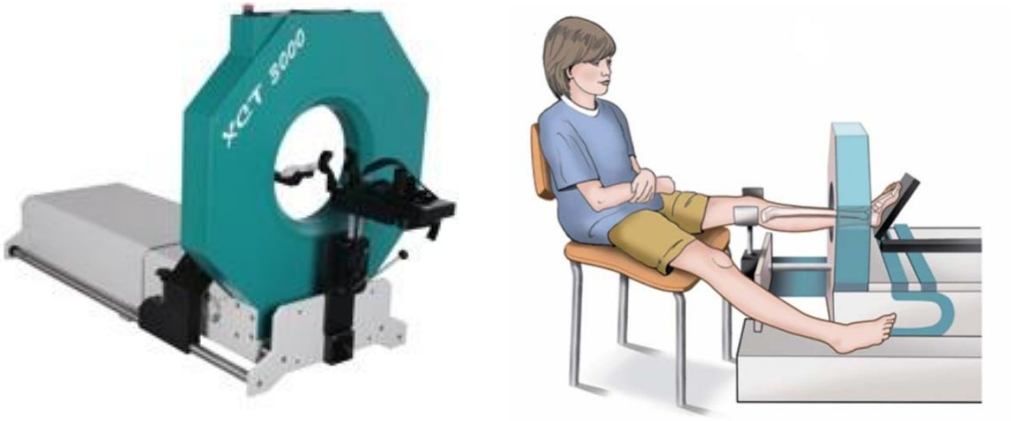


Figure 1.12. Image of peripheral quantitative computed tomography (pQCT), model XCT 3000 (Stratec Medizintechnik GmbH) and illustration of leg positioning in the pQCT. Illustration by Vicky Earle, Medical Illustrator, UBC, The Media Group.

1.2.3.2.1 Image acquisition

In contrast to DXA, pQCT image acquisition is not standardized and thus, there exists considerable variability in the current literature regarding acquisition protocols used in pediatric studies. The user can choose from various image acquisition parameters related to resolution, scan speed, reference line and scan sites. As per ISCD guidelines (137), it is vital that pQCT protocols in children and adolescents focus on the appropriate methods and calibrations to optimize results based on the research question being asked or the medical condition being evaluated so as to minimize radiation exposure.

Radiation exposure is related to the voxel (volume pixel) size used to capture the pQCT image and the scan speed. In general, a pQCT scan (per slice) takes about 3 minutes and has an effective dose of 0.43 µSv (142). Voxel sizes range from high (0.2 mm) to low (0.6 mm) resolution. High-resolution scans incur more time, thus increasing radiation exposure. Although a shorter scan time minimizes movement artifacts and effective radiation dose, scan quality is compromised using a lower resolution and a faster scan speed

(143). Several pediatric pQCT studies employed a minimum 0.4 mm voxel size and a scan speed at 30 mm/s to acquire quality pQCT images (30,32,144–146).

During scan acquisition, the choice of voxel size also influences cortical bone parameters (density, thickness) due to the partial-volume effect (PVE). The PVE refers to the presence of different densities of tissues (soft tissue and bone) in a single voxel (Figure 1.13) that results in an underestimation of true BMD. Rittweger and colleagues proposed correction equations (different detection thresholds) for the PVE based on cylindrical specimens of aluminum phantoms that corrected up to 80% of cortical density measured (147). Selecting the suitable threshold and analysis parameters, PVE can be minimized. I discuss this further in section 0.

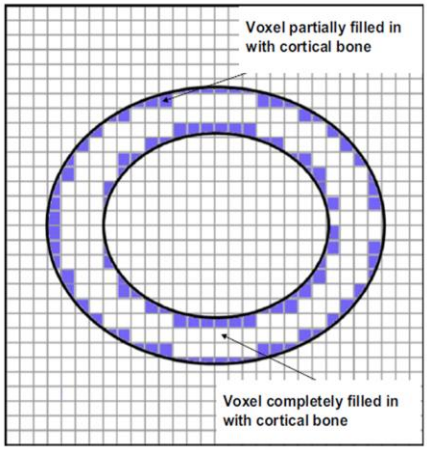


Figure 1.13. Illustration of the partial volume effect (PVE) whereby the voxels at the bone edges (blue voxels) contain both bone and soft tissue densities, resulting in a lowered density for the blue voxels. Partially filled voxels will not be accounted for during the analysis. Adapted from Zemel et al. (148), with permission from Elsevier.

Locating scan sites, especially for longitudinal studies, must be consistent in order to attribute any changes accurately to medical conditions, interventions or growth. There is currently no consensus on the

most suitable site to assess cortical and trabecular bone in youth that are still growing (137). Manufacturer's guidelines (149) suggest the reference line bisect (midline) of the most distal end of the growth plate (Figure 1.14, i) and trabecular bone be evaluated at the 4% site (of measured limb length) and cortical bone be evaluated at the 38% or 66% sites (149). However, studies have evaluated 3%, 4%, 7% and 8% for distal sites and 14%, 20%, 38%, 50% and 66% for shaft sites, in some cases using different reference lines (137). If the growth plate is fused, the manufacturer recommends the reference line bisects the mid-medial edge of the articular surface of the bone (Figure 1.14, ii) (149).

To address challenges associated with placing a reference line on a 'moving' growth plate and with accurately reproducing estimates of the mid-epiphyseal line; our research group adapted the protocol for our pediatric bone studies. That is, we position the reference line on the surface of a bony landmark that is reproducible and identifiable as children transition through maturity and into adulthood (i.e. the tibial plafond, Figure 1.14, iii) (150). To assess trabecular bone, we scan at a site 7% and 8% of the distance from this landmark, proximally, along the radius and tibia, respectively. Our measurement sites were also selected to ensure that the region of interest does not include the growth plate in most children (151). To assess cortical bone at the radius, we scan the 30% site from the medial border of the lunate fossa, also known as the medial edge of the distal radius. Ashe and colleagues demonstrated that in adults, cortical thickness at this site explained 80% of the variance in bone strength obtained from compressive failure load testing (152). At the tibial shaft, we assess cortical bone at the 50% site. Kontulainen and colleagues showed that in human cadaver bone, estimated bone strength (stress-strain index; SSI_y) at the 50% site explained 80% of the variance in failure load (four-point bending model) (153). In comparison, the manufacturer recommended site (66%) explained 76% of the variance in failure load (four-point bending model) (153). It is recommended that muscle parameters be assessed at the 66% site as it is has largest MCSA, on average (154). However, we showed that muscle cross-sectional area (MCSA) at the

50% site is highly and systematically correlated with MCSA at the 66% site (r=0.95, n=20 boys and girls, age 9-11 years; unpublished data from the Healthy Bones Study (HBS)). By also acquiring muscle parameters at the 50% site, children were not subjected to an additional pQCT scan at the 66% site.

Bone outcomes from pQCT (BMC, bone strength, structure and density) at the radius and tibia shaft sites are reproducible (coefficient of variance (CV) 0.02 to 2.2%) (157) and precise (CV 0.4% to 3.5%) from repeated scans of human cadavers (158). However, at the distal radius and tibia sites, reproducibility was less precise. For example, precision for Tt.Ar at the radius was 0.9 to 2.3 (CV%) (157) and at the tibia precision was 1.8 to 7.9 (CV%). This is due to the presence of smaller trabeculae of bone that are susceptible to the partial volume effect (158).

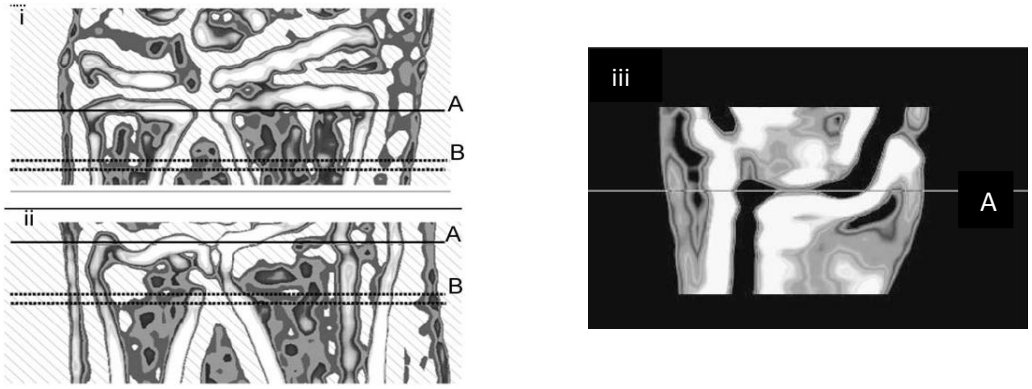


Figure 1.14. Scout scans for reference line placements in pQCT scans. Manufacturer recommended reference line (A) placements based on growth plate if (i) open, at the outer most medial border, and (ii) closed, at the medial border of the distal endplate (shown as scout scans at the radius). An alternative placement of reference line (A) at (iii) the most distal edge of bone end/surface (in this example, at the tibial plafond). Adapted from Ashby et al. (for images i and ii) (155) with permission from Springer-Verlag London and Burrows et al. (image iii) (156) with permission from Elsevier BV.

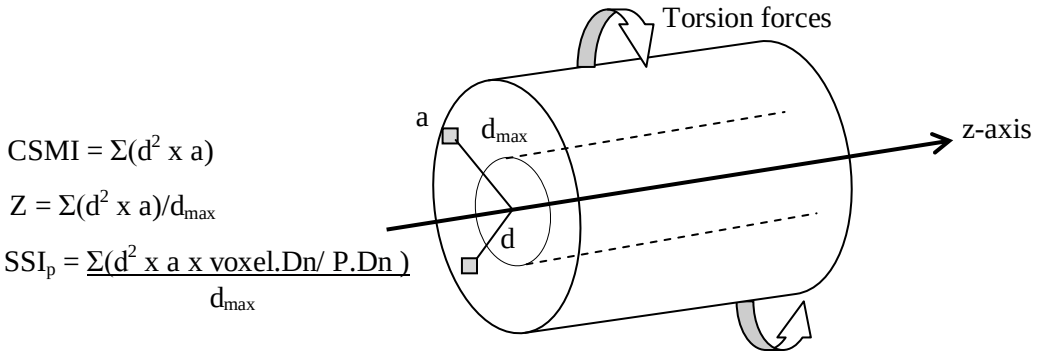


Figure 1.15. Illustration of polar stress-strain index (SSI_p) calculated from peripheral quantitative computed tomography (pQCT) that estimates torsional strength along the z-axis of the bone shaft. CSMI, cross-sectional moment of inertia (mm^4); d, distance of voxel area to the central axis; a, voxel area (mm^2); Z, section modulus (mm^3); d_{max}, maximum distance of voxel area to the central axis; voxel.Dn, voxel density; P.Dn, physiological "maximal" cortical bone density at 1,200 mg/cm^3.

1.2.3.2.2 Image analysis

Similar to image acquisition, pQCT image analysis protocols are also not standardized. Users have various options they can choose from to analyse pQCT images, using either default settings or user-defined thresholds and modes. Briefly, the image captured requires a defined region of interest for analysis, which the user can determine automatically or manually. To separate soft tissue and bone, pQCT software uses two basic techniques: 1) voxels below a researcher pre-determined threshold (outer area) are removed, then an outer bone edge is formed from remaining voxels (Contour mode 1) and 2) the software identifies the first voxel containing the specified threshold, then finds the next voxel closest in a clockwise direction until a bone edge contour is defined (Contour modes 2 and 3). The Peel mode option then separates trabecular and cortical bone at distal sites. CALCBD analysis modes are used for distal sites that require trabecular and cortical bone separation while CORTBD analysis modes are used to analyze shaft sites that are predominantly cortical bone.

Using CALCBD and CORTBD analysis modes, we are able to derive bone parameters as listed in Table 1.2 and are able to estimate bone strength. At distal bone sites, compressive bone strength is estimated based on Hooke's Law where stress is directly proportional to strain (69), and reorganized into the equation of Stress=F/A (153). As ultimate compressive stress is proportional to the square of total bone density ($Tt.Dn^2$) (126), force (F) is directly proportional to $Tt.Dn^2$ multiplied by Tt.Ar (perpendicular to the force direction). Thus, compressive bone strength can be estimated using the bone strength index (BSI (mg^2/mm^4) = Tt.Ar (mm^2) * $Tt.Dn^2$ (mg/cm^3) /10,000) (159). Stratec software incorporates an analysis of bone strength for shaft sites called the strength-strain index that addresses torsion (Figure 1.15) and bending forces. The software uses measurements of cortical bone (area and density) to produce SSI_p (strength in torsion) and $SSI_{x/y}$ (strength in bending) (153).

1.2.3.3 Strengths and limitations of pQCT

Using pQCT to assess bone strength, structure and density in children and adolescents is safe, precise and reliable. However, pQCT image acquisition and analysis would benefit from standardized methodologies related to similar populations, interventions or medical conditions. It is otherwise not possible to compare results across studies due to differences in image acquisition parameters (i.e. scan sites, reference lines, image resolution) and image analyses (i.e. modes and thresholds) (137). As pQCT measurements are limited to the peripheral skeleton it is also not possible to assess some clinically relevant sites (proximal femur, spine) and bone parameters from one site cannot be ascribed to other sites. The exception is pQCT's ability to assess the distal radius, a common fracture site in children at puberty. Further, it is not possible to accurately assess cortical density and thickness due to a PVE resulting from pQCT voxel sizes (>0.5 mm) (131,160). Despite these limitations, pQCT is able to provide structural assessment and volumetric density of cortical bone at the shaft and trabecular bone at distal sites with low

radiation exposure. Peripheral QCT also provides accurate and reliable estimations of bone strength in children and adolescents.

Table 1.2. Peripheral quantitative computed tomography outcomes, analysis sites and modes and description of bone outcomes. Adapted from Macdonald (161), with permission.

	Outcomes (unit)	Analysis site and mode	Description
Bone mass	Bone mineral content (BMC, mg/mm)	Distal & shaft: Contour mode	The amount of bone mineral within a cross-sectional slice of 1-mm thickness. Can be multiplied by the slice thickness to obtain total BMC in a 2.5 mm slice.
Bone geometry	Total bone cross-sectional area (Tt.Ar, mm^2)	Distal & shaft: Contour mode	The surface area of the entire bone cross-section including the cortex and marrow cavity. Tt.Ar directly reflects changes in bone size resulting from periosteal apposition.
	Trabecular bone cross-sectional area (Tb.Ar, mm^2)	Distal: Contour mode, Peel mode	The surface area of the trabecular bone cross-section. This area is influenced by endocortical apposition or resorption.
	Cortical bone cross-sectional area (Ct.Ar, mm^2)	Shaft: Separation mode	The surface area of cortical bone within the cross-section. Ct.Ar is influenced by periosteal apposition and endocortical apposition and resorption.
	Cortical thickness (Ct.Th, mm)	Shaft: Separation mode	The distance between the outer and inner border of the cortical shell. Ct.Th can be determined with the circular ring model that assumes a circular cross-section or through an auto-detection of the outer border.
Bone density	Total density (Tt.Dn, mg/cm^3)	Distal: Contour mode	Tt.Dn is the volumetric density averaged over the entire cross-section. It is influenced by the relative contributions and densities within both the cortical and trabecular bone compartments.
	Trabecular density (Tb.Dn, mg/cm^3)	Distal: Peel mode	Tb.Dn is the volumetric density averaged over the trabecular area of the cross-section. Tb.Dn is influenced by trabecular number and thickness and the degree of mineralization at the material level.

Table 1.2. continued

	Outcomes (unit)	Analysis site and mode	Description
Bone density (cont.)	Cortical density (Ct.Dn, mg/cm^3)	Shaft: Separation mode	Ct.Dn is the volumetric density averaged over the cortical area of the cross-section. Ct.Dn is influenced by cortical porosity and the degree of mineralization at the material level.
Bone strength	Cross-sectional moment of inertia (CSMI, mm^4)	Shaft: Separation mode	CSMI is proportional to the distribution of bone mass about the neutral axis and is an indicator of bone strength in bending or torsion (depending on which axis is used as a reference). It is calculated as the integral sum of the products of area (A) of each voxel and the squared distance (d^2) of the corresponding voxel to the bending (x, y) or torsion (z) axes.
	Section modulus (Z, mm^3)	Shaft: Separation mode	Z is CSMI divided by d_{max}, the maximum distance from the bending axis to the outer surface, in the plane of bending. Thus, Z approximates a cross section's resistance to bending in a given plane.
	Strength-strain index (SSI, mm^3)	Shaft: Separation mode	SSI is calculated as the integrated product of Z and Ct.Dn. The ration of Ct.Dn and normal physiological density (ND = 1200 mg/cm^3) provides an estimate of the modulus of elasticity. Similar to CSMI, SSI can be determined with respect to the polar (z) axis or the bending (x, y) axes.
	Bone strength index (BSI, mg^2/mm^4)	Distal: Contour mode	At distal sites, compressive strength is estimated as the square of the total density and the total cross-sectional area (the load-bearing area).

1.2.3.4 Other bone imaging technologies

Other bone imaging technologies used less frequently in pediatric bone research include HR-pQCT, MRI and QUS. I provide a brief overview of these three technologies below.

Similar to pQCT, HR-pQCT provides a 3-D analysis of bone microstructure and density. It is an accurate (151,162), reproducible (117) and safe (effective dose of 3 μSv for a single scan (163)) approach to assess these parameters in children. HR-pQCT scans encompass a bone cross-section with a thickness of 9.02 mm region of interest. Depending on the resolution desired (41, 82 or 123 μm), the region of interest is comprised of a specific number of slices – 220, 110 or 73 slices, respectively (164). From my review of literature, the most common HR-pQCT protocol uses a resolution of 82 μm and scans a total of 110 slices. In addition to the bone outcomes generated from pQCT, HR-pQCT also assesses cortical bone porosity and trabecular microarchitecture (162) (trabecular thickness (mm), number (1/mm) and separation (mm)) (164). High-resolution images enable post-acquisition finite element analyses (FEA), that simulate bone loading to provide an accurate estimate of bone strength (162). However, due to the high resolution and longer scanning time, HR-pQCT scans are more sensitive to movement artefact compared with pQCT (137). A short gantry also limits the measurement of more proximal peripheral sites (165). Lala and colleagues assessed cadavers (age unspecified) and compared tibiae outcomes obtained from HR-pQCT and pQCT at the same bone site and relative slice thickness (the first 27 slices acquired using HR-pQCT) (165). Cortical area, thickness and density were notably different (but highly correlated r = 0.96 – 0.99) at the 20% site of the tibia. These differences increased with greater cortical thickness, regardless of voxel size used in pQCT (0.2 or 0.5 mm) (165). Thus, it is important to consider the pQCT image acquisition protocols and the type of analysis conducted if bone outcomes are to be compared with results from HR-pQCT.

Two non-ionizing bone imaging technologies used to assess bone in children and adolescents are MRI and QUS. MRI bone images are created when fat and water (found in muscle mass) protons are excited after subjected to magnetic forces (bone does not have free protons) and a 'picture' (image) forms that depicts various tissues (166). In general, MR image acquisition varies based on image weightage (T1 to T2), pulse sequence (e.g., gradient or spin echo) and magnet strength (1.5 – 11.7 Tesla (T)). A 1.5 T magnet generates a resolution of about 300 μm (166). With stronger magnets, higher resolutions ranging from 150-200 μm are possible. MRI is used to assess bone structure and strength (but not density) and to assess other components such as water, fat and cartilage (e.g., erosions, effusion) (167). Imaging of bone using MRI is limited to peripheral sites such as the distal radius, humerus, distal tibia and calcaneus (168) and proximal femur (169). MRI is unable to evaluate the axial skeleton as there is insufficient contrast due to the higher volume of hematopoietic marrow (166). Cross-sectional geometry outcomes obtained using MRI to assess the 50% tibia site of 68-80 year old cadavers, was comparable to values obtained using pQCT ($r = 0.55$ to 0.85) (170).

QUS is the least expensive bone imaging tool among approaches I present. QUS provides quick scan times, is highly portable and does not require technical expertise (166). QUS measures the distance and speed that ultrasound waves travel from a source to a detector. As ultrasound waves are completely attenuated by air (166), QUS cannot examine axial sites as results are compromised by air in the lungs and bowels. Bone sites most commonly examined are calcaneus, tibia, radius and phalanx (123). Primary outcomes from QUS are speed of sound (SOS) and broadband attenuation (BUA). More dense bone results in greater ultrasound wave attenuation and loss of energy (slower velocity). Therefore, QUS outcomes are mainly linked to BMD and provide little information about bone structural changes (123). However, the central limitation of QUS is a fundamental lack of understanding of the relation of QUS outcomes to bone material and structural parameters. Thus, new paradigms are proposed for further research using QUS (171).

1.2.4 Growth and maturation

In this section, I define the terms childhood and adolescence that I have adopted in this thesis. As there are differences in chronological and biological age within an individual, I discuss various methods to assess biological maturation. Biological maturation is closely related to bone growth and development in children and adolescents.

1.2.4.1 Definition of adolescence

In order to define adolescence, I first provide a definition of *childhood*. *Childhood* is defined as the developmental period after the end of infancy and extends to the beginning of adolescence, whereby there is no development of secondary sexual characteristics. Chronologically, childhood extends until age 11 years in girls and 13 years in boys (172). Girls and boys are considered adolescents from age 12 to 18 years and 14 to 18 years, respectively (172). However, I adopt the definition from the Canadian Pediatric Society, which states that *adolescence* begins with the onset of physiologically normal puberty and ends when adult status is achieved, typically the life period between childhood and adulthood (173). Therefore, adolescence is more challenging to identify due to different maturational timing among individuals.

1.2.4.2 Assessment of puberty

Maturation is a developmental process to reach adulthood and can be divided into several domains such as physical, sexual, intellectual, emotional and even social maturation (174). Puberty consists of physical and sexual maturation that are observed through skeletal and somatic (body size) growth and development of secondary sexual characteristics (175). As bone development is more closely related to pubertal changes than chronological age, I discuss common methods to assess pubertal development in the following sections.

1.2.4.2.1 Skeletal maturation

Skeletal age, or bone age, is traditionally assessed by clinicians by comparing hand radiographs (length, width, ratio) and fusion of the epiphyseal plate to a set of sex-specific atlases with ages ranging from birth to adulthood (176). The three most popular methods are Greulich-Pyle, Tanner-Whitehouse (three versions) and Fels, which differ based on the set (and number) of hand-wrist bones assessed, scoring procedure and reference sample (177). Overall, skeletal age assessments are precise and reliable (176) but the outcomes are not comparable across methods (178). Skeletal age measurements are usually used in clinical setting, as the process itself requires highly trained personnel to obtain and interpret the radiographs. Furthermore, it involves exposure to ionizing radiation. The reference radiographs were mainly derived from a Caucasian/white cohort, which may not be suited to assess skeletal age in other ethnicities due to known ethnic differences in maturation (179).

1.2.4.2.2 Somatic maturation

In relation to skeletal growth, maturation can also be assessed based on somatic changes. For example, individual growth trajectories can be mapped with data from longitudinal studies where serial measurements of height are available. Age at PHV (APHV), an indicator of somatic maturation, can be calculated from such trajectories (Figure 1.16) (11,180). Recent Canadian data suggests that APHV occurs around 11.8±1.0 years in girls and 13.5±1.0 years in boys (114). Approximately 90-92% of adult height is achieved by APHV (9) with average gains of 8.5±1.1 cm/year and 10.4±1.3 cm/year in girls and boys, respectively at APHV (181). In relation to sexual maturation, girls reach PHV one year prior to menarche (first menstrual period), on average (182). Importantly, data from the University of Saskatchewan 7-year longitudinal Pediatric Bone Mineral Accrual Study (PBMAS) reported that peak BMC accrual followed PHV by about 7-8 months, on average (11). It has been proposed that the lag between PHV and peak BMC accrual creates a temporary period of low bone mass and transient bone fragility (183).

Epidemiological studies indicate that this corresponds to a period of peak fracture incidence in adolescent girls and boys (184–188).

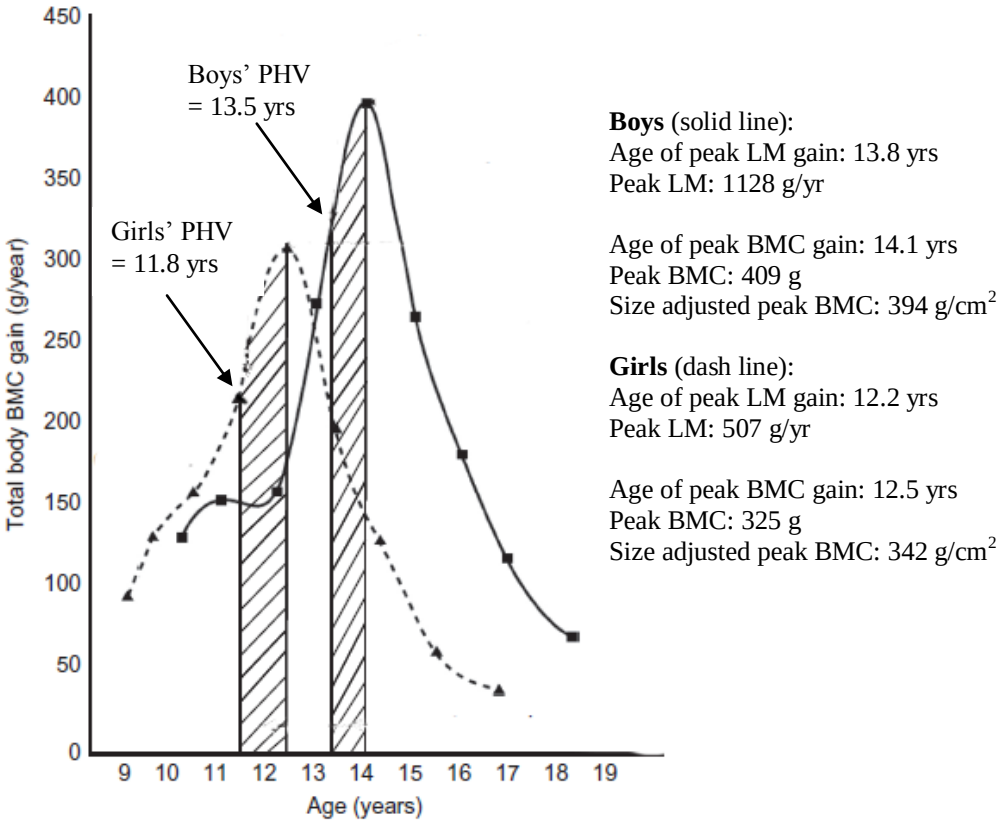


Figure 1.16. Total body bone mineral content (BMC) gain in boys and girls from longitudinal DXA data (11). Age of peak lean mass (LM) (181) and BMC gains in relation to age at peak height velocity (APHV) in boys and girls. The lag period between PHV and peak BMC (shaded area) is approximately 8 months and represents a period of low bone mass for age. Adapted from Bailey et. al, 1999 (11), with permission from the American Society for Bone and Mineral Research.

Assessment of somatic maturation is non-invasive and easy to conduct (i.e. no costly equipment or highly skilled assessors are required), but it relies upon longitudinal data and is therefore time consuming and costly to follow a cohort over a number of years. To obtain PHV, a child's height is assessed from prepuberty, through puberty, to maturity. From these data height velocities can be calculated and the growth trajectory can be mapped. Accurate measurement of height across time requires

standardized protocols and strict quality control. Longitudinal measurement also requires prolonged commitment from study participants, and thus, is often subject to high attrition rates.

If it is not feasible to collect serial measurements across growth, a model by Mirwald and colleagues that predicts sex-specific APHV has been proposed using single measures of height, sitting height, leg length, body mass and chronological age (189). The authors cross-validated the equation with two different sets of longitudinal data and set a limit to predict the mean up to a year surrounding APHV (mean at zero, SD of 0.5 yrs). The equation predicted 89% and 88% of variance in APHV from the other datasets for boys and girls, respectively (189). However, independent assessment of the equation compared with actual APHV with skeletal age using the Fels method showed poor associations with maturity status in boys aged 13-14 years (Cohen kappa = 0.13, 57% agreement, Spearman's r = 0.29) (190). Differences between predicted versus actual APHV increased with chronological age (and age from actual APHV) and tended to differ between early and late maturity groups (191,192). When tested, the predicted APHV tends to overestimate actual APHV as chronological age increases; the largest difference was observed in 11 to 13 year old girls (+0.4 to +0.6 years) (192) and 13 to 15 year old boys (+0.2 to +0.3 years) (191). As the equation was derived from a white Canadian cohort, they may have different body proportions (leg length, sitting height, etc.) compared with adolescents of other ethnicities. For example, Asians tend to have shorter limbs than their white counterparts (193,194).

1.2.4.2.3 Sexual maturation

Sexual maturation includes adrenal and gonadal maturation. Adrenarche is responsible for maturation of the gonads and pubic and axillary hair growth and contributes to behavioural and psychological changes (195). Subsequently, gonadal maturation leads to maturation of primary sex organs (ovaries and testes) and secondary sexual characteristics (breasts and genital development) through

increased levels of estrogen or testosterone (195). Thus, assessment of secondary sexual characteristics or measures of hormone levels can be used to estimate a child or adolescent's stage of maturity.

A popular method for assessing sexual maturation in children and adolescents is by the method of Tanner (195) that classifies secondary sexual characteristics into five distinct stages. When assessed by a qualified physician, the Tanner method is considered a gold standard (175). Children and adolescents are classified as pre-pubertal (Tanner stage 1), early pubertal (Tanner stage 2 and 3), peri-pubertal (Tanner stage 4) or post-pubertal (Tanner stage 5) based on standardized photographs or line drawings depicting the stages of secondary sexual maturation (breast and genital development, pubic hair growth). This is based on the work of Marshall and Tanner who studied a cohort of British children (192 girls, 228 boys) in the Harpenden Growth Study (196,197). Marshall and Tanner reported differences in both the timing of pubertal onset (anywhere between 8 to 15 years old) and the tempo of maturation. On average, a boy's pubertal development takes about 1.8 to 4.7 years to complete (197) while girls take anywhere from 1.5 to 6 years (196). Figure 1.17 illustrates the wide variation in maturation for girls at a certain chronological age, where one stage of maturation (e.g., onset of puberty) may overlap with other maturational stages (Tanner 2 to 5) of different individuals of the same age. To illustrate, a 10-year old girl might be Tanner stage 1 or Tanner stage 3 for breast development depending on the tempo and timing of her maturation. Boys' timing of maturation follows a similar trend as to girls, albeit with a lag time of one to two years compared with girls (175). Boys' tempo of growth is of greater magnitude especially at puberty, on average (198)

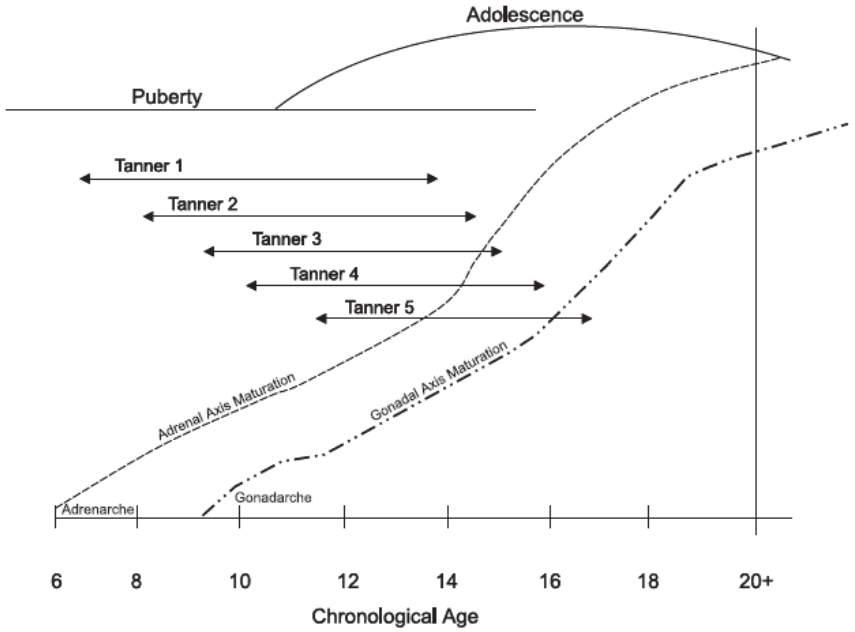


Figure 1.17. This schematic illustrates the wide variation in maturation phases as assessed by the method of Tanner across pubertal development (i.e. adrenarche and gonadarche) in girls relative to chronological age. Reproduced from Dorn et al. (175), with permission from Taylor & Francis Informa UK Ltd.

When physician-determined Tanner staging is not feasible (e.g., due to the intrusive nature of assessment, field-based research setting, resource limitations), self-assessment methods are often used. During self-assessment, participants select the closest description and picture (or line drawing) that represents their current stage of secondary sexual maturation (175). There are moderate to high correlations (0.48-0.91) between self-assessed and physician-assessed maturation with stronger correlations in normal body mass girls compared with boys (174,199). However, self-assessment may be less accurate in overweight and obese individuals, particularly in girls, as excess adipose tissue could be mistaken for breast development. Obese girls' self-assessment of breast development was only moderately correlated (r=0.37) with physician assessment (200).

Due to the sensitive nature of the Tanner method (i.e., physical examinations or graphic depictions of genitals and breasts), this method may not be appropriate in all research settings (e.g., field studies in

schools) (175). Thus, self-assessments of secondary sexual characteristics such as axillary and facial hair growth are an alternative especially for boys; however, results are less precise to the exact stage of maturation. Self-assessments of axillary hair in boys were significantly correlated with physician assessments for the appearance of axillary hair (r=0.68) (201). In a 6-year longitudinal study, 77% of boys were in Tanner stage 4 of pubic hair growth by the first appearance of axillary hair (202). In addition, age of first appearance of axillary hair in boys was moderately correlated with Tanner stage 3-5 of pubic hair growth (r=0.55-0.66) and to APHV (r=0.51) (202).

For girls, although self-assessments of axillary and leg hair growth were significantly correlated with physician assessments (r=0.74 and r=0.44, respectively), menarcheal status may be a more reliable indicator of sexual maturity (195). Menarche is considered a late event in sexual maturation; at menarche, 60% of girls reported achieving Tanner stage 4 and 10% were at Tanner stage 5 for breast development (203). Mean age at menarche (12.7±1.0 years) also coincided with the mean age at peak BMC accrual velocity (12.6±0.86 years) (11). This close association between peak BMC accrual and age at menarche provides a suitable means to evaluate maturity-related changes in bone variables in girls. Menarcheal age is usually self-reported retrospectively and therefore, accuracy of reporting depends on time since menarche (204–206). For example, among post-menarcheal girls, 66% correctly reported the month and year of menarche after an average of 323 days (205). However, accuracy decreased with time; 59% reported correctly after an average of 430 days, while only 45% correctly reported after an average of 649 days (205). Recall can be refined if prompted by occasions or conditions related to the occurrence of menarche. For example, asking about school grade, season (Spring, Summer, Fall, Winter), and/or events (e.g., Christmas, birthday, school holidays) may assist in determining the exact timing of menarche.

Hormonal assays to assess sexual maturity are challenging as they most often require a blood draw and therefore may be unappealing to healthy children. There are also only limited reference data for

children and adolescents. Although less invasive methods (e.g., saliva or blood spot) are available, the results from assays of different types (saliva, blood spot, venous blood) are not comparable and are not linked to Tanner stages (195). In addition, hormone levels follow a diurnal pattern and fluctuate by menstrual cycle phase, thus consistent timing is required when obtaining samples (195). Future advances in biochemical assays may enhance assessment of maturation using hormonal assays (195).

Overall, assessment of maturation can vary across research studies. The method selected depends largely on the aim of the study. The choice of assessment also has to be feasible based on study design (cross-sectional, longitudinal), setting (clinical, school, home) and available expertise (trained staff, health professional). A common limitation across methods is the dependence on mostly white cohorts for reference data. Yet, assessment of maturity in pediatric bone research is vital to isolate contributions of natural progression of growth from exercise and nutritional factors. In a recent study, Rantalainen and colleagues found marked site-specific sexual dimorphism of the tibia (by pQCT) and attributed these bone changes to the natural progression of growth (before and after APHV), and not from other factors such as exercise and nutrition (207).

1.2.5 Maturity- and sex-specific differences in bone

In this section, I provide an overview of the role of maturity in bone growth and development and the sex-specific differences in bone strength, structure and density. First, I discuss the findings from a seminal longitudinal study that followed children throughout growth into adulthood (11). This study provided great insight regarding differences between boys and girls in the tempo and timing of bone mineral accrual across maturity stages. Then, I focus on studies that utilized 3-D imaging tools studies to evaluate sex- and maturity-specific differences in bone strength and its contributors (structure and density) at weight-bearing and non-weight bearing sites.

1.2.5.1 Sex differences in bone mass

Bone science has benefitted tremendously from longitudinal studies that observed the process of bone development from childhood into adulthood. The PBMAS followed children from age 8 to 14 years, acquired annual DXA scans for seven consecutive years and followed up participants five years later, in adulthood (11). Findings from the PBMAS demonstrated that not unlike height, the magnitude of BMC accrual for the total body and proximal femur was 15% greater in boys (394 g/year), on average, compared with girls (342 g/year) at peak BMC velocity (PBMCV) and during the two subsequent years (11). This was not the case at the lumbar spine where there were no between-sex differences in PBMCV or accrual during the 2 years before and after APHV (11).

Evidently, there is site-specificity of bone mineral accrual between boys and girls. Overall, total body and femoral neck BMC is greater in boys compared with girls; these differences are related to stature (208). Also from the PBMAS study, girls who matured later (about a year later as assessed by APHV) had significantly less total body BMC than girls considered 'average maturers' when compared within chronological age categories. There were no differences for boys among early, average and late maturers for BMC accrual at the femoral neck or lumbar spine sites (209). This illustrates the influence of the timing and tempo of maturation on bone – and the need to account for maturity in studies of growing children.

1.2.5.2 Sex differences in bone size and strength

Sex-differences in bone size and strength can be examined through changes on the surfaces of bone. Classic cross-sectional studies by Garn and colleagues examined radiographs of the second metacarpal in children from birth to adulthood (80 years old). They reported differences between boys and girls at the periosteal and endosteal surfaces by chronological age (210,211). They concluded that sex

differences in bone area stemmed from greater apposition at the endosteal surface in adolescent girls compared with boys while boys experienced greater periosteal apposition compared with girls (210,211). Our bone research group readdressed this finding using pQCT (Gabel et al., 2015; submitted for publication) to assess the tibia of boys and girls across age 8 to 23 years and accounted for maturity using APHV. The findings supported those of Garn and colleagues (210,211) whereby periosteal apposition of bone in boys was greater than for girls. However, at the endosteal surface, a decrease in endosteal resorption (rather than increased bone apposition) in girls accounted for sex differences in the medullary area. Early planar-based imaging technologies offered a means to evaluate maturity- and sex-related differences in bone. However, the evolution of 3-D imaging (pQCT, HR-pQCT, MRI) provides a better means to evaluate the structure, strength and surfaces of bone.

1.2.5.3 Sex differences in bone at weight-bearing bone sites

Maturity- and sex-specific differences in bone strength, structure and density were reported in studies of children and adolescents that used pQCT to assess these bone outcomes. In pre- and peri-pubertal boys, BSI at the distal tibia was greater (+29%) compared with girls at the same stage of maturity (150). This may be attributed to a greater Tt.Ar and higher Tt.Dn in pre-and peri-pubertal boys compared with girls (150,212). BSI (+15%) and Tt.Ar (+6%) remained greater for boys after adjusting for tibial length and MCSA (150). In post-pubertal adolescents, there were no differences between sexes for bone strength and density at the 4% distal tibia. However, Tt.Ar and trabecular BMC (Tb.BMC) were higher (unadjusted) in boys compared with girls (213). In a study of 8 to 15 year olds, boys had higher Tb.Dn at the 4% site compared with girls (adjusted for height, Tanner stage and age) (214). This suggests that the strength advantage conferred to boys compared with girls at the distal tibia is a result of increased bone area (size) and trabecular density.

At the tibial shaft, boys had stronger bones as a function of their greater bone area, on average. Our research group reported that boys had greater bone strength (6%, SSI_p (150) and 14%, Z (215)) at postpuberty (only), as compared with girls. Importantly, peri- to post-pubertal girls demonstrated greater cortical bone density (1% to 10%) at shaft sites compared with boys at the same maturation stage (150). These sex-specific differences in cortical density persisted after adjusting for tibial length and MCSA (150). For girls, greater bone density at the tibia shaft may be one means whereby they achieve bone strength when their bones have stopped growing in size by depositing bone endosteally. In a study of 13-year old black youths, boys had greater bone strength (12%, SSI_p) at the 38% tibia compared with girls (212). This was a function of black boys greater cortical bone diameter while black girls had greater cortical density (212). Relatively, increases in bone size at shaft sites or more accurately, the displacement of bone away from the neutral axis provides exponential (third or fourth power) increases to bone strength (153) compared with bone density (second power) at distal sites (159). Two other studies reported greater bone area in boys compared with girls (213,216); however, no bone strength or density variables were assessed (216). A larger medullary area in boys compared with girls observed at early puberty was maintained into young adulthood (213,216). These sex-specific adaptations are related, in part, to the role of sex hormones. I discuss this role in greater detail in section 1.2.6.3.

Adjusting for different covariates when evaluating sex-specific differences in weight-bearing bones could provide different results. Hölger and colleagues used MRI to examine the femoral shaft (66% site) in prepubertal boys and girls and young adult men and women. After adjusting for femur length and body mass, the bone outcomes (bone area, BMC and bone strength) were not significantly different between boys and girls. These results at the femur differ from the pQCT studies mentioned previously; this may be due to differences in variables controlled for. In young adults, men (21.4±2.4 years) had significantly greater total and cortical area (5-6%), greater BMC (3%) and greater bone strength (polar second moment of area (I_p), BSI, 6-7%), compared with women (20.4±3.2 years) (217). When femur

length and muscle area were controlled for in the analyses, bone differences diminished or favoured the young women. Young women demonstrated trends toward stronger bones, thicker cortices and larger medullary area, compared with young men relative to their body size and lean body mass.

1.2.5.4 Sex differences in bone at non-weight bearing bone sites

Three pQCT studies (212,218,219) and one HR-pQCT study (220) examined sex differences in non-weight bearing bones in children and adolescents. At the distal radius, two studies examined bone strength and reported boys had greater bone strength (BSI and by FEA) compared with girls from pre- to post-puberty (212,220). Boys' greater BSI in pre-and peri-puberty was attributed to their greater Tt.Ar, Tt.Dn and Tb.Dn, compared with girls' (212). Neu and colleagues investigated maturity- and sex-related bone differences at the distal radius in a cohort aged 6 to 23 years using pQCT (218). Boys at Tanner stages 1, 2 and 5 had greater total bone area compared with girls (218). Boys at Tanner stage 1 (only), had greater Tt.Dn compared with girls (218). Kirmani and colleagues used HR pQCT to examine the distal radius (220). Boys had wider bones (larger periosteal and endosteal circumference) compared with girls during pre- (stage 1, assessed by Tanner-Whitehouse III) and post-puberty (stage 4 and 5), but not at stages 2 and 3 (220). Boys also had thicker cortices and greater Ct.Dn at stages 2 and 3 compared with girls. At stage 4, girls had thicker cortices and greater Ct.Dn (220). Thus, greater bone strength (by FEA) in boys is conferred by their larger bone size, achieved via increased periosteal apposition and higher trabecular volume. Sex-related differences in bone structure and density at non-weight bearing distal sites were highly associated with maturity. Variability across studies may be partially explained by differences in the site examined, different approaches to assess maturity and different measurement protocols.

The Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) study assessed the radial shaft (65% site) using pQCT. Their cohort was comprised of 371 healthy 6 to 23 year

olds and their parents (n=107), aged 29-40 years (219,221,222). Boys had significantly greater bone strength (+16-25%, assessed as SSI_p) compared with girls at all Tanner stages (except Tanner stage 4 where no differences were observed) (221). For Ct.Ar, boys had significantly larger bone area compared with girls at Tanner stages 1 (+11%) and 5 (+16%). Boys also had significantly greater marrow area (Me.Ar) compared with girls at Tanner stage 3 (+36%) and 5 (+26%) (219). In the same cohort, girls had greater Ct.Dn compared with boys at Tanner stages 4 (3.2%) and 5 (+3.5%) (222). Thus, boys' greater bone strength (SSI_p) compared with girls across all Tanner stages (except Tanner stage 4) may be a function of boys' larger cortex (Tanner stage 1 and 5) or Me.Ar (Tanner stage 2 and 5). These bone parameters (Ct.Ar and Me.Ar) were significantly different at certain maturational stages. However, small size differences have an exponential influence on bone strength, which may have contributed to boys' greater bone strength as compared with girls. Girls' greater bone density as compared with boys at Tanner stage 4 did not confer girls greater bone strength. Importantly, DONALD study data were not adjusted for body size or any muscle parameters.

Generally, a few cross-sectional and prospective studies support maturity- and sex-specific bone strength differences in growing children. Differences appear to be a function of 1) assessment of weight-bearing versus non-weight bearing bones, 2) maturity stage and 3) site assessed (distal or shaft). Most sex differences in bone strength were explained by differences in bone structure rather than bone density. The magnitude of the difference between sexes increased as maturity progressed. Importantly, possible confounding variables (e.g., muscle, body size) should be controlled as they can otherwise drive the outcomes observed.

1.2.6 Factors that influence bone growth and development

In this section, I introduce factors that influence bone growth and development (Figure 1.18). Specifically, I aim to provide a brief overview of inherent and extrinsic factors that influence bone strength, structure and density in children and adolescents. Inherent factors known to influence bone growth and development include genetics, ethnicity, hormones and muscle mass. Extrinsic factors include PA and dietary intake of calcium. As the influence of PA on bone is a central tenet of my thesis, I discuss this association in greater detail in section 1.2.8.

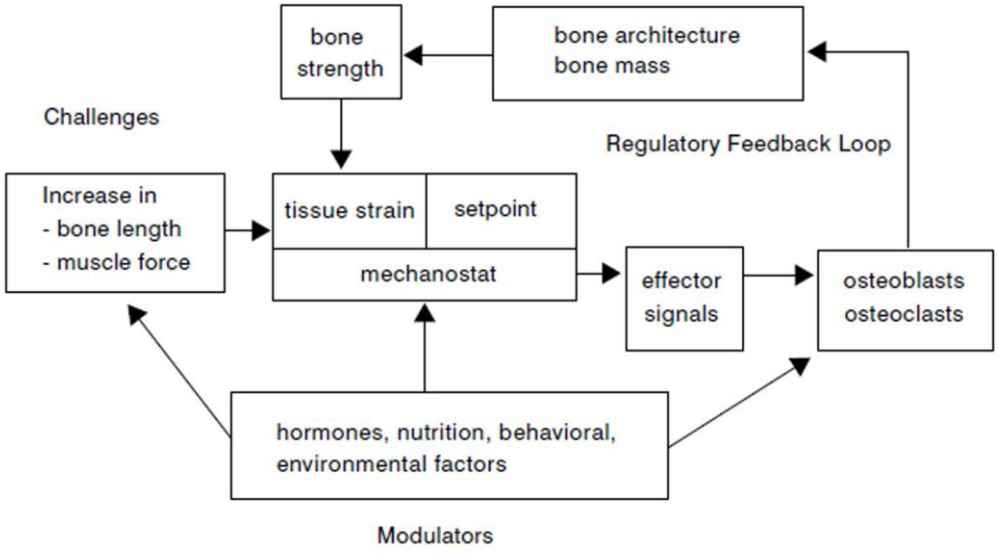


Figure 1.18. A functional model of bone development based on the mechanostat theory where bone continuously adapts to external challenges with the known modulators. Reproduced from Rauch and Schoenau (223), with permission from Lippincott, Williams & Wilkins, Inc.

1.2.6.1 Genetics

From family and twin studies, genetics accounted for 50-80% of the variability in aBMD (224–226). The heritability, i.e. inherent traits, of bone strength and structure are less well defined. Several retrospective studies used pQCT to assess adult bone strength, structure and density and demonstrated that

body length and body mass assessed in infants at 6 months to 1-year old, significantly predicted bone outcomes (r=0.12- 0.56) in adults (213,227–229). A prospective study that followed individuals across three generations (first generation: 72 to 96 years old, second generation: 40 to 80 years, third generation: 31 to 72 years) (230) and a study in twins (3,724 monozygotic and 12,050 dizygotic twin pairs, age 66 to 68 years) (231), reported that 33 to 53% of vertebral fractures were linked to genetics. While certain traits related to risk factors for fractures may be inherited, the traits may be different from genes that contribute to bone phenotype (structure, size). However, heritability of bone strength is practically unknown although several genes and loci have been associated with some bone structural components (232).

Genome-wide analysis studies (GWAS), i.e. genetic epidemiology, search widely across complete genomes to isolate single nucleopeptides (SNPs) that may be the cause of certain traits. From GWAS, researchers using pQCT and HR-pQCT, identified new loci associated with cortical and trabecular BMD, cortical thickness and bone microstructure (232–234). From large population cohort trials including the Gothenburg Osteoporosis and Obesity Determinants (GOOD) study, Avon Longitudinal Study of Parents and their Children (ALSPAC), Osteoporotic Fractures in Men (MrOS) and the Canadian MultiCentre Osteoporosis (CaMos) study, genotype samples were pooled to identify possible loci and single nucleotide proteins (SNPs) related to specific bone variables such as BMD and cortical thickness (233). Subsequently, the meta-analysis reported several SNPs associated with the Wnt16 gene (233). An example of the Wnt16 gene function is to produce receptor activator nuclear-factor kappa-β ligand (RANKL) protein that promotes differentiation and maturation of osteoclasts (233). More studies are on-going to identify the important role of genes in bone phenotype and even bone adaptation.

On top of genetic influences on bone, bone is also moulded by the environment. In a Swedish cross-sectional study of twins, environmental factors explained one third of the variance in vertebral fractures that occurred between age 50 to 70 years and up to 85% of the variance in vertebral fractures for

those age 80 years and above (231). In another cross-sectional study (226), we can see that inherent properties of height, body mass and bone density are related between mothers and daughters. Daughters by age 12.8±0.8 years, had 96% and 76% of their mothers' (age 42.4±4.2 years) height and weight, respectively (226). In addition, proximal femur aBMD of daughters was 56% and lumbar spine aBMD 70%, of their mothers' values (226). Mother-grandmother (grandmothers, 67.6±8.8 years) spine aBMD showed 66% heritability (226). There was no relation between intake of dietary calcium (across generations) and aBMD; however, low PA levels in daughter-mother-grandmother triads were associated with low aBMD and high PA levels in daughter-mother-grandmother triads were associated with greater aBMD at the femoral neck (226). This suggests that environmental factors may interact with heredity to influence bone growth and development.

1.2.6.2 Ethnicity and race

Ethnicity dictates through genetics the hair, eye and skin colour, and body build of an individual. Externally, it shapes their social-cultural diet, norms and behaviours (235). Thus, ethnicity can influence bone growth and development through various channels. To begin, I use the term *ethnicity* to mean hereditary and sociocultural influences while *race* is based on phenotype classified by a social construct prior to modern genetic studies (236). For example, the Caucasian race is identifiable with light skin and eye colour but may have stemmed from different ethnicities such as Irish, Dutch and Finnish. These terms are used interchangeably in the current bone literature. For my literature review I use terms used by authors of the studies I review; I include definitions if provided by authors. I discuss pediatric studies that are mainly cross-sectional association studies that examined the influence of ethnicity on bone health.

Studies of bone traits and fracture risk in children demonstrated distinct ethnic differences in bone structure and density, irrespective of geographical environment. Fracture risk of US white children was

twice (hazard ratio = 2.1) that of non-white children (aged 6-17 years) (237). Generally, non-white American children (i.e. blacks, Hispanics) had significantly stronger tibiae and radii (3-47%, BSI, Z, SSI_p) and greater Tt.Dn and Ct.Dn (2-23%) at distal and shaft sites (assessed using pQCT), compared with white children (controlled for age, sex, PA, muscle mass and limb length) (238–240). Our research group used HR-pQCT to examine ethnic differences in a cohort aged 14 to 22 years. Asians (East, Southeast and South Asians) had significantly smaller bone area and size compared with white (North America or European ancestry); yet, Asians had greater cortical BMD (5-8%), thicker cortices (12-27%), less cortical porosity (25%, boys only), lower trabecular number (8%, girls only) and greater trabecular separation (10%, girls only) than did whites after accounting for age, lean mass, limb length, calcium intake, PA and age of menarche (girls only) (193). Bone strength did not differ between these groups (193).

Certain factors other than genetic programming may contribute indirectly to the observed ethnic differences in bone properties in children. One factor would be pubertal timing that is different between ethnicities. Non-white girls (i.e. black, Chinese) have an earlier onset of maturation compared with white girls, on average. The mean age at menarche in Asian, black, Hispanic and white girls was 12.0, 12.1, 12.2 and 12.7 years, respectively (179,193). This relates back to the influence of maturity on bone development as discussed in section 1.2.5.

Another ethnic difference related to bone development outside the control of genetics would be calcium intake, retention and resorption. For instance, Asian children (10 years old) and adolescents (16 years old) had a lower calcium intake than their white counterparts; however, calcium intake did not contribute to total body, total hip, femoral neck and lumbar spine and BMC in either ethnicity (241). Regarding calcium retention, black girls demonstrated higher calcium retention and absorption efficiency compared with white girls, on average, across a wide range of calcium intake levels (760-2195 mg/day) (242). I discuss the role of calcium on bone development in further detail in section 1.2.6.4.

There may also be differences in muscle parameters between ethnicities – and this could influence ethnic differences in bone. For example, white boys and girls have significantly more lean mass than black, Asian and Hispanic children (243–245). Differences in muscle mass are commonly related to body size. Muscle CSA (by pQCT) at shaft sites of the radius and tibia was significantly larger in blacks compared with whites and Hispanics but these differences were no longer significant after adjusting for limb length (240). Yet, differences in bone outcomes between these ethnic groups remained after controlling for muscle (MCSA) in the analysis (193,240). I discuss the interaction of muscle and bone in detail in section 1.2.6.5.

1.2.6.3 Hormones

Endocrine and paracrine pathways that regulate bone growth and development are documented in the literature but how pathways are triggered at different phases of life is not well understood. In this section, I discuss the key role of growth hormone (GH), insulin-like growth factor-1 (IGF-1) and sex hormones in bone growth and development.

1.2.6.3.1 Growth hormone and IGF-1

GH and IGF-1 regulate metabolism and human growth across the lifespan. Levels of these key hormones remain relatively constant except during periods of accelerated growth (i.e. infancy and puberty). Secretion of GH from the anterior pituitary glands is triggered by growth-hormone releasing hormone (GHRH) from the hypothalamus. Higher levels of GH promote maturation of gonads (i.e., testes, ovaries) (246,247) and, in turn, estrogens and androgens secreted from the gonads further up regulate GH levels (248). During puberty, GH levels increase 1.5- to 3-fold from the otherwise constant levels observed during growth (249). Secretion of GH also stimulates release of IGF-1 from cartilage and hepatic

sources. This cascade of hormones orchestrates increases in height and bone mass as depicted in Figure 1.19.

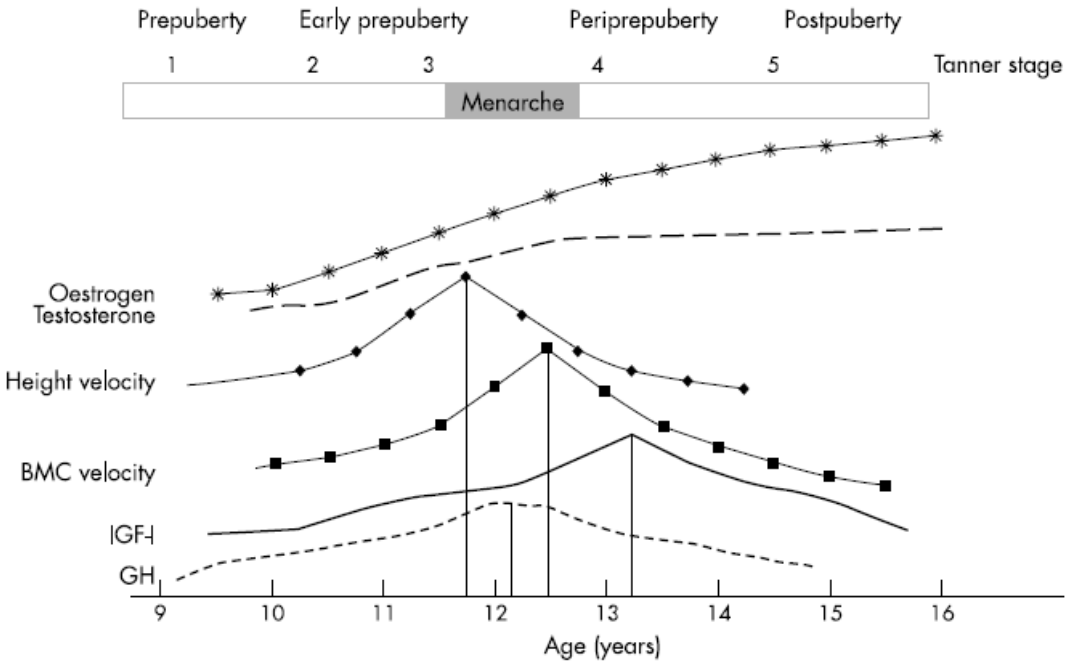


Figure 1.19. This schematic depicts trends in hormonal levels, longitudinal growth (height velocity) and bone mineral accrual (bone mineral content (BMC) velocity) in girls in relation to maturation stages and chronological age. Boys display similar patterns of hormonal and bone growth but with a delay of about a year later than girls to attain peak height and BMC levels. Reproduced from MacKelvie et al. (250), with permission from BMJ Publishing.

GH acts directly on growing bone by interacting with prechondrocytes at the epiphyseal plate to encourage longitudinal growth. GH also directs mesenchymal stem cells located in bone marrow to adopt osteoblastic and chondrocytic lineages instead of adipocytic lineage (246,247,251). Previous DXA-based studies demonstrated that children with GH deficiency had low vertebral and total body bone mass along with short stature compared with healthy counterparts (252,253). However, once body size was accounted for, children with GH deficiency had normal bone mass (BMAD), i.e. volumetric BMD, and thinner cortices thought to be related to bone length, i.e. stature (254). In excess, GH causes a very rare condition

called gigantism in children, resulting in excess height and higher incidences of vertebral fractures when they are adults (255).

Indirectly, GH acts through IGF-1 to further promote bone formation by stimulating differentiation and activity of osteoblasts. IGF-1 is known to act upon the growth plate (256) and is a prime stimulator of somatic growth and bone elongation. Primary IGF-1 deficiency, i.e. below 2.5 standard deviations (SD), is identified when children with idiopathic short stature (ISS), i.e. height-for-age below 2.5 SD, have normal GH levels but are deficient in IGF-1 (257). Anwar and colleagues described children with ISS with primary IGF-1 deficiency as shorter, with smaller hip circumference, lower BMI and less adiposity and shorter arm span than the average child with ISS. Treatment of IGF-1 deficiency involved individualized treatment of GH that resulted in marked growth in height (257,258). Both GH and IGF-1 stimulate skeletal tissue growth independently, but have additive and synergistic effects when both are present in sufficient amounts (257,258).

1.2.6.3.2 Sex hormones

The key sex hormones, testosterone and estrogen, have direct and indirect influences on skeletal development especially during puberty. I present results from in vitro studies that explain the basic cellular interactions of sex hormones and bone cells. Results from studies showed that androgens promote differentiation of osteoblast progenitor cells to osteoblasts at the epiphyseal resting zone and subsequently increase osteoblast numbers and size in the proliferation zone (259,260). Androgens also inhibit osteoblast apoptosis, further encouraging bone formation (259). Androgens increase linear bone growth by binding to androgen receptors (ARs) at the growth plate and influence transcriptional factors to form osteoblasts at the growth plate (259). Indirectly, androgens benefit bone growth through increases in lean mass (muscle), which increases mechanical loading on bone (261). Androgens also stimulate release of GH from the pituitary and IGF-1 from skeletal tissue to encourage bone growth periosteally in adolescents (259). In a

lesser, but no less vital role, aromatization of testosterones into estrogens in males is needed for healthy skeletal growth. This was illustrated in case studies of two men (aged 17 and 24 years) who had aromatase deficiency or inactive estrogen receptors (ERs), in the presence of normal levels of androgens. These men displayed thinner, smaller, and less dense bones compared with age- and sex-matched individuals as measured by DXA (lumbar spine, femoral neck and distal radius sites) (262,263). After three years of estrogen treatment, the man with aromatase-deficiency demonstrated significant gains in radius length (8.5%) and CSA (46%) and cortical thickness (12%) at the distal radius as measured by pQCT (263). This suggests that estrogen may be required for androgen action in healthy bone growth.

Estrogen has a bi-phasic function on human bone growth. Pre-puberty, low estrogen levels encourage bone growth at the epiphyseal plate by promoting osteoblast proliferation in both boys and girls (264,265). Low levels of estrogen are also thought to increase sensitivity to mechanical loads, augmenting further bone growth and periosteal apposition in boys and girls (261). Similar to testosterone, estrogen also indirectly enhances bone formation by increasing local IGF-1 secretion independent of GH action (266). As estrogen levels continue to increase during puberty, accelerated osteoblast formation exceeds the rate of chondrocyte formation and this eventually leads to fusion of the epiphyseal plate in late puberty, thus stopping longitudinal bone growth (264,265,267). Higher levels of estrogen, especially in females, appear to interact with different estrogen receptors and modulates bone apposition at the periosteal and endosteal surfaces (Figure 1.20) (261).

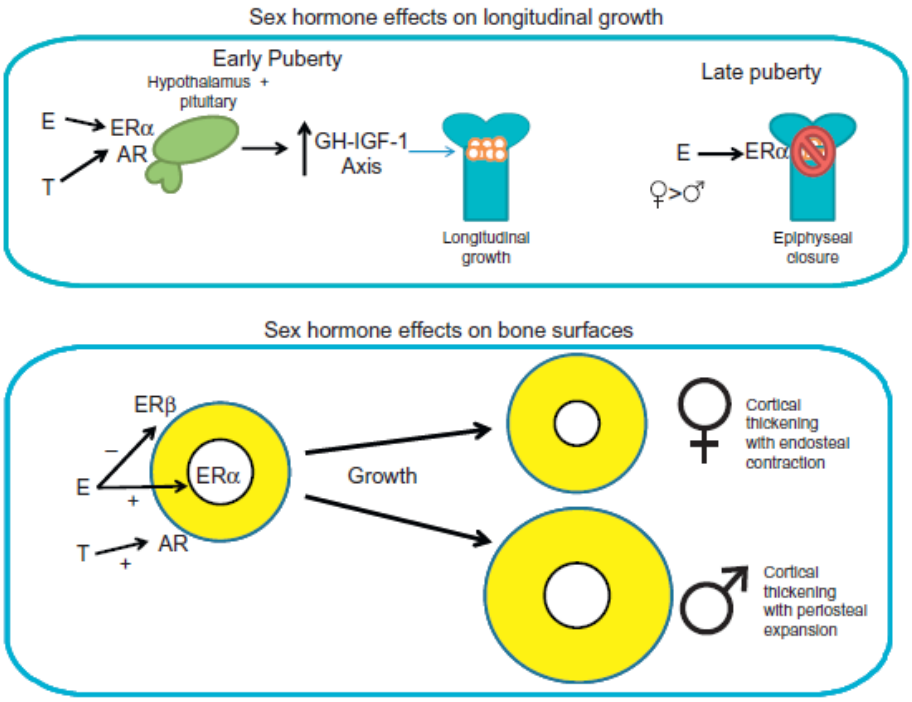


Figure 1.20 A schematic that describes the effects of sex hormones on longitudinal bone growth and bone surfaces. ♀, girls; ♂, boys; E, estrogen; T, testosterone; GH, growth hormone; IGF-1, insulin-like growth factor-1; ERα, estrogen receptor-α, ERβ, estrogen receptor-β, AR, androgen receptor. Reproduced from Bellido and Hill Gallant (251), with permission from Academic Press.

1.2.6.4 Dietary calcium

Calcium is the main building block of bone material, hydroxyapatite ($Ca_5(PO_4)_3OH$) (268). Current recommended dietary allowance (RDA) guidelines suggest that children and adolescents 9 to 18 years of age consume 1300 mg/day of dietary calcium to ensure healthy bone development (269). This RDA was derived from results of DXA-based calcium retention and calcium balance studies conducted between 1999-2009 (269).

Dietary calcium is often recommended as a public health intervention for prevention of osteoporosis. However, results of systematic reviews and meta-analyses indicate that calcium supplementation in children only has a small (but positive) effect on aBMD (by DXA) (270–272). For

example, Winzenberg and colleagues conducted a meta-analysis of 19 RCTs that assessed the effects of calcium supplementation (range: 300-1200 mg/day) on aBMD in healthy children and adolescents, 3 to 18 years of age (n= 2859). They reported a small but significant positive effect of supplementation on whole body aBMD (standardized mean difference, SMD=0.14, 95% CI: 0.01-0.27) and upper limb aBMD (SMD=0.14, 95% CI: 0.04-0.24) (270), but not proximal femur or lumbar spine aBMD. After cessation of supplementation, a sustained effect was observed only for upper limb aBMD (SMD=0.14; 95% CI: 0.01-0.28) (270). The authors suggested the small benefit in upper arm aBMD translated into a 0.2% reduction in fracture risk, which they did not consider clinically significant (271). Notably, studies included by Winzenberg and colleagues involved children who were predominantly white, healthy, mostly pre- and peri-pubertal and used DXA or single photon absorptiometry (radius sites) to examine the effects of calcium supplementation on bone mass or density (271).

While calcium supplementation alone may have a negligible effect on bone accrual, there is some evidence to suggest that calcium may act synergistically with weight-bearing PA to promote bone accrual during childhood and adolescence. In children as young as 3 to 5 years old, over an average of 50 weeks of intervention, the calcium supplementation with weight-bearing PA (gross motor activities) resulted in larger cortical area and thickness compared with children who did not receive a calcium supplementation and who participated in non-weight bearing (light motor) activities (273). Girls aged 8 to 13 years with high levels of self-reported PA (average 7.2±4.0 h/week) and high calcium supplementation (800 mg/day) demonstrated significantly greater 12-month gains in BMC at the femoral neck, one-third distal radius and total body compared with girls with calcium only, PA only or placebo and sedentary group. Benefits of calcium combined with PA were not observed at the ultra- and mid-radius or the lumbar spine (274). This suggests that bone adaptation to calcium and exercise may be site-specific, or may depend on the dose of PA and/or calcium. To date, calcium and PA trials have mostly evaluated pre- and early pubertal children;

none utilized 3-D imaging tools. Thus, there is a need to confirm the possible synergistic effects of calcium and PA on bone strength and structure in the older adolescent skeleton.

One school of thought is that calcium supplementation studies did not show significant positive effects due to insufficient vitamin D intakes. Adequate amounts of vitamin D are needed to actively transport dietary calcium. In healthy vitamin D sufficient 9-13 year old children, higher doses of vitamin D supplementation did not increase calcium gut absorption (275). A double-blind, RCT used pQCT to assess effects of calcium-vitamin D supplementation on bone in peri-pubertal twin girls (age 9 to 13 years; n=20 pairs) (276). One twin received a calcium-vitamin D supplement (800 mg calcium and 400 IU vitamin D) for 6 months while the other received a placebo. The twin receiving the calcium-vitamin D supplement demonstrated greater increases in Tb.Ar (3-5%), Tb.Dn (5%) and bone strength (5-7%) at the distal radius and tibia compared with the non-supplemented twin (276). The calcium-vitamin D supplemented twin also had greater increases in Ct.Ar (6%) and reduced Me.Ar (6-8%) at the tibia shaft compared with their placebo-control twin sibling (276). One other calcium and vitamin D RCT showed different results. Moyer-Mileur and colleagues supplemented 12-year old girls (n=71, 35 supplementation, 36 controls) with 500 mg calcium and 400 IU vitamin D daily for a year in a double-blind, RCT (277). Bone outcomes (by pQCT) between supplementation and control groups were no different (277).

Despite the multitude of nutrition and bone health studies in children and adolescents, results of supplementation trials are inconsistent. In my view, the evidence is not compelling to encourage calcium supplementation in an otherwise healthy population of children and youth. Furthermore, there is a glaring gap in bone and nutrition studies based on the absence of studies that used 3-D bone imaging modalities that are able to capture bone geometry and estimate of bone strength.

1.2.6.5 Muscle mass and force

We know that muscle development precedes peak bone mineral accrual during growth (181) as depicted in Figure 1.18. In adults, loss of muscle mass or sarcopenia is followed closely by bone loss (278). In his Utah paradigm, Harold Frost proposed that the aim of skeletal physiology is to ensure bones have sufficient mechanical competence to prevent voluntary physical loads from causing spontaneous fractures (45). Muscle forces are the biggest source of mechanical load applied to bone surfaces, as muscle contractions act upon lever arms to create movement (279). For example, muscle imparts up to 5.5 times body weight (BW) at the hip during recovery from a momentary loss of balance from a static single-leg balance, conveying a greater force than any external environmental force present (280).

Just as muscle contractions produce movement and PA, regular participation in PA increases muscle mass and function. While most bone health studies use muscle mass as a proxy for the amount of force that can be generated to influence bone adaptations, muscle function (i.e. strength/force and power) does not depend solely on muscle size or MCSA. Anliker and Toigo eloquently explained that muscle force generation is also influenced by neuromuscular factors, muscle fiber type distribution and muscle's secondary role as a metabolic tissue in the body (281). Myostatin-deficient mice are prime examples of how muscle size does not translate to the equivalent muscle strength required for bone adaptations. Myostatin-deficient mice experience muscle hypertrophy and with body mass being equal, there were no differences in femoral bone strength, geometry and mass compared with control mice (282). However, when the myostatin-deficient mice were exercised, radii strength increased more than 30% and 25% compared with non-exercised counterparts and wild-type exercised mice, respectively (283).

In a racquet sports study, examination of bone differences between playing and non-playing arm of girls (pre- to post-pubertal stages), muscle area (by MRI) explained 12% to 16% of variance in bone

mass, size and strength (by MRI) in the playing arm (284). By default, the study design (contralateral limb comparison) controlled for genetics, body size, nutrition, hormones and other possible external confounders (284). In pre- and peri-pubertal groups, muscle strength (measured using force platform or isokinetic computerised dynamometer) in addition to muscle mass (by pQCT, DXA) predicted bone strength and structure, but not bone density, at the lower extremities (150,285,286). This suggests other possible mechanisms whereby when muscles are put to use (i.e. PA), enhanced muscle function will result in stronger bones.

Assessment of muscle properties therefore becomes a focal point to better understand the muscle-bone unit. In children, three valid tests of muscle function are; 1) maximal isometric grip force (grip strength), 2) vertical jump test or Bosco test, and 3) 30-second Wingate test (287). They have been used in large European epidemiological youth studies (AVENA, EYHS, HELENA) (288). However, these protocols assess different muscle parameters. For example, the vertical jump test examines explosive strength while the Wingate examines lower limb peak power (289). Selection of the appropriate approach to evaluate muscle depends on the research question, which dictates the outcome of interest, feasibility of the measure for use in the field or laboratory as well as the validity and reproducibility of the approach. Moreover, the different ways to assess muscle function do relate to bone outcomes. Two decades ago, grip strength explained up to 87% of variance in BSI at the distal radius in children as young as 5 years and in adults as old as 57 years (290). Neu and colleagues examined the relation of grip strength and MCSA at the 65% site of the forearm in 6 to 23 year olds (n=366, 181 boys, 185 girls) (291). Grip strength per unit of MCSA, normalised for forearm length, increased by 44% from age 6 to 20 years and was similar between sexes (291). This meant that muscle function (strength) is not limited per se by the mass available during childhood growth and development. Furthermore, as muscle function is highly associated with body size, outcomes need to be adjusted for body size or be scaled allometrically (292). Other approaches to assess muscle function that were related to bone strength (and other bone outcomes) using isokinetic

dynamometers and force and power jump mechanography (force platforms) (285,286,293). Daly and colleagues reported that peak muscle force measured by an isokinetic dynamometer explained an additional 2-5% of variance in femoral neck bone area and section modulus (assessed using MRI) after accounting for limb length and total body lean mass (286).

Despite the evidence of muscle (mass and function) association to bone strength, structure and density from cross-sectional and comparison studies in human, results from RCTs are contradictory. RCTs that intervened with resistance training in adolescents did not find significant differences in bone mass changes between intervention and control groups (294,295). While muscle strength improved in the study participants, there were no improvements in bone mass and density (by DXA) in 14 to 18 year old girls after a 26-week (294) or 15-month intervention (295). The lack of evidence may be due to the use of DXA that does not capture changes in bone geometry. Also, the limited literature on the minimal effective load of resistance exercises as opposed to known minimal impact forces required to obtain bone adaptations may be the cause of the non-significant findings in this instance. Overall, more investigations on the influence of muscle, direct PA loading and bone adaptations are required. With the current advances being made using new imaging technologies such as HR-pQCT combined with reliable assessments of muscle function, future investigations may better inform us as to the association between muscle parameters and bone strength, structure and density.

1.2.7 Physical activity and sedentary behaviour

In this section, I define PA, its components and generally, how it relates to bone health. I also briefly describe commonly used PA measurement tools in relation to issues of validity, reliability, strengths and weaknesses. I also define sedentary behaviour, measurement tools for SED and discuss the

limited findings related to the influence of sedentary behaviour on bone strength, structure and/or density in children and adolescents.

1.2.7.1 Physical activity - definition, concept and components

PA and exercise are common terms that are often used interchangeably in the literature. By definition, PA refers to any bodily movement by skeletal muscles resulting in the use of energy, or incurring energy expenditure above basal metabolic rate (296). Exercise is an extension of PA whereby exercise is a structured, planned, repetitive activity with the aim of improving or maintaining physical fitness (296). I consider PA to encompass both structured and unstructured bodily movement and do not discriminate between sports, recreational or occupational activities in this thesis.

PA comprises four main components, 1) frequency, 2) intensity, 3) type (aerobic, weight-bearing, impact, flexibility), and 4) time or duration. By accounting for frequency, intensity and time/duration, we are able to quantify the volume of PA undertaken (297). This is an important concept as it enables PA to be assessed with good reproducibility and to determine the dose-response relationship between PA and health conditions by facilitating comparisons across studies (298). To better understand the influence of PA on bone strength and predict bone's response to PA, it is also important to clearly note the type of PA (weight-bearing or non-weight bearing), number of sessions, duration and number/frequency of rest intervals conducted (299).

1.2.7.2 Measurement of PA

Many methods are available to quantify children and adolescents' participation in PA (300). However, self-report questionnaires are still common in field-based studies as they are cost-effective, easy to administer, and are accepted as valid measures of PA. This is considered to be particularly so if the PA

measures are associated with physiological-based measures such as indirect calorimetry (301). However, self-reported PA is subject to recall (302) and social desirability bias (303). These measures also do not accurately capture PA intensity levels (304).

Device-based methods offer an alternative means to measure PA, as they objectively quantify motion (e.g., accelerometers, pedometers) or physiological signals (e.g., heart rate monitors) without measurement bias (301). However, device-based methods are not without limitations. Compliance issues (wearing the device for the stipulated time), inability to capture certain types of PA (water-based activities, cycling, resistance exercises) and high costs, limit the use of these methods. Depending on study aims, the PA outcome of interest and the study feasibility and logistics, self-report questionnaires or device-based methods may be employed individually or in tandem to assess PA.

1.2.7.2.1 Subjective measures of PA: questionnaires

There are many self-report questionnaires currently available for use with children and adolescents (305). I focus on the Physical Activity Questionnaire for Adolescents (PAQ-A), as it has been used extensively by our research group and allows for valid and reliable assessments of MVPA in school-based settings (20,306). The PAQ-A was designed to be self administered in large-scale studies. The questionnaire captures PA conducted in the past seven days and provides an overall mean PA score derived from eight items, each scored on a 5-point scale (307). Reliability and validity of the PAQ-A to assess general PA levels in secondary school students, are strong (307). Kowalski and colleagues examined the convergent validity of the PAQ-A with PA data from the Caltrac accelerometer, worn by adolescents between Grade 8-12 over a 7-day period (different from the 7-day period assessed by PAQ-A) (20). The study results indicated a low ($r=0.33$) but significant association between the PAQ-A score to objectively measured PA (20). Janz and colleagues compared the PAQ-A with Actigraph accelerometer data to assess concurrent validity. Participants completed the PAQ-A immediately after wearing the

accelerometer for five days (at least one weekend and at least an hour for each time segment present in PAQ-A – morning, lunch, afternoon, evening) (308). They reported a moderately high ($r=0.63$; $p<0.05$) significant association between PAQ-A score and accelerometer derived MVPA (308). Although PAQ-A is a valid and reliable tool to assess PA levels, it is unable to estimate intensity, time and frequency and its validity and reliability is specific to school day measurement. However, it is highly applicable in large research settings, easy to administer at low cost.

1.2.7.2.2 Objective measures of PA: accelerometers

Accelerometers provide an objective measure of PA by detecting acceleration in body movements (reported as counts) using piezoelectric transducers and microprocessors. Counts are arbitrary units that are translated into levels of PA intensities from calibration studies. These counts are processed to generate output of PA intensity, duration and frequency (22). Specifically, they provide categories of PA intensity based on set 'intensity' cut-off points (28,309,310). Cut-points derived from calibration studies are variable depending on the calibration methods (direct calorimetry, doubly-labelled water, laboratory or field setting) and age of those studied (21). Thus, the application of different cut-points prevents direct comparison across studies that used accelerometry to assess PA.

Accelerometers are calibrated to quantify energy expenditure. PA intensity levels are interpreted as metabolic equivalents (METs) – defined as the ratio of work metabolic rate to a standard resting metabolic rate of 1.0 (4.184 kJoule or 1 kcal per kg/hr) (311). In general, PA intensity is categorized as either light PA (LPA, <3 METs), moderate PA (MPA, 3-6 METs), vigorous PA (VPA, >6 METs) or moderate-to-vigorous PA (MVPA, ≥3 METs) (312). Sedentary behaviour typically does not exceed resting energy levels and is defined as activity levels between 1-1.5 METs (e.g., sitting, sleeping, lying down and watching television) (313).

The parameters used to collect and assess PA from accelerometry – *epoch length*, *wear time, non-wear time* and *cut points* – may be different between adults and youth (21). I briefly discuss each of these parameters below.

First, prior to accelerometer use, an epoch length is selected. An epoch is the time interval over which counts are measured and integrated. Epoch lengths can affect estimates of PA intensities (314); a 15-second epoch length is recommended to accurately capture PA of adolescents (315) compared with a 1-min epoch length for adults. Second, the amount of wear time that constitutes a valid day (including criteria for establishing non-wear time), and the number of valid days required for inclusion are set to ensure that the measurement period provides an accurate representation of general PA. Generally, at least 10 hrs/day of wear time is used to identify a valid day (316). Seven days of wear represents 'usual' PA across all ages; however, majority of studies of adolescents adopted protocols that adopted 3-4 days of wear days in their analysis (317). Third, non-wear time cut-points are used to identify and excluded as non-wear periods. A summation of continuous zero counts per minute is the typical way to identify non-wear time (317). However, this may also include sedentary periods and not true non-wear periods. Furthermore, spurious counts may occur due to slight movements while sedentary or when moved (e.g., bumped or moved from a spot to another) but not worn during PA. In studies of adolescents, investigators have used a period of 20 min and 30 min of consecutive zeros of counts per minute as a criterion of non-wear time (317). Fourth, to translate accelerometer counts into accessible measures of PA, cut-points are used to define sedentary behaviour and PA intensity based on the population of interest. Trost and colleagues compared five different accelerometer cut-points for children and adolescents to measures of intensity assessed using indirect calorimetry. They reported that cut-points published by Evenson and colleagues (28) most accurately classified PA intensity (sedentary, LPA, MPA and VPA) among children and adolescents aged 5 to 15 years (310).

Accelerometers can provide highly accurate assessments of PA level when appropriate methods are used. However, there are limitations to the use and interpretation of results from accelerometry. Accelerometers are unable to assess water based PA (as these devices are not waterproof) or PA that requires minimal trunk movement (e.g., cycling) (300). Accelerometers are also unable to account for increases in METs if a person carries an extra load (e.g., backpack) when conducting certain PA (e.g., hiking) (300). Without a consensus on the protocols to report, collect, process and analyze, results from accelerometry studies are often non-comparable (21).

1.2.7.3 Sedentary behaviour – definition and concept

There are two commonly used definitions of sedentary behaviour. The first one is limited to the intensity of the activity, whereby sedentary behaviour is any waking behaviour of ≤1.5 METs. The second definition of sedentary behaviour takes into accounts both intensity and posture, whereby sedentary behaviour is any waking behaviour of ≤1.5 METs in a sitting or reclining position (318). The latter definition takes into account the positive benefits of standing from increased muscle activation (319) including lower mortality (320) and improved cardiometabolic markers (321). As there is no consensus currently on the precise definition, the definition adopted by researchers will depend on their research focus. As there are no studies of the association between health outcomes and standing or sitting in adolescents, I adopt the first definition of sedentary behaviour in my thesis as it is more general and has been used in epidemiological studies (322–324).

1.2.7.3.1 Measuring sedentary behaviour

One novel use of accelerometers is to operationalize and quantify sedentary behaviour. Previously, sedentary behaviour was usually captured by questionnaires that depended on recall skills to estimate sedentary behaviour time, frequency and type (e.g., sitting down or lying down reading)

(325,326). Hardy and colleagues validated their sedentary behaviour questionnaire with accelerometer results in adolescents (325). They showed that the questionnaire and accelerometer results had criterion validity with less than 5% of data points outside the limits of agreement. This is despite the 3.2±11.2 hours/week discrepancy between accelerometer and questionnaire measures of sedentary behaviour. Nonetheless, as mentioned in section 1.2.7.3.1, SED can be accurately captured by accelerometry.

1.2.7.3.2 Association between measures of sedentary behaviour and bone outcomes

There are five studies, to my knowledge, that examined the influence of SED on bone properties. As 'unloading' has known detrimental effects on the skeleton (327) sedentary behaviours would theoretically be associated with low bone mass (by DXA). Results, however, are inconsistent. Some studies support this association (322,328–330) while one study from our group reported no association between SED and bone architecture and strength at the distal tibia (assessed by HR-pQCT) (331). From the National Health and Nutrition Examination Survey (NHANES), Chastin and colleagues showed that MVPA (by accelerometry) was no longer associated with BMC when sedentary activities (by self-report) were entered into regression models (329). One hour per day of screen time was associated with lower values for proximal femur BMC (0.77 g lower in girls and 0.45 g lower in boys) (329). These differences are likely associated with how sedentary behaviour was assessed, the bone site measured and the instrument used to assess bone. Thus, new evidence is emerging that highlights the need to examine the influence of PA and SED on bone outcomes during growth.

1.2.8 Studies of PA and bone strength in children and adolescents

In this section, I briefly provide an overview of how PA can influence bone health, followed by a review of studies of PA and bone health conducted in children and adolescents. I focus on the highest

levels of evidence, systematic reviews, RCTs and longitudinal studies that assessed the influence of PA on bone strength, structure and density in children and adolescents.

1.2.8.1 The influence of PA on bone health

Earlier, I discussed the mechanostat theory and how bone positively responds to loading and impact (section 1.2.2.3). I also provided an overview of the principles whereby weight-bearing exercise influences bone adaptation (section 1.2.2.3). Figure 1.21 illustrates how exercise affects bone through; 1) direct impact, (e.g., high ground reaction forces when the feet strike the ground, 2) muscle forces from contractions during PA, and 3) endocrine and paracrine related pathways (e.g., myokines that inhibit adipogenesis and positively stimulate osteogenesis and myogenesis (332). In previous child and youth studies that investigated the effect of mechanical loading on bone, it was not possible to identify pathways that played the most dominant role. However, a recent meta-analyses included RCTs conducted with children and reported a positive benefit for PA on aBMD and BMC accrual (assessed using DXA) (333,334). Importantly, PA during childhood and adolescence may impart lifelong benefits for bone health (335). The life stage when PA participation occurred influences bone mass outcomes, as illustrated in Figure 1.22.

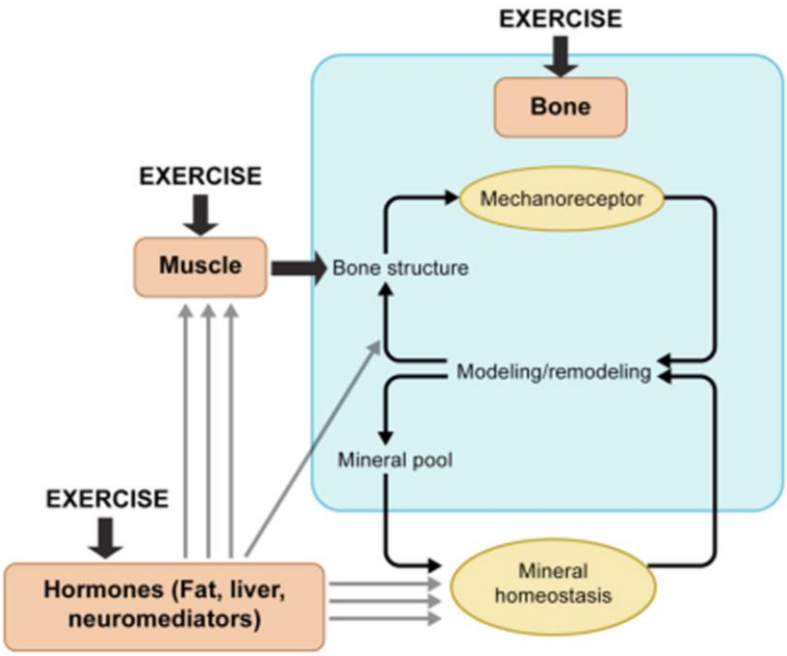


Figure 1.21. Schematic that illustrates the effect of exercise on the skeleton. During exercise, load is transmitted to the skeleton through direct stimulation on bone mechanosensors and by indirect stimulation through dynamic muscle activity. Hormones from fat and the liver modulate loading by affecting bone and muscle growth as well as muscle performance, and act indirectly through potential changes in the mineral reservoir. Reproduced from Bonnet and Ferrari (336), with permission from International Bone and Mineral Society.

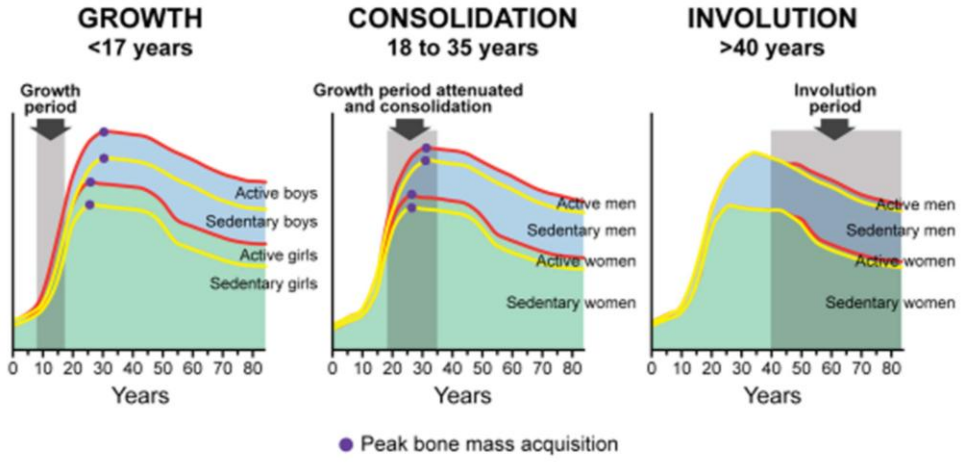


Figure 1.22. A schematic that illustrates the effect of physical activity on bone mass at different periods across the life span. The red curve represents a continuous exercise effect. Without a bone loading stimulus through exercise, bone mass accrual could be attenuated over time. The yellow curve represents a person engaging in normal activities (e.g., walking). Reproduced from Bonnet and Ferrari (336), with permission from International Bone and Mineral Society.

The University of Saskatchewan's PBMAS longitudinal study followed the growth and development of girls and boys from childhood to adulthood (11,12,337). Physically active boys and girls followed for at least six years with entry age between 8 and 14 years, had greater bone mineral accrual (7% to 17 % more total body BMC assessed using DXA) than their less active counterparts (11,12). After adjusting BMC for body size (height and body mass), the investigators found that active boys (top quartile) had 9% more total body and 7% more femoral neck BMC compared with inactive (lowest quartile) boys one year after peak bone mineral accrual velocity (11). Active girls had 16% and 11% more total body and femoral neck BMC, respectively, a year after peak BMC accrual velocity (11). Fifteen years later, PBMAS participants returned for follow up measurement as young adults. Adults who were considered active adolescents had significantly greater bone CSA (4% - 7%) and Z (5% - 8%, by DXA-HSA) (controlled for sex, height, body mass, CSA and Z at APHV, sex and relative lean mass) compared with adults who were considered as inactive during adolescence (337). This long-term study, although difficult to conduct due to high costs and attrition, provided important insights regarding the positive role of PA in bone accrual and development of bone structure at the proximal femur. However, as they used self-report measures of PA (PAQ-C), they were unable to assess the amount (frequency and duration) and type (intensity, weight-bearing, endurance) of PA that conferred these benefits. RCTs designed to evaluate the effect of PA on bone strength would confirm and advance these early findings.

A recent systematic review aimed to identify the influence of PA on bone strength across the lifespan (37). The authors focused on RCTs with a minimum 6-month intervention period and on bone strength as the primary outcome. They reported a small but significant effect of PA on bone strength in prepubertal boys (effect size = 0.17, 95% CI 0.02-0.32) from three different studies (5 publications). However, Nikander et al. grouped all bone strength parameters together regardless of skeletal site measured (radius or tibia) or instrument used to assess bone strength (DXA, pQCT). Thus, the findings may over-simplify complex and site-specific relationships. Although this first review makes an important

complication, results from the meta-analysis should be interpreted cautiously. I extended Nikander and colleagues' review by broadening the scope of eligible studies to include RCTs and observational studies that assessed the influence of PA on bone strength and structure or density in children and adolescents. I include this systematic review as a part of my thesis, in section 3.1.

1.2.8.2 Association between objectively measured PA and bone outcomes

Some studies used accelerometry to investigate the relation between PA intensity and bone outcomes in children and adolescents. They reported that MVPA and VPA were related to bone strength, structure and density in children (338–340) and adolescents (13,341). In boys who were followed longitudinally over six years (mean age 5.2 years at baseline), 40 minutes of MVPA per day was associated with a 3-5% larger CSA and greater bone strength (Z, assessed by DXA-HSA) at the femoral neck compared with boys who performed 10 min/day of MVPA (adjusted for lean mass) (339). In adolescent boys (age 12.5-17.5 years, n=189), less than 45 min/day of MVPA was associated with reduced bone mass while more than 78 min/day of MVPA or more than 28 min/day of VPA were associated with higher values for aBMD at the femoral neck (by DXA) (13). In pre- and peri-pubertal boys and girls, VPA explained 2-11% of variance in bone strength at the proximal femur (assessed by DXA) after adjusting for lean mass (338,340). In another cross-sectional study, VPA was associated with bone area and BMC at the tibial shaft (assessed using pQCT) in boys and girls (mean age 15.5 years, n=1748) (341). There was a 7-mm^2 greater bone area in those in the highest quartile of PA (VPA) compared with those in the lowest quartile of PA (341). Due to the different cut-points used to categorize MVPA and VPA, comparisons across studies should be interpreted with caution.

Accelerometers are unable to specifically record high impact PA levels that are most osteogenic (section 1.2.2.3, principles of bone adaptation). For example, Actigraph accelerometers are able to capture

accelerations as high as 2.5 G during weight-bearing PA (342). However, higher intensity activities (e.g., running at speeds > 10 km/h (equivalent to 10 METs) (343)) generate accelerations greater than 2 G, 30% to 45% of the time (344). Vertical accelerations greater than 4.2 G were not associated with higher values for femoral neck aBMD (assessed by DXA) in adolescents (344). Artistic gymnastics, a very high impact sport, generates accelerations of about 4 to 8 G (345) and has a MET value of 3.8 (343). This high-impact sport would be classified as MVPA. Thus, although accelerometers have advanced our field, an alternative means to assess the direct impact of PA on bone outcomes is still needed.

1.2.8.3 School-based intervention studies on PA and bone health

Schools are targeted settings for PA intervention trials in children and adolescents for several reasons. First, school-based PA interventions reach a large number of children and adolescents from diverse backgrounds. Second, schools are considered ideal settings for health promotion research, as nearly 50% of waking hours for the first two decade of a child's life are spent in schools (346). Third, schools provide a setting where successful interventions could be integrated into the curriculum. Targeted programs could potentially comprise a part of teacher development and training, supported by PA-promoting infrastructure.

Previous PA school-based intervention studies tended to focus on changes in physical education (PE) programs. However, multi-pronged, whole school-based interventions proved most effective (347). Importantly, these models have potential to translate research into practical application (348). A whole school-based intervention also eliminates stigmatization of specific individuals or groups (e.g., less physically active, the overweight or obese) while providing a platform to disseminate the good intentions of the intervention to all students (347). Based on the social ecological theory, changes in the social setting and environment encourage and support individuals to adopt positive health behaviours (349). Another

advantage of a whole school-based intervention is that it avoids contamination across individuals when the intervention is contained within an institution in contrast to a case where both intervention and control groups are within the same institution.

However, a meta-analysis that investigated the effect of school-based PA interventions on PA levels showed no significant effect in adolescents (38). Some studies adopted social cognitive or social action theory (350) or the transtheoretical model of behaviour change, while others used non-theoretical models. As adolescents transition to adulthood they seek to establish their independence. Thus, some studies suggested that self-determination or intrinsic motivator models may positively influence PA (351–353). Lonsdale and colleagues (354) reported that Grade 10 equivalent boys and girls who had higher self-determination performed more PA (steps/min) than students with low self-determination, regardless of whether they were in structured or free choice PE class (354). It may be that perceived competence, autonomy and sense of relatedness or social belonging (embedded within self-determination theory) (355) are factors that drive adolescents' degree of choice and empowerment related to becoming more physically active. Prescribed PA regimens provided as a part of structured interventions as in most RCTs may not appeal to adolescents. Furthermore, new findings from studies that used self-determination theory (SDT) encouraged the application within a whole school model to increase adolescent PA (356,357).

To date, only a handful of school-based interventions used 3-D imaging tools to assess the effect of PA on bone strength and structure (32,34,358). None included older adolescents (>14 to 17 years). Our research group designed and implemented Action Schools! BC – a whole school-based intervention model that sought to provide students (average age 10.1 years at baseline) with 150 minutes of PA/week across 6 Action Zones [www.actionschoolsbc.ca]. The cornerstone of the model -- 'classroom action' -- provided students 15 minutes/day, 5 days/week of classroom based PA (32,113). Within classroom action, children attending intervention schools (n= 281) participated in a progressive jumping program called Bounce at

the Bell. Students performed high-impact jumps 3 times/day (at the morning, noon and end of the day school bell), about 1 min each time, 4 days a week. Based in part on its simplicity, compliance with Bounce at the Bell was high; implementation rate by intervention teachers was 74%. After an intervention period of 16-months, investigators assessed changes in bone structure and strength using pQCT. They reported significant increases in BSI (4%) and total BMD (2%) at the 8% site of the tibia in pre-pubertal intervention boys (n=143), compared with controls (n=64) (32). Change in the maximum second moment of inertia (I_{max}) at the tibia midshaft in boys, was also significantly higher (3%) in the intervention group compared with controls (113).

Two studies in the 6 to 12 years age group found no differences in bone outcomes between intervention and control groups following 28 (34) or 36 (358) weeks of PA intervention. Specifically, Greene and colleagues used MRI to examine changes in mid-third femoral bone structure and estimated bone strength in elementary school children who participated in a three times a week, 28-week high impact loading intervention (34). Students performed 10 sets of single-leg drop landing exercises from different drop-heights (High or Low) off a step bench. There was no difference for any bone values between the exercised leg and the non-exercised leg at the mid-third of the femur. This finding might be due in part, to the short time period for the intervention and/or the small sample size (n= 13 in each of High, Low intervention and control groups) (34). Anliker and colleagues conducted a 10 min, twice a week, circuit that comprised of five jumping and sprinting exercises for 36 weeks in 8 to 12-year old boys and girls (Intervention: boys, n=12, girls, n=18; Control: boys, n=16, girls, n=14) (358). They found no differences in bone strength and structure between intervention and control groups at the end of the intervention period (358).

It appears that frequency of the intervention rather than intensity and duration of PA may be key to eliciting a positive effect in bone in this age group but each study had a different follow-up period – the

shortest was 28 weeks (34), followed by 36 weeks (358) followed by 16 months (32,113). It might also be due to a longer intervention duration. Bounce at the Bell was performed 4 day/week, three times each day (32,113). Thus, weight-bearing bones received a short bout (1 min/bout; 3 bouts/day), high impact stimulus with ground reaction force equivalent to 5 times BW (359). Other interventions delivered a 'dose' comprised of continuous 10 to 15-min PA bouts of stretching and jumping exercises, two (358) to three (34) times per week. Overall impact of jumps were similar across the three interventions as jumps were either single- or two-legged jumps (Greene and colleagues used a single-leg landing protocol). These studies were conducted in elementary schools. To my knowledge there are no PA interventions in a secondary school setting that assessed bone strength and structure (using 3-D bone imaging tools) in older adolescents.

1.2.9 Summary

Conducting studies during adolescence – when biological and physiological systems are developing rapidly at different tempos and at different times and when youth are transitioning into adulthood – is a challenge. Given the tremendous variability in maturity level, studies in this age group must account for sex and maturity levels of participants in the study design and the statistical approach. Thus, it is not surprising that few studies examined the effects of whole school-based PA interventions on bone strength and structure in adolescents. I address this gap in my thesis and incorporate a novel peer-to-peer intervention in a secondary school setting to evaluate the effect of this choice based model on adolescent bone health (strength, structure, density). I also extend the literature by assessing PA objectively using accelerometry, in conjunction with a validated PA questionnaire. Another novel component is that I assess SED by accelerometry to evaluate its independent effect on bone density, structure and strength. Given the central role of muscle in bone's response to exercise, I will examine the contribution of muscle strength to bone strength and structure at non-weight bearing bone sites. Taken

together, these novel components comprising my thesis represent the first whole school-based study of secondary school students that aimed to enhance PA and evaluate its influence on bone strength, structure and density.

1.3 Rationale, Objectives and Hypotheses

1.3.1 Part I: A systematic review of the effects of physical activity on bone strength and structure in children and adolescents

Rationale: In the past two decades, studies of the pediatric skeleton shifted from assessing the acquisition of bone mineral to assessing the acquisition of bone strength and the contribution of bone density and structure to bone strength. This is possible through the advent of safe, precise and accurate 3-D bone imaging instruments such as pQCT and HR-pQCT. However, we still know surprisingly little about how bone strength adapts in the rapidly changing adolescent skeleton and even less about the role of PA in those adaptations. Therefore, I extend the literature by conducting a systematic review (including RCTs and observational studies) that evaluates the effect of PA on bone strength and structure in adolescents.

Objective: To systematically evaluate the effect of PA on bone strength and structure (i.e. bone density, total area, cortical area, cortical thickness) in children and adolescents.

Contribution: This systematic review (360) is a comprehensive and thorough examination of RCTs and observational studies that evaluated the effects of PA on bone strength and structure in children and adolescents. This is the first systematic review of PA studies in children and youth that focused on bone

structure and estimated bone strength as primary outcomes. I extend the scope of previous reviews to include observational studies with recreational PA and athletic populations.

1.3.2 Part II: Determinants of bone strength and structure in adolescent boys and girls

Rationale: Several factors are known to influence bone strength in children – PA is among them. However, we know relatively little about factors that influence bone strength accrual in adolescents. Thus, I aim to identify the role of modifiable factors (with a focus on PA) on bone outcomes (bone strength, density and structure) in adolescents. To do so I will account for non-modifiable traits such as ethnicity, sex and maturity in my analyses. I also examine relationships between bone outcomes and muscle strength, MVPA, SED and VPA.

Objectives:

1. To identify PA factors (with a focus on intensity) that predict estimated bone strength in adolescent boys and girls (accounting for sex, maturation, ethnicity, limb length and muscle mass).
2. To identify PA factors (with a focus on intensity) that predict bone density and structure in adolescent boys and girls (accounting for sex, maturation, ethnicity, limb length and muscle mass differences).

Hypotheses

H_1: PA is positively associated with bone strength (adjusting for sex, maturation, ethnicity, limb length and muscle mass).

H_2: PA is positively associated with bone structure (total area, cortical area and medullary area) but not bone density (adjusting for sex, maturation, ethnicity, limb length and muscle mass)

Contribution: This study is novel from several aspects other than the less studied 15-year old cohort. First, this is the first study on the association of objectively measured PA *and* SED on bone strength, structure and density *assessed by a 3-D bone imaging tool (pQCT)* at the distal *and* shaft sites of the tibia. Second, my investigations on the association of *muscle function* (i.e. grip strength) on bone strength, structure and density (by pQCT) at the distal *and* shaft sites of the radius, *while controlling for muscle mass*, will provide insights on the muscle-bone unit interaction. Overall, my findings will inform intervention studies on the determinants of bone strength, structure and density from PA-related factors in 15-year old boys and girls.

1.3.3 Part III: Effect of a secondary school physical activity intervention on bone strength and structure in adolescents.

Rationale: School-based PA intervention programs may be the best means to access youth across a range of ethnicities and socioeconomic strata. Historically, schools represent a setting where strong compliance and an effective delivery system hold promise for PA interventions (361,362). As adolescents transition into adulthood, choice-based models may represent the best (and a novel) method to encourage PA and decrease SED. Increased engagement in PA may, in turn, provide positive bone health benefits. Finally, we need to know more about the kinds of PA programs youth are willing to engage in and the effect of novel models on PA and bone outcomes. From this starting place, we can then design more effective school-based programs that target bone health.

Objectives:

1. To assess the effect of a choice-based PA intervention (Health Promoting Secondary School (HPSS)) on estimated bone strength at the tibia and radius in adolescent boys and girls.
2. To assess the effect of a choice-based PA intervention (Health Promoting Secondary School (HPSS)) on bone density and structure at the tibia and radius in adolescent boys and girls.

Hypotheses

H_1: Students who participate in the intervention (HPSS) will have greater bone strength at the tibia and radius at the end of the 8-month study, compared with students randomly assigned to the control group.

H_2: Students who participate in the intervention (HPSS) will have greater bone structure (total area, cortical area and medullary area) and density at the tibia and radius at the end of the study, compared with students randomly assigned to the control group

H_3: Students who participate in the intervention (HPSS) will demonstrate a greater increase in PA at the end of the study, compared with students randomly assigned to the control group.

Contribution: I extend the sparse literature by conducting a school- and choice-based RCT that examines the effect of PA on bone health in adolescents. This is the only study that examined both a weight-bearing *and* non-weight bearing bone together in the same 15-year old boys and girls. My findings will shed light on how bone strength, structure and density adapt to PA in this age group and will guide design of effective secondary school-based interventions in future.

Chapter 2: Methods

In this chapter, I present the methods I used to address my research questions (described in section 1.3). Specifically, in section 2.1, I describe the protocol adopted to conduct the systematic review (Part I). In sections 2.2 and 2.3, I provide an overview of the Health Promoting Secondary Schools (HPSS) intervention and the methods used to examine my research questions (Parts II and III), respectively.

2.1 Part I - Systematic Review Protocol[3]

I conducted the systematic review as per PRISMA (363) and Centre for Reviews and Dissemination (University of York) (364) guidelines. Before starting the systematic review, I conducted a search online in the Cochrane Databases and PROSPERO to ensure that there were no similar reviews in progress. Under the guidance of Drs. Maureen Ashe and Heather McKay, I established a systematic review protocol and I registered the systematic review in PROSPERO (No: CRD42013003948, http://www.crd.york.ac.uk/PROSPERO/) to avoid duplication of work. PROSPERO provides an efficient use of resources through their online consolidation of completed and in-progress reviews.

[3] Section 2.1 is published as a condensed version in the methods section along with working definitions of bone terms as part of my systematic review manuscript (Chapter 3).

2.1.1 Search strategy

I drafted the search strategy and consulted information specialist, Madeline (Mimi) Doyle-Waters, from the Centre for Clinical Epidemiology and Evaluation (C2E2), to fine-tune the literature search. We ensured the search terms and commands used were sensitive to locate relevant articles within respective databases. I had four search concepts 1) bone physiology, 2) bone anatomy, 3) PA and 4) imaging techniques. These concepts were selected based on our outcomes of interest and after considering how librarians coded information; we were able to efficiently search for relevant articles to include in the systematic review. I outline key words and subject headings in Figure 2.1. I used search filters from the Scottish Intercollegiate Guidelines Network (SIGN) for Medline and EMBASE databases to target peer-reviewed RCTs and observational studies (365). Lastly, I used age-specific phrase filters in addition to individual database age-limiters to enhance the search sensitivity for studies on children and adolescents (366). To consolidate the outcomes of the search concepts, I used Boolean operators "AND" and "OR" as displayed in Figure 2.2. To be inclusive I did not restrict searches to English publications.

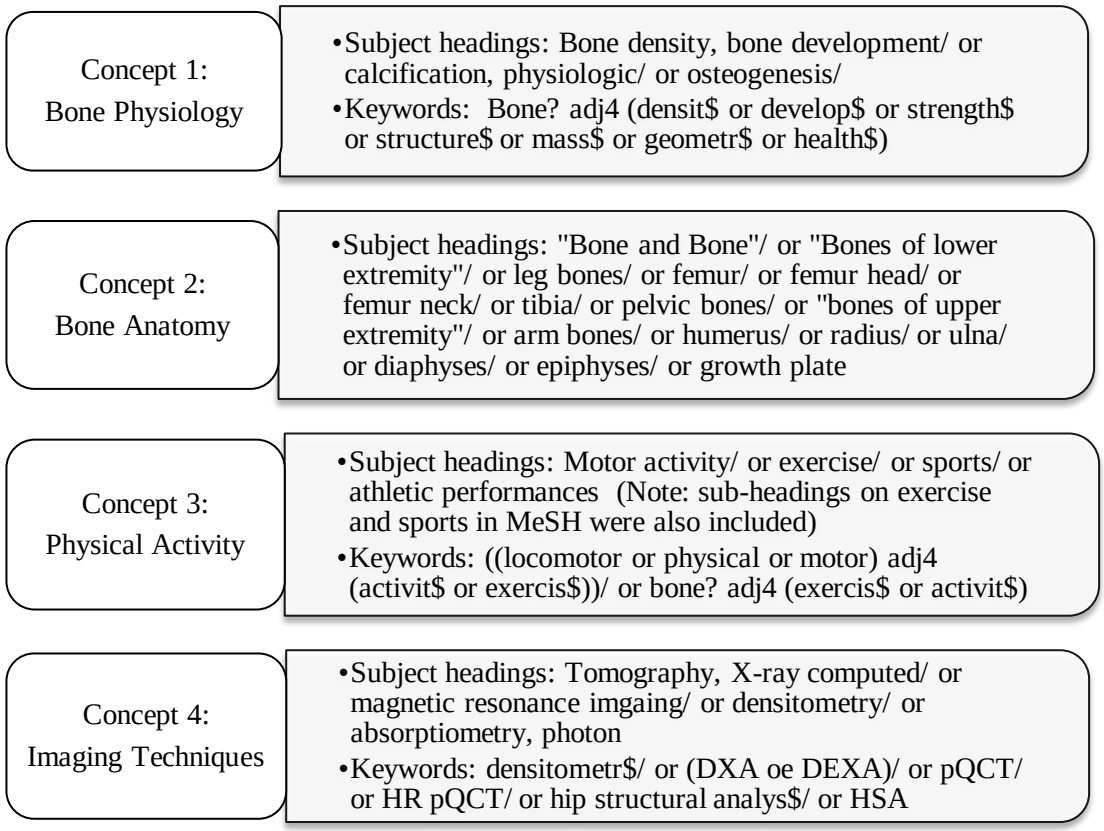


Figure 2.1. Key words and subject headings specific to the search concepts applied in electronic databases searches.

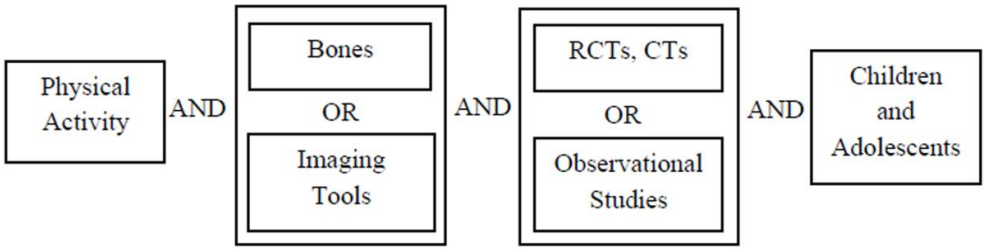


Figure 2.2. Search concepts, the use of Boolean operators and search filters to identify relevant articles for the systematic review. *RCTs, randomized controlled trials; CTs, controlled trials*

I searched Medline (1946 to 17 January 2013), EMBASE (1974 to 17 January 2013), CINAHL (1982 to 17 January 2013), Sport Discus (1921 to 17 January 2013), PEDro (1929 to 17 January 2013), Informit (1977 to 17 January 2013) and Cochrane Central Register of Controlled trials (up to January 2013) for relevant studies. I exported search results into Refworks (Bethesa, MD), an online database

management and citation system, and removed duplicates using the 'close match' and 'exact match' function.

I screened eligible studies by title and abstract based on inclusion and exclusion criteria (Table 2.1) with assistance from Dr. SoJung Kim. Then, we obtained full-text articles and divided the number of articles between us. Based on a predetermined template, we extracted data from the studies. We both counter checked all the information obtained in data extraction tables and included studies upon mutual agreement; Dr. Ashe resolved any discrepancies.

Table 2.1. Inclusion and exclusion criteria for systematic review studies.

Inclusion criteria
• Study participants who are healthy and aged between 5 and 18 years. • Study reports bone strength and/or bone geometry/structure. • PA a component of the study (measured, intervention program or sports-related training). • Full-text, published in peer-reviewed journals. • Randomized-controlled, controlled- and observational studies (prospective and cohort studies).
Exclusion criteria
• Studies with participants over 18 years old grouped into the analysis. • Studies with co-interventions such as nutrition supplementation and muscle function. • Case studies and validation studies. • Non-English full-text publications.

2.1.2 Quality assessment

We assessed the quality of all included studies using the validated Quality Assessment Tool for Quantitative Studies (Appendix A**:** Effective Public Health Practice Project Tool & Dictionary), developed by the Effective Public Health Practice Project (EPHPP), Canada (367). Although previous studies used the Cochrane Collaboration Risk of Bias Tool (CCRBT) to assess quality, we chose the EPHPP tool because our systematic review included both RCTs/CTs and observational studies. We also noted other benefits of the EPHPP tool compared with the CCRBT. Both tools provide different quality

scores due to the different constructs measured by each tool (368). However, the EPHPP tool uses a generic scale to assess study design while CCRBT was designed specifically to assess RCTs (e.g., sequence generation, allocation concealment and blinding of participants/researchers are heavily scored in the overall assessment). Inter-rater agreement was higher in the EPHPP tool (ICC=0.77, 95% CI: 0.51-0.90) versus CCRBT (ICC=0.58, 95% CI: 0.20-0.81) due to the relative subjectivity in the guidelines used to score the domains in the CCRBT (368). The Guidelines for Systematic Reviews in Health Promotion and Public Health Taskforce (369) also recommended the use of the EPHPP tool due to its ability to assess not only RCTs but also quasi-experimental and uncontrolled studies to be used in systematic reviews.

The EPHPP tool has eight domains and scores are assigned for six of the domains which are 1) selection bias, 2) study design, 3) confounders, 4) blinding, 5) data collection methods and 6) withdrawals and dropouts. We did not assess the intervention integrity or the statistical approach used, as these two domains do not contribute to the overall score generated from the EPHPP tool. The overall score for each article was rated STRONG, MODERATE or WEAK based on the scoring instructions. A STRONG study consisted of at least four STRONG domains and no WEAK domains. A MODERATE study had less than four STRONG domains and no more than one WEAK domain. WEAK studies had two or more WEAK domains. The EPHPP tool and dictionary is provided in Appendix A**:** Effective Public Health Practice Project Tool & Dictionary. Dr. Kim and I independently assessed all included articles using the EPHPP tool. We determined inter-rater precision using 10 randomly selected studies. The intraclass correlation coefficient (ICC) for the global score assessment was 0.90 (95% CI: 0.74 – 0.96). Before we attained the final global score for each study, we compared individual scores for all the six domains and resolved any differences in scoring through further discussions.

2.1.3 Narrative synthesis

Based on our initial data extraction, we concluded that a meta-analysis of estimated bone strength outcomes was not viable due to substantial variability in imaging tools (at least two different DXA manufacturers and a variety of pQCT, HR-pQCT and MRI systems) and protocols used to acquire images (differences in bone scan sites, reference lines, voxel size). Analysis methods used to derive estimated bone strength were also vastly different. DXA-based measurements used different algorithms to generate estimates of bone strength and tomography scans used different thresholds and analysis parameters that generated non-comparable results. Maturity assessment varied across studies, adding to the complexity of conducting a meta-analysis. Thus, we elected to conduct a narrative synthesis (364).

The Centre for Reviews and Dissemination's guidelines outlined four main elements of the narrative synthesis framework. These elements are: 1) theory development, 2) preliminary synthesis development, 3) exploration of within and between group relationships and 4) assessment of robustness. Rodgers et al. (370) evaluated these four elements by comparing outcomes from a guided narrative synthesis with same study data derived from the meta-analysis. Use of the guide increased transparency of narrative methods. I adopted the four elements as steps to compile and generate data from studies included in the review.

The first step of the narrative synthesis framework is theory development. Therefore, we theorized that *weight-bearing or impact-related PA increases estimated bone strength in children and adolescents.* For the second element of the narrative synthesis framework (preliminary synthesis development), I grouped the studies based on study design. RCTs/CTs were grouped together; observational studies were categorized into recreational PA or organized sports studies. I extracted key study components and results from each individual study and compiled them in a table.

For the third element of the guided synthesis (exploration of within and between group relationships), I formed sub-groups of studies based on similarities among sex and maturity characteristics of study participants. For organized sports studies, I further grouped studies based on the type of sport. To conduct within group analyses, I examined PA moderator variables defined in RCT/CT studies. These included frequency, intensity, type and duration of PA conducted, the total intervention period, compliance reported and study setting (e.g., school-based). For leisure PA observational studies, moderator variables were frequency, intensity, type and duration of PA, methods of assessing PA either objectively or subjectively and quantified PA levels or scores. For organized sports studies, the moderator variables were starting age in the sport, type of sport (weight-bearing or non-weight bearing) and training history (hours/week, years trained, years stopped). I conducted between-group analyses with a focus on positive bone strength outcomes derived from each sub-group. I also evaluated specific bone variables (structure, mass, density) that may explain or contribute to changes in bone strength. In addition to PA related variables, I examined the possible influence of anatomical sites, duration of PA/training exposure and muscle function on bone outcomes across studies.

For the fourth element of the guided synthesis (to assess the robustness of the synthesis conducted), I highlighted the strengths and limitations of the evidence generated from the synthesis in the discussion for Part I (the systematic review) in section 3.1.

2.2 Part II – Health Promoting Secondary Schools

In this section, I discuss development and implementation of the HPSS intervention model. HPSS was a whole-school model that promoted PA and healthy eating in secondary schools and discouraged sugar-sweetened beverage intake and excessive screen time. This was achieved through active

participation of students and teachers who planned and implemented changes for health promoting behaviours through curricula (Planning 10 and PE 10), school activities and policies. HPSS targeted Grade 10 students in British Columbia secondary schools, as that is the last year that PE is mandatory and health-related education is provided through Planning 10 lessons.

2.2.1 Development of the HPSS model

The HPSS team developed the model using community-based research principles (371) that encouraged study participants to be actively involved in the design and implementation of the intervention. HPSS was therefore referred to as a 'Real Community Trial' (ReaCT) whereby the intervention was feasible and tailored to the specific needs of the school and its students. Translating research studies into real-life programmes involves participation of stakeholder groups and is more likely if a study design is geared towards gathering practice-based evidence rather than evidence elicited from more rigidly controlled efficacy trials (372). This, therefore, was the premise adopted for HPSS, which is considered an effectiveness rather than an efficacy trial.

The HPSS intervention model was context-sensitive and guided by the self-determination theory (SDT) framework (373). The SDT guided the intervention structure around three main components related to adolescent behaviour: 1) autonomy (a sense of choice), 2) competence (a sense of self-efficacy), and 3) relatedness (a sense of connectedness and social belonging) (374). Design of the HPSS intervention encouraged self-initiation and youth engagement by focusing on intrinsic motivation to sustain the intervention model (373). By engaging youth in planning and implementation, it fostered competence and belonging. This recognizes that adolescents experience increased autonomy when transitioning into adulthood.

2.2.2 Implementation of the HPSS model

The components of the HPSS intervention and the implementation process were tailored to meet school and student needs and focused upon four critical areas: 1) increasing students' PA levels, 2) increasing students' intake of fruits and vegetables, 3) decreasing students' physical inactivity and screen time, and 4) decreasing students' intake of sugar-sweetened beverages. Outcomes from the four focus areas were evaluated on top of the voluntary enrolment into Grade 11 PE class upon completion of the intervention. HPSS was a "For Youth With Youth" model and is depicted in Figure 2.3.

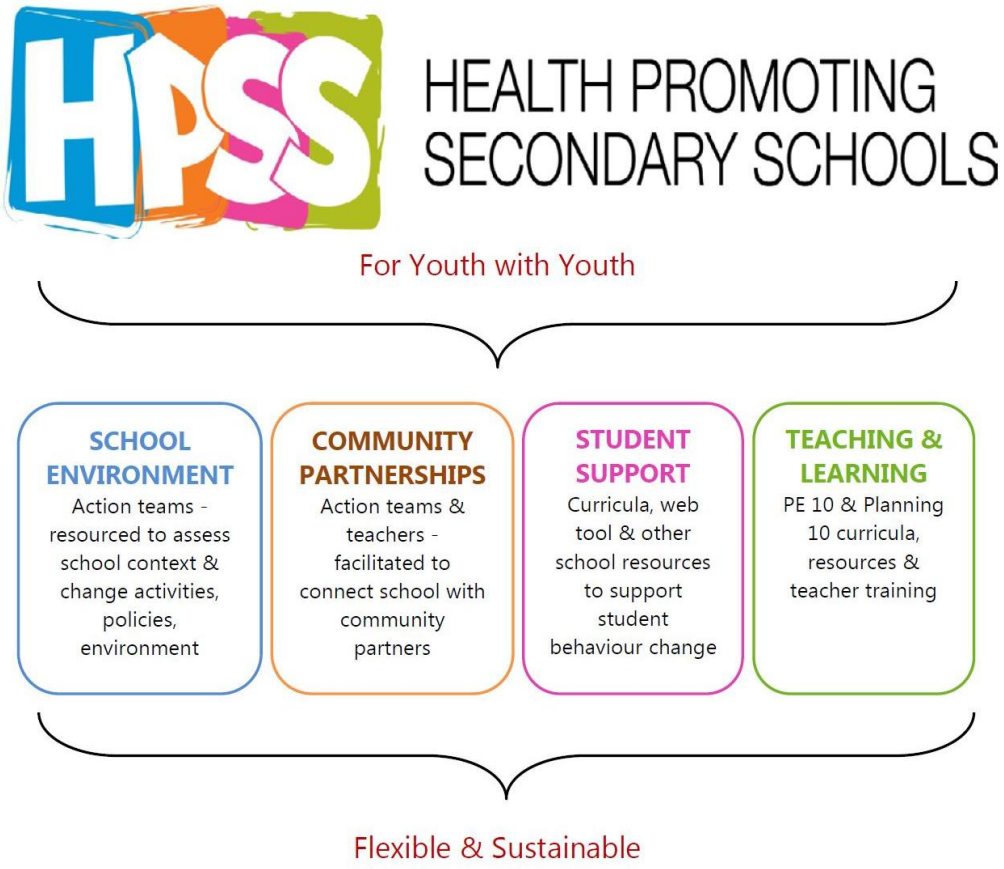


Figure 2.3. The HPSS model with four Action Zones - school environment/culture, community partnerships, student support and teaching & learning to enable a flexible and sustainable program.

The **CORE** components (curricula, opportunities, resources, engagement) of the HPSS intervention implemented in schools were supported by recommendations from Murillo Pardo et al. (375). Briefly, from their systematic review, Pardo and colleagues recommended strategies that results in successful intervention outcomes for adolescents: 1) incorporate multi-component interventions that empower the school community, 2) implement changes to PE programs, 3) design non-curricula programs and activities, 4) use computer-tailored components to implement and monitor interventions, and 5) focus on the needs and interests of girls. Of those recommendations, the HPSS model embedded four of the five recommendations. I elaborate on the **CORE** components of the HPSS intervention, below:

C – Curricula for PE 10 and Planning 10 incorporated choice-based learning activities. In British Columbia, Grade 10 is the last year when health-related lessons delivered through PE 10 and Planning 10 classes are mandatory. After receiving teachers' input, the HPSS curriculum team created a comprehensive curriculum comprised of detailed lesson plans, hand-outs, assignments, tracking tools and background information about guidelines related to PA, screen time, fruit and vegetable intake and sugar-sweetened beverages. Lesson plans incorporated various components with the concept of choice, competence and relatedness embedded, as per SDT principles.

O – Opportunities to increase knowledge and skills beyond the classroom through school-wide events and policies. Each intervention school was asked to implement a minimum of two school-wide events/activities and develop two health-related policies within the academic year. This addresses the role of schools as a larger setting that influences the health behaviour of students (376). In the long run, schools have the authority to maximize ownership and sustainability of the HPSS model if desired.

R – Resources were provided to support teaching and learning among students. Intervention schools received support through grants ($1,400) provided at the beginning of the school year. These funds

covered teacher-on-call expenses (substitute teachers) to release classroom teachers for training, individual needs related to PE 10 and Planning 10 classes and to sponsor school-wide events. Intervention schools also received a one-time grant ($2,500) to support 'health-positive' infrastructure and environment changes within the school. The HPSS school liaison facilitated teacher training workshops for PE 10 and Planning 10. The HPSS team also created a youth-orientated, theory-based website resource with interactive tracking and goal setting tools to support the Planning 10 curricula (http://www.healthyactiveschools.ca).

E – Engagement of youth in design and delivery of school-wide activities, events and policies. A school-based Action Team was formed (6-10 members) with 50% adult and 50% youth representation. The Action Team (guided by the HPSS school liaison) conducted a systematic assessment of the school environment for PA and healthy eating opportunities and identified areas of action. The Action Team used a Healthy Schools Planner to create an Action Plan that clearly stated healthy eating and PA goals (minimum requirement of 2 events/activities and 2 policies within one academic year).

2.2.3 HPSS organizational structure

HPSS was a collaborative project between the University of Victoria (principal investigators: Dr. Joan Wharf Higgins and Dr. Patti-Jean Naylor) and the University of British Columbia (principal investigator: Dr. Heather McKay), funded by the Canadian Cancer Society (CCS, grant no: 227967). The HPSS Bone Health Study (principal investigators: Dr. Heather McKay and Dr. Heather Macdonald) that comprises my thesis was a sub-project within the larger HPSS study and was funded by the Canadian Institutes of Health Research (CIHR, Catalyst grant no: CBO-109634). As the CIHR award came ten months following the CCS award, much of the curricular modules had been developed, and lacked a direct emphasis on bone building physical activities. However, we collaborated with the central program on all

aspects of the implementation and data collection of the intervention and the larger study but I focussed my attention most specifically on those elements and outcomes related to adolescent bone health. Thus, for my thesis I focus only on the bone health part of a larger program of research related to HPSS. Below, I briefly describe the organizational structure for the larger HPSS program of research and specify my role within the Bone Health Study (BHS).

The HPSS Central Team. The HPSS Central Team (comprised of staff school liaisons, Sandy Courtnall and Lauren Sulz, who were HPSS staff members with teacher training) conducted school and student recruitment including participants for the BHS. School contact was limited to interaction with these individuals to ensure consistent and clear communication channels and to avoid confusion within schools. Given their background and training in education, the HPSS Central Team was responsible for implementing the intervention model within schools. The Central Team also evaluated all other outcomes identified as important related to the intervention model that were not related to bone health (e.g., fruit and vegetable intake, screentime levels, motivation for healthy eating).

The HPSS BHS Team. I led the HPSS BHS team and provided input into study design and outcomes. I scheduled schools for measurement and the BHS team conducted measurement sessions independent of the Central Team. Specifically, the BHS team focused on assessing anthropometry, PA, dietary intake of calcium and bone measures of HPSS Grade 10 students that volunteered for the BHS component. I trained and supervised the four-person data collection team, scheduled measurement times with study participants, and personally collected all anthropometry and DXA data. I analyzed all DXA and pQCT bone scans. I also cleaned all data, with the exception of accelerometry data, related to the BHS. Douglas Race, our research team member, imported all BHS data into the FileMaker database. Douglas Race, who is also the HPSS accelerometer coordinator, calibrated, uploaded, cleaned and analyzed all accelerometry

data. I conducted all analyses of the BHS data. These parts and specific methods are described in more detail below.

2.3 HPSS BHS

2.3.1 Study design

The BHS evaluated specific outcomes related to the HPSS RCT, which as stated previously, adopted community-based research principles to create a ReaCT. Schools were recruited as paired-clusters and matched based on geographical and socioeconomic factors (Appendix B). Schools within the same cluster were randomly allocated into intervention or control arms by random draw conducted by Central HPSS Team research coordinator. The intervention took place over one academic year (September 2011 to June 2012). Control schools were offered the opportunity to implement the intervention program and access resources after the intervention study period. I provide the study timeline in Figure 2.4. The UBC Behavioural Research Ethics Board (H10-01917) and the University of Victoria Research Ethics Board (10-168) provided ethics approval for all parts of the study.

2.3.2 Sample size calculation

I used the results of our group's intervention study in elementary school children (32) to calculate the sample size required for the BHS. From the study, prepubertal intervention boys had a 3% greater increase in BSI compared with control boys. Based on 80% power, Type 1 error rate of 5% and a SD of 4% for a two-sided test (377), a total of 28 participants per group were required. To account for 20% attrition rate across a school year and within-sex comparison, we aimed to recruit 200 students (100

intervention, 100 control). This calculation does not account for the clustered study design; however, I accounted for clustering in the statistical analysis.

2.3.3 Recruitment

2.3.3.1 School and teacher recruitment

The HPSS Central Team identified 18 school districts across Vancouver's lower mainland and southern Vancouver Island that were considered within feasible travelling distance. All 18 school districts received information packages that contained the overall purpose, methods and tools of the HPSS core and BHS that they were encouraged to review. After securing school district approval, the HPSS Central Team identified and contacted schools that offered elective Grade 11 PE classes to look at voluntary enrolment to PE. I detail the timeline of HPSS recruitment and BHS outcome variables of interest in Figure 2.4 and provide more details regarding recruitment of teachers and students.

The Central HPSS school liaisons sent out information packages regarding HPSS core and BHS to relevant schools inviting them to participate in the research. Schools had an option to participate in HPSS core only or both, core and HPSS BHS. HPSS school liaisons visited interested schools (n= 6) to address questions of school administrators and teachers in Planning and PE 10. Depending on the school, there were two to six teachers who were briefed about the HPSS and BHS. I was present for three school visits with HPSS school liaisons and answered questions pertaining to the BHS. HPSS school liaisons later followed up with a teacher/staff appointed by the school if they were willing to participate in the HPSS and/or BHS study. School recruitment and participant flow diagram is presented in Chapter 5.

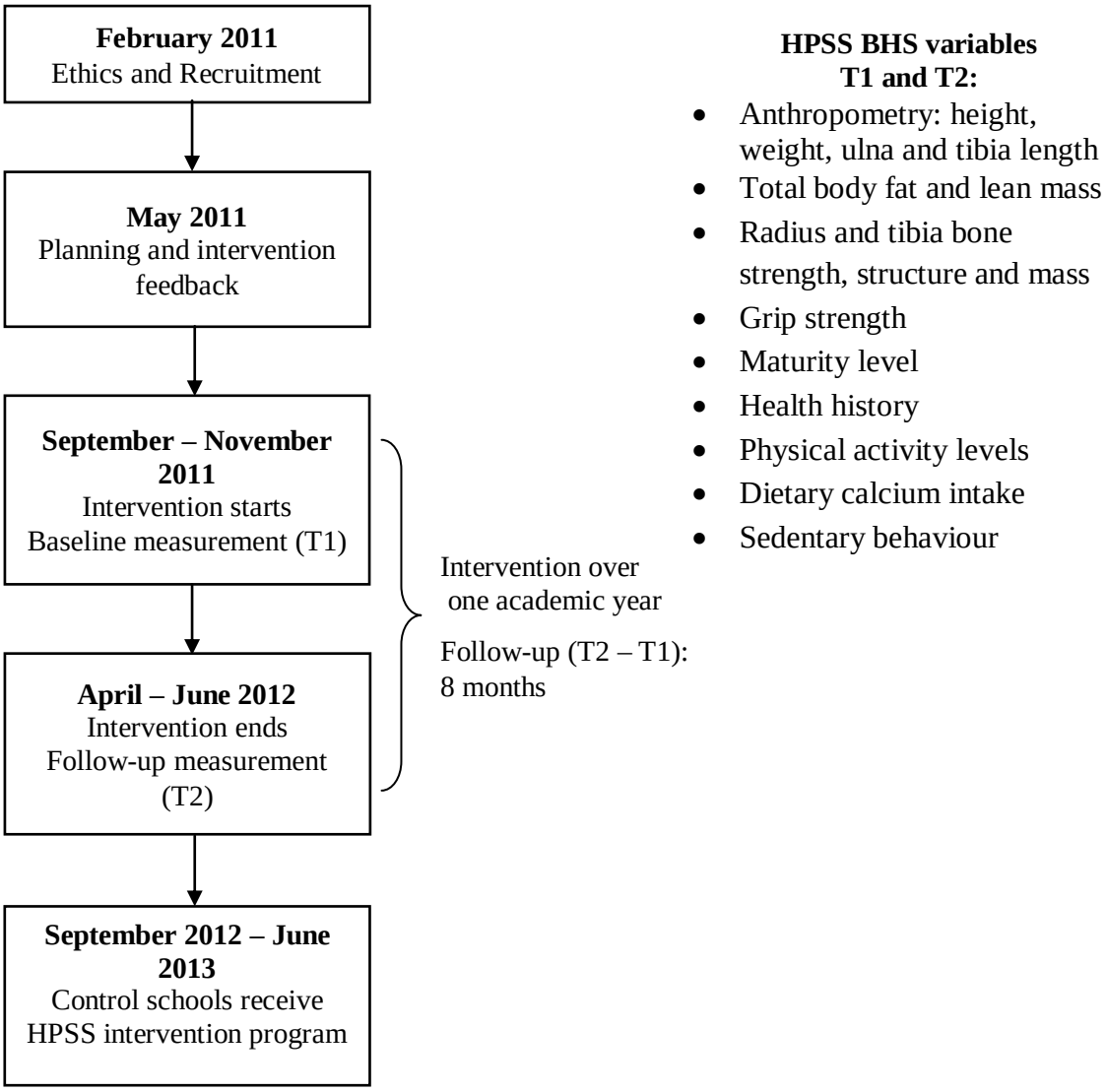


Figure 2.4. Timeline of Health Promoting Secondary Schools (HPSS) study and Bone Health Study (BHS) measurements at baseline (T1) and at follow-up (T2)

For the 2011/12 academic year, 1914 Grade 10 students attended nine schools whose principals volunteered to participate in the BHS. This represented 3% of all Grade 10 students across BC (63,818 students; C Ministry of Education, 2012: http://www.bced.gov.bc.ca/apps/imcl/imclWeb/Home.do).

2.3.3.2 Student recruitment

Eligible participants in the BHS were all Grade 10 students (boys and girls) (n=1914) who were healthy and able to participate in regular PA. Inclusion and exclusion criteria are as listed in Table 2.2. Teachers sent information packages with consent forms (Appendix C) home with students and a BHS research assistant collected these forms from the school after two weeks. We obtained signed consent forms from the participant and also from a parent or guardian. Of 1914 eligible students, 244 students (13%, 95 boys and 149 girls) volunteered to participate in the BHS.

Table 2.2. Participant inclusion and exclusion criteria for Health Promoting Secondary Schools (HPSS) Bone Health Study (BHS).

Inclusion criteria
• Boys and girls in Grade 10
Exclusion criteria
• Disorders and/or disabilities that restricted or changed participation in PA during study period. • Pregnant or at risk of being pregnant. • Use intravenous contraceptive methods, i.e. medroxyprogesterone acetate (Depo). • Have medical conditions (e.g., thyroidism, cancer, anorexia nervosa) or use medication that interfere with PA or bone metabolism (e.g., glucocorticoids, thyroid medication). • Special needs student (e.g., physical disability such as cerebral palsy or a mental disability such as autism)

Near the end of the intervention period, we contacted baseline participants by phone to schedule their follow-up measurements in school. We also followed-up with a reminder phone call the day before the scheduled appointment to those participants who requested it. For the participants that we were unable to contact by phone, we contacted the participants in school to schedule their follow-up measurement. We

also rescheduled participants that missed their appointments to the next available measurement session while we were parked on the school grounds.

2.3.4 Data collection

2.3.4.1 Measurement training

To standardize data collection methods, I conducted six hours of in-house training at the Centre for Hip Health and Mobility (CHHM) one week prior to measurement. CHHM is located on the Vancouver General Hospital campus in Vancouver, BC. Training included instruction related to anthropometry, administration of all questionnaires and assessment of grip strength.

I was responsible for overseeing all parts of training with the exception of scan acquisition and analyses. I have prior training and Level 1 certification from the International Society for the Advancement of Kinanthropometry. The measurement team was comprised of eight full or part time research staff, of whom two were postdoctoral fellows. Although some of the measurement team had prior measurement experience all were trained using standard methods to assess stretch stature (height, cm), body mass (kg), waist and hip circumference (cm) and ulnar and tibial length (cm). I describe all measurement protocols and equipment (models, versions) used from section 2.3.4.3 onwards.

pQCT and DXA imaging:

Scan Acquisition: A qualified CHHM imaging technologist (Dr. Danmei Liu) trained three research staff (including myself) to operate DXA and pQCT instruments and to acquire and then analyse all scans following acquisition. For each imaging tool, Dr. Liu demonstrated and explained the standard positioning and protocols before obtaining at least two scans while observed by the research staff. Then, each research

staff member conducted at least two practice runs on each imaging instrument under Dr. Liu's supervision and guidance until they were familiar with the equipment. Finally, each research staff acquired three scans independently and Dr. Liu checked the images for quality and positioning.

Scan Analysis: Dr. Liu explained and instructed the same three research staff on both DXA and pQCT image analysis with examples of images obtained. We then processed the five images obtained individually as practice. Then, we analyzed a set of 10 images that were pre-selected by Dr. Liu to compare the precision of our analysis with outcomes previously obtained by Dr. Liu.

Questionnaires: During training, I first explained the purpose and application of each questionnaire. I trained research staff to administer the questionnaires and to cross check answers between instruments. For example, one question in the health history questionnaire (HHQ) inquired about daily consumption of milk; a similar question was included in the food frequency questionnaire (FFQ). Measurers were trained to ensure participants' answers were consistent between these questions. They practiced administering questionnaires in a one-on-one interview setting to ensure quality control of data. At the end of the training, measurers were able to perform all measures, describe the purpose of the study, how data would be used and assure participants that data would be kept confidential.

Grip strength: We used a hand dynamometer to assess grip strength according to standard protocols (378). All research staff familiarized themselves with the operations of our digital hand dynamometer and I demonstrated the correct method to assess grip strength and shared the verbal instructions provided to each participant. Everyone practiced the protocol until they were comfortable with it and could provide consistent verbal encouragement, as maximal effort is required to elicit accurate results.

The measurement team: Of the eight trained research personnel, the same three measurers worked throughout the baseline and follow-up data collection period (myself included). We assessed bone using

pQCT and DXA, collected anthropometry and grip strength measures and administered all questionnaires. One specially licensed CHHM staff member drove the CHHM Mobile Research Laboratory (Mobile Lab) for the duration of the study. Three other research assistants worked in rotation with us 30% of the time to assist in questionnaire administration. We had research staff trained as "back-ups", should any of the main operators fall ill or be unable to attend measurement.

Measurement practice sessions: We used the Mobile Lab (Figure 2.5) to assess all participants at baseline and follow-up, which spanned a 6-week period each time. Approximately two weeks prior to data collection, we drove the Mobile Lab to an off-site location to conduct a measurement session that simulated on-site school measurement and included the entire BHS protocol. This practice session was designed to identify possible issues so that they could be addressed prior to start of data collection. We identified and solved issues such as timing for each measurement station, mechanical issues with the trailer and generator noise. Volunteers, two to four at each session, met us at the practice location and were rotated through all measurement stations, described in detail in section 2.3.4.3 onwards.

Figure 2.5. The Centre for Hip Health and Mobility Mobile Research Laboratory; uncoupled truck and trailer (top); entrance to the Mobile Lab (bottom left) and deployed rooms for DXA and pQCT measurements (bottom right).

2.3.4.2 School-site measurement

Siting the Mobile Lab: The CHHM driver sited the Mobile Lab a day or two prior to measurement at a designated place in the school precinct. The Mobile Lab driver conducted pre-measurement site visits to visually inspect the location whenever possible. He otherwise used Google Earth to visually inspect the school grounds. We sited the CHHM Mobile Lab at each school for data collection.

Scheduling participants: I scheduled appointments directly with students by phone prior to data collection dates. Two other research staff based at CHHM took over scheduling when I was on-site collecting data and assisted with cancellations and rescheduling. I provided the school office with a list of participant names and our appointment schedule so they would be aware when a student would be absent from class. Participants were asked to meet us at the Mobile Lab for their scheduled appointment.

Participant flow: The Mobile Lab has two levels. The lower level houses the bone imaging stations (DXA and pQCT), the anthropometry station, and a hand grip dynamometer. There is also a change room for study participants should they need one. The upper level is equipped with comfortable seating behind a screened partition to diminish distraction for students and is where we administer questionnaires. There is a single entrance and exit (Figure 2.5, bottom left picture) that leads to all levels.

When we first arrived at the school the whole measurement team signed-in at the school office. We prepared for data collection by warming-up the lab interior, setting-up and calibrating equipment and preparing participant packages. If participants did not show up for their scheduled appointment after 10 minutes, I visited the school office to request assistance to locate and remind participants of their appointment.

Two participants arrived together; one would complete a questionnaire to provide demographic data while sitting at the pQCT station. The other participant would be measured at the anthropometry station. Anthropometry required about 8-10 minutes to complete and was completed first, as height, body mass and limb lengths were required prior to beginning imaging procedures. From the anthropometry station, one participant proceeded to the pQCT station; we required about 20 minutes to position the participant and complete image acquisition. The second participant moved to the anthropometry station and then immediately to the DXA station; we required about 10 minutes to position the participant and complete image acquisition. Participants then switched stations and when finished proceeded to the questionnaire station where questionnaires were administered by one of our measurement team. We assessed grip strength last (about 5 minutes). One of our measurement team was tasked with checking each questionnaire and data form for completeness and would cross-check questionnaires before participants returned to class.

With a 3-person measurement team, we assessed 10 participants per day between approximately 9am and 4pm, on average. We allocated about 1.5 hours to complete all measures for each participant. With a 4-person measurement team, we assessed three participants at a time (during each 1.5-hour time period); 15 participants per day, on average. With a 4-person measurement team, the third participant would begin at the questionnaire station immediately after anthropometry was completed and we could cover up to 20 participants per day.

2.3.4.3 Anthropometry and body composition

Height and body mass: I measured stretch stature in duplicate to the nearest 0.1 cm using a wall-mounted stadiometer (Model 242, Seca, Germany) with the participant's shoes off. The head was positioned in the Frankfort plane (Figure 2.6) and the heels remained flat on the floor. I applied gentle traction to the mastoid process to obtain a 'stretch'. An assistant lowered the stadiometer headboard onto the apex of the participant's head. Participants who were unable to remove their headdress or turbans were measured as closely as possible; this was noted in the measurement form. I obtained a third measurement if two measurements differed more than 0.4 cm. Final height was the mean of two closest measures. If three measurements were taken, I used the median of three for analyses. We adopted this approach for all anthropometric measurements. I assessed body mass using an electronic scale (BWB 800, Tanita, Japan) to the nearest 0.1 kg. Participants emptied their pockets, removed heavy clothing (e.g., coats, sweatshirts) and shoes before stepping onto the scale. If two measurements differed by more than 0.2 kg, a third measurement was obtained.

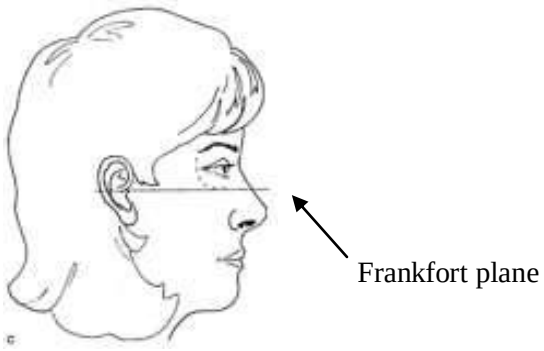


Figure 2.6. A schematic diagram that illustrates the horizontal Frankfort plane and the position of the head for measurement of stretch stature.

Limb lengths: I measured the length of the forearm (mm) on the non-dominant side (defined as the side not used for writing). I palpated the lateral ulnar process and marked the edge of the bone with a marker. Ulnar length (mm) was the distance from the proximal olecranon tip to the lateral edge of the ulna, measured using a steel ruler (mm) adapted on a forearm measurement tool. I assessed left tibial length (mm) using a steel anthropometric tape (Rosscraft Inc, Canada). Tibial length was the distance from the palpated points of the medial malleolus of the tibia to the tibial plateau. I measured both limbs in duplicate to the nearest 1 mm. If the measured differed by more than 4 mm, I took a third measure. I used the mean of two or the median of three measures for analyses.

Lean and fat mass: I determined total body (bone mineral free) lean mass (LM; kg) and total body fat mass (FM; kg) from a whole body scan acquired using DXA (Discovery-A model; Hologic Inc., Waltham, USA). I conducted daily calibration and quality control using the vertebral phantom provided by the manufacturer. Before scanning participants, I asked questions to rule out pregnancy and to ascertain any recent radiation exposure (outlined in the DXA form, Appendix D) Specifically, I asked post-menarcheal girls the date of their last menstrual period and if there was any possibility of pregnancy to determine if the participant should be excluded from DXA scans.

For the scan, participants wore light clothing without metal zips, snaps or buttons and removed jewellery, watches and glasses (Figure 2.7). I briefed each participant on the DXA procedure, the importance of being stationary throughout the scan and answered any questions prior to positioning participants for the whole body scan as per standard protocol (379). The effective dose equivalent for a whole body scan on a 15-year old was 4.2µSv for the Discovery-A DXA model (120). To put this in perspective this is a dose equivalent to one-fifth of the dose of a chest X-ray (380). I analysed scans with Apex 2.3 software using the standard procedures (379).

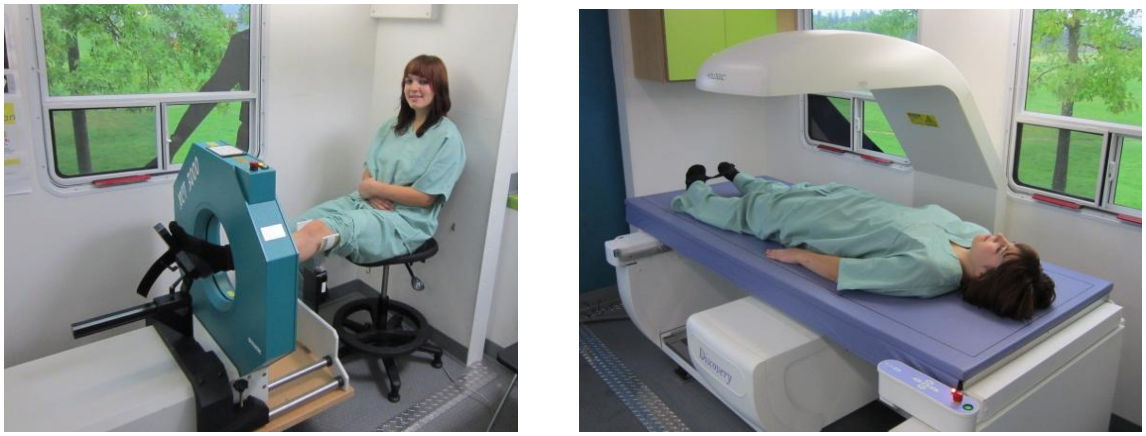


Figure 2.7. The figure illustrates a participant's bone health being assessed by pQCT (left) and DXA (right) in the Mobile Lab.[4]

2.3.4.4 Bone strength, structure and density

Image acquisition*:* To assess bone strength and structure, we used pQCT (model XCT3000; Stratec Medizintechnik, Pforzheim, Germany) and standard techniques. The pQCT operator performed daily calibration and quality assurance using a cone phantom from the manufacturer. As room temperature can

[4] *We obtained informed consent for pictures to be used for media publication from the 16-year old participant.*

affect pQCT image acquisition, during baseline data collection during Fall (September – November), we heated the Mobile Lab above 18°C before calibrating the instruments. The same trained pQCT operator acquired all pQCT images following standard manufacturer protocol (149).

The pQCT operator first performed a 30-mm planar scout scan to identify key landmarks on the non-dominant radius and the left tibia. Using the scout view, the operator placed the reference line at the medial edge of the radius or the tibial plafond, for radial and tibial scans, respectively. Radius sites of interest were 7% and 30% of measured ulna length, proximal to the reference line (Figure 2.8). Tibia sites of interest were 8% and 50% of tibial length, proximal to the reference line (Figure 2.9).

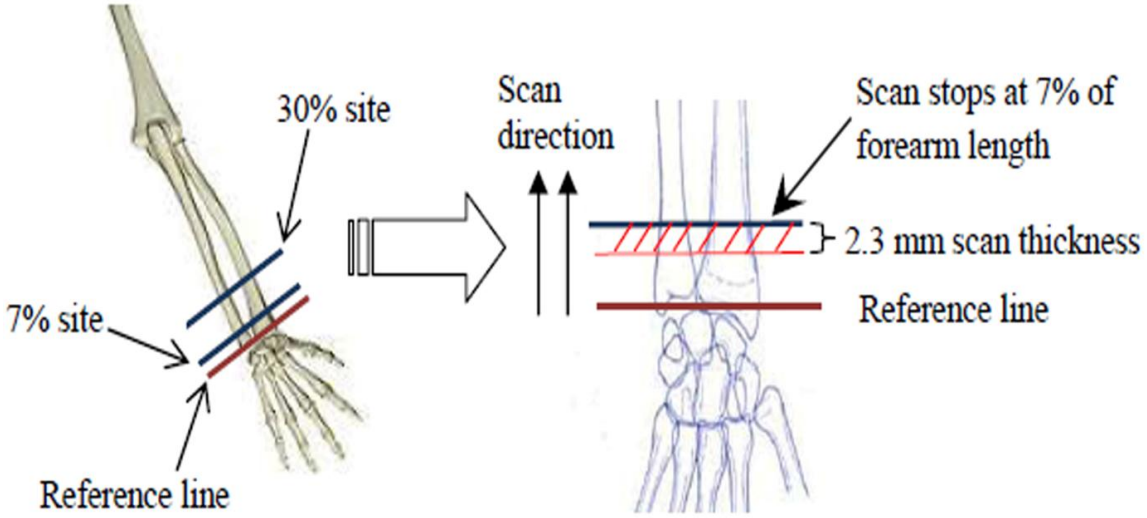


Figure 2.8. Position of the reference line and scan sites (7% and 30%) assessed at the radius using peripheral quantitative computed tomography (pQCT). Region of interest, scan direction and start point at the 7% site are also identified.

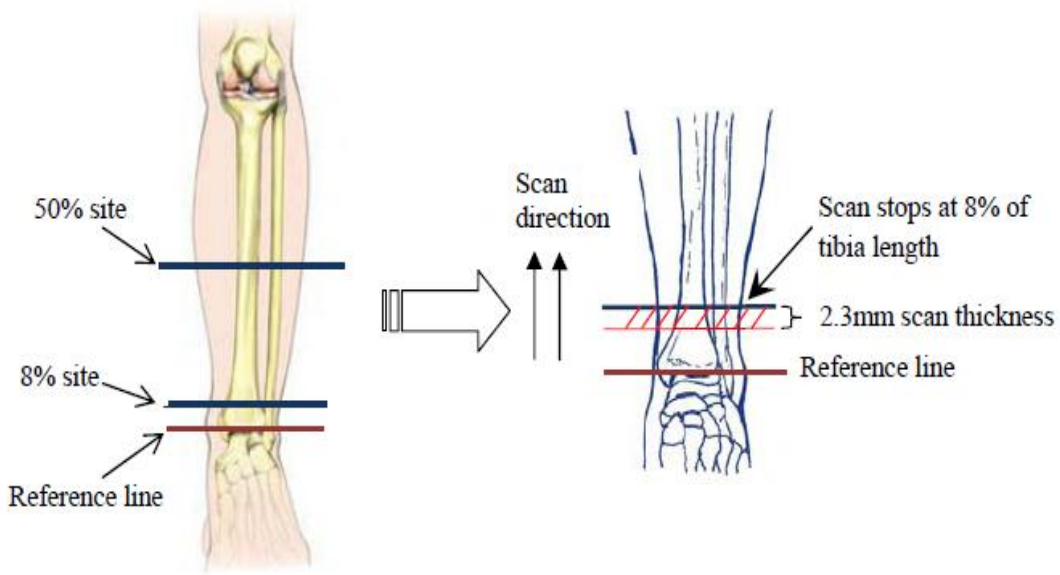


Figure 2.9. Positioning of the reference line and scan sites (8% and 50%) sites at the tibia using peripheral quantitative computed tomography (pQCT). Region of interest, scan direction and start point at the 8% site are also identified.

As per other studies in our lab, we used a scan speed of 30 mm/sec and a sampling resolution of 0.40 mm voxel size. 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. There is a minimal amount of scatter radiation from pQCT as the beam is tightly collimated (<1 μSv). Health Canada recommends that radiation exposure to not exceed 1 mSv per year (381), thus one pQCT scan is responsible for 1/1000 of the recommended yearly exposure.

Prior to imaging, our pQCT operator screened participants to rule out pregnancy, to ascertain prior exposure to ionizing elements and to determine each participant's fracture history (pQCT screening form in Appendix E). The screening questions were similar to those asked during DXA screening and were briefly repeated and answers reconfirmed. We noted if participants reported a prior fracture of the limb of interest during the past six months; if yes, the operator assessed the contralateral side. No participants reported sustaining fractures of both radii or both tibiae during the study.

The pQCT operator briefly explained to participants how the pQCT worked and asked participants to remove any objects from the measured side (e.g., watches, bracelets and anklets) that might interfere with the scan. The operator then requested that participants extend their arm or leg into the pQCT gantry where they rested it on a supported platform lined with bubble wrap. The bubble wrap functioned to produce a gap between the limb and the platform to assist with image analyses. The operator secured the limb firmly with Velcro straps to minimize movement during the scan. If movement occurred in the first pQCT scan, the operator stopped the scan or if the scan was complete, she obtained a second scan. The operator did not conduct a third scan regardless of motion artefact. Each scan required approximately three minutes to complete. I provide sample pQCT images that illustrate the reference lines and sites scanned for the radius (Figure 2.10) and tibia (Figure 2.11) below.

I analysed all pQCT images using Stratec software version 6.0 and followed standardized analysis procedures (149) supported by Dan Schiferl who provided training and service for Stratec imaging instruments at CHHM. Briefly, an automatic region of interest was generated after I placed the cursor at the centre of the image. Subsequently, I saved the images using a set file name and adopted analyses modes and thresholds as outlined in Table 2.3. As analysis protocols for pediatric pQCT studies are not standardized (discussed in section 0), I adopted analysis modes and thresholds used in our previous studies (32,150).

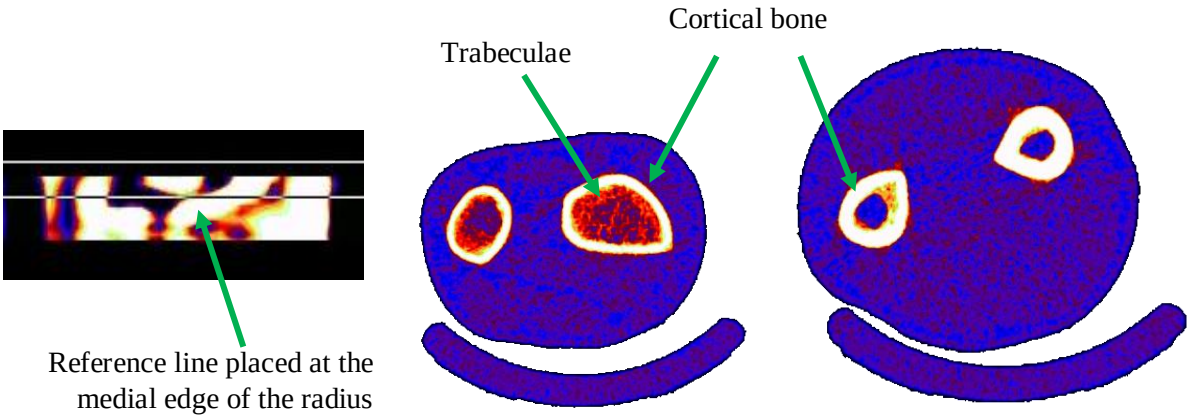


Figure 2.10. Images of the radius acquired using peripheral quantitative computed (pQCT); scout view that shows the reference line position (left). Images (middle) acquired at the 7% site with trabecular (orange) and cortical (white) bone compartments and at the 30% site (right) that demonstrates the high proportion of cortical bone. Images also illustrate the separation using bubble wrap between the measured limb and the support platform. This assists with accuracy when drawing the region of interest for analyses.

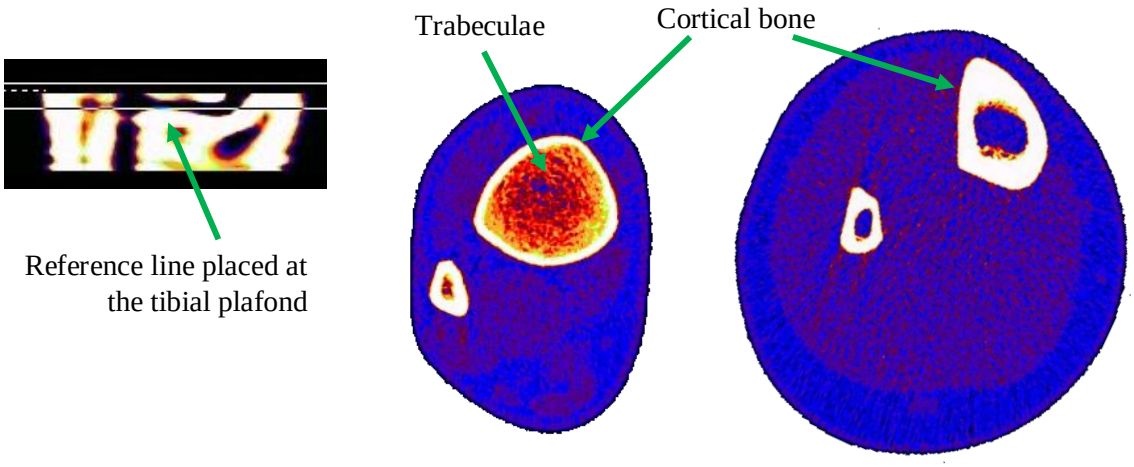


Figure 2.11. Tibial images from peripheral quantitative computed (pQCT); scout view of reference line position (left), tomographic image at the 8% site (middle) with trabeculae (orange) and cortical (white) sections and 50% site (right) with mainly cortical bone.

Table 2.3 Analysis modes, thresholds and outcome variables for pQCT assessments at the radius (7% and 30% sites) and tibia (8% and 50% sites).

Site	**Analysis Mode (Threshold, mg/cm^3)**	**Variables**
Distal	Contour mode 3 (200)	Total bone mineral density (Tt.Dn, mg/cm^3)
	Peel mode 5	Total bone cross-sectional area (Tt.Ar, mm^2)
7% & 8%	Cort mode 3 (200)	Trabecular BMD (Tb.Dn, mg/cm^3)
	Nofilters	Trabecular bone cross-sectional area (Tb.Ar, mm^2)
		BSI = Tt.Ar * Tt.Dn^2/10,000 (mg^2/mm^4)
Shaft	Contour mode 1 (710, 540)	Total bone cross-sectional area (Tt.Ar, mm^2)
	Peel mode 2	Cortical density (Ct.Dn, mg/cm^3)
30% 50%	Filter 1	Cortical area (Ct.Ar, mm^2)
	Cort mode 1 (710)	Cortical thickness, (Ct.Th, mm)
	No filters	
	Contour mode 1 (480, 480)	Polar strength-strain index (SSI_p, mm^3)
	Peel mode 2	
	Filter 1	
	Cort mode 1 (480)	
	Contour mode 1 $_{line1 \& line2}$ (-100, 40)	Muscle cross-sectional area (MCSA, mm^2)
	Peel mode 2 $_{line1 \& line2}$	
	Filters= 0 $_{line1}$, 1 $_{line2}$	
	Cort mode 1 $_{line1}$, (710, 0)	
	Cort mode 4 $_{line2}$ (-100 $_{line2}$, 2000 $_{line2}$)	
	No filters	

2.3.4.5 Physical activity

Questionnaire: We (BHS measurement team) assessed each participant's habitual PA using the PAQ-A (Appendix F: Physical Activity Questionnaire for Adolescents (PAQ-A)). The PAQ-A is a validated tool (20,306) designed to assess the amount of PA performed in the past seven days. Results are generated as a composite score that range from 1 (low PA) to 5 (high PA). The PAQ-A is comprised of nine items; our bone research group modified the questionnaire to include questions regarding screen time and organized activities (sports and non-sports) outside of school. The PAQ-A identifies spare time activity (Item 1) and

our research group added a time component, inquiring on the time taken, on average, for each spare time activity. Other items include specific activity times (Item 2 to 7, PE/lunch/after school/evenings/weekday/weekends), PA composite score (Item 10) and not usual activity (Item 9). The additional items captured the amount of screen time during the past seven days (Item 8), organized sports activities (Item 11) and other organized activities (Item 12). From the original PAQ-A, three activities (aerobics, rowing/canoeing and tag) of the 22 activities in Item 1 were replaced by martial arts, gymnastics and skiing/snowboarding, to reflect more common activities in our local settings. For Item 1 of the questionnaire we added a component of time spent (min) in each activity so as to estimate average moderate-to-vigorous PA (MVPA) performed.

To determine the association between MVPA as assessed by questionnaire with accelerometry-assessed MVPA, I examined the correlation between accelerometer-derived MVPA (min/day) and PAQ-A MVPA (min/day) from a different cohort of boys (n= 89) and girls (n= 117) who participated in the HBS, conducted in our research centre. The protocol used to assess PA (PAQ-A and accelerometry) was the same for the HBS and HPSS BHS. Participants in the HBS were aged 9 to 20 years (mean age = 14.5 years). The association between boys' MVPA (minutes) between measures was 0.43 ($p<0.001$). For girls, the association was 0.19 ($p= 0.037$; unpublished data). These findings were similar to a systematic review that reported measurement properties of PA questionnaires where associations between PAQ-A and accelerometer/direct observations MVPA ranged from 0.18 to 0.49 (305).

Accelerometry: I also assessed PA objectively by accelerometry to obtain duration, frequency and intensity of PA. The HPSS accelerometer coordinator distributed uniaxial accelerometers (GT1M, Actigraph, Pensacola, U.S.A.) to participants during school-based measurement sessions. We asked participants to wear the accelerometer on their right hip during waking hours for seven consecutive days except when participating in water-related activities (e.g., bathing, swimming), as accelerometers are not

waterproof. To monitor sessions of non-wear, we provided participants with a log sheet to note times they removed their accelerometers and to report any activities undertaken during this non-wear time. We used 15-second epochs for data recording. The accelerometer coordinator used Actilife version 5.9.2 (ActiGraph, Pensocola, U.S.A.) to initialize accelerometers, set the data capture for 15-second epochs, and download data and processed raw data using KineSoft version 3.3.67 (KineSoft, Sasketchewan, Canada).

The accelerometer coordinator analyzed all accelerometry data using Kinesoft software. For data to be included, participants had to wear their accelerometer for a minimum of 10 hours/day, across at least 3 valid (10 h) days, regardless of whether they were weekends or weekdays. The hours required for a valid day were similar to the criterion used in NHANES (316). I defined non-wear time as a minimum of 60 minutes of continuous zero counts. Using cut-points defined by Evenson et al. (28), the accelerometer coordinator analyzed time spent (min/day) in SED (<100 counts per minute, cpm), MPA (2296 – <4012 cpm), VPA (≥4012 cpm) and MVPA (≥2296 cpm).

2.3.4.6 Muscle strength

At least two members of our BHS measurement team conducted the upper arm muscle strength assessment using a hand grip dynamometer (Model EN-120604 Jamar Plus+, Lafayette Inc., U.S.A.) to the nearest 0.1 kg. We followed the protocol as described by Roberts and colleagues (378). First, a measurement team member explained and demonstrated the measurement protocol to participants. Second, participants held the hand grip dynamometer as we adjusted the hand grip so that the proximal interphalangeal joint rested on the adjustable bar of the dynamometer. Third, participants stood with their legs shoulder-width apart, with their arms flexed at 90-degrees without any shoulder or elbow abduction. Similarly, each participant's wrist was held in a neutral position along the sagittal plane, with no abduction or adduction. Participants performed three trials with each arm, alternating between dominant

and non-dominant arms. Both measurement team members provided verbal encouragement to participants to elicit a maximal effort. All participants received the same verbal encouragement. I recorded measures of muscle strength (kg) and used the maximum score for each hand in my analyses.

2.3.4.7 Maturity

For girls, we administered a maturity questionnaire that asked participants to recall the date of their first menstrual period (age of menarche), the date of their most recent menstrual period and whether they used contraception (usage and brand/type; Appendix G). Female research staffs were available to assist the girls with completion of the maturity questionnaire as needed. They also used prompts as needed to help girls recall the approximate date of their first menstrual period. For example, girls were asked to recall events that occurred around the time of menarche – what grade they were in at school, which school term, before or after Spring break or festive events such as Hallowe'en, Christmas and so forth. Research staff aimed to assist each girl to pinpoint the month and day of menarche (if possible) or the approximate time period within a month timeframe. If the exact date was unknown, the 15^{th} of the month was used instead.

For boys, we assessed maturity with a self-administered questionnaire that asked the question; "How much underarm hair do you have now?" followed by five possible answers (Appendix H). Studies that used axillary hair to assess maturity highlighted that axillary hair (AH) growth represents a later stage of maturation (201). The appearance of axillary hair coincides with Tanner stage 4 (across 5 Tanner stages) of pubic hair development in boys (202). I reported the outcomes as a 5-point Likert scale but considered AH stage 2 onwards as peri- to post-pubertal in boys.

In addition to the assessment of sexual maturity via questionnaire, I also used a maturity offset equation. The equation was initially based on the Mirwald equation that estimates the years from APHV

(189) and was recently recalibrated by our research group (382). Moore and colleagues simplified the equation and tested it on three different epidemiological cohorts – the Harpenden Growth Study, PBMAS and HBS (382). Results showed that by using height and age, the new maturity offset equation accurately predicted about 90% of the actual PHV (382). With the availability of the recalibrated maturity offset equation, I chose to use this measure of maturity in boys versus the use of self-reported axillary stages. For white and Asian participants, I estimated APHV using the sex- and ethnic-specific equations provided below. For participants of other and mixed ethnicities I estimated APHV using the equation for white boys and girls based on the recommendation of the author (Sarah Moore, personal communication).

Maturity offset equation (with age in years and height in cm) (382),

1) White boys: -7.99994 + (0.0036124 x age x height)
2) Asian boys: (-7.99994 + 0.8036223) + (0.0036124 x age x height)
3) White girls: -7.709133 + (0.0042232 x age x height)
4) Asian girls: (-7.709133 + 0.7303442) + (0.0042232 x age x height)

2.3.4.8 Health history

We administered a HHQ to determine each participant's ethnicity, fracture history and/or limb immobilization, medication and/or any other medical condition that may affect bone health, development or the participant's ability to participate in PA (Appendix I). Using the HHQ we also assessed tobacco use, alcohol consumption and immediate family health history related to bone health. Using Statistics Canada census classifications for ethnicity (383), I used each participant's self-reported ethnicity and grouped participants into white, Asian and Other categories. Participants who self-reported to be of mixed ethnicity, a minority with small representation (e.g., Pacific Islander, Black) or of aboriginal descent were grouped as Other.

2.3.4.9 Dietary calcium intake

I used a validated FFQ (384) (Appendix J) to identify consumption of foods that contribute to dietary calcium intake (mg/day). The FFQ also captured the use of nutrition supplements. Participants reported how often they consumed 20 calcium-rich food items (times per week, times per month) and the how much they consumed each time (number of servings as per serving size described in the FFQ). They also reported information regarding food allergies and type of milk consumed.

2.3.5 Statistical analysis

I used Stata version 10.1 (StataCorp, College Station, Texas, USA) to conduct all statistical analyses. I checked data for normality and outliers using scatter plots and strip plots, and checked for possible data entry errors (corrected when found). I removed outliers due to known measurement errors or if the value recorded was not physiologically plausible, (e.g., leg length entered as 247 mm). I provide a detailed description of the statistical analysis specific to each research objective within the relevant parts of the thesis.

Chapter 3: The Influence of Physical Activity on Bone Strength, Structure and Density in Children and Adolescents: A Systematic Review and Narrative Synthesis[5]

SYNOPSIS: PA is known to enhance bone mineral accrual in children and adolescents but less is known about PA's effect on bone strength and structure. Studies with advanced methods that analyzed bone strength and structure in children and adolescents with PA as an intervention or as an associated factor are examined in this chapter. This chapter explores the influence of bone strength and structure through a systematic review process and is presented in its published format with minor modifications.

3.1 Introduction

The antecedents of adult bone health problems develop during the growing years when more than one third of adult bone mass is accrued during two key years around peak adolescent growth (114). The convergence of this critical period of bone accrual *and* bone loading through weight-bearing, high-impact and/or muscle enhancing PA is thought to confer a 'window of opportunity' for development of a healthy skeleton (385). As osteoporosis and fractures impart a high social, emotional and financial levy (4), it is important that children and adolescents adopt physically active behaviours as one potential means to prevent these problems from occurring later in life.

Tempered by genetics, the evidence that weight-bearing PA plays a central role in promoting bone mass accrual, especially during the growing years, is irrefutable (334). Specifically, over the past two

[5] I retained the content of my published manuscript (360) as Chapter 3.

decades many high quality intervention and longitudinal studies provided strong evidence to support a positive effect of PA on bone mass accrual in boys and girls. Results from these studies are summarized in several excellent reviews (42,250,334,386). Early findings advanced our understanding of how bone accrues in the healthy skeleton and how the skeleton adapts to PA during growth (11,12).

Ultimate bone strength is comprised of bone's material properties, quantity, dimensions (size and material distribution), quality and microarchitecture (8). Although it would be ideal to measure each of these bone parameters, it is not possible to do so in clinical studies. For example, current non-invasive techniques cannot assess bone at the material level (degree of bone "mineralization") per se. Thus, we define bone strength as the ability to resist fractures including bone's extrinsic properties such as the quantity of bone mineral available (mass) and the distribution of bone mass from the neutral axis (structure) as captured by current in vivo bone imaging technologies.

Muscle force is generated during PA, and concentric and eccentric muscle contractions incur high loads on the skeleton (50). The mechanostat theory describes muscle as a mediator that transfers ground reaction forces and forces generated during muscle contractions, to bone (45). Thus, muscle and bone comprise an inextricably linked unit that must be considered together if we are to better understand the influence of PA on bone strength (336).

Pioneering bone health studies used DXA to generate 2-D measures of bone mineral content (BMC, grams) and areal bone mineral density (aBMD, g/cm^2). However, DXA-derived images have a number of well-known limitations such as their planar nature and low spatial resolution (387). DXA is unable to distinguish between cortical and trabecular bone compartments or to differentiate the specific elements of bone macro- and micro-structure that adapt to mechanical loading associated with PA to give bone its strength. As bone strength has been considered the 'bottom line' regarding fracture prevention

(118), algorithms were developed that provide estimates of bone structure and strength at the proximal femur using DXA (122,126,127,388).

Despite these limitations, bone-imaging technology has come a long way. Instruments such as pQCT, HR-pQCT and MRI enable safe and precise examination of many aspects of bone structure that contribute to bone strength. These tools are sensitive enough to characterize key differences in cortical and trabecular bone between boys and girls (389); HR-pQCT (390) and MRI (217) also provide a 3-dimensional characterization of how the peripheral skeleton of boys and girls adapts to weight-bearing PA. However, advanced imaging modalities provide only *estimates* of bone strength. These estimates were validated against a direct measure of bone strength using mechanical testing (170,391), commonly assessed as the force required to cause a material to fail under certain loading conditions.

Nikander and colleagues recently conducted a systematic review that focused on the effect of targeted exercise regimens on bone strength across childhood, adulthood and later adult life (37). The authors reported a positive sex- and maturity-related effect for exercise in children, but not in adolescents. Specifically, load-bearing exercise interventions had a small but significant effect on bone strength at the lower extremities in pre-pubertal boys (effect size=0.17; 95% CI: 0.02-0.32). Their conclusion was based on comparisons across four publications from two RCTs that used different exercise interventions, imaging tools, measured different skeletal sites and reported different outcomes; making it difficult to draw any firm conclusions. The critical role of muscle as a mediator between PA and bone strength, as proposed in the mechanostat theory (45), was not addressed.

In this systematic review we aimed to extend the work of Nikander and colleagues (37) and the knowledge base generally, beyond what is known from a few published RCTs that reported bone strength as the outcome. To do so, we drew upon findings from RCTs but also from observational studies (392) of

children and adolescents that evaluated the role of recreational PA and organized sports participation on bone strength. We also considered the influence of muscle by noting when studies adjusted for muscle or surrogates of muscle function (e.g., lean mass, muscle strength, MCSA). Finally, we examined the influence of PA on components of bone mass and structure that contribute to bone strength.

Thus, our primary aim was to determine the influence of PA and participation in organized sports on bone strength in children and adolescents. Our secondary aims were to; i) identify sex- or maturity-related differences regarding the influence of PA and participation in organized sports on bone strength, and ii) assess the influence of PA and participation in organized sports on specific bone parameters (such as bone structure and density) that contribute to bone strength. Where muscle parameters were evaluated, we describe the influence of muscle power or its surrogates on bone strength.

3.2 Methods

We were guided by the Centre for Reviews and Dissemination's guidelines (364) and the work of Popay and colleagues (393) in conducting this systematic review. We used the PRISMA guideline (363) to create our protocol for this systematic review. The protocol of this systematic review is registered with the PROPERO International Prospective Register of Systematic Reviews (http://www.crd.york.ac.uk/prospero) under the registration number: CRD42013003948.

3.2.1 Search strategy and resources

We searched electronically in MEDLINE (1946 to 17 January 2013), EMBASE (1974 to 17 January 2013), CINAHL (1982 to 17 January 2013), Sport Discus (1921 to 17 January 2013), PEDro (1929 to 17 January 2013), Informit (1977 to January 2013) and Cochrane Central Register of Controlled Trials (up to January 2013) for relevant articles. We combined text and MeSH terms for our search

concepts (Figure 3.1) to create a detailed list of search terms (Appendix K). We applied search filters from the Scottish Intercollegiate Guidelines Network (SIGN) to identify RCTs and observational studies in MEDLINE and EMBASE. To enhance sensitivity, we used an age-specific phrase filter (366) with individual database age-limits; we restricted our search to human studies. We did not apply any language restrictions so as to be more inclusive. We conducted forward searches from reference lists to identify other relevant studies.

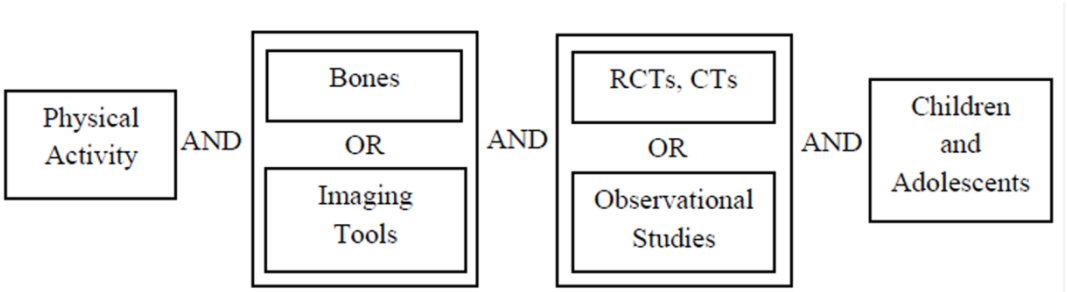


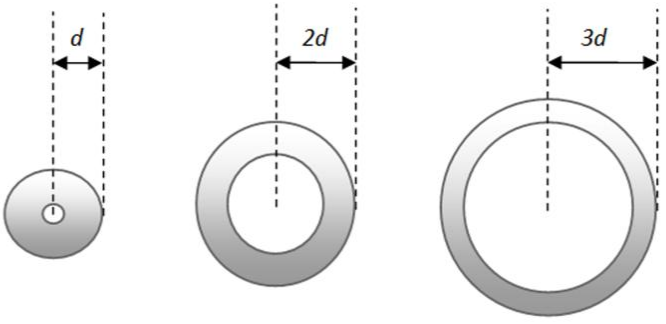
Figure 3.1. Search concepts and the use of Boolean operators within databases. RCTs, randomized-controlled trials; CTs, Controlled trials.

3.2.1.1 Working definitions

For the purpose of this review, we define bone strength as the ability to resist fractures including bone's extrinsic properties such as the quantity of bone mineral (mass) and the distribution of bone mass from the neutral axis (structure), as captured by current in vivo bone imaging technologies (394). Intrinsic properties of bone strength such as the amount of hydroxyapatite mineralization and collagen structure in bone composite cannot currently be assessed in vivo. We define bone mass as quantified bone tissue (bone mineral content, g) or amount of bone tissue per unit volume (bone mineral density). Bone mineral density (BMD) is reported as volumetric BMD (vBMD, g/cm^3) using pQCT or HR-pQCT or as areal BMD (aBMD, g/cm^2) using DXA. We define bone structure as being comprised of bone geometry and macroarchitecture (e.g., cortical thickness, trabecular and cortical bone area, periosteal and endosteal

circumference). We use bone microarchitecture to represent measures of trabecular thickness, trabecular separation and cortical porosity (35).

Importantly, bone strength can only be assessed directly using invasive approaches *ex vivo*, thus all studies we included in our review used surrogates of bone strength. For example, compressive strength or bone strength index [(BSI) = Total bone area (Tt.Ar) x Total bone density (Tt.Dn) ^{2}] was used to represent bone strength at distal sites of long bones assessed using pQCT (153). DXA-based algorithms such as hip structural analysis (HSA) (388) were also reported as surrogates of bone strength at the proximal femur. Finally, cross-sectional moment of inertia (CSMI) and section modulus (Z) represent distribution of bone away from its neutral axis and were also used to estimate bone strength as defined by its ability to resist bending forces (153). Figure 3.2 is a schematic that illustrates how bone mass and structure are related to bone strength.



aBMD (by DXA)	:	1	1	1
Compressive strength	:	1x	2x	3x
Bending strength	:	1x	4x	9x

Figure 3.2. Schematic of the effects of bone mass (areal bone mineral density, aBMD) and structure (cross-sectional) on bone strength. The influences of bone mass distribution from the neutral axis are not captured by DXA-based bone density measures that greatly affect bone strength. With the increase in *d*, distance of the outer bone mass to the neutral axis or plane of bending, approximate bone strength increases are compared with the values in the left column (Adapted from Bouxsein and Krasik, 2006, with permission from Springer).

3.2.2 Study selection and inclusion criteria

We included studies that; 1) evaluated healthy participants aged between 5 and 18 years, 2) reported bone strength and/or bone mass and structure, 3) measured PA either subjectively or objectively, conducted PA interventions or sports-related training programs, 4) provided full-text, published in peer-reviewed journals, and 5) were RCTs, controlled trials (CTs) or observational studies (prospective studies, cohort studies). We excluded studies with co-interventions such as a nutrition arm, case studies or validation studies. We excluded non-English publications (obtained through title and abstract screening) if the full-text article did not have an English version. We imported all studies into RefWorks (Bethesda, MD) and removed duplicates using the 'close match' and 'exact match' functions. Two reviewers (SK and VT) screened all titles and abstracts before retrieving full-text articles. These same reviewers independently assessed each article, reached agreement on exclusions wherever possible and discussed discrepancies (n=3) with a co-investigator (MA).

3.2.3 Assessment and data extraction

We used the validated Quality Assessment Tool for Quantitative Studies developed by the Effective Public Health Practice Project (EPHPP) to assess RCTs, CTs and observational studies (367). We allocated a WEAK, MODERATE or STRONG global score for quality based on six components: 1) selection bias, 2) study design, 3) confounders, 4) blinding, 5) data collection methods, and 6) withdrawals and dropouts. Using these criteria, if a study had four STRONG components (equal weights) and no WEAK components, the global rating was STRONG. If a study had less than four STRONG components and one WEAK component, the global rating was MODERATE. If a study had two or more WEAK components, the global rating was WEAK. We did not assess the appropriateness of statistical methods

within studies. The intraclass correlation coefficient (ICC) for the global score between our reviewers (SK, VT) for 10 randomly selected papers was 0.90 (95% CI: 0.74, 0.96).

Data were extracted and cross-checked by two reviewers (VT and SK) regarding study design, study reference (authors, year of publication, country), participants (sex, sample size, mean age), the PA intervention and measured PA outcomes (duration, frequency, type, intensity, sports training initiation), bone imaging tools used, imaging sites, bone variables reported (density, structure, estimated strength) and significant statistical outcomes.

3.2.4 Data synthesis and analysis

We were unable to conduct a meta-analysis due to the diversity of measures used to assess bone strength and structure (e.g., different imaging tools, measurement sites and analysis protocols) across studies. Thus, *we conducted a narrative synthesis* based on study design (intervention or observational studies). RCTs and CTs were considered intervention studies. Within observational studies of leisure-time PA, we subdivided studies based on their focus on either recreational PA or organized sports. Observational studies that assessed recreational PA examined the influence of PA intensity, duration and frequency on bone outcomes. Studies of organized sports examined the influence of participation in a specific sport (training hours, type of training, age of training initiation) on bone outcomes.

Specifically, to avoid bias and encourage transparency, we adopted the methodological framework of Rodgers et al. (370) that includes elements of a narrative synthesis when a statistical meta-analysis is not possible or advisable. We used our aims to guide the synthesis process -- akin to the first element of theory development. We produced a preliminary synthesis by study design (intervention or observational studies) and identified trends within and between study groups (intervention, observational recreational

PA and observational organized sports). Within our narrative, we interpreted and described the relationships we observed by further sub-grouping studies based on sex and maturity levels, as these factors have a strong influence on bone development during growth (215). We looked into 'within' study group relationships including patterns of PA (e.g., characteristic of intervention programs, observational study PA assessment methods, sports training history) and bone outcomes. For 'between' study group analysis, we focused on the incidence of positive bone strength outcomes and related measures of bone mass and structure. We also considered other influences on outcomes such as anatomical sites, duration of PA/training exposure, type of PA (e.g., weight-bearing, high-impact or low-impact PA) and identified muscle/lean mass as a potential confounder across studies. We define weight-bearing PA as those activities where at least one foot and leg is in contact with the ground and is supporting at least full body weight. Weight-bearing was often used by authors as an umbrella term to encompasses high-impact activities; however the definition of high-impact activity varied across the studies we reviewed – but generally referred specifically to activities that imposed a ground reaction force 2-5 times BW. We adopt the specific descriptors for PA used by authors of the papers we reviewed. (e.g., weight-bearing or high/low impact). Whenever possible we provide ground reaction forces associated with the physical activities of focus in the studies. We categorized interventions/recreational PA/sports that described load bearing on examined bones as weight-bearing PA (e.g., running, tennis, walking, jumping, resistance/weight training) to highlight the type of PA addressed by the study. Other mixed type (e.g., triathlon) or non-loaded physical activities (e.g., swimming, cycling) are considered as Mixed PA. We summarized possible factors (study design and quality, program or analysis) that contributed to the outcome. Finally, we considered key findings in light of study quality (global rating, potential confounders, PA components reported).

As per the aims of this review, bone strength was the outcome of primary interest, while bone mass, structure and microarchitecture were secondary outcomes. We report and discuss secondary

outcomes only if bone strength results were statistically significant (as per the definition of statistical significance within each study). We report significant differences (percent) between groups if provided by authors and otherwise estimate percent change from mean values. We report the variance explained (R^2) by PA in multivariable regression models designed to predict bone variables, rather than β-coefficients. We report β-coefficients if R^2 values were not reported. We name bone variables using a recently updated nomenclature list (395) that provides standardized terms and abbreviations.

3.3 Results

3.3.1 Search results

Our search yielded 4389 potentially relevant articles. We reviewed titles and abstracts to identify 63 articles for full-text review. Of these, we included 37 studies in our systematic review – 14 intervention trials (RCTs/CTs), 10 observational studies of recreational PA and 13 observational studies of organized sports. Figure 3.3 presents a brief summary of the review process and our rationale for excluding 26 full-text articles.

3.3.2 Assessing studies based on 'quality'

We included all 37 studies in the review regardless of their quality rating and provide independent component scores and overall ratings for all studies in Table 3.1. Of the 14 intervention studies (RCTs/CTs), five received a STRONG global rating and nine a MODERATE global rating. Of the 23 observational studies, 21 received a MODERATE global rating. Based on their study design, no

observational study received a STRONG global rating. Two organized sports studies were given a WEAK global rating due to selection bias and their cross-sectional study design.

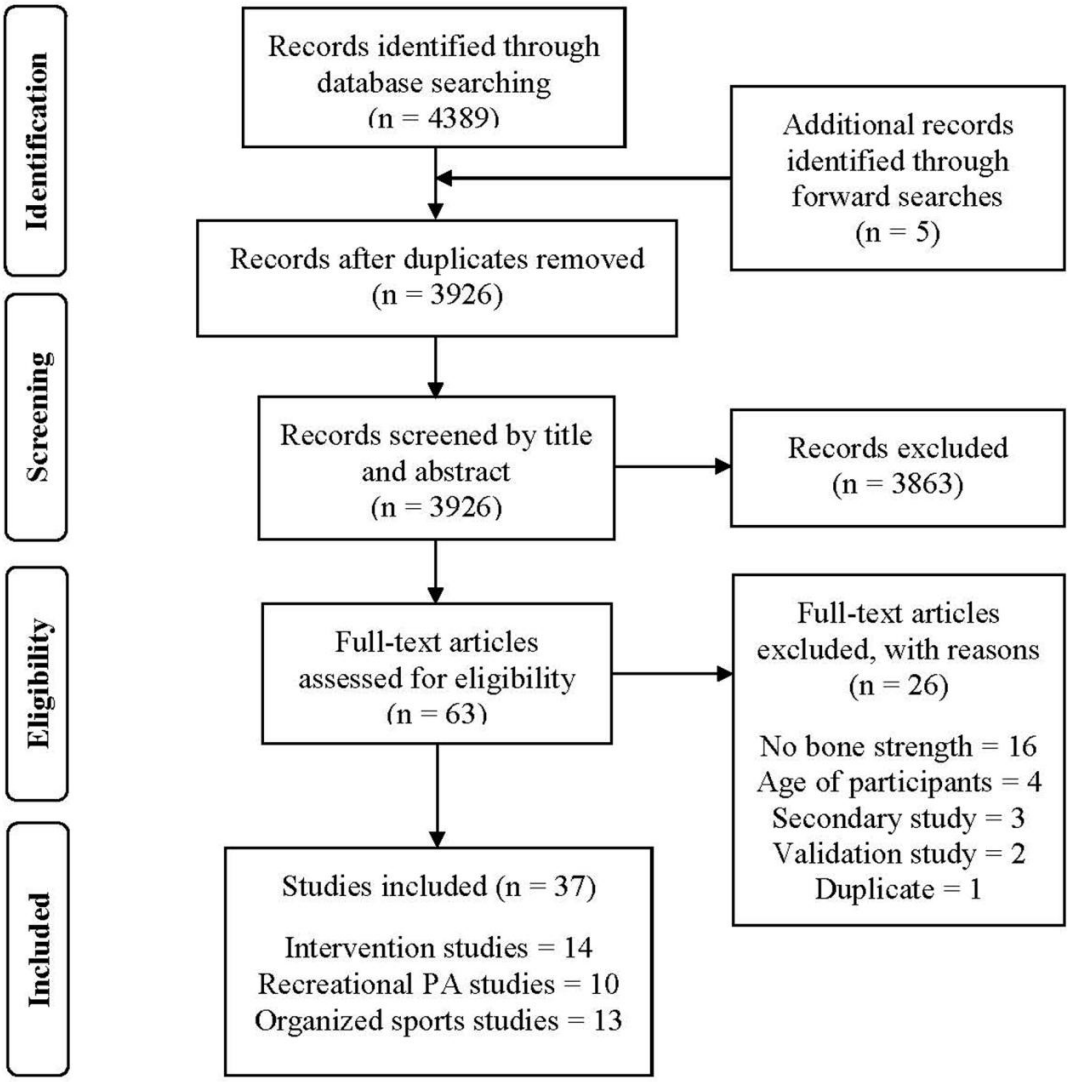


Figure 3.3. Flow diagram of study selection.

Table 3.1. Quality assessment of intervention studies and observational studies of recreational physical activity (PA) and organized sport (n=37).

	Selection bias	Study design	Con-founders	Blind-ing	Data collection method	With-drawals /dropouts	Global Rating
Intervention Studies							
1. Alwis et al., 2008a (396)	**	**	**	**	***	***	**
2. Alwis et al., 2008b (397)	**	**	***	**	***	***	**
3. Alwis et al., 2008c (398)	**	**	***	**	***	**	**
4. Bradney et al., 1998 (399)	**	***	**	**	***	***	**
5. Anliker et al., 2012 (358)	*	***	***	**	***	**	**
6. Greene et al., 2009 (34)	**	***	***	**	***	***	***
7. Heinonen et al., 2000 (30)	**	***	***	**	***	***	***
8. Macdonald et al., 2007 (32)	**	***	***	**	***	**	**
9. Macdonald et al., 2008 (400)	**	***	***	**	***	**	**
10. Macdonald et al., 2009 (113)	**	***	***	**	***	***	***
11. Mackelvie et al., 2004 (401)	**	***	***	**	***	*	**
12. McKay et al., 2005 (359)	**	**	***	**	***	***	**
13. Petit et al., 2002 (31)	**	***	***	**	***	***	***
14. Weeks et al., 2008 (33)	**	***	***	**	***	***	***
Recreational PA Studies							
1. Bhattacharya et al., 2008 (402)	**	*	***	**	***	**	**
2. Farr et al., 2011 (145)	**	**	***	**	***	**	**
3. Forwood et al., 2006 (403)	**	**	***	**	***	*	**
4. Janz et al., 2004 (338)	**	*	***	**	***	**	**
5. Janz et al., 2007 (339)	**	**	***	**	***	*	**
6. Kardinaal et al., 2000 (404)	**	*	***	**	***	**	**
7. McKay et al., 2011 (405)	**	*	***	**	***	**	**
8. Moyer-Mileur et al., 2001 (406)	**	*	***	**	***	**	**
9. Sardinha et al., 2008 (340)	**	*	***	**	***	***	**
10. Wang et al., 2005 (407)	**	*	***	**	***	**	**
Organized Sport Studies							
1. Bass et al., 2002 (107)	**	*	***	**	***	**	**
2. Burt et al., 2012 (146)	**	*	***	**	***	**	**
3. Dowthwaite et al., 2007 (408)	**	*	***	**	***	**	**
4. Dowthwaite et al., 2011a (409)	**	*	***	**	***	**	**
5. Dowthwaite et al., 2011b (410)	**	*	***	**	**	**	**
6. Dowthwaite et al., 2012 (411)	**	*	***	**	***	**	**
7. Duncan et al., 2002 (412)	**	*	***	**	***	**	*
8. Erlandson et al., 2011 (413)	**	*	***	**	***	**	**
9. Faulkner et al., 2003 (414)	**	*	***	**	***	**	**
10. Ferry et al., 2011(415)	**	*	***	**	***	**	*

Table 3.1. continued.

	Selection bias	Study design	Con-founders	Blind-ing	Data collection method	With-drawals /dropouts	Global Rating
Organized Sport Studies (cont.)							
11. Greene et al., 2005 (416)	**	*	***	**	***	**	****
12. Tournis et al., 2010 (417)	**	*	***	**	***	***	****
13. Ward et al., 2005 (418)	**	*	***	**	***	**	****

Ratings = *: Weak; **: Moderate; ***: Strong.

3.3.2.1 Characteristics of intervention and observational studies

3.3.2.1.1 Intervention studies

Of the 14 intervention studies, 12 were conducted in elementary schools and one was conducted in a secondary school (33). Only one study was not school-based, although participants were recruited from one elementary and one middle school (30). Twelve studies used 'school' as the unit of randomization for intervention and control groups; one study randomized participating students into age-category intervention and control groups (34), while another only recruited and randomized grade 9 students (33). Sample size within group by sex and/or maturity levels ranged from 10 to 151 participants. The length of interventions ranged from 28 weeks to 2 years. Attrition ranged from less than 10% to more than 60%. Intervention programs were diverse – some focused on providing 30 min/week of weight-bearing PA during physical education class (31,401); others added 140 min/week of different kinds of PA within the school day (396–398). Six studies failed to report intensity of PA interventions while eight studies reported specific weight-bearing types of PA (31,32,34,113,358,359,400,401). Compliance rates for participation in intervention programs ranged from 65 to 100% across ten studies (30,32,34,113,358,359,396,399–401).

Key findings regarding design of intervention studies: Interventions studies were primarily conducted in elementary schools; treatment groups were mostly randomized by school. However, there

were substantial differences in the specific intervention, which varied by the type, frequency, intensity and duration of PA delivered and the overall length of the intervention.

3.3.2.1.2 Observational studies of recreational PA

For the 10 studies that focused on recreational PA, participants were 5 to 16 years of age and sample size ranged from n=18 boys (pilot study) to n=1116 girls (multicenter study across six European countries). Median sample size across all studies was n=143. Eight studies were cross-sectional and two were prospective.

One prospective and two cross-sectional studies used accelerometers to objectively measure PA (338–340). Accelerometry data were based on three days of wear time, 8-10 hours/day of valid data and 1 min epochs. The three PA studies that used accelerometers (338–340) adopted different classifications for sedentary behaviour (<152 counts per minute (cpm) vs. ≤ 100 cpm), moderate PA (≥527 cpm, > 3000 cpm or 2001-3999 cpm) and vigorous PA (VPA) (≥ 2818 cpm or > 4000 cpm).

Seven studies used self-report questionnaires to assess PA. Of these, four specifically assessed weight-bearing (min/wk (405), MET hr/wk (406), past-year PA questionnaire (PYPAQ) scores (145)) or loaded PA (High (≥2 times BW) or Low (<2 times BW) **i**mpact groups) (407). One study used the Physical Activity Questionnaire for Children (PAQ-C) (306) which provided a general PA score (1 (low) to 5 (high)) but did not specifically describe PA intensity, frequency or duration (403). One study used a questionnaire that focused on farm tasks to represent PA (402). The multicenter study (404) reported minutes of metabolic equivalents (METs) acquired in a week from a PA questionnaire (PAQ). As is evident, PA was assessed in many different ways in these observational studies.

3.3.2.1.3 Observational studies of organized sports

Of the 13 studies that focused on organized sports, most (n=11) assessed girls and of those most (n=9) focused on gymnastics. Artistic and rhythmic gymnastics were included under the umbrella of gymnastics (6/9). Other studies assessed athletes who participated in tennis (107), running (416), soccer *and* swimming (415) or running *and* swimming *and* cycling *and* triathlon (412). Sample size of different sport groups ranged from n=9 to 60 per group; participants ranged in age from 5 to 18 years.

For more than half of organized sports studies (7/13) (146,408,410,413,414,417,418), participants began training during pre-puberty. All participants in organized sports acquired at least one year of sport-specific training before study entry. Levels of participation and hours of training were derived mainly from training logs and ranged from recreational/pre-competitive (mean=2 h/week) to elite (mean=24 h/week). We were unable to report compliance to filling out training logs, as this was not reported in any study. No studies reported training intensity, nor were we able to discern specific types of training (strength, endurance or flexibility).

Key findings regarding design of observational studies: Studies of recreational PA were mostly cross-sectional. Very few (three studies) used objective measures (accelerometry) to assess PA while most (seven studies) reported a range of outcomes (total scores, min/week of PA, hours/week PA to METs min/week) from PA questionnaires. The preponderance of organized sports studies focused on gymnasts, most of whom started training during pre-puberty. Information about training (training hours and years of training) was derived mainly from training logs; intensity and type of training was not specified.

3.3.3 Differences in research methods across studies

Studies varied as to how bone strength was estimated. In 13 DXA-based studies, bone strength was derived using HSA (125) (Table 3.2, Table 3.3 and Table 3.4). Five studies used algorithms devised by Carter et al. (126) (n=1) (399), Sievanen et al. (122) (n=2) (33,408) or Karlamangla et al.(127) (n=2) (340,402). Approximately 14 different analysis protocols were used to estimate bone strength across pQCT (n=12) and MRI studies (n=4). Single studies used HR-pQCT (405), DXA (Osteopar) (404) and paired vertebral analysis (410) for measurement and analysis. We did not critically evaluate whether analyses protocols adopted to estimate bone strength were appropriate but accepted all as valid estimates of bone strength.

Studies also varied as to the anatomical site assessed; 21 DXA studies reported results at the proximal femur (n=16) (31,33,338–340,359,396–403,414,415), radius (n=4) (404,408,409,411) or the lumbar spine (n=3) (33,402,410). Twelve studies used pQCT and reported findings at the tibia (n=9) (30,32,113,145,358,406,407,413,417), radius (n=5) (146,409,411,413,418) or femur (n=1) (145); one study used HR-pQCT to assess bone strength at the tibia (405). Four other studies used MRI to assess bone strength at the femur (n=2) (34,293), tibia (n=1) (416) or humerus (n=1) (107).

RCTs tended to focus on prepubertal (n=7) (34,113,396–399,401) or mixed pre- and peri-pubertal groups (n=5) (31,32,359,400,419); only two studies included both pre- and post-pubertal participants (30,33). Recreational PA and organized sports studies more often assessed bone outcomes in pre-pubertal (n=2) (338,418) or mixed pre- and peri-pubertal groups .

Table 3.2. Randomized controlled trials and controlled trials (n = 14) of physical activity effects on bone strength, structure and mass in children and adolescents. Ratings are indicated as: *WEAK, **MODERATE, ***STRONG.

Reference & Rating	Population		Study design	Intervention	Control	Duration	Com-pliance	PA measure-ment	Imaging methods	Results (EX > CON) §	
	n (EX, CON)	Age (yrs) ¶								Bone strength	Bone mass & structure
Prepubertal boys											
Alwis et al. (2008a) (396) **	137 (80,57)	EX: 7.8±0.6 CON: 8.0±0.6	CT: 1 EX school, 3 CON schools	Extend PE to 200 min/wk	Usual PE of 60 min/wk	2 yrs	Not reported	Accelero-meter	DXA (DPX-L, Lunar) HSA	FN **Z**, **CSMI**: NS	FN: NS
Bradney et al. (1998) (399) **	38 (19, 19)	EX: 10.4±0.2 CON: 10.3±0.2	CT: 1 EX school, 1 CON school	Regular PE with additional 30 min, 3x/wk weight-bearing activities	Regular PE: 2hr/wk	8 mo	96%	Not reported	DXA (DPX-L, Lunar) Carter	FS: **CSMI**, **Z**, **Strength index:** NS	FS: NS
Macdonald et al. (2009) (113) ***	202 (139, 63)	EX: 10.2±0.6 CON: 10.3±0.6	RCT: 7 EX schools, 3 CON schools	1) Usual PE with extra 15 min PA, 5x/wk, 2) Bounce at the Bell: Progressive jumps, 3min x 3x/day, 4x/wk	Regular PE: 40 min x 2x/wk	11 mo	74%	PAQ-C	pQCT (XCT 2000, Stratec)	Left 50% tibia: $\mathbf{I_{max}}$ = 3.0	Left 50% tibia: Structure NS vBMD not reported

Table 3.2. continued

Reference & Rating	Population		Study design	Intervention	Control	Duration	Com-pliance	PA measure-ment	Imaging methods	Results (EX > CON) §	
	n (EX, CON)	Age (yrs) ¶								Bone strength	Bone mass & structure
Prepubertal boys (cont.)											
Mackelvie et al. (2004) (401) **	64 (31, 33)	EX: 10.2±0.5 CON: 10.1±0.5	RCT: 7 EX schools, 7 CON schools	Progressive, 10-12 min weight-bearing warm-up during PE, 3x/wk	10 min warm-up stretches	2 school yrs of 7mo/yr	Not reported	PAQ-C	DXA (QDR 4500W, Hologic) HSA	NN: **CSMI** = 12.4 **Z** = 7.4	NN: **BMC** = 4.3 Structure NS
Prepubertal girls											
Alwis et al. (2008b) (397) **	103 (53, 50)	EX:7.7±0.6 CON:7.9± 0.6	CT: 1 EX school, 3 CON schools	Extend PE to 200 min/wk	Usual PE of 60 min/wk	1 yr	90%	Validated questionn aire	DXA (DPX-L, Lunar) HSA	FN **Z**, **CSMI**: NS	FN: NS
Alwis et al. (2008c) (398) **	85 (42,43)	EX:7.7±0.6 CON:7.9± 0.6	CT: 1 EX school, 3 CON schools	Extend PE to 200 min/wk	Usual PE of 60 min/wk	2 yrs	Not reported	Accelerom eter and validated questionna ire	DXA (DPX-L, Lunar) HSA	FN **Z,** **CSMI**: NS	FN: NS
Greene et al. (2009) (34) ***	39 (HD: 13, LD: 13, 13) HD: EX high-drop LD: EX low-drop	EX HD: 7.89±1.1 EX LD: 7.79±0.9 CON:7.87 ±0.8	RCT: School-based, 1 school	ND single-leg drop, 5-step bench landing exercises (HD = 28cm step, LD = 14cm step), 10 sets, 3x/wk	Less than 3hr/wk of organized sports outside of school	28 wks	100%	Past-Year Leisure-Time Physical Activity Question naire	MRI (1.5 tesla, Philips)	Mid-third femur $\mathbf{I_{max}}$, $\mathbf{I_{min}}$, $\boldsymbol{J_0}$: NS (Adj. LM)	Mid-third femur: NS

Table 3.2. continued

Reference & Rating	Population		Study design	Intervention	Control	Duration	Com-pliance	PA measure-ment	Imaging methods	Results (EX > CON) [§]	
	n (EX, CON)	Age (yrs) [¶]								Bone strength	Bone mass & structure
Pre- and peri-pubertal girls											
Petit et al. (2002) (31) ***	177 PRE (44, 26) PERI (43, 64)	EX PRE:10.0 ±0.6 PERI:10.4 ±0.7 CON PRE:10.1 ±0.5 PERI:10.5 ±0.6	RCT: 7 EX schools, 7 CON schools	Progressive, 10-12 min high-impact (3.5-5x body weight) during PE, 3x/wk	Not reported	7 mo	Not reported	PAQ-C	DXA (QDR 4500, Hologic) HSA	Left NN: PERI **Z** = 4.0 Left IT and FS **Z:** NS	Left NN: PERI **CSA** = 2.3 **Ct.Th** = 3.2 **aBMD** = 2.6 Left IT and FS: NS
Pre-menarcheal and post-menarcheal girls											
Heinonen et al. (2000) (30) ***	126, PreM (25, 33) PostM (39, 29)	EX PreM: 11.7±1.3 PostM: 13.7±0.9 CON PreM: 11.0±0.9 PostM: 13.7±1.0	CT: 2 EX schools, 3 CON	Step aerobics program, 2x/wk; 50 min/session, with extra jumps	Maintain regular PA	9 mo	65%	Physical activity diary, fitness tests	pQCT (XCT 3000, Stratec) Sievanen	Right 50% tibia $\mathbf{Z}_{\mathbf{density}}$: NS	Right 50% tibia: NS

Table 3.2. continued

Reference & Rating	Population		Study design	Intervention	Control	Duration	Com-pliance	PA measure-ment	Imaging methods	Results (EX > CON) [§]	
	n (EX, CON)	Age (yrs) [¶]								Bone strength	Bone mass & structure
Pre- and peri-pubertal boys and girls											
Anliker et al., (2012) (358) **	45, Boys (12,11), Girls (10,12)	EX: 10.±1.2 CON: 10.8±1.1	CT: 2 EX schools, 1 CON school	Progressive, 10-min PE jumping, 2x/week	Regular PE	9 mo	100%	PAQ-C	pQCT (XCT 3000, Stratec)	ND 14%, 38% 66% tibia **SSI_p**: NS	ND 4%, 14%, 38% 66% tibia: NS
Macdonald et al. (2007) (32) **	410, PRE (145, 64), PERI (136, 65)	EX Boys:10.2 ±0.6 Girls:10.2 ±0.6 CON Boys:10.3 ±0.6 Girls:10.3 ±0.5	RCT: 7 EX schools, 3 CON schools	1) Usual PE with extra 15 min PA, 5x/wk, 2) Bounce at the Bell: Progressive jumps, 3min x 3x/day, 4x/wk	Regular PE: 40 min x 2x/wk	11 mo	74%	PAQ-C	pQCT (XCT 2000, Stratec)	Left 8% tibia PRE Boys: **BSI** = 3.7 (Adj. MCSA) Left 50% tibia: **SSI_p** = NS	Left 8% tibia PRE Boys: **Tt.vBMD** = 2.2 Structure NS Left 50% tibia: NS
Pre- and peri-pubertal boys and girls											
Macdonald et al. (2008) (400) **	410 PRE (151, 62) PERI (142, 55)	EX Boys:10.2 ±0.5 Girls:10.2 ±0.6 CON Boys:10.3 ±0.7 Girls:10.2 ±0.5	RCT: 7 EX schools, 3 CON schools	1) Usual PE with extra 15 min PA, 5x/wk, 2) Bounce at the Bell: Progressive jumps, 3min x 3x/day, 4x/wk	Regular PE: 40 min x 2x/wk	11 mo	74%	PAQ-C	DXA (QDR 4500W, Hologic) HSA	Per protocol analysis Girls FN: **Z** = 5.4 (Adj. LM)	Per protocol analysis Girls FN: **CSA** = 3.7 **SPW** = NS **BMC** = 3.7

Table 3.2. continued

Reference & Rating	Population		Study design	Intervention	Control	Duration	Com-pliance	PA measur e-ment	Imaging methods	Results (EX > CON) §	
	n (EX, CON)	Age (yrs) ¶								Bone strength	Bone mass & structure
Pre- and peri-pubertal boys and girls											
McKay et al. (2005) (359) **	124 (51, 73) Matched for race, sex, height and maturity	EX: 10.1±0.5 CON: 10.2±0.4	CT: 3 EX schools, number of CON schools not stated	10 counter movement jumps, 3min/day, 3x/day, 4x/wk	Regular PE	8 mo	97%	PAQ-C	DXA (QDR 4500W, Hologic) HSA	NN and FS **Z**: NS	NN and FS: NS
Boys and girls Tanner 1-5											
Weeks et al. (2008) (33) ***	99, Boys (22, 24), Girls (30, 23)	EX Boys: 13.8±0.4, Girls13.7±0.4 CON: Boys 13.8±0.4 Girls13.7±0.5	RCT: 1 school	Progressive, 10-min PE jumping, 2x/wk, occasional upper body strengthening activities	Usual PE	8 mo	80%	Bone-specific physical activity questio nnaire (BPAQ)	DXA (XR-36, Norland) Sievanen	Intent-to-treat analysis Boys and girls LS: **IBS** =3.5 Boys and girls FN **CSMI:** NS	Intent-to-treat analysis Boys and girls LS and FN: NS

¶Reported as mean±SD unless stated otherwise.

§Results are displayed as percent differences that are calculated from mean values in studies if no reports were provided in tables or text.

EX, intervention group; CON, control group; yrs, years; PA, physical activity; CT, controlled trial; PE, physical education; min/wk, minutes per week; DXA, dual energy X-ray absorptiometry; HSA, hip structural analysis; FN, femoral neck; Z, section modulus (cm^3); CSMI, cross-sectional moment of inertia (cm^4); NS, not significant; NR, not relevant; x/wk, times per week; hr/wk, hours per week; FS, femoral shaft; Strength index, section modulus divided by femur length; RCT, randomized-controlled trial; PAQ-C, physical activity questionnaire for children; I_{max}, largest area moment of inertia from the central axis (mm^4); vBMD, volumetric bone mineral density (g/cm^3); mo/yr, months per year; ND, non-dominant; wks, weeks; MRI, magnetic resonance imaging; I_{min}, smallest area moment of inertia from the central axis (mm^4); J_0; polar moment of inertia (mm^4); Adj, adjusted; LM, lean mass (kg); PRE, pre-pubertal; PERI, peri-pubertal; CSA, cross-sectional area (cm^2); Ct.Th, cortical thickness (mm); aBMD, areal bone mineral density (g/cm^2); PreM, pre-menarcheal; PostM, post-menarcheal; mo, months; $Z_{density}$, density-weighed section modulus (mm^3); BSI, bone strength index (mg^2/mm^4); MCSA, muscle cross-sectional area (mm^2); SSI_p, polar strength strain index (mm^2); Tt.BMD, total bone mineral density (mg/cm^3); SPW, sub-periosteal width (cm); IBS, index of bone structural strength (g^2/cm^4).

Table 3.3. Observational studies (n=10) of the relationship between recreational physical activity and bone strength, structure and mass in children and adolescents. Ratings are indicated as: *WEAK, **MODERATE and ***STRONG

Reference & Rating	Population		Study design	Physical Activity		Imaging methods	Results[§]	
	n (♂, ♀)	Age (yrs) [¶]		Measurement	Outcomes		Bone strength	Bone mass & structure
Prepubertal boys and girls								
Janz et al. (2004) (338)**	467 (218, 249)	Boys: 5.2±0.4 Girls: 5.3±0.4	Cross-sectional, Iowa Bone Development Study	Accelerometer Averaged 3 days, 8h/day, 1-min epoch	PA intensity levels (♂, ♀; min/day): Sed = 244±43, 251±48 Mod = 267±44, 262±44 Vig = 38±19, 28±14	DXA (QDR 2000, Hologic) HSA	Vig PA, R^2 (%) Left hip (Boys and girls): NN **Z**= 2.6 IT **Z**= 4.0 FS **Z**= 2.1 (Adj. LM)	Vig PA, R^2 (%) Left hip (Boys and girls): NN **CSA** = 4.9 IT **CSA** = 4.5 FS **CSA** = 3.9 aBMD not reported
Pre- and peri-pubertal girls								
Wang et al. (2005) (407)**	242, Tan1 (Low: 43, Mod: 43, High: 42, High-Impact: 30, Low-Impact: 98) Tan2 (Low: 38, Mod: 38, High: 38, High-Impact: 19, Low-Impact: 95)	Tan1 Low: 10.7±0.6 Mod: 10.8±0.5, High: 10.9±0.7 Tan2 Low: 11.4±0.7, Mod: 11.5±0.7, High: 11.5±0.6)	Cross-sectional	Questionnaire scores = $\Sigma_{1\text{-}3}$ (Freq (times/wk) x Intensity index x Duration (hrs) x Load (none=1, load=2)) for Low, Mod and High PA High (≥2x body weight) or Low (<2x body weight) Impact groups based on most favourite activity	PA scores of Low, Mod and High PA not provided High Impact (at least 2 times body weight)	pQCT (XCT 2000, Stratec)	High Impact > Low Impact Tan1 Left 60% tibia: $\mathbf{I_p}$ = 7.0	High Impact > Low Impact Tan1 Left 60% tibia: **CSA** = 3.4 **Ct.Th** = 5.5 **Ct.vBMD** = 1.4 **BMC** = 5.5

Table 3.3. continued

Reference & Rating	Population		Study design	Physical Activity		Imaging methods	Results[§]	
	n (♂, ♀)	Age (yrs) [¶]		Measurement	Outcomes		Bone strength	Bone mass & structure
Pre- and peri-pubertal boys and girls								
Sardinha et al. (2008) (340) **	293 (150, 143)	Boys: 9.7±0.3 Girls: 9.7±0.3	Cross-sectional	Accelerometer Averaged 3 days, ≥ 600min data, 1-min epoch	Total PA (♂, ♀; counts per min/day) = 732±272, 587±190 PA intensity levels (♂, ♀; min/day): Sed = 962.3±89.9, 982.4±77.8 Light = 278.9±49.6, 297.9±47.7 Mod = 169.4±55.4, 141.7±47.3 Vig = 29.4±21.2, 18.0±14.4 Vig PA quartiles (♂,♀; min/day) Q_1 = 6.4±3.8, 3.2±2.4 Q_2 = 19.0±4.3, 11.8±2.4 Q_3 = 32.6±5.1, 19.4±2.9 Q_4 = 57.4±12.9, 37.7±13.2	DXA (QDR 1500, Hologic) Karlamangla	Vig PA, SR^2 (%) Left hip (♂, ♀) FN **Compressive**= 6.3, 5.7 FN **Bending**= 3.2 (Mod PA), 6.8 FN **Impact**= 9.3, 4.7 (Adj. LM) Vig PA $Q_4 > Q_1$, % difference Left hip, (♂, ♀) FN **Compressive**= 12, NS FN **Bending**= 10, 11 FN **Impact**= 14, NS	No data reported
Pre-PHV girls								
Farr et al. (2011) (145)**	465 Each quintile, n=93	10.6±1.1	Cross-sectional Jump-In: Building Better Bones Study	PYPAQ scores = $\Sigma_{1\text{-}n}$ Freq ([mo/12]*day/wk) x Duration (min/session) x Load (peak strain score); n=number of activities	Quintiles PYPAQ scores (mean±SD): Qu_1 = 192.8±77.8 Qu_2 = 401.8±51.6 Qu_3 = 618.9±79.7 Qu_4 = 952.8±119.7 Qu_5 = 2130.6±1346.6	pQCT (XCT 3000, Stratec)	$Qu_5 > Qu_1$ % ND 20% femur: **SSI** = 7.7 ND 4% tibia: **BSI** = 8.7 ND 66% tibia: **SSI** = 8.1	$Qu_5 > Qu_1$ % ND 20% femur: **Es.Pm** = 3.6 **Ps.Pm** = 3.2 ND 4% tibia: **Ps.Pm** = 2.8 ND 66% tibia: **Es.Pm** = 5.9 **Ps.Pm** = 3.7

Table 3.3. continued

Reference & Rating	Population		Study design	Physical Activity		Imaging methods	Results[§]	
	n (♂, ♀)	Age (yrs) [¶]		Measurement	Outcomes		Bone strength	Bone mass & structure
Pre- and post-menarcheal girls								
Moyer-Mileur et al. (2001) (406)**	84	12.8±0.8	Cross-sectional	Modified PYPAQ for weight-bearing PA	Weight-bearing PA (MET-h/wk) = 36.8±25.7 (range: 5-107)	pQCT (XCT 2000, Stratec)	ND 10% and 66% tibia: **SSI**: NS	ND 10% and 66% tibia: NS
Peri- and post-pubertal boys and girls								
McKay et al. (2011) (405)**	278, (146, 132)	Boys: 16.4±1.7 Girls: 16.9±1.7	Cross-sectional, Healthy Bones Study III	PAQ-A Impact PA (min/wk) Non-impact PA (min/wk)	PAQ-A (♂, ♀; min/wk) Impact PA = 344±300, 219±249 Non-impact PA = 166±209, 164±199	HR-pQCT (Xtreme CT, Scanco Medical)	Impact PA, R^2 (%) ND 8% tibia: $\mathbf{I_{max}}$ Boys = 12.0 $\mathbf{I_{min}}$ Boys = 10.0 (Adj. MCSA)	Impact PA, R^2 (%) ND 8% tibia: **Tt.Ar** Boys = 6.0 vBMD NS (Adj. MCSA)
Two years around PHV boys and girls								
Forwood et al. (2006) (403) **	230, (109, 121)	Ages 8 to 15 (2yrs before and after PHV)	Longitudinal (7 yrs), PBMAS	PAQ-C scores (Lowest = 1, Highest = 5)	Tertiles PAQ-C score (details not available) T_1 = lowest tertile, T_3 = highest tertile	DXA (QDR 2000, Hologic) HSA	PA scores, β±SE NN **Z** Boys: 0.015±0.007 NN **Z** Girls: 0.009±0.004 (NS after adj. LM)	PA scores, β±SE NN **CSA** Boys: 0.024±0.011 NN **CSA** Girls: 0.015±0.007 aBMD not reported (NS after adj. LM)

Table 3.3. continued

Reference & Rating	Population		Study design	Physical Activity		Imaging methods	Results[§]	
	n (♂, ♀)	Age (yrs)[¶]		Measurement	Outcomes		Bone strength	Bone mass & structure
Girls Tanner 1-5								
Kardinaal et al. (2000) (404)**	1116	Tan1: 11.7±0.7 Tan2: 11.8±0.7 Tan3: 12.3±0.9 Tan4: 13.4±1.3 Tan5: 14.3±1.2	Cross-sectional, CALEUR study	Questionnaire scores = $\Sigma_{four\ intensity\ levels}$ (min/wk × METs)	PA scores (min/wk x METs) Tan1 = 8221±4164 Tan2 = 7729±3341 Tan3 = 7660±4528 Tan4 = 7207±5898 Tan5 = 6875±7569	DXA (p-DXA, Osteoscan) Osteopar software	ND radius **BBRI**: NS	ND radius: NS
Boys, maturity not assessed								
Bhattacharya et al. (2008) (402)**	36 (18, 18) boys	Farm: 15.3±0.4 Non-farm: 16.0±0.44	Cross-sectional	Questionnaire on 22 farm tasks	QA score Farm = 10.8±1.0 Non-farm = 2.7±0.6	DXA (Hologic – no model) Karlamangla	Lumbar and left PF **Compressive, Z, BR**: NS	Lumbar and left PF: NS
Boys and girls, maturity not assessed								
Janz et al. (2007) (339)**	Baseline: 393 (185, 208) T-2: 445 (212, 233) T-3: 387 (184, 203)	Boys Baseline: 5.2±0.4 T-2: 8.7±0.6 T-3: 11.2±0.3 Girls Baseline: 5.3±0.4 T-2: 8.7±0.6 T-3: 11.2±0.3	Longitudinal (6 yrs), Iowa Bone Development Study	Accelerometer Avg 3days, 8h/day, 1-min epoch	MVPA (♂, ♀; min/day): Boys Baseline = 31.7±16.1 T-2 = 38.6±21.3 T-3 = 41.6±22.0 Girls Baseline = 31.7±16.1 T-2 = 38.6±21.3 T-3 = 41.6±22.0	DXA (QDR 2000 & 4500, Hologic) HSA	10 min of MVPA, β±SE Left FN **Z** Boys = 0.004±0.002 (Adj. LM)	10 min of MVPA, β±SE Left FN **CSA** Boys = 0.012±0.003 aBMD not reported

[¶]Reported as mean±SD unless stated otherwise.
[§]Percent differences are calculated from mean values in studies if no reports were provided in tables or text.
h/day, hours per day; min, minute; PA, physical activity; min/day, minutes per day; Sed, sedentary; Mod, moderate PA; Vig, vigorous PA; DXA, dual energy X-ray absorptiometry; HSA, hip structural analysis; R^2, coefficient of determination; NN, narrow neck; Z, section modulus (cm^3);IT, inter-trochanter; FS, femoral

shaft; Adj, adjusted; LM, lean mass (kg); CSA, cross-sectional area (mm^2 or cm^2); aBMD, areal bone mineral density (g/cm^2); Tan1, Tanner stage 1; Tan2, Tanner stage 2; Freq, frequency; times/wk, times per week; hrs, hours; pQCT, peripheral quantitative computed tomography; Ip, polar moment of inertia (g/mm); Ct.Th, cortical thickness (mm); Ct.vBMD, cortical volumetric bone mineral density (mg/cm^3), BMC, bone mineral content (g); min, minutes; Light, light PA; Q_1, first quartile; Q_2, second quartile; Q_3, third quartile; Q_4, fourth quartile; SR^2, squared semi-partial correlation (%); FN, femoral neck; Compressive; compressive strength (g/kg); Bending, bending strength (g/kg); Impact, impact strength (g/kg); PHV, peak height velocity; PYPAQ, past-year physical activity questionnaire; mo, month(s); Qu_1, first quintile; Qu_2, second quintile; Qu_3, third quintile; Qu_4, fourth quintile; Qu_5, fifth quintile; ND, non-dominant; SSI, strength-strain index (mm^3); BSI, bone strength index (mg^2/mm^4); Es.Pm; endosteal perimeter/circumference (mm); Ps.Pm; periosteal perimeter/circumference (mm); h/wk, hours per week; NS, not significant; PAQ-A, physical activity questionnaire for adolescents; min/wk, minutes per week; HR-pQCT, high resolution peripheral quantitative computed tomography; R2, adjusted regression variance; I_{max}, largest area moment of inertia from the central axis (mm^4); I_{min}, smallest area moment of inertia from the central axis (mm^4); MCSA, muscle cross-sectional area; Tt.Ar, total area (mm^2); vBMD, volumetric bone mineral density (g/cm^3) PBMAS, Pediatric Bone and Mineral Accrual Study; PAQ-C, physical activity questionnaire for children; Tan3, Tanner stage 3; Tan4, Tanner stage 4; Tan5, Tanner stage 5; METs, metabolic equivalents; BBRI, breaking bending resistance index (mm^3); QA, questionnaire; PF, proximal femur; BR, buckling ratio; T-2, second time point; T-3, third time point; MVPA, moderate-to-vigorous physical activity; β, regression coefficient; SE, standard error; FN, femoral neck.

Table 3.4. Observational studies (n=13) of the relationship between participation in organized sports and bone strength, structure and mass in children and adolescents. Ratings are indicated as: *WEAK, **MODERATE and ***STRONG.

Reference & Rating	Population		Characteristics of PA		Imaging methods	Results (% differences) [§]	
	n (EX, NON)	Age (yrs) [¶]	Sports group	Non-sports group		Bone strength	Bone mass & structure
Gymnastics – Pre-menarcheal girls							
Dowthwaite et al. (2007) (408)**	56: Tan1 (12, 10), Tan2 (16, 18)	7 to 12 yrs	Artistic gymnastic training: min. 6 h/wk training for the past 2 yrs	Weight-bearing PA min. 5h/wk	DXA (QDR 4500W, Hologic) Sievanen	GYM > NON Left UD radius (Tan1, Tan2): **IBS** = 56, 41 **FSR** = 45.3, 61.6 Left 1/3 radius (Tan1, Tan2): **Z** = 38, 24 **FSR** = 59.4, 34.3 (Adj. LM)	GYM > NON Left UD radius (Tan1, Tan2): **Ps.Wi** ~ 12.5, NS **BMC** =34.5, 26.7 **aBMD** ~24.8, 18.8 **BMAD** ~ 11.0, 13.2 Left 1/3 radius (Tan1, Tan2): **Ps.Wi** ~ 11.6, 7.3 **CSA** ~ 22.8, 15.9 **Ct.Th** ~ 9.8, NS **BMC** = 20, 17.5 **aBMD** ~ 10.0, 7.6
Faulkner et al. (2003) (414)**	60 (30,30)	GYM: 11.7±1.9 NON: 11.5±1.8	GYM (h/wk): 15 Years trained: 5.2 PAQ-C score: 3.17±0.5	PAQ-C score: 3.06±0.65	DXA (QDR 2000, Hologic) HSA	GYM > NON Shaft: **Z** ~ 21.7 **S-SI** ~ 20.0 **CSMI** ~ 23.7 NN: **Z** ~ 9.3 NS after adjusting for lean body mass	GYM > NON Shaft: **CSA** ~ 16.7 **SPW** ~ 3.8 **aBMD** ~ 11.7 NN: **CSA** ~ 12.6 **SPW** ~ -4.9 **Es.Dm** ~ -8.6 **aBMD** ~ 18.9 NS after adjusting for lean body mass

Table 3.4. continued

Reference & Rating	Population		Characteristics of PA		Imaging methods	Results (% differences) [§]	
	n (EX, NON)	Age (yrs) [¶]	Sports group	Non-sports group		Bone strength	Bone mass & structure
Gymnastics – Pre-menarcheal girls (cont.)							
Tournis et al. (2010) (417)**	49 (26, 23)	R-GYM: 11.3±0.2 NON: 10.9±0.1	R-GYM (h/wk): 24 Training age (yrs): 4.4±0.3 PA (METs/day): 46.8±1.4	PA (METs/day): 34.4±1.2	pQCT (XCT 2000, Stratec)	R-GYM > NON ND 14% tibia: **SSI_p** ~ 19.0	R-GYM > NON ND 14% tibia: **Tt.Ar** ~ 11.0 **Ct.Ar** ~ 16.6 **Tb.Ar** ~ 11.0 **Tt.BMC** ~ 15.7 **Ct.BMC** ~ 18.0 **Tb.BMC** ~ 39.8
						ND 38% tibia: **SSI_p** ~ 31.5	ND 38% tibia: **Tt.Ar** ~15.5 **Ct.Ar** ~ 30.1 **Ct.Th** ~ 25.8 **Ps.Pm** ~ 7.7 **Tt.vBMD** ~ 7.8 **Ct.BMC** ~ 30.3 **Tt.BMC** ~ 25.0
Gymnastics – Pre- and post-menarcheal girls							
Dowthwaite et al. (2011b) (410)**	114, (60, 54)	X-GYM/GYM: 14.2 (CI: 13.7, 14.7) NON: 13.8 (CI: 13.3, 14.4)	Artistic GYM (h/wk): 10.5 (CI: 8.7, 12.6) General PA (h/wk): 7.4 (CI: 6.1,.0)	General PA: 3.0 (CI: 2.4, 3.7)	DXA (Discovery A, Hologic)	X-GYM/GYM > NON L3: $_{PA}$ **IBS** = 17.6 $_{LAT}$ **IBS** = 16.4 $_{PALAT}$ **FRI** = -10.4	X-GYM/GYM > NON L3: $_{PA}$ **Wi** ~ 8.5 $_{PA}$ **CSA** ~ 17.5 $_{PA}$ **Vol** ~10.6 $_{LAT}$ **Ar** ~ -3.8 $_{LAT}$ **Ht** ~ -6.1 $_{PALAT}$ **CSA** ~ 10.5 $_{PA}$ **BMC** ~ 10.7 $_{PA}$ **aBMD** ~ 8.4 $_{LAT}$ **aBMD** ~ 8.0 $_{LAT}$ **BMAD** ~ 5.9

Table 3.4 continued

Reference & Rating	Population		Characteristics of PA		Imaging methods	Results (% differences) [§]	
	n (EX, NON)	Age (yrs) [¶]	Sports group	Non-sports group		Bone strength	Bone mass & structure
Gymnastics – Pre- and peri-pubertal girls							
Burt et al. (2012) (146)**	88 (HGYM: 30, LGYM: 29, 29)	HGYM: 8.09 (CI: 8.1, 9.0) LGYM: 8.3 (CI: 7.8, 8.8) NON: 8.6 (CI: 8.1, 9.0)	Artistic GYM (h/wk) HGYM: 10.6 (CI: 9.2, 12.0) LGYM: 3.0 (CI: 2.5, 3.5) Years trained HGYM: 3.1(CI: 2.6, 3.5) LGYM: 2.7 (CI: 2.2, 3.3)	Total PA (h/wk): 2.2 (CI: 1.5, 2.6)	pQCT (XCT 2000, Stratec)	HGYM > NON ND 4% radius: **BSI** = 21 **S/W** = 41 ND 66% radius: **SSIp** = 10 **S-W** = 19 LGYM > NON ND 4% radius: **S/W** = 23 S/W 4% radius HGYM remained significant after adjusting for MCSA.	HGYM > NON ND 4% radius: **Tt.Dn** = 10 ND 66% radius: **Tt.Ar** = 6 NS after adjusting for MCSA.
Gymnastics – Pre- and post-menarcheal girls							
Dowthwaite et al. (2011b) (410)**	114, (60, 54)	X-GYM/GYM: 14.2 (CI: 13.7, 14.7) NON: 13.8 (CI: 13.3, 14.4)	Artistic GYM (h/wk): 10.5 (CI: 8.7, 12.6) General PA (h/wk): 7.4 (CI: 6.1,.0)	General PA: 3.0 (CI: 2.4, 3.7)	DXA (Discovery A, Hologic)	X-GYM/GYM > NON L3: $_{PA}$ **IBS** = 17.6 $_{LAT}$ **IBS** = 16.4 $_{PALAT}$ **FRI** = -10.4	X-GYM/GYM > NON L3: $_{PA}$ **Wi** ~ 8.5 $_{PA}$ **CSA** ~ 17.5 $_{PA}$ **Vol** ~10.6 $_{LAT}$ **Ar** ~ -3.8 $_{LAT}$ **Ht** ~ -6.1 $_{PALAT}$ **CSA** ~ 10.5 $_{PA}$ **BMC** ~ 10.7 $_{PA}$ **aBMD** ~ 8.4 $_{LAT}$ **aBMD** ~ 8.0 $_{LAT}$ **BMAD** ~ 5.9

Table 3.4. continued

Reference & Rating	Population		Characteristics of PA		Imaging methods	Results (% differences) [§]	
	n (EX, NON)	Age (yrs) [¶]	Sports group	Non-sports group		Bone strength	Bone mass & structure
Gymnastics – Girls mixed maturity							
Dowthwaite at el. (2012) (411)**	96, 77 Tan1 (26, 20) Tan2 (49, 31) PostM (21, 26)	X-GYM/GYM Tan1: 9.9±1.4 Tan2: 11.8±1.2 PostM: 14.6±1.5 NON Tan1: 9.6±1.1 Tan2: 11.1±1.7 PostM: 14.1±1.0	GYM (h/wk) Tan1: 11.0±4.6 Tan2: 12.4±4.2 PostM: 16.1±4.1 General PA (h/wk) Tan1: 11.7±4.8 Tan2: 12.6±4.3 PostM 17.0±4.4	General PA (h/wk) Tan1: 3.1±2.5 Tan2: 2.9±1.8 PostM: 5.0±3.6	DXA (QDR 4500W, Hologic) HSA	GYM > NON ND UD radius: **IBS** Tan1 = 44 Tan2 = 62 PostM = 96 1/3 radius: **Z** Tan2 = 20 PostM = 96 Left NN: **Z** = 9.9 **BR** All = -19.0 Tan1 = -25 Tan2 = -13 **CSMI** = 3.4 (Adj. LM)	GYM > NON ND UD radius: NS **BMC** Tan1 = 24 Tan2 = 35 PostM = 55 1/3 radius: **Wi** PostM = 15 **Ct.Ar** PostM = 40 **Es.Dm** PostM = 18 **BMC** Tan2 = 13 PostM = 21 Left NN: **Ct.Ar** = 9.4 **Es.Dm** = -7.2 **Ct.Th** = 16.4 **Wi** = -4.1 **aBMD** = 14.5
Gymnastics – Prepubertal boys and girls							
Ward et al. (2005) (418)**	86 Boys (17,20) Girls (27, 22)	Boys: GYM: 9.4±1.2 NON: 8.9±1.6 Girls: GYM: 8.7±1.7 NON: 8.6±1.2	Boys PA (h/wk): 15.0±3.3 Girls PA (h/wk): 14.7±5.1	Boys PA (h/wk): 8.4±3.7 Girls PA (h/wk): 5.3±2.6	pQCT (XCT 2000, Stratec)	GYM > NON (♂ & ♀) ND 50% radius: **SSI** = 13.6	GYM > NON (♂ & ♀) ND 50% radius: **Tt.Ar** = 9.2 **Ct.Ar** = 8.2 vBMD NS

Table 3.4. continued

Reference & Rating	Population		Characteristics of PA		Imaging methods	Results (% differences) [§]	
	n (EX, NON)	Age (yrs) [¶]	Sports group	Non-sports group		Bone strength	Bone mass & structure
Gymnastics – Pre-PHV boys and girls							
Erlandson et al. (2011) (413)**	120 Boys (GYM: 9, X-GYM: 26, NON:19) Girls (GYM: 20, X-GYM: 20, NON: 26)	Boys GYM: 5.7±1.5 X-GYM: 6.6±1.2 NON: 6.8±1.2 Girls GYM: 7.1±1.1 X-GYM: 7.4±1.0 NON: 6.9±1.5	Artistic GYM (h/wk) Boys GYM: 2.0±2.3, X-GYM: 1.0±0.2 Girls GYM: 5.0±5.0, X-GYM: 1.5±1.8 Years trained Boys GYM: 2.9±1.1, X-GYM: 1.9±0.9 Girls GYM: 3.2±0.9, X-GYM: 2.1±0.9	No gymnastics training	pQCT (XCT 2000, Stratec)	GYM > NON (♂ & ♀) Left 4% radius: **BSI** = 25 X-GYM > NON Left 4% radius: **BSI** = 24 Left 4% tibia: **BSI** = 22 X-GYM > GYM Left 66% tibia: **SSI_p** = 8 NS after adjusting for lean body mass	GYM > NON (♂ & ♀) Left 4% radius: NS **BMC** = 18 **Tt.vBMD** = 6 X-GYM>NON Left 4% radius **BMC** = 14 **Tt.vBMD** = 9 Left 4% tibia **BMC** ~ 12.1 X-GYM > GYM Left 66% tibia: **BMC** ~ 7.0 Structure NS NS after adjusting for lean body mass
Running – Post-menarcheal girls							
Greene et al. (2005) (416)**	40 (20,20)	RUN: 15.9±1.6 NON: 16.0±1.8	PA (h/wk): 8.9±4.1 Trained for the past 2 yrs	PA (h/wk): 2.0±.07	MRI (1.5T, Philips)	RUN > NON ND 20-30% tibia **BSI** ~ 43.2 **CSMI** ~ 24.8 PA (h/wk), R^2 **BSI** = 0.46 BSI NS after adjusting for lean body mass	RUN > NON ND 20-30% tibia **Ct.Ar** ~ 23.6 **Me.Ar** ~24.4 **Ct.Vol** ~ 28.1 **BMC** ~ 11.3 **Tt.vBMD** ~ 14.7

Table 3.4. continued

Reference & Rating	Population		Characteristics of PA		Imaging methods	Results (% differences) [§]	
	n (EX, NON)	Age (yrs) [¶]	Sports group	Non-sports group		Bone strength	Bone mass & structure
Tennis –Mixed maturity girls							
Bass et al. (2002) (107)**	47 PRE: 17 PERI: 11 POST: 19	PRE: 10.4±0.3 PERI: 12.2±0.3 POST: 14.5±0.4	Tennis (h/wk): PRE = 6.0±0.5 PERI = 7.4±0.9 POST = 10.8±1.0 Years trained: PRE = 4.9±0.3 PERI = 5.6±0.8 POST = 7.0±0.4	Not applicable	MRI (1.5T, Signa)	PLAY > NON side 30-40% humerus: **I_p** PRE = 14.6 PERI = 19.2 POST = 23.3	PLAY > NON side 30-40% humerus: **Ct.Ar** PRE = 11.2 PERI = 16.5 POST = 14.5 **Ps.Ar** PRE = 6.7 PERI = 8.4 POST = 7.5 **Me.Ar** POST = -8.9
						PLAY > NON side 50-60% humerus: **I_p** PRE = 11.3 PERI = 16.9 POST = 17.0	PLAY > NON side 50-60% humerus: **Ct.Ar** PRE = 7.7 PERI = 11.9 POST = 12.2 **Ps.Ar** PRE = 6.2 PERI = 8.9 POST = 7.5
					DXA (DPX-L, Lunar)		PLAY > NON side Humerus : **BMC** PRE ~10.8

Table 3.4. continued

Reference & Rating	Population		Characteristics of PA		Imaging methods	Results (% differences) [§]	
	n (EX, NON)	Age (yrs) [¶]	Sports group	Non-sports group		Bone strength	Bone mass & structure
Mixed sports – Post-menarcheal girls							
Duncan et al. (2002) (412)**	50 (10/group SWIM, CYC, RUN, TRI, NON)	SWIM:16.7±1.3 CYC: 16.5±1.4 RUN: 17.8±1.4 TRI: 17.7±1.1 NON: 16.9±0.9	Training (h/wk) SWIM: 15.0±4.8 CYC: 15.0±4.9 RUN: 8.4±1.2 TRI: 16.2±4.7 Years trained SWIM: 6.9±2.3 CYC: 6.1±2.0 RUN: 6.2±1.7 TRI: 7.4±2.2 Years specialized (YS) SWIM: 6.1±2.7 CYC: 3.1±1.8 RUN: 5.0±1.6 TRI: 2.5±1.2	No training hours	MRI (1.5T, Philips)	RUN > x group Mid-third femur region: **BSI** NON ~ 83.8 SWIM ~ 90.3 CYC ~ 93.4 **CSMI** NON ~ 60.2 SWIM ~ 66.6 CYC ~ 93.4	RUN > x group Mid-third femur region: **Ct.Ar** NON ~ 11.8 SWIM ~ 15.3 CYC ~ 13.3 **aBMD** CYC ~ 14.7

Table 3.4. continued

Reference & Rating	Population		Characteristics of PA		Imaging methods	Results (% differences) [§]	
	n (EX, NON)	Age (yrs) [¶]	Sports group	Non-sports group		Bone strength	Bone mass & structure
Mixed sports – Post-menarcheal girls (cont.)							
Ferry et al. (2011) (415)*	73 (SWIM: 26, SOC: 32, NON: 15) NON matched for age, wt, ht	SWIM: 15.9±2.0 SOC: 16.2±0.7 NON:16.3±1.2	SWIM: At least 6 yrs of 10 h/wk SOC: Averaged 7 yrs of 10 h/wk	Non-elite athletes	DXA (QDR 4500, Hologic) HSA	[†]Z-scores differences from NON (zero value) Dom NN: **BR** SWIM = 1.2 **Z** SWIM = 1.3 SOC = 1.5 **CSMI** SOC = 1.0 Dom FS: **BR** SWIM = 1.2 **Z** SOC = 1.9 **CSMI** SOC = 1.7 Dom IT: **BR** SWIM = 1.2 **Z** SOC = 1.5 **CSMI** SOC = 1.9	[†]Z-scores differences from NON (zero value) Dom NN: **CSA** SWIM = -1.5 SOC = 1.0 **Ct.Th** SWIM = -1.3 **Wi** SOC = 1.2 **aBMD** SWIM = -1.4 Dom FS: **CSA** SOC = 2.6 **Ct.Th** SOC = 2.2 **aBMD** SOC = 2.9 Dom IT: **CSA** SWIM = -0.8 SOC = 1.8 **Ct.Th** SWIM = -1.0 SOC = 1.0 **aBMD** SWIM = -1.1 SOC = 1.1

[¶]Reported as mean±SD unless stated otherwise.
[§]Percent differences are calculated from mean values in studies if no reports were provided in tables or text. Displayed as ~.
[†]Results are not percent differences between groups but comparisons to Z-scores from NON.
PA, physical activity; EX, sport-specific group; NON, non-sport specific or control group; yrs, years; ; Tan1, Tanner stage 1; Tan2, Tanner stage 2; h/wk, hours per week; DXA, dual energy X-ray absorptiometry; GYM, gymnasts/gymnastics; UD, ultradistal; IBS, index of bone strength (g^2/cm^4) ; FSR, fall strength ratio; Adj, adjusted; LM, lean mass (kg); Z, section modulus (mm^3); Ps.Wi, periosteal width (mm); ~, calculated percent differences; NS, not significant; BMC, bone

mineral content (g); aBMD, areal bone mineral density (g/cm^2); BMAD, bone mineral apparent density (g/cm^2); CSA, cross sectional area (cm^2); Ct.Th, cortical thickness (mm); PAQ-C, physical activity questionnaire for children; HSA, hip structural analysis; S-SI, strength strain index (cm^2); CSMI, cross-sectional moment of inertia (cm^4); NN, narrow neck; SPW, sub-periosteal width (cm); Es.Dm, endosteal diameter (cm); R-GYM, rhythmic gymnasts/gymnastics; METs, metabolic equivalents; pQCT, peripheral quantitative computed tomography; ND, non-dominant; SSI_p, polar strength strain index (mm^3); Tt.Ar, total area (mm^2); Ct. Ar, cortical area (mm^2); Tb.Ar, trabecular area (mm^2); Tt.BMC, total bone mineral content (g); Ct.BMC, cortical bone mineral content (g); Tb.BMC, trabecular bone mineral content (g); Ps.Pm, periosteal perimeter/circumference (mm); Tt.vBMD, total volumetric bone mineral density (g/cm^2); HGYM, high volume gymnastics training; LGYM, low volume gymnastics training; S/W, strength weight index; MCSA, muscle CSA (mm^2) X-GYM/GYM, ex-gymnasts and gymnasts; CI, 95% confidence interval; L3, third lumbar; $_{PA}$, posterior-anterior; $_{LAT}$, lateral; $_{PALAT}$, paired postero-anterior and lateral dimensions; FRI, fracture risk index; Wi, width (cm); Vol, volume (cm^3); Ar, area (cm^2); Ht, height (cm); X-GYM, ex-gymnasts; h/wk/yr, annual mean hours per week; PostM, post-menarcheal; BR, buckling ratio (more negative values equate to stronger bones); SSI, stress-strain index (mm^3); BSI, bone strength index (mg^2/mm^4); PRE, pre-pubertal; PERI, peri-pubertal; POST, post-pubertal; MRI, magnetic resonance imaging; PLAY, playing arm; I_p, second polar moment of inertia (mm^4); Ps.Ar, periosteal area (mm^2); Me.Ar, medullary area (mm^2); RUN, runners/running; R^2, coefficient of determination; SWIM, swimmers/swimming; CYC, cyclists/cycling; TRI, triathletes/triathlon; SOC, soccer players/soccer sport; Dom, dominant; IT, intertrochanter; FS, femoral shaft.

groups (n=8) (145,146,340,407,408,413,414,417). Fewer were conducted in post-menarcheal (n=4) (293,409,415,416) or with mixed (pre-, peri- and post-pubertal) groups (n=7) (107,403–406,410,411). Two studies did not assess maturity (339,402). Maturity was assessed in several different ways. Most studies used ratings as per Tanner stages (presence of secondary sex characteristics – development of pubic hair, breast and/or genitalia) and others used menarche status (n=10), (30,293,406,408–410,414–417) skeletal age (Greulich-Pyle method; n=1) (418) or APHV (n=3) (145,403,413). For every study conducted with boys, there were two studies conducted with girls. This ratio was strongly influenced by the organized sports group studies, as only two included boys.

Key findings related to methodological differences across studies: There was substantial heterogeneity in imaging tools used, anatomical sites measured, analysis procedures adopted and algorithms developed to estimate bone strength. Most studies evaluated pre- and peri-pubertal children while studies of girls exceeded studies of boys by a ratio of 2 to 1.

3.3.4 Influence of PA on bone strength, mass and structure.

3.3.4.1 Effects of PA on bone strength – Intervention studies

Bone strength adaptations to weight-bearing PA were maturity- and sex-specific and related to the global quality rating of the study. Three (of five) weight-bearing PA intervention studies with a STRONG rating for quality reported significantly greater gains in bone strength for the intervention group (3% to 4%) compared with controls. Notably, these studies did not adjust their analyses for the influence of muscle (31,33,113). Three (of nine) studies with a MODERATE rating showed a positive effect for the intervention (3-12% gains in bone strength compared with controls) (32,400,401). Two of these studies adjusted MCSA (32) or lean mass (400) in their analyses. Generally, studies that reported positive outcomes implemented weight-bearing PA programs with ground reaction forces 3-5 times BW, for a

minimum of 7 months, 3-12 sessions/week for 1-12 min each session. To illustrate the nature of effective interventions, we briefly describe results from three STRONG rated studies below and provide a summary of results for all RCTs in Table 3.2.

The POWER PE study implemented an 8-month, 10-min/session, 2x/week jumping intervention within physical education (PE) (33). Boys (13.8±0.4 yrs) and girls (13.7±0.5 yrs) randomly assigned to the intervention arm demonstrated significant gains (3.5%) in lumbar spine bone strength index (by DXA) compared with controls (intent-to-treat analysis). There was no difference in response to the intervention between boys and girls (33). The HBS (31) targeted pre-and peri-pubertal girls to assess the effect of a 7-month, 10-12 min/session, 3x/week high-impact (3.5-5 times BW) intervention. Peri-pubertal girls in the intervention arm demonstrated greater gains in bone strength (Z, 4%) compared with controls at the femur narrow neck (HSA by DXA) (31). Action Schools! BC also targeted pre- and early pubertal boys and girls and implemented a 7-month whole school PA model that aimed to provide children with 150 min/week of PA that included a short, daily high-impact (3.5-5 times BW) jumping regimen (113). Pre-pubertal boys (n=139), but not girls, who attended intervention schools demonstrated greater gains (3%) in tibial bone strength (I_{max} by pQCT) compared with controls (113).

3.3.4.2 Effects of PA on bone mass and structure – Intervention studies

We also sought to identify specific properties of bone that could potentially contribute to reported changes in bone strength. Four of six studies that demonstrated an effect of a PA intervention on bone strength reported significant changes in these properties (31,32,400,401). Specifically, in the HBS peri-pubertal girls assigned to the intervention arm increased bone strength (Z) 4% more than girls in the control group (31). This was attributed to a greater increase in bone cross-sectional area (CSA; 2%), cortical thickness (3%) and areal BMD (3%) alone or in combination (31). The increased bone strength

(CSMI 12% and Z 7%) reported for pre-pubertal boys in the HBS was attributed to a 4% increase in BMC (401). The 5% greater increase in bone strength (Z) reported for pre- and peri-pubertal girls in the Action Schools! BC intervention arm (400) was driven by a 4% greater increase in CSA and BMC at the femoral narrow neck (400). Finally, the significantly greater increase in bone strength (BSI, 4%) for pre-pubertal boys who participated in Action Schools! BC was explained by a 2% greater increase in total BMD (32).

Key findings from intervention studies related to the effect of PA on bone strength, mass and structure: Most intervention studies that received a STRONG rating reported a significant effect for weight-bearing exercise on bone strength in pre-pubertal boys and pre- and peri-pubertal girls. Of these, two-thirds demonstrated increases in bone mass and structure. Only two studies that demonstrated a positive effect of the intervention adjusted for muscle in the analyses.

3.3.4.3 Associations between recreational PA and bone health

Of the ten recreational PA studies (Table 3.3), six developed regression models (338–340,403,405,406) to assess the contribution of PA to bone outcomes (others compared differences between groups). Of these, four studies reported a significant contribution (2 to 12%) of vigorous (VPA), moderate-to-vigorous (MVPA) or loaded PA to bone strength in girls and boys (338–340,405). Weight-bearing PA explained 10-12% ($p < 0.001$) of the variance in bone strength (minimum area moment of inertia, I_{min} and maximum area moment of inertia, I_{max} by HR-pQCT) in peri- and post-pubertal boys (only) at the 8% tibia (adjusted for ethnicity, body size, calcium intake, MCSA and maturity) (405). Studies that used objective measures of PA (accelerometers), showed positive associations between VPA (338,340) or MVPA (339) and bone strength (Z by DXA; R^2=2–11%, $p<0.05$) in pre- and peri-pubertal boys and girls at the proximal femur (adjusted for age, lean mass and body size). Strong associations between PA and bone strength (R^2= 2–9%, $p<0.05$) were noted for pre- to peri-pubertal boys and girls

who engaged in 30-38 and 18-28 min/day of VPA, respectively (338,340). When lean mass was entered into the regression model, VPA explained less of the variance (2-4%) in bone strength (338). In a 6-year prospective study there was no association between MVPA and bone strength at the femoral neck (adjusted for lean mass) in girls. However, this association was significant for boys (339). Similarly, in a 7-year prospective study there was no association between PA and femoral neck bone strength (adjusted for leg lean mass and length) in boys and girls (403).

Several studies that focused on girls reported mixed results for the association between bone strength and PA. In some cases, the association appeared to be maturity specific and related to dose of PA. For example, pre-pubertal girls (n=30) who participated in high-impact PA demonstrated 7% greater bone strength (polar moment of inertia, I_p by pQCT) at the 60% site of the tibia compared with those (n=98) who participated in low-impact PA; no association was observed for peri-pubertal girls (407). For girls in advance of peak height velocity (considered prepubertal), those who were more physically active had significantly stronger bones (8-9%; BSI and SSI by pQCT) (145). Conversely, a different study that assessed the tibia of pre- and post-menarcheal girls demonstrated no association between weight-bearing PA and bone strength (SSI by pQCT) (406). Finally, the largest observational study of girls conducted to date (n = 1116) showed no association between PA and radius strength (breaking bending resistance index, BBRI by DXA) across maturity groups (Tanner stages 1 to 5) (404).

3.3.4.4 Associations between recreational PA and bone mass and structure

We also sought to identify the association between PA and the specific properties of bone that may contribute to changes in bone strength. In five studies (338,339,405,407,420), CSA, Ct.Th, endosteal and/or periosteal circumference were significantly associated with higher levels of PA. We provide details of a few key studies below; results for all studies are provided in Table 3.3.

In a study of prepubertal boys and girls, VPA explained 4 to 5% ($p<0.05$) of variance in CSA at the narrow neck, intertrochanter and proximal femur shaft (338). Pre-pubertal girls in a high-impact PA group had 6% greater cortical thickness and 1% higher cortical BMD compared with girls in a low-impact PA group (407). In peri- and post-pubertal boys, there was a positive association ($R^2 = 6\%$, $p=0.003$) between weight-bearing PA (mean 344±300 min/week) and bone area at the distal tibia (405). Finally, girls across maturity groups who had the highest PA scores had 3-4% greater periosteal circumference compared with girls who had the lowest PA scores (145).

Key findings from recreational PA observational studies related to the influence of PA on bone strength, mass and structure: For most studies, high intensity exercise explained a significant amount of a variance (4-14%) in bone strength. The independent contribution to bone strength (2-12%) diminished when muscle covariates were included in the model. For those that compared differences between groups, 50% of studies showed that those in high (compared with low) PA groups had stronger bones (7-9%; not controlled for muscle). Studies that reported significant associations between PA and bone strength also reported positive associations with bone structure. Associations were observed across maturity groups for boys (pre-, peri- and post-pubertal) and for prepubertal girls.

3.3.4.5 Association between organized sports participation and bone strength

All 13 studies showed a positive association between participation in organized sports (across 6 sports; 70% gymnastics) and bone strength. Specifically, athletes had significantly greater bone strength (16 – 96%; unadjusted for muscle) compared with non-athletes across different measured sites (4 of the radius (146,408,409,418), 2 of the tibia (416,417) or proximal femur (414,415) and one study each of the radius and proximal femur (411), radius and tibia (413), humerus (107), femur (412) or lumbar spine (410)). Greater bone strength for athletes was noted across all maturity groups. Muscle (e.g., lean mass

and MCSA) (146,413,414,416) significantly contributed to bone strength in studies that assessed this (5/13). Athletes began participating in sport during pre- or peri-puberty. We highlight results from two studies of gymnasts and two studies of other sport groups and provide results for all studies in Table 3.4.

In gymnasts, bone strength was compared between recreational (<5 h/week) (413) and elite (>10h/wk) (411) athletes. Pre-pubertal gymnasts who trained a minimum of 1-5 hours/week, for two to three years had 22-25% greater bone strength (BSI by pQCT) at the radius and tibia (413). Differences in bone strength disappeared after adjusting for lean mass. In pre- and peri-pubertal gymnasts who trained 11-16 hours/week, bone strength at the ultradistal radius was significantly greater (44-96%) compared with non-gymnasts; this result persisted after adjusting for lean mass (411).

Post-menarcheal runners had greater bone strength (BSI, CSMI by MRI) at the femoral midshaft compared with swimmers (90% greater), cyclists (93% greater) and controls (84% greater) but were not different from triathletes (412). In the classic study of young female tennis players, bone strength (I_p by MRI) in the dominant playing arm (humerus) was significantly greater (11-23%) compared with the non-playing arm across all measured sites (107). Neither study assessed the contribution of muscle to bone strength.

3.3.4.6 Association between organized sports participation and bone mass and structure

All studies (but one (146)) that demonstrated a bone strength advantage for sport groups, reported positive adaptations in both bone mass and structure. Generally, bone strength was conferred to athletes based on a larger bone area (6-80%; total, cortical and medullary) (107,409,412,416–418) or significantly larger cortices (e.g., up to 26% greater cortical thickness at 38% tibia (417)). Overall, athletes had higher BMD and BMC (6 to 40% more). In one study gymnasts and ex-gymnasts had bone strength at the radius

(33% site by pQCT) that was 47% higher than non-gymnasts but significantly lower total volumetric BMD (about 7% lower) (409).

Key findings from organized sports observational studies related to the influence of PA on bone strength, mass and structure: Athletes had significantly stronger bone (16-96%) compared with non-athletes across all studies. This association was mediated by muscle. Bone structure was superior in athletes compared with non-athletes (values from 6 to 80% higher). Similarly, bone mass was 6 to 40% higher in athletes compared with non-athletes. Bone strength advantages for boys and girls engaged in sport were evident across all maturity groups.

3.4 Discussion

It is well established that when subjected to loading, bones' ability to resist fracture is a function of its strength. Bone strength, in turn, depends upon it mass, material properties, geometry and tissue quality. Although animal studies illustrate greater structural and material adaptations in growing bone in response to exercise compared with adult bone (91), we know relatively little about how bone *strength* characteristics adapt to exercise during childhood. Thus, we sought to review the literature that examined the contribution of PA to bone strength during the critical years of child and adolescent growth and development.

We extend previous reviews that assessed the role of PA on bone in a number of ways. First, we move beyond the preponderance of studies that reported bone *mas*s (by DXA) (42,334,386) to examine bone *strength*. We update the only previous review (37) of RCTs that reported estimated bone *strength* (by pQCT or DXA; n=9 new studies). Second, we expand previous reviews to include observational studies

(n=23) that assessed the influence of PA on bone strength. Prospective observational studies especially provide vital information on relationships and trends when included in the context of a systematic review (392). Finally, we sought to identify the contribution of bone mass and structure to reported changes in bone strength. While we acknowledge and briefly discuss the central role that muscles (and its surrogates) play in the developing and mature skeleton, it was not the focus of the current review.

Compelling evidence from our review supports that PA has a significant positive association with bone strength in the growing skeleton (26/37 studies support this). Specifically, our findings support 5 key outcomes; 1) weight-bearing PA enhanced bone *strength* in children and adolescents, 2) changes in bone *structure* rather than bone *mass* were most often associated with gains in bone strength, 3) adaptations in bone structure and strength related to PA were most often observed in *pre- and peri-pubertal groups*, 4) although some adaptations in bone structure and strength related to PA were sex related -- PA was generally associated with improved bone strength in both boys and girls, and 5) muscle mediated the influence of PA on bone strength. Given the diversity of studies we presented key findings at the end of each part of the Results and briefly discuss these key findings, below.

3.4.1 Weight-bearing PA enhanced bone strength in children and adolescents

Relatively recent and with the advent of advanced imaging tools, studies that assess the role of weight-bearing PA on bone *strength* have begun to emerge. We note that the quality of studies varied considerably; however, those of the highest quality (of sufficient duration and intensity, adequate sample size and with limited attrition) most often showed a positive effect of PA on bone strength in children and adolescents. Our key findings support previous reviews that focused on bone *mass* as an outcome (42,250,334,386).

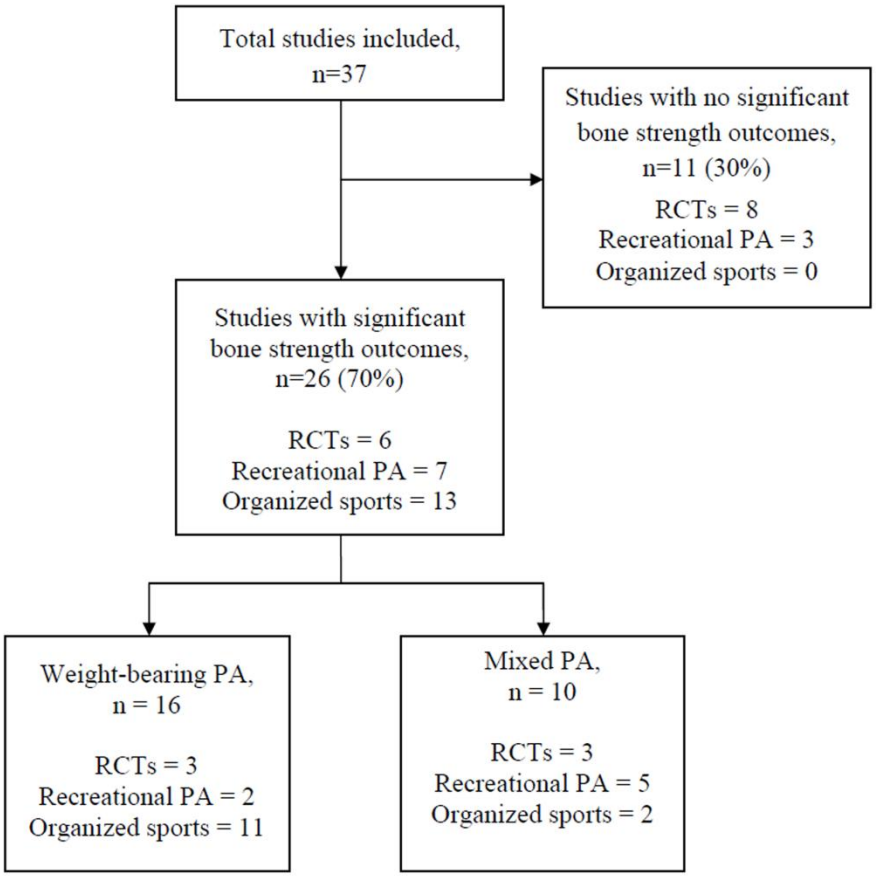


Figure 3.4. Overall view of bone strength, mass and structure outcomes based on weight-bearing physical activity (PA) or mixed type of PA (Mixed PA). RCTs, randomized-controlled trials.

We know from landmark animal studies and the basic tenets of mechanotransduction that activation of new bone formation requires a dynamic load beyond a theoretical threshold -- modulated by strain rate and amplitude (421). Further, short bouts of exercise followed by rest insertions are an effective means to elicit new bone formation (94).

Although it is not possible to directly translate these results to studies of children, most of the effective interventions we reviewed adopted these principles, and our findings support these basic tenets (77). That is, effective studies utilized dynamic loads of short (113) or longer (31,33) duration. Given the central role that muscle plays in bones' response to loading (336), it was surprising how few studies we

reviewed discerned the specific contribution of muscle strength (or surrogates) to bone strength. This seems an important consideration for future studies.

Of note, observational studies used a range of tools to assess PA; PA was compared across intensities (VPA, MVPA, METs min/day, etc.) or presented as a score (high versus low scores). Activity was often assessed using questionnaires (10/26 studies) or reported as training regimen (13/26 studies). As few studies (3/26) employed objective measures to assess PA (i.e. accelerometry), future studies should standardized methods and adopt these more robust techniques.

3.4.2 Changes in bone structure rather than bone mass most often accompanied gains in bone strength

Although we are unable to directly assess bone's material properties in vivo, imaging tools permit us to better understand the contribution of bone mass (or density) and structure to bone's overall strength in response to PA. In most studies, adaptations to both bone mass and structure contributed to bone strength (Table 3.5). A few exceptions highlighted the independent contribution of bone mass (32,401,413) or bone structure (107,145,338,339,403,405,418) to bone strength. Interestingly, the contribution of bone structure was in some cases double the contribution of bone mass (Table 2, 3 and 4). This highlights the importance of assessing bone structure as well as bone mass in studies of bone strength.

Table 3.5. Bone mass and structure outcomes from studies with positive bone strength (n=26) grouped into weight-bearing physical activity (PA) and mixed type of PA (Mixed PA) studies.

Study category	Significant bone structure & bone mass	Significant bone structure	Significant bone mass	No significant secondary outcomes
Intervention/RCTs				
Weight-bearing PA (n=3)	Petit et al., 2002 (31)[***]	-	Mackelvie et al., 2004 (401)	Weeks et al., 2008 (33) [***]
Mixed PA (n=3)	Macdonald et al., 2008 (400)[ϕ]	-	Macdonald et al., 2007 (32)[ϕ]	Macdonald et al., 2009 (113)[ŧ***]
Observational: recreational PA				
Weight-bearing PA (n=2)	Wang et al., 2005 (407)	McKay et al., 2011(405)[ϕ]	-	-
Mixed PA (n=5)	-	Farr et al., 2011(145); Forwood et al., 2006 (403)[ŧΨ]; Janz et al., 2004 (338) [ϕ ŧ]; 2007 (339)[ϕ ŧ]	-	Sardinha et al., 2008 (340) – not reported
Observational: organized sports				
Weight-bearing PA (n=11)	Burt et al., 2012 (146)[Ψ:] Dowthwaite et al., 2007 (408)[ϕ]; 2011a (409); 2011b (410); 2012 (411)[ϕ]; Greene et al., 2005 (34) [Ψ]; Faulkner et al., 2003 (414)[Ψ]; Tournis et al., 2010 (417)	Bass et al., 2002 (107); Ward et al., 2005 (418)	Erlandson et al., 2011 (413)[Ψ]	
Mixed PA (n=2)	Duncan et al., 2002 (412); Ferry et al., 2011(415)	-	-	-

[***]*STRONG rating studies.* [ϕ] *Studies that adjusted for muscle.* [ŧ] *Bone mass not reported/assessed.* [Ψ] *No longer significant after adjusting for muscle. No studies examined non-weight-bearing PA on its own.*

The specific pattern whereby bone structure adapts to mechanical stresses was described in a landmark study by Robling and colleagues (93). They loaded the right ulna of female rats (360 loading cycles per day; one uninterrupted bout or four bouts of 90 cycles each) and demonstrated a substantial increase in ultimate strength (64-87%) but only small changes in bone mass (BMC; 5-12%). Enhanced bone strength was a function of changes in bone geometry; specifically, bone apposition on the medial and lateral sections of the ulna (69-96% more in the exercised ulnae) (93).

Warden and colleagues also illustrated the selective adaptation of bone structure to enhance bone strength, using a rat ulna loading model across 7 weeks (335). After training they reported a two-fold increase in second moment of inertia due to periosteal bone apposition. After detraining, bone structure (minimal second moments of area) accounted for 76% of the variance in ultimate force compared and BMC accounted for 29% (335). Importantly, the intervention induced lifelong benefits in bone structure, strength and fracture resistance (8,16). Thus, taken together key animal studies support our findings for children regarding the components of bone structure that make significant contributions to bone strength. Researchers should be encouraged to move beyond sole measures of BMD to assess structural parameters in PA studies of children and adolescents.

3.4.3 PA-related adaptations in bone structure and strength were related to maturity

A secondary aim of our review was to identify the proposed 'window of opportunity' related to maturity when PA enhanced bone strength responses in children and/or adolescents. Our findings generally support the notion of pre and peri-puberty as key times to intervene with exercise; however, only one intervention study compared across maturity stages (and showed a positive result) (33). Most high quality intervention studies and all observational studies that focused on younger children showed a positive influence of exercise on bone. Importantly, there were very few high quality intervention studies

in more mature boys and girls; this hampered our ability to draw conclusions in this maturity group. This speaks to the notion that intervention studies with adolescents were either not conducted or not published, given the reported publication bias of negative results (422).

We were also unable to clearly discern from the published literature whether age of starting sport-specific training (from 2 to 6 years prior to assessment) contributed to an enhanced bone response to training. However, a contralateral controlled study that reported a dramatic side-to-side difference in bone strength in the humeri of pre-menarcheal (26% difference) versus post-menarcheal (11% difference) racquet sports players is notable (106). A significantly larger cortex in players who started training earlier explained the side-to-side bone strength difference between maturity groups.

In our view, there is no perfect approach to classify maturity status; all have strengths and limitations (195). Therefore, it was not surprising that approaches to assessing maturity varied and included Tanner staging (most common), APHV, menarcheal status and skeletal age. Indeed, the overall landscape as to interrelated factors that influence the growing skeleton is complex and encompasses the influence of different endocrine environments across maturity groups, the interplay between levels of sex steroids, growth hormones and insulin-like growth factors (423), and the independent influence of muscle and mechanical loading on growing bone at different maturity time points (48). Bone adaptations, especially during post-puberty, most likely traverse a sex-specific pathway towards increasing bone strength (424,425). However, high quality PA intervention studies in boys and girls during later stages of maturity, comparisons across maturity groups in the same study, studies that investigate the interaction between the endocrine environment and mechanical loading and that describe how this interplay influences bone strength accrual, are currently missing from the literature.

3.4.4 PA-related adaptations in bone structure and strength were sex related -- but generally PA was associated with improved bone strength in both boys and girls

Our findings generally support that PA was beneficial for bone strength in both boys and girls. Maturity, most often assessed as pubertal stage (as per the method of Tanner) was strongly associated with bone apposition, especially in girls. This is likely related to the biphasic role that estrogen plays in bone modeling (423). We were unable to elucidate sex differences in the specific bone parameters that contributed to bone strength (i.e., bone mass and structure). Notably, as osteoporosis and related fracture more commonly affect women than men (1), most studies were of girls. This was especially true in observational studies of organized sports where all but two of thirteen studies focused on female gymnasts. Given that osteoporosis is often overlooked and related fracture will affect 20% of men (426), it seems important that studies of both girls and boys be conducted in future, to discern the role that bone structure plays in the adaptation of growing bone to PA.

3.4.5 The contribution of muscle to bone strength

The Utah Paradigm describes how mechanical loads on bone arise from muscle forces, and stimulate bone apposition when loads exceed a certain strain threshold (45). Timing of peak muscle mass accrual that precedes peak BMC accrual also speaks to the role of muscle on bone development (48). In addition to its role in mechanotransduction, muscle also regulates bone modeling through paracrine and endocrine signaling pathways (50). Thus, it was somewhat surprising that 65% of studies we reviewed did not evaluate the role that muscle played in the bone strength response to PA. Of those that demonstrated a significant role for PA on bone strength, only 46% considered the contribution of muscle to bone outcomes. Of these, 5 observational studies (146,403,413,414,416) showed that once a measure of muscle was added to the model, the independent role of PA diminished. This illustrates the specific mechanical

influence of muscle on bone strength. Although, we deliberately chose to focus our review on PA and bone strength and its parameters, it seems important that the independent role of muscle on bone and its association with sex, maturity and PA be considered in studies of children and adolescents. This is especially important as a means to paint a more comprehensive picture of the mechanisms that drive bone adaptations to PA (427,428).

3.4.6 PA prescription for bone strength

In keeping with the global related literature, weight-bearing PA was a more effective means to increase bone strength compared with non-weight-bearing PA. Exercise intervention studies that did not report a significant effect of PA on bone strength were hampered by a relatively small sample size (34,358,399), low magnitude and frequency of PA (30,34,359) and/or a relatively short intervention period (as low as 28 weeks) (34). Although of lower quality, observational studies we reviewed (338,339,405,407) demonstrated that children who participated in more vigorous weight-bearing PA, achieved the greatest bone strength benefit. Extreme loading sports like gymnastics elicit loads that may exceed 10 times BW (10 BW vs. runners with loads at 3 BW) (336). Thus, it was of no surprise that bone strength of gymnasts was enhanced at every level of participation (recreational to elite). This association was most often mediated by muscle mass in recreational gymnasts (trained <5 h/wk) (413) whereas an independent influence of the sport was noted in more highly trained gymnasts (trained >6h/wk) (408,411).

It was not possible to discern, with confidence, the specific components of PA (magnitude/intensity, frequency, duration, rest periods) that contributed most to enhanced bone strength in children and adolescents. Duration, frequency and/or loads related to PA, explained a significant amount of variance (adjusted for maturity, body size and ethnicity), although their contribution to strength overall was meagre (R^2 of about 0.4 at the femur (20% site) and 0.5 at the tibia (66% site)) (145). The nature of

PA interventions varied considerably but almost all incorporated load-bearing components, some of very short duration. For example, Action Schools! BC intervened with a program of countermovement jumps, three times a day (max 36 jumps/day), four times a week during school hours. These activities demanded a minimal investment of teachers' time and training and elicited a significant osteogenic response (32,113,400).

Turner and Robling proposed calculating an OI (97) to estimate the magnitude and cycles of loading and categorize the intensity of exercise regimens. Shorter bouts of PA with higher frequencies were more osteogenic compared with longer bouts of PA and fewer sessions– although the volume of exercise was the same (94,98). A 'desensitization' period after a certain amount of loading stimulus, as described in animal studies (94,98) may partially explain this. Specifically, a brief 10-second rest insertion between loads increased bone formation rate (2-4 times) in animals (95). As a means to standardize the 'dose' of PA to compare across studies, calculating the OI for PA interventions may offer one approach. This could also be utilized in studies to allow us to better understand the dose-response of bone to weight-bearing PA, as this is currently not clear.

3.4.7 Limitations to conducting our systematic review

The Oxford Centre for Evidence-Based Medicine suggests that systematic reviews, such as the one we conducted, provide the highest level of evidence to guide the overall understanding of complex relationships (429).[99] However, we were unable to conduct a meta-analysis due to different approaches across studies in (for example); 1) imaging equipment used, 2) bone scan acquisition and analysis protocols (percentages from limb length, reference lines), 3) anatomical site assessed (dominant vs. non-dominant side, weight-bearing vs. non-weight-bearing bone, various distal and mid-shaft sites), 4) bone outcomes reported, 5) method of assessing maturity, 6) method of assessing PA (questionnaire,

accelerometry), and 7) reported PA outcomes (PA type, frequency, etc). These differences also highlight some barriers to comparing results across studies.

Importantly, we found very few methodological studies of children or adolescents that compared measurement differences using pQCT (hardware, software, site scanned or analysis protocol used). However, differences in the protocol adopted across studies may partially explain differences in outcomes (148). It is of note that consensus has not been reached regarding standard measurement sites or analysis protocols for assessment of trabecular and cortical bone in studies of children and adolescents (148). This seems important to consider in future, while accepting that the measurement approach adopted will always be driven by the research question being addressed.

Although researchers reported a range of PA interventions and used different PA assessment tools, most showed a significant effect of PA or associations of weight-bearing PA with bone strength. Studies where objective measures were used to assess PA were largely absent. Thus, where relevant, a standard means to quantify PA (magnitude, rest-load cycles, frequency, type, etc.) might provide enable researchers to more readily compare outcomes across studies, in future.

3.5 Conclusions

Our findings extend previous reviews to support a central role for weight-bearing PA to enhance bone strength in children and adolescents. However, we have much to learn about the specific response of bone mass versus bone structure to PA and the relative contribution of each to bone strength in the child and adolescent skeleton. More specifically, studies that differentiate how bone compartments (trabecular versus cortical) respond to different loading conditions and how maturation influences this response are

needed to advance the field of pediatric bone health. Animal studies suggest that structure rather than mass drives the bone strength adaptation to loading -- a response that persists across the life span (335). Our finding that early maturity may be the most opportune time for children to engage in PA is tempered by the fact that very few studies were conducted in more mature children and adolescents. Where possible, a standardized approach using pQCT and HR-pQCT to measure children and adolescents would enable researchers to compare and conduct meta-analyses across studies. Similarly, should studies develop standard and objective measures to assess PA, it would permit us to more readily discern the specific exercise prescription (type, frequency, duration and load of PA) that best promotes bone strength accrual in the growing skeleton. Finally, long-term follow up studies that investigate whether bone strength advantages persist into the later years are a crucial and currently missing piece of the puzzle.

Chapter 4: The Influence of Physical Activity, Sedentary Time and Muscle Strength on Bone Strength, Structure and Density in Older Adolescents.

SYNOPSIS: Adolescence is a complex, transitional stage from childhood to adulthood. The influence of modifiable factors, such as PA, sedentary behaviour and muscle strength, on adolescent bone strength and structure are not well understood. This study investigates these relationships in adolescent boys and girls.

4.1 Introduction

Adolescence is a complex, transitional stage from childhood to adulthood. As children mature they undergo a period of accelerated skeletal growth during which time they accrue more than one third of their adult bone mass (114). Following the peak in bone accrual (which occurs, on average, at age 12.5 years in girls and age 14.1 years in boys (9)), gains in bone mass and strength continue during the later stages of adolescence, but to a lesser degree. Further, whereas weight-bearing PA has its most profound osteogenic effects during peak adolescent growth (32,401,430), we know relatively little about the how the older adolescent skeleton adapts to mechanical challenges associated with PA.

This knowledge gap is likely due, in part, to the reliance in previous studies on 2-D bone imaging using DXA. Similar to the mature, adult skeleton (431) bone adaptations in the adolescent skeleton in response to PA may involve subtle modifications to bone structure rather than significant gains in bone mass. Due to its planar technology and low image resolution, DXA is unable to evaluate adaptations in bone size through periosteal apposition or detect changes in bone density of cortical and trabecular compartments, all of which contribute to bone strength. Thus, 3-D imaging tools such as pQCT and HR-pQCT are increasingly used to investigate the influence of PA on bone (micro)structure, vBMD and

estimates of bone strength. However, to date only one study used 3-D imaging to describe the relationship between weight-bearing PA and trabecular microstructure at the distal tibia in older adolescents (and young adults) (432).

Weight-bearing PA is a major mechanical stimulus on bone growth. Less physically active and increasingly sedentary pursuits by adolescents may translate into poor bone health outcomes. In a recent report only 7% of 15 to 17 year old Canadians were meeting recommendations of 60 minutes of moderate-to-vigorous PA (MVPA) daily (17). MVPA of less than 50 min/day may result in reduced bone mass while a higher duration of MVPA or higher intensities of PA may be required to optimize bone growth in adolescents (13). Of particular concern to adolescent health is that today's youth spend 62% of their waking hours in sedentary pursuits and SED increases in the later stages of adolescence (323). The potentially deleterious effects of sedentary behaviours on bone structure and strength during growth are not well known. However, evidence from four DXA-based studies suggests that SED (measured either by questionnaire or accelerometry) is negatively associated with bone mineral content (BMC) in children and adolescents (322,328–330). In contrast, recent evidence from our laboratory indicates that SED is not associated with bone microarchitecture or strength (by HR-pQCT) in children, adolescents and young adults (331). Most PA and bone studies do not consider the role of SED, yet daily living represents a combination of both types of activities – MVPA and SED. Thus, further studies are required to clarify these relationships (MVPA, SED) on bone outcomes.

In the context of Harold Frost's mechanostat theory (75), PA and SED are considered modulating factors that may act upon bone development through their influence on muscle (mass, force, power) - the source of the greatest physiological loads on the skeleton (75,223). This is evident in several studies whereby the strength of relationships between PA and bone mass or estimates of bone strength (by DXA hip structural analysis (HSA) and pQCT) was diminished (338,339) or negated (403,413) when muscle

mass was added to the prediction models. In recent years, the importance of muscle-bone relationships gained momentum in musculoskeletal research; findings of cross-talk between these two tissues are redefining the way we perceive the relationship (433,434). Muscle not only stimulates bone adaptation through biomechanical pathways, but also through endocrine and paracrine pathways that scientists are only starting to comprehend (336). Consequently, muscle mass and muscle function may contribute independently to bone strength, structure and density in the growing years. Therefore, it is important to appropriately consider muscle or its surrogates when examining the relation between PA and bone outcomes.

Thus, I identify four objectives and three related hypotheses that I will address in this cross-sectional study.

Objective 1. To describe bone strength, structure and density (referred to from here as *bone outcomes*) at the tibia and radius in adolescent boys and girls.

Objective 2. To identify sex differences in bone outcomes (controlling for limb length and total body lean mass) in adolescents.

Hypothesis 1: Adolescent boys will have greater bone outcomes compared with girls after adjusting for limb length and total body lean mass.

Objective 3. To identify the influence of PA and SED on tibial bone outcomes (controlling for muscle mass, ethnicity, tibial length and maturity status) in adolescent boys and girls

Hypothesis 2: MVPA will positively predict bone strength and structure, but not density, at the distal and midshaft of the tibia, after adjusting for muscle mass, ethnicity, tibial length and maturity status.

Hypothesis 3: SED will negatively predict bone strength and structure, but not density, at the distal and midshaft of the tibia, after adjusting for muscle mass, ethnicity, tibial length and maturity status.

Objective 4: To identify the influence of muscle strength on radius bone outcomes (controlling for muscle mass, ethnicity, ulnar length and maturity status) in adolescent boys and girls.

Hypothesis 4: Muscle strength will positively influence bone strength and structure, but not bone density, at the distal and midshaft sites of the radius after adjusting for muscle mass, ethnicity, ulnar length and maturity status.

4.2 Methods

I conducted a cross-sectional analysis of baseline data of the Health Promoting Secondary School (HPSS) Bone Health Study (BHS). I provide a detailed description of participant recruitment and methodology in Chapter 2 (Section 2.3 onwards). The primary outcome of my study was bone strength (BSI for distal site and SSI_p for shaft site); secondary outcomes were bone structure – Tt.Ar, Ct.Ar, and Me.Ar, and density –Tt.Dn and Ct.Dn. I briefly describe methods below.

4.2.1 Participants

From the nine eligible secondary schools, 210 Grade 10 adolescents (94 boys, 116 girls) provided parental/guardian consent to participate in baseline BHS measurement. All participants were healthy with no known medical conditions or disabilities. Figure 4.1 displays the included and excluded participants in the study as well as the number and reasons for excluded pQCT scans

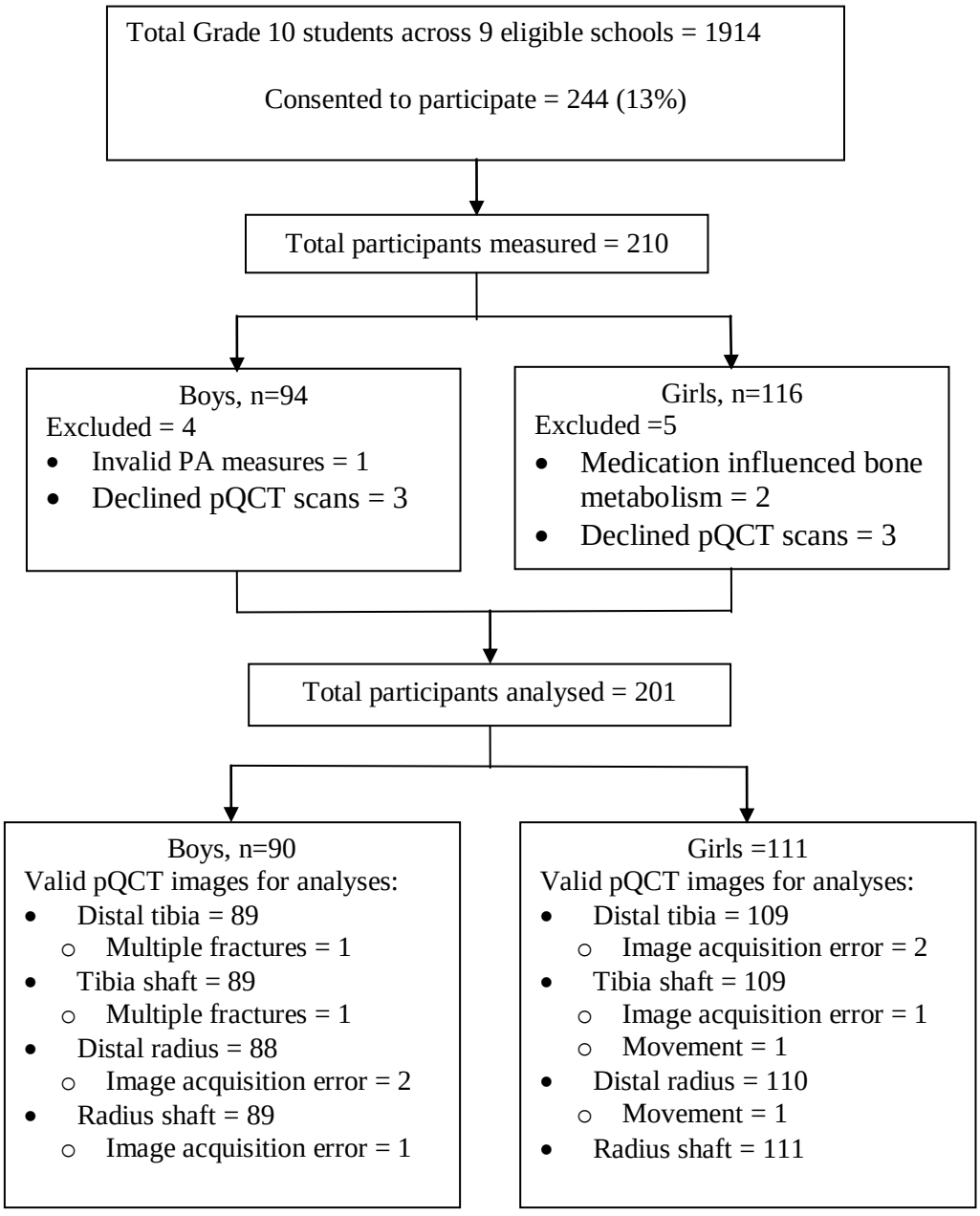


Figure 4.1.Flow chart of participants recruited, measured, excluded and the number of pQCT images analysed.

4.2.2 Measurements

4.2.2.1 Anthropometry

I assessed standing height (stretch stature), body mass, and limb lengths (tibia and ulnar) using standard anthropometric protocols. I used a wall-mounted stadiometer (Model 242, Seca, Germany) and a digital scale (BWB 800, Tanita, Japan) to assess standing height (cm) and body mass (kg), respectively. Using an anthropometric tape, I measured participants' tibia length (mm) as the distance from the medial malleolous to the tibial plateau. Ulnar length (mm) was measured from the lateral ulna process to the proximal tip of the olecranon. I adjusted for limb length in regression analysis as bone outcomes are relative to body size, and also to account for the role of limbs as lever arms (150).

4.2.2.2 Bone image acquisition

One trained technician conducted daily calibrations and acquired all tomographic images at the 7% and 30% sites of the non-dominant radius and the 8% and 50% sites of the left tibia by pQCT (XCT3000, Stratec Medizintechnik, Pforzheim, Germany). Measurement sites were determined as a percentage distance from the reference line, proximally. The reference line for the radius was placed at the medial edge of the distal radius. The reference line for the tibia was placed at the tibial plafond. Short-term precision for repeated scans using pQCT to acquire all bone outcomes had a coefficient of variance (CV) of 0.23-1.56 (%, UBC Bone Health Research Group, unpublished data).

4.2.2.3 Bone image analysis

I used Stratec software (version 6.0) to analyze all pQCT scans. For the distal radius and tibia, I used Contour mode 3, Peel Mode 5 with a threshold of 200 mg/cm^3 to obtain Tt.Ar (mm^2) and Tt.Dn (mg/cm^3), used to calculate BSI (mg^2/mm^4, BSI= Tt.Ar * $Tt.Dn^2$/10,000). BSI provides an estimate of

bone strength in compression. For the radius and tibia shaft, I used Cort mode 1, Peel mode 2 with a threshold of 710 mg/cm^3 to obtain Tt.Ar (mm^2), Ct.Ar (mm^2) and Ct.Dn (mg/cm^3), and Cort mode 1, Peel mode 2 with a threshold of 480 mg/cm^3 to evaluate SSI_p (mm^3) that provides an estimate of bone strength in torsion. I calculated Me.Ar (mm^2) at the radial and tibial shaft as the difference between total bone area and cortical bone area (Tt.Ar - Ct.Ar).

4.2.2.4 Physical activity

All participants completed the validated self-report PA questionnaire for adolescents (PAQ-A) (306) that provides a general PA score for the past seven days and an estimate of MVPA (min/day).

During data collection, our research team members inquired if participants would be keen to wear a PA-measurement device for seven consecutive days during waking hours (except for during water-based activities such as bathing or swimming). A subset of participants (n = 146, 63 boys, 83 girls) volunteered to wear the uniaxial accelerometers (GT1M, Actigraph, USA). Included participants had at least 3 valid days where a valid day consisted of 10 hours or more of wear time (316). I defined non-wear time as 60 consecutive minutes of zero counts without exception, and used cut-points as defined by Evenson and colleagues (28) to analyze SED (<100 count per minute, cpm), MPA (2295 – <4012 cpm), MVPA (≥2296 cpm) and VPA (≥4012 cpm) from raw counts collected at 15-second epochs. MVPA and SED are my independent variables of interest related to Objective 3.

4.2.2.5 Body composition

I used DXA (Discovery A, Hologic, USA) to obtain whole body scans as per standard protocols (379). I conducted daily calibration and quality control procedures as per standard operating protocol. I personally acquired all DXA scans and analyzed all images. Body composition outcomes were whole

body fat mass (FM, kg) and bone mineral free lean mass (LM, kg) minus the head, as skull measures are known to be highly variable (435). Short-term precision (CV) in our laboratory for total body fat mass and total body lean mass (with repositioning) was 1.9 and 0.3%, respectively (UBC Bone Health Research Group; unpublished data). Total body lean mass (LM, without bone) is another potential covariate to represent the role of muscle in the analyses for Objectives 2, 3 and 4.

4.2.2.6 Muscle outcomes

Strength: One of two trained research assistants used a hand grip dynamometer with a known reliability of 0.23 kg (436) to assess participants' muscle strength. Specifically, to assess isometric grip strength (kg) participants completed three trials with each arm, alternating arms between tests; this provided a 15-20 second rest between trials. Two research assistants provided consistent verbal encouragement at each test. I used the maximum value achieved from the non-dominant arm as the independent variable for Objective 4 analyses.

Cross-sectional area: I used pQCT to acquire scans at the 30% radius and 50% tibia using Contour mode 1 (-100 mg/cm^3), Peel mode 2 (40 mg/cm^3) and Cort mode 1 (710 mg/cm^3) analysis parameters. I provide precision values for this measure as CV (%) in section 4.2.2.2.

Muscle as a covariate: Given the known relation between muscle mass and bone outcomes (47,48,286,437), I resolved to identify a muscle variable that served as the best representation of muscle mass. To do so I examined the strength of the association between muscle mass variables (LM by DXA; tibia or radius MCSA by pQCT) and bone outcomes. From the correlation analysis, LM was more strongly associated with bone outcomes (e.g., distal tibia, r =0.33-0.66, p<0.01) compared with tibia or radius MCSA (e.g., distal tibia, r =0.32-0.56, p<0.01).

4.2.2.7 Health history, dietary calcium intake and maturity

Participants completed a health history questionnaire (HHQ) that captured current health status, previous bone fractures, use of alcohol, smoking, and use of medication(s) known to interfere with bone metabolism (438). Participants also reported their ethnicity that can account for differences in bone mass and structure (193). Thus, ethnicity is a covariate in the analyses for Objective 3 and 4. Participants completed a validated food frequency questionnaire (FFQ) to assess dietary calcium intake (mean; mg/day) (384). Maturity status for boys was an estimate of years post age at peak height velocity (APHV) or maturity offset (382) while girls provided the approximate date of menarche (month, year). As maturity is driven by levels of sex hormones that also influence bone growth (238,425), maturity status is included as a covariate in the analyses for Objectives 3 and 4.

4.2.3 Statistical analysis

I used Stata, version 10.1 (StataCorp, College Station, Texas) for all data analyses. Across all analyses, I set the significance level at $p<0.05$.

For data cleaning, I first generated histograms with normality curves to inspect for normal distribution for all variables. Second, I visually inspected the distribution and general relationship between descriptive (age, height, weight, limb lengths, muscle strength, MCSA, total body fat mass, LM, maturity, all PA variables) and bone variables using scatter-plots. Scatter-plots provided a visual pattern (positive or negative trend) and identified potential outliers. I consider outliers as data points falling outside the expected range of more than 2.5 SD (439). I further investigated potential outliers to determine if they were systematic or random errors (e.g., data entry errors) or acceptable values (e.g., higher body mass

participants with high body fat mass) and cleaned the data as needed. A research team member entered all data into a FileMaker database (FileMaker Pro, Version 11, Santa Clara, CA, U.S.A.).

To address Objective 1, I generated mean and standard deviations to describe bone outcomes at the tibia and radius in adolescent boys and girls. To address Objective 2, I used analysis of covariance (ANCOVA) and adjusted for limb length and LM to determine sex-specific differences in bone outcomes. To address Objectives 3 and 4, I developed sex-specific hierarchical multivariable regression models to determine the influence of MVPA, SED and muscle strength on bone outcomes, respectively. First, I conducted sex-specific pairwise correlations (Appendix L) to determine associations between bone outcomes and continuous descriptive variables. I used Spearman's rank correlation to examine associations between bone outcomes and categorical descriptive variables (i.e. ethnic group and boys' axillary hair stages). Then, I used the nested regression function (xi:nestreg) in Stata to conduct the regression analysis, and controlled for ethnicity, limb length, maturity (age at menarche for girls, years post APHV for boys) and LM. I constructed regression models in blocks: Block 1 controlled for ethnicity (dummy variables: 0= White, 1= Asian, 2= Other), maturity (boys: years post APHV, girls: age at menarche) and limb length (tibial length), Block 2 included LM while the last block included the predictor variable of interest. Specifically, for Objective 3 (influence of MVPA and MVPA with SED on bone outcomes at the tibia), the variable of interest was MVPA (min/day) or MVPA with SED (min/day). Briefly, I incorporated SED together with MVPA into my models as SED on its own was not a significant predictor of any bone outcomes in adolescent boys and girls from my multivariable regression analyses. In the models where MVPA with SED was a significant predictor of bone outcomes, I further examined the influence of SED, MPA and VPA (delineated from MVPA) on bone outcomes and blocks were added in the order of – average wear time (min/day), SED, followed by MPA (min/day) and the final block was VPA (min/day). To address Objective 4 (influence of muscle strength on bone outcomes at the radius) I

used a modeling approach described above for Objective 3 (the same covariates and order of blocks). However, I replaced tibial length with ulnar length and MVPA (or SED) with grip strength. To check for assumptions in all my regression analyses (440), I generated and visually inspected partial-regression plots. I identified unusual and influential points using Cook's distance values defined as having a value above 4/N, where N refers to the number of observations. I checked residual plots for normality of distribution (kernel density plots, probability plots and quintile plots). I also inspected residual-variance plots for homoscedasticity and conducted variance inflation (VIF) tests to check for multicollinearity (if VIF scores were above 10) (440).

4.3 Results

4.3.1 Descriptive characteristics of participants

I provide descriptive characteristics and mean differences for boys and girls in Table 4.1. My sample included 201 participants (90 boys, 111 girls); 53% white, 37% Asian and 10% of other ethnicity. Participants were of similar age; however, boys were taller (6%), heavier (7%), and had longer limbs (8%) compared with girls. Boys also had greater LM (21%) and MCSA at the tibial (9%) and radial shaft (26%), compared with girls. Boys' and girls' grip strength values fell within normal age- and sex-specific reference ranges (441). Girls had more fat mass (39%) compared with boys. Boys reported consuming more dietary calcium (26%) compared with girls.

For PA, boys had a higher PA score (7%) and more MVPA min/day (32.6%) compared with girls (by PAQ-A). In participants with valid accelerometer data (n=103; 40 boys, 63 girls), boys were more physically active compared with girls. Specifically, boys spent approximately 14 minutes more per day engaged in MVPA compared with girls. Girls tended to spend more time being sedentary than did boys;

however, the difference in SED did not achieve statistical significance. There were no differences in height, body mass or dietary calcium between participants who had and those without valid accelerometer data (Appendix L).

4.3.2 Sex differences in bone outcomes

I present bone outcomes at the tibia and radius for boys and girls in Table 4.2. Overall, boys had significantly larger and stronger bones compared with girls, while girls had higher bone density compared with boys (except for the radial shaft). However, after controlling for limb length and LM, apparent advantages conferred to boys did not persist (except Tt.Ar at the distal radius). The bone density advantage conferred to girls remained intact.

Table 4.1. Descriptive characteristics, physical performance measures and physical activity levels for boys and girls (mean differences; 95% CI).

	Boys			Girls			Mean difference[ϕ]
	n	Mean (SD)	Range	n	Mean (SD)	Range	(95% CI)
Age (yrs)	90	15.3 (0.4)	14.7 – 16.5	111	15.4 (0.4)	14.8 – 16.8	-0.03 [-0.13, 0.07]
White/Asian/Other	90	49/34/7	-	111	59/40/12	-	na
Years post APHV	90	1.9 (0.6)	0.7 – 3.2	111	3.1 (0.6)	1.8 – 4.9	**-1.2 [-1.3, -1.0]**
Age at menarche (yrs)	-	-	-	103	12.4 (1.1)	9.6 – 15.0	na
Height (cm)	90	174.0 (6.9)	160.25 – 188.0	111	162.9 (6.3)	149.8 – 180.1	**11.2 [9.3, 13.0]**
Body mass (kg)	90	63.4 (11.2)	46.2 – 105.2	111	58.9 (10.5)	38.5 – 99.2	**4.5 [1.5, 7.6]**
Tibial length (cm)	89	40.4 (2.2)	35.1 – 45.7	110	37.0 (2.0)	33.4 – 42.4	**3.4 [2.8, 4.0]**
Ulna length (cm)	89	27.7 (1.4)	24.5 – 31.1	111	25.4 (1.3)	23.1 – 29.1	**2.3 [2.0, 2.7)]**
Fat mass (kg)	86	9.6 (5.6)	2.8 – 31.1	107	15.9 (6.4)	5.0 – 43.7	**-6.2 [-8.0, -4.5]**
Lean mass (kg)	86	45.6 (7.2)	32.3 – 64.0	107	36.2 (4.9)	26.6 – 48.6	**9.4 [7.6, 11.2]**
Grip strength (kg)	90	34.7 (7.6)	21.5 – 52.4	111	26.6 (4.9)	17.3 – 39.8	**8.0 [6.2, 9.9]**
Tibia MCSA (cm^2)	89	4170.1 (744.1)	2569.6 – 6305.8	109	3792.0 (604.7)	2335.8 – 5398.9	**378.0 [184.8, 571.2]**
Radius MCSA (cm^2)	89	1691.5 (276.7)	1144.5 – 2416.0	110	1249.8 (201.5)	782.9 – 1735.4	**445.2 [375.8, 514.6]**
Dietary calcium (mg/day)	90	1301 (752)	180 – 4408	111	961 (625)	124 – 3904	**340.3 [148.7, 531.9]**
PAQ-A							
PA Score	90	2.8 (0.6)	1.1 – 3.7	111	2.6 (0.5)	1.4 – 4.0	**0.17 [0.02, 0.32]**
MVPA (min/day)	90	113.1 (75.6)	0.0 – 320.4	111	76.2 (51.8)	0.0 – 219.3	**36.9 [18.4, 55.4]**
Accelerometer							
Total wear time (min/day)	40	841.5 (55.5)	698.8 – 946.5	63	833.4 (50.8)	745.4 – 939.8	8.1 [-13.0, 28.2]
Average counts (counts/min)	40	458.7 (137.1)	414.9 – 502.6	63	375.3 (146.3)	338.4 – 412.1	**83.5 [26.2, 140.7]**
MVPA (min/day)	40	57.9 (21.5)	11.9 – 105.4	63	44.0 (20.7)	5.2 – 108.6	**13.8 [5.4, 22.3]**
Sedentary (min/day)	40	591.6 (64.5)	435.6 – 774.7	63	610.7 (69.4)	450.8 – 772.5	-19.2 [-46.3, 7.9]
Moderate PA (min/day)	40	32.9 (11.9)	10.5 – 68.9	63	28.1 (11.5)	4.4 – 68.9	**4.8 [0.1, 9.5]**
Vigorous PA (min/day)	40	25.0 (12.0)	1.4 – 54.6	63	16.0 (12.5)	0.8 – 68.8	**9.1 [4.1, 14.0]**

[ϕ] *absolute mean difference (boys minus girls); CI, confidence interval; APHV, age at peak height velocity; MCSA, muscle cross-sectional; MVPA, moderate-to-vigorous physical activity; na, not applicable; PA, physical activity; PAQ-A, physical activity questionnaire for adolescents; SD, standard deviation.*

Bold values are significant at $p<0.05$.

Table 4.2. Summary statistics for tibia and radius bone strength, structure and density in boys and girls and mean difference (boys – girls) unadjusted and adjusted for limb length and total body lean mass.

	Boys		Girls		Unadjusted difference, boys-girls (95% CI)	Adjusted difference, boys-girls (95% CI)
	Mean (SD)	Range	Mean (SD)	Range		
8% Tibia	n= 89		n= 109			
BSI (mg^2/mm^4)	9454.8 (2137.1)	4191.5 – 14587.4	8609.8 (2145.2)	3617.4 – 16453.2	**845.1 [241.7, 1448.4]**	**-847.4 [-1492.2, -202.6]**
Tt.Ar (mm^2)	748.8 (134.3)	495.8 – 1293.3	615.0 (93.2)	421.9 – 902.1	**133.8 [101.8, 165.7]**	21.6 [-15.0, 58.2]
Tt.Dn (mg/cm^3)	356.7 (48.3)	216.5 – 477.1	373.5 (46.7)	235.2 – 527.9	**-16.8 [-30.1, -3.5]**	**-19.0 [-36.8, -1.2]**
50% Tibia	n= 89		n= 109			
SSI_p (mm^3)	2012.5 (405.2)	1120.2 – 2841.1	1547.4 (302.1)	1008.8 – 2454.3	**465.1 [362.9, 564.3]**	-13.8 [-97.9, 70.2]
Tt.Ar (mm^2)	474.6 (66.1)	315.7 – 613.4	382.1 (51.9)	282.6 – 522.6	**92.4 [75.9, 109.0]**	12.2 [-2.0, 26.4]
Ct.Ar (mm^2)	326.2 (48.0)	229.6 – 434.4	270.6 (38.2)	177.3 – 375.7	**55.6 [43.5, 67.7]**	-3.4 [-13.2, 6.5]
Me.Ar (mm^2)	148.4 (31.7)	77.8 – 218.9	111.6 (25.6)	59.0 – 186.1	**36.8 [28.8, 44.9]**	**15.6 [5.4, 25.8]**
Ct.Dn (mg/cm^3)	1073.2 (27.3)	1000.6 – 1128.9	1127.2 (22.7)	1027.1 – 1088.2	**-54.0 [-61.0, -46.9]**	**-44.9 [-54.3, -35.5]**
7% Radius	n = 88		n = 110			
BSI (mg^2/mm^4)	3156.8 (1065.3)	1401.9 – 7575.7	3397.8 (1025.7)	1077.4 – 6503.5	-241.0 [-535.3, 53.3]	**-921.6 [-1271.4, -571.7]**
Tt.Ar (mm^2)	244.8 (49.3)	149.3 – 402.7	196.1 (30.5)	128.0 – 305.9	**48.7 [37.4, 60.0]**	**15.0 [2.5, 27.6]**
Tt.Dn (mg/cm^3)	357.5 (55.9)	229.4 – 543.0	415.0 (70.2)	233.6 – 594.0	**-57.5 [-75.6, -39.4]**	**-69.1 [-94.1, -44.2]**
30% Radius	n= 89		n=111			
SSI_p (mm^3)	238.8 (58.1)	133.0 – 388.6	189.6 (37.0)	112.8 – 299.8	**49.2 [35.9, 62.6]**	1.3 [-12.1, 14.6]
Tt.Ar (mm^2)	104.6 (18.3)	67.0 – 143.0	86.5 (12.2)	60.5 – 123.0	**18.0 [13.8, 22.3]**	2.8 [-1.5, 7.1]
Ct.Ar (mm^2)	82.7 (13.3)	56.2 – 114.6	69.4 (9.1)	47.8 – 93.4	**13.3 [10.2, 16.5]**	1.6 [-1.4, 4.6]
Me.Ar (mm^2)	21.8 (8.1)	5.3 – 43.7	17.1 (5.6)	6.6 – 37.0	**4.7 [2.8, 6.6]**	1.2 [-1.3, 3.7]
Ct.Dn (mg/cm^3)	1105.6 (29.7)	972.7 – 1158.3	1161.0 (21.8)	1068.5 – 1198.7	-55.5 [-62.6, 48.3]	**-52.8 [-62.8, -42.7]**

CI, confidence interval; BSI, bone strength index; Ct.Ar, cortical area; Ct.Dn, cortical density; Me.Ar, medullary area; SD, standard deviation; SSI_p, polar strength strain index; Tt.Ar, total area; Tt.Dn; total density.
Bold values are significant at $p<0.05$.

Unadjusted values at the tibia: At the *distal tibia*, bone strength (represented by BSI) was greater (9%) for boys compared with girls (Table 4.2). Bone size (represented by Tt.Ar) was greater (18%) in boys compared with girls. Tt.Dn for girls was 5% greater compared with boys. At the *tibial shaft*, bone strength (represented by SSI_p) was 23% greater in boys compared with girls. Bone size was greater (Tt.Ar: 20%, Ct.Ar: 17%) in boys compared with girls. The endosteal compartment (represented by Me.Ar) was 25% greater in boys compared with girls. Finally, girls' Ct.Dn at the *tibial shaft* was 5% greater compared with their male counterparts.

Adjusted values at the tibia: After adjusting for tibial length and LM, a different picture emerged. At the *distal tibia*, BSI was 10% greater in girls compared with boys. Differences in Tt.Ar were no longer significant; however, between-sex differences in Tt.Dn persisted, being greater (5%) in girls compared with boys. At the *tibial shaft*, SSI_p was no longer different between the sexes. Me.Ar remained significantly larger (11%) in boys as compared with girls. Girls' Ct.Dn at the *tibial shaft* was 4% greater compared with boys. There were no other significant differences in any bone outcomes between sexes after adjusting for covariates.

Unadjusted values at the radius: At the *distal radius*, BSI was not significantly different between boys and girls. Boys' Tt.Ar was 20% greater compared with girls while Tt.Dn was 14% greater in girls compared with boys. At the *radial shaft*, bone strength (represented by SSI_p) was greater (21%) in boys compared with girls. Bone size (Tt.Ar and Ct.Ar) was greater (17% and 16%, respectively) in boys compared with girls. Boys also had a greater Me.Ar (22%) as compared with girls. There were no significant differences in Ct.Dn between sexes at the *radial shaft*.

Adjusted values at the radius: After adjusting bone outcomes at the *distal radius* for ulnar length and LM, girls had significantly greater bone strength (BSI: 27%) compared with boys. This was despite

boys having larger bones (Tt.Ar: 6%) compared with girls. Girls had greater Tt.Dn (17%) compared with boys. At the *radial shaft,* there were no sex differences among bone strength or structure outcomes after adjusting for covariates. However, girls had more dense cortices (5%) compared with boys.

4.3.3 Determinants of tibial bone strength, structure and density in boys and girls

Boys: Due to the small number of boys with valid accelerometer data (n=40), I was unable to fit multivariable regression models to examine the influence of objectively measured MVPA on bone outcomes. I adopted the rule of thumb that 10 participants per independent (PA) and/or control (ethnicity, limb length, maturity offset and LM) variable are required to conduct valid multivariable regression analysis (442). Thus, for boys (n=90) I represented PA using MVPA (min/day; from the PAQ-A) and entered it as an independent variable into the regression models. At the *distal tibia,* after controlling for covariates (ethnicity, tibial length, maturity offset and LM), boys' MVPA did not significantly predict any bone outcome (Table 4.3). However, at the *tibial shaft*, MVPA was a positive predictor of bone strength (SSI_p) and structure (Ct.Ar) (Table 4.3). Specifically, MVPA explained 2-4% of the variance in these outcomes after control variables were entered into the model (Figure 4.2). At the *tibial shaft,* MVPA was not a significant predictor of boys' Tt.Ar, Me.Ar or Ct.Dn.

Girls: At the *distal tibia,* after controlling for covariates (ethnicity, tibial length, age at menarche and LM), MVPA (by accelerometry) was a positive predictor of bone strength (BSI; 7% of variance explained) and density (Tt.Dn; 6% of variance explained) (Figure 4.2). However, MVPA did not significantly predict bone structure (Table 4.3). At the *tibial shaft,* MVPA did not significantly predict bone strength, structure or density (Table 4.3). As a second step I added SED and controlled for accelerometer wear time in the MVPA regression models for BSI and Tt.Dn; MVPA was no longer a significant predictor of BSI or Tt.Dn. As a third step, I investigated the independent influence of moderate

and vigorous PA. Vigorous PA positively predicted BSI and explained an additional 4% of variance in girls (only) after adjusting for covariates, total wear time, MPA and SED (Table 4.4, Figure 4.3). Vigorous PA was not a significant predictor of Tt.Dn and moderate PA did not significantly predict BSI or Tt.Dn in girls.

Table 4.3. Hierarchical multivariable regression models to demonstrate the independent contribution of moderate-to-vigorous physical activity (MVPA) to bone strength, structure and density at the distal tibia (8% site) and tibial shaft (50% site) in boys and girls (controlled for ethnicity, tibial length, age (boys), maturity (girls) and lean mass).

		Boys					Girls			
DV	IV	β (SE)	p value	ΔR^2	Overall R^2	IV	β (SE)	p value	ΔR^2	Overall R^2
8% site										
BSI	Asian [a]	349.7 (639.7)	0.550	0.181[τ]		Asian [a]	1259 (430.4)	0.005	0.121[τ]	
	Other ethnicity	546.5 (689.6)	0.430			Other ethnicity	1159 (596.2)	0.057		
	Tibial length	-34.0 (11.4)	0.004			Tibial length	-15.2 (11.5)	0.193		
	Years post APHV	667.0 (583.8)	0.255			$Age_{MENARCHE}$	-506.4 (166.3)	0.004		
	Lean mass	0.23 (0.04)	<0.001	0.265		Lean mass	0.32 (0.06)	<0.001	0.447	
	$MVPA_{PAQ-A}$	3.1 (2.5)	0.209	0.011		**$MVPA_{ACCEL}$**	**33.8 (10.5)**	**0.002**	**0.073**	
	Constant	11078 (3879)	0.005		0.457	Constant	7084 (3816)	0.069		0.640
Tt.Ar	Asian	-4.9 (43.9)	0.912	0.217[τ]		Asian	-44.8 (21.9)	0.046	0.244[τ]	
	Other ethnicity	-77.7 (47.4)	0.105			Other ethnicity	-5.6 (30.4)	0.854		
	Tibial length	0.17 (0.78)	0.833			Tibial length	-0.52 (0.59)	0.378		
	Years post APHV	-38.6 (40.8)	0.338			$Age_{MENARCHE}$	11.0 (8.5)	0.201		
	Lean mass	0.01 (0.003)	<0.001	0.146		Lean mass	0.012 (0.003)	<0.001	0.228	
	$MVPA_{PAQ-A}$	0.16 (0.17)	0.336	0.008		**$MVPA_{ACCEL}$**	0.16 (0.54)	0.770	0.0	
	Constant	263.0 (266.3)	0.326		0.371	Constant	252.0 (194.3)	0.200		0.473
Tt.Dn	Asian	7.7 (18.5)	0.680	0.093[τ]		Asian	40.8 (11.4)	0.001	0.230[τ]	
	Other ethnicity	30.7 (19.9)	0.127			Other ethnicity	26.9 (15.8)	0.096		
	Tibial length	-0.84 (0.33)	0.012			Tibial length	-0.19 (0.31)	0.533		
	Years post APHV	23.8 (16.9)	0.163			$Age_{MENARCHE}$	-14.3 (4.4)	0.002		
	Lean mass	0.002 (0.001)	0.096	0.034		Lean mass	0.003 (0.002)	0.034	0.142	
	$MVPA_{PAQ-A}$	0.03 (0.07)	0.719	0.001		**$MVPA_{ACCEL}$**	**0.66 (0.28)**	**0.022**	**0.062**	
	Constant	557.7 (112.0)	<0.001		0.129	Constant	466.7 (101.4)	<0.001		0.434

Table 4.3. continued

DV	Boys IV	β (SE)	p value	ΔR^2	Overall R^2	Girls IV	β (SE)	p value	ΔR^2	Overall R^2
50% site										
SSI_p	Asian [a]	84.4 (88.1)	0.341	0.440[τ]		Asian [a]	25.5 (61.8)	0.681	0.192[τ]	
	Other ethnicity	-198.6 (94.9)	0.040			Other ethnicity	-12.7 (87.3)	0.885		
	Tibial length	1.8 (1.6)	0.254			Tibial length	1.7 (1.5)	0.279		
	Years post APHV	-3.8 (80.4)	0.962			$Age_{MENARCHE}$	-27.6 (24.3)	0.261		
	Lean mass	0.04 (0.005)	<0.001	0.254		Lean mass	0.03 (0.008)	<0.001	0.284	
	$MVPA_{PAQ-A}$	**0.74 (0.34)**	**0.033**	**0.017**		**$MVPA_{ACCEL}$**	2.5 (1.5)	0.111	0.025	
	Constant	-687.2 (534.0)	0.202		0.711	Constant	-32.9 (510.9)	0.949		0.501
Tt.Ar	Asian	14.0 (14.8)	0.348	0.452[τ]		Asian	-6.6 (10.7)	0.537	0.238[τ]	
	Other ethnicity	-38.4 (16.0)	0.019			Other ethnicity	-14.7 (15.0)	0.334		
	Tibial length	0.50 (0.26)	0.061			Tibial length	0.28 (0.26)	0.284		
	Years post APHV	-7.4 (13.5)	0.584			$Age_{MENARCHE}$	-3.0 (4.2)	0.483		
	Lean mass	0.007 (0.0009)	<0.001	0.232		Lean mass	0.006 (0.001)	<0.001	0.257	
	$MVPA_{PAQ-A}$	0.10 (0.06)	0.087	0.012		**$MVPA_{ACCEL}$**	0.25 (0.27)	0.354	0.008	
	Constant	-23.0 (89.9)	0.799		0.695	Cons	99.3 (88.1)	0.265		0.503
Ct.Ar	Asian	8.4 (10.0)	0.402	0.419[τ]		Asian	-1.00 (7.6)	0.895	0.254[τ]	
	Other ethnicity	-8.0 (10.8)	0.460			Other ethnicity	-3.3 (10.7)	0.758		
	Tibial length	0.35 (0.18)	0.055			Tibial length	0.30 (0.19)	0.114		
	Years post APHV	-8.8 (9.2)	0.337			$Age_{MENARCHE}$	-3.6 (3.0)	0.234		
	Lean mass	0.005 (0.0006)	<0.001	0.271		Lean mass	0.004 (0.001)	<0.001	0.283	
	$MVPA_{PAQ-A}$	**0.13 (0.04)**	**0.001**	**0.041**		**$MVPA_{ACCEL}$**	0.35 (0.19)	0.066	0.029	
	Constant	49.0 (142.3)	0.732		0.731	Constant	-36.8 (62.6)	0.559		0.567

Table 4.3. continued

		Boys				Girls				
DV	IV	β (SE)	p value	ΔR^2	Overall R^2	IV	β (SE)	p value	ΔR^2	Overall R^2
50% site										
Me.Ar	Asian [a]	5.6 (11.0)	0.614	0.225[τ]		Asian [a]	-5.6 (8.2)	0.496	0.051[τ]	
	Other ethnicity	-30.4 (11.8)	0.012			Other ethnicity	-11.4 (11.6)	0.331		
	Tibial length	0.15 (0.20)	0.434			Tibial length	-0.02 (0.001)	0.940		
	Years post APHV	1.4 (10.3)	0.889			$Age_{MENARCHE}$	0.6 (3.2)	0.847		
	Lean mass	0.002 (0.0006)	0.018	0.049		Lean mass	0.002 (0.001)	0.153	0.032	
	$MVPA_{PAQ-A}$	-0.04 (0.04)	0.413	0.006		**$MVPA_{ACCEL}$**	-0.1 (0.2)	0.680	0.005	
	Constant	16.1 (66.6)	0.810		0.280	Constant	62.5 (67.8)	0.361		0.088
Ct.Dn	Asian	-9.1 (9.5)	0.340	0.259[τ]		Asian	19.4 (5.0)	<0.001	0.455[τ]	
	Other ethnicity	15.2 (10.2)	0.141			Other ethnicity	27.2 (7.0)	<0.001		
	Tibial length	-0.71 (0.17)	<0.001			Tibial length	-0.17 (0.12)	0.184		
	Years post APHV	32.8 (8.7)	<0.001			$Age_{MENARCHE}$	-4.5 (2.0)	0.024		
	Lean mass	0.0002 (0.0006)	0.787	0.0009		Lean mass	-0.0004 (0.0006)	0.526	0.0	
	$MVPA_{PAQ-A}$	0.007 (0.04)	0.857	0.0003		**$MVPA_{ACCEL}$**	0.12 (0.12)	0.319	0.010	
	Constant	1292 (57.6)	<0.001		0.260	Constant	1247 (41.1)	<0.001		0.466

β, unstandardized coefficient; ΔR^2, change in the proportion of variance explained by independent variable(s); $Age_{MENARCHE}$, age at menarche (year); BSI, bone strength index (mg^2/mm^4); Cons, constant; Ct.Ar, cortical area (mm^2); Ct.Dn, cortical density (mg/cm^3); Ct.Th, cortical thickness (mm); DV, dependent variable; IV, independent variable; Me.Ar, medullary area (mm^2); $MVPA_{ACCEL}$, moderate-to-vigorous physical activity by accelerometer (min/day); $MVPA_{PAQ-A}$, moderate-to-vigorous physical activity by questionnaire (min/day); APHV, age at peak height velocity; SE, standard error; SSI_p, polar strength strain index (mm^3); Tt.Ar, total area (mm^2); Tt.Dn, total density (mg/cm^3).

Bold variables are independent variable of interest and bold values are significant at $p<0.05$.

Units: tibial length (cm) and lean mass (kg).

[a] Dummy variable: 0 = White, 1 = Asian, 2 = Other ethnic group

[τ] , represents the R^2 contribution from Block 1 (ethnicity, tibial length and years post APHV/age at menarche)

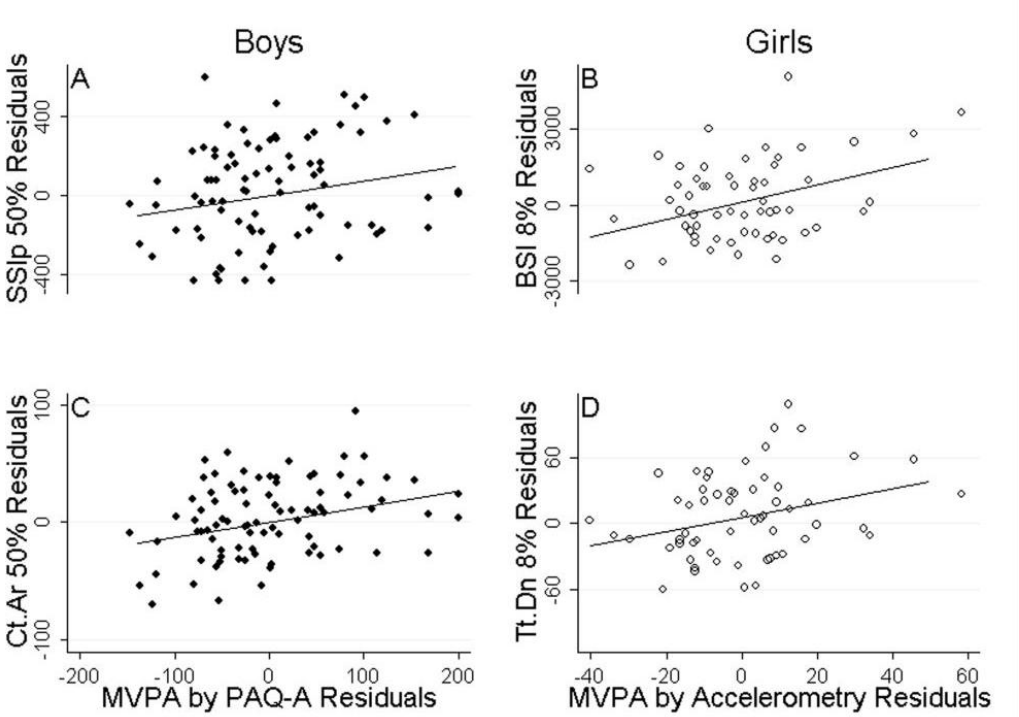


Figure 4.2. Significant relationships ($p<0.05$) of moderate-to-vigorous physical activity (MVPA) regression residuals and bone strength (SSI_p, BSI), structure (Ct.Ar) and density (Tt.Dn) at the 8% and 50% tibia sites in boys and girls. SSI_p, polar strength strain index; BSI, bone strength index; Ct.Ar, cortical area and Tt.Dn, total density.

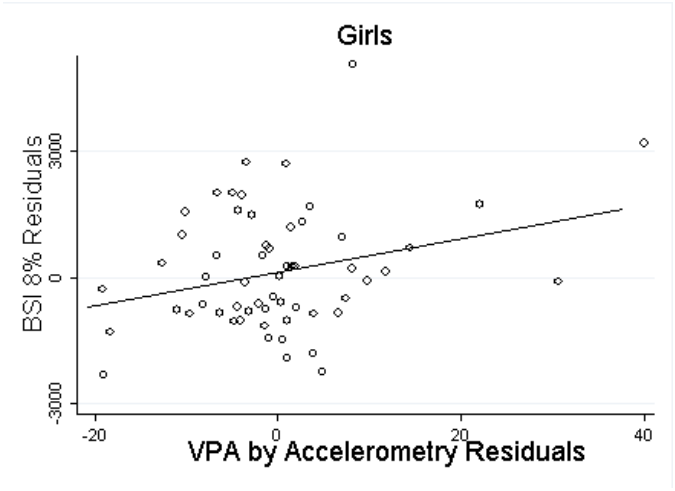


Figure 4.3 Significant relationships ($p<0.05$) of vigorous physical activity (VPA) regression residuals and bone strength index (BSI) at the 8% tibia site in girls ($p<0.05$).

Table 4.4. Hierarchical multivariable regression models that show the contribution of moderate physical activity (MPA), vigorous PA (VPA) and sedentary (SED) time to bone strength and density at the distal tibia (8% site) in girls.

DV	IV	β (SE)	p value	ΔR^2	Overall R^2
8% tibia					
BSI	Asian [a]	1346 (434.8)	0.003	0.121[τ]	0.674
	Other ethnicity	1429 (613.0)	0.024		
	Tibial length	- 16.6 (11.8)	0.164		
	$Age_{MENARCHE}$	-538.3 (174.3)	0.003		
	Lean mass	0.35 (0.06)	<0.001	0.447	
	Wear time	-8.6 (6.5)	0.189	0.044	
	SED_{ACCEL}	1.2 (6.4)	0.851	0.018	
	MPA_{ACCEL}	23.6 (26.6)	0.379	0.008	
	VPA_{ACCEL}	**39.1 (16.9)**	**0.025**	**0.037**	
	Constant	13382 (4762)	0.007		
Tt.Dn	Asian	41.8 (11.7)	0.001	0.230[τ]	0.476
	Other ethnicity	32.5 (16.4)	0.054		
	Tibial length	-0.14 (0.32)	0.665		
	$Age_{MENARCHE}$	-16.0 (4.4)	0.001		
	Lean mass	0.004 (0.002)	0.023	0.142	
	Wear time	-0.23 (0.17)	0.186	0.051	
	SED_{ACCEL}	0.05 (0.17)	0.766	0.020	
	MPA_{ACCEL}	1.0 (0.7)	0.158	0.025	
	VPA_{ACCEL}	0.42 (0.46)	0.364	0.009	
	Constant	609.4 (127.7)	<0.001		

β, unstandardized coefficient; ΔR^2, change in the proportion of variance explained by independent variable(s); $Age_{MENARCHE}$,, age at menarche (year); BSI, bone strength index (mg^2/mm^4); Cons, constant; DV, dependent variable; IV, independent variable; MPA_{ACCEL}, moderate physical activity (min/day); SED_{ACCEL}, sedentary time (min/day), SE, standard error; Tt.Dn, total density (mg/cm^3); VPA_{ACCEL}, vigorous physical activity (min/day).

Bold variables are independent variable of interest and bold units are significant at p<0.05.

Units: tibial length (cm) and lean mass (kg).

[a] Dummy variable: 0 = White, 1 = Asian, 2 = Other ethnic group

[τ] , represents the R^2 contribution from Block 1 (ethnicity, tibial length and age at menarche)

4.3.4 Determinants of radial bone strength, structure and density in boys and girls

Boys: At the *distal radius*, after controlling for covariates (ethnicity, ulnar length, age and LM), grip strength was a positive predictor of bone strength (BSI; 6% of variance explained) and size (Tt.Ar;

6% of variance explained) (Figure 4.4, Table 4.5). Grip strength did not predict Tt.Dn. At the *radial shaft*, grip strength explained additional variance in bone strength (SSI_p; 7%), size (Tt.Ar; 6%) and structure (Ct.Ar; 10%) (Figure 4.5, Table 4.5). Grip strength was not a significant predictor of Me.Ar or Ct.Dn at the radial shaft.

Girls: At the *distal radius*, after controlling for covariates (ethnicity, ulnar length, age at menarche and LM), grip strength explained 6% of the variance in Tt.Ar after adjusting for covariates, but did not predict BSI or Tt.Dn (Figure 4.4, Table 4.5). At the *radial shaft*, grip strength was a positive predictor of bone size (Tt.Ar; explained 5% of variance), strength (SSI_p) and structure Ct.Ar (explained an additional 4% of variance, both) (Figure 4.5, Table 4.5). Grip strength was not a significant predictor of Me.Ar or Ct.Dn at the radial shaft in girls.

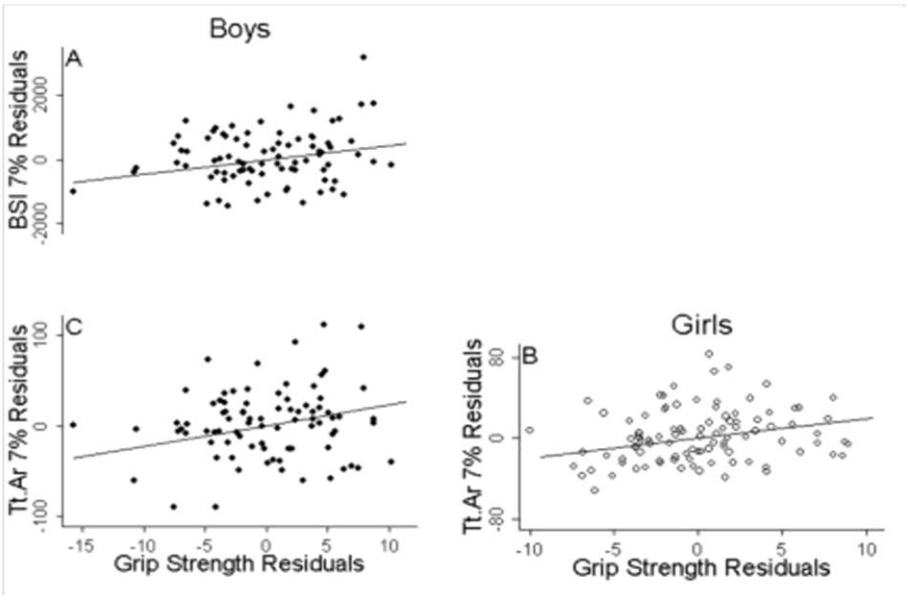


Figure 4.4. Significant relationship ($p<0.05$) of grip strength regression residuals and bone strength (BSI) and structure (Tt.Ar) at the 7% radius site ($p<0.05$) in boys and girls. BSI, bone strength index and Tt.Ar, total bone area.

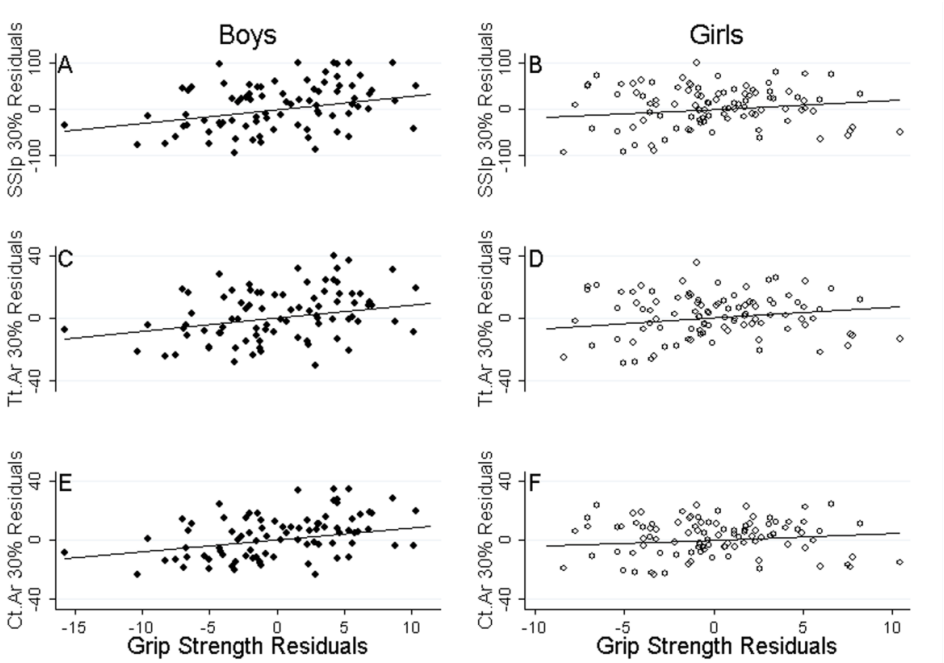


Figure 4.5. Scatterplots of grip strength regression residuals and bone strength (SSI_p) and structure (Tt.Ar , Ct.Ar) at the 30% radius site ($p<0.05$) in boys and girls. SSI_p, polar strength strain index; Tt.Ar, total bone area and Ct.Ar, cortical area.

Table 4.5. Hierarchical multivariable regression models to demonstrate the independent contribution of grip strength to bone strength, structure and density at the distal radius (7% site) and radial shaft (30% site) in boys and girls

		Boys					Girls			
DV	IV	β (SE)	p value	ΔR^2	Overall R^2	IV	β (SE)	p value	ΔR^2	Overall R^2
7% site										
BSI	Asian [a]	-42.94 (319.1)	0.893	0.166[τ]		Asian [a]	576.0 (186.4)	0.003	0.246[τ]	
	Other ethnicity	392.6 (349.6)	0.265			Other ethnicity	482.5 (270.6)	0.078		
	Ulnar length	-33.1 (10.5)	0.002			Ulnar length	-9.7 (7.3)	0.185		
	Years post APHV	511.0 (290.6)	0.083			$Age_{MENARCHE}$	-306.1 (71.4)	<0.001		
	Lean mass	0.08 (0.03)	0.002	0.223		Lean mass	0.11 (0.02)	<0.001	0.221	
	Grip strength	**48.0 (17.4)**	**0.007**	**0.055**		**Grip strength**	34.3 (19.8)	0.087	0.017	
	Constant	6039 (2269)	0.009		0.444	Constant	4688 (1638)	0.005		0.483
Tt.Ar	Asian	-12.2 (13.6)	0.376	0.282[τ]		Asian	-6.6 (5.9)	0.265	0.195[τ]	
	Other ethnicity	-19.2 (15.0)	0.204			Other ethnicity	12.3 (8.5)	0.153		
	Ulnar length	-0.93 (0.45)	0.041			Ulnar length	-0.29 (0.23)	0.209		
	Years post APHV	-0.6 (12.4)	0.961			$Age_{MENARCHE}$	4.6 (2.3)	0.042		
	Lean mass	0.004 (0.001)	0.001	0.209		Lean mass	0.002 (0.0007)	0.002	0.177	
	Grip strength	**2.3 (0.7)**	**0.003**	**0.055**		**Grip strength**	**1.9 (0.6)**	**0.003**	**0.058**	
	Constant	268.0 (97.0)	0.007		0.546	Constant	79.35 1.6 (51.5)	0.128		0.430
Tt.Dn	Asian	5.0 (21.3)	0.813	0.076[τ]		Asian	44.5 (13.9)	0.002	0.293[τ]	
	Other ethnicity	37.3 (23.3)	0.114			Other ethnicity	17.5 (20.2)	0.389		
	Ulnar length	-1.2 (0.7)	0.083			Ulnar length	-0.24 (0.55)	0.661		
	Years post APHV	32.3 (19.4)	0.100			$Age_{MENARCHE}$	-24.3 (5.3)	<0.001		
	Lean mass	0.002 (0.002)	0.285	0.004		Lean mass	0.004 (0.002)	0.022	0.050	
	Grip strength	1.1 (1.2)	0.367	0.009		**Grip strength**	-0.01 (1.5)	0.994	0.0	
	Constant	511.5 (151.4)	0.001		0.126	Constant	618.8 (1 to 2.4)	<0.001		0.343

Table 4.5. continued

DV	Boys IV	β (SE)	p value	ΔR^2	Overall R^2	Girls IV	β (SE)	p value	ΔR^2	Overall R^2
30% site										
SSI_p	Asian [a]	0.2 (13.9)	0.987	0.304[τ]		Asian [a]	4.0 (7.1)	0.571	0.139[τ]	
	Other ethnicity	3.2 (15.3)	0.837			Other ethnicity	10.1 (10.4)	0.330		
	Ulnar length	-1.1 (0.5)	0.020			Ulnar length	-0.38 (0.28)	0.174		
	Years post APHV	14.8 (12.7)	0.247			$Age_{MENARCHE}$	-3.0 (2.7)	0.278		
	Lean mass	0.005 (0.001)	<0.001	0.271		Lean mass	0.004 (0.0009)	<0.001	0.270	
	Grip strength	**3.0 (0.8)**	**<0.001**	**0.072**		**Grip strength**	**2.0 (0.8)**	**0.010**	**0.041**	
	Constant	190.0 (99.1)	0.059		0.646	Constant	122.3 (60.0)	0.052		0.450
Tt.Ar	Asian	0.06 (4.5)	0.990	0.313[τ]		Asian	1.1 (2.4)	0.643	0.141[τ]	
	Other ethnicity	-0.8 (4.9)	0.867			Other ethnicity	6.5 (3.4)	0.063		
	Ulnar length	-0.29 (0.15)	0.058			Ulnar length	-0.15 (0.09)	0.096		
	Years post APHV	3.5 (4.1)	0.399			$Age_{MENARCHE}$	-0.41 (0.90)	0.653		
	Lean mass	0.002 (0.0004)	<0.001	0.256		Lean mass	0.001 (0.0003)	<0.001	0.269	
	Grip strength	**0.85 (0.25)**	**0.001**	**0.058**		**Grip strength**	**0.69 (0.25)**	**0.007**	**0.045**	
	Constant	78.6 (32.1)	0.017		0.626	Constant	63.7 (20.5)	0.003		0.454
Ct.Ar	Asian	-0.29 (3.0)	0.922	0.334[τ]		Asian	1.6 (1.6)	0.331	0.133[τ]	
	Other ethnicity	3.7 (3.3)	0.259			Other ethnicity	3.8 (2.4)	0.113		
	Ulnar length	-0.16 (0.10)	0.104			Ulnar length	-0.10 (0.06)	0.108		
	Years post APHV	2.9 (2.7)	0.286			$Age_{MENARCHE}$	-0.23 (0.63)	0.711		
	Lean mass	0.001 (0.0002)	<0.001	0.250		Lean mass	0.001 (0.0002)	<0.001	0.318	
	Grip strength	**0.82 (0.16)**	**<0.001**	**0.102**		**Grip strength**	**0.44 (0.18)**	**0.014**	**0.035**	
	Constant	51.9 (21.3)	0.017		0.686	Constant	46.7 (14.4)	0.002		0.486

Table 4.5. continued

		Boys					Girls			
DV	IV	β (SE)	p value	ΔR^2	Overall R^2	IV	β (SE)	p value	ΔR^2	Overall R^2
30% site										
Me.Ar	Asian [a]	0.35 (2.9)	0.903	0.127$^{\tau}$		Asian [a]	-0.5 (1.4)	0.707	0.068$^{\tau}$	
	Other ethnicity	-4.6 (3.2)	0.153			Other ethnicity	2.6 (2.0)	0.192		
	Ulnar length	-0.12 (0.10)	0.197			Ulnar length	-0.05 (0.05)	0.349		
	Years post APHV	0.56 (2.6)	0.831			$Age_{MENARCHE}$	-0.17 (0.52)	0.743		
	Lean mass	0.0006 (0.0002)	0.010	0.105		Lean mass	0.0002 (0.0002)	0.176	0.057	
	Grip strength	0.03 (0.16)	0.842	0		**Grip strength**	0.25 (0.15)	0.087	0.027	
	Constant	26.7 (20.5)	0.197		0.233	Constant	17.0 (11.9)	0.157		0.152
Ct.Dn	Asian	-9.2 (11.2)	0.411	0.117$^{\tau}$		Asian	2.8 (4.8)	0.560	0.245$^{\tau}$	
	Other ethnicity	16.4 (12.3)	0.187			Other ethnicity	5.1 (7.0)	0.469		
	Ulnar length	-0.60 (0.37)	0.106			Ulnar length	0.27 (0.19)	0.145		
	Years post APHV	27.9 (10.2)	0.008			$Age_{MENARCHE}$	-9.7 (1.8)	<0.001		
	Lean mass	-0.0004 (0.0009)	0.616	0		Lean mass	-0.0003 (0.0006)	0.626	0.019	
	Grip strength	0.41 (0.61)	0.499	0.005		**Grip strength**	-0.93 (0.51)	0.072	0.025	
	Constant	1227 (79.7)	<0.001		0.123	Constant	1245 (41.7)	<0.001		0.288

β, unstandardized coefficient; ΔR^2, change in the proportion of variance explained by independent variable(s); $Age_{MENARCHE}$, age at menarche (year); BSI, bone strength index (mg^2/mm^4); Cons, constant; Ct.Ar, cortical area (mm^2); Ct.Dn, cortical density (mg/cm^3); Ct.Th, cortical thickness (mm); DV, dependent variable; IV, independent variable; Me.Ar, medullary area (mm^2); APHV, age at peak height velocity; SE, standard error; SSI_p, polar strength strain index (mm^3);Tt.Ar, total area (mm^2); Tt.Dn, total density (mg/cm^3).

Bold variables are independent variable of interest and bold values are significant at $p<0.05$.

Units: tibial length (cm), lean mass (kg) and grip strength (kg).

[a] Dummy variable: 0 = White, 1 = Asian, 2 = Other ethnic group

$^{\tau}$, represents the R^2 contribution from Block 1(ethnicity, tibial length and years post APHV/age at menarche).

4.4 Discussion

To my knowledge, this is the first study to report bone outcomes at the distal tibia and tibial midshaft (assessed using pQCT) and measure MVPA *and* sedentary behaviour objectively, in adolescent boys and girls. I advance knowledge of bone strength, structure and density in this age group and characterize key relationships between bone strength (and structure and density) and PA and muscle function. Specifically, results supported Hypothesis 1, as I report sex differences in bone outcomes that varied across skeletal sites before and after adjusting for limb length and LM. Results did not support Hypothesis 2, as MVPA did not significantly predict bone strength across every site in boys and girls. Importantly, there were site-specific differences in the relationship between PA and bone outcomes in boys and girls; MVPA positively predicted boys' bone strength and structure (but not bone density) at the tibial midshaft and girls' bone strength and density (but not bone structure) at the distal tibia. Results did not support Hypothesis 3, as SED did not significantly predict a negative correlation with bone outcomes. With SED and MVPA in the analyses, the previously positive influence of MVPA on bone strength and Tt.Dn was no longer significant. Results support Hypothesis 4 as muscle strength positively predicted bone strength and structure (but not bone density) at the radius (distal and shaft sites) in boys and girls after adjusting for ethnicity, limb length, maturity and LM. In the following sections, I discuss these findings and embed them in the context of the current literature.

4.4.1 Sex differences in bone strength, structure and density

A number of studies reported sex differences in bone strength, structure and density in children and adolescents (216,150,212,215,217–220,222,424,443). Generally, published results compare favourably with my findings. That is, boys tended to have larger bone area and greater bone strength

across all measured sites compared with girls (212,215,220,443–445). Girls consistently had greater Ct.Dn at distal (by HR-pQCT) and shaft sites (by pQCT) of long bones compared with boys (222,424,443). However, it is difficult to compare results across studies given the different instruments used to assess bone (DXA versus pQCT/HR-pQCT), different age and maturity levels of participants and different covariates used in analyses. Therefore, I focus on studies that used 3-D imaging tools to avoid discrepancies that may arise from comparing my results with those from DXA-based studies. Furthermore, although results from pQCT studies are often non-comparable based on different protocols and scan sites used (137), I provide general bone sites assessed – distal or shaft sites – and I assumed protocols were appropriate based on peer-reviewed process of the publications.

Sex differences at the tibia: Sex differences (unadjusted) I observed at the tibial shaft were similar to those reported for a large cohort of 15-year old boys and girls (n = 1748) (341). That is, boys had larger tibiae (greater Tt.Ar and Ct.Ar by pQCT) but lower Ct.Dn compared with girls (unadjusted values at the midshaft). In studies of pre-and early pubertal children that adjusted for limb length and MCSA (150,215,216) or height (212), boys had greater tibial bone strength (distal and shaft sites) and bone area (shaft site only) but lower Ct.Dn (shaft site only) (by pQCT) compared with girls. In a well-designed study across maturity stages by Leonard and colleagues that adjusted for tibial length, age, ethnicity and muscle variables, boys had greater tibial (shaft) bone strength due to their larger bones compared with girls only at late maturation stage (Tanner stage 5) (238). In contrast, greater tibial Ct.Dn in girls compared with boys was observed from an earlier maturation stage (from Tanner stage 3 to Tanner stage 5) (238). These findings reiterate my results in post-pubertal boys and girls after adjusting for covariates (limb length and LM) where no sex differences in bone size (Tt.Ar and Ct.Ar) were observed but girls had greater bone strength (BSI) at the distal site and bone density (Tt.Dn and Ct.Dn) at both distal and shaft sites compared with boys. The higher bone densities observed in girls, without a larger Tt.Ar, would reflect bone mineral

apposition on trabecular bone at distal sites and at the endosteal bone surface at shaft sites (girls had a smaller Me.Ar compared with boys). Thus, bone strength at the weight-bearing tibia in 15-year olds is conferred by different bone properties, whereby bone density seems to be the main contributor to bone strength in girls and bone size contributes to bone strength in boys.

Sex differences at the radius: It is important to approach comparisons at the radial shaft, as it is a non-weight bearing site, a little differently. While the tibia experiences forces primarily from compression during weight bearing, the radius is subject to multitude of forces (compressive, tensile, bending, torsion) dependent on the action required (446). At the distal radius (unadjusted), sex differences in bone density partially aligned with observations from two studies of maturity-matched 15-year old participants (218,220). Neu and colleagues found that 14- to 15-year old boys had larger bone CSA, higher Tb.Dn but lower Ct.Dn (unadjusted) at the distal radius compared with girls of similar age (by pQCT) (218). However, contradicting my findings, Tt.Dn at the distal radius was significantly higher in boys compared with girls at the prepubertal stage, only (218). Kirmani and colleagues found that girls at Tanner stage 4 (15-17 year olds) had higher Ct.Dn at the distal radius (by HR-pQCT) compared with boys (similar to my findings, both unadjusted studies) but differences were no longer significant when participants matured (i.e. advanced to Tanner stage 5) (220). For the radial shaft, a longitudinal study in adolescents (219) displayed the same trends in unadjusted values as I report. That is, boys (15 years old, on average) had larger bones (total area) compared with girls at the radial shaft. Boys also had a greater Me.Ar compared with girls, which indicates less endosteal and more periosteal apposition in boys (219). However, Ct.Dn at the radial shaft was higher in girls compared with boys after Tanner stage 4 and upwards (222). In my findings, Ct.Dn was higher in girls compared with boys only after adjustments and this speaks to the possible endosteal apposition of bone mineral in girls as reported in the aforementioned studies.

Adjusted versus unadjusted sex differences: Given that limbs function as a lever arm (also as a surrogate measure of stature) and muscles impose the primary stimulus for osteogenesis – I adjusted for these variables in my analyses. When I adjusted for limb length and LM, my results differed from other studies that adjusted for other factors. For example, a study of pre- and early-pubertal children showed that boys' distal tibiae were stronger, larger and had greater density compared with girls, after accounting for limb length and MCSA (150). In a peri-pubertal group of boys and girls assessed by HR-pQCT in a previous study in our lab, Nishiyama et al. found that boys tended to have larger bones (bone area) than did girls at the distal tibia and radius after adjusting for limb length and height (443). Consistent with my findings, girls in their study had greater Ct.Dn than did boys. However, bone strength (ultimate stress estimated using FEA) did not differ between sexes (443).

The choice of covariates may result in different outcomes as demonstrated by Hölger and colleagues who used MRI to assess femoral shaft (66% site) in pre- and post-pubertal boys and girls (217). Investigators controlled for three sets of covariates to demonstrate how this influenced sex differences in bone outcomes. Specifically they used: i) dimensional scaling, ii) controlled for limb length and body mass; and iii) controlled for limb length and muscle mass (217). Using the first two approaches, post-pubertal boys had greater bone strength (I_{max}, I_{min}, I_p, BSI), larger bone area (Tt.Ar, Ct.Ar and Me.Ar) and greater Ct.Dn compared with post-pubertal girls (217). However, when limb length and muscle mass were controlled, results favoured post-pubertal girls who now had larger bone area and Ct.Dn compared with post-pubertal boys. This contributed to girls' greater bone strength. This effect was maturity specific and was not observed in pre-pubertal children. Hence, Hölger and colleagues aptly demonstrated what we know to be true – once scaled for size and muscularity, girls tibial bone strength, area and density would be comparable to (if not slightly greater than) boys. Thus, it is size and muscle mass differentials that drive sexual dimorphism in bone strength and this may not be evident before puberty.

Maturation is an important confounding factor when examining sex differences in bone outcomes. However, maturity levels between boys and girls in my study were different, as girls are known to mature earlier than boys (447). I used different maturational indicators for boys and girls in my study, as menarche is quantified easily and reliably, it is a key indicator of sexual maturation while boys' maturity offset (i.e. years post APHV) is an indicator of somatic maturation. Menarche is a late maturational event in girls (generally about one year following PHV and coincides with PBMCV (182). However, given that sexual maturation occurs at an earlier chronological age in girls as compared with boys, more girls in my study (average 3.0±1.2 years post-menarche) would have reached peak BMC accrual compared with boys (9). This maturation difference might account (at least in part) for greater bone density in girls as compared with boys. As PHV occurs approximately eight months before peak bone mineral accrual (11), boys may have achieved growth in size (length and width) but lagged behind in mineral apposition, which may explain their larger yet less dense bone. Proximal radius peak velocity gain in bone structure assessed by pQCT occurred earlier in girls (Ct.Ar, age 11years) while boys had peak velocity gains in Ct.Ar around age 16 years (219). These quite profound maturational differences specific to bone parameters underscore the complexity of studying children especially during the adolescent period of rapid growth and development and importance of accounting for biological age when comparing sex differences in bone variables, wherever possible. I recognize not having comparable maturational indices as a limitation of my study.

4.4.2 Influence of MVPA, VPA and SED on tibial bone strength, structure and density

The contribution of weight-bearing exercise (loading) to bone development and bone strength is well known (32,33,113,385). Bones adapt to mechanical stimulation through mechanotransduction (60,79)

and physically active boys and girls accrue more bone mineral (12,341,448) and have larger bone areas (31,105,106,400) that result in stronger bones compared with less active boys and girls.

I used objective measures of PA (accelerometry) to assess the influence of MVPA on bone strength in girls. To my knowledge, this is the first study to report a positive relationship between MVPA (by accelerometry) *and* pQCT-derived bone strength at the distal tibia in adolescent girls. The influence of MVPA on bone strength appeared to be manifest through enhanced bone density in the more active girls. As a next step, I evaluated the independent contribution of vigorous and moderate PA to bone outcomes. In keeping with Frost's mechanostat theory (75), VPA that yields higher strains through greater muscle activation was the strongest predictor of girls' bone strength.

As there is a dearth of studies that used accelerometers together with 3-D imaging equipment in children, I included known DXA-based studies and their findings to compare and contrast my results. Current evidence suggests, and not surprisingly, that VPA is more strongly related to boys' bone structure (by pQCT, (341)) and strength (by DXA-HSA, (338,340)) than is MPA. Sayers and colleagues reported a positive influence of VPA (≥ 6200 cpm, β (95% CI): 0.059 [0.034, 0.084]) on Ct.Ar at the tibial shaft (by pQCT) in 778 adolescent boys (341). In contrast, MPA (≥3200 – 6199 cpm) was not significantly related to Ct.Ar (341). Similar results were observed in earlier DXA-based studies of pre- and peri-pubertal boys. That is, objectively measured VPA was a stronger predictor of femoral neck bone strength (Z) and CSA (by HSA) than was MPA (338,340). Janz and colleagues defined MPA as ≥ 527 cpm and VPA as ≥ 2818 cpm (338). Sardinha and colleagues defined MPA as ≥ 2001-3999 cpm and VPA as ≥ 4000 cpm (340). One study showed that MVPA (≥ 3000 cpm) was positively associated with femoral neck bone strength and CSA in boys (339). Importantly, comparisons across these studies are limited by differences in bone imaging tools and by differences in accelerometry cut-points. One other study indicated that measures of ground reaction forces are more relevant to bone outcomes versus the level of PA in adolescents (344).

Other than higher loads or larger strain magnitude, number of loads per day or strain rates are related to successful adaptation of bone to mechanical stimulus (385). The reverse is true where reduced PA could result in bone resorption due to the lack of mechanical stimulus. A decrease in PA among former athletes (age 17 years at baseline) followed prospectively for 12 years showed a loss of aBMD at the pelvis and hip compared with active athletes that continued to gain or maintain their aBMD (449). Other than a decrease in PA, studies are suggesting that increased SED may also result in lower BMD in children and youth.

The independent effect of SED has gained much attention in PA research in recent years (450,451). However, this concept has only recently been applied to growing bone (322,329,331,452). The few cross-sectional studies that examined associations between SED and bone mass (by DXA) showed a negative relationship (322,328,329). Chastin and colleagues conducted the largest of these studies and examined sedentary activities (by self-report) and SED (by self-report or accelerometry) in 8 to 22 year old boys (n = 671) and girls (n = 677) and controlled for MVPA (329). Each additional hour per day of screen time was associated with less BMC at the proximal femur (-0.77 g in girls and -0.45 g in boys) (329). This negative association between more time spent sedentary (screen time) and bone health can be understood in a context similar to the detrimental physiological response of bone to bed rest or mobility limitations (453). For example, in a study of adults who were confined to bed-rest, ultradistal tibia total vBMD measured by HR-pQCT decreased by 1.2% in a short span of six weeks (454).

In my cohort, the relationship between VPA and bone strength in girls persisted after accounting for SED (Table 4.4). In contrast, SED muted the positive influence of MVPA on girls' bone strength and density at the distal tibia. Similarly, data from NHANES showed the negative association between femoral BMC and SED (by accelerometer) and behaviour (screen time) remained when MVPA (by accelerometry) was introduced to the analysis but the association was no longer significant with the introduction of VPA

into the analysis (329). Thus, it is likely that sedentary behaviour evokes an independent mechanism (to weight-bearing PA) that alters strain thresholds in growing bones. These findings suggest two things; first, SED may be a more powerful negative influence than PA (or more precisely MVPA) is a positive one and, second as adolescent girls spend a large part of each day being sedentary (>10 hrs/d, on average), weight-bearing PA of higher intensity may be required to offset these behaviours and to preserve or enhance bone strength.

Although it seems intuitive that sedentary adolescents may be less physically active – this was not the case in my study. To illustrate, girls in the present study were generally more sedentary than the average 15-19 year old Canadian girl (611 min/day vs. 582 min/day (323)). However, they engaged in more MVPA (44 min/day of MVPA, defined as >2296 cpm vs. 39 min/day of MVPA, defined as >1500 cpm) (323). In a recent study from our group, SED was not a significant predictor of bone microarchitecture, strength or density at the distal tibia (assessed using HR-pQCT) in 206 adolescents and young adults (331). Relatively little is known about the implications of the rising rates of child and youth SED on bone health, generally and bone strength, specifically. Furthermore, the decline in PA as children transition to adolescence is of particular concern. Janz and colleagues reported that MVPA, measured by accelerometer, declined by 34% and 65% in boys and girls, respectively, between the ages of 5 and 17 years (455). Therefore, further investigation is required to understand this important relationship better particularly with objectively measured PA and SED.

I was unable to determine the independent contribution of objectively measured PA to bone outcomes in boys. This was due to the relatively small number of boys from whom I collected valid accelerometry data (n=40). I therefore entered MVPA estimated from their questionnaires (PAQ-A) into my regression models. MVPA from the PAQ-A is a valid estimate of PA. It was moderately associated with accelerometry-derived MVPA ($r= 0.43$, $p<0.001$) in 89 boys and young men aged 9 to 20 years in

our HBS III (UBC Bone Health Research Group, unpublished data) and weakly to moderately associated (r=0.18 to 0.49) with MVPA by accelerometry or direct observations in previous studies (20,308,456). I acknowledge that self-report questionnaires are subject to bias, especially for duration and intensity of PA (457). In my study, MVPA estimated using the PAQ-A was almost two-fold higher than MVPA assessed objectively (113 vs. 58 min/day). This suggests that boys may have overestimated their duration of MVPA; however, my sample size was too small to draw any firm conclusions from objectively measured MVPA on bone outcomes.

While acknowledging these limitations, self-reported MVPA was a significant predictor of boys' bone strength (SSI_p) at the tibial shaft. The influence of MVPA on bone strength was mediated through an apparent influence on cortical bone structure (Ct.Ar) rather than Ct.Dn. These findings align with other studies that used pQCT or MRI to assess bone structure. For example, in young racquet sport players mechanical loading drove structural bone changes that enhanced bone strength at the distal and shaft sites of the radius and humeral (105–107). Bone structure also mediated the positive influence of PA on tibial (distal and shaft) and femoral (shaft) bone strength in children and adolescents (145,407,432). Small amounts of bone accrued at the periosteal surface have an exponential influence on bone strength, given that bone strength is a product of the distribution of bone material away from its neutral axis (the radius of bone to the fourth power) (70). As long ago as 1638 Galileo showed how structural adaptations, such as a hollow diaphysis, adapt bones for strength and lightness (458). However, in a randomized-controlled PA intervention study of pre-pubertal children, total bone density rather than bone structure (assessed by pQCT) accompanied significant increases in bone strength in the intervention group (32). Perhaps due to the maturational stage of growth (pre- to post-pubertal), bone adapts more structurally to mechanical stimuli post-puberty in boys as can be seen by the associations of PA to bone size rather than density.

4.4.3 Independent contribution of muscle strength to radial bone strength and structure

Muscle plays a profound role as the major source of physiological loading, generated through muscle contractions, to stimulate adaptation in growing bones. In my study, muscle strength appeared to influence bone strength at the radius (distal and shaft sites) through increased periosteal apposition (Tt.Ar and Ct.Ar and no difference in Me.Ar) rather than via enhanced bone density. These findings align with previous studies in which muscle (mass, strength, force or power) was a powerful predictor of bone structure and strength at the radius (105–107,290,459), tibia (285) and proximal femur (286). Almost two decades ago Schoneu and colleagues showed that as much as 87% of BSI at the distal radius was explained by grip strength alone (290).

The muscle-bone unit describes the close relation of muscle and bone, whereby muscle growth precedes bone mineral accrual and muscle forces applied to bone drive bone adaptation. Intriguingly, there appears to also be an independent influence of muscle mass and muscle force on bone outcomes. In the present study muscle strength explained an additional (to muscle mass) amount of the variance in bone strength and structure when both muscle variables were entered into the regression model. Daly and colleagues studied the contralateral limb of female tennis players (n=47, age 8 to 17 years) and found that training-induced muscle size accounted for 12% of bone's adaptive responses (mass, size, bending strength) in the playing arm compared with the non-playing arm (284). As the study design (comparison of contralateral limbs) automatically accounts for genetics, nutrition, hormonal and other intrinsic factors, authors suggests that the unifying hypothesis that muscle size proportionally influences bone adaptation to be overly simplistic (284). Similarly, in a study of prepubertal girls, muscle force (by isokinetic dynamometer) and power (by ground reaction forces) explained an additional 2% to 5% of the variance in bone strength and structure at the femoral neck after accounting for leg lean mass (by DXA) (286). In a study of adults, Lorberg and colleagues reported that grip strength explained additional (to MCSA, by

pQCT) variance in bone strength (BSI) at the distal radius (460). Therefore, while muscle mass is a dominant predictor of bone outcomes, there might be other muscle-related factors that encourages adaptations in growing bones such as muscle force or strength.

To elaborate, with training, muscle force or strength can increase without concomitant muscle hypertrophy (441,461). Other aspects related to force generation such as neuromuscular activity, motor unit recruitment rate, muscle pennation angle, muscle length, fiber type and even direct impact of vibration loading (e.g., impact from racket sports) contribute to muscle strength (172,281,462), independent of muscle mass. The independent influence of muscle function versus muscle mass deserves greater study across all ages.

Early intervention studies that evaluated the effect of resistance training on aBMD (by DXA) in adolescent girls reported small (295) or no (294) significant gains in aBMD, despite significant increases in muscle strength. As there was substantial variability in maturity and as bone was assessed using planar DXA technology in these studies, we still do not know definitively whether resistance training leads to gains in bone strength in the adolescent skeleton. Therefore, studies that intervene with resistance training (especially applied to non-weight bearing bones) and use 3-D imaging techniques to assess bone are needed to shed more light on adaptations of bone structure and strength to changes in muscle function.

4.4.4 Strengths and limitations

My study has a number of strengths. First, I used accelerometers to objectively measure levels of PA and SED in adolescents. Second, the use of 3-D imaging, pQCT, provided measures of bone structure, density and estimates of bone strength that are reliable, valid and precise (143,152,153). Third, I studied an age cohort where information on sex differences in bone outcomes, while controlling for body size and

muscle mass, was limited in the literature. Fourth, I analysed the influence of a typical pattern of sedentary and PA time in girls on their bone outcomes. Lastly, I examined the contribution of muscle strength, independent of muscle mass, to bone outcomes, providing possible insights for the design of resistance-training interventions aimed at increasing bone strength in adolescents.

I acknowledge that my study has several limitations. First, the cross-sectional study design does not permit evaluation of cause-effect relationships. Second, I used different maturation assessments for boys and girls, as my main focus was to obtain a key maturational landmark related to bone development and not to compare bone differences between sexes based on maturity level. Third, I was unable to use objective measures of PA to assess the association between MVPA and bone outcomes in boys due to the small number of boys with valid accelerometry data. Future studies might incorporate text-based reminders to improve compliance to wearing accelerometers across a full day. Fourth, although an advanced imaging technology, pQCT measures are limited to a non-clinically relevant site in the weight-bearing lower extremities (the tibia). Ideally, 3-D technologies that capture bone strength adaptations at the more clinically relevant proximal femur are needed. Fifth, partial volume effects associated with the resolution of pQCT imaging may underestimate total density at distal sites in participants with thin cortices (222).

4.5 Conclusion

In conclusion, boys are conferred a bone strength advantage as compared with girls based on their larger bone size whereas bone strength in girls is conferred by consistently more dense bones. More importantly, knowing that bone outcomes are relative to body size and musculature, accounting for these parameters in future pediatric bone studies is vital to understand the independent contribution of other

modulators (e.g., PA, nutrition) to bone adaptation. Despite the known influence of PA on bone strength, there is a need to consider the extent to which SED may mitigate the osteogenic effects of MVPA. In the meantime, it is important to promote VPA in adolescents to ensure adequate mechanical stimuli are provided to obtain optimal bone strength at weight-bearing sites. Further, the role of muscle strength or force (shown to be independent of muscle mass) in bone strength accrual at non-weight bearing sites could be studied in resistance training interventions in adolescents. Finally, it seems important for experts to reach consensus regarding standard analysis and acquisition protocols for pQCT, as these currently do not exist. Similarly, independent validation studies that identify the 'best' accelerometer cut-points to represent MVPA in different age groups and the relation of these PA intensities to impact forces on the skeleton would greatly advance our field.

Chapter 5: Effect of the Health Promoting Secondary School (HPSS) Intervention on Bone Strength, Structure and Density in Adolescent Boys and Girls.

Synopsis: School-based PA intervention studies are abundant in the literature, yet few address the effects of PA on bone strength in older adolescents. This chapter examines the effectiveness of a school-based, theory-driven PA intervention on optimizing bone strength in adolescents.

5.1 Introduction

Physical activity influences many health-related outcomes including bone strength in children (8). From well-designed intervention studies, pre and peri-pubertal children who performed weight-bearing PA gained greater bone strength, structure and density compared with controls (32,34,358). A review of young athletes also presented similar positive effects of PA on bone strength (463). Furthermore, these skeletal benefits conferred during childhood from PA and sports participation were also related to better bone health in older adults (464). This level two evidence (i.e. RCTs and prospective studies (429)) is one indicator that bone gains during youth may last a lifetime. However, less is known about the influence of PA on bone strength in adolescents (14 to 17 years). This is likely due to the challenge of unravelling the complexities of bone development intermingled with sex-specific stages of maturation or pubertal growth. Yet, it is critical to understand the relationship between PA and bone strength in this age group as bone structure and/or density may continue to adapt during this active period of growth and development before the skeleton fully matures. Unfortunately, PA levels in adolescents decline substantially from childhood through adolescence (323,465). A lack of mechanical stress during this period of bone development may prove to be detrimental to bone health in the short and longer term.

DXA-based studies provided insight regarding bone mineral accrual in adolescents (125,139), However, its limitation as a planar instrument and its inability to adequately represent a 3-D skeletal structure, are well known (123). Peripheral QCT as a 3-D imaging modality aptly assesses bone structure and differentiates between bone compartments (e.g., trabecular and cortical area) and vBMD (137). During adolescence, bone size and mass increase and these bone parameters contribute to bone strength. Outcomes from pQCT are used to estimate bone compressive strength using the BSI, which takes into account the Tt.Ar of bone multiplied by the squared value of Tt.Dn (153). Therefore, increased bone strength can be attributed to changes in bone structure and density. However, the influence of PA on bone strength at different times across the developmental continuum needs further investigations. A better understanding of how PA affects bone strength seems imperative, as bone strength constitutes the primary risk for fracture at any age. In particular, studies of adolescence that used pQCT or other 3-D bone imaging tools are extremely limited.

Sedentary behaviour has received much less attention than high intensity PA for its effect on bone. However, withdrawal of osteogenic stimuli (with physical inactivity) may well be detrimental to bone health. The NHANES found that self-reported total screen time was negatively associated with femoral neck BMC in 671 boys and 677 girls (age 8 to 22 years) (329). The negative association was maintained **regardless of the amount of MVPA** conducted (by accelerometry) (329). Importantly, more VPA attenuated the negative influence of screen time (329). This is similar to my findings (section 4.1 onwards), which demonstrated that SED (by accelerometry) in girls attenuated the positive influence of MVPA on distal tibia bone strength and density. VPA positively influenced distal tibia bone strength after accounting for SED but the influence on distal tibia bone density was not maintained. However, results from other studies are mixed. A different study of the distal tibia in 9 to 20 year old boys and girls using HR-pQCT by our research group found no associations between SED, bone strength and

microarchitecture (331). The influence of SED on bone strength in adolescents clearly warrants further investigation.

Physically active adolescents benefit from more lean mass and less body fat mass and lean mass is highly and positively associated with bone strength (437,466,467). Indeed, the positive associations between muscle and bone are irrefutable. The muscle-bone unit and its functional relationship was eloquently presented in the 'Utah Paradigm', whereby Frost proposed two plausible explanations of muscle and bone interaction based on available evidence (45). Further, muscle is influenced by the hormonal environment at puberty (291). This adds another layer of complexity to understanding the link between PA and bone adaptations. Several investigators assessed the response of bone strength to PA in intervention studies (32,34,400). However, some did not control for muscle in this growing population (33,358,359). Thus, it is unclear the extent to which bone augmentation was influenced by increased muscle mass due to growth independent of increased PA levels. The amount of muscle alone without functional use through PA did not confer greater bone strength and structure in animal studies (282,283).

Schools are an ideal setting to deliver a PA intervention in youth for a number of reasons (362). Among them; 1) schools are able to reach students from diverse ethnic and social backgrounds that reflects the composition of a general population, 2) adolescents spend about half of their waking time in school with instructional and focused learning (346), 3) as structured institutions, schools provide the opportunity, human resources and infrastructure to carry out a targeted intervention program, and 4) an intervention program incorporated into the school setting provides the opportunity to continue the program should the intervention prove successful (348).

For the most part, intervention studies that evaluated the effect of PA on bone strength using 3-D bone imaging modalities were conducted in elementary and middle schools (360). Studies based in

secondary schools are sorely lacking and there are very few intervention studies that aimed to increase PA in adolescents. A recent systematic review of RCTs across age groups focussed on PA as the main outcome (38). Only one of five studies reported results for elementary and secondary school students. All others focused on PA interventions in elementary schools (38).

This focus on elementary school children is ironic given that older adolescents are less physically active compared with younger children. For example, Janz and colleagues recently published a longitudinal study of PA (by accelerometry) in children aged 5 to 17 years. Children were classified as active, moderately active and persistently inactive during early childhood, age 5 years. By age 17 years PA declined by 50-65% in girls and 26-54% in boys (455). More importantly, by age 15 years, less than 6% of girls accrued more than 60 min/day of MVPA. Investigators concluded that physical inactivity in girls was endemic (455). The Canadian Health Measures Survey reported that fewer than 6% of boys and 2% of girls age 15 to 19 years performed at least 60 min/day or more of MVPA, six days per week (323). These sobering statistics speak to the need for effective PA interventions in adolescents if we are to stem the decline in PA levels and the postulated negative consequence of inactivity on bone health (468).

My study aims to fill current gaps in the literature and focuses upon whether the Health Promoting Secondary Schools (HPSS) intervention positively affects bone strength, structure and mass through changes in PA levels in adolescent boys and girls. My study is novel and among the first to examine the effectiveness of a choice-based PA intervention on tibial *and* radius bone strength, structure and density (by pQCT) in older adolescents.

Objective 1. To examine whether a choice-based PA intervention (HPSS) is an effective means to increase bone strength (primary outcome) at the tibia and radius in adolescent boys and girls.

Hypothesis 1: Adolescent boys and girls who participate in HPSS (intervention) will demonstrate greater gains in bone strength at the tibia and radius compared with adolescents of the same age and sex who do not participate in HPSS (controls).

Objective 2. To examine whether a choice-based PA intervention (HPSS) is an effective means to enhance bone structure and density (secondary outcomes) at the tibia and radius in adolescent boys and girls.

Hypothesis 2. Adolescent boys and girls who participate in HPSS (intervention) will demonstrate positive changes in bone structure and density at the tibia and radius compared with adolescents of the same age and sex who do not participate in HPSS (controls).

5.2 Methods

I provide a detailed description of the HPSS intervention, methods for school and participant recruitment and data collection procedures in Chapter 2 (section 2.3.3). I briefly summarize my methods below.

I conducted a cluster-randomized controlled study of schools where individuals (students) were the outcome of interest. The Central HPSS Team sent invitations to schools that offered alternative PE programs after Grade 10 (n=48) to participate in the study, through their school. From 48 eligible secondary schools in Greater Vancouver and the southern school district on Vancouver Island, 10 schools agreed to participate in the HPSS study. Prior to randomization, schools were matched based on geographic location and socio-economic standings (i.e. annual household income, number of minority students). Schools were randomized through a random draw to intervention (INT; participation in HPSS)

or control (CON; did not participate in HPSS) groups. Then, we invited all 10 schools to participate in the bone health component. Of these, nine schools (four INT, five CON) agreed to participate in BHS. The school that did not agree to participate in HPSS BHS declined due to the initial tools used to assess maturity levels, which were pictorial depictions of genital areas of boys and girls. In all participating districts, we received School Board ethics to conduct our study.

Grade 10 students in all nine schools were invited to participate. Of the 1914 eligible students from the nine schools, 210 volunteered to participate in the study. Of that group 201 individuals (90 boys, 111 girls) met my inclusion criteria of being healthy with no known medical conditions or disabilities. My inclusion and exclusion criteria are outlined in detail elsewhere (section 4.2.1). I received informed consent (for those older than 16 years) or parental consent and participant assent (for those younger than 16 years). Ethnicity was classified by participants' self-report based on classifications used for the Canadian Statistics Survey.

Baseline measurements (T1) were conducted between September and November, 2011. HPSS was implemented from September 2011 to June 2012. Follow-up measurements (T2) were performed between April and June, 2012. The average follow-up time was 30.9±2.6 (range: 16.7 to 34) weeks for INT and 29.0±2.4 (range: 27.1 to 34.3) weeks for CON schools. Length of follow-up was significantly longer (+13 days, 95% CI: 7.8, 18.1) for INT compared with CON schools to allow for maximal intervention exposure.

5.2.1 HPSS Intervention

The Central HPSS Team designed the HPSS study to build upon self-determination theory (SDT) ((373). HPSS was a whole-school model that targeted Grade 10 students in their last year of mandatory PE

classes. The HPSS model applied a 'For Youth By Youth' strategy whereby youth were empowered and encouraged to be agents of change by increasing autonomy, relatedness and competency to adopt a more active lifestyle (469). By applying a choice-based program in a whole-school setting, HPSS addressed the key intra- and inter-personal relationships that students face in being more physically active in school (353).

Briefly, HPSS adopted a whole-school concept and integrated changes within the curriculum, provided opportunities for student engagement and resource building across four Action Zones; 1) increased PA levels, 2) increased fruit and vegetable consumption, 3) decreased screentime and 4) decreased intake of sugar-sweetened beverages. HPSS targeted students in Planning 10 and PE 10 – both are compulsory classes in the Grade 10 curriculum (469). Teachers of Planning 10 and PE 10 were provided lesson plans, handouts, tracking tools and information related to each Action Zone. Within the PE 10 classroom resource guide, a section on skills, knowledge and leadership contained three (of 32) sections on jumping and skipping activities that benefit bone health. Teachers were required to select one of 32 options for every PE class, conducted twice a week in schools. The Planning 10 resource guide provided information regarding the role of PA in optimizing bone health (adapted from Public Health Agency Canada, www.publichealth.gc.ca).

As per SDT, adolescents were engaged and given the opportunity to design and deliver at least two school-wide events and to influence school policy by forming Action Teams (50% students and 50% teachers). HPSS provided financial resources, about CAD$4,000, to each school, to run school-wide events, workshops for teachers on how to use HPSS materials and website support for students inside and outside the classroom. As HPSS was a choice-based intervention, bone health enhancing activities were optional. I provide additional details of the HPSS intervention and the theoretical framework in section 2.2.1.

The HPSS school liaison conducted focus groups (in Feb and May 2012) to determine what activities Action Teams (comprised of teachers and students) selected. The HPSS school liaison reviewed Action Team monthly meeting minutes and year-end reports as well as teachers' logs to determine the type of activities being conducted. Control schools conducted their usual activities without any monitoring or reporting of school activities.

5.2.2 Descriptive variables

The same research personnel who collected baseline data conducted follow-up measurements using the same protocols. We assessed the same outcome variables at each time point – height, body mass, ulnar and tibial length, body composition, maturity, grip strength, dietary calcium intake, health history and PA (by PAQ-A and accelerometer). I assessed height (stretch stature), weight and limb lengths using standard anthropometry protocols. For body composition, I used DXA to measure total body lean mass (LM, kg) and fat mass (FM, kg) at baseline and follow up. I collected all DXA scans at baseline and at follow-up and implemented the same body positioning to avoid measurement errors. I also obtained muscle cross-sectional area (MCSA, cm^2) at the 50% tibia site and 30% tibia site using pQCT that is explained in detail in section 0.

To assess boys' maturity status, I estimated years from age at APHV based on a maturity offset equation recalibrated by our research group using data from two longitudinal pediatric studies (382). For girls, I used self-reported age at menarche (years) as a maturity indicator. Grip strength was assessed using a handgrip dynamometer, with alternate testing between dominant and non-dominant arms (section 2.3.5.6).

I used questionnaires to assess dietary calcium intake, health history and PA. I averaged participants' dietary calcium intake (ICC=0.61, p<0.01) and PA scores (ICC=0.53, p<0.01) from baseline and follow-up to provide a better assessment. However, I also collected objective measures of PA using accelerometry at baseline and follow-up.

5.2.3 Bone outcomes

For bone measures, the same technician acquired all pQCT scans of the distal and shaft sites of the radius (7% and 30% sites, respectively) and tibia (8% and 50% sites, respectively) at baseline and follow-up (XCT-3000, Stratec Medizintechnik, Pforzheim, Germany). I analysed all pQCT scans using the manufacturer's software (Version 6.0). I provide details of the image acquisition and analyses protocols in section 1.2.3.2. Bone outcomes that I obtained at the distal bone sites were bone strength index (BSI, mg^2/mm^4), total bone area (Tt.Ar, mm^2) and total bone density (Tt.Dn, mg/cm^3). Bone outcomes that I obtained at the shaft sites were polar strength strain index (SSI_p, mm^3), total bone area (Tt.Ar, mm^2), cortical area (Ct.Ar, mm^2), medullary area (Me.Ar, mm^2) and cortical density (Ct.Dn, mg/cm^3).

With bone strength as the primary outcome of this study, a 3% change in bone strength (BSI) with a standard deviation of 4% was considered significant from the Action Schools! BC intervention study (32). To achieve a power of 80% with a type 1 error at 5% (two-sided), a minimum of 28 participants per group were required for the study.

5.2.4 Statistical analysis

I used Stata, Version 10.1 (StataCorp, College Station, Texas) for all analyses. I considered results significant at the $p<0.05$ level. I stratified all analyses by sex due to known differences in bone accrual and in the tempo and timing of maturation, between boys and girls (208,425). Prior to conducting my primary analyses, I visually inspected data for normality using histograms and scatterplots of all continuous variables (age (y), height (cm), body mass (kg), limb lengths (cm), LM (kg) and FM (kg), PA outcomes (MVPA min/wk and PA score), non-dominant arm grip strength (kg), dietary calcium intake (mg/day), muscle cross-sectional area (MCSA; cm^2) and bone outcomes (at the radius and tibia)) at T1 and T2. I calculated absolute change for each outcome (T2-T1) and plotted these values. I generated means and standard deviations (SD) for all continuous variables and frequencies for categorical variables (i.e. ethnicity, axillary hair stage) to examine their distribution and change, for INT and CON groups. To determine differences between INT and CON groups at baseline, I conducted two-tailed Student's t-test (equal variance) or Welch's t-test (unequal variance) for continuous variables and used Pearson's chi-square test for categorical variables. To determine the association between descriptive variables and bone outcomes, I examined pair-wise associations between change in each bone outcome with *baseline and change* for descriptive variables (Appendix M: Additional Data for Chapter 5). I conducted Spearman's rank correlations to assess the relation between bone changes and baseline categorical descriptive variables. For the subset of study participants who had valid accelerometer data, I compared their baseline values with participants who *did not* have valid accelerometer data using two-tailed Student's t-test; I did not account for clustering.

To address Objectives 1 and 2, I fit multivariable linear regression models. Change in each bone outcome was the dependent variable and group (INT or CON) was the independent variable. For each model, I adjusted for covariates known to have biological and/or biomechanical influence on bone

outcomes and those variables significantly associated with dependent variables. I included baseline bone values as covariates to account for changes that might be influenced by (high or low) baseline values. For boys' tibia and radius, covariates were baseline bone value, change in tibial or ulnar length, years post APHV and change in tibia or radius MCSA. For girls' tibia, covariates were baseline bone values, change in tibial length, age at menarche and baseline tibia MCSA. For girls' radius, covariates were baseline bone value, change in ulnar length, age at menarche, baseline FM and radius MCSA. I used residual plots to check assumptions of normality (kernel density plots, probability plots and quintile plots), linearity (augmented partial residual plots) and homoscedasticity (residual-versus-fitted values plots) of the regression models. I conducted variance inflation tests (VIF) to check for multicollinearity (non-collinear when values of VIF>10) and removed influential points using Cook's distance values defined as having a value above 4/N, where N refers to the number of observations (440). I removed influential points by running a sensitivity analysis whereby regression outcomes with all participants were compared with regression outcomes that excluded the highest Cook's distance values, one at a time. The influential point was omitted if conclusions of regression models were different after exclusion (470).

To account for the clustered study design, I multiplied the standard error of the estimated intervention effect by the square root of the design effect (D) (471). I calculated D as follows (471),

$$D = 1 + (m' - 1)*ICC$$

where m' is the adjusted mean cluster size when cluster sizes are not equal. In the present analysis the cluster size ranged from 11 to 45 at baseline. I calculated the adjusted mean cluster size, m', as,

$$m' = 1/(k-1)*[n - (\Sigma_k m_k^2/n)]$$

where k = number of clusters (nine schools – four INT, five CON), m = size of k-cluster (range: 10 – 45 per cluster) and n = number of participants (baseline, n=201). As there were no published ICC values for radius or tibia bone outcomes by pQCT in older adolescents, I obtained baseline ICC values for all radius and tibia outcomes by sex using the standard one-way analysis of variance (ANOVA) with the *loneway* command in Stata, where ICC provides the total variance explained by the cluster design by taking into consideration within-school, s_w, and between-school, s_b, variance for each bone outcome.

$$ICC = s_b^2 / (s_b^2 + s_w^2)$$

5.3 Results

All INT and CON schools at baseline completed the study. The average study duration was 30 weeks.

5.3.1 Participants

At follow-up, 91% of participants (n=184) assessed at baseline returned for measurement. Participants with baseline measurements who were lost at follow-up (n=17); 1) changed schools (6 boys, 2 girls), 2) were absent on measurement days, e.g., sick (4 boys, 1 girl) or 3) or voluntarily withdrew without providing reasons (4 boys). Additionally, I excluded one girl (INT, white) who reported use of medroxyprogesterone acetate (i.e. Depo-Provera/Provera) injections at follow-up. Eight participants (four boys, four girls) did not have DXA scans at baseline due to concerns about radiation exposure. I excluded them from DXA analyses. I provide the flow of participants through the study in Figure 5.1 as per the CONSORT guidelines for cluster randomized controlled trials (472).

I present the flowchart for accelerometer wear by INT and CON boys and girls in Figure 5.2. At baseline, 78% of INT and 65% CON participants who wore accelerometers completed at least 3 valid days, where a valid day was defined as having a minimum wear time of 10 hours. At follow-up, 77% of INT and 62% of CON participants who wore accelerometers had achieved the valid accelerometry criteria.

A total of 183 participants had bone scans at follow-up. Those that were excluded are noted in Figure 5.1. Overall, at the *tibia,* five participants had scans at one of the two sites at baseline (two had a distal scan only; three had a shaft scan only). I excluded two scans due to image acquisition errors (one distal, one shaft). At the *radius,* two participants did not have baseline scans (distal site). I also excluded ten images due to the presence of motion artifacts (four distal, six shaft) and five images (two distal, three shaft) due to image acquisition errors.

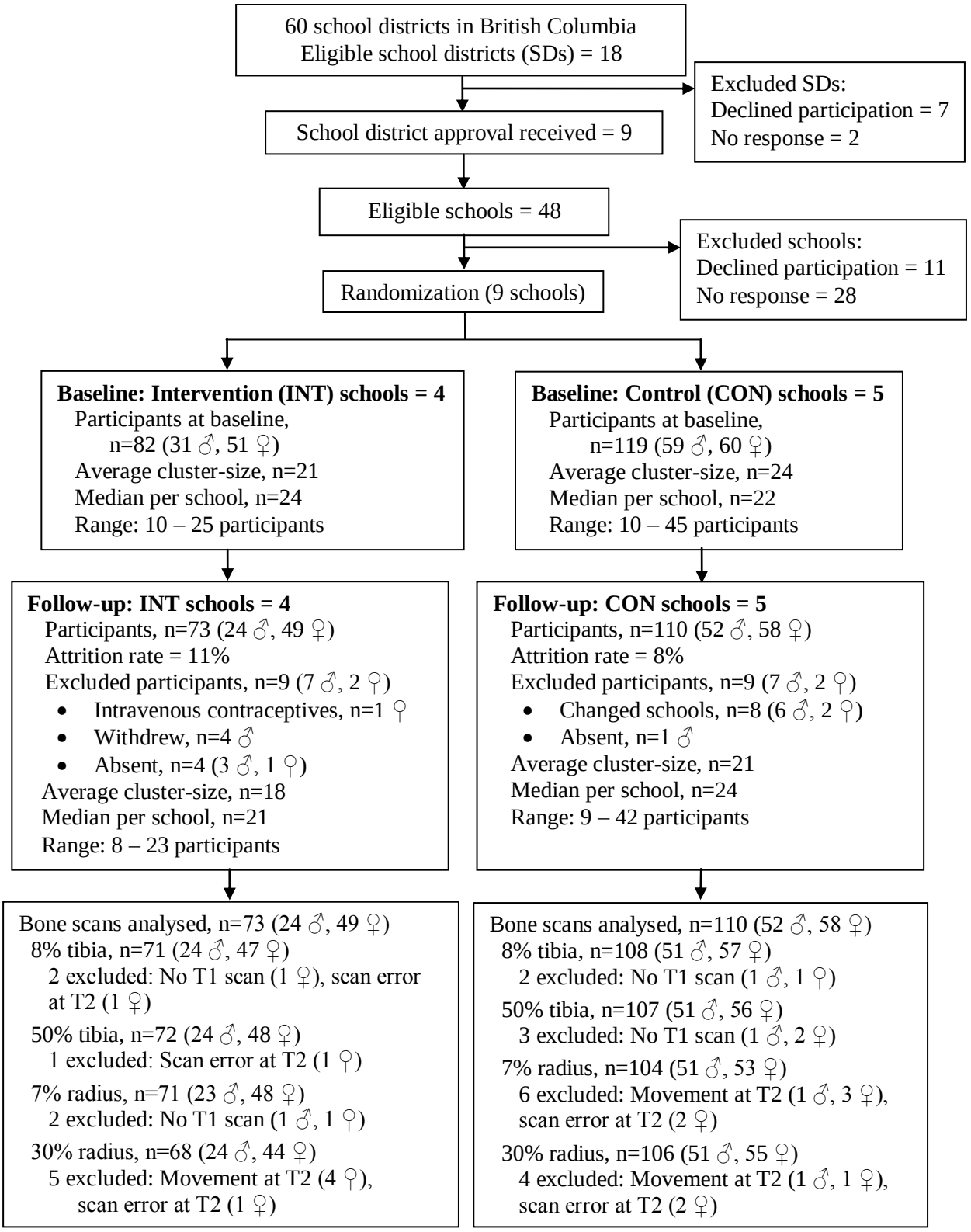


Figure 5.1. Flow diagram of school and participant recruitment based on CONSORT guidelines for clustered randomized trials.

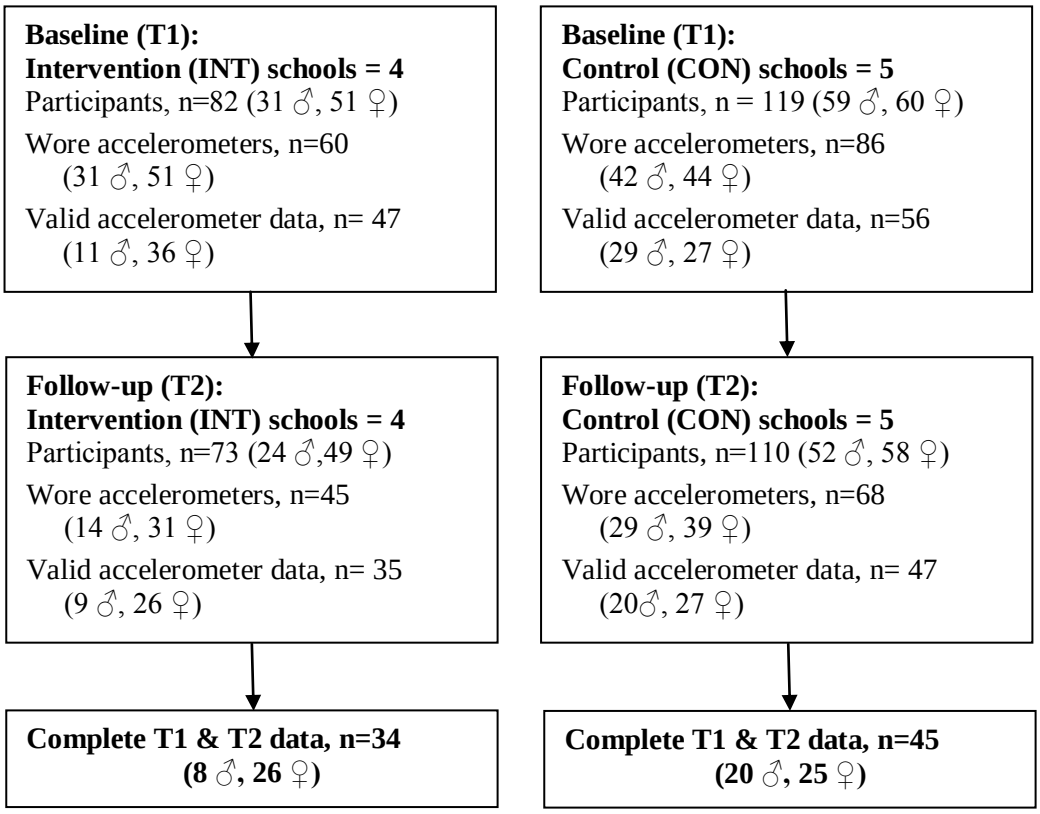


Figure 5.2. Flowchart of accelerometer wear and valid accelerometry based on a minimum three 10-hour days criterion.

5.3.2 Descriptive variables

At baseline, INT and CON *boys* (Table 5.1) were of similar age, height, body mass, limb length, LM, FM, grip strength, MCSA and ethnic distribution. At follow-up, INT and CON boys demonstrated similar increases in height, body mass, tibia length, LM, grip strength and MCSA (Table 5.1). Average dietary calcium intake was similar between INT and CON boys. FM for INT boys increased by 7% while CON boys had no significant changes in FM, although the difference in change between INT and CON boys was not significant. Ulnar length was significantly greater (+6%) at the end of intervention for INT boys compared with CON boys

There was also no difference in PA levels (by PAQ-A; averaged PA score and estimated MVPA) between INT and CON boys. For accelerometry results, there were very few (8 INT and 20 CON) boys with valid accelerometer data at T1 and T2 to provide an accurate representation of the study sample. However, descriptive variables were similar between the accelerometer group and those without accelerometer data (Appendix M: Additional Data for Chapter 5).

At baseline, INT and CON *girls* were of comparable age, age at menarche, maturity offset, height, body mass, limb lengths, LM, FM, grip strength, tibia and radius MCSA and ethnic distribution (Table 5.2). At follow-up, INT and CON girls experienced similar significant increases in height, body mass, limb lengths, LM, FM, grip strength and tibia MCSA. Three girls (2 INT, 1 CON) who were pre-menarcheal at baseline reached menarche during follow-up, but this did not influence the average age at menarche in either group. Average dietary calcium intake was similar between INT and CON girls.

There were also no differences in PA levels (by PAQ-A) between INT and CON girls. In the subset of girls with valid accelerometer data (26 INT, 25 CON), overall accelerometer counts, MVPA and MPA decreased by 18-23% in INT girls, but did not change significantly in CON girls (Table 5.3).

Table 5.1 Boys' baseline and follow-up mean (SD) values for age, maturity, anthropometry, body composition, muscle cross-sectional area (MCSA) with within-group absolute change (follow-up - baseline) with 95% confidence intervals (CI) and averaged (baseline and follow-up) dietary calcium and Physical Activity Questionnaire for Adolescents (PAQ-A) outcomes in Intervention and Control groups.

	Intervention (n= 24)			**Control (n= 52)**		
	Baseline	**Follow-up**	**Change (95% CI)**	**Baseline**	**Follow-up**	**Change (95% CI)**
Age (yrs)	15.3 (0.4)	15.9 (0.3)	**0.58 [0.56, 0.60]**	15.3 (0.3)	15.9 (0.3)	**0.54 [0.53, 0.55]**
White/Asian/Other	13/10/1 (%: 54/42/4)		-	28/18/6 (%: 54/35/12)		-
Years post APHV	2.0 (0.5)	2.5 (0.5)	-	1.9 (0.6)	2.3 (0.8)	-
Height (cm)	174.8 (6.0)	177.1 (6.1)	**2.3 [1.5, 3.0]**	173.4 (6.8)	175.0 (6.7)	**1.6 [1.3, 1.9]**
Body mass (kg)	64.3 (11.4)	67.5 (12.1)	**3.3 [2.2, 4.4]**	62.6 (11.7)	65.5 (12.1)	**2.9 [2.1, 3.7]**
Tibial length (cm)	41.1 (1.8)	41.4 (2.0)	**0.3 [0.2, 0.5]**	40.2 (2.3)	40.4 (2.4)	**0.3 [0.2, 0.4]**
Ulnar length (cm)	28.1 (1.3)	28.6 (1.2)	**0.5 [0.3, 0.6]**	27.6 (1.4)	27.8 (1.4)	**0.3 [0.2, 0.4]**
DXA scans		*n=22*			*n=50*	
Lean mass (kg)	46.3 (7.8)	47.8 (8.8)	**1.6 [0.7, 2.5]**	44.9 (7.1)	46.5 (7.5)	**1.6 [1.1, 2.0]**
Fat mass (kg)	9.6 (5.3)	10.3 (5.7)	**0.7 [0.2, 1.3]**	9.5 (5.7)	9.9 (6.0)	0.4 [-0.2, 0.9]
Grip strength (kg)	36.6 (8.7)	40.6 (8.0)	**4.0 [2.5, 5.6]**	34.0 (6.8)	37.0 (7.7)	**3.0 [2.0, 4.1]**
Tibia MCSA (cm^2)†	4191.3 (858.9)	4436.7 (891.0)	**245.5 [192.1, 298.8]**	4179.7 (728.3)	4378.2 (787.7)	**198.5 [150.1, 246.8]**
Radius MCSA (cm^2)†	1720.9 (276.4)	1784.0 (282.5)	**63.1 [25.2, 101.0]**	1666.6 (268.5)	1699.9 (274.9)	**32.8 [14.6, 51.0]**
Dietary calcium (mg/day)	1392 (751)		-	1230 (600)		-
PAQ-A score	2.6 (0.5)		-	2.6 (0.4)		-
PAQ-A MVPA (min/day)	120.3 (74.5)		-	100.3 (57.4)		-
Accelerometer		*n=8*			*n=20*	
Valid counts (counts/min)	409.6 (93.1)	326.0 (101.0)	-83.5 [-212.8, 45.7]	462.7 (153.0)	438.4 (164.2)	-24.2 [-99.4, 50.9]
Total wear time (min/day)	843.4 (34.4)	859.8 (56.2)	16.4 [-36.0, 68.8]	838.3 (58.7)	838.8 (68.5)	0.5 [-37.3, 38.4]
Sedentary (min/day)	595.3 (43.6)	612.8 (56.4)	17.6 [-36.5, 71.6]	589.6 (71.2)	595.4 (99.1)	5.8 [-36.4, 47.8]
MVPA (min/day)	49.3 (13.4)	36.2 (13.7)	-13.1 [-30.1, 3.9]	57.5 (20.1)	54.7 (22.0)	-2.8 [-12.6 ,7.1]
Moderate PA (min/day)	27.9 (7.8)	25.5 (8.4)	-2.4 [-12.0, 7.2]	33.2 (10.4)	31.2 (10.9)	-2.0 [-6.9, 3.0]
Vigorous PA (min/day)	21.4 (7.0)	10.7 (6.4)	**-10.7 [-18.9, -2.5]**	24.3 (11.8)	23.5 (13.6)	-0.8 [-7.5, 5.9]

APHV, age at peak height velocity; PA, physical activity; MVPA, moderate-to-vigorous physical activity.

Bold values highlight significant change ($p<0.05$) from baseline for Intervention and Control groups.

†Tibial MCSA (Intervention=24; Controls=51) and radius MCSA (Intervention=24; Controls=52) measures.

Table 5.2. Girls' baseline and follow-up mean (SD) values for age, age at menarche, anthropometry, body composition, muscle cross-sectional area (MCSA) with within-group absolute change (follow-up - baseline) with 95% confidence intervals (CI) and averaged (baseline and follow-up) dietary calcium and Physical Activity Questionnaire for Adolescents (PAQ-A) outcomes in Intervention and Control groups.

	Intervention (n=49)			**Control (n=58)**		
	Baseline	**Follow-up**	**Change (95% CI)**	**Baseline**	**Follow-up**	**Change (95% CI)**
Age (yrs)	15.3 (0.5)	15.9 (0.3)	**0.60 [0.58, 0.61]**	15.4 (0.5)	15.9 (0.4)	**0.57 [0.55, 0.58]**
White/Asian/Other	24/18/7 (%: 49/37/14)		-	33/21/4 (%: 57/36/7)		-
Age at menarche (yrs)‡	12.5 (1.0)	12.7 (1.2)	-	12.2 (1.2)	12.3 (1.3)	-
Years post APHV	3.1 (0.6)	3.5 (0.6)		3.2 (0.5)	3.6 (0.5)	
Height (cm)	162.1 (6.8)	162.4 (6.8)	**0.3 [0.1, 0.4]**	163.4 (6.0)	163.8 (6.2)	**0.4 [0.3, 0.6]**
Body mass (kg)	58.9 (10.5)	60.4 (10.7)	**1.5 [0.9, 2.0]**	58.4 (10.1)	59.9 (10.6)	**1.5 [0.9, 2.1]**
Tibial length (cm)	36.9 (2.2)	37.0 (2.2)	**0.1 [0.05, 0.2]**	37.1 (1.9)	37.2 (2.0)	**0.1 [0.07, 0.2]**
Ulnar length (cm)	25.4 (1.4)	25.5 (1.3)	**0.1 [0.04, 0.2]**	25.4 (1.2)	25.5 (1.1)	**0.07 [0.03, 0.1]**
DXA scans		*n=47*			*n=56*	
Lean mass (kg)	36.3 (4.9)	36.7 (5.1)	**0.4 [0.1, 0.7]**	35.9 (4.7)	36.3 (4.9)	**0.4 [0.1, 0.7]**
Fat mass (kg)	15.6 (6.9)	16.1 (7.0)	**0.4 [0.1, 0.7]**	15.7 (5.8)	16.6 (6.3)	**0.9 [0.5, 1.3]**
Grip strength (kg)	26.3 (3.7)	27.5 (4.4)	**1.2 [0.3, 2.1]**	26.6 (5.6)	28.0 (5.7)	**1.4 [0.8, 2.0]**
Tibia MCSA (cm^2)†	3773.4 (93.7)	3891.0 (692.4)	**117.6 [78.1, 157.1]**	3802.2 (562.8)	3896.8 (617.5)	**94.6 [55.3, 133.9]**
Radius MCSA (cm^2)†	1244.6 (177.5)	1256.8 (176.9)	12.2 [-0.9, 25.3]	1255.8 (207.6)	1247.9 (222.2)	-7.9 [-21.9, 6.0]
Dietary calcium (mg/day)	851 (483)		-	914 (471)		-
PAQ-A score	2.5 (0.5)		-	2.5 (0.5)		-
PAQ-A MVPA (min/day)	75.8 (44.3)		-	74.2 (47.8)		-
Accelerometer		*n=26*			*n=25*	
Valid counts (counts/min)	410.2 (169.0)*	317.9 (118.6)	**-92.3 [-137.5, -47.1]**	350.5 (124.7)	334.1 (139.4)	-16.4 [-56.5, 23.7]
Total wear time (min/day)	835.3 (41.8)	833.8 (57.2)	-1.4 [-28.4, 25.6]	826.9 (58.4)	836.5 (61.0)	9.6 [-20.7, 39.9]
Sedentary (min/day)	602.2 (75.3)	631.1 (65.3)	28.9 [-3.7, 61.6]	612.9 (66.4)	639.5 (71.2)	26.6 [-7.1, 60.2]
MVPA (min/day)	49.2 (23.7)*	36.3 (17.8)	**-12.9 [-19.2, -6.6]**	39.7 (17.4)	37.9 (17.7)	-1.8 [-6.5, 3.0]
Moderate PA (min/day)	32.1 (13.4)*	24.4 (11.4)	**-7.7 [-11.1, -4.4]**	24.4 (8.6)	24.0 (10.5)	-0.3 [-3.4, 2.8]
Vigorous PA (min/day)	17.1 (13.8)	11.9 (10.7)	**-5.2 [-9.4, -1.0]**	15.3 (11.2)	13.9 (9.8)	-1.5 [-4.3, 1.4]

**Significant differences (p<0.05) at baseline, not analyzed by clusters, between Intervention and Control girls. APHV, age at peak height velocity; PA, physical activity; MVPA, moderate-to-vigorous physical activity.*

Bold values highlight significant change (p<0.05) from baseline for Intervention and Control groups.

‡Premenarcheal at baseline: Intervention=3, Control=3. Premenarcheal at follow-up: Intervention=1, Control=2.

†Tibial MCSA (Intervention=48; Control=56) and radius MCSA (Intervention=48; Control=55) measures.

Table 5.3. Differences in unadjusted 30-week change between groups (Intervention - Control) with 95% confidence intervals (CI) and p-value for age, anthropometry, grip strength, body composition, and physical activity by accelerometer outcomes in boys and girls.

	Boys (INT=24, CON=52)		**Girls (INT=49, CON=58)**	
	Difference in change (95% CI)	**p value**	**Difference in change (95% CI)**	**p value**
Age (yrs)	**0.04 [0.02, 0.06]**	**<0.001**	**0.03 [0.01, 0.05]**	**0.004**
Height (cm)	0.66 [-0.1, 1.5]	0.103	-0.17 [-0.37, 0.04]	0.106
Body mass (kg)	0.4 [-1.0, 1.7)]	0.594	-0.03 [-0.84, 0.78]	0.941
Tibial length (cm)	0.7 [-1.0, 2.5]	0.406	-0.14 [-1.1, 0.8]	0.763
Ulnar length (cm)	**1.8 [0.2, 3.5]**	**0.033**	3.5 [-2.8, 9.8]	0.266
DXA scans	***INT=22, CON=50***		***INT=47, CON=56***	
Lean mass (kg)	0.02 [-0.86, 0.91]	0.957	-0.03 [-0.42, 0.36]	0.876
Fat mass (kg)	0.35 [-0.39, 1.1]	0.345	-0.44 [-0.96, 0.07]	0.092
Grip strength (kg)	1.0 [-0.8, 2.8]	0.281	-0.23 [-1.3, 0.9]	0.676
Tibia MCSA (cm^2)†	47.0 [-31.5, 125.4]	0.237	23.0 [-32.3, 78.4]	0.411
Radius MCSA (cm^2)†	30.3 [-6.1, 66.6]	0.101	14.0 [-5.1, 33.0]	0.149
Accelerometer	***INT=8, CON=20***		***INT=26, CON=25***	
Valid counts (counts/day)	-59.3 [-195.9, 77.4]	0.381	**-75.9 [-134.9, -16.9]**	**0.013**
Total wear time (min/day)	15.9 [-49.8, 81.6]	0.624	-11.0 [-50.6, 28.5]	0.577
Sedentary (min/day)	11.8 [-60.4, 84.0]	0.740	2.4 [-43.3, 48.1]	0.917
MVPA (min/day)	-10.4 [-28.3, 7.6]	0.246	**-11.1 [-18.8, -3.7]**	**0.006**
Moderate PA (min/day)	-0.4 [-9.8, 8.9]	0.926	**-7.4 [-11.8, -2.9]**	**0.002**
Vigorous PA (min/day)	-9.9 [-21.3, 1.5]	0.085	-3.7 [-8.7, 1.3]	0.139

INT, intervention; CON, controls; MCSA, muscle cross-sectional area; PAQ-A, physical activity questionnaire for adolescents; PA, physical activity; MVPA, moderate-to-vigorous physical activity.

Bold text highlights significant differences in change ($p<0.05$) between Intervention and Control groups.

†Tibia MCSA (Boys INT=24, CON=51; Girls INT=48, CON=56) and radius MCSA (Boys INT=24, CON=52; Girls INT=48, CON=55).

5.3.3 Primary outcomes

I present bone strength by sex for tibia and radius sites and include bone structure and density outcomes. There were no differences in the sensitivity analyses conducted on statistical models with and without influential data points (one to two only in for each model) on bone strength outcomes for boys and girls. Therefore, I left the influential data points in the statistical models.

5.3.3.1 Boys

At the distal and shaft sites of the *tibia*, bone strength (BSI, SSI_p) was similar between INT and CON boys at baseline (Table 5.4). After 30 weeks, both INT and CON boys demonstrated comparable gains in BSI (INT +8%, CON +7%) and SSI_p (INT +5%, CON +3%) (Table 5.5). A similar picture emerged at the *radius*, as bone strength at the distal and shaft sites was not significantly different between INT and CON boys at baseline (Table 5.4) and gains in BSI (INT +12%, CON +14%) and SSI_p (INT +6%, CON +4%) were not significantly different between groups (Table 5.5).

5.3.3.2 Girls

At the distal and shaft sites of the *tibia*, bone strength (BSI, SSI_p) was similar between INT and CON girls at baseline (Table 5.6). After 30 weeks, both INT and CON girls had significant increases in BSI (INT +4%, CON +4%) and SSI_p (INT +2%, CON +1%) (Table 5.6) and changes were similar between groups. At the *radius*, bone strength at both sites was comparable between INT and CON girls at baseline (Table 5.6). At follow-up, BSI at the distal radius increased significantly in INT (+6%) and CON (5%) girls whereas SSI_p at the radial shaft increased significantly in INT girls (+2%) but not in CON girls. However, the difference in change in SSI_p between INT and CON girls was not statistically significant (Table 5.7).

Table 5.4. Boys' baseline and follow-up mean (SD) values for tibial and radius bone strength, structure and density by Intervention and Control groups and within-group change (follow-up - baseline) with 95% confidence intervals (CI).

	Intervention (n = 24)			Controls (n = 51)		
	Baseline	**Follow-up**	**Change (95% CI)**	**Baseline**	**Follow-up**	**Change (95% CI)**
8% tibia		***n = 24***			***n = 51***	
BSI (mg^2/mm^4)	9855.9 (2216.0)	10607.5 (2394.2)	**751.1 [550.5, 951.8]**	9282.4 (1974.4)	9924.6 (2005.8)	**642.3 [492.2, 792.4]**
Tt.Ar (mm^2)	770.5 (117.8)	761.7 (118.2)	-8.8 [-18.5, 0.9]	727.5 (141.4)	721.9 (138.3)	-5.6 [13.6, 2.3]
Tt.Dn (mg/cm^3)	357.8 (46.2)	373.4 (48.1)	**15.6 [11.0, 20.2]**	359.4 (47.2)	372.7 (44.1)	**13.3 [9.3, 17.3]**
50% tibia		***n = 24***			***n = 51***	
SSI_p (mm^3)	2077.1 (357.8)	2177.9 (371.5)	**100.8 [70.3, 131.3]**	1970.2 (437.2)	2033.4 (448.8)	**63.2 [44.4, 82.0]**
Tt.Ar (mm^3)	489.8 (57.6)	500.4 (59.2)	**10.6 [6.4, 14.8]**	465.2 (10.1)	473.1 (73.3)	**7.9 [5.6, 10.2]**
Ct.Ar (mm^2)	338.2 (50.7)	349.9 (52.3)	**11.7 [7.6, 15.8]**	320.6 (46.8)	328.9 (48.1)	**8.3 [6.4, 10.2]**
Me.Ar (mm^3)	151.7 (26.4)	150.5 (28.2)	-1.2 [-3.4, 1.1]	144.6 (34.7)	144.1 (34.5)	-0.4 [-1.4, 0.5]
Ct.Dn (mg/cm^3)	1060.7 (30.9)*	1076.2 (28.3)	**15.5 [11.8, 19.2]**	1077.3 (24.3)	1087.3 (24.0)	**10.1 [7.3, 12.9]**
7% radius		***n = 23***			***n = 51***	
BSI (mg^2/mm^4)	3280.3 (879.1)	3661.8 (970.1)	**381.5 [238.2, 524.8]**	2951.8 (904.1)	3367.5 (955.2)	**415.7 [338.1, 493.3]**
Tt.Ar (mm^2)	252.5 (45.4)	255.6 (46.0)	3.1 [-2.5, 8.7]	239.0 (50.9)	237.5 (51.5)	-1.5 [-4.9, 1.9]
Tt.Dn (mg/cm^3)	360.5 (49.8)	379.1 (58.8)	**18.7 [10.6, 26.7]**	350.5 (48.3)	376.8 (51.4)	**26.3 [20.6, 31.9]**
30% radius		***n = 24***			***n = 51***	
SSI_p (mm^3)	244.1 (47.6)	257.4 (46.0)	**13.3 [9.0, 17.7]**	231.2 (63.2)	240.2 (64.0)	**9.0 [6.1, 11.9]**
Tt.Ar (mm^2)	106.7 (15.6)	110.0 (15.1)	**3.2 [2.1, 4.3]**	101.9 (19.6)	104.4 (20.1)	**2.5 [1.8, 3.3]**
Ct.Ar (mm^2)	84.1 (11.9)	87.3 (11.3)	**3.2 [2.2, 4.2]**	81.3 (13.4)	83.8 (13.8)	**2.5 [1.9, 3.2]**
Me.Ar (mm^2)	22.7 (7.1)	22.7 (7.0)	0.05 [-0.35, 0.44]	20.6 (8.3)	20.6 (8.4)	0.001 [-0.30, 0.30]
Ct.Dn (mg/cm^3)	1097.7 (30.4)	1112.4 (27.7)	**14.7 [9.8, 19.6]**	1107.3 (31.2)	1117.6 (28.8)	**10.3 [6.2, 14.3]**

BSI, bone strength index; Tt.Ar, total area; Tt.Dn; total density; SSI_p, polar strength strain index; Ct.Ar, cortical area; Me.Ar, medullary area; Ct.Dn, cortical density.

Bold values highlight significant change ($p<0.05$) from baseline for Intervention and Control groups.

**Significant differences ($p<0.05$) at baseline, not analyzed by clusters, between Intervention and Control boys.*

Table 5.5. Differences in change of tibial and radius bone outcomes between groups (Intervention - Control) in boys with adjusted variance estimates for 95% confidence intervals (CI) and p-value based on the interclass correlation (ICC) values.

	Tibia†				Radius‡				
	n	**Difference in change (SE)**	**95% CI**	**p value**	**ICC**	**n**	**Difference in change (SE)**	**95% CI**	**p value**	**ICC**
			8% site					***7% site***		
BSI (mg^2/mm^4)	75	58.9 (123.8)	-185.0, 302.8	0.638	<0.001	74	-30.8 (83.5)	-210.1, 148.5	0.734	0.02
Tt.Ar (mm^2)	75	-2.82 (6.8)	-24.3, 18.7	0.802	0.16	74	2.04 (3.1)	-7.6, 11.7	0.682	0.15
Tt.Dn (mg/cm^3)	75	1.86 (3.2)	-6.1, 9.8	0.652	0.06	73	-3.36 (4.8)	-20.5, 13.7	0.696	0.23
			50% site					***30% site***		
SSI_p (mm^3)	75	25.5 (14.1)	-2.3, 53.3	0.074	<0.001	75	3.39 (2.7)	-1.9, 8.7	0.212	<0.001
Tt.Ar (mm^3)	75	0.98(1.5)	-2.0, 3.9	0.516	<0.001	75	0.12 (0.6)	-1.1, 1.3	0.842	<0.001
Ct.Ar (mm^2)	75	2.25 (1.5)	-1.2, 5.7	0.204	0.04	75	0.05 (0.6)	-1.2, 1.3	0.424	0.008
Me.Ar (mm^3)	75	-1.24 (0.8)	-3.0, 0.4	0.140	<0.001	75	0.09 (0.27)	-0.5, 0.7	0.764	0.02
Ct.Dn (mg/cm^3)	75	3.03 (2.4)	-3.6, 9.7	0.374	0.10	75	1.35 (3.2)	-5.1, 7.8	0.682	0.006

SE, standard error; BSI, bone strength index; Tt.Ar, total area; Tt.Dn; total density; SSI_p, polar strength strain index; Ct.Ar, cortical area; Me.Ar, medullary area; Ct.Dn, cortical density.

† Adjusted for baseline bone outcome, maturity offset (years), tibial length change (cm) and tibia muscle cross-sectional area change (cm^2).

‡ Adjusted for baseline bone outcome, maturity offset (years), ulnar length change (cm) and radius muscle cross-sectional area change (cm^2).

Table 5.6. Girls' baseline and follow-up mean (SD) values for tibial and radius bone strength, structure and density by Intervention and Control groups and within-group change (follow-up - baseline) with 95% confidence intervals (CI).

	Intervention			Controls		
	Baseline	**Follow-up**	**Change (95% CI)**	**Baseline**	**Follow-up**	**Change (95% CI)**
8% tibia site		***n = 47***			***n = 57***	
BSI (mg^2/mm^4)	8829.9 (2418.1)	9184.2 (2474.8)	**354.4 [264.0, 444.8]**	8459.1 (1909.3)	8784.2 (1878.6)	**325.1 [261.2, 389.1]**
Tt.Ar (mm^2)	612.3 (104.6)	609.0 (103.7)	-3.3 [-7.4, 0.9]	612.2 (73.5)	610.0 (74.4)	-2.2 [-5.8, 1.5]
Tt.Dn (mg/cm^3)	378.8 (48.2)	387.6 (48.5)	**8.8 [5.9, 11.6]**	370.8 (43.8)	378.8 (42.4)	**8.0 [5.8, 10.3]**
50% tibia site		***n = 48***			***n = 56***	
SSI_p (mm^3)	1529.6 (315.1)	1559.9 (314.7)	**30.2 [16.6, 43.9]**	1563.9 (275.5)	1582.0 (273.4)	**18.1 [8.4, 27.8]**
Tt.Ar (mm^2)	376.8 (51.3)	382.0 (52.3)	**5.2 [3.5, 7.0]**	386.6 (49.9)	388.3 (49.7)	**1.7 [0.3, 3.1]**
Ct.Ar (mm^2)	270.1 (39.9)	275.5 (41.9)	**5.3 [3.5, 7.2]**	271.1 (34.7)	273.4 (35.4)	**2.3 [1.1, 3.5]**
Me.Ar (mm^2)	106.7 (23.8)	106.5 (23.7)	-0.1 [-1.0, 0.7]	115.5 (26.7)	114.9 (26.3)	**-0.6 [-1.2, -0.1]**
Ct.Dn (mg/cm^3)	1132.3 (21.4)*	1134.9 (21.0)	2.6 [-0.3, 5.5]	1122.9 (23.0)	1130.4 (20.4)	**7.5 [5.5, 9.6]**
7% radius site		***n = 48***			***n = 53***	
BSI (mg^2/mm^4)	3405.8 (1039.8)	3605.8 (981.6)	**200.0 [131.8, 268.2]**	3355.1 (1070.1)	3529.1 (1059.3)	**174.0 [111.1, 236.9]**
Tt.Ar (mm^2)	198.4 (32.4)	199.0 (33.7)	0.6 [-2.2, 3.3]	195.1 (29.6)	197.1 (30.2)	2.1 [-0.4, 4.5]
Tt.Dn (mg/cm^3)	414.2 (76.6)	426.6 (71.1)	**12.4 [6.7, 18.1]**	412.0 (67.9)	421.3 (66.3)	**9.3 [3.9, 14.7]**
30% radius site		***n =44***			***n = 55***	
SSI_p (mm^3)	187.6 (35.9)	191.0 (35.1)	**3.4 [1.5, 5.4]**	192.2 (39.2)	192.9 (40.0)	0.8 [-1.0, 2.5]
Tt.Ar (mm^2)	85.9 (12.0)	86.2 (12.0)	0.3 [-0.2, 0.7]	87.3 (12.6)	87.4 (12.8)	0.2 [-0.2, 0.5]
Ct.Ar (mm^2)	69.8 (9.1)	70.1 (8.9)	0.36 [-0.04, 0.75]	69.4 (9.3)	69.7 (9.5)	0.22 [-0.15, 0.59]
Me.Ar (mm^2)	16.2 (5.2)	16.1 (5.1)	-0.09 [-0.26, 0.07]	17.9 (5.7)	17.8 (6.0)	-0.08 [-0.30, 0.14]
Ct.Dn (mg/cm^3)	1163.9 (19.7)	1173.3 (15.9)	**9.4 [6.0, 12.8]**	1159.5 (24.2)	1163.4 (22.0)	**3.9 [1.1, 6.7]**

BSI, bone strength index; Tt.Ar, total area; Tt.Dn; total density; SSI_p, polar strength strain index; Ct.Ar, cortical area; Me.Ar, medullary area; Ct.Dn, cortical density.

Bold values highlight significant change ($p<0.05$) from baseline for Intervention and Control groups.

*Significant differences ($p<0.05$) at baseline, not analyzed by clusters, between Intervention and Control girls.

Table 5.7. Differences in change of tibia and radius bone outcomes between groups (Intervention - Control) in girls with adjusted variance estimates for 95% confidence intervals (CI) and p-value based on the interclass correlation (ICC) values.

	Tibia†					Radius‡				
	n	**Difference in change (SE)**	**95% CI**	**p value**	**ICC**	**n**	**Difference in change (SE)**	**95% CI**	**p value**	**ICC**
			8% site					***7% site***		
BSI (mg^2/mm^4)	100	-13.1 (51.6)	-141.0, 114.8	0.842	0.05	94	-5.80 (43.7)	-110.0, 98.4	0.912	0.04
Tt.Ar (mm^2)	98	1.87 (2.4)	-4.5, 8.3	0.568	0.07	94	-0.96 (2.0)	-6.6, 4.7	0.742	0.09
Tt.Dn (mg/cm^3)	99	-1.70 (1.5)	-4.7, 1.3	0.262	<0.001	94	0.56 (3.7)	-7.5, 8.6	0.888	0.02
			50% site					***30% site***		
SSI_p (mm^3)	101	6.37 (7.3)	-14.9, 27.6	0.556	0.10	92	1.98 (1.4)	-1.9, 5.8	0.312	0.08
Tt.Ar (mm^2)	101	2.82 (1.0)	-0.17, 5.8	0.064	0.11	92	-0.06 (0.28)	-0.8, 0.7	0.880	0.08
Ct.Ar (mm^2)	99	1.66 (0.89)	-1.3, 4.6	0.276	0.16	92	-0.07 (0.29)	-0.8, 0.6	0.842	0.04
Me.Ar (mm^2)	99	0.65 (0.37)	-0.1, 1.4	0.080	0.001	92	0.02 (0.16)	-0.4, 0.5	0.928	0.07
Ct.Dn (mg/cm^3)	101	-2.83 (1.7)	-1.7, 7.4	0.218	0.07	90	**5.72 (1.9)**	**1.4, 10.1**	**0.010**	0.03

SE, standard error; BSI, bone strength index; Tt.Ar, total area; Tt.Dn; total density; SSI_p, polar strength strain index; Ct.Ar, cortical area; Me.Ar, medullary area; Ct.Dn, cortical density.

Bold text highlights significant differences in change ($p<0.05$) between Intervention and Control groups.

† Adjusted for baseline bone outcome, age of menarche (years), tibial length change (cm) and baseline tibia muscle cross-sectional area (cm^2).

‡ Adjusted for baseline bone outcome, age of menarche (years), ulnar length change (cm), baseline body fat mass (kg) and radius muscle cross-sectional area change (cm^2).

5.3.4 Secondary outcomes

I present results for bone structure and density by sex, below. I present results for the tibia followed by results for the radius. Primary and secondary outcomes are presented together in Table 5.4 (boys) and Table 5.6 (girls). For boys, from sensitivity analyses of other tibial and radial bone structure and density models, results were no different with or without influential points (one to two in each model) and thus, I present the data without any points removed. I present these results in Table 5.5 (boys). For girls, sensitivity analyses of distal tibia Tt.Ar and Tt.Dn, and tibial shaft Ct.Ar and Me.Ar demonstrated large changes in means and SE (>10% differences) between models with and without influential points (maximum of two in each model) but was not different statistically. From the sensitivity analyses, changes to the mean and standard error (SE) for Tt.Dn at the distal radius were 17% and 11%, respectively, after removing an influential point in girls. I present these results for between group differences after removal of the single influential point in the distal radius Tt.Dn model in Table 5.7 (girls).

5.3.4.1 Boys

At the distal and shaft sites of the *tibia*, baseline bone structure and density were similar between INT and CON boys. The exception was Ct.Dn at the shaft of the tibia where INT boys had lower bone density (-2%) compared with CON boys (Table 5.4). At follow-up, Tt.Dn at the *distal tibia* in INT and CON boys increased (INT +4%, CON +4%) but Tt.Ar did not change in either group (Table 5.4). At the *tibial shaft*, boys showed gains in Tt.Ar (INT +2%, CON +2%), Ct.Ar (INT +4%, CON +3%) and Ct.Dn (INT +2%, CON +1%) but Me.Ar did not change over the 30-week period. Change in bone structure and density at the distal and shaft sites of the tibia was similar between INT and CON boys (Table 5.5).

At the distal and shaft sites of the *radius*, bone structure and density were similar between INT and CON boys at baseline (Table 5.4). At follow-up, Tt.Dn at the *distal radius* increased in INT (+5%) and CON (+8%) boys. Tt.Ar did not change significantly over time. At the *radial shaft site*, INT and CON boys increased their Tt.Ar (INT +3%, CON 3%), Ct.Ar (INT +4%, CON +3%) and Ct.Dn (INT +1%, CON +1%). Me.Ar did not change in either group (Table 5.4). Overall, changes in bone structure and density at the distal and shaft sites of the radius were not significantly different between INT and CON boys (Table 5.5).

5.3.4.2 Girls

At baseline, for distal and shaft sites of the *tibia*, INT and CON girls had similar bone structure and density (distal site only). The exception was Ct.Dn at the tibial shaft, where INT girls had higher Ct.Dn (+1%) compared with CON girls at baseline (Table 5.6). At follow-up, Tt.Dn at the *distal tibia* increased similarly in INT and CON girls (+2%). The change in Tt.Ar was not significant within and between groups (Table 5.6). At the *shaft site* of the *tibia*, Tt.Ar (INT +1%, CON 0.3%) and Ct.Ar (INT +2%, CON +1%) increased similarly in INT and CON girls. Ct.Dn increased in CON girls (+1%) but change was negligible for INT girls. Change in Me.Ar at the tibia shaft was not significant in INT or CON girls. In sum, changes in bone structure and density at distal and shaft sites of the tibia were not statistically different between INT and CON girls (Table 5.7).

At baseline, bone structure and density at distal and shaft site of the *radius* was similar between INT and CON girls (Table 5.6). At follow-up, *distal radius* Tt.Dn for INT and CON girls increased similarly (INT +3%, CON 2%); there was no change in Tt.Ar in either group (Table 5.6). At the *radial shaft*, Ct.Dn increased in both INT (+0.8%) and CON (+0.3%) girls. Tt.Ar, Ct.Ar and Me.Ar did not change significantly over 30-weeks in either group (Table 5.6). Most changes in radius bone structure and

density were comparable between INT and CON girls. However, change in Ct.Dn was significantly greater in INT girls (+0.5%) compared with CON girls (Table 5.7).

Overall, ICC values ranged from less than 0.001 to 0.23 for primary and secondary bone variables in boys and < 0.001 to 0.16 in girls. In boys, the largest ICC value was 0.23 for Tt.Dn at the distal radius site. For girls, the largest ICC value was 0.16 for Ct.Ar at the tibial shaft.

5.4 Discussion

This cluster randomized controlled trial in adolescents is unique in several ways. First, HPSS was designed based on a real-community trial (ReaCT) concept using a whole-school intervention model guided by socio-ecological and self-determination theories. Second, this is the first study to use pQCT to evaluate the bone response to a PA intervention in adolescents at both the weight-bearing tibia and non-weight bearing radius. This study provides novel insight on bone strength changes in adolescents as a function of growth. It also demonstrates the challenges when conducting a choice-based PA intervention designed to increase PA in adolescent boys and girls. I disproved my hypotheses. HPSS was not an effective model for increasing bone strength, structure or density in boys or girls (with the exception of Ct.Dn at the radial shaft in girls). I outline several possible explanations for my findings, below.

5.4.1 HPSS: a choice-based, theory-driven intervention

HPSS was designed to provide each school the autonomy to adopt and implement activities that would work well and be suited to their environment (469). Thus, each school created their own programs (e.g., walking groups, run workshops, jumping events) and these programs varied between schools. It was

a challenge for schools to effectively track and account for participation in all activities. With that in mind, at the end of the intervention, the HPSS Central Team assessed teacher logs to ascertain what parts of the curriculum were adopted in PE and Planning 10 lessons, what programs/projects were implemented by Action Teams and how monetary grants were expended (469). However, only two of four schools completed the logs and reports appropriately and they were therefore unable to adequately assess implementation of the model. In general, activities conducted in each intervention school varied. Students and teachers tried many selections from the resource guide; some of these activities were not physically challenging. For example, one INT school used 22/60 resources for PE 10 classes. Activities ranged from touring a fitness facility, discussions regarding the PA pyramid, breakdown of sports-related skills for skills assessment and student-lead fitness sessions. Given the curricular nature of PE, it was not wholly surprising that HPSS was more about learning and transfer of information than about participation in PA. It was not possible from the logs to ascertain the scope of activities undertaken or to discern what components of HPSS were successfully adopted.

Further, the Action Team was asked to complete monthly meeting minutes to track the progress of the intervention. These records were also incomplete. Therefore, it was not possible to determine to what extent study participants participated in activity sessions conducted in classes or school-wide events. I feel it is safe to assume that if participants were present, they would participate in HPSS class activities, as these classes were compulsory for Grade 10 students. The process evaluation was unable to fully counter poor compliance to logging and tracking or to completely capture dose and fidelity of the intervention. In future, earlier and more frequent feedback regarding implementation during the intervention may be an important step towards improving intervention outcomes, such as increased PA (473). Feedback would provide an opportunity to confront any challenges and to adapt the intervention so as to enhance its implementation. For example, teachers voiced concerns about how difficult it was to complete the

paperwork required to log and track implementation of HPSS. In hindsight, this could have been simplified or we could have used other means (e.g., a research assistant, or frequent phone interviews) to gather information. Thus, the results of my study most likely reflect challenges to implementation of the HPSS model rather than to ineffective design of the model. However, there is no way to assess this.

The researchers who developed the HPSS model in collaboration with the teachers in participating schools, used SDT, as per findings on the applicability of SDT (and the socio-ecological model) to increase PA in Grade 10 students (353). Stuntz and Weiss recommended this approach as an effective means to encourage children and adolescents to adopt and sustain a physically active lifestyle (355). Furthermore, application of a socio-ecological model to support and build the environment for increased PA levels in youth is also best practice (375). Indeed, this approach is one of five recommended strategies in a narrative review on strategies to effectively increase PA in adolescents (375).

Despite adoption of these best and supported practices, HPSS did not encourage INT students to become more physically active as compared with CON (469). One key hindrance to implementation of HPSS was a province-wide teacher strike where teachers withdrew voluntary and administrative services for the academic year. Thus, teachers had only limited and progressively declining involvement in extra-curricular activities as the school year proceeded, did not participate in the HPSS Action Teams nor fully comply with the study's request to complete and submit process evaluation data. Action Teams were designed to be comprised of 50% students and 50% teachers (474). However, this was most often not the case as teachers declined to participate. While we adopted an intact theoretical framework designed to increase PA in adolescents through self-driven, intrinsic motivation, the model's reliance upon school/ teacher involvement likely rendered it an ineffective means to enhance PA in these secondary school students.

5.4.2 Changes in bone strength, structure and density

Over the study period, changes in bone strength, structure and density were similar at the distal sites (tibia and radius) and tibia shaft between INT and CON groups. The exception was the radial shaft where INT girls had greater Ct.Dn compared with CON girls post-intervention. Below, I discuss bone changes by sex. As bone changes are site-specific, I call upon in my discussion, studies that used 3-D bone imaging tools and examined the same bone sites as evaluated in the current study.

5.4.2.1 Boys

Bone strength increased at the distal and shaft sites of the tibia in all boys as a normal consequence of growth and development. At the *distal tibia*, gains in bone strength were a function of increased bone density -- as total bone area decreased slightly but not significantly. A previous study from our lab also demonstrated a slight decrease in bone area at the distal tibia in boys aged 15 – 16 years. This was followed by increased bone area in subsequent years (follow up annually to age to 20 years) (390). Burrows and colleagues noted that bone density rather than bone area was the main contributor to bone strength in the early adolescent age group (390). Accelerated bone growth slows after PHV is achieved and as boys approach full maturity. Walsh and colleagues reported that distal tibia cortical perimeter (assessed using HR-pQCT) was similar between young adults (average age 32 years, range: 30-32 years) and older adolescents (average age of 17.5 years, range: 16-18 years) (475). This suggests that most boys may have achieved adult bone size by age 18 years – although there is tremendous variability in growth rate and timing among boys (197). The ICC value that is close to 0.20 (Tt.Ar at 8% tibia in boys) represents a large degree of variability between groups. Tt.Ar at the distal tibia was highly variable in boys and that may be related to different growth rates with little variability for bone strength and total density at

the same site in boys. In future, a larger sample size would provide more definitive answers regarding patterns of bone structural change in adolescent boys.

At the *tibial shaft*, gains in bone strength were conferred by increments of Tt.Ar, Ct.Ar and Ct.Dn. Change in Me.Ar was not significantly different among *boys* and all boys demonstrated a trend toward decreased Me.Ar. This suggests that endosteal apposition occurred at this shaft site. The reverse was true in a previous study from our lab in a younger cohort. Kontulainen and colleagues reported increased Me.Ar over 20 months in boys who were 12.3±0.4 years, on average, at baseline (216), which suggests that bone was being resorbed rather than accrued on the endosteal surface. The same cohort also showed gains in bone strength, Tt.Ar, Ct.Ar and Ct.Dn (215,216). As APHV occurs at age 13.5 years, on average in boys (11), the boys were close to APHV, around 14 years old, at the end of the 20-month long follow-up (216). Participants in the current study were two years post PHV, on average, at baseline. Thus, differences in change in Me.Ar between studies reflect boys at different stages of maturity. A longitudinal study of boys demonstrated that two years post PHV, bone area (by DXA) at the femoral neck has achieved 95% of adult values and this plateaus at 97% in the next few years (114). Thus, bone growth slows substantially and bone size at the tibial shaft is almost its maximum. In a large cross-sectional study using pQCT, investigators found that by the age of 19 years, there was no periosteal apposition in young men (476), which to an extent agreed with the findings from the previous longitudinal study that used DXA. The field would benefit from a longitudinal study that uses 3-D bone imaging equipment to capture bone strength, structure and density across childhood and adolescence.

Gains in bone strength at the *distal radius* in *boys* were also conferred by increased bone density rather than increased bone area. In a cross-sectional study, Neu and colleagues measured total bone area at the distal radius in boys (by pQCT) and reported that bone area was two times higher in boys aged 15 years compared with boys at age 6 years (218). Bone size differences was around 16% to 20% greater in

boys, prior to age 14 to 15 years and subsequently, the increments were much smaller, 1% to 5% until age 23 years. However, bone density differences were much larger after age 15 years, compared with younger boys aged 13 years and below where changes were negligible (218). Hence, greater bone strength at the distal radius in boys after age 15 years appears to be conferred by higher bone density to a greater extent than larger bone size. As the appendicular skeleton, particularly the upper limbs, is the first to experience a growth spurt, it is plausible that the greater increase in density and lesser increase in size for 15-year old boys are due to the timing of growth. Thus, by age 19 years, Lorentzon and colleagues found that age no longer correlated with periosteal circumference of the radial shaft but still significantly correlated with Ct.Dn and endosteal circumference (476). Lorentzon and colleagues interpreted this as stagnant growth in bone size by late adolescence into young adulthood. If so, this adequately explains the phenomenon observed in my study cohort. Prospective studies that use 3-D bone imaging tools to track changes in bone size from adolescence to adulthood can shed more light into this issue.

Bone strength, structure and density increased at the *radial shaft in boys* over the course of the study. However, Me.Ar did not change significantly suggesting that endosteal apposition was in balance with endosteal resorption. Gains in bone strength at the shaft sites were a function of increased Tt.Ar, Ct.Ar and Ct.Dn. Garn conducted a landmark cross-sectional study where he used radiogrammetry (and planar radiographs of the second metacarpal shaft) to study bone growth in 5735 males, aged 1 to 80 years. He reported that periosteal bone area increased into the third decade of life (mid-twenties) (210). Other cross-sectional studies of the radial shaft reported greater bone strength, structure and density for boys aged 15 years and older compared with younger boys (219,221). The trend for decreased Me.Ar that I noted was also reflected in similar age groups in other studies (210,211,219). Neu and colleagues showed that boys who were 14-15 years old had a smaller Me.Ar compared with 16-17 year old boys, on average (219). This suggests either decreased endosteal resorption or increased bone apposition during

this stage of puberty. Garn and colleagues reported similar patterns for Me.Ar in boys aged 14 to 16 years. This period was followed by increased medullary area suggesting the reverse occurred – whereby either bone apposition slowed and/or bone resorption increased (210,211). Although of some interest, changes in Me.Ar were small in my study and did not appear to contribute overall to increased bone strength at the radial shaft.

5.4.2.2 Girls

Despite known sex differences in bone strength, size and density, we observed similar patterns for change in these outcomes at the distal tibia and radius in girls as compared with boys over 30 weeks. However, the magnitude of change was greater for boys compared with girls. I discuss bone adaptations at both distal sites as a function of growth and at the tibial and radial shaft in INT compared with CON groups for girls.

Girls' bone strength at the *distal tibia and radius* increased across the study period and this was conferred by an increase in Tt.Dn while bone area did not change. In late adolescence (age 15 years), cortical perimeter at the distal tibia (by HR-pQCT) was close to adult size in girls (475). Not surprisingly and in keeping with tenets of growth, girls achieve near adult bone size at the distal tibia and radius at a younger age compared with boys. To illustrate, Völgyi and colleagues conducted a 7-year longitudinal study of daughters and their mothers. By age 18 years, daughters had achieved 100% of their mother's value for radial and tibial length (477). While growth in length slows or stops, periosteal expansion proceeds, albeit at a slower rate (218,477). For example, Walsh and colleagues found that 17.5-year old girls had comparable cortical perimeter at the distal tibia (by HR-pQCT) to 31-year old women, on average (475). However, cortical thickness was larger in adults compared with adolescents and this was attributed to higher Ct.Dn. at both the distal tibia and radius. Walsh and colleagues attributed the age-

related variability in the distal radius cortical perimeter and thickness to endocrine factors, i.e. insulin-like growth factor-1 (IGF-1) and estrogen, age and sex. As for the distal tibia, bone strength adaptations in both adolescent and young women were more likely a function of mechanical forces in compression than endocrine factors (475).

Bone strength at the *tibial shaft* in girls increased over the study period, predominantly as a result of increased cortical area. The ICC value for Ct.Ar at the 50% tibia site is very close to the 0.20 value that indicates large variability between schools. Interestingly, other factors that contributed to increased bone strength differed between girls in INT and CON groups. In CON girls we noted slight gains ($p>0.05$) in cortical bone density whereas INT girls demonstrated increased periosteal apposition as estimated by the gain in Tt.Ar. This suggests that changes in bone size in INT girls may be due to higher levels of MVPA (by accelerometer) at baseline compared with CON girls. Due to the small sample size of valid accelerometer data in my study, I interpret these findings with caution. Given the exponential influence of increased bone size through periosteal apposition on bone strength (478), the 2% increase in bone strength in both INT and CON was not a surprise. Granted this gain in bone strength was not the result of the HPSS intervention, this demonstrates the potential for late adolescent girls (3.5 years post APHV, on average) to enhance their bone strength at the tibia. Although girls in late adolescence demonstrate only very small increases in bone area, it appears possible to further enhance bone structure and subsequently improve bone strength in this population. Further evidence in support of this comes from a cross-sectional study by Rantalainen and colleagues who examined tibial bone characteristics (by pQCT) in relation to PA (207). Peri- and post-pubertal boys and girls appeared to benefit from bone loading exercises (by questionnaire) that enhanced bone structure (207).

The radial shaft, a non-weight bearing bone site, presents a quite different scenario. Bone structure (total, cortical and medullary area) changed only slightly over the intervention period. However, SSI_p

(density-weighted bone strength estimate, +1.8%) and Ct.Dn (+0.8%) increased significantly in INT girls, albeit by a smaller magnitude than what was observed in pre and early pubertal girls (219,221). This is not surprising as rapid structural growth at the radius happens prior to puberty. Growth at the radius is minimal after age 14 years in girls (218,219). More specifically, bone mineral accrual parallels maturation (209,479). As mechanical stress at the non-weight bearing radius is minimal, it is expected that structural changes would follow suit. Interestingly, INT girls demonstrated significantly greater increases in Ct.Dn at the radial shaft as compared with CON girls. This was not likely a function of the intervention, as HPSS did not increase PA levels in the INT group. Dietary calcium intake was also not a factor, as INT and CON girls did not differ in this regard. One possible influence on the positive change in INT girls' Ct.Dn may have been the higher levels of PA in this group at baseline. Biologically, secondary bone mineralization can take from six months to 30 months to achieve maximum mineralization (480).

5.4.3 Changes in PA levels

Sadly, the decline I observed in PA levels in both INT boys (VPA only) and girls (MPA and MVPA) as they matured mimics the national trend (316,455,481). From the main HPSS study, MVPA in both the INT and CON groups declined to an average 32 min/day (by self-reported PA questionnaire, unpublished).

In general, regardless of whether PA questionnaires (481) or devices (316) are used to assess PA, as adolescents mature, scores decrease and less time is spent in moderate and vigorous physical activities. From the small sample of valid accelerometer data, INT boys engaged in 50% less MVPA (11 min/day, on average) at follow-up compared with baseline. Similarly, INT girls performed 26% less MVPA and 30% less VPA compared with baseline in the short span of 30 weeks. Although my small accelerometer sample is not a reliable representation of the overall BHS cohort, the average MVPA of 37 min/day (combined

boys and girls) at follow-up was similar to values from other studies. Nelson and colleagues conducted a 5-year longitudinal study on PA levels of two cohorts – junior high (mean age 12.8 years at baseline) and secondary school (mean age 15.8 years at baseline) students (481). Boys in secondary school decreased their MVPA from 6.5 to 5.1 hours/week (56 to 44 min/day, by questionnaire) while girls in secondary school decreased their PA from 5.1 to 3.5 hours/week (44 to 30 min/day) (481). Janz and colleagues reported that active girls (>60 min/day of MVPA) who performed, on average (by accelerometry), 85 min/day of PA at age 5 plummeted by 65% to 30 min/day of MVPA by age 17 (455). At age 17 years there was no difference in PA between previously active versus previously inactive (< 60 min/day MVPA) in girls (455). This disturbing trend of decreased PA as maturity advances is substantiated by population level Canadian data (323).

Thus, the trend I observed with decreased PA levels from baseline to follow-up in boys and girls is not surprising. The significantly greater decline in MPA and MVPA in INT compared with CON girls is somewhat perplexing. INT and CON schools both followed a semester system and during the 2011-2012 academic year, PE classes were held in the first semester (September-January). Thus, there were no differences in scheduled PE between groups. There were also no seasonal differences that might have influenced baseline or final measures between groups. Although seasonality might explain higher PA for the INT girls at baseline compared with follow-up, we would also expect a similar influence in CON girls. INT and CON schools were also matched to reflect geographic regions and socio-economic background (e.g., average family income, suburban/rural/semi-rural living area, number of native students). Therefore, these environmental influences were also similar between INT and CON groups. It is possible that attitude and motivation level toward PA may play a role in these PA patterns. Perceived competence may represent one barrier to PA in adolescent girls (482). More will be known after further analyses of data from the main HPSS study.

The negative consequences of inadequate PA on bone health are well documented. Garcia-Marco and colleagues reported that less than 45 min/day of MVPA or less than 20 min/day of VPA was negatively associated with BMC at the femoral neck (by DXA) in adolescent boys and girls aged 12 to 17 years (13). Janz and colleagues assessed the left tibia of 17 year olds at the 4%, 38% and 66% sites (by pQCT) having tracked their PA levels (by accelerometry) since age 5 years (455). Teens categorized as previously active, moderately active or persistently inactive during childhood had similar MVPA levels by age 17 years (boys: 30-50 min/day and girls: 20-30 min/day) (455). In addition, bone strength at all three sites was similar between previously active (76 min/day of MVPA) and moderately active (64 min/day of MVPA) boys. Importantly, both groups had greater bone strength compared with persistently inactive boys (46 min/day of MVPA). For girls, bone strength at the 4% and 66% sites of the tibia was similar between previously active (85 min/day of MVPA) and moderately active (55 min/day of MVPA) girls at age 17 years (455). However, persistently inactive girls (40 min/day of MVPA) had lower bone strength at all three tibial sites compared with previously active and moderately active girls (455). These findings suggested that PA likely influenced bone strength during early childhood. Although this cross-sectional study of bone with links to prospective tracking of PA is important, it was not possible for the investigators to attribute changes in bone strength to declines in PA. Longitudinal studies that track both bone strength and PA are needed to more clearly delineate this relationship.

Also of concern in adolescents, is increasing SED that may result in a concomitant decrease in PA. Sisson and colleagues surveyed 53,562 children and youth (27,499 boys and 26,063 girls) aged 6 to 17 years in the United States and found that leisure-time screen time of more than 2 hr/day was linked with less daily PA (483). They theorized that PA were ‘displaced’ by SED. However, increases in SED and decreases in MVPA were not significant (except for MVPA for INT girls) in my study. On average, girls in my study increased their SED by about 30 min/day while MVPA decreased by 2-13 min/day. Only

a paucity of studies have investigated the influence of SED on bone strength. However, to lend credence to the theory that SED displaces MVPA, adolescents must be followed over a longer period of time. This is also needed to ascertain the effects of SED on bone strength in adolescents.

5.4.4 Strengths and Limitations

This study contributes substantially to the sparse literature that evaluates PA and bone strength in adolescents. Outcomes provide insight regarding change in PA levels and change in bone strength, structure and density in this less studied age group. There are no previous studies that adopted a randomized controlled design, used pQCT to assess bone and assessed maturity longitudinally (growth data) in this age group. Furthermore, no previous RCTs investigated both weight-bearing and non-weight bearing bones in secondary school aged adolescents.

Several limitations and challenges arose during the course of the study. The province-wide teacher job action affected facilitation of the intervention and extracurricular activities that were a crucial part of HPSS. Further, the job action hindered teachers' ability to adequately log and track implementation of the intervention. Thus, we lack information regarding uptake of the HPSS intervention generally, and the provision of weight-bearing PA known to improve bone strength, specifically in this age group. Despite this, there appears to be a need to revisit strategies and methods used to encourage PA in adolescents. I attempted to objectively assess PA but compliance to wearing the device for the required duration was poor. Thus, improving compliance to objective assessment of PA in future would add great value. Finally, there is no consensus regarding protocols used to acquire and analyse pQCT scans in children and youth (137).

5.5 Conclusion

HPSS was an ambitious whole-school, theory-driven, real-community trial that aimed to increase PA levels in Grade 10 students. The study was hampered by a province-wide teacher strike and teacher compliance to both delivery and tracking of the intervention. Although differences in bone outcomes between INT and CON groups were unremarkable, we demonstrated trends for decreased PA and increased SED, similar to those reported globally (18,323,465,484) – both have potentially dire consequences on growing bone. Exercise interventions and longitudinal studies that focus on this age and maturity group are sorely needed if we are to fully understand the consequences of PA levels and SED on bone health in adolescents.

Chapter 6: Integrated Discussion

In this final chapter, I present an overview of the findings from this thesis and highlight the contributions of Parts I - III to the literature. I follow with discussions related to the challenges associated with the study of the PA-bone strength relationship in adolescents and with school-based interventions. I also discuss the limitations of my research and provide recommendations for future studies in the area of PA and bone strength in adolescents. I close with the public health implications of the findings from my thesis and a summary and conclusions for Parts I, II and III.

6.1 Overview of Findings

6.1.1 Systematic review of studies on PA and bone strength in children and adolescents

In Part I, I conducted a systematic review to determine the effect of PA on bone strength in children and adolescents. This review extended the recent review by Nikander and colleagues (37) who conducted a meta-analysis that included only four publications (three from our research group). Their focus was RCTs that investigated the influence of PA at different bone sites (distal and shaft sites of the tibia, femoral neck and shaft) assessed using different imaging modalities (pQCT, DXA with HSA). I reviewed 10 recent RCTs in addition to those included in the Nikander et al. review. I also reviewed observational studies (n=23) as I viewed their contribution to the literature as meaningful. I noted great variability across these studies in bone imaging acquisition and analysis protocols used, thus, I was unable to conduct a meta-analysis. Therefore, I presented a narrative synthesis to summarize key aspects of the relevant literature.

Briefly, 70% of studies reported a positive association or effects of PA on bone strength. Results were mainly from studies of younger children (81% pre- to peri-pubertal) and the ratio of studies that focused on girls versus boys was 2:1. All RCTs examined weight-bearing bones (i.e. tibia, femur). In response to PA and at this younger age, bone structure rather than bone density contributed to bone strength in most cases. I discuss below how my outcomes in a less studied adolescent cohort varied from these findings. The systematic review highlighted the need to: 1) standardize bone imaging protocols *within* studies of children and youth to enable comparisons of results *across* studies, 2) focus on adolescents to better understand the relation of PA and bone *strength*, as few studies have done this, 3) investigate the effect of PA on non-weight bearing bones as no studies have targeted an upper limb PA intervention in this age group, 4) delineate the role of muscle function and its surrogates on bone, and 5) establish the independent influence of muscle on bone versus loading (axial, compressive, torsional) through PA.

6.1.2 Determinants of bone strength in adolescents

In Part II, I compared sex differences in bone strength, structure and density and examined determinants of bone strength, structure and density by sex at distal and shaft sites of the radius and tibia. To my knowledge, this is the first study to use pQCT to investigate these differences in weight-bearing *and* non-weight bearing bones in the adolescent skeleton. Data from my study showed that in youth who were 15-years old, on average, sex differences in bone outcomes were a function of body size (i.e. limb length, height) and body mass. After adjusting for differences in size and mass, bone strength and structure were similar between boys and girls. Bone density was the exception whereby girls had greater bone density compared with boys after adjusting for size and mass. This is consistent throughout the literature (220,222,424,443) and may be a due to 1) earlier maturation in girls compared with boys that

resulted in more bone mineral accrual (209); 2) estrogen's role to increase bone mass rather than size as a possible reservoir of calcium for reproductive purposes (485,486), and 3) compensation for smaller bone size with density to increase bone strength (487). These findings reiterated the importance of accounting for body dimensions and mass when interpreting bone outcomes in a growing population.

This cross-sectional study is the first to demonstrate, using pQCT: 1) the positive association between PA and bone strength, structure and density in adolescent boys and girls, and 2) the potential of SED to negate the positive association between PA and bone strength and density in adolescent girls. I acknowledge the limitations of cross-sectional studies; however, these associations are promising and warrant conducting intervention studies to ascertain the influence of PA on bone strength in this age group. As PA levels decline in adolescents, especially girls as they mature (323,455), and SED tends to increase in adolescents (323), effective interventions in this age group seem imperative.

Importantly, a new finding from my study was that SED attenuates the positive influence of MVPA. Thus, it is important that investigators be aware of the contributions of PA and SED to bone outcomes and consider patterns of SED in their analyses. I consider my findings in a relatively small sample as preliminary. Well-designed, weight-bearing PA intervention studies of at least one year duration that use 3-D bone imaging tools and objectively measured PA are needed to more clearly ascertain the effect of PA and SED on bone strength in adolescents.

I also provided novel data that showed the association between grip strength and radial bone strength in adolescent boys and girls. Specifically, in boys, grip strength was a significant predictor of bone strength at the distal and shaft sites of the radius. In girls, grip strength significantly predicted bone strength at the radial shaft only (but also predicted bone area at the distal and shaft sites). The positive influence of grip strength on bone strength remained after accounting for muscle mass (total body lean

mass). It would be interesting to conduct an RCT to determine if resistance training effectively increases bone strength in older adolescents who are experiencing decelerated bone growth.

6.1.3 Effects of the HPSS intervention on bone strength, structure and density in adolescents

In Part III of my thesis, I found that the HPSS intervention was not associated with significant gains in bone strength, structure or density in adolescent boys or girls, with the exception of cortical bone density in girls. The lack of a significant effect of the intervention on bone outcomes was likely due, in part, to the fact that the 30-week intervention failed to increase adolescents' PA levels. In addition, the intervention itself did not contain a prescribed/mandatory bone-loading component as the study was built around a choice-based concept guided by the SDT. The choice of this model was deliberate and based on theory. Our central tenet for the intervention was that adolescents should be delivered an adult-based (choice-based) rather than a child-based prescriptive model. Overall, HPSS met with implementation challenges such as lack of teacher participation and reporting during the province-wide teacher strike. Thus, this study was reliant on measures of (and limited by) implementation as well as effectiveness. I feel that the question of whether a targeted program of exercise delivered through schools to adolescents could increase bone strength remains unanswered. To address the question of whether prescribed exercise can influence bone structure at adolescence, I suggest a 12-month (minimum duration) RCT with quantifiable, structured weight-bearing PA and/or resistance training. To address the question of whether an effective model can be implemented within schools requires a thorough evaluation of implementation (focus on the role of teachers) within a choice-based model with some weight-bearing (prescribed) PA components. Exploring additional community portals to reach youth (e.g., sports teams/clubs, recreation centres), may also be warranted given the often political and unstable nature of the public education sector as a research site. In heeding Berliner's caution of the challenges inherent in conducting educational research, " . . . *the*

power of contexts, the ubiquity of interactions and the short half-life of our findings" (488), quality evaluation measures are required. This will address the question of whether an effective whole school model can be implemented within the secondary school settings. It will also further inform on the influence of a choice-based model upon prescribed weight-bearing PA components. Furthermore, a look into the sedentary patterns within schools during the intervention study would be an interesting next step based on my findings in Part II.

I described sex- and site-specific changes in bone outcomes over the 30-week study. Although our research group has described these changes in a younger group previously (215), this is the first study to describe changes in bone outcomes using pQCT in an older group of adolescents. Importantly, gains in bone strength at distal sites of the radius and tibia were mainly due to increases in total bone density whereas at the shaft sites bone strength was conferred by increases of both bone area and cortical density. In the natural progression of bone growth, deceleration in linear growth occurs after PHV (63,489). As all participants had surpassed PHV (by an average of 1.9 years) at baseline, bone growth would have slowed down. This deceleration of growth was reflected in the PBMAS study, as gains of total body bone area (by DXA) were 7% versus 3% of adult values in boys who were a year after PHV and two years post PHV, respectively (114). Within a short 30-week period, changes in bone size at the *distal bone sites* may not be prominent enough to be significant. The measure of bone area, however, is 2-D measure of bone size. At the *shaft sites* of the tibia and radius in boys or girls, total bone area increased. However, medullary area did not change significantly in either sex. This highlights the surface-specific adaptations of bone during growth where bone is accrued on the periosteal surface (rather than the endosteal surface) preferentially. This finding also dispels an often held belief based on an early cross-sectional radiogrammetry study (210) that bone is accrued moreso at the endosteal surface during adolescence in girls compared with preferred accrual at the periosteum in boys, but this concept is closely related to the stage of maturity (487).

These changes relate to the tempo and sequence of growth. As the growth plate begins to fuse after PHV is achieved, increases in bone length at the epiphyses diminish. Furthermore, major contributions (about 60%) to growth in bone length at the tibia stems from the proximal growth plate (490). However, increased bone size through appositional growth occurs into adulthood in response to mechanical forces (from loading and through muscle) and a balance between the action of osteoblasts (bone apposition) and osteoclasts (bone resorption) on the surfaces of bone (63). In addition, while linear bone growth slows, the skeleton continues to accrue bone mineral as reflected by increased aBMD up to five years post APHV (114). Decrements in the magnitude of growth related to bone in length, size and structure have been reported from traditional orthoroentgenogram studies that followed children from as early as age 8 years until growth plate closure (490,491). Similar long-term prospective trials with more accurate methods would be valuable to characterize these events more clearly, particularly in bone geometry in growing children and adolescents.

6.2 Challenges Associated with the Study of the PA-Bone Strength Relationship in Adolescents

I provided insights regarding the determinants of bone strength and bone development in 15-year olds. Intervention and prospective studies in this age group using 3-D bone imaging are limited in the pediatric bone literature. There are many more studies of children and younger adolescents (below age 15 years). There are a number of factors that might influence this; 1) adolescents are a more difficult population to recruit and retain; 2) the varied changes related to maturity within the sample, which make it difficult to discern the independent effects of an intervention; 3) the school environment is more variable as students select courses and change classes; 4) the classroom teacher is less involved on an on-going

basis with activities of students; and 5) PE is not mandatory after a certain grade in secondary schools, e.g., Grade 10 is the last mandatory year for PE for students in British Columbia, Canada.

Changes in the skeleton at this age are more subtle – so the myriad of influences (above) may mask them. I compared bone changes in elementary school children (aged 12 years at baseline) from our research group's HBS. Briefly, after adjusting the 20-month change to reflect a shorter duration change over 30 weeks, I found that bone strength (section modulus) at the tibial shaft site in HBS boys increased by 12% (increased 32% in 20 months) (215). I noted only a 3% increase in bone strength (SSI_p) in boys aged 15 years in the present study. In girls, bone strength at the tibial shaft increased by 4% in the HBS cohort (215) whereas bone strength increased by 2% at the tibial shaft in 15-year old girls in my study. The lower magnitude of change observed within the HPSS cohort is expected, as on average, most if not all the participants were two years past their APHV.

I am unable to clearly discern from my data the role of PA in bone accrual in boys and girls. I am also unable to clearly resolve whether PA introduced as a choice-based model within schools can be effective as my study was hampered by intervention delivery during teacher job action. Therefore, there is still a need for intervention studies that investigate effective PA models, designed to optimize bone strength in older adolescents.

I turn now to a brief discussion of other key factors that came into play in my study of bone health and PA in adolescents (imaging modality, assessment of PA, the role of maturity and the role of muscle).

6.2.1 pQCT imaging

As discussed elsewhere (section 1.2.4.3), pQCT offers several advantages over traditional DXA imaging. Of particular relevance to my thesis is the ability of pQCT to estimate bone strength and

investigate the components of bone (structure and density) that contribute to bone strength. However, there was great variability among studies regarding how pQCT images were acquired and analyzed; therefore, comparisons across studies were not possible (137). There is a need for researchers to come together to standardize pQCT image acquisition and analysis protocols, specific to pediatric bone and clinical pediatric populations.

Between-study differences in pQCT protocols include – placement of the reference line, sites of measurement and voxel size; all of these factors influence measurement outcomes and interpretation of bone images (137). Previous studies that assessed accuracy and precision of pQCT were conducted with adults or using bones from adult cadavers (139,152,153,492,493). Similar pediatric studies have not been conducted due to concerns related to unnecessary radiation exposure and ethical issues in obtaining pediatric cadaver bones. Importantly, pQCT is unable to examine the clinically relevant proximal femur. However, one could argue that bone fracture is much more common at the distal radius in children and adolescents (187). Despite its limitations, pQCT offers a plethora of information about how bones develop and adapt (structure, volumetric density) to enhance bone strength toward adulthood.

Virtually absent from the pediatric bone imaging (pQCT) literature are measurements of sex and growth hormones, bone biomarkers and specific skeletal-pathway related proteins such as sclerostin at the cellular level. There are many reasons for this, among them the challenge and ethics of collecting blood samples from healthy children and the variable nature of hormone levels related to time of day/month, maturation and diet. However, these data would establish a more complete picture of bone physiology (bone modeling/remodeling) as bones mature. The few longitudinal (423) and short-term intervention (101) studies that assessed the endocrine environment provided great insight regarding bone development. In a 7-year longitudinal study, investigators followed girls from an average age of 11 years to examine IGF-1, estrogen and testosterone levels in relation to changes in bone size (length, circumference), density

(vBMD) and mass (total BMC) (by pQCT)at the 60% tibia site (423). Not surprisingly, measured hormone levels increased dramatically prior to menarche. These increases coincided with increases in bone outcomes. After menarche, sex hormones plateaued (estrogen) or increased slowly (testosterone) and the associations with bone outcomes observed prior to menarche were no longer present. Lester and colleagues examined the effectiveness of an 8-week resistance-training program in young women aged 20.3±1.8 years. Bone formation markers increased throughout the intervention while bone resorption markers decreased at mid-intervention and increased back to baseline levels at the end of the intervention. This resulted in small but significant changes in BMD at the distal 4% tibia site (by pQCT).

Thus, it would be ideal to conduct individual endocrine assessments during growth, as they are currently missing from most studies of pediatric bone health. Finally, changes related to each individual's hormonal assays rather than using group means for analyses, while controlling for many other known confounding variables (including maturity), is key to efficiently incorporate biological markers with imaging data to further understand bone adaptations.

6.2.2 Assessment of maturity

Adolescence represents a complex stage of growth and development. These complexities are not limited to biology and physiology but extend to individual adolescent behaviours and attitudes per se towards health and PA. Adolescent girls are concerned with their appearance, femininity and issues with menstruation during PA that deters them from being more active (494).

In pediatric bone health studies, precise assessment of maturity and identifying a standard maturational time point is a challenge. In this thesis, I used age at menarche in girls as a standard maturational time point. Although age at menarche is subject to recall bias (205), it is an objective

biological indicator of sexual maturity in girls. An equivalent indicator of sexual maturation does not exist for boys. Sexual maturity is most often self-assessed using the method of Tanner. Given the subjective nature of these measures, their broad categories and the difference in timing between sexes regarding the appearance of secondary sexual characteristics – it is not easy to compare between sexes.

If longitudinal height data are available, it may be preferable to determine APHV, as a measure of somatic maturity. Our research group recently recalibrated the highly-cited maturity offset prediction equation (382) originally developed by Mirwald and colleagues (189). Thus, I used this equation to predict maturity offset in boys in Parts II and III of my thesis. For girls, I used age of menarche versus the maturity offset equation as; 1) it is a direct measure of biological maturity, and 2) comparison of bone outcomes between boys and girls matched for maturity was not the focus of my thesis. In future studies that compare sex differences in bone growth and development that controlled for maturity, I would recommend using the recalibrated maturity offset equation (382). This would provide a comparable maturational time point that would enable comparisons in bone adaptations between sexes.

6.2.3 PA assessment

Based on the mechanostat theory (75), PA is the primary mechanical stimuli required to optimize bone strength, Thus, there is a need for accurate tools that objectively quantify PA (and especially weight-bearing PA) in adolescents. I focus my integrated discussion on objective measurements that are able to determine the contribution of intensity, duration, frequency to overall PA.

Objective measures of PA (accelerometers) provide valid and reliable PA data (316) and are able to delineate the intensity and duration of PA across a known time span. However, compliance to wearing accelerometers remains a challenge (495). This was evident in Part III of my thesis – although I attempted

to assess PA objectively. In INT boys, compliance with wearing accelerometers was very poor (44% with valid accelerometer data). In other studies, compliance with accelerometer wear has been much better -- 60-62% in adolescents (aged 12 to 19 years) and 84% in adults > age 60 years (2003-2004 NHANES) (316). NHANES used several strategies to improve participant compliance. For example, in NHANES (2012 protocol) accelerometers are worn at the wrist instead of at the hip (496). NHANES used a waterproof accelerometer and encouraged participants to wear the monitor for 24 hours a day, seven days a week. They included an incentive (USD $40) so that participants returned accelerometers at the end of seven days. Similar monetary incentives as well as phone/text reminders and PA logs were used in previous studies to improve compliance in children and adolescents (495,497). Reports of compliance to wear time for NHANES 2012 accelerometer protocol was not available at the time of my writing this thesis, but would be interesting to follow up on.

Importantly, accelerometers (e.g., Actigraph, Actical) do not measure impact forces that are relevant to bone outcomes (344,498). Needless to say, if accelerometry could objectively and accurately assess PA intensity and impact forces, this would indeed be ideal for PA studies in bone health. In the Iowa Bone Development Study, Janz and colleagues pioneered the use of accelerometers to examine PA intensities and patterns/trajectories in relation to bone strength (339,455) and structure/density (338,448) outcomes in children. Currently, however, there are still limitations to using accelerometry to assess the impact of weight-bearing PA on bone. To illustrate, gymnastics elicits an energy expenditure of approximately 3.8 METs (499) and would therefore be classified as moderate PA using commonly applied definitions to accelerometry data. However, this would underestimate the actual impact of the ground reaction forces experienced by gymnasts (4 to 8 G) (345) on bone.

In addition, objective measures that accurately capture SED are important. There is currently no consensus on the definition of SED. However, two commonly used definitions are; energy expenditure

≤1.5 METs or energy expenditure ≤1.5 METs in a sitting or reclined posture (318). The latter definition is more relevant to pediatric bone and PA studies as the removal of weight-bearing forces will have measured negative effects on the growing skeleton. Thus, future studies need to also incorporate body-posture measurement devices if possible.

6.2.4 Muscle-bone relationship

The role of muscle as a mediator in bone adaptation is key to how bones develop. Therefore, muscle or its surrogates should be included in models that assess the relation of PA to bone strength. Muscle growth and development is related to sex, maturity level (hormones), nutrition, PA and genetics – similar to factors related to growth and development of bone. Thus, better understanding the contribution of muscle to bone accrual would in turn delineate the independent contribution of PA to bone strength accrual. In Part I, I reported that the influence of PA on bone outcomes was attenuated (or disappeared) once a surrogate of muscle force was included in analysis models. In Part II, after controlling for muscle (total body lean mass or muscle cross-sectional area), the contribution of MVPA, VPA and grip strength to bone strength, structure and density in adolescents persisted. I interpreted this to suggest that external forces from PA and muscle *function* confer benefits to bone independent from muscle mass. I suggest that studies that examine the PA-bone relationship should also assess the contribution of muscle power (or its surrogates) to better understand the independent contribution of PA to bone adaptations. This is particularly important in children and adolescents as in hormones, dietary and levels/types of PA may all contribute to individual variability in muscle mass (279).

As adolescents have yet to plateau in growth, the opportunity to optimize bone strength is likely substantial and these benefits may persist throughout life into older adulthood (335). Thus, there is a need

for well-designed studies in adolescents that differentiate the contributions of maturity, muscle and PA to bone strength.

6.3 Challenges Associated with School-Based Interventions (Community Trials)

HPSS was a unique school-based intervention, built upon a solid theoretical foundation and designed by teachers in collaboration with our research team to maximize knowledge exchange and potential for sustainability. First, the HPSS intervention was guided by SDT. This approach was deemed appropriate for a population on the brink of adulthood, independence and at a time when they more fully participate in decision-making. Second, HPSS was a whole-school model, designed to benefit and enhance positive health habits of the larger school community. Based on the socio-ecological model an individual's health behaviours are adaptable and influenced by family and peer support and the environments (school, government policies and politics) within which they enact healthy behaviours. Third, HPSS included an implementation plan to translate research outcomes into real world settings had the intervention proved successful. This plan was based on the real community-based trials as described by Katz and colleagues (371). However, province-wide teacher job action during the academic year interrupted vital intervention strategies (i.e. Action Teams did not form and function as intended in all INT schools) (500). Furthermore, we were unable to collect quality implementation data, as teachers were instructed by their union to withdraw voluntary services that included completing reports for HPSS.

In my view, HPSS did not affect bone strength accrual in boys or girls for several reasons. First, increased PA was one of four actions that INTschools could *choose* related to school programs and policies. Second, choice of weight-bearing PA was limited in the HPSS PE 10 resource guide. Of the 60 types of activities, only four were weight-bearing or resistance exercises. In addition, the HPSS resource

guide provided only limited information on the benefits of PA to improve bone health. These all represent limitations of a choice-based model and the fine balance between designing for effectiveness (on bone) and feasibility to implement in a real-world secondary school setting. A balanced approach might include offering an array of physical activities for students and teachers to select among within prescribed and required areas of exercise (e.g., bone health, aerobic health, muscular strength, flexibility etc.).

School-based intervention trials are clustered by nature and this study design must be accounted for in analyses (501). If not, type 1 errors due to inflated p values might occur (501). Previous PA studies in elementary school children (that used DXA (502) or pQCT (32)) to assess bone, reported minimal cluster-related effects. Regardless, I accounted for the cluster study design in my statistical analysis, as per methods described previously (32,400). From Part III, I noted that ICC values for total bone area at the distal tibia and radius were highly variable between school clusters (close to 0.20). Inter-cluster variability for tibial total and cortical bone area in intervention girls, was also high. Although ICC data were not available to me when I designed my study, ICC values should be considered when designing future studies. A small sample size within a cluster, few clusters and variance in bone area between clusters, all potentially influence study outcomes.

Overall, investigating school-based interventions present many challenges: "*Conducting field-based evaluations of social and behaviour change interventions is difficult – very difficult*" (503). However, despite these, we need to better understand what will work in 'real-world' settings. Schools represent important community portals to reach students (504) and translate effective interventions into pragmatic and sustainable health practice and policies through appropriate implementation and knowledge transfer processes (473). It is important where possible to anticipate limitations and to design strategies to overcome them to minimize their effect in school-based intervention studies.

6.4 Public Health Implications of Adolescence PA and Sedentary Time on Bone Health

In Part II, I demonstrated the associations between MVPA, VPA, SED and grip strength with bone strength and structure in adolescents. In Part III, PA trended downwards in boys while previously active girls demonstrated significant declines in PA over one school year. Together, these illustrate the importance of identifying solutions to prevent the diminishing levels of PA as children mature. I demonstrated this in my relatively small study, but these disturbing trends have been reported globally in much larger trials (323,505,506). There is also a need to identify an effective PA prescription to improve bone strength in adolescents. Current research not only emphasizes the need for appropriate involvement in PA, but also, in later life, attributes mortality and morbidity to physical *inactivity* (507) and sedentary behaviour (326,505). We must counter low levels of PA and high levels of sedentariness in youth (323,455) as it is vital to "…*get those already not choosing health (PA) on their own to join with those who are*" (508). Thus, a two-pronged approach that encourages positive PA and deters sedentary habits during the crucial years of growth and development is important to consider in future studies.

6.5 Summary and Conclusions

6.5.1 Part I: Influence of PA on bone strength in children and adolescents: A systematic review and narrative synthesis

Summary (Primary Objective – Bone Strength):

a) From the narrative synthesis, 70% of all studies included showed a positive influence or effect of PA on bone strength in children and adolescents.

b) Benefits of PA on bone strength are evident in both girls and boys.

c) Benefits of PA on bone strength were mainly observed in pre- and peri-pubertal children; however, few studies were conducted with adolescents in the later stages of maturity (i.e. pubertal and post-pubertal).

d) Based on weight-bearing PA interventions (RCTs alone), 60% of strong-rated studies found that bone strength increased by 3 to 4% in children; 30% of moderated-rated studies found significant effects of PA; 3 to 12% gains in bone strength in pre- and peri-pubertal children were reported, on average.

e) Of the 10 observational studies, four studies (three of children younger than 12 years, one study of adolescent aged 14 years) reported that weight-bearing MVPA and VPA explained 2 to 12% of the variance in bone strength at the tibia and proximal femur.

f) All 13 observational studies of organized sports (60% of studies were in gymnastics), most assessed girls, documented positive relations between sport participation and bone strength.

Summary (Secondary Objective – Bone Structure and Density):

a) Gains in bone strength through PA were more often associated with changes in bone structure than bone mass.

b) Muscle mediates the influence of PA on bone strength and thus, future PA studies should assess the muscle-bone relationship.

Conclusions:

a) Weight-bearing PA positively influences bone strength in children and adolescents.

b) Bone strength gains associated with PA are more often due to changes in bone structure rather than bone mass.

c) PA benefits bone strength in both boys and girls, particularly during pre- and peri-puberty.

6.5.2 Part II: Determinants of bone strength, structure and density in adolescent boys and girls

Summary (Primary Objective – Bone Strength):

GENERALLY

a) *Distal radius and tibia:* Girls had greater bone strength compared with boys (after adjusting for limb length and body mass).
b) *Shaft sites - tibia and radius:* Bone strength was similar between boys and girls (after adjusting for limb length and body mass).

BOYS:

c) *Tibial shaft*: MVPA (by questionnaire) explained 2% of the variance in bone strength (after adjusting for ethnicity, years post APHV, tibial length and total body lean mass).
d) *Radius – distal and shaft sites:* Grip strength explained 6% and 7% of the variance in bone strength (after adjusting for ethnicity, years post APHV, ulnar length and total body lean mass).

GIRLS

a) *Distal tibia*: MVPA (by accelerometry) explained 7% of the variance in bone strength (after adjusting for ethnicity, age of menarche, tibial length and total body lean mass). When SED was included in the analysis, the influence of MVPA on bone strength in girls was no longer significant.

VPA explained 4% of the variance in bone strength (after adjusting for SED, wear time, ethnicity, age of menarche, tibial length and total body lean mass).

b) *Radial shaft*: Grip strength explained 4% of the variance in bone strength (after adjusting for ethnicity, age at menarche, ulnar length and total body lean mass)

Summary (Secondary Objective – Bone Structure and Density):

BOYS

a) *Tibial shaft*: MVPA by questionnaire explained 4% of the variance in cortical bone area (after adjusting for ethnicity, years post APHV, tibial length and total body lean mass)
b) *Distal radius:* Grip strength explained 6% of the variance in total bone area (after adjusting for ethnicity, years post APHV, ulnar length and total body lean mass)
c) *Radial shaft*: Grip strength explained 6% and 10% of the variance in total and cortical bone area, respectively (after adjusting for ethnicity, years post APHV, ulnar length and total body lean mass).

GIRLS

a) *Distal tibia*: MVPA (by accelerometry) explained 6% of the variance in total bone density (after adjusting for ethnicity, age of menarche, tibial length and total body lean mass). Once SED was included in the model, MVPA was no longer a significant predictor of total bone density in girls.
b) *Distal radius:* Grip strength explained 6% of the variance in total bone area (after adjusting for ethnicity, age of menarche, ulnar length and total body lean mass).
c) *Radial shaft*: Grip strength explained 5% and 4% of the variance in total and cortical bone area, respectively (after adjusting for ethnicity, age at menarche, ulnar length and total body lean mass).

Conclusions:

a) Comparison of sex-specific bone strength, structure and density differences need to account for body size and mass in adolescent boys and girls.
b) Adolescent boys who are physically active (average 113 min/day of MVPA) may have a larger cortical bone area that contributes to increased bone strength at the tibial shaft.
c) Adolescent girls who are physically active (average 44 min/day of MVPA) could potentially increase their bone strength at the distal tibia through increased bone density if they are also not SED for more than 10 hours/day.
d) In order to have a positive PA influence on bone strength while having >10hr/day of SED, girls need to participate in VPA (average 16 min/day).
e) Improvements in muscle function (grip strength) in boys and girls could infer stronger radius (for both distal and shaft sites) through increased bone size (total area at the distal and cortical area at the shaft).
f) There may still be a window of opportunity to optimize bone strength in 15-year old adolescent boys and girls through proper management of PA and SED and improved muscle strength.

6.5.3 Part III: Effect of a school-based, community-trial intervention on bone strength in adolescents

Summary (Primary Objective – Bone Strength):

BOYS

a) T*ibia* and *radius*: Change in bone strength was comparable between INT and CON. PA was similar between groups at final measurement.

b) *Tibia – distal and shaft* sites*:* Bone strength increased by 7 to 8% at the distal site and 3 to 5% at the shaft site in both INT and CON.

c) *Radius – distal and shaft sites:* Bone strength increased by 12 to 14% (distal) and 4 to 6% (shaft) in both INT and CON.

GIRLS

a) *Tibia* and *radius*: Change in bone strength was comparable between INT and CON groups. INT girls had larger declines, -23% and -22%, in MPA and MVPA, respectively, compared with CON girls.

b) *Tibia – distal and shaft sites:* Bone strength increased by 4% (distal) and 1 to 2% (shaft) in both INT and CON groups.

c) *Distal radius*: Bone strength increased by 5 to 6% in both INT and CON groups.

d) R*adial shaft*: INT girls had a 2% increase in bone strength; CON girls had negligible bone strength gains.

Summary (Secondary Objective – Bone Structure and Density):

BOYS

a) *Distal tibia:* Total bone density increased by 4%; bone size did not change significantly.

b) *Tibial shaft*: Total and cortical bone area increased by 2 to 4%; cortical density increased by 1 to 2%; medullary area did not change significantly.

c) *Distal radius*: Bone density increased by 5 to 8%; bone area did not change significantly.

d) *Radial shaft:* Total and cortical bone area increased by 3 to 4%; cortical density increased by 1%; medullary bone area did not change significantly.

GIRLS

a) *Distal tibia:* Bone density increased by 2%; bone area did not change significantly.
b) *Tibial shaft*: Total and cortical bone area increased by about 1 to 2% with negligible increases in medullary area and cortical density.
c) D*istal radius:* Bone density increased by 2 to 3%; total bone area did not change significantly.
d) R*adial shaft*: Cortical bone density increased by 1%; bone structure (total, cortical and medullary area) did not change significantly.

Conclusions:

a) The HPSS intervention did not have a significant effect on change in bone strength, structure or density in adolescent boys or girls.
b) In 15-year old boys and girls, gains in bone strength at the *distal tibia and radius* appear to be due to greater bone mineral accrual rather than increases in bone size.
c) In 15-year old boys and girls, gains in bone strength at the *tibial shaft* seem to be attributed to periosteal apposition with no significant change at the endosteal surface.
d) In boys, gains in bone strength at the non-weight bearing *radial shaft* seemed to be from periosteal apposition without any significant changes at the endosteal surface.

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Appendices

Appendix A: **Effective Public Health Practice Project Tool & Dictionary**

McMaster University
School of Nursing

EFFECTIVE PUBLIC HEALTH PRACTICE PROJECT (EPHPP)

Ref ID: _____
Author: _____
Year: _____
Reviewer: _____

QUALITY ASSESSMENT TOOL FOR QUANTITATIVE STUDIES

COMPONENT RATINGS

A) SELECTION BIAS

(Q1) Are the individuals selected to participate in the study likely to be representative of the target population?

1 Very likely
2 Somewhat likely
3 Not likely
4 Can't tell

(Q2) What percentage of selected individuals agreed to participate?

1 80 - 100% agreement
2 60 – 79% agreement
3 less than 60% agreement
4 Not applicable
5 Can't tell

RATE THIS SECTION	STRONG	MODERATE	WEAK
See dictionary	1	2	3

B) STUDY DESIGN

Indicate the study design

1 Randomized controlled trial
2 Controlled clinical trial
3 Cohort analytic (two group pre + post)
4 Case-control
5 Cohort (one group pre + post (before and after))
6 Interrupted time series
7 Other specify _____
8 Can't tell

Was the study described as randomized? If NO, go to Component C.

No Yes

If Yes, was the method of randomization described? (See dictionary)

No Yes

If Yes, was the method appropriate? (See dictionary)

No Yes

RATE THIS SECTION	STRONG	MODERATE	WEAK
See dictionary	1	2	3

C) CONFOUNDERS

(Q1) Were there important differences between groups prior to the intervention?

1 Yes
2 No
3 Can't tell

The following are examples of confounders:

1 Race
2 Sex
3 Marital status/family
4 Age
5 SES (income or class)
6 Education
7 Health status
8 Pre-intervention score on outcome measure

(Q2) If yes, indicate the percentage of relevant confounders that were controlled (either in the design (e.g. stratification, matching) or analysis)?

1 80 – 100%
2 60 – 79%
3 Less than 60%
4 Can't Tell

RATE THIS SECTION	STRONG	MODERATE	WEAK
See dictionary	1	2	3

D) BLINDING

(Q1) Was (were) the outcome assessor(s) aware of the intervention or exposure status of participants?

1 Yes
2 No
3 Can't tell

(Q2) Were the study participants aware of the research question?

1 Yes
2 No
3 Can't tell

RATE THIS SECTION	STRONG	MODERATE	WEAK
See dictionary	1	2	3

E) DATA COLLECTION METHODS

(Q1) Were data collection tools shown to be valid?

1 Yes
2 No
3 Can't tell

(Q2) Were data collection tools shown to be reliable?

1 Yes
2 No
3 Can't tell

RATE THIS SECTION	STRONG	MODERATE	WEAK
See dictionary	1	2	3

F) WITHDRAWALS AND DROP-OUTS

(Q1) Were withdrawals and drop-outs reported in terms of numbers and/or reasons per group?

1 Yes
2 No
3 Can't tell

(Q2) Indicate the percentage of participants completing the study. (If the percentage differs by groups, record the lowest).

1 80 -100%
2 60 - 79%
3 less than 60%
4 Can't tell

RATE THIS SECTION	STRONG	MODERATE	WEAK
See dictionary	1	2	3

G) INTERVENTION INTEGRITY

(Q1) What percentage of participants received the allocated intervention or exposure of interest?

1 80 -100%
2 60 - 79%
3 less than 60%
4 Can't tell

(Q2) Was the consistency of the intervention measured?

1 Yes
2 No
3 Can't tell

(Q3) Is it likely that subjects received an unintended intervention (contamination or co-intervention) that may influence the results?

4 Yes
5 No
6 Can't tell

H) ANALYSES

(Q1) Indicate the unit of allocation (circle one)

community organization/institution practice/office individual

(Q2) Indicate the unit of analysis (circle one)

community organization/institution practice/office individual

(Q3) Are the statistical methods appropriate for the study design?

1 Yes
2 No
3 Can't tell

(Q4) Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?

1 Yes
2 No
3 Can't tell

GLOBAL RATING

COMPONENT RATINGS

Please transcribe the information from the gray boxes on pages 1-4 onto this page.

		RATE THIS SECTION See dictionary	STRONG 1	MODERATE 2	WEAK 3
A	**SELECTION BIAS**				
B	**STUDY DESIGN**				
C	**CONFOUNDERS**				
D	**BLINDING**				
E	**DATA COLLECTION METHODS**				
F	**WITHDRAWALS AND DROPOUTS**				

GLOBAL RATING FOR THIS PAPER (circle one):

1	STRONG	(four STRONG ratings with no WEAK ratings)
2	MODERATE	(less than four STRONG ratings and one WEAK rating)
3	WEAK	(two or more WEAK ratings)

With both reviewers discussing the ratings:

Is there a discrepancy between the two reviewers with respect to the component (A-F) ratings?

No Yes

If yes, indicate the reason for the discrepancy

1 Oversight
2 Differences in interpretation of criteria
3 Differences in interpretation of study

Final decision of both reviewers (circle one):

1 STRONG
2 MODERATE
3 WEAK

Quality Assessment Tool for Quantitative Studies Dictionary

The purpose of this dictionary is to describe items in the tool thereby assisting raters to score study quality. Due to under-reporting or lack of clarity in the primary study, raters will need to make judgements about the extent that bias may be present. When making judgements about each component, raters should form their opinion based upon information contained in the study rather than making inferences about what the authors intended.

A) SELECTION BIAS

(Q1) Participants are more likely to be representative of the target population if they are randomly selected from a comprehensive list of individuals in the target population (score very likely). They may not be representative if they are referred from a source (e.g. clinic) in a systematic manner (score somewhat likely) or self-referred (score not likely).

(Q2) Refers to the % of subjects in the control and intervention groups that agreed to participate in the study before they were assigned to intervention or control groups.

B) STUDY DESIGN

In this section, raters assess the likelihood of bias due to the allocation process in an experimental study. For observational studies, raters assess the extent that assessments of exposure and outcome are likely to be independent. Generally, the type of design is a good indicator of the extent of bias. In stronger designs, an equivalent control group is present and the allocation process is such that the investigators are unable to predict the sequence.

Randomized Controlled Trial (RCT)

An experimental design where investigators randomly allocate eligible people to an intervention or control group. A rater should describe a study as an RCT if the randomization sequence allows each study participant to have the same chance of receiving each intervention and the investigators could not predict which intervention was next. If the investigators do not describe the allocation process and only use the words 'random' or 'randomly', the study is described as a controlled clinical trial.

See below for more details.

Was the study described as randomized?

Score YES, if the authors used words such as random allocation, randomly assigned, and random assignment.

Score NO, if no mention of randomization is made.

Was the method of randomization described?

Score YES, if the authors describe any method used to generate a random allocation sequence.

Score NO, if the authors do not describe the allocation method or describe methods of allocation such as alternation, case record numbers, dates of birth, day of the week, and any allocation procedure that is entirely transparent before assignment, such as an open list of random numbers of assignments.
If NO is scored, then the study is a controlled clinical trial.

Was the method appropriate?

Score YES, if the randomization sequence allowed each study participant to have the same chance of receiving each intervention and the investigators could not predict which intervention was next. Examples of appropriate approaches include assignment of subjects by a central office unaware of subject characteristics, or sequentially numbered, sealed, opaque envelopes.

Score NO, if the randomization sequence is open to the individuals responsible for recruiting and allocating participants or providing the intervention, since those individuals can influence the allocation process, either knowingly or unknowingly.

If NO is scored, then the study is a controlled clinical trial.

Controlled Clinical Trial (CCT)
An experimental study design where the method of allocating study subjects to intervention or control groups is open to individuals responsible for recruiting subjects or providing the intervention. The method of allocation is transparent before assignment, e.g. an open list of random numbers or allocation by date of birth, etc.

Cohort analytic (two group pre and post)
An observational study design where groups are assembled according to whether or not exposure to the intervention has occurred. Exposure to the intervention is not under the control of the investigators. Study groups might be non-equivalent or not comparable on some feature that affects outcome.

Case control study
A retrospective study design where the investigators gather 'cases' of people who already have the outcome of interest and 'controls' who do not. Both groups are then questioned or their records examined about whether they received the intervention exposure of interest.

Cohort (one group pre + post (before and after)
The same group is pretested, given an intervention, and tested immediately after the intervention. The intervention group, by means of the pretest, act as their own control group.

Interrupted time series
A time series consists of multiple observations over time. Observations can be on the same units (e.g. individuals over time) or on different but similar units (e.g. student achievement scores for particular grade and school). Interrupted time series analysis requires knowing the specific point in the series when an intervention occurred.

C) CONFOUNDERS

By definition, a confounder is a variable that is associated with the intervention or exposure and causally related to the outcome of interest. Even in a robust study design, groups may not be balanced with respect to important variables prior to the intervention. The authors should indicate if confounders were controlled in the design (by stratification or matching) or in the analysis. If the allocation to intervention and control groups is randomized, the authors must report that the groups were balanced at baseline with respect to confounders (either in the text or a table).

D) BLINDING

(Q1) Assessors should be described as blinded to which participants were in the control and intervention groups. The purpose of blinding the outcome assessors (who might also be the care providers) is to protect against detection bias.

(Q2) Study participants should not be aware of (i.e. blinded to) the research question. The purpose of blinding the participants is to protect against reporting bias.

E) DATA COLLECTION METHODS

Tools for primary outcome measures must be described as reliable and valid. If 'face' validity or 'content' validity has been demonstrated, this is acceptable. Some sources from which data may be collected are described below:

Self reported data includes data that is collected from participants in the study (e.g. completing a questionnaire, survey, answering questions during an interview, etc.).

Assessment/Screening includes objective data that is retrieved by the researchers. (e.g. observations by investigators).

Medical Records/Vital Statistics refers to the types of formal records used for the extraction of the data.

Reliability and validity can be reported in the study or in a separate study. For example, some standard assessment tools have known reliability and validity.

F) WITHDRAWALS AND DROP-OUTS

Score **YES** if the authors describe BOTH the numbers and reasons for withdrawals and drop-outs.

Score **NO** if either the numbers or reasons for withdrawals and drop-outs are not reported.

The percentage of participants completing the study refers to the % of subjects remaining in the study at the final data collection period in all groups (i.e. control and intervention groups).

G) INTERVENTION INTEGRITY

The number of participants receiving the intended intervention should be noted (consider both frequency and intensity). For example, the authors may have reported that at least 80 percent of the participants received the complete intervention. The authors should describe a method of measuring if the intervention was provided to all participants the same way. As well, the authors should indicate if subjects received an unintended intervention that may have influenced the outcomes. For example, co-intervention occurs when the study group receives an additional intervention (other than that intended). In this case, it is possible that the effect of the intervention may be over-estimated. Contamination refers to situations where the control group accidentally receives the study intervention. This could result in an under-estimation of the impact of the intervention.

H) ANALYSIS APPROPRIATE TO QUESTION

Was the quantitative analysis appropriate to the research question being asked?

An intention-to-treat analysis is one in which all the participants in a trial are analyzed according to the intervention to which they were allocated, whether they received it or not. Intention-to-treat analyses are favoured in assessments of effectiveness as they mirror the noncompliance and treatment changes that are likely to occur when the intervention is used in practice, and because of the risk of attrition bias when participants are excluded from the analysis.

Component Ratings of Study:

For each of the six components A – F, use the following descriptions as a roadmap.

A) SELECTION BIAS

Strong: The selected individuals are very likely to be representative of the target population (Q1 is 1) **and** there is greater than 80% participation (Q2 is 1).

Moderate: The selected individuals are at least somewhat likely to be representative of the target population (Q1 is 1 or 2); **and** there is 60 - 79% participation (Q2 is 2). 'Moderate' may also be assigned if Q1 is 1 or 2 and Q2 is 5 (can't tell).

Weak: The selected individuals are not likely to be representative of the target population (Q1 is 3); **or** there is less than 60% participation (Q2 is 3) **or** selection is not described (Q1 is 4); and the level of participation is not described (Q2 is 5).

B) DESIGN

Strong: will be assigned to those articles that described RCTs and CCTs.

Moderate: will be assigned to those that described a cohort analytic study, a case control study, a cohort design, or an interrupted time series.

Weak: will be assigned to those that used any other method or did not state the method used.

C) CONFOUNDERS

Strong: will be assigned to those articles that controlled for at least 80% of relevant confounders (Q1 is 2); **or** (Q2 is 1).

Moderate: will be given to those studies that controlled for 60 – 79% of relevant confounders (Q1 is 1) **and** (Q2 is 2).

Weak: will be assigned when less than 60% of relevant confounders were controlled (Q1 is 1) **and** (Q2 is 3) **or** control of confounders was not described (Q1 is 3) **and** (Q2 is 4).

D) BLINDING

Strong: The outcome assessor is not aware of the intervention status of participants (Q1 is 2); **and t**he study participants are not aware of the research question (Q2 is 2).

Moderate: The outcome assessor is not aware of the intervention status of participants (Q1 is 2); **or** the study participants are not aware of the research question (Q2 is 2); **or b**linding is not described (Q1 is 3 and Q2 is 3).

Weak: The outcome assessor is aware of the intervention status of participants (Q1 is 1); **and** the study participants are aware of the research question (Q2 is 1).

E) DATA COLLECTION METHODS

Strong: The data collection tools have been shown to be valid (Q1 is 1); **and** the data collection tools have been shown to be reliable (Q2 is 1).

Moderate: The data collection tools have been shown to be valid (Q1 is 1); **and** the data collection tools have not been shown to be reliable (Q2 is 2) **or** reliability is not described (Q2 is 3).

Weak: The data collection tools have not been shown to be valid (Q1 is 2) **or** both reliability and validity are not described (Q1 is 3 and Q2 is 3).

F) WITHDRAWALS AND DROP-OUTS - a rating of:

Strong: will be assigned when the follow-up rate is 80% or greater (Q2 is 1).

Moderate: will be assigned when the follow-up rate is 60 – 79% (Q2 is 2) **OR** Q2 is 5 (N/A).

Weak: will be assigned when a follow-up rate is less than 60% (Q2 is 3) or if the withdrawals and drop-outs were not described (Q2 is 4).

Appendix B: HPSS Schools with Geographical and Socioeconomic Factors

Table B.1. Schools matched based on socioeconomic and geographical setting and randomized into intervention (INT) or control (CON) schools

School	Grade 10 pop.	Minorities %	Aboriginals %	Avg. Family Income	% Low income	School Setting
Frank Hurt Secondary (INT)	248	44%	8.5%	$82,572	13.5	Suburban
Heritage Woods (CON)	308	32.9%	1.0%	$87,091	14.0	Suburban
Panorama Secondary (INT)	305	44%	2.6%	$82,572	13.5	Suburban
Sullivan Heights (CON)	262	44%	1.5%	$82,572	13.5	Suburban
Stelly's Secondary (INT)	262	7.5%	11.1%	$96,013	5.6	Semi-residential/rural
Parklands Secondary (CON)	173	7.5%	1.7%	$96,013	5.6	Semi-resid/rural
Edward Milne (INT)	162	5.0%	20.4%	$80,556	6.0	Rural/Resi-dential
Lake Cowichan (CON)	43	4.5%	18.6%	$72,060	7.9	Rural
Pleasant Valley (CON)	151	1.6%	13.2%	$64,039	6.7	Rural

Appendix C: HPSS BHS Participant Information and Consent Package

Student Assent Form

You are invited to participate in a study entitled: **Health Promoting Secondary Schools (HPSS) - Pilot Project** that is being conducted by Drs. Joan Wharf Higgins, PJ Naylor, Sandy Gibbons, and Ryan Rhodes who are Professors from the School of Exercise Science, Physical and Health Education at the University of Victoria.

Drs. Heather McKay and Heather Macdonald from the Centre for Hip Health & Mobility at the University of BC are also part of the research project.

This consent form is specific to the measurement of your bone health.

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 program or wish further information please contact
In Victoria:

- Karen Strange
- Sandy Courtnall
- Jan Wharf Higgins

In Vancouver:

- Dr. Heather McKay
- Dr. Heather Macdonald
- Ms. Alix Howard

You may verify ethical approval of this study or raise any concerns you might have about your rights or treatment as a participant in this study by contacting the "Human Research Ethics" at UVic and/or UBC Office of Research Services

Physical activity, inactivity and bone health in adolescents: A novel pQCT study

Principal Investigator: Heather McKay Ph
Co-Investigators: Heather Macdonald PhD, Patti-Jean Naylor PhD, Joan Wharf Higgins PhD

Information to Participants:

We would like to thank you for taking the time to read this information. We are inviting you to participate in a health-related research project to answer questions about how physical activity influences bone health in adolescents.

This study is being taken on as a joint investigation between the University of British Columbia; Department or Orthopaedics, Faculty of Medicine and the Center for Hip Health and Mobility. The researchers in this study are extensively experienced in working with children's health and have conducted several large research projects over the years.

Physical activity is essential for growth and development, but we know little about bone adapts its mass and structure to physical activity during the later stages of adolescent growth. We are interested in knowing how the physical activity that you do affects your bone health. Your school is participating in the Health Promoting Secondary Schools Study (HPSS) and as a part of this study, we would like to look more closely at the relationship between your bone health and the physical activity that you do. We are inviting Grade 10 students to participate in our study. Should you choose to participate in our study, you will have your bone structure and muscle strength assessed at the **beginning** of the school year (**September** 2011). The total time you will be in our measurement lab will be approximately 1 hour.

If you agree that you want to participate, please sign the attached consent form and return it to the research assistant. Should you have any questions about this study please contact Thank you for your interest in this study. We look forward to hearing from you.

Sincerely,

Heather McKay, Ph.D.
Professor
University of British Columbia
Department of Orthopaedics

HPSS Bone Health Study Student Assent Form

Procedures:

Your participation in the project will involve one measurement session at either the Bone Health Research Group laboratory at Vancouver General Hospital Research Pavilion or in the Mobile Research Lab that will be located at your school (Vancouver Island schools only). The measurement sessions will take 1 hour (one at the start of the school year and one at the end). You will undergo the following assessments;

1. **Anthropometry:** Measures of height, calf girth, hip girth, waist girth, forearm girth and weight will be taken. Trained staff will take 10 minutes to compete these measures.

2. **Questionnaires:** You will be asked to complete questionnaires that will assess your health history 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. 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 no participant can be identified. All questionnaires will be administered in private (without the presence of your parents/guardians). You do not need to answer any questions which make you feel uncomfortable. All questionnaires will be administered by trained staff and will take approximately 20 minutes.

3. **Muscle strength:** Muscle strength will be assessed by performing a maximal grip strength test on your non-dominant arm and a maximal jump test using both legs. The grip strength test will require you to maximally grip a hand-held instrument for a few seconds, and repeat this test 3 times. The jump test will require you to stand with both feet together, bend your knees and jump as far as high as you can vertically using only your legs. The test will administered by a trained operator and will take 5 minutes.

4. **Bone Densitometer:** Your whole body bone, lean and fat mass will be evaluated by a bone densitometer. This is a machine which takes a picture of 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 10 minutes. Although the bone measurement is X-ray based, the total patient effective dose per session will be 41.2μSv which is less than the background radiation one would receive by taking a return flight from Vancouver to Halifax. To put this in perspective, the annual background radiation in Vancouver due to natural sources is around 1769μSv per year. All bone density measurements will be conducted by a trained operator and take 10 minutes. Please note, if you are pregnant you will be excluded from this part of the study.

5. **pQCT:** Analysis of bone geometry of your non-dominant forearm and left lower leg will be performed using images generated by peripheral quantitative computerized tomography (pQCT). The pQCT involves a minimal amount of radiation (less than 6.0 μSv), that's less than 1 tenth the radiation of a normal chest x-ray, and less than the radiation exposure one would receive flying to Toronto from Vancouver. You will have to remain still with your arm or leg extended into the device for two scans of each limb. A trained operator will conduct the scan which will take 10 minutes. Please note, if you are pregnant you will be excluded from this part of the study.

Possible Harms:
Any doses of radiation could be potentially harmful, but the risks that you will be exposed to are so small that they are difficult to measure. If you have had a lot of x-rays already, you should discuss this with your Study Doctor. Although the bone densitometer scan is x-ray based, the total patient effective dose per session will be 39.5μSv (boys) and 41.2 μSv (girls) which is less than the background radiation one would receive by taking a return flight from Vancouver to Halifax. To put this in perspective, the annual background radiation in Vancouver due to natural sources is around 1769μSv per year. The pQCT scan involves a minimal amount of radiation (6.0 μSV), that's 1 tenth the radiation of a normal chest x-ray, and less than the radiation exposure one would receive flying to Toronto from Vancouver. "The risks from all sources of radiation are cumulative over a lifetime."

Benefits:
If you choose to participate in the HPSS Bone Health Study, you will learn more about how physical activity and muscle strength can contribute to your bone 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. We will provide your parents/guardians with $15 to help offset transportation and parking costs if necessary. In addition, following each measurement session, you will be entered into a draw for an iPod Nano and the winner will be notified once all students have been measured.

Rights and Welfare of the Individual:
Your participation in this research is entirely voluntary. You may withdraw from this study at any time without penalty and without providing reasons for your decision. If you decide to enter the study and to withdraw at any time in the future, there will be no penalty or loss of benefits to which you are otherwise entitled, and your future medical care will not be affected. If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrolment in the study will be retained for analysis. By law, this data cannot be destroyed. Your confidentiality will be respected. However, personal information may be disclosed if required by law. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research 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 BHRG study 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. 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

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

HPSS Bone Health Study Student Assent Form

Please sign this assent form and return it along with the parental consent form on the next page

Student's Assent:

I _____ **voluntarily agree** to participate in the bone health study (anthropometry, questionnaires, muscle strength and bone). I understand that I may withdraw at anytime. 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 results will only be used for scientific objectives.
- I understand that participation in this study is voluntary and 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 shall receive.
- I understand that I don't have to answer any questions that make me uncomfortable.
- 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 me any benefits (if applicable).
- I have read this form and freely consent to participate in this study.
- I have been told that I will receive a dated and signed copy of this form.
- I understand that if I am pregnant, I will not have the bone pictures taken.
- I have as much time as I want to decide to be part of the study,I have also been asked to talk about my choice to be in this study with my parents.

I have had the opportunity to read this consent form, to ask questions about my participation in this research, and to discuss my participation with my parents/guardians.** All my questions have been answered. I understand that I may withdraw from this research at any time, and that this will not interfere with the availability to me of other health care. I have received a copy of this consent form. I assent to participate in this study.

Signatures

_____	_____	_____
Printed name of child	Signature	Date
_____	_____	_____
Printed name of Witness	Signature	Date

The child 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.

_____	_____	_____
Printed name of Principal Investigator	Signature	Date

Parent's Consent Statement:

I, _____

(please print the name of one or both parents/guardians) the parent/guardian of

_____
(please print child's first and last name) understand the purpose and procedures of this program as described.

_____	_____
Signature of Parent/Guardian	**Date**

Appendix D: Dual Energy X-ray Absorptiometry Screening Form

HEALTH PROMOTING SECONDARY SCHOOLS

ID:
Date: _____

Bone Health Study

DXA Information Sheet

Fall 2011

SCAN TECHNICIAN: _____

DXA Checklist:

1. Any X-rays within the last 6 months?
2. Any metal implants?
3. Removed ALL metal items – watches, underwire brassiere, jewelry such as rings, ear-rings, piercings?
4. Properly attired – no zippers, buttons, metal eyelets on clothing?
5. Any possibility of pregnancy?
    a. Methods of birth control (OCP brand, injections, IUDs, others)

Comments:

_____

_____

_____

_____

_____

Appendix E: pQCT Screening Form

HEALTH PROMOTING SECONDARY SCHOOLS

ID:
Date: _____

Bone Health Study
pQCT Information Sheet
Fall 2011

SCAN TECHNICIAN: _____

Required medical information:

1. Do you have a history of bone fracture?
2. Have you had X-rays within the last 6 months?
3. Are you on any medication that could affect the bone scan results?
4. Are you at risk of being pregnant?

Scan site	Limb scanned	Radiation dose (μSV)	If scan has been repeated, please detail why

NOTE: Please complete the information in the table for each scan performed. If you repeat a scan, you will need to complete the table for each scan completed.

Total radiation dose:

Appendix F: Physical Activity Questionnaire for Adolescents (PAQ-A)

HEALTH PROMOTING SECONDARY SCHOOLS

ID: _____
Checked by: _____

Bone Health Study
Physical Activity Questionnaire – Adolescents
Fall 2011

Date : _____dd_____mm_____yyyy

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 does not 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	_____
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. Last week, how many hours per day, on average, did you watch television, movies, played video games or sit in front of a computer or smart phone? 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!

Appendix G: Girl's Maturity Questionnaire

ID#: _____ Checked By: _____

HEALTH PROMOTING SECONDARY SCHOOLS

Self-Assessment of Maturity Survey for Girls - Spring 2012

Today's Date: _____dd _____ mm _____ yyyy

1. Have you had your first menstrual period?

___ Yes ____No

If Yes ...

a) Do you know the date of your first menstrual period?

_____

b) What was the date of your last menstrual period?

_____

c) Is there a possibility that you might be pregnant?

_____Yes _____No

2. Do you take oral contraceptives (birth control pills or injections)?

_____Yes _____No

If Yes ...

a) Do you know the brand name of oral contraceptives that you take?

_____

Appendix H: Boy's Maturity Questionnaire

HEALTH PROMOTING SECONDARY SCHOOLS

Self-Assessment of Maturity Survey for Boys – Fall 2011

Today's Date: _____ dd _____ mm _____yyyy

1. How much underarm hair do you have now? Circle the one that describes you best.

   a. None at all

   b. I have a small amount of fine, light, underarm hair

   c. I have hair under my arms that is slightly coarse and dark and covers a small area

   d. I have hair under my arms that is as dark and coarse as that of an adult male, but the hair doesn't cover my entire underarm

   e. As much underarm hair as I will probably ever have (dark and coarse, covers entire underarm)

Appendix I: Health History Questionnaire (HHQ)

HEALTH PROMOTING SECONDARY SCHOOLS

ID: _____
Checked by: _____

Bone Health Study
Health History Questionnaire
Spring 2012

Date: _____dd_____mm_____yyyy

We would like you to answer the following questions so that we can better understand your current health and family history.

REMINDER ANY INFORMATION CONTAINED HEREIN WILL BE TREATED AS CONFIDENTIAL

1. In which country were your **parents** born?

   Mother: _____ Father: _____

2. In which country were your **grandparents** born?

   Mother's mother: _____ Mother's father: _____

   Father's mother: _____ Father's father: _____

3. How long have you lived in North America? _____ years

4. How long have your parents lived in North America? Mother: _____ years

   Father: _____ years

   a) If relevant, in which country did your parents lived before moving to North America?

   Mother: _____ Father: _____

5. What is your family's ethnicity? *(Please tick more than one, if applicable)*

   - ○ White
   - ○ Métis
   - ○ Inuit
   - ○ Arab
   - ○ Black
   - ○ Filipino
   - ○ Korean
   - ○ Other – Specify _____
   - ○ Latin America
   - ○ North American Indian
   - ○ Chinese
   - ○ West Asian (e.g., Iranian, Afghan, etc.)
   - ○ South Asian (e.g., East Indian, Pakistani, Sri Lankan, etc.)
   - ○ Southeast Asian (e.g., Vietnamese, Cambodian, Malaysian, Laotian, etc.)
   - ○ Japanese

6. Are you right handed or left handed? O Right O Left

7. Are you on a special diet? O Yes O No

If **yes:** _____ vegetarian
_____ dairy-free
_____ low sodium
_____ low cholesterol
_____other (Please specify: _____)

8. Do you drink milk every day? O Yes O No

If **yes:** How many cups per day? _____

9. Do you currently take any medications? O Yes O No

If **yes:** What medication(s) are you taking? _____

10. Have you ever broke any bones, were hospitalized, confined to bed or had a limb immobilized (i.e. arm in a cast)?

O Yes O No

If **yes:** List the affected area or condition, approximate date and duration involved.
(Example: Fracture right wrist (or lower arm) July 2010 6 weeks)

Condition **Date** **Duration**

_____

_____

_____

If relevant, 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)

11. Have you ever been sick for more than a month?

O Yes O No

If yes, what did you have? _____

12. Has any member of your family been diagnosed with osteoporosis?

O Yes O No

If **yes**, who? _____

13. Has any member of your family been diagnosed with cardiovascular disease or stroke?

O Yes O No

If **yes**, who? _____

14. Do you drink cola/ pop/ fizzy/ carbonated drinks? O Yes O No

- O Sometimes
- O One to two cans per day
- O Three cans or more per day

15. Do you drink coffee? O Yes O No

- O One or two cups a day
- O Three or more cups a day
- O Sometimes

16. Have you ever drunk some kind of alcoholic beverage? O Yes O No

16.1 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 Don't know
O I've only tasted or had a sip from someone else's drink.

16.2 At what age did you start to drink alcohol? _____

17. Have you ever smoked tobacco products (cigarettes, cigars, or nicotine based products)? O Yes O No

17.1 Have you ever smoked for six months or more? O Yes O No

17.2 How long did you smoke? _____

17.3 Do you still smoke?

O Yes, daily
O Yes, occasionally
O No, not at all

17.4 When you are/were smoking how many cigarettes do/did you usually smoke per day?

About _____ per day

17.5 At what age did you start to smoke daily? _____

18. Have you, or your immediate family(mother, father and / or siblings), ever had:

		WHO
18.1 Rheumatoid arthritis	O Yes O No	_____
18.2 Osteoporosis	O Yes O No	_____
18.3/18.4 An overactive/underactive thyroid Or parathyroid gland	O Yes O No	_____
18.5 Alcoholism	O Yes O No	_____
18.6 Chronic liver disease	O Yes O No	_____
18.7 Cancer	O Yes O No	_____
18.8 Stomach ulcers	O Yes O No	_____
18.9 Lactase deficiency (inability to digest milk)	O Yes O No	_____
18.10 Eating Disorders (anorexia nervosa or bulimia)	O Yes O No	_____

19. For the following medications, please circle whether you have taken them in the past, currently taking them, or have never taken the drug:

19.1 Cortisone or similar drug	O Past	O Currently	O Never
19.2 Anabolic steroids	O Past	O Currently	O Never
19.3 Thyroid hormone pills	O Past	O Currently	O Never
19.4 Asthma medication	O Past	O Currently	O Never
19.5 Medication for heartburn or indigestio (eg Tums, Rolaids, Maalox or Mylanta antacids)	O Past	O Currently	O Never
19.6 Lithium (for over one year) (a mood stabilizing medication)	O Past	O Currently	O Never
19.7 Anticonvulsant drugs	O Past	O Currently	O Never

Appendix J: Food Frequency Questionnaire (FFQ)

HEALTH PROMOTING SECONDARY SCHOOLS

ID: _____
Checked by: _____

Bone Health Study

Food Frequency Questionnaire
Fall 2011

_____ *dd* _____ *mm* _____ *yyyy*

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

I usually drink (check ✔ ***one***):

O milk
O flavoured milk (chocolate, strawberry, etc.)
O soy milk
O rice milk
O almond 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.

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.

Please Note: This question is **not about any medications you may be taking.*

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

Appendix K: Systematic Review Search Terms

Example of database search from Medline.

Database: Ovid MEDLINE(R) 1948 to Present with Daily Update
Search Strategy:
---

1 Bone Density/ (33670)
2 (bone? adj3 densit$).mp. (44991)
3 bone development/ or calcification, physiologic/ or osteogenesis/ (31885)
4 (bone? adj3 develop$).mp. (21671)
5 (bone? adj3 strength$).mp. (4097)
6 bone microstructure.mp. (226)
7 (bone? adj3 structure$).mp. (4377)
8 (bone? adj3 mass$).mp. (13262)
9 (bone? adj4 geometr$).mp. (1033)
10 (bone? adj4 health$).mp. (4011)
11 "bone and bones"/ or "bones of lower extremity"/ or leg bones/ or femur/ or femur head/ or femur neck/ or fibula/ or tibia/ or pelvic bones/ or acetabulum/ or ilium/ or ischium/ or pubic bone/ or "bones of upper extremity"/ or arm bones/ or humerus/ or humeral head/ or radius/ or ulna/ or olecranon process/ or diaphyses/ or epiphyses/ or growth plate/ (150561)
12 or/1-11 (212451) [Bone Concepts]
13 Motor Activity/ (63436)
14 exercise/ or muscle stretching exercises/ or resistance training/ or movement/ or locomotion/ or running/ or jogging/ or swimming/ or walking/ or musculoskeletal development/ or bone development/ or calcification, physiologic/ or osteogenesis/ or chondrogenesis/ or muscle development/ (188536)
15 exercise therapy/ or motion therapy, continuous passive/ or muscle stretching exercises/ or resistance training/ (23015)
16 ((locomotor or physical or motor) adj4 (activit$ or exercis$)).tw. (77226)
17 sports/ or athletic performance/ or physical endurance/ or baseball/ or basketball/ or bicycling/ or boxing/ or football/ or golf/ or gymnastics/ or hockey/ or martial arts/ or tai ji/ or mountaineering/ or racquet sports/ or tennis/ or running/ or jogging/ or skating/ or snow sports/ or skiing/ or soccer/ or swimming/ or diving/ or "track and field"/ or volleyball/ or walking/ or weight lifting/ or wrestling/ (91158)
18 (bone? adj4 (exercis$ or activit$)).tw. (7594)
19 or/13-18 (349093) [Physical activity]
20 Tomography, X-Ray Computed/ (238285)
21 Magnetic Resonance Imaging/ (225683)
22 densitometry/ or absorptiometry, photon/ (25220)
23 densitometr$.tw. (12150)
24 Absorptiometry, Photon/ (14022)
25 (DXA or DEXA).mp. (7245)
26 absorptiometry, photon/ or tomography, x-ray/ or tomography, x-ray computed/ or x-ray microtomography/ (256410)

27 pQCT.mp. (940)
28 HRpQCT.mp. (8)
29 HR pQCT.mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] (80)
30 MRI.mp. (99312)
31 hip structural analys$.mp. (49)
32 or/20-22,24,26 (447336) [Key imaging tools]
33 (child$ or youth or adolescen$ or pediatr$ or paediatr$).mp. (2391639)
34 limit 32 to ("preschool child (2 to 5 years)" or "child (6 to 12 years)" or "adolescent (13 to 18 years)") (83586)
35 or/33-34 (2391639) [Specific age filter]
36 Randomized Controlled Trials as Topic/ (73551)
37 randomized controlled trial/ (308680)
38 Random Allocation/ (71716)
39 Double Blind Method/ (110673)
40 Single Blind Method/ (15068)
41 clinical trial/ (463340)
42 clinical trial, phase i.pt. (11252)
43 clinical trial, phase ii.pt. (17849)
44 clinical trial, phase iii.pt. (6188)
45 clinical trial, phase iv.pt. (614)
46 controlled clinical trial.pt. (82595)
47 randomized controlled trial.pt. (308680)
48 multicenter study.pt. (131496)
49 clinical trial.pt. (463340)
50 exp Clinical Trials as topic/ (242227)
51 or/36-50 (859949)
52 (clinical adj trial$).tw. (155365)
53 ((singl$ or doubl$ or treb$ or tripl$) adj (blind$3 or mask$3)).tw. (108007)
54 PLACEBOS/ (29747)
55 placebo$.tw. (129660)
56 randomly allocated.tw. (12604)
57 (allocated adj2 random$).tw. (14841)
58 or/52-57 (327045)
59 51 or 58 (955341)
60 case report.tw. (158507)
61 letter/ (716707)
62 historical article/ (275243)
63 or/60-62 (1140604)
64 59 not 63 (929222) [RCT search filter]
65 Epidemiologic studies/ (5035)

66 exp case control studies/ (508092)
67 exp cohort studies/ (1100847)
68 Case control.tw. (55247)
69 (cohort adj (study or studies)).tw. (52861)
70 Cohort analy$.tw. (2460)
71 (Follow up adj (study or studies)).tw. (31463)
72 (observational adj (study or studies)).tw. (26366)
73 Longitudinal.tw. (101411)
74 Retrospective.tw. (193988)
75 Cross sectional.tw. (109554)
76 Cross-sectional studies/ (125340)
77 or/65-76 (1462658) [Observational search filter end]
78 and/12,19,32 (3109) [Combination bones, PA, key measures]
79 and/64,78 (422) [With RCT filter]
80 and/77-78 (982) [With Observational studies filter]
81 and/35,79 (118) [Medline age limits RCTs]
82 and/35,80 (455) [Medline age limits Observationals]
83 limit 82 to humans (453) [Medline human limits Observationals]
84 limit 81 to humans (117) [Medline human limits RCTs]

****

Appendix L: Additional Data for Chapter 4

Table L.1. Baseline descriptive variables of participants with and without accelerometry data in boys and girls, reported as mean (SD).

	Boys		Girls	
	Without accel n=50	**With accel n=40**	**Without accel n=63**	**With accel n=48**
Age (yrs)	15.4 (0.4)	15.2 (0.3)	15.3 (0.3)	15.4 (0.4)
White/Asian/Other				-
Years post APHV	2.0 (0.6)	1.8 (0.5)	3.2 (0.6)	3.0 (0.5)
Age at menarche (yrs)	-	-	12.5 (1.1)	12.3 (1.1)
Height (cm)	173.4 (7.5)	174.9 (6.0)	164.1 (6.9)	162.0 (5.7)
Weight (kg)	62.0 (11.9)	65.3 (10.1)	58.6 (11.0)	59.1 (10.2)
Tibial length (cm)	40.0 (2.3)	40.9 (18.9)	37.3 (2.0)	36.9 (2.1)
Ulna length (cm)	27.6 (1.6)	27.9 (1.3)	25.7 (1.3)	25.2 (1.2)*
Fat mass (kg)	9.3 (5.7)	10.0 (5.5)	15.9 (6.1)	15.8 (6.6)
Lean mass (kg)	44.2 (7.7)	47.3 (6.3)	36.4 (5.5)	36.1 (4.4)
Grip strength (kg)	34.2 (7.9)	35.4 (7.4)	26.7 (5.5)	26.6 (4.4)
Tibia MCSA (cm^2)	4003.8 (707.2)	4383.3 (744.6)	3745.8 (649.9)	3825.8 (572.4)
Radius MCSA (cm^2)	1678.2 (298.2)	1707.9 (250.7)	1240.1 (228.2)	1251.0 (185.2)
Dietary calcium (mg/day)	1300 (720)	1302 (799)	992 (629)	938 (626)
PAQ-A				
PA Score	2.7 (0.6)	2.8 (0.5)	2.6 (0.5)	2.6 (0.6)
MVPA (min/day)	104.7 (75.6)	123.6 (82.2)	73.6 (50.6)	78.1 (53.1)

APHV, age at peak height velocity; MCSA, muscle cross-sectional area; PAQ-A, physical activity questionnaire for adolescents; PA, physical activity;
MVPA, moderate-to-vigorous physical activity.
*Significant at $p<0.05$

Table L.2. Baseline correlations of bone and dependent variables in boys (n=89) and girls (n=109) at the 8% tibia.

Tibia	**Boys**			**Girls**		
8% site	**BSI**	**Tt.Ar**	**Tt.Dn**	**BSI**	**Tt.Ar**	**Tt.Dn**
Age (years)	0.21	0.002	0.18	0.04	0.05	-0.006
Ethnic	-0.17	-0.39**	0.13	0.11	-0.25**	0.26**
Years post APHV	0.26*	0.08	0.15	0.10	0.23*	-0.04
Age at menarche (years)	-	-	-	0.30**	-0.19	0.42**
Axillary hair stages	0.29**	0.06	0.21	-	-	-
Height (cm)	0.39**	0.40**	0.03	0.12	0.51**	-0.19*
Weight (kg)	0.51**	0.48**	0.08	0.57**	0.40**	0.33**
Calcium (mg/day)	0.22*	-0.14	0.28**	-0.06	-0.14	0.02
PA score	0.04	0.21*	-0.10	0.18	0.08	0.13
$MVPA_{PAQ}$ (min/day)	0.25*	0.17	0.10	0.24*	0.15	0.15
MCSA 50% (cm^2)	0.45**	0.32**	0.16	0.56**	0.35**	0.33**
Grip strength (kg)	0.43**	0.42**	0.08	0.45**	0.42**	0.18
Tibial length (cm)	0.18	0.28**	-0.08	0.10	0.43**	-0.17
Lean mass (kg)	0.60**	0.58**	0.10	0.66**	0.56**	0.33**
Fat mass (kg)	0.18	0.17	0.007	0.35**	0.15	0.27**
Total counts (cpm)	-0.12	0.01	-0.08	0.36**	0.26*	0.118
Wear time (min/day)	-0.08	0.03	-0.13	0.08	0.04	0.04
SED_{accel} (min/day)	0.16	0.11	0.03	-0.12	-0.23	0.01
MPA_{accel} (min/day)	-0.15	0.17	-0.24	0.31*	0.35**	0.10
VPA_{accel} (min/day)	0.04	0.03	-0.006	0.38**	0.35**	0.14
$MVPA_{accel}$ (min/day)	-0.06	0.11	-0.14	0.40**	0.41**	0.14

BSI, bone strength index (mg^2/mm^4); Tt.Ar, total area (mm^2); Tt.Dn, total density (mg/cm^3); APHV,age at peak height velocity, MVPA, moderate-to-vigorous physical activity; $_{PAQ}$,using the physical activity questionnaire for adolescents; MCSA 50%, tibial muscle cross-sectional area; SED , sedentary time;$_{accel,}$ by accelerometer;MPA, moderate physical activity; VPA, vigorous physical activity.

Note: Correlations did not account for clustering

* $p<0.05$, ** $p<0.01$.

Table L.3. Baseline correlations of bone and dependent variables in boys (n=89) and girls (n=109) at the 50% tibia.

Tibia			**Boys**					**Girls**		
50% site	**SSI_p**	**Tt.Ar**	**Ct.Ar**	**Me.Ar**	**Ct.Dn**	**SSI_p**	**Tt.Ar**	**Ct.Ar**	**Me.Ar**	**Ct.Dn**
Age (years)	0.16	0.13	0.11	0.10	0.26*	0.04	0.02	0.06	-0.05	0.7
Age at menarche (years)	-	-	-	0.06	-	0.11	0.05	0.12	-0.08	0.40**
Years post APHV	0.41**	0.38**	0.32**	0.31**	0.24*	0.21*	0.18	0.17	0.10	0.07
Ethnic	0.35**	0.32**	0.18	-0.26	0.32**	-0.16	-0.15	0.38**	-0.03	-0.09
Axillary hair stages	0.27*	0.25*	0.26*	0.12	0.17	-	-	-	-	-
Height (cm)	0.65**	0.65**	0.64**	0.38**	0.007	0.40**	0.40**	0.39**	0.23*	-0.18
Weight (kg)	0.74**	0.73**	0.71**	0.45**	0.008	0.64**	0.64**	0.65**	0.33**	-0.14
Calcium (mg/day)	0.16	0.13	0.19	-0.004	0.04	0.10*	-0.10	-0.07	-0.11	-0.04
PA score	0.14	0.14	0.16	0.06	-0.03	0.14	0.12	0.20*	-0.05	-0.01
$MVPA_{PAQ}$ (min/day)	0.31**	0.28**	0.34**	0.07	0.12	0.18	0.18	0.22*	0.04	-0.12
MCSA 50% (cm^2)	0.56**	0.55**	0.60**	0.24*	-0.11	0.60**	0.61**	0.58**	0.37**	-0.07
Grip strength (kg)	0.59**	0.58**	0.58**	0.27*	-0.04	0.52**	0.51**	0.53**	0.25**	-0.24*
Tibial length (cm)	0.55**	0.57**	0.55**	0.36**	-0.28	0.39**	0.40**	0.39**	0.24*	-0.28*
Lean mass (kg)	0.80**	0.79**	0.82**	0.41**	-0.11	0.77**	0.76**	0.80**	0.34**	-0.20
Fat mass (kg)	0.38**	0.37**	0.29**	0.34**	0.05	0.35**	0.36**	0.36**	0.19	-0.11
Total counts (cpm)	-0.12	-0.10	-0.18	0.08	-0.04	0.23	0.20	0.27*	0.001	-0.07
Wear time (min/day)	0.002	-0.003	-0.06	0.08	-0.14	0.03	0.02	0.05	-0.04	-0.03
SED_{accel} (min/day)	0.21	0.16	0.14	0.12	0.001	-0.16	-0.18	-0.21	-0.03	0.19
VPA_{accel} (min/day)	0.05	0.06	-0.001	0.13	-0.02	0.35**	0.30*	0.41**	0.02	-0.04
$MVPA_{accel}$ (min/day)	0.11	0.12	0.04	0.19	-0.01	0.38**	0.35**	0.44**	0.03	-0.14

SSI_p, polar strength strain index (mm^3), Tt.Ar, total area (mm^2); Ct.Ar, cortical area (mm^2); Me.Ar, medullary area (mm^2); Ct.Dn, cortical density (mg/cm^3); PHV, age at peak height velocity, MVPA, moderate-to-vigorous physical activity; $_{PAQ}$,using the physical activity questionnaire for adolescents; MCSA 50%, tibial muscle cross-sectional area; SED , sedentary time;$_{accel,}$ by accelerometer;MPA, moderate physical activity; VPA, vigorous physical activity.

Note: Correlations did not account for clustering

* $p<0.05$, ** $p<0.01$.

Table L.4. Baseline correlations of bone and dependent variables in boys (n=88) and girls (n=110) at the 7% radius.

Radius		**Boys**			**Girls**	
7% site	**BSI**	**Tt.Ar**	**Tt.Dn**	**BSI**	**Tt.Ar**	**Tt.Dn**
Age (years)	0.27*	0.07	0.25*	0.06	0.08	0.008
Ethnic	-0.17	-0.47**	0.10	0.20*	-0.23*	0.27**
Years post APHV	0.24*	0.03	0.24*	0.05	0.08	0.008
Age at menarche (years)	-	-	-	0.46**	-0.14	0.50**
Axillary hair stages	0.33**	0.07	0.33**	-	-	-
Height (cm)	0.36**	0.39**	0.13	-0.04	0.37**	-0.21*
Weight (kg)	0.42**	0.55**	0.09	0.46**	0.34**	0.26**
Calcium (mg/day)	0.25*	0.04	0.12	-0.07	0.001	-0.05
PA score	0.05	0.16	-0.07	0.007	0.21*	-0.08
$MVPA_{PAQ-A}$ (min/day)	0.26**	0.11	0.23*	0.07	0.24*	-0.05
MCSA 30% (cm^2)	0.42**	0.54**	0.33**	0.65**	0.57**	0.13
Grip strength (kg)	0.33**	0.64**	0.21	0.58**	0.50**	0.12
Ulnar length (cm)	-0.02*	0.29**	0.10	0.27*	0.20*	0.002
Lean mass (kg)	0.37**	0.22**	0.17	0.56**	0.51**	0.18
Fat mass (kg)	0.20*	0.27*	-0.07	0.07	0.11	0.25**
Total counts (cpm)	-0.04	-0.12	0.04	0.05	0.27*	-0.06
Wear time (min/day)	-0.06	-0.03	-0.06	0.07	-0.02	0.07
SED_{accel} (min/day)	-0.10	0.12	0.02	0.10	-0.32*	0.21
MPA_{accel} (min/day)	-0.05	-0.05	-0.01	-0.05	0.41**	-0.20
VPA_{accel} (min/day)	0.11	-0.06	0.12	0.10	0.24	-0.01
$MVPA_{accel}$ (min/day)	0.04	-0.06	0.06	0.03	0.37**	-0.12

BSI, bone strength index (mg^2/mm^4); Tt.Ar, total area (mm^2); Tt.Dn, total density (mg/cm^3); PHV, age at peak height velocity, MVPA, moderate-to-vigorous physical activity; $_{PAQ}$,using the physical activity questionnaire for adolescents; MCSA 30%, radial muscle cross-sectional area; SED , sedentary time;$_{accel,}$ by accelerometer;MPA, moderate physical activity; VPA, vigorous physical activity.

Note: Correlations did not account for clustering

* $p<0.05$, ** $p<0.01$.

Table L.5. Baseline correlations of bone and dependent variables in boys (n=89) and girls (n=111) at the 30% radius

Radius			**Boys**					**Girls**		
30% site	**SSI_p**	**Tt.Ar**	**Ct.Ar**	**Me.Ar**	**Ct.Dn**	**SSI_p**	**Tt.Ar**	**Ct.Ar**	**Me.Ar**	**Ct.Dn**
Age (years)	0.18	0.16	0.16	0.10	0.32**	0.01	0.05	0.04	0.04	0.17
Ethnic	-0.32**	-0.34**	-0.28**	-0.31*	0.15	-0.06	-0.05	-0.05	-0.05	0.20*
Years post APHV	0.26*	0.25*	0.25*	0.15	0.23	0.10	0.08	0.08	0.04	0.11
Age at menarche (years)	-	-	-	-	-	0.15	0.11	0.12	0.06	0.51**
Axillary hair stages	0.26*	0.21*	0.25*	0.08	0.14	-	-	-	-	-
Height (cm)	0.53**	0.53**	0.54**	0.31**	-0.03	0.30**	0.29**	0.27**	0.18	-0.15*
Weight (kg)	0.63**	0.65**	0.63**	0.43**	0.01	0.46**	0.42**	0.47**	0.15	-0.03
Calcium (mg/day)	0.21*	0.16	0.20	0.02	0.11	-0.07	-0.07	-0.08	-0.01	-0.01
PA score	0.08	0.08	0.08	0.07	-0.005	0.19*	0.19*	0.23*	0.05	-0.05
$MVPA_{PAQ}$ (min/day)	0.26*	0.21	0.27*	0.03	0.13	0.24*	0.24*	0.28**	0.08	-0.14
MCSA 30% (cm^2)	0.70**	0.66**	0.77**	0.23*	-0.06	0.61**	0.65**	0.68**	0.31**	-0.19*
Grip strength (kg)	0.72**	0.70**	0.75**	0.36**	0.02	0.53**	0.53**	0.53**	0.29**	-0.19
Ulnar length (cm)	0.45**	0.46**	0.50**	0.23*	-0.07	0.19	0.16	0.20*	0.02	-0.05
Lean mass (kg)	0.74**	0.74**	0.76**	0.43**	-0.05	0.60**	0.59**	0.63**	0.26**	-0.11
Fat mass (kg)	0.24*	0.28**	0.21	0.29**	0.06	0.19	0.14	0.19*	0.007	0.03
Total counts (cpm)	-0.23	-0.22	-0.29	-0.07	0.03	0.20	0.23	0.28*	0.05	-0.40**
Wear time (min/day)	-0.01	0.04	-0.07	-0.19	-0.22	-0.11	-0.05	-0.09	0.03	-0.07
SED_{accel} (min/day)	0.24	0.27	0.21	0.25	-0.14	-0.19	-0.19	-0.24	-0.004	0.25*
VPA_{accel} (min/day)	-0.11	-0.10	-0.13	0.008	-0.01	0.22	0.24	0.25	0.10	-0.31*
$MVPA_{accel}$ (min/day)	-0.11	-0.09	-0.16	0.07	0.06	0.25	0.27*	0.29*	0.10	-0.40**

SSI_p, polar strength strain index (mm^3), Tt.Ar, total area (mm^2); Ct.Ar, cortical area (mm^2); Me.Ar, medullary area (mm^2); Ct.Dn, cortical density (mg/cm^3); APHV, age at peak height velocity, MVPA, moderate-to-vigorous physical activity; $_{PAQ}$,using the physical activity questionnaire for adolescents; MCSA 30%, radial muscle cross-sectional area; SED , sedentary time;$_{accel,}$ by accelerometer;MPA, moderate physical activity; VPA, vigorous physical activity.

Note: Correlations did not account for clustering

* p<0.05, ** p<0.01.

Appendix M: Additional Data for Chapter 5

Table M.1. Boys' (n=76) Pearson correlations of descriptive variables (baseline and 30-week changes) – age, maturity-offset, height, weight, tibial length, lean and fat mass, muscle cross-sectional area (MCSA) at 50% tibial site, 30-week average physical activity questionnaire for adolescents (PAQ-A) score, PAQ-A moderate-to-vigorous physical activity (MVPA) and dietary calcium with change in bone strength index (BSI), total bone area (Tt.Ar) and total bone density (Tt.Dn) at the distal tibia (8% site, n=75) and polar stress-strain index (SSI_p), total bone area (Tt.Ar), cortical bone area (Ct.Ar), medullary area (Me.Ar) and cortical bone density (Ct.Dn) at the tibial shaft (50%, n=75) and bivariate correlations (using Spearman's rank correlation) with ethnic grouping and axillary hair stage.

	Distal tibia (8% site)			**Tibial midshaft (50% site)**				
	BSI Δ	**Tt.Ar Δ**	**Tt.Dn Δ**	**SSI_p Δ**	**Tt.Ar Δ**	**Ct.Ar Δ**	**Me.Ar Δ**	**Ct.Dn Δ**
Age	-0.15	0.97	-0.15	-0.29*	-0.31**	-0.27*	-0.13	-0.11
Ethnic group	-0.17	0.16	-0.08	-0.29*	-0.26*	-0.26*	0.0003	0.11
Baseline years post APHV	-0.09	0.11	-0.15	-0.21	-0.32**	-0.35**	-0.01	0.17
Baseline axillary hair stage	0.05	0.17	-0.10	-0.16	-0.19	-0.18	-0.04	-0.16
Final axillary hair stage	0.14	0.16	-0.07	0.06	-0.01	-0.02	0.005	-0.14
Baseline height	0.17	0.03	0.04	0.12	-0.05	-0.07	0.03	0.12
Height change	0.03	-0.03	0.05	0.67**	0.66**	0.55**	0.36**	0.16
Baseline weight	0.18	0.06	0.01	0.08	-0.0008	0.13	-0.25*	-0.21
Weight change	0.12	0.06	0.06	0.17	0.20	0.21	0.03	-0.05
Baseline tibial length	0.04	0.09	-0.02	0.21	0.12	0.07	0.13	0.07
Tibial length change	0.01	-0.06	0.06	0.52**	0.71**	0.49**	0.58**	0.02
Baseline lean mass	0.31**	0.01	0.10	0.20	0.06	0.16	-0.19	-0.09
Lean mass change	0.02	0.29**	-0.13	0.22	0.15	0.26**	-0.17	-0.04
Baseline fat mass	-0.07	0.10	-0.10	-0.08	-0.03	0.07	-0.22	-0.30*
Fat mass change	0.02	0.02	-0.10	-0.07	-0.06	-0.03	-0.07	-0.06
Baseline MCSA 50% site	0.32**	-0.007	0.13	0.02	-0.08	0.06	-0.28*	-0.08
MCSA 50% site change	0.39**	-0.04	0.29*	0.50**	0.51**	0.56**	0.003	-0.06
Average PAQ score	0.04	0.11	-0.09	0.12	0.11	0.15	-0.04	-0.15
Average PAQ MVPA	-0.03	0.14	-0.17	0.06	-0.02	0.06	-0.14	0.13
Average dietary calcium	-0.09	0.08	-0.10	0.10	0.16	0.09	0.19	-0.11
Sedentary time change[†]	0.08	0.03	0.03	0.24	0.21	0.10	0.22	0.17
MVPA change[†]	0.29	0.07	0.25	0.11	0.04	0.05	0.004	0.03
MPA change[†]	0.28	0.05	0.21	0.17	0.01	0.02	0.004	0.16
VPA change[†]	0.23	0.07	0.22	0.04	0.05	0.06	0.004	-0.08

Note: Correlations do not account for clustering
*p<0.05, **p<0.01
[†]n=28

Table M.2. Boys' (n=76) Pearson correlations of descriptive variables (baseline and 30-week changes) – age, maturity-offset, height, weight, tibial length, lean and fat mass, muscle cross-sectional area (MCSA) at 30% radius site, 30-week average physical activity questionnaire for adolescents (PAQ-A) score, PAQ-A moderate-to-vigorous physical activity (MVPA) and dietary calcium with change in bone strength index (BSI), total bone area (Tt.Ar) and total bone density (Tt.Dn) at the distal radius (7% site, n=74) and polar stress-strain index (SSIp), total bone area (Tt.Ar), cortical bone area (Ct.Ar), medullary area (Me.Ar) and cortical bone density (Ct.Dn) at the radius shaft (50%, n=76) and bivariate correlations (using Spearman's rank correlation) with ethnic grouping and axillary hair stage.

	Distal radius (7% site)			**Radius shaft (30% site)**				
	BSI Δ	**Tt.Ar Δ**	**Tt.Dn Δ**	**SSI_p Δ**	**Tt.Ar Δ**	**Ct.Ar Δ**	**Me.Ar Δ**	**Ct.Dn Δ**
Age	-0.14	-0.13	-0.11	-0.24*	-0.32**	-0.26*	-0.22	-0.03
Ethnic group	0.03	-0.03	0.13	-0.01	-0.06	-0.04	-0.06	0.16
Baseline maturity	-0.09	-0.19	-0.008	-0.14	-0.23*	-0.15	-0.24*	0.16
Baseline axillary	-0.27*	-0.17	-0.14	-0.35*	-0.24*	-0.18	-0.23*	-0.0006
Final axillary	-0.21	-0.15	-0.15	-0.30*	-0.26*	-0.21	-0.20	-0.02
Baseline height	-0.01	-0.23	0.0009	-0.15	-0.14	-0.09	-0.16	0.13
Height change	-0.07	0.49**	-0.24**	0.35**	-0.51**	0.42**	0.33**	-0.02
Baseline weight	0.03	-0.08	-0.07	-0.05	-0.12	-0.09	-0.11	0.05
Weight change	-0.17	0.12	-0.17	0.08	0.14	0.15	0.001	0.009
Baseline radius length	-0.09	-0.01	-0.11	-0.11	-0.10	-0.06	-0.11	0.10
Radius length change	-0.03	0.39**	-0.21	0.27*	0.38**	0.39**	0.05	0.04
Baseline lean mass	0.04	-0.14	-0.06	-0.06	-0.08	-0.03	-0.14	0.10
Lean mass change	-0.21	-0.03	-0.13	-0.02	0.03	0.08	-0.12	-0.003
Baseline fat mass	-0.02	0.07	-0.09	0.0007	-0.11	-0.09	-0.06	-0.07
Fat mass change	-0.18	-0.13	-0.08	0.007	-0.03	-0.02	-0.02	0.11
Baseline MCSA 30% site	0.20	-0.16	0.07	-0.09	-0.08	-0.04	-0.12	0.11
MCSA 30% site change	-0.03	0.30*	-0.18	0.17	0.30**	0.26*	0.14	0.22
Grip strength change	-0.11	0.03	-0.10	0.02	0.13	0.12	0.07	0.05
Average PAQ score	-0.16	0.11	-0.23	-0.02	0.06	0.05	0.04	-0.11
Average PAQ MVPA	-0.25*	-0.02	-0.26	-0.07	-0.03	-0.03	-0.004	0.05
Average dietary calcium	-0.10	-0.10	-0.10	-0.06	-0.02	-0.006	-0.04	-0.06
Sedentary time change[†]	-0.24	-0.12	-0.17	0.25	0.22	0.20	0.07	0.16
MVPA change[†]	0.28	-0.19	0.14	0.05	0.29	0.31	-0.05	-0.25
MPA change[†]	0.18	0.009	0.08	0.07	0.21	0.30	-0.26	-0.09
VPA change[†]	0.28	0.29	0.15	0.03	0.28	0.24	0.12	-0.31

Note: Correlations do not accout for clustering

*p<0.05, **p<0.01

[†]n=28

Table M.3. Pearson correlations of anthropometric, body composition, grip strength with baseline values and 30-week change, average of physical activity questionnaire for adolescents (PAQ-A) scores and minutes of moderate-to-vigorous physical activity (MVPA), average dietary calcium and accelerometer changes in boys (n=76).

	Maturity offset	**Height**	**Weight**	**Tibial length**	**Radius length**	**Lean mass**	**Fat mass**	**MCSA 50%**	**MCSA 30%**	**Grip strength**
Baseline height	0.60**	-	0.63**	0.81**	0.81**	0.74**	0.29*	0.34**	0.53**	0.53**
Height change	-0.27*	-0.14	-0.15	0.19	-0.05	-0.11	-0.08	-0.22	-0.27*	-0.20
Baseline weight	0.27*	0.63**	-	0.51**	0.67**	0.90**	0.80**	0.66**	0.68**	0.63**
Weight change	0.07	0.07	0.08	0.15	0.18	0.14	0.05	0.04	-0.02	0.16
Baseline tibial length	0.55**	0.81**	0.51**	-	0.85**	0.52**	0.38**	0.10	0.23	0.31**
Tibial length change	-0.26*	-0.05	-0.12	0.09	-0.02	-0.08	-0.08	-0.15	-0.16	-0.08
Baseline radius length	0.53**	0.81**	0.67**	0.85**	-	0.66**	0.50**	0.24*	0.45**	0.47**
Radius length change	-0.18	-0.30**	-0.31**	-0.38	-0.27*	-0.30*	-0.18	-0.38**	-0.33**	-0.19
Baseline lean mass	0.23	0.74**	0.90**	0.52**	0.66**	-	0.46**	0.76**	0.83**	0.74**
Lean mass change	0.07	0.22	0.26*	0.31**	0.35**	0.24*	0.19	0.09	0.09	0.18
Baseline fat mass	0.19	0.29*	0.80**	0.38**	0.50**	0.46**	-	0.30*	0.24*	0.26*
Fat mass change	0.19	0.13	0.06	-0.01	0.12	0.08	0.03	0.11	0.02	0.06
Baseline MCSA 50%	-0.07	0.34**	0.66**	0.10	0.24*	0.76**	0.30*	-	0.64**	0.51**
MCSA 50% change	-0.25*	0.04	0.12	0.12	0.11	0.21	-0.02	0.22	0.17	0.15
Baseline MCSA 30%	0.13	0.53**	0.68**	0.23	0.45**	0.83**	0.24*	0.64**	-	0.76**
MCSA 30% change	-0.005	0.02	0.02	0.08	0.05	0.08	-0.09	-0.07	-0.03	0.10
Grip strength	0.20	0.53**	0.63**	0.31**	0.47**	0.74**	0.26*	0.51**	0.76**	-
Grip strength change	-0.11	-0.03	-0.05	-0.07	-0.03	0.01	-0.14	-0.12	0.005	-0.13
Average PAQ score	0.16	0.15	0.09	0.10	0.08	0.16	-0.09	-0.05	0.10	0.16
Average PAQ MVPA	0.28*	0.23*	0.21	0.20	0.19	0.24*	0.06	0.01	0.28*	0.22
Average calcium intake	0.04	0.06	-0.05	0.13	0.04	-0.07	-0.04	-0.10	-0.08	0.03
Sedentary time change[†]	0.25	0.26	-0.20	0.32	0.13	-0.04	-0.31	-0.13	-0.24	0.006
MVPA change[†]	-0.17	-0.02	0.09	-0.05	0.18	0.16	-0.03	0.17	0.31	0.21
MPA change[†]	-0.13	0.003	-0.01	-0.004	0.10	0.20	-0.26	0.11	0.37	0.19
VPA change[†]	-0.16	-0.03	0.15	-0.08	0.19	0.10	0.16	0.17	0.19	0.16

Note: Correlations do not account for clustering

*p<0.05, **p<0.01

[†]n=28

Table M.4. Girls' (n=107) Pearson correlations of descriptive variables (baseline and 30-week changes) – age, age at menarche, maturity-offset, height, weight, tibial length, lean and fat mass, muscle cross-sectional area (MCSA) at 50% tibial site, 30-week average physical activity questionnaire for adolescents (PAQ-A) score, PAQ-A moderate-to-vigorous physical activity (MVPA) and dietary calcium with change in bone strength index (BSI), total bone area (Tt.Ar) and total bone density (Tt.Dn) at the distal tibia (8% site, n=104) and polar stress-strain index (SSIp), total bone area (Tt.Ar), cortical bone area (Ct.Ar), medullary area (Me.Ar) and cortical bone density (Ct.Dn) at the tibial shaft (50%, n=104) and bivariate correlations (using Spearman's rank correlation) with ethnic grouping.

	Distal tibia (8% site)			**Tibial midshaft (50% site)**				
	BSI Δ	**Tt.Ar Δ**	**Tt.Dn Δ**	**SSI_p Δ**	**Tt.Ar Δ**	**Ct.Ar Δ**	**Me.Ar Δ**	**Ct.Dn Δ**
Age	-0.14	-0.09	-0.06	-0.14	-0.05	-0.03	-0.05	-0.07
Age at menarche	0.04**	-0.11	0.33**	0.36**	0.33**	0.33**	0.02	0.14
Ethnic group	-0.15	0.10	-0.16	-0.21*	-0.15	-0.19	0.08	-0.06
Baseline maturity	-0.03	-0.17	0.04	-0.21*	-0.11	-0.14	0.06	0.08
Baseline height	0.11	-0.12	0.08	0.04	0.09	0.06	0.07	0.10
Height change	0.34**	-0.15	0.32**	0.27**	0.21*	0.16	0.13	0.17
Baseline weight	-0.09	0.006	-0.17	-0.06	0.09	0.14	-0.12	-0.14
Weight change	-0.009	0.07	-0.06	-0.05	-0.008	0.07	-0.19	-0.09
Baseline tibial length	0.05	-0.06	-0.003	0.06	0.08	0.04	0.10	0.17
Tibial length change	0.21*	-0.18	0.29**	0.21*	0.30**	0.13	0.43**	0.11
Baseline lean mass	-0.02	-0.004	-0.15	0.07	0.14	0.21*	-0.16	-0.13
Lean mass change	0.04	-0.004	0.01	-0.06	-0.02	0.07	-0.20*	-0.05
Baseline fat mass	-0.15	0.01	-0.16	-0.14	-0.02	0.006	-0.07	-0.10
Fat mass change	-0.009	0.07	-0.07	0.006	0.04	0.14	-0.22*	-0.07
Baseline MCSA 50% site	-0.08	0.05	-0.17	0.09	0.08	0.22*	-0.33**	-0.18
MCSA 50% site change	0.11	-0.13	0.15	0.007	0.05	0.04	0.01	0.03
Average PAQ score	0.13	-0.12	0.12	0.11	0.11	0.08	0.08	-0.03
Average PAQ MVPA	0.18	-0.08	0.12	0.10	0.15	0.16	-0.03	-0.04
Average dietary calcium	0.04	-0.02	0.02	0.02	-0.02	-0.02	-0.01	0.08
Sedentary time change[†]	0.08	0.07	-0.008	-0.15	-0.06	0.04	-0.21	0.04
MVPA change[†]	-0.10	0.04	-0.001	0.01	-0.17	-0.22	0.02	0.28
MPA change[†]	-0.09	0.07	-0.04	0.02	-0.15	-0.15	-0.06	0.15
VPA change[†]	-0.08	-0.004	0.03	-0.002	-0.14	-0.22	0.09	0.30*

Note: Correlations do not account for clustering

*p<0.05, **p<0.01

[†]n=51

Table M.5. Girls' (n=107) Pearson correlations of descriptive variables (baseline and 30-week changes) – age, age at menarche, maturity-offset, height, weight, tibial length, lean and fat mass, muscle cross-sectional area (MCSA) at 30% radius site, 30-week average physical activity questionnaire for adolescents (PAQ-A) score, PAQ-A moderate-to-vigorous physical activity (MVPA) and dietary calcium with change in bone strength index (BSI), total bone area (Tt.Ar) and total bone density (Tt.Dn) at the distal radius (7% site, n=101) and polar stress-strain index (SSIp), total bone area (Tt.Ar), cortical bone area (Ct.Ar), medullary area (Me.Ar) and cortical bone density (Ct.Dn) at the radius shaft (30%, n=103) and bivariate correlations (using Spearman's rank correlation) with ethnic grouping.

	Distal radius (7% site)			**Radius shaft (30% site)**				
	BSI Δ	**Tt.Ar Δ**	**Tt.Dn Δ**	**SSI_p Δ**	**Tt.Ar Δ**	**Ct.Ar Δ**	**Me.Ar Δ**	**Ct.Dn Δ**
Age	-0.20	-0.06	-0.10	0.008	-0.10	-0.02	-0.17	-0.06
Age at menarche	0.38**	0.02	0.33**	-0.02	0.06	0.09	-0.05	0.26**
Ethnic group	0.04	-0.12	0.05	-0.11	-0.11	0.03	-0.25*	-0.08
Baseline maturity	0.01	0.04	-0.02	-0.21*	-0.26**	-0.14	-0.27**	-0.09
Baseline height	0.17	0.10	0.07	-0.09	-0.09	-0.04	-0.10	0.06
Height change	0.10	0.07	0.10	0.09	0.07	0.0002	0.16	0.23*
Baseline weight	-0.27**	0.12	-0.32**	-0.14	-0.29**	-0.27**	-0.04	0.18
Weight change	-0.05	-0.12	0.02	0.08	0.008	-0.12	0.23*	0.09
Baseline radius length	0.06	0.15	-0.05	-0.13	-0.15	-0.13	-0.05	0.13
Radius length change	0.27**	0.04	0.22*	-0.005	0.05	0.06	-0.02	0.04
Baseline lean mass	-0.12	0.12	-0.22*	0.0007	-0.19	-0.17	-0.04	0.24*
Lean mass change	-0.002	-0.14	0.07	-0.08	0.02	-0.004	0.05	-0.11
Baseline fat mass	-0.34**	0.08	-0.33**	-0.20	-0.28**	-0.28**	-0.02	0.10
Fat mass change	0.001	0.02	-0.04	0.05	0.02	-0.11	0.26*	0.12
Baseline MCSA 30% site	-0.09	0.05	-0.18	0.03	-0.11	-0.15	0.13	0.18
MCSA 30% site change	0.05	-0.26**	0.17	0.19	0.22*	0.20*	0.06	0.17
Grip strength change	-0.08	0.17	-0.11	0.08	0.04	0.03	0.02	0.0001
Average PAQ score	0.03	-0.11	0.09	0.21*	0.08	0.13	-0.08	0.03
Average PAQ MVPA	0.02	-0.05	0.04	0.09	0.05	0.09	-0.06	0.04
Average dietary calcium	0.07	-0.16	0.11	-0.0004	-0.12	-0.11	-0.02	0.05
Sedentary time change[†]	0.17	0.02	0.15	-0.22	-0.12	-0.02	-0.16	-0.07
MVPA change[†]	-0.02	-0.04	0.02	-0.02	0.11	0.03	0.12	-0.14
MPA change[†]	-0.19	-0.005	-0.18	-0.0002	0.08	0.003	0.13	-0.15
VPA change[†]	0.16	-0.06	0.22	-0.03	0.10	0.05	0.08	-0.09

Note: Correlations do not account for clustering
*p<0.05, **p<0.01
[†]n=51

Table M.6. Pearson correlations of anthropometric, body composition, grip strength with baseline values and 30-week change, average of physical activity questionnaire for adolescents (PAQ-A) scores and minutes of moderate-to-vigorous physical activity (MVPA), average dietary calcium and accelerometer changes in girls (n=107).

	Maturity offset	**Age of menarche**	**Height**	**Weight**	**Tibial length**	**Radius length**	**Lean mass**	**Fat mass**	**MCSA 50%**	**MCSA 30%**	**Grip strength**
Baseline height	0.64**	0.25*	-	0.30**	0.85**	0.73**	0.48**	0.02	0.05	0.14	0.33**
Height change	-0.13	0.30**	0.20*	-0.12	0.17	0.04	0.02	-0.22*	-0.03	0.13	0.03
Baseline weight	0.16	-0.23*	0.30**	-	0.33	0.42**	0.83**	0.89**	0.53**	0.54**	0.44**
Weight change	-0.28**	-0.007	-0.12	0.06	-0.13	-0.19*	0.09	0.07	0.12	0.13	-0.06
Baseline tibial length	0.63**	0.28**	0.85**	0.33**	-	0.79**	0.38**	0.18	-0.04	0.006	0.24*
Tibial length change	-0.10	0.17	0.04	-0.09	0.02	-0.03	-0.12	-0.08	-0.12	-0.09	-0.03
Baseline radius length	0.61**	0.15	0.73**	0.42**	0.79**	-	0.42**	0.30**	0.004	0.08	0.34**
Radius length change	0.13	0.11	0.11	0.05	0.25**	0.10	-0.05	0.11	-0.15	-0.02	-0.07
Baseline lean mass	0.17	-0.12	0.48**	0.83**	0.38**	0.42**	-	0.49**	0.63**	0.73**	0.61**
Lean mass change	-0.10	0.05	0.02	0.01	0.06	-0.05	0.05	-0.03	0.18	0.13	0.06
Baseline fat mass	0.09	-0.26*	0.02	0.89**	0.18	0.30**	0.49**	-	0.30**	0.22*	0.17
Fat mass change	-0.20*	0.04	0.03	0.18	-0.009	-0.02	0.21*	0.13	0.18	0.28**	0.08
Baseline MCSA 50%	-0.06	-0.24*	0.05	0.54**	-0.04	0.004	0.63**	0.30**	-	0.69**	0.29**
MCSA 50% change	0.02	0.01	0.06	0.14	0.08	0.02	0.10	0.09	0.24*	0.22*	-0.006
Baseline MCSA 30%	-0.17	-0.10	0.14	0.54**	0.006	0.08	0.73**	0.22*	0.69**	-	0.61**
MCSA 30% change	-0.05	-0.14	-0.02	0.006	-0.04	-0.06	0.09	-0.06	0.08	0.05	0.008
Grip strength	0.08	-0.04	0.33**	0.44**	0.24*	0.34**	0.61**	0.17	0.29**	0.61**	-
Grip strength change	-0.08	-0.10	-0.08	-0.0009	-0.13	-0.14	0.10	-0.11	0.03	0.28**	-0.16
Average PAQ score	-0.05	-0.03	0.15	0.13	0.05	-0.03	0.31**	0.0002	0.14	0.24*	0.22*
Average PAQ MVPA	-0.02	0.02	0.21*	0.12	0.12	0.03	0.31**	-0.04	0.11	0.25*	0.19
Average calcium intake	0.03	0.24*	0.08	-0.03	0.11	0.06	0.12	-0.03	-0.17	-0.04	0.17
Sedentary time change[†]	-0.17	0.21	-0.07	0.03	-0.01	-0.002	0.06	0.01	0.08	0.15	0.007
MVPA change[†]	0.09	-0.14	-0.08	-0.19	-0.20	-0.07	-0.30*	-0.08	-0.05	-0. 17	-0.14
MPA change[†]	0.10	-0.27	-0.08	-0.17	-0.16	0.004	-0.21	-0.11	-0.02	-0.08	-0.04
VPA change[†]	0.04	0.04	-0.06	-0.15	-0.17	-0.12	-0.29*	-0.02	-0.06	-0.20	-0.19

Note: Correlations do not account for clustering

*p<0.05, **p<0.01

[†]n=51