BONE MASS IN PRE- AND PERI-PUBERTAL CANADIAN CHILDREN: EFFECTS OF A HIGH IMPACT EXERCISE INTERVENTION, MATURITY, AND ETHNICITY

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ABSTRACT

INTRODUCTION: Osteoporosis has, in part, been attributed to physical inactivity. The direct health care costs of osteoporosis in Canada exceed 650 million dollars annually. Targeted physical activity may be an effective and feasible primary prevention strategy to lessen the burden of this debilitating disease.
AIM: The primary aim was to determine the effects of a randomized, controlled, school-based, bone-loading exercise intervention on bone mineral accrual.

METHODS AND RESULTS:
Subjects: Children were a mixed ethnic group of 383 initially 9-11 year old boys and girls.
Ethnic comparisons at baseline: For prepubertal girls (n=56), general physical activity, calcium intake, and total body (TB) and femoral neck (FN) bone mineral content (BMC) (adjusted for body size) were significantly ($p<0.05$) lower in Asians than Caucasians. For early pubertal girls (n=75), loaded physical activity and sport nights, calcium intake, TB, proximal femur (PF) and FN BMC and areal bone mineral density (aBMD) (adjusted for body size) were significantly lower (~10%) in Asians than Caucasians.

7-month change (girls): Fourteen schools were randomized to intervention (n=7) or control (n=7). I evaluated the effect of a 10-minute, 3x/week, exercise program on bone accrual at the TB, LS, PF, FN and trochanter (TR) in girls. Early pubertal (n=43), but not prepubertal (n=44) intervention girls gained significantly more (+~2%) adjusted BMC, aBMD, and vBMD (FN only) at the FN and LS than same-maturity controls (pre: n=26, early: n=63).

7-month change (boys): Prepubertal Asian and Caucasian boys in the exercise schools (n=61) gained significantly more adjusted TB BMC and PF aBMD (+1-2%) than prepubertal controls (n=60) over 7 months. Accrual was similar between ethnicities.

20-month change (girls): I investigated the continued bone health benefits from exercise over 20 months in girls. Intervention girls (N=32) gained significantly more adjusted FN and LS BMC (+4-5%) than controls (N = 43).

CONCLUSIONS: Risk factors for osteoporosis were more prevalent in early pubertal Asian girls compared with Caucasians. A targeted, inexpensive and feasible school-based exercise program, implemented over 7 or 20 months, offered an effective strategy to enhance bone mineral accrual during growth. The skeletal response to loaded physical activity was sex-, site- and maturity-specific.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GLOSSARY OF TERMS AND ABBREVIATIONS USED IN THIS THESIS

Bone Definitions	
DXA:	Dual energy x-ray absorptiometry.
BA	Bone area (cm^2), as measured by DXA.
BMC	Bone mineral content (grams), as measured by DXA.
ABMD	Areal bone mineral density (grams/cm^2, or BA/BMC), as measured by DXA.
Bone mass	An alternate way of expressing BMC (grams).
Bone mineral	Refers to either BMC or aBMD, as measured by DXA.
VBMD	Volumetric bone mineral density (grams/cm^3), calculated from BA and BMC values obtained by DXA.
BMAD	Bone mineral apparent density; an alternate way of expressing vBMD, used in previous studies (calculated from DXA values of BA and BMC).
PQCT	Peripheral quantitative computed tomography.

Maturity Definitions	
Maturity	The state of development of the reproductive system. Within this thesis, maturity is generally discussed in terms of Tanner stage.
Tanner Staging	A method of assessing the stage (1 through 5) of reproductive maturity in a girl or boy, by judging breast and pubic hair growth in girls, and genitalia and pubic hair growth in boys. Tanner stage 1 corresponds to prepuberty, Tanner stages 2 and 3 represent early puberty (and peri-puberty), Tanner stage 4 is considered peri-puberty (and late puberty), and Tanner stage 5 represents the post- or late-pubertal adolescent. Throughout this thesis *Tanner stage for girls* refers to *breast stage*, and *Tanner stage for boys* refers to *pubic hair stage*.
Prepuberty	Tanner stage 1.
Early Puberty	Tanner stages 2 and 3.
Peri-Puberty	Tanner stages 2, 3 and 4.
Late Puberty or Post-Puberty	Tanner stage 5.
Premenarcheal	Refers to a girl who has not yet experienced her first menstrual period; includes prepubertal (Tanner stage 1) and early pubertal (Tanner stage 2 and 3) girls, as menarche typically occurs after the achievement of Tanner stage 3.
Postmenarcheal	Refers to a girl who has had her first menstrual period; typically includes girls in Tanner stage 4 and 5, but may include girls in Tanner stage 2 and 3.

PREFACE: PUBLICATIONS ARISING FROM THIS THESIS

Sections of this thesis have been published as multi-authored papers in refereed journals, which are indicated with a * beside the publications below. Details of authors' contributions are provided, where relevant. I agree with the stated contributions of the thesis author, as indicated below.

Dr. Heather McKay (thesis supervisor)

Published Papers

***MacKelvie KJ**, McKay HA, Petit MA, Khan KM, Moran O. 2002. Bone mineral response to a 7-month randomized controlled, school-based jumping intervention in 121 prepubertal boys: associations with ethnicity and body mass index. Journal of Bone and Mineral Research. 17 (5); 834-844.
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MA Petit, HA McKay, **KJ MacKelvie**, A Heinonen, KM Khan, TJ Beck. 2002. A randomized school-based jumping intervention confers site and maturity-specific benefits on bone structural properties in girls: a hip structural analysis study. Journal of Bone and Mineral Research. 17 (3); 363-372.

***MacKelvie KJ**, Khan KM, McKay HA. Systematic review: Is there a critical period for bone response to weight-bearing exercise intervention in children and adolescents? 2002. British Journal of Sports Medicine. 36 : 250-7.
Authors' contributions: Kerry MacKelvie and Karim Khan generated the original ideas behind the paper. Kerry MacKelvie researched, wrote and edited the original paper. Karim Khan was the key editor on this paper. Heather McKay provided further editorial assistance.

Papers Submitted

***MacKelvie KJ**, Khan KM,Petit MA, Janssen P, McKay MA. A school-based exercise intervention elicits substantial bone health benefits: A 2-year randomized controlled trial in girls. Submitted to The British Medical Journal, July 2002.
Authors' contributions: Kerry MacKelvie was responsible for the original ideas behind the paper, analysis and presentation of findings, and writing and editing of the original paper. Heather McKay was the key editor on this

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HA McKay, MA Petit, **KJ MacKelvie**, O Moran, KM Khan, TJ Beck. Bone structural response to a 7-month randomized controlled exercise trial in prepubertal boys: A Hip Structural Analysis (HSA) Study. Submitted to Bone, July 2002.

Abstracts

I presented first authored abstracts orally or as a poster at the conference indicated.

KJ MacKelvie, HA McKay, MA Petit, KM Khan. 2002. Continued bone mass accrual benefits are conferred to 9-10 year old girls participating in a 20-month, randomized, controlled, school-based exercise intervention. 2nd International Conference of Child Bone Health, Sheffield Hallam University, June 2002. Young Investigator Award Recipient.

KJ MacKelvie, HA McKay, MA Petit, KM Khan. 2001. Bone mineral change in prepubertal Asian and Caucasian boys: Effects of jumping intervention, body mass, and ethnicity. Journal of Bone and Mineral Research; 16 (S1): 173. Presented orally at the American Society for Bone and Mineral Research Annual Meeting, October 2001, Phoenix AZ.

MA Petit, HA McKay, **KJ MacKelvie**, A Heinonen, KM Khan, TJ Beck. 2001. Bone structural adaptation to exercise in pre- and early-pubertal girls: A randomized intervention trial. Journal of Bone and Mineral Research; 16 (S1): 173.

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1 - Introduction

Osteoporosis related fractures present a serious health problem (2). Annually, there are 8 million low-trauma fractures worldwide, with a 3-fold increase predicted by the year 2050 (3). There are over 24 000 hip fractures in Canada each year (4), that result in an annual direct health care cost of 650 million dollars (2). Over the long term, prevention, rather than treatment of osteoporosis, will be the most cost effective solution for reducing the consequences of this disease (1). One area of preventative medicine that has recently garnered increasing attention is exercise science. Exercise habits developed during childhood may offer the greatest potential for promoting lifetime bone health as well as preventing osteoporosis (5). Hip fractures in the elderly have a multifactorial pathophysiology, and thus the various benefits of exercise across the lifespan– related to balance, proprioception, and strength, as well as augmented bone mineral accrual and bone strength – may contribute to primary prevention of osteoporotic fractures.

In studies of growing animals (6-10), weight-bearing exercise influenced the amount of bone mineral accrued, and the ultimate breaking strength of bone. However, there are few prospective studies of children that have examined exercise as a modulator of bone mineral accrual during growth (11,12). Investigations in retired, elite female dancers (13) and gymnasts (14) suggest exercise training in childhood is associated with greater areal bone mineral density in adulthood. These initial findings suggest that childhood exercise may well be related to a reduced risk of osteoporotic fracture in the elderly (5).

The predisposition to osteoporosis is likely linked to the habits assumed during childhood (15). Evidence supports the theory that osteoporosis may be better classified as a disease of inadequate bone accrual than accelerated bone loss (5,16). The amount of bone gained during the adolescent growth spurt approximates that lost throughout adult life (11). Seizing the opportunity to reach the highest possible peak in bone mass in childhood and early adolescence may substantially reduce the impact of inevitable bone loss in later life.

Many factors, both intrinsic (i.e., heredity and race) and extrinsic (i.e., nutrition and physical activity), contribute to bone mass or areal density at any specific age. Heredity explains 46 – 62% of the population variance in areal bone mineral density (16-18), the best single predictor of osteoporotic fracture (19). A component of heredity is race, which provides a template for bone size and possibly density, as evidenced by well-documented differences between Asians, Caucasians, and Blacks (20). Other factors, such as mechanical loading, nutrient intakes, and lifestyle choices, explain as much as 54% of adult population variation in bone mineral density (18). Whether these extrinsic contributions override or enhance the genetic template for bone development is unknown. However, the possibility that approximately half of the variation in peak bone mass is amenable to modifiable factors provides a strong argument for investigating the environmental influence on growth and development of the skeleton. Thus, this thesis explores primarily the influence of exercise on bone mineral accrual during the critical transition from prepuberty to adolescence, while secondarily investigating the important contributions of ethnicity and physical maturation.

Previous work in Richmond, British Columbia (21) suggested that ethnic differences in lifestyle factors related to bone health, such as physical activity and calcium intake, exist in prepuberty in young Asian and Caucasian children. Further investigation as to when maturational and ethnic differences in bone mass or areal density emerge is warranted, along with a characterization of the contributing lifestyle and body composition factors.

Results from cross-sectional (22) and retrospective (13) athlete studies, and one recent exercise intervention study in healthy young girls (23) raised the possibility of a 'critical maturational period' when bone responds optimally to physical loading. Should such a critical period exist for girls and boys, this would provide a cost-effective and feasible focus for large-scale health programs targeting osteoporosis prevention. Richmond, BC has a diverse demographic that provides an ideal research setting where the differential bone response to exercise between ethnic groups living in a common environment can be examined.

Therefore, the primary focus of my thesis is to define the bone mineral response to high impact exercise in a young cohort, with special reference to sex, maturity, and ethnicity. Part I compared the lifestyle factors related to bone health, as well as bone parameters between Asian and Caucasian girls at two stages of physical maturity. Part II quantified the magnitude of the skeletal response to a 7-month (1 school year), high impact exercise intervention in girls belonging to two distinct maturity groups. Part III investigated the effects of the 7-month exercise intervention on bone mineral accrual in Asian and Caucasian prepubertal boys. Finally, Part IV compared bone changes over 20 months between girls who participated in the exercise intervention over two school years, and their controls.

To provide salient background literature, Chapter 2 summarizes bone biology and mechanics, bone mineral measurement, maturational effects on normal bone mineral accrual, determinants of bone mineral accrual, and the role of physical activity on bone and bone mineral accrual in children and adolescents. Chapter 3 provides the rationale, specific objectives and hypotheses for the four studies I undertook. I then detail the methods (Chapter 4), and I describe the cohort of children who participated in this study (Chapter 5). In Chapters 6 through 9, I provide papers that have been published (Parts I – III) or submitted (Part IV). In Chapter 10, I discuss the 4 studies as an integrated whole, draw conclusions, and suggest areas of future research in pediatric exercise and bone health.

2 - Literature Review

2.1 *Bone Biology and Mechanics*

In this section, I begin with an overview of the major anatomical components of bone, followed by a definition of the processes of change in, and adaptation of, bone. Bone mechanics is then discussed as it relates to bones' material and geometric properties, and the specific adaptations in response to mechanical loading. The section concludes by detailing the animal studies that investigated the bone response to mechanical loading in the growing skeleton.

2.1.1 ***Bone Tissue***

In the human body, bone serves many purposes: it provides mechanical support, permits lever action and soft tissue protection, is a vehicle for calcium homeostasis and a reservoir for phosphate, magnesium, potassium and bicarbonate storage. In addition, it hosts hematopoiesis (24). Bone tissue and its structure reflect all of these functions, but I will focus primarily on bone's role in mechanical support.

Bone exists in four typically distinct forms: woven, lamellar, Haversian system, and laminar bone (25). Fine-fibered woven bone is generally laid down quickly, as in the growing fetus or the callus that forms after a bone fracture. It has a random orientation, in contrast to the precise arrangement typical of lamellar bone that is laid down more slowly in sheets (lamellae). Within the lamellar plane, fibrils tend to be oriented in one direction. The preferred direction changes from one lamellar sheet to the next, and this property has mechanical consequences. Haversian systems are made up of a different form of lamellar bone. They are formed by the erosion of bone around a blood vessel, with a subsequent smoothing of the wall and deposition of bone in concentric layers. Laminar bone is typically found in large mammals where bones have grown quickly in diameter. The result is a bone wrapped in alternating layers of parallel-fibered bone and lamellar bone tissue (25).

There are three types of bone cells: osteocytes, or mature bone cells, osteoblasts, which have a primary function of bone formation, and osteoclasts, the bone resorbing cells. Osteocytes are osteoblasts that have become encased in calcified bone (26). Osteoblasts exist in groups on the bone surface, and synthesize, secrete, and mineralize collagen, noncollagen, matrix proteins, and regulatory factors (26). Osteoclasts are large, multi-nucleated cells, that resorb bone by producing proteolytic enzymes and hydrogen ions (27). The function of bone cells in relation to bone processes is outlined in Section 2.1.2 (Bone Turnover).

At a higher level of structure, two types of bone (cortical and trabecular), that have identical molecular and cellular compositions but differ in porosity and function, make up the human skeleton. All cortical and trabecular bone is composed of protein in the form of collagen, and hydroxyapatite mineral (28). Thirty-two percent of the mineral within bone is calcium (29). Cortical bone forms the hard, dense outer layer found in the walls of bone shafts and on external bone surfaces, and makes up approximately 80% of total bone mass (30). This type of bone plays a major role in mechanical support (24).

Trabecular bone is named for the thin plates, or trabeculae, that surround the connective collagen tissue. This type of bone is more porous than cortical bone, is spongier and lighter in weight, and is more metabolically active (24,31). Porosity in trabecular bone can range from almost complete, where there is only the occasional strut penetrating the marrow, to less than 50% porosity, at which point it becomes difficult to differentiate trabecular from cortical bone (25). While cortical bone appears fairly uniform, trabecular bone structure varies by location. Trabecular bone made of cylindrical struts and lacking a particular orientation is found deep within bones, while that made of plates with specific orientations is located in close proximity to loaded surfaces that experience relatively constant patterns of stress (25).

Cortical and trabecular bone represent different proportions of separate bones and different proportions within sections of a single bone. In the adult lumbar spine, trabecular bone makes up approximately 38% of the vertebral body, while it only contributes 7.3% to the vertebral arch (32). Trabecular bone makes up approximately 25% of the adult femoral neck, 50% of the intertrochanteric region of the proximal femur, and 5% of the femoral shaft (33). Tremendous variation exists within the femoral neck, depending on the region considered (34). QCT analysis of several 2 mm slices along the femoral neck in adults showed that total bone mass was constant, regardless of location. However, cortical content clearly increased and trabecular content decreased with closer proximity to the intertrochanteric region (34).

2.1.2 *Bone Turnover*

Three distinct processes modulate bone growth and maintenance throughout human life: growth, modeling and remodeling. *Growth* refers to the increase in bone length and width from birth to the end of adolescence. This process is mediated by hormones and genetically predetermined (35). Periosteal apposition leads to growth in bone width and the production of cortical bone, while endochondral ossification leads to growth in bone length and trabecular bone production (5).

Modeling is the process by which bone adapts, at the macro level, to the loads experienced during growth. The strength of bone is altered by increasing mass and improving local architecture. Bone is formed without prior resorption, resulting in a net gain of bone (15). Modeling is active on periosteal, cortical-endosteal, and trabecular surfaces (36). The outcome of this process serves to decrease bone strain under subsequent loading. Goodship, Lanyon and McFie (37) demonstrated this adaptation by removing part of the ulna in young pigs. They measured strain directly with strain gauges during walking, and initially, the strain in the altered limb was double that of the intact limb. After 3 months of rapid bone growth, bone strain was equal between limbs (37). A similar study in sheep demonstrated a lower peak strain in the radius of sheep 12 months after the ulna had been removed than in sheep with intact forelimb bones (38).

Remodeling is the primary process by which mature bone, within a specific region, renews and maintains itself, although this process also occurs during growth. It differs from modeling as remodeling is initiated with an activation phase, followed by bone resorption and formation (15). Bone resorption is carried out by specialized surface areas on osteoclasts. Part of the cell membrane adheres tightly to the mineralized bone and forms a pocket, the resorption space. Lysosomal enzymes then degrade the matrix and dissolve the mineral. Osteoblasts carry out bone formation by secreting bone matrix that is mineralized extracellularly (24). Fatigue microdamage in bone significantly contributes to the initiation of the remodeling process (39). Over time, bone is lost with age, when bone resorption exceeds formation, especially on endosteal surfaces in contact with marrow (15).

2.1.3 *Bone Mechanics*

The next sections outline the material and geometric properties of bone that contribute to its strength. This is followed by an overview of bone adaptation to mechanical loading and its components, and specific adaptations in growing, as compared to adult, animals.

2.1.3.1 Mass, Density, Stiffness and Strength

The intrinsic material properties of bone include mineral mass, density, stiffness and strength (40). Mineral mass, density, and stiffness influence strength. In humans, bone *mass* is often measured as bone mineral content (grams) by dual energy x-ray absorptiometry (DXA). Bone d*ensity* is more difficult to measure in humans, as 'true' density is defined as the mass of a substance per unit volume of its own bulk (20). As noninvasive measurement instruments cannot separate bone mineral from the other components of bone, the best measures available are estimates of bone density. DXA provides a measure of areal (2-dimensional) density (aBMD, g/cm^2) of cortical and trabecular bone combined, and quantitative computed tomography (QCT) provides a measure of volumetric (3-dimensional) density of trabecular and cortical bone separately. The density value of cortical bone as measured by QCT is considered the best estimate of 'true density' currently available, due to the absence of non-bone space and material in cortical bone (20).

Carter and colleagues (41) demonstrated that areal density, as measured by DXA, is directly proportional to the square root of the strength index of bone. On this basis, we should expect a strong relationship between aBMD and whole bone fracture strength. Although the contribution of bone size to bone strength has not been adequately quantified, it is likely an independent determinant. Areal BMD is significantly influenced by bone size, a factor that might actually *enhance* its ability to predict fracture (19). The amount of bone present remains the single most important factor in determining the likelihood of fracture (42). There is a strong association between DXA measures of bone mineral content and areal density and fracture risk in humans (19,42). However, it is important to note that 90% of hip fractures result from falling (43). Hip fractures in the elderly clearly have a multifactorial pathophysiology, and bone density is an important contributor (44).

Stiffness refers to the force required to deform a whole bone by a specific amount (45). When considering whole bone, stiffness is dependent on the combination of its particular material and geometric properties (40). Stiffness will vary depending on the orientation of the load, so load direction must be specified when reporting stiffness. Stress is defined as the intensity of a force acting across a particular plane (in

Newtons/meter^2), while strain refers to the change in the length of a bone in a particular direction, in response to a force and dependent on stiffness (expressed as the fraction (or %): change in bone dimension/original bone dimension) (25,40). Strain is greatest at the point of the applied load, and diminishes with increasing distance from this point (40).

The *elastic modulus* of a bone segment represents its inherent resistance to loading (40). It is a measure of stiffness for a particular bone region (33) (whereas stiffness refers to whole bone). The elastic modulus is related to apparent density and is proportional to bone strength (46). Over 80% of the variation in the elastic modulus of cortical bone can be explained by mineralization and porosity (47). The other 20% of the variation is attributable to bone orientation, and will be discussed under 'geometric properties'. The elastic modulus for cortical bone of the mid-femur loaded in transverse tension is about 2/3 the elastic modulus of the same bone loaded in longitudinal tension. The ultimate strength of the bone under the transverse load is approximately 1/3 of the same bone, with constant apparent density, loaded longitudinally (48).

2.1.3.2 Geometric Properties and Bone Strength

The geometric properties of bone include shape, size, cross sectional area, cortical thickness, and trabecular/collagen fibre architecture (40). Several of these properties are interrelated, and all of them influence bone strength. The geometry of bone is strongly influenced by locomotion and strength requirements: bone structure is optimal if it is light as well as strong. The geometric properties of bone refer to the amount and distribution of mineralized tissue in a specific configuration (49). Although cortical and trabecular bone have identical molecular and cellular compositions (28), variations in mineral distribution within a whole bone or bone segment (50) can account for strength differences.

For long bones, a hollow cylinder configuration maximizes the greatest bending strength/lightest weight ratio. A greater cross sectional moment of inertia (CSMI) confers a greater bending strength in bone (40). This factor is related to the ratio between the diameter of the cylinder (bone) and the thickness of the wall (cortical thickness) (25). When bone is distributed as far as possible from the central neutral axis, maximum CSMI is achieved, and less bone area and mass are needed to maintain bone strength (40). (This applies until the sleeve of bone is extremely thin, in which case bone may buckle (51)). In long bone, architectural redistributions that occur with age increase bones' ability to resist torsion and bending, two loads that impose the greatest skeletal stress (45).

Bone ends and vertebrae, on the other hand, are broad and filled with porous trabecular bone, which helps to absorb axial compression and impact. Trabecular architecture is better suited to energy absorption than cortical bone (25).

The elastic properties of bone depend somewhat on its orientation with respect to the loading direction, which reflects its load-bearing function. Two bones having the same apparent density can nonetheless differ in strength depending on the trabecular orientation (41). Most bones are strongest in their primary loading direction:

for example, cortical bone from the femoral diaphysis is strongest when loaded transversely, and trabecular bone in the vertebral bodies is strongest when loaded vertically (45).

In cortical bone, the collagen fiber orientation greatly influences bone strength, although to a lesser extent than apparent density (52). Tensile strength is maximum under longitudinal orientations of collagen fibers, while compressive strength is optimized when the fibers run in a variety of directions (52,53).

Trabecular architecture, described by the basic tissue structure (i.e., number of trabeculae in a given volume, degree of connectivity, thickness) and orientation (with respect to loading directions), contribute significantly to the mechanical properties in this type of bone, and varies with region, age, density, and disease (45). At any site, trabeculae tend to be preferentially oriented in the direction of principle stresses, allowing the trabecular bone to be both stronger and stiffer in these particular directions (41). The importance of trabecular orientation in relation to principle stresses was illustrated when vertebrae of similar apparent density were loaded in the superior-inferior and medial-lateral directions (54). The superior-inferior trabecular orientation provided vertebrae with two times as much strength when they were loaded in that direction as compared to medial-lateral loading.

2.1.3.3 Bone Adaptation to Mechanical Loading

The shape and size of every bone is, to some extent, a result of adaptation to its particular mechanical environment. The genetic blueprints for basic bone characteristics have evolved due to natural selection acting on mechanically functional skeletons (25). The loads encountered during an individual's lifetime superimpose adaptation upon this skeletal blueprint. The skeleton's ability to adapt to functional demands is well-recognized and the basic theory describing this phenomenon - Wolff's Law (55), was proposed over a hundred years ago. Generally speaking, the premise of this law is that bone seeks to optimize its structure in terms of mass and morphology by responding to the level of activity to which it is subjected.

Bone responds to strain resulting from external loading via four steps, and this integrated process is called mechanotransduction (56,57). A load is signalled to bone cells, via mechanocoupling, from fluid flow creating shear stress on osteocytes' cell membranes (57). The effect elicited by bone cells is brought about through the linking of the mechanical signal to a biochemical signal (biochemical coupling), and the subsequent transmission of the biochemical signal to the bone lining cells (and osteocytes) (56). Loading increases IGF-1 in osteocytes, which in turn, stimulates bone matrix formulation (57). In response to a dynamic load, prostaglandins and prostacyclin are released within the osteoblast layer of bone, which elicits anabolic effects and stimulates upregulation of growth factors (57,58). Prostaglandin E2 and applied load have an additive effect for bone formation at endocortical surfaces, and a synergistic effect at periosteal surfaces (59). Strains are sensed and elicit responses according to three primary variables: strain magnitude, strain rate, and strain distribution.

Strain Magnitude. Animal studies show that bone adapts to optimize strain. There is remarkable uniformity in the magnitude of bone strains experienced by different animals during the activities of daily living, with peak strains in the range of 2000 to 3500 microstrain (uE) (60); 1000 uE is equivalent to a 0.1% deformation (61). Harold Frost proposed the theory of the Minimum Effective Strain (MES) (62) to explain bone system overload and the adaptive response to high strains. In this theory, there exists a range of strain, (~200 to 3000 uE), within which bone does not respond, and bone mass is unchanged. Strains greater than ~3000 uE overload the system and elicit an adaptive response where bone mass increases. By applying loads to turkey ulnae, Rubin and Lanyon better defined the bone response thresholds (63). Complete immobilization for 8 weeks resulted in a 20 percent decrease in cross sectional area (CSA), 100 load cycles per day in the range of 1000 to 2500 uE maintained or increased CSA through periosteal and endosteal bone formation, and strains exceeding 4000 uE elicited a 40 percent increase in CSA.

Several animal studies demonstrated that strain magnitude influences bone more than does strain cycle frequency. In rats, a program of 5 jumps/day was as effective as 40 jumps/day in increasing femur fat-free dry weight and tibial cortical area (64), and alternate day loading produced similar results to daily loading (65). A 36-cycle regime elicited a 40 percent increase in BMC in rats, while regimes of 360 or 1800 cycles did not cause substantially different responses (66). Fifteen minutes of running with a 50 gram weight elicited the greatest increase in lower extremity BMC in rats compared to three other groups: rats running with an identical 50 gram weight for 30 minutes, rats running with no weight for 30 minutes, and sedentary rats (67). A recent study published by Cullen and colleagues (68) provided strong evidence of the greater relative importance of strain magnitude over strain cycle frequency in tibial periosteal and endosteal bone formation in rats. Rats were subjected to two levels of strain: 800 uE and 1000 uE in four-point bending at 4 different strain cycle frequencies: 0, 40, 120, and 400 cycles/day. At the lower strain magnitude (800 uE), a 2.8-fold increase in periosteal bone formation rate was observed in response to the highest cycle frequency only (400 cycles/day). At the higher level of strain (1000 uE), periosteal bone formation rate increased 8-10 fold, formation surface increased 2–3 fold, and endosteal formation surface increased 1-fold compared to controls at *both* the 120 and 400 cycle frequencies. Thus, at higher strain magnitudes, bone formation occurred at both the periosteal and endosteal surfaces, when the strains were applied at moderate *or* high frequencies (68).

Strain Rate. Strain rate is also of critical importance to the skeletal response to loading. Strain rate is defined by the time over which strain develops following load application (60). Dynamic, longitudinal compressive strains (4000 uE) applied to the rat ulnae at three different strain rates resulted in 54 and 67 percent greater periosteal bone formation in the group loaded at the highest strain rate as compared to those loaded at moderate and low rates, respectively (69). New periosteal bone was deposited on the compression side of immature and mature rabbit tibiae that were loaded either continuously or dynamically (70). However, more bone was laid down in the immature as compared to the mature rabbits, and in the dynamically (higher rate) rather than continuously loaded limbs. Judex and colleagues (10) reported a significant correlation between sector-specific strain rate (measured directly by strain gauges) in the immature rooster tarsometatarsus and the increase in endosteal bone formation rate in birds that experienced 200 drop jumps/day. That is, the sector of bone that experienced the greatest strain rate also demonstrated the greatest endosteal formation rate (10).

Strain Distribution. In combination with strain magnitude and strain rate, strain distribution during loading plays a significant osteogenic role (58). Strains that pose a unique loading distribution to the skeleton may elicit the greatest bone response. Bone responds to strain distributions that are perceived to differ from normal by making architectural adaptations to reduce the error, a theory known as the 'error strain distribution hypothesis' (58). This hypothesis has been tested in animal studies. When normally distributed strains typical of locomotion were applied to the rat ulna with a controlled, low magnitude load (1300 uE), there was no osteogenic response until strains reached 4000 uE (71). However, low strain magnitudes typical of daily activity applied in an unusual distribution elicited a substantial osteogenic response in both sheep (38) and avian ulnae (63). The combination of unusual strain distributions with high strain magnitude and high strain rate appear to elicit the greatest osteogenic response. However, a large increase in either strain magnitude or strain rate, without a change in strain distribution, may also elicit a bone formation response. This was elegantly illustrated by the direct measurement of strain distribution, magnitude and rate in roosters performing drop jumps and walking (10). Strain distribution was similar between the 2 activities, strain magnitude differed marginally (+30%), but strain rate was augmented 740% in drop jumping. This resulted in 40% and 370% increases in periosteal and endosteal formation rates, respectively, in young roosters that performed 200 drop jumps/day.

2.1.3.4 The Response to Mechanical Loading in Growing and Mature Animals

Modeling Changes. In many studies of low to moderate intensity exercise interventions in young animals, cortical bone parameters (measured from excised bone) increased at a variety of skeletal regions (8,9,72-74). Specific geometric changes (increased cross-sectional area and moment of inertia) were evident in response to a swimming regimen (74). Different levels of bone modeling in young rats (5 weeks old) were observed when the animals were randomized to loaded swimming (n=20), unloaded swimming (n=10) and sedentary (n=10) groups. The swimming rats, regardless of load, had greater humeral bone weight, volume, length, cortical area, density and content after 20 weeks of training (8). Daily running training in 6 week old rats (n=38 runners, n=22 sedentary) resulted in similar changes as compared to swim training: greater femoral and tibial lengths, weights and volumes in the runners (9). However, mineralization changes may be specific to loading site, as tibial, but not femoral, BMD and BMC were greater in the runners than controls. In these running and swimming studies the BMC difference between trained and untrained animals ranged from 15 to 25% (8,9).

Surface-Specific Changes. Generally, bone geometry can change with loading by increasing apposition at the periosteal and/or endosteal surfaces. Bone adapts with surface-specific changes that cause overall change in bone structure (75). This was illustrated in recent studies. Mosley and colleagues demonstrated the mechanisms underpinning the osteogenic response to high peak strains (~4000 uE) resulting from compressive (bending) loads applied to the growing rat ulna. Lamellar bone was laid down due to an increase in mineral apposition rate on the lateral face of the ulna and active formation of new bone on the previously resorbing medial surface (76). These regions corresponded to the areas experiencing the highest local strain.

Similarly, Hilliam and Skerry described the pattern of bone deposition in the immature rat ulna subjected to 6 days of axial compressive (bending) loads approximately two times greater than normal locomotion (7). Results showed an inhibition of modeling-related resorption accompanied by an enhancement of bone formation on the medial periosteal surface. During growth in the rat, bone is typically resorbed from this surface to form the normal curvature of the ulna, as was the case on this surface in unloaded bones. However, when compressive loads were applied, this surface was lined with osteoid and osteoblasts, and there were lower levels of biochemical markers of osteoclast activity.

Finally, after 8 weeks of jump training (10 sets x 15 reps/day), growing rats improved bone strength and structure at the mid femur. This was attributed to cortical drift caused by expansion of the periosteal envelope with a reduction in endosteal mineral apposition (6).

Response in Immature vs. Mature Bone. Aging increased the minimum mechanical loading threshold that will elicit a periosteal bone response in rats (77). The bone response to 300 cycles of 3000 uE magnitude strains over 8 weeks was also age-dependent in the turkey ulna (78). In 1-year-old turkeys, the ulnar periosteal mineralizing surface was increased, resulting in significant gains in bone area. Geometric bone properties were unchanged in 3-year-old turkeys. A daily (4 sessions/day) treadmill running program resulted in significantly longer, larger and heavier bones in 4 week old rats, as compared to their sedentary counterparts, by the end of week one (79). The authors described the increased bone mineral density and cortical thickness of the femur and tibia observed on x–rays of the excised bones in young, exercised animals compared to controls by week three of training. Bone weight was increased by week 6 in the mature exercising rats, with no discernible differences between bone length, density or cortical thickness between the mature exercised and control rats.

As previously noted, two studies that investigated the effects of exercise interventions (swimming and running) in young (5-6 week old) rats showed significantly greater gains in both bone morphometric and mineralization characteristics in the exercising as compared to the sedentary animals (8,9). Similar exercise studies in adult rats suggested that the osteogenic response to exercise differed with stage of maturity. Although BMD and BMC of the humerus and tibia were greater in swim-trained adult rats (n=14, trained for 4 months, 5 times/week) than sedentary rats (n=14), bone morphometry was not different between the two groups (80). This provides evidence that adult animals may respond to loads induced through exercise (over a minimum of 4 months) by increasing mineralization without the accompanying modeling-related changes in bone morphometry noted in growing animals.

Changes in trabecular bone architecture in response to loading may depend on age and maturity. Greater gains in trabecular number and thickness were observed in exercising (treadmill and resistance training) 9-week (81) and 20-week (82) old rats as compared to their respective controls of similar maturity. However, there was no difference in trabecular changes in elderly treadmill-trained and sedentary 56-week-old rats (83).

Overall, results from studies of growing and mature animals provide substantial evidence that skeletal adaptations to exercise during growth are more substantial than those achieved after maturity.

2.2 *Bone Mineral Measurement*

In this section, I define bone parameters measured by dual energy x-ray absorptiometry (DXA). I then discuss the utilization and limitations of this technique. Finally, I provide an overview of other tools used to assess pediatric bone.

2.2.1 ***Dual Energy X-Ray Absorptiometry (DXA)***

Dual energy x-ray absorptiometry (DXA) is widely used to investigate material properties (mass, and areal density) of trabecular and cortical bone combined. It is relatively inexpensive (as compared to, for example, magnetic resonance imaging), precise, safe, and has a short scanning time. In DXA systems, photons originate from an x-ray tube and are measured as they exit the region of interest (total body, lumbar spine, proximal femur, or forearm), and bone mineral content is determined by computer calibration materials (84). Bone measurements are based on the attenuation of the x-ray beam photons, which is, in turn, related to the energy of the photons, the length of the beam path, and the material properties of the tissue (85). *In vivo* areal bone mineral density precision values (% CV) are consistently excellent and were 0.66% for the total body, 0.56% for the lumbar spine, 0.53% for the total proximal femur, 0.84% for the trochanteric region, and 1.1% for the femoral neck (Hologic QDR 4500) in the University of British Columbia Bone Health Research lab. However, this instrument also has some limitations. For this thesis, the most significant limiting factor is with the interpretation of change in bone mineral in two, rather than three, dimensions. As well, recent research suggests that body composition, and specifically, fat distribution, influences DXA measurement of bone (86,87). Further, conventional DXA measurements provide no indication of bone architecture, a factor closely related to bone strength. Finally, traditional DXA measures do not have the capacity to separate trabecular and cortical compartments, although this approach has recently been reengineered to provide an evaluation of bone geometry (51).

DXA provides a measure of bone mineral content (BMC) in grams, often referred to as 'bone mass', that is strongly associated with bone strength (19). Bone area (cm^2), and areal bone mineral density (aBMD, g/cm^2) are the other two parameters measured by DXA. Areal BMD represents bone in only two dimensions and may misrepresent true 3 dimensional density. Wider, longer bones also tend to be deeper. Correcting the measured BMC by length and width (area) only, results in overestimated aBMD in tall people and underestimated aBMD in short people (88). Thus, aBMD only partially corrects for bone size, which is an important consideration in growing children (35). In children, BMC is often corrected for height or region of interest (ROI) alone (by expressing values as the quotient of whole body BMC/height, or regional BMC/ROI) (23,89), or for height, weight and/or body composition (through the use of covariates in statistical models) to control for the influence of body size on bone (11).

Researchers have developed mathematical equations in attempts to adjust for the third dimension of bone. The resultant 'bone mineral apparent density' (BMAD), or estimated volumetric density (vBMD, used in this thesis) (35), of a bone region is defined as the mineralized tissue mass per total tissue *volume* (90). As volume cannot be determined from the two-dimensional data provided by DXA, it must be estimated. To do this, it is assumed that the cross sectional shape of a bone region is geometrically similar between subjects and that bone thickness within the projection area is uniform (91). These assumptions stand up well at the femoral neck, as it is quite consistently cylindrical between people (91). However, these assumptions do not apply as well to the lumbar vertebrae.

In addition, precision errors in the range of 1% are critical to the interpretation of results, as reported changes in aBMD due to exercise programs often fall within this range (92). A lower percent reproducibility combined with a greater number of measurements in several subjects narrows the confidence interval for results. Cummings and Black demonstrated that the 95% confidence interval for 4 measurements that show a 2% loss in bone using a technique with 3% reproducibility is +0.6% to –4.6%. The confidence interval for the same loss with a 1% reproducibility would be –1.1 to –2.9 (93). As the reproducibility of results is largely attributable to operator variability, the experience and skill of a single operator within a longitudinal study is critically important to maintain excellent precision and permit accurate interpretations of bone gain or loss. Errors in observed changes in DXA parameters can also occur when analysis protocols are inconsistent over time.

2.2.2 *Other Assessments of Bone Mineral*

Other techniques used to assess components of bone strength, that are suitable for pediatric use, include *peripheral* computed quantitative tomography (pQCT), magnetic resonance imaging (MRI), the hip structural analysis program (94), and quantitative ultrasound (QUS). As these outcomes are not reported in this thesis, they will be outlined only briefly.

QCT is used to assess peripheral bone at the tibia or radius (pQCT), and directly evaluates 3-dimensional cortical and trabecular densities (grams/cm^3) and bone cross sectional areas. Periosteal and endosteal dimensions can also be derived from pQCT. Thus, although pQCT cannot quantify bone at clinically important sites (i.e., hip and spine), this technique provides important information about bone structure and geometry at highly loaded sites, that is unattainable by DXA (85).

Utilizing MRI to evaluate pediatric bone is a relatively new approach. It provides the radiation-free assessment of bone and muscle volume and area. Quantification and standardization of MRI outcomes are still in development, however this modality has the potential to safely assess the magnitude of bone changes with growth and exercise at a range of skeletal sites (95).

The hip structural analysis (HSA) program was designed by Dr. Tom Beck at Johns Hopkins University School of Medicine, and offers an alternative analysis method for traditional DXA scans of the proximal femur (51,94,96). This method assesses bone structural parameters in three regions of the proximal femur (femoral neck, intertrochanteric region, and femoral shaft), and directly measures subperiosteal width (cm), bone cross-sectional area exclusive of soft-tissue spaces (CSA, cm^2), and calculates cross-sectional moment of inertia (CSMI, cm^4), cortical thickness and section modulus (an indicator of bone bending strength). HSA provides the opportunity to quantify changes in bone structure as well as bone mass. However, HSA suffers from the same limitations inherent in all DXA data, as the measurements are derived from a 2-dimensional assessment of a 3-dimensional structure, and therefore bone volume and density can only be estimated.

Although radiation-free QUS is an attractive option for pediatric bone assessment, its role in the evaluation of bone change with growth and with exercise remains ill-defined. Perhaps this stems from the fact that QUS measurement variables are *related* to bone mechanical competence, density and strength, but do not directly assess density and strength (97). In QUS systems, information is derived as bone progressively alters the shape, intensity and speed of a propagating sound wave. The wave is altered to a greater or lesser extent, depending on bone's mechanical and physical properties (98). Velocity is reported as speed of sound (SOS, m/second) and is related to elasticity and density (84,98). Attenuation values depend on architecture and density, as the ultrasonic wave is attenuated by spreading and scattering in cortical bone, and by absorption in trabecular bone, marrow and soft tissue (98). Ultrasound parameters had the same predictive ability for hip fracture as DXA measures of femoral neck aBMD in a large (n=5662) prospective study of women aged 75 and older (99). This supports an association between ultrasound parameters and aspects of bone strength. The use of ultrasound to evaluate children is in a relatively early stage of development. Thirty-one pre- and early-pubertal male gymnasts (Tanner Stage $\leq$ 2) and 50 normally active, matched controls were compared for change in ultrasound attenuation and velocity over 18 months (100). The gymnasts had a minimum competitive training history of 1.5 years. Ultrasound velocity was significantly greater in the gymnasts as compared to controls at baseline (suggesting superior density), but attenuation values did not differ between groups (suggesting similar bone structure). Attenuation increased significantly in the gymnasts over the 18-month observation period, but did not change significantly in controls; velocity did not change over this time. These results provide a platform for the future use of ultrasound in monitoring change in response to a bone loading exercise program in pediatric subjects.

2.3 *Maturation and Bone Mineral Accrual*

In this section, I discuss methods used to assess physical maturity in children and provide an overview of the important hormones that influence bone mineral accrual during growth. I also present studies that explored childhood bone mineral accrual with special reference to timing, regional specificity, and sexual dimorphism.

2.3.1 ***Assessing Maturity***

Chronological age does not adequately represent the diversity among children in the timing and intensity of the physiological changes that occur during growth (101). Early and late maturing children of identical chronological ages can differ developmentally by as much as much as 6 years (102). Further, children at the same initial developmental or maturity stage may advance at vastly different rates (103). Therefore, it is crucial that pediatric studies define the participants by both age and maturity. Throughout this thesis, the terms 'immature' and 'mature' refer to the state of development of the reproductive system. Childhood is defined as the period between infancy and the onset of puberty, while adolescence refers to the time after pubertal onset during which a mature reproductive system is developed (102).

There are a number of radiological and clinical methods to assess a child's maturity. By employing one of the Fels, Tanner Whitehouse, or Greulich-Pyle methods, radiographs can be used to compare a child's skeletal development against normative data (102). Skeletal age by standard radiography, although safe, is used infrequently because it involves the use of ionizing radiation. Although the advent of computerized skeletal rating systems has improved the accuracy of bone age assessments, the manual method, which is still commonly employed, has a between-observer error rate as high as 33% (104).

Tanner Staging was originally developed for use by clinicians to rate girls' and boys' reproductive maturity against established standards (101), and to ascertain normal, delayed or advanced development. This technique is currently used frequently in pediatric research studies, however, due to its invasive nature (the child must remove his/her clothing), self-assessment is more common in non-clinical studies. Self-assessment of Tanner stage is non-invasive, and has good agreement with physicians' ratings (105). Children classify themselves as Tanner stage 1 through 5 (based on developmental diagrams of breasts (girls), pubic hair (for both girls and boys) and genitalia (boys)) (101). Duke and colleagues (105) reported that in 66 boys and girls aged 9 through 18, the self- and physician-ratings agreed in 93% of cases for female pubic hair, 86% of cases for breast stage, and 91% of cases for combined pubic hair and genital stage in males. By this method, children in Tanner stage 1 are considered prepubertal, Tanner stages 2 and 3 represent the early pubertal phases, Tanner stage 4 adolescents are considered to be in the late stages of puberty, whereas Tanner stage 5 represents reproductive maturity. Self-reported Tanner staging was the method used to categorize maturity in this thesis. Throughout this thesis, breast stage is reported for girls, and pubic hair stage is reported for boys. Breast stage, as compared to pubic hair stage, may be more reflective of gonadal activity in girls (106). Assessment of pubic hair stage in boys appears to be more reliable in duplicate assessments than genital stage

(107). Breast/genital and pubic hair stages were similarly correlated with testosterone (girls: r=0.5, boys: r=0.8, P<0.05) and estradiol (girls: r=0.6, boys: r=0.5, P<0.05) levels in a cross-sectional study of 108 early adolescents (108).

There is a wide range of chronological ages within each Tanner stage. Tanner staging indicates the sequence of pubertal events more aptly than does chronological age. In most girls, menarche is achieved after Tanner breast stage 3, following peak height velocity. These events can occur any time between 10.5 and 15.5 years of age (101), but are most commonly observed in girls at age 12.7 years (11,109) (Figure 2).

Probably the most precise way of measuring physical maturity in children is relative to peak height velocity (PHV). This, however, requires that a minimum of three years of longitudinal data be collected across the peak linear growth period. With PHV, a child can be positioned along his/her individual growth continuum, whereas by Tanner staging, a child must be represented by one of five categories. On average, peak height velocity occurs at 11.8 years (Tanner stage 3, Figures 1 and 2) in girls and 13.4 years (Tanner stage 4) in boys (11,101).

2.3.2 *Hormonal Regulation of Bone Mineral Change*

As children enter puberty, a number of biochemical changes occur that relate to and enhance bone development. At approximately 10.5 years in girls and 11.5 years in boys, a significant change occurs in the amplitude of the hypothalamic 'gonadostat'. At this time, the primary systemic hormones involved in skeletal growth are estrogens and androgens, growth hormone (GH), and insulin-like growth factor-I (IGF-1).

Sex Steroids. Sex steroids enhance GH and growth factor production, and work in concert with GH to promote linear growth (110,111). In both females and males, estrogens have particular influence on bone accrual, mineralization, linear growth and epiphyseal closure, while androgens likely have stronger ties to cortical bone size (111-113). The apposition of bone on the endosteal surface, unique to the pubertal period, is influenced by levels of estrogen. Testosterone, on the other hand, likely exerts a stronger effect on bone apposition at the periosteal surface (114).

Around Tanner Stage 2, estrogen levels in girls are high enough to promote breast development, and subsequent to this, linear growth rate increases (115). Estrogen levels increase sharply between Tanner stages 2 and 4 (116,117), coinciding with the timing of peak bone mineral accrual velocity (Tanner stage 3) (11), as well as menarche (Tanner stage 3 – 4) (109) (Figure 1). In a 4-year longitudinal study of 30 girls, the steepest slope of increase for testosterone in girls was noted between Tanner stages 2 and 3 (116).

In maturing boys, estrogen levels correlate with pubertal stage, and testosterone and IGF-1 levels (118). The greatest absolute change in estrogen occurred between Tanner Stages 4 and 5 in a longitudinal study of boys (118). Hormone concentrations were measured every 4 months for a minimum of 5 years in 23 normally growing boys, who were between 8 and 12 years old at first measurement. Between Tanner Stages 4 and 5, estrogen levels increased from 10.7±2.13 pmol/L to 42.5±3.27 pmol/L, while total testosterone climbed from

13.9±0.66 nmol/L to 20.0±0.36 nmol/L during this time. As with girls, the boys' surge in estrogen corresponds to the timing of peak bone mineral accrual velocity (Tanner stage 4-5) (11). Longitudinal studies (118,119) demonstrated a surge in testosterone levels following Tanner stage 2 in boys.

Growth Hormone (GH). Growth hormone stimulates linear growth in a dose dependent manner, and in normally growing children, the peak amplitude of GH pulsatile secretion coincides with peak height velocity (115,118). The GH influence on bone is likely mediated through local and systemic insulin-like growth factors (5), that have anabolic effects on longitudinal growth and possibly bone mass (120,121). In prepubertal children, GH is the primary contributor to bone growth, while sex steroids act in concert with GH to exert a significant influence on bone during and beyond puberty.

Insulin-like growth factor-1 (IGF-1). Serum levels of IGF-1 reflect growth hormone secretion in healthy children (122). The simultaneous, and possibly synergistically anabolic, rise in GH and IGF-1 levels is unique to the pubertal growth period (123). Insulin-like growth factor levels increase more abruptly after age 10 in girls and age 11 in boys (around the Tanner stage 1-2 transition), with peaks occurring around age 13.5 years in girls (Figure 1) and 16 years in boys (Tanner stage 4-5). After these peaks, serum IGF-1 levels decline quickly (124). In one large cross-sectional study of boys and girls, IGF binding protein-1 decreased from prepuberty to peripuberty, and then rose after age 17 years, indicating a higher level of free, biologically active IGF during puberty (124).

Recent research has linked serum IGF level to BMC and aBMD in Tanner stage 2 and 3 girls (114), and to cross sectional and cortical bone areas in 7 to 18 year old girls and boys (125). In sixty-five 9 to 14 year-old-girls, the correlation between serum IGF-1 and metacarpal cortical thickness was strongest in Tanner stage 2 girls ($r=0.76$, $P<0.05$) (114). An 18-month longitudinal study of 18 Tanner stage 1 and 2 male gymnasts demonstrated a strong correlation ($r=0.67$, $P<0.05$) between change in calcaneal ultrasound bone parameters and baseline serum IGF-1 (100). Thus, the bone change resulting from extreme high impact activity may be mediated by high levels of IGF-1.

The relative timing of events associated with normal maturation are depicted in Figure 1, that shows peak height and bone mineral content velocities, and GH and IGF-1 levels, as well as major increases in estrogen and testosterone levels in girls during early to peri-puberty (Tanner stage 2 through 4). There are considerable changes in bone during this time, owing to synergistic contributions from numerous sources.

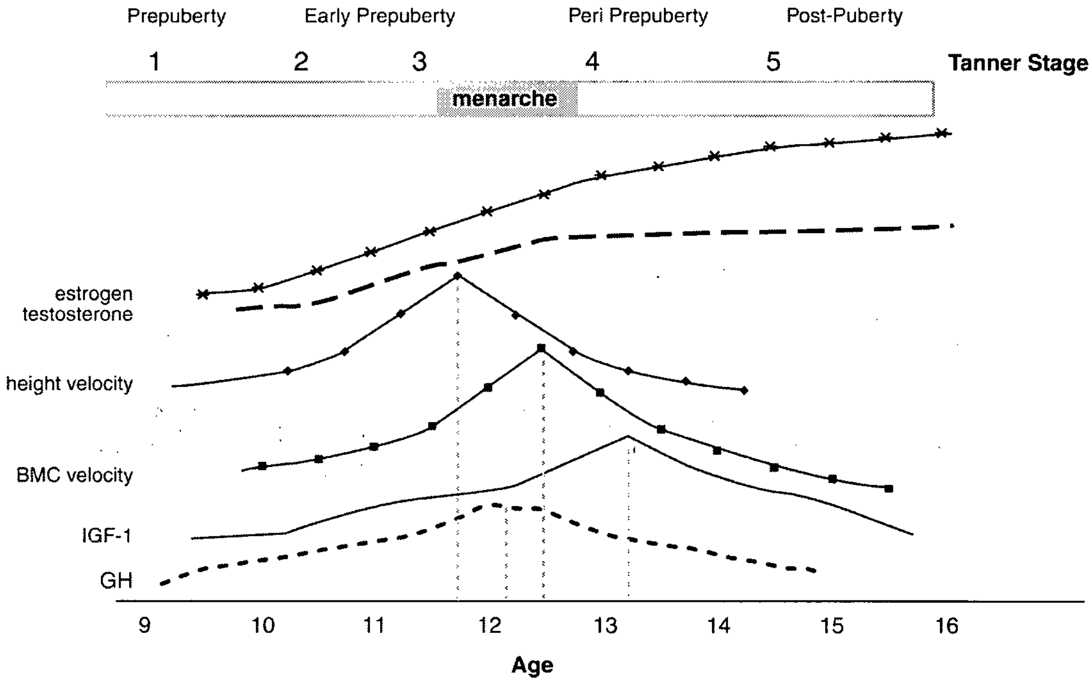


Figure 1: Peaks for height velocity, BMC velocity (bone mineral content velocity), GH (growth hormone) amplitude, IGF-1 (insulin-like growth factor-1) amplitude, and trends for estrogen and testosterone levels in girls relative to average age and Tanner Stage.

Peaks (connected to age by dotted lines) for height velocity, BMC velocity, GH and IGF-1 show the average age/Tanner Stage at which maximum gains in height or BMC are made, and when maximum levels of GH and IGF-1 occur. In boys, peak height velocity and peak bone mineral content velocity occur about 1.5 years later than in girls (at 13.4 years (Tanner stage 3) and 14.0 years (Tanner stage 4), respectively). Relationships between Tanner stages and peaks for GH and IGF-1 are similar for boys and girls. Derived from references (11,101,102,118,126)

2.3.3 *Bone Mineral Accrual*

2.3.3.1 Timing

Generally, periosteal apposition accounts for growth in bone width and the formation of cortical bone, while endochondral ossification is responsible for growth in bone length and trabecular bone formation (5). From birth to the onset of puberty skeletal weight increases fairly uniformly, when more rapid gains occur (127). A recently published, six-year longitudinal study of bone mineral accrual in 53 girls and 60 boys provides a unique record of the timing of bone mineral gain though critical stages in the maturational process (11). Figure 2 demonstrates that peak bone mineral content velocity (PBMCV) occurs approximately one year after peak height velocity in both boys and girls. The timing of PBMCV occurred at 12.5 ± 0.9 years in girls and 14.1 ± 0.95 years in boys. During the two years surrounding PBMCV (~age 11.5 –13.5 in girls and 13.1 – 15.1 in boys), approximately 26% of total body bone mineral was accrued (11). The dissociation between peak height velocity (PHV) and PBMCV may represent a transient period of weakness in the adolescent skeleton (11), when bone mineral accrual is catching up with linear growth. The correlation between height and bone mass, which is strong during childhood, weakens slightly during adolescence, at around the same time that PHV and PBMCV dissociate (128,129). After puberty, there is a slower gain in bone mineral, that contributes 15 to 20% of peak bone mass (130). Thus, late childhood through adolescence represents the most critical time for skeletal development, as bone modeling and turnover are greatest (127). Bone formation dominates over resorption from birth to the end of adolescence, and on average, the majority of skeletal mass is accumulated by age 18 (127).

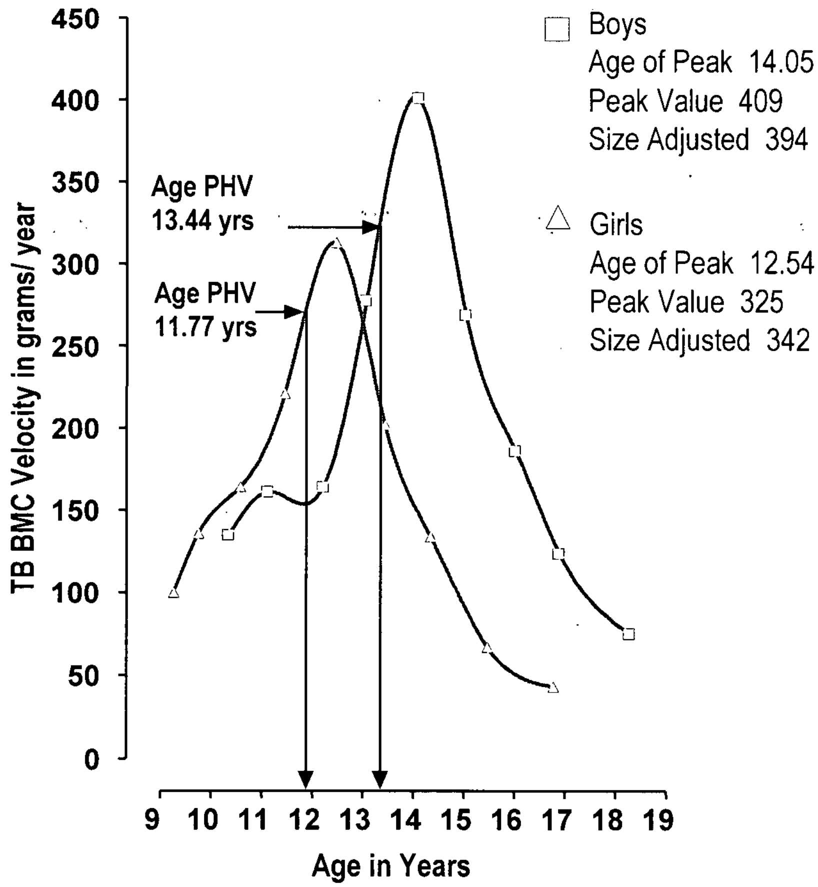


Figure 2: Timing of peak height velocity (PHV) and peak bone mineral accrual velocity relative to age in boys (□) and girls (△).

2.3.3.2 *Site-Specificity of Bone Mineral Accrual*

A 2-year, mixed longitudinal study of bone mineral accrual in 109 Australian Caucasian girls (6.9 – 16.6 years old) provided evidence for a different timing of bone accumulation in the legs and spine (131). At the onset of the study, 36 girls were prepubertal (Tanner Stage 1, 6.9 – 11.9 years old), 26 girls were peripubertal (Tanner Stage 2 to 4, 10.1 – 15.1 years old) and 36 girls were postmenarcheal (11.4 – 16.6 years). The greatest gains in spine BMC were made between 11 and 14 years (72 grams), with substantially less bone accumulated from 7 to 11 years (38 grams) and 14 to 17 years (46 grams). Equivalent gains in leg BMC were made between 7 to 11 years (240 grams) and 11 to 14 years (241 grams); the gain dropped to 149 grams during the 14 to 17 year age interval. In this study, the discrepant gains in bone mineral were attributed to a more rapid growth velocity for sitting height between 12 and 13 years, than at any time during pre or post puberty. Thus, the relatively greater gains in spinal BMC between 11 and 14 years of age reflect a period of rapid trunk growth velocity, which is consistent with previous work (102,132). The girls experienced a rapid growth velocity in the legs during the 7 to 11 year age interval (femur length increased, on average, approximately 19.0%), which slowed after age 11 (9.9% increase in femur length between 11 and 14 years). The equivalent gain in bone mineral content during the 7 to11 year interval and 11 to 14 year interval, despite a slower growth velocity in the latter interval, suggests that childhood bone mineral gain is related to growth in bone length and periosteal apposition, while in puberty, significant gains in BMC can be made by apposition of bone at the periosteal surface and contraction at the endosteal surface (131,133).

The results of Bass and colleagues (131) are similar to those reported from a 3-year, mixed-longitudinal study of bone gain at the lumbar spine and femoral neck in 90 Caucasian children between the ages of 6 and 14 (12). Children were classified as prepubertal if they displayed no evidence of sexual maturation over the 3-year study, peripubertal if they had some evidence of sexual maturation, and postpubertal if they were Tanner stage 5 at the first visit. Peripubertal children made much greater gains in lumbar spine BMC over the 3 years as compared to prepubertal children. In prepuberty, the average accrual rate was 0.027 g/cm^2 per year, while the corresponding value was 0.077 g/cm^2 per year in peripuberty (an approximately 3-fold greater gain in peri-puberty). In constrast, bone gains at the femoral neck were more similar during prepuberty (0.030 g/cm^2 per year) and peripuberty (0.047 g/cm^2 per year) (a 1.6-fold greater gain) (12). These two studies provide evidence that slower accumulation in cortical bone of the legs may span from mid-childhood to puberty, whereas a more hormonal-dependent surge in trabecular (spinal) bone accrual, appears to occur later, and may occur during a shorter, more defined 'critical period'.

2.3.3.3 Sexual Dimorphism in Growing Bone

There seems to be little difference in bone mineral content (134), aBMD (135), appendicular bone size (136) and both spinal cancellous and femoral cortical density by QCT (136) between the *prepubertal* male and female skeletons. During and after puberty, significant differences begin to emerge between the sexes for total body and regional BMC. Significant gender differences in peak velocities for bone mineral accrual and the 2-year BMC gain around peak were observed in The University of Saskatchewan Bone Mineral Accrual Study (11). Total body peak bone mineral accrual velocity was 52 grams/year greater in boys, which conferred a BMC advantage of 104 grams after the 2 years of peak accrual (~Tanner stage 4-5), after controlling for height and weight. This pattern was similar for the femoral neck, but was not observed for accrual at the lumbar spine.

Results from cross-sectional studies that compared aBMD in pubertal and post-pubertal boys and girls conflict. In a study of 98 Caucasian girls and 109 Caucasian boys aged 9 – 18 years, *lumbar spine* aBMD did not differ significantly between girls and boys when compared by chronological age or maturational stage (129). In this same sample, aBMD of the *femoral shaft* was greater in boys than girls at all pubertal stages after pre-puberty. When femoral shaft aBMD was compared between boys and girls based on chronological age, boys were higher at age 17 only. The discrepancy in findings based on pubertal stage and chronological age illustrates the importance of interpreting bone parameters relative to maturity in both sexes. A weakness of the Bonjour et al.(129) study was the small sample sizes within some age groups (i.e., n = 8 for 17-18 year old females). Another cross-sectional study in 205 boys and 295 girls reported that, on average, girls had higher lumbar spine aBMD at all ages from 4 through 20 (137). This sample was a mixed-ethnic group (Caucasian, Black and Asian), with unequal ethnic distributions between male and female groups. As the between-race differences in aBMD are likely greater than the between-sex differences (20), this factor may have unduly influenced the results. Furthermore, as girls and boys were compared at the same chronological age, differences at younger ages were likely due to the earlier maturation of girls.

A 1-year prospective study examined BMC and aBMD of the lumbar spine and femoral neck in 37 girls and 28 boys between 7 and 20 years of age (138). The mean annual gains in aBMD at both sites were similar between boys and girls. Boys had greater annual gain in BMC and bone size at the femoral neck compared with girls, which agrees with other findings (11). Interestingly, one large cross-sectional study of 469 males and females (6 to 40 years of age) demonstrated greater volumetric BMD (by pQCT) at the radial diaphysis in the adult females than males (139). On the other hand, the discrepancy between sexes in BMC and cross-sectional bone area was much greater, in favour of the males, in the adults than in children. Although limited in its cross-sectional design, these data suggest that periosteal apposition is greater in males than females in the later stages of growth, which, in this study, explained sex differences in BMC. The greater volumetric BMD in females was attributed to less endosteal expansion than in males (139).

The simultaneous accumulation of bone at both the periosteal and endosteal surfaces is unique to the pubertal growth spurt, and confers a sizable gain in cortical width in both girls and boys during this time (140). This critical time for bone mineral gain was further defined by Bass and colleagues (131) in their study of 109 schoolgirls, previously described under 'Site-Specificity of Bone Mineral Accrual'. Their data was obtained by assessing femoral midshaft periosteal and medullary diameters using the ruler function on a Lunar densitometer (Lunar, Inc.), and the same diameters of the third metacarpal midshaft with radiogrametry. They reported increases in cortical width at the midshaft of the metacarpal and femur between Tanner stages 1 and 2, due to periosteal apposition only. The increase in cortical width was further enhanced after Tanner Stage 3 at the metacarpals and at menarche (~12.7 years, Tanner Stage 4+) for the femoral midshaft due to contraction of the medullary canal. A similar longitudinal or mixed-longitudinal study in boys spanning several maturational stages is warranted.

In summary, in pubertal and post-pubertal children, differences in aBMD, *by DXA*, between the sexes appear to reflect size differences (20) rather than actual discrepancies in density. There is some evidence for greater volumetric BMD at the radial diaphysis, by pQCT, in adult females than males which may be due to greater endosteal apposition (20), however, mature females have lower cross-sectional bone area due to less periosteal apposition in the latter stages of growth (139,141). As yet, we have no informative longitudinal studies that have employed pQCT to evaluate sex differences in volumetric BMD across the stages of growth. To date, cross sectional studies suggest that differences between males and females in spine or femoral vBMD (by QCT) are quite subtle, if they exist at all (142). Longitudinal studies that utilize pQCT measures of bone in pre-, peri- and post-pubertal youth are warranted to ascertain the sex differences in bone volume and structural changes that occur with growth.

2.4 Determinants of Bone Mineral Accrual: Heredity, Race, and Dietary Calcium Intake

There are numerous influences on bone mineral accrual. Mechanical loading through physical activity is one such influence, but it should be considered in light of other major determinants: heredity, race, and dietary calcium intake. In this section, I first discuss heredity as a determinant of bone mineral accrual with respect to familial resemblance and polygenic influences. Second, I discuss differences in fracture rates, bone parameters, and lifestyle factors between Asians, Caucasians and Blacks. I then give consideration to calcium requirements, its threshold behaviour, and the association between childhood calcium intakes and bone mineral accrual.

2.4.1 *Heredity: Familial Resemblance*

The family unit reflects the combined influence of genetics and environment. Heritability is not a constant, but an estimate that is relative to the age and situation of family members (143). To quantify the proportion of aBMD variation attributable to genetic inheritance, some authors have attempted to tease out external factors (i.e., similarities in physical activity, calcium intake, and smoking and drinking habits) in their analyses of adult family groups (18,144,145). After controlling for environment, reports of familial influence on aBMD range from 46 – 62%, depending on skeletal site (18), and 39 – 84% (for total body aBMD) depending on age (145). Two investigations of aBMD resemblance between growing children and their parents revealed significant relationships as early as age 8 (146,147). Thus, the family influence on absolute aBMD is apparent at a very early stage.

Twin studies provide some of the strongest evidence that aBMD is a product of environmental and lifestyle modulation of the genetic template. Although monozygotic twins have identical genetic compositions, within-pair variability in aBMD is 10 to 20% (148). In 112 female twin pairs (57 MZ, 55 DZ) with a mean age of 52 years, genetic heritability was estimated at 78, 76, and 79% for lumbar spine, femoral neck, and total body aBMD, respectively (148). An extensive longitudinal twin study involving 215 female pairs aged 10 to 26 years demonstrated that peak femoral neck aBMD similarity in monozygotic twins occurred between the ages of 10 and 13 (149). Environmental components were more influential on lumbar spine and femoral neck aBMD in twins 13 to 17 years old than in younger or older twins (up to 26 years old).

It is significant that in twin studies, lean mass is repeatedly the best independent correlate of variation in aBMD (149-151) and change in aBMD during growth (152). This indicates strong genetic control over lean (muscle) mass (153), but at the same time emphasizes a common environmental component where twins might be expected to participate in similar types and amounts of physical activity that affect muscle mass development.

2.4.2 *Heredity: Polygenic Influence on Bone Mineral Accrual*

A phenotypic trait is defined as polymorphic if more than one allelic form exists in a normal population (154). Subtle genetic polymorphisms that influence height, weight, growth, timing of maturation, and the intricacies of bone (re)modeling likely work in concert to influence bone area, content, and density. Bone densitometry allows for the study of the genetics of bone in terms of a defined phenotype - (areal) bone density (155).

Significant associations have been reported between polymorphisms of several genes and BMD in children and adolescents (156-164). Investigators have focused on several genes that may contribute to the polygenic control of bone during growth. These include the vitamin D receptor gene, estrogen receptor gene, and genes coding for type I collagen, transforming growth factor-B, insulin-like growth factors, and interleukin-6. The relationships between polymorphic genes and bone outcomes are likely largely mediated by bone size, maturity, gene interactions, and race. This was highlighted in a recent study that investigated aBMD, age at menarche (retrospectively), and the polymorphic Apal site of the vitamin D receptor gene in 120 Japanese girls, 18-19 years old (164). Age at menarche was significantly earlier, and BMI higher (NS) in heterozygotes (Aa) than homozygotes (aa), and there was a significant association between age at menarche and forearm aBMD in the heterozygotes only. Thus, the genetics of peak bone mass is part of a complex picture, integrated with the genetics of maturation and body size. The studies within this thesis did not examine the genetic contribution to bone mineral gain during growth, and thus, this complex area will not be reviewed here.

2.4.3 *Race*

2.4.3.1 Race and Fracture Rates

Race, by definition, refers to three major categories of humans: Asian, Black and Caucasian (165). Ethnicity, on the other hand, refers to the myriad of factors that define a human group, including shared environment, language, religion, country of origin, diet, and typical activities (165). The impetus for comparing bone parameters between racial (or ethnic) groups arises from the different fracture rates among races. There is a greater difference in fracture rates between races than between males and females within a race. Fracture rates are lower in non-Caucasians than Caucasians, with the lowest incidence reported in South African Blacks

(166). Industrialization appears to have influenced fracture trends within countries. In Asia (especially Hong Kong), there has been a dramatic increase in hip fracture rates since 1960, and this rate is now approaching that of Caucasians in North America. Epidemiological data show that the vertebral fracture rate is higher in Asians from Hong Kong, China and Taiwan than in Caucasians living in North America (167,168).

2.4.3.2 Race and Bone Parameters in Children and Adolescents

In general, studies in adults have reported that areal BMD, at several skeletal regions, follows a pattern of Blacks < Caucasians < Asians, a trend which is only partly explained by bone size (20). Other observations of greater trabecular thickness in Black as compared to White adults in the U.S. (169), and greater trabecular density (by QCT) in Caucasian-American as compared to Japanese adults (170), point towards a sound structural basis for racial differences in aBMD measurements in adults.

There have been few studies comparing bone mineral between children of various ethnicities. McKay and colleagues (21) studied prepubertal (8.9 ± 0.7 years) Asian (n = 58) and Caucasian (n = 110) boys and girls in Vancouver, BC. Although there were large discrepancies in physical activity (15%) and calcium (35%) intake in favour of the Caucasian children, ethnic differences in proximal femur aBMD were only apparent for boys (+8% for Caucasian boys). Furthermore, when body size, sex, and lifestyle factors were controlled in hierarchical regression, ethnicity was not a significant factor in explaining variance in bone mineral. The prospective follow-up to this study revealed similar 8-month changes in aBMD at a range of skeletal sites in Asian and Caucasian children (92).

Results from a recently published study of 336 prepubertal (6 –11 years old) children (n = 135 Asian, n = 79 Black, n = 122 Caucasian) demonstrated an ethnic difference in total body BMC, where Black children had significantly greater BMC (+ 31 grams, $P<0.01$) than non-Black children (171). Although the report did not present statistical comparisons between BMC of Asian and Caucasian children, it appeared that Caucasian girls (TB BMC = 1240 grams) more closely resembled Black girls (TB BMC = 1258 grams) than Asian girls (TB BMC = 1156 grams). In boys, means for total body BMC in the three ethnic groups were quite similar (Caucasian = 1251 grams, Black = 1273 grams, and Asian = 1272 grams). In this cohort, total body BMC was most closely related to body size and bone area, thus, the racial differences were largely a function of body size.

Cross-sectional studies in Black and Caucasian children at a range of maturational stages provided initial evidence that racial differences may become more apparent with pubertal advancement (172,173). Volumetric bone density of the lumbar spine (by QCT) was no greater in Black as compared to age- and maturity-matched Caucasian girls until puberty (172). At sexual maturity, the femoral cross-sectional area (by QCT) was 5.7% greater in Black children as compared to age-, maturity- and size-matched Caucasians, with little difference at lesser maturational stages (173). To my knowledge, no studies have compared volumetric bone mineral density, bone cross-sectional areas (by pQCT), or bone structural parameters (by pQCT or HSA) between Asian and Caucasian children.

The results of a cross-sectional study of lumbar spine and femoral neck aBMD and calculated bone mineral apparent density (BMAD) in Black (n = 115), Caucasian (n = 103), Hispanic (n = 102) and Asian (n = 103) youths living in California (174) are presented in Table 1. For girls, there was an overall trend for similar aBMD and BMAD in early puberty among ethnic groups, with greater differences between Black girls and Asians and Caucasians noted at maturity (Tanner Stage 5). For boys, there were more differences in early puberty (Black children had greater BMD and BMAD than other groups), than in mid-puberty. In the mature group, FN BMAD was significantly greater in Black than Asian boys only.

Table 1: Results of a cross-sectional study comparing lumbar spine and femoral neck areal bone mineral density (aBMD) and estimated bone mineral apparent density (BMAD) in Black, Asian, Caucasian and Hispanic youths. (174).

	Early puberty (Tanner Stage I - II)	**Mid-Puberty (Tanner Stage III - IV)**	**Maturity (Tanner Stage IV +)**
Lumbar Spine aBMD	**Girls:** no differences **Boys:** Blacks > Hispanics	**Girls:** no differences **Boys:** no differences	**Girls:** Blacks > Asians **Boys:** no differences
Lumbar Spine BMAD	**Girls:** no differences **Boys:** Blacks > Hispanics, Caucasians	**Girls:** no differences **Boys:** no differences	**Girls:** Blacks > Asians, Caucasians **Boys:** no differences
Femoral Neck aBMD	**Girls:** no differences **Boys:** Blacks > Asians	**Girls:** no differences **Boys:** no differences	**Girls:** Blacks > all others **Boys:** no differences
Femoral Neck BMAD	**Girls:** no differences **Boys:** Blacks > all others	**Girls:** Blacks > Whites **Boys:** no differences	**Girls:** Blacks > Asians, Caucasians **Boys:** Blacks > Asians

Although the total sample size for this study was large (N= 423), when the group was divided into 3 maturity groups, 4 ethnic groups, and two sexes for analyses, there were low numbers in some categories (174). The authors suggested that because BMAD (which attempts to correct for bone depth) was greater, in some cases, in Black children compared with other ethnic groups, aBMD differences between races should not be solely attributed to differences in bone size.

The same research group recently published a 4-year mixed longitudinal study comparing 423 Asian, Black, Hispanic and Caucasian males and females between (initially) 9 and 25 years (89). The most consistent differences were observed between Black and non-Black subjects, such that Blacks had significantly higher mean aBMD at the spine, femoral neck, total hip, and whole body, and greater lumbar spine and femoral neck BMAD as compared to all other subjects (males and females separately). Fewer differences were noted between other ethnic groups. Asian females had lower femoral neck and whole body aBMD than Caucasian and Hispanic females, and femoral neck BMAD was lower in Asian and Caucasian females as compared to Hispanics. Among males, lumbar spine aBMD was lower in Hispanics as compared to Asians or Caucasians, and total proximal femur aBMD was greater in Caucasians than Asians and Hispanics. For the total proximal femur and spine, Asian boys and girls tended to reach a lower peak aBMD earlier than Caucasian boys and girls, respectively. Unfortunately, the statistical approach utilized in this study did not allow for a comparison of bone accrual by ethnic group within specific age or maturity groups.

Hip axis length and vertebral bone size also vary between Asian and Caucasian youths (174,175). The larger vertebrae observed in Caucasian adults are likely stronger than the smaller ones in Asians, accounting for the lower fracture rate in Caucasians. A shorter hip axis length, typical of the Asian population (174), may be responsible for their lower incidence of hip fracture as compared to Caucasians (176,177).

Although it is uncertain where along the maturational spectrum racial or ethnic differences become apparent, current evidence from the limited cross-sectional and longitudinal studies suggest two things: (1) ethnic differences in areal bone mineral density are at least partly related to bone size, and (2) ethnic differences in aBMD may increase with growth in girls, and may be greater closer to maturity. The relationships between race-specific bone geometry, structure, and material properties have not been investigated, and would provide great insight into the bone strength differential, and thus, fracture incidence, between populations.

2.4.3.3 Ethnicity, Lifestyle and Implications for Bone Health

Ethnic-specific differences in lifestyle among different groups of Asians may modulate, to some extent, the racial templates for bone. Bone mineral accrual and growth were modified by diet in a study of 243, 5-year-old Jiangmen-Chinese and Hong Kong-Chinese children (178). Dietary practices differ as part of the culture in these two geographically close regions. Children in Jiangmen consume 50% less dietary calcium than Hong Kong children. There were no between-sex differences in body size or bone parameters, but the Hong Kong boys and girls were significantly taller, heavier, had greater radial BMC and bone-width corrected BMD than the Jiangmen boys and girls.

Lifestyle practices of young Asians and Caucasians living in a similar environment were different in the few studies that examined this issue (21,137,179-181). Lower physical activity levels were reported in Asian than Caucasian girls in the Netherlands (137) and United States (181). Caucasian-American boys reported taking more activity lessons and participated in more vigorous activity both inside and outside of school than Asian-American boys (180). In British Columbia, Canada, Asian high school students consumed less calcium than their Caucasian peers (179), and Asian school children consumed 35% less calcium and were 15% less active than Caucasian school children (21). Thus, it appears that Asian children living in North America may present with greater risk factors for poor bone health than their Caucasian peers. The long-term impact of activity and nutritional habits on bone health in various ethnic groups has yet to be ascertained in a prospective study.

2.4.4 *Calcium Requirements and Retention in Childhood*

In the following sections I will examine the current recommendations for calcium intake in light of evidence pertaining to calcium requirements and its threshold behaviour during growth. I then present evidence surrounding the association between childhood calcium intakes and bone mineral accrual, examining, specifically, retrospective studies, longitudinal studies and randomized, controlled trials.

2.4.4.1 Calcium Requirements

Thirty-two percent of the adult skeleton is comprised of calcium (29). From birth to the end of adolescence, 150 mg/day of calcium, on average, must be retained from the diet (182). The highest requirements for calcium are during infancy and adolescence- the two periods of greatest skeletal growth and calcium absorption (183). The 6-year longitudinal Saskatchewan Bone Mineral Accrual study of 113 pubertal children (60 boys) reported peak calcium accretion rates of 359 $\pm$ 81 mg/day for boys and 284 +/- 58 mg/day for girls (184). These values were calculated from peak bone mineral accrual rates (as measured by DXA) by assuming a 32.2% fraction of calcium in bone (184). The calcium intakes in this group were 1113 $\pm$ 378 mg/day and 1140 $\pm$ 392 mg/day in girls and boys, respectively. The accretion rates greatly exceeded the maximum calcium retention estimated in young adults (114 $\pm$ 133 mg/day) in a meta-analysis of 519 individuals from 34 different studies (185), and illustrate the increased physiological drive to retain calcium during growth. The recently published Dietary Reference Intakes (DRIs) reflect the high calcium requirements (and a greater capacity to retain calcium) for adolescent males and females- the current recommendation is a lifetime high of 1300 mg per day (186).

2.4.4.2 Threshold Behaviour of Calcium

An intake threshold for calcium is defined as the point below which intake is directly related to retention, and above which there is little increase in retention with an increased intake (185). This threshold may be genetically determined, as two studies in children have shown that VDR gene polymorphisms are related to large differences in calcium retention (159,187). A calcium threshold behaviour was demonstrated in growing rats (188), but because of appropriate ethical limitations this cannot be studied definitively in children. A meta-analysis of 34 studies that included 519 individual calcium balances in subjects from infancy to 30 years of age, showed a slope (from balance vs. intake) close to zero in all groups at the highest intake levels (185). At lower intakes, the slope was uniformly positive. In adolescents, the maximum balance was in the range of 396 mg/day, which corresponded to an intake of 1480 mg/day. Intakes in excess of this value did not elicit further increases in calcium balance. In another study, greater amounts of calcium were excreted at intakes of approximately 1500 mg/day compared with lower intakes (189). Intakes of calcium at the threshold should provide maximum skeletal retention, and promote the potential to achieve peak bone mass (183), however, there is no evidence that intakes in excess of the threshold will offer increased benefits to bone.

2.4.4.3 Association Between Childhood Calcium Intakes and Bone Mineral Accrual

A retrospective study of adult women indicated an association between milk and dairy products consumed over adolescence and current bone mineral density (190). Ecological research that compared adult populations with different lifetime calcium intakes in both China and Yugoslavia suggested that high calcium intakes provided a benefit for peak bone mass (191,192). Measurements of 5-year old Chinese children accustomed to very low and average calcium intakes, respectively, showed 14% greater BMC in the average intake group (178). These studies support the theory that calcium is a required element for bone mineral accrual. However, definitive data, from prospective intervention trials, to suggest a strong link between calcium intake, retention, and bone accrual are scarce.

Cross-sectional studies of children and adolescents provide support for a positive relationship between calcium intakes and a single measurement of BMC or BMD (137,178,189,193,194). There have been few longitudinal studies of calcium intake and bone accretion (184,195,196). One of these demonstrated that bone accrual continued after cessation of longitudinal growth in 156 college women studied for 5 years. In these young women, rate of bone accrual for the total body, lumbar spine and forearm was positively correlated with calcium intake (195). Fehily and colleagues performed a 14 -year follow-up study of 20-year-old women (n = 371), who, as children (7-9 years old) had been randomized to either a calcium intervention or control group for 2 years (196). There was a trend (non-significant) for higher BMC and aBMD in the intervention group at age 20, and a significant relationship between *current* calcium intake and aBMD.

In the 6-year Saskatchewan study described earlier, the correlations between peak calcium accretion rate and calcium intake were 0.05 for boys (average intake was 1140 $\pm$ 392 mg/day) and 0.07 for girls (average intake was 1113 $\pm$ 378 mg/day), and explained less than 1% of the variance (184). These data suggest that calcium retention in children is adaptable to calcium availability. On the other hand, a recently published, 2-year study of bone mineral content accretion in 45 premenarcheal Caucasian girls in British Columbia demonstrated that a small, but significant proportion (4.7%, P<0.05) of the variance in total body BMC gain was explained by habitual calcium intake (197).

Double blind, randomized, placebo-controlled calcium intervention studies in pre- and peri-pubertal children provide some evidence for a direct, positive relationship between intake and bone accretion (198-203). In these studies, calcium supplementation ranged from 300 mg/day (202) to 1000 mg/day (198). The confounding influences of growth and genetics were well controlled in one study that randomized twins to either a control or intervention group (198). This twin study (198) had the highest supplementation (1000mg), the longest intervention period (36 months), and the greatest differences in bone mineral change between *prepubertal* twins at the radial midshaft (+5.1% in supplemented twins) and distal radius (+3.8% in supplemented twins). The differences in gain at the lumbar spine (+2.8%) and femoral neck (<1%, NS) were less, and were in agreement with other intervention studies (199-203). Girls who were postpubertal by the end of the 3-year study did not receive any benefit from the supplements (198). Bonjour and colleagues reported the greatest gains for those girls who had pre-supplementation calcium intakes below 880 mg/day (199).

Follow-up studies to intervention trials are rare, but are a necessary step in establishing a causal relationship between calcium intakes and peak bone mass, and in later life, fracture. In 2 follow-up studies ranging from 18 to 36 months after intervention cessation, the between-group differences were no longer apparent (204,205). However, after another intervention study (199), at 1 and 3 ½ year follow-ups after supplementation cessation, the between-group differences in bone gain appeared to persist (206). Girls were originally supplemented by fortified foods with calcium from milk extract, while the majority of other studies supplemented with non-food sources of calcium (i.e., calcium carbonate in pill form (207)). The 3½ -year follow-up study brought maturational imbalances between control and intervention groups to the fore (206). Although girls in the intervention group seemed to maintain a bone mineral advantage, they were also 2.4 cm taller than controls, and tended to be at more advanced Tanner stages (which was not controlled in the analysis). These factors are associated with increased bone mineral, independent of the intervention. Thus, we lack conclusive evidence that the effects of calcium supplementation, which generally appear positive, are maintained when the supplement is withdrawn.

2.5 Physical Activity and Bone in Growing Children

Exercise during human growth is a predominant factor in determining bone's strength. This was recognized over 100 years ago and formed the major tenet of Wolff's Law (1892)- *"Bone will optimize structure, so as to withstand functional loading, and to ensure the metabolic efficiency of locomotion"* (55). The topic of physical activity and bone in growing children has been reviewed in several places (35,126,130). In this section, I briefly discuss how physical activity is assessed young people, with special reference to the Physical Activity Questionnaire for Children (PAQ-C). Next, I examine the association between generalized physical activity and bone parameters in children, discuss cross-sectional studies with an emphasis on elite young athletes, present the contribution of unilateral control studies (racquet sport studies) to the exercise-bone literature, and focus on an in-depth examination of all published exercise intervention studies in children and adolescents. Finally, I assess the current level of knowledge regarding what is known about whether the benefits of childhood physical activity persist into adulthood.

2.5.1 *Assessment: The Physical Activity Questionnaire for Children*

Studies that examine the contribution of physical activity to bone mineral accrual in large groups of children require an efficient, reliable method to assess typical exercise. As questionnaires can be administered to large groups at one time, self-administered recalls of physical activity are efficient and inexpensive (208). For these reasons, self-report is the most common method used to assess physical activity in exercise studies related to bone outcomes (209). However, these investigations are limited in that children must read and interpret the questionnaire items themselves. Thus, Sallis does not recommend recall questionnaires for children younger than 9 years (210).

The Physical Activity Questionnaire for Older Children (PAQ-C) is a self administered, 7-day recall questionnaire, designed to measure general, habitual physical activity in a moderate to vigorous range (208). In the Saskatchewan Pediatric Bone Mineral Accrual Study (11), it was administered 3 times a year to over 200 children for six years. This questionnaire was designed to discriminate by activity score between children who have high, average and low activity levels. It is a valid assessment that distinguishes between these groups (211). The PAQ-C was significantly, moderately related to other self-administered physical activity measures, motion sensors, and interview-assisted recall ($r = 0.39 – 0.63$, $P<0.05$) (208,211). In addition, the questionnaire was sensitive to differences in activity levels between girls and boys and to seasonal variation in activity (208). These factors affect validity related to bone health, as there may be some basis for a sex by activity interaction for absolute BMD (212), and different rates of bone accrual by season (213). From a bone health perspective, the PAQ-C is limited in that it can only relate general physical activity to bone accrual, and cannot specify types of beneficial activity or assist in recommending frequencies.

2.5.2 *General Physical Activity*

Studies of general physical activity in children show a positive relationship between activity levels and bone accumulation at most sites (11,214,215). The most recent longitudinal observational study of physical activity and bone accretion in children (11) showed that active children (with scores in the highest quartile on the PAQ-C) not only had significantly higher *absolute* values for bone mineral one year after peak accumulation, but also had significantly higher peak bone mineral accrual *velocities* than inactive children. One year after peak bone mineral accrual velocity, total body BMC in active girls and boys was 17 and 9% higher, respectively, than that of their inactive peers (after controlling for body size). The boys and girls in this study were initially 8 to 14 years of age (11).

2.5.3 *Cross Sectional Studies of Young Athletes*

Athlete studies provide a convincing model of the bone response to high levels of chronic mechanical loading. Young gymnasts sustain some of the greatest mechanical loads in sport. Young male gymnasts who performed common training manoeuvres on a force platform sustained peak forces of 3.6 (upper body) and 10.4 (lower body) times body weight (100). These loads incorporate the essential factors for bone adaptation as shown in animal studies: a combination of high magnitude strains (216), high strain rate (69), and unusually distributed strains (38,63). Cross sectional studies comparing young (7-11 years old) female gymnasts to swimmers (217,218), and nonathletic controls (217-220), consistently show significantly higher mean whole body (217), radial (218), lumbar spine (218,220), femoral neck (218,219) and trochanteric (219) aBMD in the gymnasts (Figure 3). Peripheral QCT measures in one study demonstrated that both trabecular and cortical components of radial BMD were significantly higher in gymnasts (219).

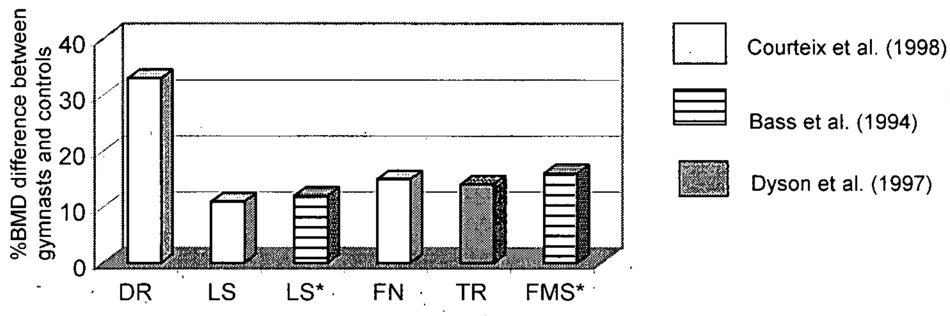


Figure 3: Percent differences in aBMD at the distal radius (DR), lumbar spine (LS), femoral neck (FN), trochanter (TR), femoral mid-shaft (FMS) between pre/early pubertal gymnasts and non-athletic controls (by DXA). *estimated volumetric BMD.

In these studies, lumbar spine, proximal femur, and femoral mid-shaft aBMD percent differences between gymnasts and controls tended to be approximately 10% (Figure 3). One study that reported >30% differences at the distal radius between gymnasts and controls (218) is indicative of the importance of 'unusual' strains to elicit an osteogenic response (58). While non-athletic controls likely experienced little loading at the radius, the gymnasts sustained high impact, unusual strains at this site. The distributions of the gymnasts' lower segment loading activities could be similar to the lower magnitude activities, i.e., running and jumping games, of the controls, and therefore, the gymnast-control differences at the femoral neck, trochanter, and femoral midshaft (~10%) were much less than at the distal radius (30%). Similarly, in boys, a cross-sectional study of 59, 15 – 20 year old, male junior Olympic weight lifters suggested that the osteogenic effects of extreme loading are great. The lifters had forearm bone mineral content more than 2 standard deviations greater than age-matched controls (221).

There are two important limitations in these cross-sectional studies that interfere with our interpretation of the effects of skeletal loading in children. First, there is a selection bias in favour of the athletes in cross-sectional studies. It is possible that athletes choose to compete in sport based on a positive physical predisposition to the requirements of the sport. That is, the bones of young athletes may have been stronger than their non-athletic counterparts *prior* to elite level training. Second, gymnastics and Olympic weight lifting studies demonstrate large differences in bone mass between children and adolescents sustaining extremely high skeletal loads over long time periods, compared with non-athletic controls. However, cross-sectional studies of elite young athletes provide little insight into the minimal effective loading that can elicit a positive bone response. The typical duration of their training programs (218,219,221) far exceed what would be recommended for a non-elite athlete population.

2.5.4 *Racquet Sport Studies and Starting Age*

The problem of self-selection in athlete studies is largely avoided in unilateral control studies of racquet sport athletes, as the non-dominant arm is used as the control. Two initial studies of adult racquet sport athletes in Finland set the foundation for exploring the influence of starting age of training on bone mineral accrual (222,223). In these studies, large differences in aBMD between the playing and non-playing humeri were observed in male (n=20) and female (n=19) adult squash and tennis players (+14% to +25%) (222,223), and greater side-to-side differences were noted in players who started training in childhood (+22%) versus adulthood (+9%) (223). In male and female controls, the side-to-side differences were small, in the range of 0 to 6%.

The same research group then studied female players (16 – 48 years old) with special consideration of starting age of training relative to menarche (224). The results clearly showed that players who began training before or at menarche had side-to-side aBMD differences that were two to four times higher than those who began training >15 years after menarche. The side-to-side differences were twice as large if training commenced 5 or 3 years before menarche, or at menarche, as compared to 5 years (or more) after menarche.

These Finnish researchers also measured side-to-side differences at the proximal humerus, humeral shaft and distal radius in 91 young female players, aged 7 to 17 years, who had been training regularly for at least a year, and 58 age matched controls (22). Girls were grouped by Tanner stage (1 through 5). Proximal humerus, humeral shaft and distal radius side-to-side aBMD differences were significantly greater in players as compared to controls at Tanner stage 3, 4, and 5. There were no differences between Tanner stage 1 players and controls; Tanner stage 2 girls differed only at the humeral shaft, the most heavily loaded site. A limitation of this observational study was that training volumes also increased with Tanner stage and critics have suggested this may have influenced greater bone gains in Tanner 3 – 5 girls, independent of maturity (130). Haapasalo and colleagues (22) demonstrated that training time did not correlate with side-to-side aBMD differences in the Tanner stage 1 or 2 players, while these variables correlated moderately in Tanner stage 3-5 players. Thus, frequent training did not associate significantly with bone gain in Tanner stages 1 and 2. The side-to-side aBMD differences between peri-pubertal players and controls (~ 10% at Tanner stage 3 and beyond) raise the possibility of a synergism between advancing pubertal status and loading-induced bone gain. However, only prospective studies that intervene with similar amounts and intensities of activity across maturity levels can truly investigate the presence off a critical period for peripubertal bone gain.

2.5.5 *Longitudinal Studies of Specific Physical Activity*

There are very few longitudinal studies that tracked bone changes in young athletes. Three studies of young gymnasts have recently been completed, one in boys (100), and two in girls (14,225). Pre- and peripubertal (</= Tanner Stage 2 throughout study) male gymnasts had significantly higher ultrasound velocity at the calcaneus, distal radius and phalanx at baseline than normally active controls (100). Over 18 months, ultrasound attenuation values in gymnasts increased to over 3 times the baseline value, but did not change

significantly in controls. Ultrasound velocity scores did not change significantly in either group. These velocity values reflect the resistance to deformation and are determined by elasticity and density of bone. Attenuation relates more closely to the quality of architecture within bone, as more bone with tighter connections will cause a greater attenuation of the sound wave (98). Thus, this study suggested that prepubertal bone architecture improved more than density in response to extreme loading. In 45 prepubertal female gymnasts (10.4 years old) aBMD increased at an average rate 30 to 85% greater than nonathletic controls over 12 months (14). In the most recent study (225), a small group of prepubertal 10-year-old female gymnasts (n=10) and same age controls (n=14) were followed for 3 years. The gymnasts had greater absolute and greater yearly change in aBMD (adjusted for lean mass) at the femoral neck, trochanter, and radius than controls. Thus, it appears that young gymnasts, both male and female, have higher absolute, and greater change in, bone parameters related to bone strength than less active controls just prior to puberty.

2.5.6 *Exercise Intervention Studies in Children and Adolescents*

As it is unlikely that any parent, clinician, researcher, or teacher would recommend that pre- and peripubertal children undertake elite gymnastics to increase bone strength, there is a clear need for studies of exercise interventions implemented in the community and school settings. Well-planned intervention studies should test and further define the 'critical period' hypothesis. That being said, it remains that "for an imposed load to effect a change in mass, it must materially alter the daily stress stimulus from its baseline equilibrium" (60). This presents a challenge for those designing bone loading programs for large groups of children, with diverse exercise histories and current levels of physical activity.

2.5.6.1 Exercise Intervention Studies in Pre-Pubertal Children

There have been two exercise intervention studies in children who were classified as prepubertal at both baseline and post-intervention (Table 2) (226,227). The difference in 8-month bone mineral gain between intervention and control groups ranged from 1.2 to 5.6% by BMC and/or aBMD, depending on measurement site (Table 2). With respect to bone structure (226), cortical thickness increased more in intervention boys than controls. This change was due to greater endosteal apposition, not increased periosteal diameter (226). Further work is needed to make the link between increases in BMC or aBMD and increased bone strength.

Although the bone response over 8-months was similar between the two studies, the intervention programs were distinct (Table 2). One study prescribed a 30-minute, 3 times/week extracurricular weight-bearing exercise program involving a range of games and activities (226). The other implemented a high impact, repetitive, box jumping program (227) (Table 2). This elicited peak ground reaction forces of 8.5 times body weight (228), compared with typical pediatric ground reaction forces of 2.5 times body weight during running (229), and 10 times body weight in elite gymnastics training (100).

From these studies it appears that for prepubertal children, the format or specificity of intervention may be less important than the magnitude of increase in weight-bearing activity. The activities in both interventions supplemented the regular 2-hours/week (226) and 30-minutes/week (227) physical education curricula. There were no differences in extracurricular physical activity or sports participation between intervention and control groups in either study. Thus, the interventions represented substantial 75% (226) and 150% (227) increases, respectively, in weight-bearing activity as compared to that undertaken by controls in their regular physical education classes and extracurricular activities.

These data demonstrate that the prepubertal skeleton is responsive to vigorous exercise programs that introduce a significant amount of mechanical loading, beyond that which is typically provided in physical education classes (226,227). Bone mineral change and growth in prepuberty are largely influenced by growth hormone, and occur independently of the input of sex steroids (in contrast to early and peri-puberty, as discussed in Section 2.3.2). Short bouts of intense exercise may activate the growth hormone-IGF-1 axis in both pre- (230) and peri-puberty (117). Long term activation and elevation of these hormones may mediate an effect on bone over time. Support for this theory comes from a recent study in growth hormone-deficient rats (231). Injections of growth hormone promoted bone formation under loading conditions; rats that were loaded in the absence of growth hormone did not show positive skeletal adaptations. Skeletal loading intervention studies in children thus far have not investigated the hormonal mechanisms underlying modelling changes in immature bones. It is unclear whether increases in growth hormone are necessary to promote bone gain, and whether less vigorous programs - ones that take less time, and incorporate a variety of moderate impact activities - could be effective in promoting bone mineral accrual in prepubertal children.

McKay and colleagues (92) implemented a less vigorous, school-based, bone-loading program, facilitated by the participants' regular classroom teacher within the physical education curriculum. The group was largely (89%) prepubertal at baseline. During each physical education class, children performed games and circuits involving jumping or skipping, and did 10 tuck jumps (92) (Table 2). The intervention did not require children to participate in any extra physical activity outside of school, and therefore the program represented a change in activity *type* (rather than an increase in duration) as compared to that of children at control schools. Changes in aBMD at the trochanter were significantly greater (1.4%, $P<0.01$) in the intervention children. Other studies of prepubertal children did not measure (226) or did not report (227) change at the trochanter. The less vigorous nature of the school-based intervention may explain the nonsignificant difference in change at other measured sites. As many of the activities involved running with jumping, muscle pull at the trochanter may have been substantial, resulting in greater change in this highly trabecular area (232,233).

2.5.6.2 Exercise Intervention Studies in Early Pubertal Children

Using a similar intervention to Bradney and colleagues (226) (Table 2), Morris et al. investigated the bone response at several skeletal sites in pre- and early pubertal (all premenarcheal) exercise intervention (N = 33) and control (N =38) girls (234). After controlling for differences in height and weight, girls in the intervention group gained significantly more total body, lumbar spine and proximal femur bone mineral (2.3 - 5.5%), depending on bone site and parameter (Table 2), than controls. A 10% difference in aBMD change at the femoral neck (in favour of the intervention group) was not statistically significant once change in height and weight were controlled for, which suggests a possible imbalance in either maturity or body size between groups. Furthermore, Tanner staging was expressed as an average for each group (Intervention 1.61 vs Control 1.50), instead of describing the numbers of girls within each Tanner stage at baseline. An imbalance in the number of Tanner stage 2-3 girls between intervention and control groups could greatly affect results, as girls who achieve Tanner stage 3 would be expected to make greater gains in height and BMC than girls in lesser Tanner stages (Figure 1). Despite these limitations, this was the first study to intervene with exercise in immature girls. The greater differences in bone mineral gain between intervention and control groups in this study as compared to previous exercise studies in adults (235-238), highlighted the growing years as a potentially important time for intervention. Prior to menarche, estrogen, growth hormone, IGF-1 and testosterone reach higher levels (Figure 1) – all factors that enhance bone formation. In combination with accelerating growth velocity, bone modelling and remodelling may occur more readily under the influence of mechanical loading.

2.5.6.3 Exercise Intervention Studies in Adolescents

Two exercise intervention studies in postmenarcheal girls failed to significantly increase bone mineral compared with control groups (Table 2) (239,240). In both studies, the intervention and control girls were well-matched for maturational status, and the exercise programs involved substantial amounts of training. The first study implemented a progressive, 6.5-month weight-training program, involving exercises on hydraulic machines targeting both the trunk and limbs (239). Changes in lumbar spine bone parameters did not differ significantly between intervention and control girls at post-intervention. The use of hydraulic machines may not have created an optimal strain environment (in terms of impact and novel distributions) (241) to elicit changes in bone. However, the role of maturity becomes apparent when intervention girls failed to show greater changes in BMC than their maturity-matched controls after completing a complex, high impact, intense, 9-month, plyometric training program (240) (Table 2). As shown in Figure 1, during postmenarche (Tanner stages 4-5) the velocities of height and bone gain decrease, and absolute levels of GH, and IGF-1 decline. As these pivotal bone-enhancing factors decrease, it likely becomes more difficult to promote modelling changes in bone. Furthermore, as has been shown in animal studies, the bone response to a given level of strain decreases with maturity (77,79).

2.5.6.4 Exercise Intervention Studies in Two Distinct Maturational Groups

A recently published prospective intervention addressed the issue of maturity-related skeletal responsiveness to mechanical loading by *simultaneously* intervening with an intense jumping program (Table 2) in premenarcheal (N = 25, Tanner stage 1 –3) and post-menarcheal girls (N = 38 Tanner stage 3 – 5, N = 1 Tanner stage 2) (23). BMC changes at the lumbar spine and femoral neck were 3 to 4% greater in premenarcheal intervention girls as compared to premenarcheal controls; changes did not differ by intervention and control group in the postmenarcheal girls.

Premenarcheal exercisers tended to have greater, although not significant, gains in cortical cross sectional area (relating to a change in bone structure) and cortical density, at the tibial midshaft, as measured by peripheral quantitative computed tomography (23). Changes in section modulus (an estimate of bone strength) at the tibial shaft did not differ significantly between groups. By using pQCT future studies can compare changes in both bone mass and structure between more highly trabecular areas (i.e., distal tibia) and the highly cortical tibial midshaft (23).

Heinonen and colleagues provided some evidence that the osteotrophic potential may be greater in the premenarcheal years as compared with postmenarche (23). However, it is critical to note that premenarche includes both pre- and early pubertal children, who, biologically, have different mechanisms of bone change.

2.5.7 *Exercise During Childhood and Bone in Later Life*

The goal of exercise intervention to promote bone health during childhood is to prevent fracture in adults and the elderly. Whether this can be achieved or not is a controversial issue - the 'Achilles heel of exercise' (242-244). Currently, there is very little research to support either side of the argument, as follow-up studies are rare.

One recent prospective study examined side-to-side differences in proximal humerus, humeral shaft and distal radius BMC in 64 elite adult female racquet sports athletes when they were competing frequently, and 5 years later, when their training was reduced to approximately 1/3 of the former commitment (245). The side-to-side differences were compared to the same healthy controls at each evaluation. The differences in humeral shaft BMC between playing and non-playing arms were well-maintained despite the reduction in training, in women who had started playing prior to menarche (+21%, both times) and those who had started later (+9%, both times); controls maintained a 3.5% difference between arms. This report lends strength to the theories that greater gains in BMC can be made when training is initiated prior to the end of puberty, and that exercise-induced gains do not dissipate with decreased training 5 years later (245). Earlier findings in 13 retired male tennis players (246), complement these comparisons. Athletes began their tennis careers at mean age 11 years. BMC was initially measured (time 1) when players were competing nationally (mean age 26 years). A four-year follow-up (time 2) was conducted after athletes had retired for 2.3 ± 0.6 years, and training levels were half those at time 1. Side-to-side BMC differences were comparable from time 1 to time 2 in the humeral shaft (25% at time 1, and 26% at time 2), proximal humerus (19% and 18%), and distal radius (13% both times). In 13 controls, BMC differences were less than 5%, and did not change from time 1 to 2.

A study of 99 retired female ballet dancers (51 ± 14 years old), showed that ballet lessons taken between ages 10 and 12 *only* (ie, Tanner stages 1 – 3) were related to the current difference between proximal femur aBMD of the dancers and their age-, height-, weight-, and menopausal status-matched controls. Current activity was not related to aBMD in these women (13). In another study, the effect of gymnastics training *before* puberty on total body and regional aBMD in 36 retired elite female gymnasts (25 years old) was profound (14). Areal BMD at the spine, legs and total body was 0.5 to 1.5 standard deviations higher in the retired athletes than in 50 active controls. The current mean weekly exercise was similar in retired gymnasts and controls (1.8 hours).

To my knowledge, there have been no follow-up studies of exercise interventions involving children. However, in twenty-nine 30-45 year old women, 6 months of detraining diminished the positive effects on aBMD that were apparent after a 12-month resistance training program (247). A cross-sectional study of retired male soccer players (19 – 85 years old) demonstrated that leg aBMD of 70 year- old retired athletes (n =51) was 6.5% higher than controls (adjusted for current activity and body composition) (248). Differences at the femoral neck and arms did not differ between 70-year-old former soccer players and control groups. In a separate analysis, the differences between retired athletes and controls dissipated with longer retirement, and became nonsignificant. However, this study was criticized (242) for small numbers (n = 128 former soccer players, in 5 age categories) and for obtaining training details retrospectively.

Further work linking childhood exercise to bone health in later life is critical. Future studies need to incorporate diverse measures of bone strength/health, such as magnetic resonance imaging and peripheral quantitative computed tomography at several bone regions, to approximate the full impact of an intervention on the immature skeleton. There is a need for controlled exercise interventions that test the magnitude of skeletal effects separately across the various stages of maturation to fully assess the existence of a 'window of opportunity' when the skeletal response to loaded exercise is optimized.

Table 2: Skeletal loading exercise intervention studies in children and adolescents.

First Author	Participants and Design	Exercise Intervention	Statistical Approach	Results (% Difference in gain between Ex and Con, after statistical adjustments)
Prepubertal				
Bradney 1998(226)	BOYS. Caucasian. N=20 Con, N=20 Ex; mean age 10.4 ± 0.2; age range 8.4-11.8 yr. All TS 1 throughout. **Randomized** by school: 1 Con + 1 Ex. DXA system: Hologic QDR 2000.	**Program:** One P.E. teacher supervised intervention program outside of school time. Included aerobics, soccer, volleyball, dance, gymnastics, basketball, weight training. **Frequency & Duration:** 3 times/week; 30 minutes/session; 8 months duration. **Progression:** Not stated.	Independent t-tests to compare bone changes between groups.	****TB:** + 1.2% aBMD. **LS:** + 2.8% aBMD. **PF:** Not measured. **FN:** Not measured. **GT:** Not measured. **#Femoral Mid-shaft:** + 5.6% BMC; + 5.6% aBMD, vBMD NS, +6.4% cortical thickness.
Fuchs 2001(227)	GIRLS & BOYS. Caucasian. N=44 Con; N=45 Ex; mean age 7.5 ± 0.2; age range 5.9 – 9.8 yr. All TS 1 throughout. Children were **randomized** to Con or Ex within one elementary school. DXA system: Hologic QDR 4500.	**Program:** Intervention took place outside of regular P.E. classes, supervised by research team. Each session: 100 2-footed drop landings from 61 cm height onto a wooden floor. Average GRFs= 8.5 x body weight (228). **Frequency & Duration:** 3 times/week; 10 minutes jumping/session; 7 months. **Progression:** Week 1-4: Progressed from 50 jumps per session (no box), to 80 jumps per session (from box). Week 5 - end : 100 jumps from 61 cm box.	1 factor ANCOVA; baseline bone, change in height, change in weight, and age as covariates.	**TB:** Not measured. **LS:** +3.1% BMC; +2.0% aBMD. **PF:** Not reported. **FN:** + 4.5% BMC; aBMD not significant. **GT:** Not reported.
McKay 2000(92)	GIRLS & BOYS. Asian & Caucasian. N=81 Con; N=63 Ex; mean age 8.9 ± 0.7 yr; age range 6.9 – 10.2 yr. Boys were TS 1 throughout; 89% of girls were TS1 and 11% were TS 2 at baseline; ~ 30% of girls were TS 2 by follow up. **Randomized** by school: 5 Con + 5 Ex. DXA system: Hologic QDR 4500.	**Program:** School-based; teachers chose activities from a variety of games, circuits, dances which incorporated jumping. 10 tuck jumps performed before P.E. class, and one time in classroom each week. **Frequency & Duration:** 3 times/week; 10 – 30 minutes/session; 8 months. **Progression:** As per fitness level of class; more challenging activities added after 3 months.	2 (Ex, Con) x 2 (Asian, Caucasian) x 2 (male, female) ANOVA to examine bone changes. Regression to examine intervention effect when baseline bone, height change, lean mass change, physical activity, calcium, sex, and ethnicity controlled.	**TB:** Not significant. **LS:** Not significant. **PF:** Not significant. **FN:** Not significant. **GT:** BMC not significant; + 1.4% aBMD.

Early Pubertal				
Morris 1997(234)	GIRLS. Ethnicity not stated, schools matched for ethnicity. N=33 Con; N=38 Ex girls; mean age 9.5± 0.9 yr; age range 9 – 10 yr. All premenarcheal throughout; TS 1 – 3. **Not randomized**; schools self selected: 1 Con + 1 Ex. DXA system: Hologic QDR 2000	**Program**: One P.E. teacher supervised intervention program outside of school time. Included aerobics, soccer, football, step aerobics, dance, skipping, ball games, weight training. **Frequency & Duration**: 3 times/week; 30 minutes/session; 10 months. **Progression**: In 10 week weight training session only.	Independent t-tests to compare bone change between groups. ANCOVA (with change in height and weight as covariates) to compare adjusted change in bone between groups.	**TB**: +5.5% BMC; +2.3% aBMD. **LS**: BMC not significant; +3.6% aBMD; +2.9% vBMD. **PF**: BMC not significant; +3.2% aBMD. **FN**: +4.5% BMC; aBMD not significant; vBMD not significant. **GT**: Not reported.
Adolescents				
Blimkie (239)	GIRLS. Ethnicity not stated. N=16 Con, N=16 Ex girls. Mean age 16.2 ± 0.2 yr; age range 14 –18 yr. All postmenarcheal (TS 4 - 5) at baseline. Girls were **randomized** to Con or Ex within 1 high school. DPA system not stated.	**Program**: Resistance training using hydraulic machines (13 exercises, 4 sets with 10-12 reps of each). Sessions supervised by researchers. **Frequency & Duration**: 3 times/week; session duration not stated; 6.5 months. **Progression**: Resistance increased every 6 weeks.	Con and Ex matched for age, body mass, level of habitual physical activity. Two-way repeated measures ANOVA used to compare change in bone between groups.	**TB**: Not significant. **LS**: Not significant. **PF**: Not measured. **FN**: Not measured. **GT**: Not measured.
Witzke 2000(240)	GIRLS. All Caucasian. N=29 Con; N=27 Ex; mean age 14.6 ± 0.5 yr; age range13 – 15 yr. All postmenarcheal at baseline. Con and Ex matched for age and months postmenarche. **Not randomized**; exercisers partici-pated for P.E. credit at 2 high schools. DXA system: Hologic QDR 1000	**Program**: First 3 months: resistance training + plyometrics. Next 6 months: plyometrics, including jumps, depth jumps, bounding and hopping on soft surfaces. **Frequency & Duration**: 3 times/week; 30-45 minutes/session; 9 months. **Progression**: Weight training progressed from months 1-3: repetitions, sets, and weight gradually increased. Plyometric training progressed in jump difficulty and number of reps.	Repeated measures ANOVA.	**TB**: Not significant. **LS**: Not significant. **PF**: Not significant. **FN**: Not significant. **GT**: Not significant. **#Femoral Mid-shaft:** Not significant.

Two Maturity Groups				
Heinonen 2001(23)	GIRLS. All Caucasian. N = 58 (33 Con + 25 Ex) Premenarcheal (TS 1 – 3, mean age 11.0 ± 0.9 yr (Con), 11.7 ± 1.3 yr (Ex)). N = 68 (29 Con + 39 Ex) Postmenarcheal (TS 2 –5; mean age 13.7 ± 0.9 yr (Con & Ex)). **Not randomized**; schools self-selected to 2 Ex + 3 Con. DXA system: Norland XT 26	**Program:** Jump training sessions incorporating single and 2-foot jumps from floor, and on and off a 30 cm box. **Frequency & Duration**: 2 times/week; 20 minutes jump training/session; 9 months. **Progression**: Progressed gradually from 2-foot floor jumps (100 jumps) to combination 1- and 2-foot jumps from box (200 jumps).	Individual BMC values normalized by the length of the ROI. ANCOVA's performed within each maturity group, (baseline bone values and age as covariates).	*Premenarcheal: **TB:** Not measured. **LS:** + 3.3% BMC. **PF:** Not significant. **FN:** + 4.0% BMC. **GT:** Not significant. **^Tibial Mid-shaft**: Not significant. *No significant differences between postmenarcheal Ex and Con.

Con = Controls, Ex = Exercisers; TS = Tanner Stage; DXA = dual energy x-ray absorptiometry; DPA = dual photon absorptiometry; ROI = region of interest; ANOVA = Analysis of Variance; ANCOVA = Analysis of Covariance; TB = Total Body; LS = Lumbar Spine; PF = Proximal Femur; FN = Femoral Neck; GT = Greater Trochanter; BMC = Bone Mineral Content (by DXA); aBMD = areal Bone Mineral Density; vBMD = volumetric Bone Mineral Density (estimated from DXA).

** Calculated 8-month change from reported% change / month.

#Bradney et al. and Witzke et al. were the only 2 studies that measured and reported change at the femoral midshaft (DXA).

^Heinonen et al. was the only study that measured and reported change at the tibial midshaft (pQCT).

2.6 *Summary of Directions for New Research on Bone Mineral in Pre- and Peri-Pubertal Children: Effects of Ethnicity, Maturation, and Exercise.*

The literature review that precedes this section highlighted the need for further studies in pre- and peri-pubertal children. In particular, there are unanswered questions regarding the effects of ethnicity, maturation and exercise on bone mineral gain.

2.6.1 ***Ethnicity, Maturation and Bone Mineral Accrual***

Results from geographically separate studies (89,172,173) have indicated that the ethnic or racial effect on bone may be influenced by physical maturation. However, this has not been tested in a large cohort of Asian and Caucasian children living in a similar geographical environment. The literature suggests a relationship between physical activity and bone health (11,35), however, how this integrates with advancing maturity and is influenced by ethnicity, is unknown. Lifestyle factors related to bone health, such as calcium intake and physical activity, vary with age and/or maturity (249) as well as ethnicity (21). No study has yet considered the combination of these factors on bone mass or aBMD in a young cohort. Furthermore, there is a paucity of data characterizing the skeletal response to exercise intervention in any ethnicity other than Caucasian.

2.6.2 ***Exercise, Maturation and Bone Mineral Accrual***

Several different studies in children and adolescents have reported great variation in the skeletal effects attributable to exercise interventions (23,92,226,227,234). This variation may be due to differences in maturational status, sex, or intervention type or duration between studies. Only *one* exercise intervention study has examined skeletal outcomes in boys – and that was in a relatively small cohort of Caucasian boys (226). Evidence from animal studies suggests that bone responds more favourably to mechanical loading during growth (77-79), and this has been substantiated by unilateral control studies in female racquet sport athletes (22).

Only one study (23) has attempted an exercise intervention in 2 distinct maturational groups (pre and postmenarcheal girls) but using menarche to define maturation inherently includes a range of pubertal status in the premenarcheal group, that is, premenarche includes Tanner stage 1, 2, and 3. The optimal time during growth, when it may be most sensible to promote community and school-based exercise in children to promote osteogenesis has not yet been defined. We need a single intervention study in a large cohort of *boys and girls* of various maturational levels to address this question.

2.6.3 ***Exercise Duration, Maturation and Bone Mineral Accrual***

The bone health literature offers no pediatric exercise studies longer than 10 months duration (1 school year). Further, published literature to date offers no evidence of the lasting benefits of continued participation in exercise throughout growth. We do not know whether exercise intervention effects conferred over a 10-month period are maintained, augmented, or lost over the long term. Finally, prospective data pertaining to bone mineral gain in relation to pubertal stages, change in body composition, and physical activity is lacking. Thus, there is an urgent need to: 1) conduct an exercise study with an intervention period longer than 10 months; 2) propose long term follow-up studies of young adults who participated in an exercise intervention as children; and 3) conduct a study of children at different stages of maturity. These investigations would allow us to characterize the contributions of maturation, physical activity and change in body size or composition to adequately assess normal bone mineral accrual in healthy children.

3 - *Rationale, Objectives and Hypotheses*

In this chapter, I outline the rationale underlying the new studies that make up thesis. For each part, I provide objectives and hypotheses, followed by the scientific contribution each study will make.

3.1 Part I: Lifestyle Risk Factors For Osteoporosis in Asian and Caucasian Girls

Rationale. The rates of osteoporotic fractures differ between races. In general, hip fracture rates are greater in Caucasians than other racial groups (166). Asians have a higher incidence of vertebral fracture than do Caucasians (167,168). As aBMD is the best single predictor of the likelihood of fracture (42), it is important to examine BMC and aBMD between races to ascertain a physiological basis for differences in fracture rates. Lifestyle determinants of bone mineral, such as calcium intake and physical activity, may influence the attainment and maintenance of peak bone mass. Very few studies have investigated bone parameters in pre- and peri-pubertal Asian and Caucasian children. As yet, it is unknown whether differences exist between these groups before and/or during puberty. Only one study has examined a large prepubertal cohort of Asians and Caucasians living in a similar geographic environment. The authors reported greater aBMD in Caucasian boys, and greater calcium intake and physical activity in 8-year-old Caucasian boys and girls, as compared to their Asian counterparts (21).

Objectives. The *primary objective* of this cross-sectional study is to compare BMC and aBMD (total body, lumbar spine, proximal femur, femoral neck, trochanter) in Asian and Caucasian girls at two stages of physical maturity (Tanner stages 1 and 2). The *secondary objective* is to compare lifestyle factors (physical activity and dietary calcium intake) associated with BMC and aBMD in these Tanner 1 and Tanner 2 girls. Finally, I aim to identify the relative contributions of body size, lean mass and fat mass, dietary calcium intake, physical activity and ethnicity to aBMD at the femoral neck.

***Primary Hypothesis**:* BMC and aBMD will be similar between Asian and Caucasian girls in both Tanner stage 1 and Tanner stage 2.

***Secondary Hypothesis**:* Physical activity and dietary calcium intakes will be significantly lower in Asian than Caucasian girls in both Tanner stage 1 and Tanner stage 2.

Contribution. This study will characterize the relationships between ethnicity and dietary calcium intakes, physical activity, maturity and BMC / aBMD in pre- and early pubertal Asian and Caucasian girls. Physical activity levels and nutrition are likely associated with both ethnicity and puberty in girls, thus, there is a need to examine these associations as they relate to bone health. Results from this study will assist in the identification of young girls who present with greater risk factors for low peak bone mass and osteoporosis. Exercise and nutrition interventions could then target this group.

3.2 *Part II: Seven-Month Longitudinal Study of a High Impact Exercise Intervention in Pre- and Early Pubertal Girls*

Rationale. As much as 50% of the population variance in bone mineral density is attributable to differences in lifestyle factors, such as physical activity (18). Four separate studies found an association between current BMC or aBMD in retired elite athletes and childhood training (13,14,245,246). There are many questions surrounding bone-loading exercise intervention in children and, specifically, these questions have largely focused around a '*critical period hypothesis*': a time during maturation when bone may be more likely to adapt to exercise. Unilateral control studies (22,224) provide evidence that peri-pubertal children may adapt more strongly to exercise than pre-pubertal children. Only one exercise intervention study has compared the skeletal response in 2 distinct maturity groups (pre and postmenarcheal girls) (23). This study found a 3–4% augmented gain in premenarcheal exercisers and no skeletal response to exercise in the postmenarcheal group. This begs the question: is there a time during *premenarche,* which spans pre to early puberty, when bone might be more responsive to loaded exercise?

Objectives. The objective is to investigate the changes in BMC, aBMD (total body, lumbar spine, proximal femur, femoral neck, trochanter) and vBMD (femoral neck) in prepubertal and early pubertal girls who participate in 7 months of school-based, jumping activities, and to compare these changes to similar-maturity controls.

Hypothesis. Changes in BMC, aBMD and vBMD will be greater at all sites in exercise intervention girls than respective controls in both prepubertal and early pubertal groups. Differences in bone mineral changes between intervention and control groups will be similar in pre- and early pubertal groups.

Contribution. Defining a *critical period* has implications for how we target our resources for community and school-based exercise interventions. Although ideally we would like to see *all* children and adolescents enjoy an active lifestyle, public funding to increase activity with a goal of increasing peak bone mass and bone strength in youth must have a well-defined target population.

3.3 *Part III: Seven-Month Longitudinal Study of a High Impact Exercise Intervention in Prepubertal Asian and Caucasian Boys*

Rationale**.** As osteoporosis has been traditionally viewed as a disease of women, research efforts primarily target women. This is also true for studies that investigated factors related to bone mineral accrual in children – the overwhelming majority of studies target girls. However, ¼ of hip fractures in Canada occur in men, and male hip fracture patients tend to be younger (~6 years) than female hip fracture patients (2). Exercise during growth is an important modulator of peak bone mass and strength (11,75), and is likely related to bone health, and possibly fracture incidence in adulthood. There has been only one exercise intervention that evaluated skeletal outcomes in boys (226), and this was in a small cohort of prepubertal Caucasian boys (N = 40 total).

Objectives. The *primary objective* is to compare the changes in BMC, aBMD (total body, lumbar spine, proximal femur, femoral neck, trochanter), and vBMD (femoral neck) between prepubertal boys who participate in a 7-month, school-based, jumping intervention with same-maturity, non-exercising controls. (During primary analyses, I identified that body mass and ethnicity may have modified intervention effects on bone mineral accural. I completed secondary analyses after categorizing boys as either high body mass index (hiBMI) or average/low BMI (avBMI). My *secondary objective,* therefore, was to investigate how a jumping intervention and ethnicity affected 8-month bone mineral change in Asian and Caucasian boys of average/low BMI, and in boys with high BMI.)

Primary Hypothesis. Changes in BMC, aBMD and vBMD will be greater at all sites in exercise intervention boys than controls.

Contribution. Prepubertal Asian boys in Richmond schools had greater risk factors for low peak bone mass, and significantly lower aBMD at the femoral neck than Caucasian boys (21) and, thus, represent an important target population for lifestyle intervention strategies. Thus, In Part III, I seek to characterize the skeletal response to exercise intervention in both Asian and Caucasian boys, who comprise a grossly understudied population in the bone health field.

3.4 Part IV: Twenty Month Longitudinal Study of a High Impact Exercise Intervention in Pubertal Girls

Rationale. All published exercise interventions that evaluated skeletal outcomes in children and adolescents have been less than 10 months duration. Skeletal effects observed over this short time period may be a result of the bone remodeling transient (56), and although this represents real change in bone, we cannot be certain whether these effects persist. It is unknown whether immature bone can continue to adapt to exercise interventions implemented over longer periods of time.

Objectives. The *primary objective* is to assess the magnitude of BMC change (total body, lumbar spine, proximal femur, femoral neck, trochanter) in girls who participate in a school-based, high impact exercise intervention over 2 school years and their controls. The *secondary objective* is to examine the contribution of initial body size, maturation, change in body size and composition, extracurricular physical activity, and intervention participation to variance in BMC change over 20 months.

Primary Hypothesis. The 20-month changes in BMC will be significantly greater at all sites in exercise intervention girls than controls.

Secondary Hypothesis*:* Change in lean body mass and maturation (advancing a Tanner stage) will account for the greatest amount of variance in BMC change over 20 months.

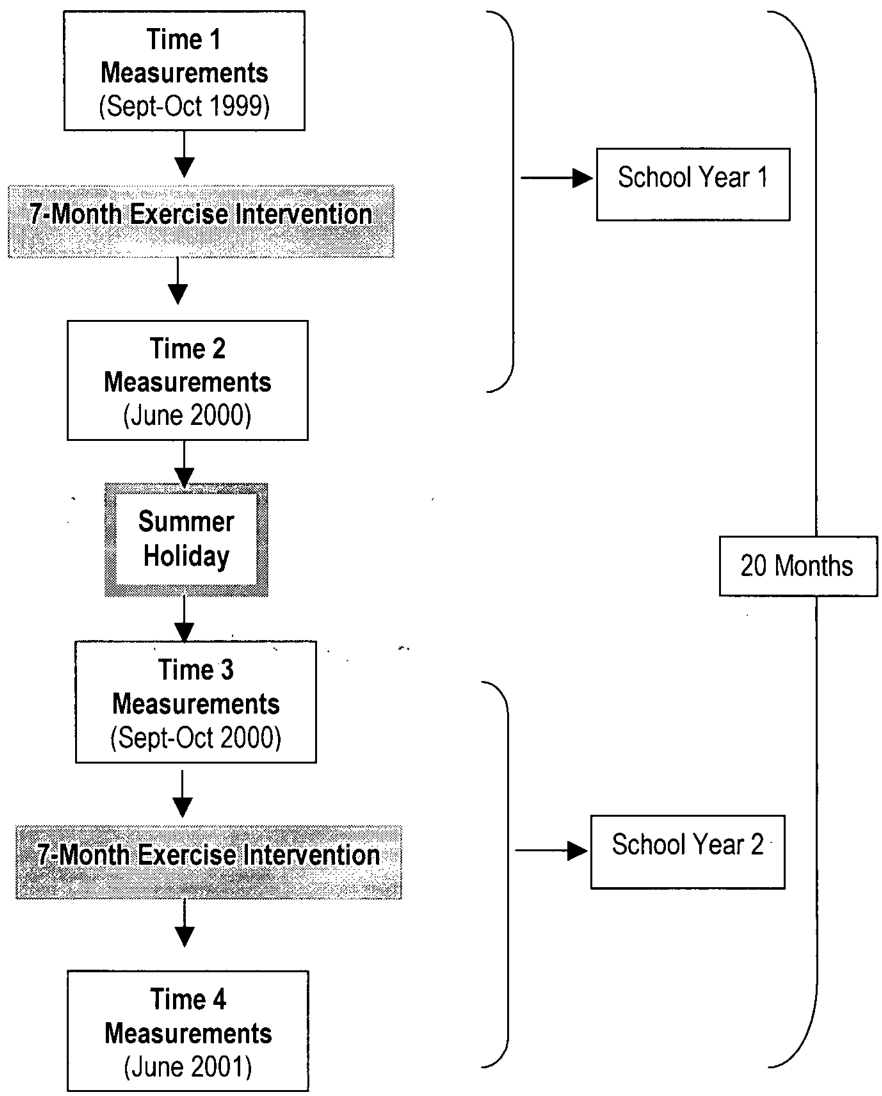
Contribution. This is the first study of girls to investigate the skeletal effects of exercise intervention over 20 months (2 school years). To prescribe bone-loading interventions, we need to understand if young bone continues to respond over a longer term.

4 - *Methods*

4.1 Study Design and Overview

We conducted a randomized, controlled, 20-month prospective study of an exercise intervention trial involving 14 elementary schools. The several measurement timepoints (Figure 4) allowed for multiple investigations (Parts I – IV) within this design. Part I, a cross-sectional evaluation, reports on data collected in September and October 1999 (Time 1), prior to the start of the intervention trial. The first follow-up measurement, Spring 2000 (Time 2), was conducted after the first 7- month intervention period, and provided follow-up data for Parts II and III. Measurements were conducted during Fall 2000 (Time 3) and Spring 2001 (after the 2nd, 7-month intervention period, Time 4) and provided the final data for Part IV.

Figure 4: Schematic of study timeline.



4.2 *Recruitment of Schools, Teachers, and Students*

Our research group established a rapport with several principals and teachers in the Richmond School District during a previous study that was conducted in the 1997-98 school year (21,92). Recruitment and many aspects of the current study benefited from this relationship. We approached recruitment at three levels. First, schools were recruited by presenting the project at a board-wide principals' meeting (May 1999). Interested principals were asked to meet with combined Grades 4/5 and 5/6 and Grade 5 teachers in their schools. Second, interested teachers then contacted the principal investigator (Heather McKay) for further information. I arranged meetings at 15 schools in June 1999, and we presented the project, with a proposed timeline and an outline of responsibilities provided for appropriate teachers. Over the summer, one school decided not to participate due to major building renovations that would affect their gymnasium. Third, during the first 2 weeks of the school year (September 1999), we conducted classroom presentations in Grades 4/5, 5, and 5/6 classes at the 14 schools where principals and teachers had volunteered to participate. Students in these grades were between 8 and 12 years old (the majority were 9-11 years old). These presentations (16 in total between September 7th and September 21st ,1999) facilitated interaction and information transmission to potential student participants, and allowed us to distribute information and consent forms in English and Chinese (Appendix 1) to be taken home to parents. I distributed approximately 780 consent forms during this time. During the presentations, and with assistance from the classroom teacher, the ethnic breakdown within the classroom was estimated. Approximately 34% of the population in Richmond report either Mandarin or Cantonese as their first language, however, the ethnic distribution is not consistent between schools in Richmond. We expected approximately 30 - 40% of our sample to be of Asian descent. All children who returned signed consent forms were included in the study; however, children were screened for inclusion prior to data analyses.

Incentives for children to participate included 4 trips to the University of British Columbia for measurements, snacks, skeleton stickers and pencils, printouts of their own skeleton image after the 2nd and 4th measurements and individual and group growth and physical performance results (mailed over the summer, Appendix 4). For teachers, incentives included detailed manuals of the physical education intervention program with cross-curricular activities (new each year), intervention posters and musical tapes, the opportunity to have a 4th year UBC-HKIN student volunteer to assist them in physical education classes, a Healthy Bones Study t-shirt, and two Healthy Bones Study training sessions (paid inservices with lunch provided).

Prior to completion of the Year 1 data collections, Dr. McKay and I met with participating intervention teachers to invite their continued participation for Year 2. We also asked them to recommend the program to the teachers at their school who would most likely be teaching Healthy Bones Study participants during the next school year. Information letters were sent out to all principals at intervention schools to facilitate recruitment of teachers for the next school year. Parents and students were invited to join the program for a second year when results were sent home over the summer holiday. We then met with all potential Year 2 intervention teachers

(September 2000), to provide information and answer questions about the intervention program, and to ask these teachers to assist in sending out and collecting Year 2 consent forms from Year 1 participants. We did not recruit any *new* participants in Year 2.

4.3 *Consent, Randomization, Health History and Exclusion*

Once all the Year 1 consent forms within a class were returned (Fall 1999), students were scheduled for baseline measurements. The schedule allowed for a maximum of 18 participants to be measured per day, and baseline measurements (details to follow) were concluded in 27 weekdays. All consenting children, regardless of medical history, were measured.

Following baseline measurements, schools were stratified by number participating (small: < 20 students; or large: > 20 students) and estimated ethnic breakdown (>50% or <50% Caucasian), and assigned to control or intervention groups, by random draw.

Parents completed a Health History Questionnaire (Appendix 3) for their child. This questionnaire also asked parents to classify their own, and their child's ethnicity. Children were also asked to identify the language(s) spoken at home, and the birthplace of their parents (Personal Data Form, Appendix 3). We classified children as '*Asian*' if both parents or all 4 grandparents were born in Hong Kong, China, Japan, Taiwan, the Philippines, or Korea; '*Caucasian*' providing both parents or all 4 grandparents were born in North America or Europe; and '*Other*' if the child had parents of other origins (ie, Africa, India) or had parents of 2 distinct races (ie, Caucasian-Black, Asian-Black, Caucasian-Asian). We identified medical conditions that interfered with normal physical activity or bone metabolism through the Health History Questionnaire, and/or on the Personal Data Form (Appendix 3, completed by child on each visit to the laboratory), and excluded children from data analyses *only*, as appropriate.

4.4 *Exercise Intervention*

The exercise intervention program was designed and modified with input from several sources: Judy Notte (Curriculum Specialist and Richmond School District teacher), Dr. Moira Petit (Director of the first *Healthy Bones* exercise intervention study in Richmond), Dr. Heather McKay (Principal Investigator, Healthy Bones Studies), Dr. Ari Heinonen (Postdoctoral fellow specializing in biomechanics and bone) and Garry Tsang (Ground Reaction Force Study). We designed the program to provide a brief (10-12 minute), high impact, weight-bearing exercise session during the twice-weekly scheduled physical education class, and on one other occasion (supervised in the classroom or outside) during the week. We applied basic principles of bone loading to provide an exercise program that incorporated *high impact* (i.e., jumping, hopping), *dynamic*, and *progressive* activities with *various strain rates*. We provided teachers with manuals detailing intervention procedures in Year 1 and 2, and posters to set up in the gym to remind children of correct actions. The intervention manuals also included a range of cross-curricular activities focused on aspects of bone health, which could be implemented in nutrition, health, spelling, art, and science lessons (Appendix 2, sample manuals).

4.4.1 *Year 1 Intervention Program*

For each session, teachers chose a circuit made up of 5 activities from a menu of 9 different exercises, and were encouraged to choose different activities from session to session. Students rotated through the 5 activity stations, taking approximately 1.5 to 2 minutes per station. All stations were comprised of jumping exercises (i.e., jumping jacks, lunge jumps, hopping, jumping over various obstacles, drop jumps from a platform). The program generated progressively greater impact-loading over the school year and the 3 levels (each ~2.5 months long), were made increasingly difficult over time. For example, a simple jump using both feet was changed to a tuck jump, and later to a plyometric jump. The height of the platform was increased progressively for drop jumps, from 10 cm (Level 1) to 30 cm (Level 2) to 50 cm (Level 3). Within each level, the number of jumps at every station increased each week (starting with 10 to a maximum of 20 jumps). By this design, students jumped a minimum of 50 times each session at the beginning of a level, and progressed to approximately 100 jumps by the end of a level. Ground reaction forces for these activities were typically 3.5 – 5.5 times body weight, as measured in a subset (N = 70) of children from the original cohort (250). Following the circuit intervention, the regular physical education class continued normally.

The teachers who provided the intervention (N=15) undertook a 4-hour training session (October 1999) and this was supplemented by a 3-hour inservice during the school year (February 2000). Trained, 4th-year Human Kinetics student-volunteers from the University of British Columbia visited each intervention class once per month, to ensure that the program was implemented consistently. These 4th-year physical education majors completed this volunteer work as part of a directed study project (HKIN 499). Volunteers and intervention teachers were matched so that each class was observed and assisted as required once every 4 weeks. I met with volunteers throughout the school year to discuss general trends within schools, and problems and/or issues with the intervention. Teachers completed a log detailing the date, time spent, and circuit stations chosen each time their class performed the circuit (Appendix 2). Teachers at control schools (N=18) were asked to implement a 10-minute stretching warm-up at the beginning of their physical education classes, and a 'stretch break' during class time on one other day during the week. The 4th Year Human Kinetics student volunteers introduced this stretching (low impact) program to each control school. We interviewed teachers to ensure that the same amount of time was allocated to physical education as the intervention schools (two x 40 minute sessions). Teachers at control and intervention schools all used the standard School Board Instructional Resource Package for Physical Education.

4.4.2 *Year 2 Intervention Program*

Teachers followed a simplified version of the same basic format for the second year of intervention, utilizing a higher proportion of high impact jumps (plyometric jumps) as determined from our ground reaction force study (250). Twice each week at the start of the physical education class, 3 'step' stations (from which girls completed plyometric jumps), one alternating-foot jump station (2 jumps/leg, over a line or in and out of a hoop), and one 2-foot obstacle jump station (4 jumps over obstacles) were set up around the gym. Participants completed running or skipping (alternating week to week) laps of the gym, stopping at each station to complete the jump(s). The number of laps was increased each week from a minimum of 5 (55 jumps) to a maximum of 12 (132 jumps), within a level. 'Levels' were advanced every 2 – 2.5 months, when the step height (step station) and the obstacle height (2-foot jump station) increased from 10 cm (Level 1) to 30 cm (Level 2) to 50 cm (Level 3). Teachers continued with regular physical education following the 10 to 12 minute intervention session. On one other day during the week, teachers implemented a jump session in the classroom or outside where participants performed progressive (minimum 15 to maximum 50) plyometric jumps (Level 1), half-tuck jumps (Level 2), or full tuck jumps (Level 3).

During Year 2, we (2 trained research assistants and myself) conducted in-school training of teachers (N=15) and demonstrated the set up of the circuits and the activities to be performed at each station. We visited each intervention class twice during the school year to monitor the consistency of the intervention between schools, and to advise teachers as required. For each session, teachers completed a log detailing the date, attendance of study participants, and number of laps or jumps performed (Appendix 2). Teachers at control schools were asked to maintain the regular physical education curriculum provided by the School Board.

4.5 Data Collection: Overview

Each child that participated in the study was released from school for about 2 ½ - 3 hours during each data collection (Fall and Spring of 2 school years, Figure 4). An additional physical activity and food frequency questionnaire was administered to children in their school classroom in the winter. For Fall and Spring measures, participants were transported to the University of British Columbia Bone Health Research Laboratory via minivan in groups of 5-6 and supervised en route by a chaperone (in addition to the driver). The daily transportation schedule is outlined in Table 3.

Table 3: Transportation schedule of study participants to and from the UBC laboratory.

	Pick-Up From School	At UBC	Return to School
Group 1*	9:00 am	9:40 –11:15	11:50
Group 2*	10:30	11:15 – 12:35	1:10
Group 3*	11:45	12:20 – 1:45	2:20

*4 – 6 students per group.

At the lab, children rotated through 4 stations: Bone densitometry, Anthropometry (height, weight), Performance Measures (vertical and long jumps), and Questionnaires (physical activity, nutrition, maturity). Students were supervised by study staff personnel at all times. Prior to each measurement period, a team of 6-10 researcher assistants were trained to administer questionnaires, and to collect standardized anthropometric and physical measures. The drivers and chaperones were instructed to adhere to the transportation schedule, and to drive safely.

4.6 Bone Measurements

Bone area (BA, cm^2), bone mineral content (BMC, grams) and areal density (aBMD, grams/cm^2) were measured for the total body, lumbar spine, and proximal femur (and its femoral neck and trochanteric subregions), in late September to late October (Time 1), late May to late June (Time 2), and during the same times in Year 2 (Times 3 and 4) using a Hologic QDR 4500 bone densitometer. Trained operators, Dr. Moira Petit and I performed all scans. Bone densitometry is a safe, painless, extremely low dose radiation procedure, that is routinely used in clinical practice. The total radiation exposure per session was less than 10 millirem, which is similar to the background radiation one would receive making a one-way flight from Vancouver to Halifax on a commercial airline. The simple procedure required that the child lay on the padded examination table for positioning and measurement (~ 15 minutes). The children wore light clothing (without metal zips and snaps) and removed jewellery for measurement. Spine and anthromorphic phantoms were scanned daily for quality assurance. *In vitro* spine precision for the Hologic QDR 4500 was consistent (between 0.3 and 0.6% CV for all bone parameters) across the 4 measurement timepoints (Table 4).

Table 4: Lumbar spine in vitro precision (% CV) for the Hologic QDR 4500 densitometer over 4 data collection periods.

	Fall 1999	Spring 2000	Fall 2000	Spring 2001
Bone area (cm^2)	0.49	0.49	0.42	0.46
Bone mineral content (grams)	0.60	0.52	0.52	0.63
Areal bone mineral density (grams/cm^2)	0.32	0.28	0.39	0.40

We conducted a short-term *in vivo* precision study by measuring total body (TB), lumbar spine (LS), proximal femur (PF) (and femoral neck (FN), greater trochanter (TR)) BMC and aBMD three times within one hour in 17 healthy young adults. The coefficients of variation (CV) for BMC and aBMD for the TB and LS were less than 0.7%. The CV for BMC at the PF, FN, and TR was 1.4, 2.6, and 3.5%, respectively. Similarly, the CV for aBMD was 0.5, 1.2, and 0.8%, respectively, at these sites. For femoral neck vBMD, the precision was 2.7% CV. Precision studies in children on other DXA systems result in similar variation: between 0.8 and 2.3% CV for aBMD, depending on the site measured (251). I analyzed all scans at each timepoint (N = 3309 total) using standard Hologic analysis protocol (252) (Figure 5 a–c).

(a) (b) (c)

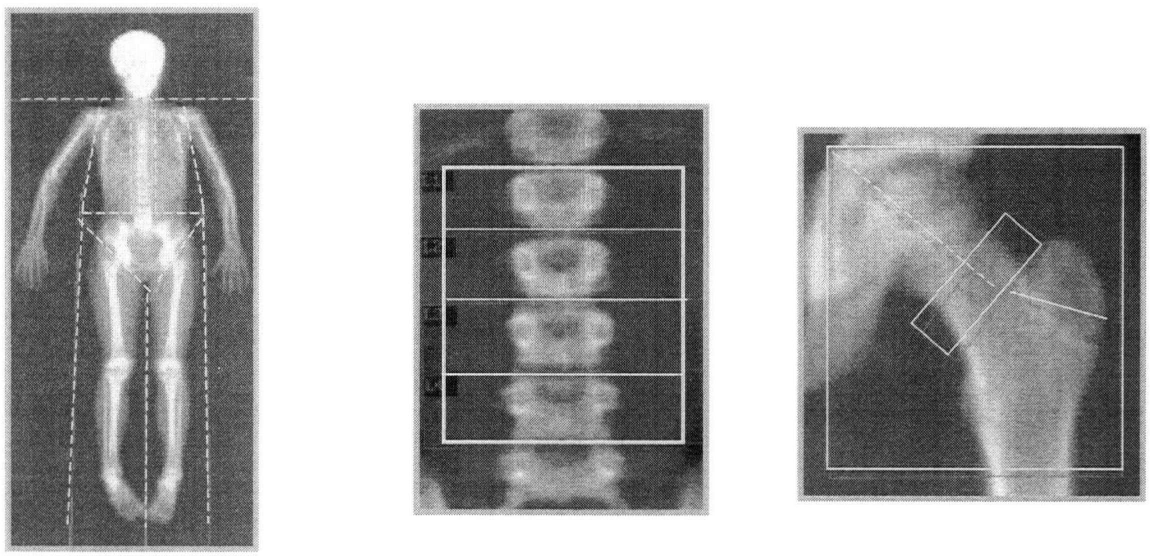


Figure 5: (a) Total body scan, (b) lumbar spine scan, (c) proximal femur scan, showing standard, default analyses.

BMC and aBMD are reported in Parts I, II, and III. As the interpretation of BMC (bone mass in grams) is more straightforward than that of aBMD (a ratio variable, g/cm^2), and the greater % changes over 20 months are outside the precision error of BMC, I reported only BMC (adjusted for size) in Part IV. Several researchers have recently outlined the benefits of reporting adjusted BMC in favour of aBMD (253), (T. Beck and A. Heinonen, personal communication).

Estimated volumetric bone mineral density (vBMD) was calculated for the femoral neck (for Parts II and III), assuming this region approximates a cylinder, by the equation: $vBMD = (4BMC*(\text{height of FN box}))/ \Pi * (\text{FN area})^2$ (91). The height of the femoral neck box was held constant at the manufacturer's default of 1.5 cm (254). These assumptions provide a good approximation of true femoral neck geometry (91).

4.7 Anthropometry

Body composition estimates of bone mineral free lean mass and fat mass were generated from DXA total body scans. *In vivo* precision of our bone densitometer was $0.30 \pm 0.15\%$ CV for total body lean mass and 1.42 ± 0.78 % CV for total body fat mass.

Duplicate measures of sitting height and standing height (without shoes) to the nearest mm, using a customized wall-mounted stadiometer were taken. Stretch statures for both sitting and standing height were measured by the standard method, by applying gentle upward traction from the base of the mastoid processes. Leg length equalled the difference between standing height and sitting height. Weight measures with the children dressed in light clothing, were taken with an electronic scale to the nearest 0.1 kg. For both height and weight, duplicate measures were taken unless measures differed by ±0.4 cm (height) or ±0.2 kg (weight), when a third measurement was made. The average of 2 values or the median of 3 values was used for statistical analyses.

4.8 Performance Measures

Vertical and horizontal jump tests were used to assess muscle strength. These performance measures were significantly correlated ($r=0.7$, $P<0.01$) to strength of the hip, knee, and ankle extensor muscles in young women (255). For the vertical jump, a maximum two-foot takeoff jump was performed from a standing position near a wall. Distance, to the nearest mm, was measured from the highest reaching point with feet flat on the ground to the highest point touched (with ink-marked fingers) during the jump (256). For the horizontal jump, children performed a barefoot, two-foot take-off from a matted surface. The child's feet were chalked to mark the landing, and the recorder measured distance, to the nearest mm, from the starting line to the most proximal heel mark. For both vertical and horizontal jumps, children took 2 practice jumps, followed by 2 recorded performances. The average of the 2 performances was reported.

4.9 Questionnaires

4.9.1 ***Maturity***

Stage of pubertal maturity was self- or parent-assessed using Tanner Staging (101). This method consisted of a series of line drawings depicting 5 stages of development (breast and pubic hair for girls and pubic hair for boys). The child picks the drawings she or he feels most closely resemble her/his physical appearance (Appendix 3). As many of the Grade 4 and 5 children were not yet introduced to human development and sexuality within school family life classes at the commencement of the study, the Time 1 assessments were sent home for parents to complete with their child. Forms were returned to the investigators by mail. We sought parental permission to administer the questionnaire in the laboratory during subsequent measurement occasions. When there was a discrepancy between breast and pubic hair rating in girls, breast stage was used to represent the level of maturity. Overall, self-assessed Tanner stage agrees well with physician ratings (105). Tanner Stage 1 represents pre-puberty, Tanner Stage 2 and 3 correspond to early puberty, Tanner Stage 4 represents late/peri-puberty, and Tanner Stage 5 is considered post-puberty.

4.9.2 *Physical Activity*

General physical activity was assessed seasonally (3 times/school year) by questionnaire (PAQ-C) (Appendix 3). This is a self administered, 7-day recall questionnaire, designed to measure general, habitual physical activity in a moderate to vigorous range (208). It was developed through a multi-step process that incorporated item analysis and feedback from children and research assistants (208). The final result was a ten item questionnaire: one item is a specific activity recall from a list of common sports and activities, six items focus on physical activity throughout different parts of the day (P.E. classes, recess, lunch time, after school, evening) and weekend, one item asks the child to select a statement which best describes his/herself from low to highly active, one item asks how often the child was active on each day of the week, and one item asks whether they were sick or prevented from regular physical activity during the past 7 days. Final general physical activity scores were calculated as an average of the first nine PAQ-C items, in a continuous range between 1 (low activity) and 5 (high activity) *(physical activity score).*

This questionnaire was designed to discriminate by activity score between children who have high, average and low levels of general physical activity. The PAQ-C was validated against other self-administered physical activity measures, motion sensors, and interview-assisted recall (r = 0.39 – 0.63) (211). Other self-or interviewer-administered physical activity assessments had similar relationships to heart rate index (r = 0.5 – 0.6) and accelerometer score (r = 0.3) (257).

For the purposes of this study, this questionnaire was modified to include an estimate of time per session in item # 1, and the activities were designated as *'loaded'* if they involved impact greater than walking. Time spent in loaded physical activity was calculated (*load time*, hours/week). Children were also asked to describe extracurricular activities (sports, music lessons, tutoring, language lessons, etc.), and the number of nights per week they were involved in sporting activities (*sport nights*, scored as 0 through 7). Research assistants used cues to facilitate children's recollection of time in item # 1 (i.e., recess is 15 minutes long, lunch time is 30 minutes long, etc.). I derived average physical activity scores, load time (hours per week in loading activities), and sports nights for each child as a mean of 3 assessments (Parts II and III) or 6 assessments (Part IV).

Reliability of questionnaire items improved with multiple assessments. Intraclass correlations of a single assessment of *physical activity score, load time, or sport nights* were alpha = 0.41- 0.55 for boys, and alpha=0.34 – 0.59 for girls. The average intraclass correlation coefficients for 3 measures (Fall, Winter and Spring X 1 year) for *physical activity score, load time, and sport nights* were alpha = 0.79, 0.73, and 0.61 in boys, respectively, and alpha = 0.74, 0.61, and 0.76 in girls, respectively. Over the 6 assessments (Fall, Winter and Spring X 2 years), correlation coefficients for *physical activity score, load time, and sport nights* were alpha = 0.88, 0.82, and 0.80 in boys, respectively, and alpha = 0.90, 0.75, and 0.87 in girls, respectively. Thus, using the PAQ-C, the average of multiple assessments was the most reliable indicator of physical activity in this cohort.

The correlation between the averaged (fall, winter, and spring) *physical activity score* and *load time* was 0.61, P<0.01, while *sport nights* had a weaker relationship with both *physical activity score* (r = 0.34, P<0.01) and *load time* (r = 0.30, P<0.01).

4.9.3 *Dietary Intake*

A food frequency questionnaire (FFQ) was administered seasonally (3 times) to assess dietary intake of calcium (Appendix 3). The FFQ was adapted from a questionnaire validated in Asian and Caucasian adolescents living in Vancouver (179). Children completed the questionnaire in small groups (maximum 3) facilitated by a research team member. Certain cues were used to assist children in food frequency recall, such as "Do you eat this food every morning?" or "How many times since school began this year did you eat this food?" A bilingual (Chinese-English) measurer was available to assist Chinese children. Pictures of food items in appropriate serving sizes were mounted on the laboratory walls. In comparison to food records, this questionnaire has a low respondent burden and is relatively easily administered in children. Questionnaires were analyzed by calculating a daily calcium intake (mg) based on the calcium content of food items.

The reliability (intraclass correlation coefficient) of a single assessment of calcium intake using the Food Frequency questionnaire was alpha=0.41 in boys, and alpha=0.55 in girls. The average of 3 assessments (Fall, Winter, Spring) during Year 1 had a reliability of alpha=0.71 in boys and alpha=0.77 in girls. The reliability of the average of 6 assessments over 2 school years was alpha=0.81 in boys and alpha=0.88 in girls.

4.10 Statistical Analysis

The statistical analyses specific to Parts I – IV are presented in the Methods section for each study (Chapters 6 – 9). However, the approach taken followed a similar format for each study. Initially, data were checked with scatterplots to eliminate errors and examine outliers (< 1SD above or below the mean). For example, in Part III, one boy had bone mineral changes > 3 SD above the mean, that could not be explained by scan artifact or analysis errors and therefore his data was removed to avoid distorting the distribution. Dependent variables were checked for normality by calculating skewness and kurtosis, where values less than 2 indicated a normal population distribution (258).

Analysis of covariance was used in all studies, and the choice of covariates was based on the biological relationship between body size, maturation and bone mineral change, discrepancies in baseline characteristics between intervention and control groups, and/or consistent prediction of variance in the dependent variable in (exploratory) stepwise regression. Specific covariates used to adjust bone outcomes varied between Parts I – IV, but were consistently based on their theoretical and actual relationships to bone mineral status or bone mineral change (specific to the analysis group, i.e., some body size-bone or physical activity-bone relationships were different between boys and girls, and therefore covariates differed between Studies). Covariates were generally selected from the following categories: body/bone size at baseline, rate of linear growth (change in height), maturity (age or Tanner stage), and physical activity (physical activity score, load time, or sport nights). As a measure of effect size, I report eta squared with between-groups comparisons for bone outcomes (where eta squared is the proportion of the dependent variable (absolute or change in BMC or aBMD) explained by the independent variable (generally, ethnic or intervention group)). Where regression was used (Parts I and IV) assumptions of linearity, normality and constant variance were checked. As well, independent variables were checked for multicollinearity. Standardized residual values were checked for the fit of the regression model (for a good fit, 99% of standardized residuals should be within –2.58 and +2.58 (259)) and to ensure that they were of random pattern.

The power to show the main effect of the intervention (the primary independent variable of interest) was calculated based on a 3% (SD 4%) difference in aBMD change between intervention and control groups, a conservative estimate, based on the previous publication in early pubertal girls (234). Power = 0.83 with 30 participants per group. We anticipated dividing the cohort into groups by sex, maturity and ethnicity and thus, we recruited a large group of children (N = 383) to allow for these divisions, and to have enough power to analyze smaller groups of well-matched children. Recruitment of this large cohort also allowed for attrition (estimated at approximately 10%/year).

Data were analyzed using SPSS statistical software, Windows version 8.0 (SPSS Inc., Chicago, IL, 1993). Significance was set at an alpha level of 0.05.

5 - Overview of the Cohort

5.1 *Participants*

We recruited and measured 383 Richmond children in Grades 4, 5 and 6 in the Fall, 1999. School randomization is shown (Table 5). The breakdown of this cohort by sex and ethnicity is given (Table 6). There were even numbers of boys (n = 192) and girls (N = 191) at baseline, and a balanced number of children in Intervention (n = 181) and Control (n = 202) schools. The school with the greatest number of participants was Brighouse with 44 participants, and the lowest number was at Steves with 12 participants.

Of the original 383 participants, 366 (96%) returned for follow-up measurements at Time 2 (Spring 2000). In Year 2, 181 of the original participants consented again, with 173 (96%) of the children from that group returning for the final measurements in Spring 2001 (Table 6). Thus, attrition within each year of the study was very low (4%). The primary reason for attrition between the two years of the study was the withdrawal of Rideau Park and Steves schools (attrition is described in more detail in Part IV, Section 9.2.2 Subjects).

Table 5: Schools randomized to intervention and control groups, and number of participants at each school.

Intervention	**N**	**Control**	**N**
Ferris	21	Wowk	41
Steves	12	Errington	14
Garden City	17	Diefenbaker	19
Tait	25	Walter Lee	33
Westwind	41	Brighouse	44
Rideau Park	40	Kilgour	32
Mitchell	25	Thomas Kidd	19

5.2 *Characteristics of the Cohort*

An overview of descriptive characteristics and bone parameters at baseline is provided (Table 7). The children in this cohort were, on average, 10 years old (girls: 10.3 ±0.7 years; boys: 10.4 ± 0.7 years). The youngest child was 8.8 years old, and the oldest was 12.2 years old. The ethnic distributions were similar for girls (45% Caucasian, 35% Asian, 20% Other) and boys (44% Caucasian, 39% Asian, 17% Other). Girls were generally in Tanner stage 1 (39%) or 2 (50%) at baseline, with a smaller percentage in Tanner stage 3 (10%), and 1% in Tanner stage 5. Nearly all boys were in Tanner stage 1 (87%) at baseline, with a smaller group in Tanner stage 2 (12%) and Tanner stage 3 (1%).

Correlations between *baseline* bone area, BMC, and aBMD and age, Tanner stage, body size and composition, and lifestyle factors, in girls and boys, respectively are presented (Tables 8 and 9). Correlations for change in bone parameters with baseline characteristics as well as change in body size and composition in girls and boys is also provided (Tables 10 and 11). The bivariate relationships between lumbar spine and proximal femur BMC versus height, weight and lean mass at baseline, physical activity and age are plotted (Figures 6 to 10). Scatterplots for change in lumbar spine and proximal femur BMC against the variables noted above (as well as change in height and lean mass) are also provided (Figures 11 to 18).

By design, our study was quasi-experimental, in that we randomized children to the intervention or control group by school (not at the level of the individual child). This approach was taken because of the school-based nature of the program – we could not expect teachers to intervene with only part of their class. As well, randomization by school avoided the possibility of contamination of the control group by observing their classmates performing the intervention. To provide evidence of the homogeneity in change in body size and bone parameters across all 14 schools, the absolute and percent 8-month change in height, weight and total body BMC *by school* is given (Table 12). There was a narrow range in the change in BMC between the 14 schools, 9.2% - 11.9%. There was no evidence of a school effect for changes in body size or BMC, physical activity or dietary calcium intake in this cohort. Detailed comparisons of body size and composition, maturity, and lifestyle factors are presented in greater detail within the chapters pertaining to specific studies.

Table 6: Girls and boys by ethnicity and intervention/control group at all timepoints.

			GIRLS	BOYS	TOTAL
TIME 1 Fall 1999	Asian	Intervention	36	34	70
		Control	30	41	71
	Caucasian	Intervention	39	44	83
		Control	47	40	87
	Other	Intervention	16	12	28
		Control	23	21	44
			191	**192**	**383**
TIME 2 Spring 2000	Asian	Intervention	35	33	68
		Control	29	39	68
	Caucasian	Intervention	38	43	81
		Control	44	39	83
	Other	Intervention	14	11	25
		Control	21	20	41
			181	**185**	**366**
TIME 3 Fall 2000	Asian	Intervention	14	14	28
		Control	14	24	38
	Caucasian	Intervention	15	22	37
		Control	29	22	51
	Other	Intervention	5	4	9
		Control	9	9	18
			86	**95**	**181**
TIME 4 Spring 2001	Asian	Intervention	12	13	25
		Control	14	24	38
	Caucasian	Intervention	15	20	35
		Control	27	22	49
	Other	Intervention	5	3	8
		Control	9	9	18
			82	**91**	**173**

Table 7: Girls' and boys' age, maturity, ethnic distribution, lifestyle variables, body size and composition, physical performance and bone area, bone mineral content (BMC) and areal bone mineral density (aBMD) at baseline (Fall 1999).

	GIRLS			BOYS		
	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum
N	191*			192**		
Number Caucasian/Asian/Other	86 / 66 / 39			84 / 75 / 33		
Number Tanner Stage 1/2/3/4/5	74 / 96 / 19 / 0 / 2			167 / 23 / 2 / 0 / 0		
Age (years)	10.3 (0.7)	8.8	11.8	10.4 (0.6)	8.8	12.2
Height (cm)	142.2 (7.8)	116.8	158.6	142.4 (7.5)	122.3	172.6
Weight (kg)	36.9 (8.9)	20.4	68.0	37.7 (10.0)	22.0	80.8
Leg Length (cm)	67.0 (4.3)	51.4	77.7	67.2 (4.3)	53.8	84.1
Sitting Height (cm)	75.3 (4.2)	65.4	87.3	75.3 (3.8)	65.9	88.6
Calf Girth (cm)	29.2 (3.1)	22.3	37.5	29.4 (.3)	23.5	42.1
Physical Activity Score (/5)	2.8 (0.6)	1.6	4.6	3.1 (0.6)	1.6	4.6
Load Time (hr/wk)	5.0 (4.2)	0	24.1	7.6 (6.4)	0	33.1
Sport Night (#/wk)	1.7 (1.8)	0	7.0	1.8 (2.0)	0	7.0
TV Time (hr/day)	2.3 (1.2)	1.0	5.0	2.7 (1.3)	0	5.0
Lesson Nights (#/wk)	0.4 (0.9)	0	7.0	0.4 (1.0)	0	7.0
Long Jump (cm)	124.3 (16.4)	81.9	173.2	136.9 (18.5)	81.3	200.7
Vertical Jump (cm)	22.1 (5.1)	7.3	34.6	24.8 (5.3)	11.6	43.8
Calcium Intake (mg/day)	819 (428)	0	2463	853 (442)	97	2768
Total Body Area (cm^2)	1281(197)	801	1767	1289 (196)	841	1841
Total Body BMC (grams)	1104 (229)	577	1819	1118 (206)	673	1808
Total Body aBMD (g/cm^2)	0.86 (0.06)	0.72	1.08	0.86(0.05)	0.74	1.01
Lumbar Spine Area (cm^2)	39.4 (5.5)	23.6	61.2	39.7 (4.8)	29.9	56.5
Lumbar Spine BMC (grams)	26.0 (6.9)	12.7	57.6	24.5 (4.7)	14.3	44.9
Lumbar Spine aBMD (g/cm^2)	0.65 (0.09)	0.44	0.97	0.61 (0.06)	0.44	0.80
Proximal Femur Area (cm^2)	23.2 (3.2)	15.1	32.3	23.1 (3.5)	15.2	36.4
Proximal Femur BMC (grams)	16.1 (3.9)	8.9	30.0	16.8 (4.0)	8.8	31.0
Proximal Femur aBMD (g/cm^2)	0.69 (0.09)	0.52	1.03	0.72 (0.09)	0.45	1.03
Femoral Neck Area (cm^2)	4.2 (0.4)	3.1	5.0	4.2 (0.4)	3.3	5.5
Femoral Neck BMC (grams)	2.7 (0.5)	1.7	4.3	2.9 (0.5)	1.7	5.0
Femoral Neck aBMD (g/cm^2)	0.65 (0.09)	0.47	0.91	0.69 (0.08)	0.50	1.00
Trochanter Area (cm^2)	5.8 (1.4)	2.3	9.4	5.0 (1.3)	2.7	10.1
Trochanter BMC (grams)	3.2 (1.2)	1.0	7.2	2.8 (1.0)	1.1	6.5
Trochanter aBMD (g/cm^2)	0.54 (0.08)	0.38	0.85	0.56 (0.07)	0.37	0.77
Lean Mass (kg)	25.6 (4.5)	15.9	39.7	27.0 (4.7)	17.5	42.7
Fat Mass (kg)	10.0 (4.9)	2.6	28.4	9.3 (5.8)	2.7	34.7

*means for descriptive variables for girls calculated with N = 189 (due to 2 exclusions: 1 Down's syndrome, 1 polycystic kidney removal + precocious puberty).

** means for descriptive variables for boys calculated with N = 187 (due to 5 exclusions: 1 Down's syndrome, 1 cerebral palsy, 1 casted club foot, 1 recent heart surgery, 1 refusal to do baseline bone measurements).

Table 8: Pearson Product Moment Correlations of age, Tanner stage, body size, body composition, and lifestyle factors with bone area, bone mineral content (BMC) and areal bone mineral density (aBMD) for girls at baseline (N = 189, 2 exclusions).

	Age	Tanner Stage	Height	Leg Length	Sitting Height	Weight	Lean Mass	Fat Mass	BMI	PA Score	Load Time	Sport Nights	Calcium Intake
Total Body area	0.42**	0.58**	0.88**	0.75**	0.87**	0.88**	0.93**	0.67**	0.65**	-0.04	0.09	0.19*	0.07
Total Body BMC	0.39**	0.53**	0.81**	0.67**	0.81**	0.79**	0.88**	0.55**	0.57**	-0.001	0.09	0.21**	0.10
Total Body BMD	0.22**	0.27**	0.42**	0.32**	0.45**	0.37**	0.50**	0.16*	0.23	0.06	0.08	0.19*	0.12
Lumbar Spine area	0.43**	0.48**	0.82**	0.65**	0.85**	0.63**	0.79**	0.35**	0.34**	-0.02	0.09	0.17*	0.10
Lumbar Spine BMC	0.41**	0.53**	0.75**	0.56**	0.82**	0.67**	0.81**	0.39**	0.42**	0.02	0.07	0.17*	0.07
Lumbar Spine aBMD	0.32**	0.46**	0.58**	0.41**	0.65**	0.59**	0.68**	0.37**	0.42**	0.06	0.05	0.15*	0.03
Proximal Femur area	0.47**	0.54**	0.81**	0.72**	0.77**	0.72**	0.81**	0.49**	0.48**	-0.08	0.13#	0.15*	0.05
Proximal Femur BMC	0.39**	0.52**	0.75**	0.64**	0.73**	0.75**	0.84**	0.52**	0.55**	0.05	0.17*	0.23**	0.14#
Proximal Femur aBMD	0.22**	0.33**	0.48**	0.39**	0.50**	0.57**	0.62**	0.40**	0.46**	0.17*	0.16*	0.23**	0.19*
Femoral Neck area	0.41**	0.50**	0.73**	0.63**	0.71**	0.67**	0.71**	0.49**	0.47**	-0.12	0.10	0.13#	0.08
Femoral Neck BMC	0.33**	0.46**	0.69**	0.58**	0.70**	0.76**	0.80**	0.57**	0.60**	0.08	0.17*	0.21**	0.17*
Femoral Neck aBMD	0.18*	0.29**	0.46**	0.38**	0.48**	0.58**	0.60**	0.44**	0.49**	0.19*	0.17*	0.21**	0.18*
Trochanter area	0.54**	0.53**	0.79**	0.66**	0.78**	0.67**	0.80**	0.41**	0.42**	-0.03	0.09	0.15*	0.01
Trochanter BMC	0.47**	0.52**	0.75**	0.61**	0.77**	0.69**	0.83**	0.41**	0.45**	0.04	0.11	0.19*	0.07
Trochanter aBMD	0.25**	0.35**	0.52**	0.40**	0.56**	0.53**	0.64**	0.32**	0.39**	0.15*	0.13#	0.22**	0.14#

**P<0.01; *P<0.05; #P<0.1; TB=total body, LS=lumbar spine, PF=proximal femur, FN=femoral neck, TR=trochanter, PA score=physical activity score, load time=time in loaded physical activity, sport nights=number of extracurricular sport nights per week.

Table 9: Pearson Product Moment Correlations of age, Tanner stage, body size, body composition, and lifestyle factors with bone area, bone mineral content (BMC) and areal bone mineral density (aBMD) for boys at baseline (N = 187, 5 exclusions)

	Age	Tanner Stage	Height	Leg Length	Sitting Height	Weight	Lean Mass	Fat Mass	BMI	PA Score	Load Time	Sport Nights	Calcium Intake
Total Body area	0.40**	0.28**	0.86**	0.76**	0.84**	0.87**	0.93**	0.69**	0.69**	0.13#	0.15*	0.26**	-0.07
Total Body BMC	0.42**	0.31**	0.79**	0.71**	0.77**	0.77**	0.87**	0.58**	0.59**	0.16*	0.17*	0.29**	-0.04
Total Body BMD	0.28**	0.24**	0.28**	0.25**	0.26**	0.19*	0.32**	0.04	0.11	0.16*	0.17*	0.26**	0.07
Lumbar Spine area	0.36**	0.23**	0.73**	0.63**	0.74**	0.48**	0.68**	0.24**	0.25**	0.21**	0.18*	0.28**	-0.05
Lumbar Spine BMC	0.40**	0.20*	0.69**	0.60**	0.70**	0.56**	0.72**	0.33**	0.37**	0.16*	0.13#	0.29**	0.00
Lumbar Spine aBMD	0.31**	0.11	0.43**	0.36**	0.45**	0.48**	0.55**	0.35**	0.41**	0.06	0.04	0.22**	0.05
Proximal Femur area	0.43**	0.28**	0.84**	0.77**	0.79**	0.67**	0.82**	0.44**	0.43**	0.16*	0.19*	0.26**	-0.11
Proximal Femur BMC	0.42**	0.30**	0.77**	0.70**	0.74**	0.70**	0.83**	0.48**	0.51**	0.22**	0.24**	0.29**	-0.05
Proximal Femur aBMD	0.28**	0.20*	0.45**	0.39**	0.45**	0.53**	0.59**	0.39**	0.45**	0.23**	0.23**	0.25**	0.03
Femoral Neck area	0.33**	0.25**	0.68**	0.62**	0.65**	0.52**	0.66**	0.33**	0.34**	0.15*	0.11	0.23**	-0.06
Femoral Neck BMC	0.36**	0.31**	0.67**	0.60**	0.64**	0.60**	0.73**	0.40**	0.44**	0.25**	0.21**	0.31**	0.01
Femoral Neck aBMD	0.29**	0.26**	0.48**	0.43**	0.47**	0.50**	0.58**	0.35**	0.40**	0.26**	0.23**	0.29**	0.04
Trochanter area	0.47**	0.25**	0.78**	0.71**	0.74**	0.63**	0.78**	0.41**	0.40**	0.20*	0.19*	0.23**	-0.08
Trochanter BMC	0.45**	0.27**	0.73**	0.66**	0.71**	0.62**	0.77**	0.40**	0.42**	0.25**	0.23**	0.27**	-0.05
Trochanter aBMD	0.25**	0.19**	0.38**	0.31**	0.40**	0.39**	0.49**	0.25**	0.31**	0.27**	0.24**	0.24**	0.004

**P<0.01; *P<0.05; #P<0.1; TB=total body, LS=lumbar spine, PF=proximal femur, FN=femoral neck, TR=trochanter, PA score=physical activity score, load time=time in loaded physical activity, sport nights=number of extracurricular sport nights per week.

Table 10: Pearson Product Moment Correlations of age, Tanner stage, body size and body composition (and their changes), and lifestyle factors with 8-month change in bone area, bone mineral content (BMC) and areal bone mineral density (aBMD) for girls at Time 2 (N = 179, 2 exclusions)

Change In:	TB AREA	TB BMC	TB BMD	LS AREA	LS BMC	LS BMD	PF AREA	PF BMC	PF BMD	FN AREA	FN BMC	FN BMD	FN vBMD	TR AREA	TR BMC	TR BMD
Age	0.21*	0.31**	0.18*	0.31**	0.42**	0.37**	-0.07	0.20*	0.27**	0.03	0.22**	0.27**	0.14#	-0.07	0.21**	0.30**
Baseline Tanner Stage	0.30**	0.48**	0.37**	0.26**	0.41**	0.35**	-0.13#	0.14#	0.25**	0.02	0.24**	0.29**	0.13#	-0.12	0.13#	0.23**
Final Tanner Stage	0.34**	0.50**	0.33**	0.29**	0.43**	0.36**	-0.05	0.22**	0.29**	-0.05	0.19*	0.29**	0.18*	-0.08	0.19*	0.24**
Baseline Height	0.28**	0.51**	0.36**	0.31**	0.49**	0.37**	0.03	0.33**	0.29**	0.05	0.24**	0.20*	0.07	-0.42	0.31**	0.29**
Height Change	0.42**	0.33**	0.12	0.53**	0.46**	0.42**	0.30**	0.41**	0.40**	0.24**	0.34**	0.24**	-0.04	0.35**	0.45**	0.40**
Baseline Sit Height	0.34**	0.57**	0.38**	0.36**	0.57**	0.46**	0.01	0.32**	0.31**	0.06	0.25**	0.22**	0.07	-0.05	0.30**	0.29**
Sit Heightt Change	0.32**	0.30**	0.14#	0.37**	0.31**	0.24**	0.08	0.21**	0.25**	0.08	0.18*	0.19*	0.04	0.04	0.16*	0.19*
Baseline Leg Length	0.16*	0.36**	0.27**	0.22**	0.34**	0.22**	0.04	0.28*	0.22*	0.03	0.19*	0.15*	0.05	-0.04	0.26**	0.24**
Leg Length Change	0.08	0.02	-0.03	0.15#	0.13#	0.17*	0.22**	0.21*	0.16*	0.16*	0.15*	0.05	-0.08	0.32**	0.30**	0.22**
Baseline Weight	0.24**	0.48**	0.35**	0.19*	0.41**	0.35**	-0.05	0.23**	0.20*	0.07	0.25**	0.17*	0.02	-0.03	0.26**	0.20*
Weight Change	0.47**	0.46**	0.21**	0.27**	0.32**	0.28**	-0.09	0.19*	0.33**	0.11	0.27**	0.25**	0.05	0.012	0.27**	0.37**
BMI Change	0.29**	0.24**	0.08	0.03	0.04	0.02	-0.23**	-0.04	0.14#	0.00	0.08	0.13#	0.06	-0.125#	0.02	0.17*
Baseline BMI	0.17*	0.34**	0.27**	0.07	0.25**	0.24**	-0.08	0.12	0.10	0.07	0.20*	0.11	-0.02	-0.003	0.17*	0.10
Baseline Fat Mass	0.12	0.26**	0.19*	0.04	0.20*	0.20*	-0.06	0.09	0.03	0.04	0.14#	0.05	-0.04	0.02	0.15#	0.05
Fat Mass Change	0.20*	0.07	-0.10	-0.20*	-0.19*	-0.16*	-0.27**	-0.22**	-0.10	-0.03	-0.05	-0.04	-0.02	-0.15*	-0.14#	-0.04
Baseline Lean Mass	0.33**	0.62**	0.46**	0.32**	0.54**	0.43**	-0.02	0.34**	0.34**	0.08	0.32**	0.27**	0.07	-0.06	0.33**	0.32**
Lean Mass Change	0.47**	0.60**	0.44**	0.60**	0.64**	0.55**	0.15*	0.49**	0.56**	0.20*	0.43**	0.39**	0.08	0.20**	0.53**	0.53**
Baseline TB Area	0.24**	0.56**	0.45**	0.28**	0.52**	0.41**	-0.04	0.33**	0.31**	0.06	0.30**	0.26**	0.08	-0.09	0.32**	0.31**

**P<0.01; *P<0.05; #P<0.1; TB=total body, LS=lumbar spine, PF=proximal femur, FN=femoral neck, TR=trochanter, vBMD=(calculated) volumetric bone mineral density, PA score=physical activity score, load time=time in loaded physical activity, sport nights=number of extracurricular sport nights per week.

Table 10 (continued)

Change In:	TB AREA	TB BMC	TB BMD	LS AREA	LS BMC	LS BMD	PF AREA	PF BMC	PF BMD	FN AREA	FN BMC	FN BMD	FN vBMD	TR AREA	TR BMC	TR BMD
Baseline TB BMC	0.19*	0.53**	0.39**	0.25**	0.51**	0.39**	-0.07	0.31**	0.29**	0.02	0.29**	0.28**	0.11	-0.14	0.28**	0.27**
Baseline TB aBMD	0.03	0.29**	0.16*	0.12	0.32**	0.23**	-0.07	0.20*	0.18*	-0.05	0.18*	0.25**	0.15*	-0.17*	0.14#	0.13#
Baseline PF Area	0.32**	0.54**	0.38**	0.34**	0.51**	0.40**	-0.07	0.28**	0.28**	0.12	0.30**	0.21**	0.02	-0.07	0.32**	0.34**
Baseline PF BMC	0.24**	0.55**	0.41**	0.28**	0.51**	0.40**	-0.14#	0.27**	0.27**	0.04	0.28**	0.24**	0.07	-0.13#	0.31**	0.29**
Baseline PF aBMD	0.09	0.39**	0.30**	0.13#	0.35**	0.27**	-0.18*	0.18*	0.17*	-0.05	0.17*	0.19*	0.10	-0.16*	0.21*	0.16*
Baseline FN Area	0.35**	0.54**	0.37**	0.31**	0.46**	0.37**	0.08	0.35**	0.32**	-0.15#	0.12	0.24**	0.25**	0.08	0.37**	0.32**
Baseline FN BMC	0.23**	0.52**	0.35**	0.23**	0.45**	0.36**	-0.02	0.32**	0.25**	-0.11	0.16*	0.22**	0.16*	-0.03	0.34**	0.23**
Baseline FN aBMD	0.09	0.35**	0.23**	0.11	0.31**	0.23**	-0.08	0.21*	0.13#	-0.06	0.14#	0.14#	0.06	-0.08	0.22**	0.10
Baseline vBMD	-0.15#	-0.03	-0.03	-0.10	-0.02	-0.04	-0.13	-0.04	-0.09	0.04	0.04	-0.03	-0.10	-0.13#	-0.03	-0.11
Baseline TR Area	0.37**	0.61**	0.42**	0.42**	061**	0.50**	-0.06	0.34**	0.38**	0.11	0.34**	0.29**	0.08	-0.21**	0.27**	0.38**
Baseline TR BMC	0.31**	0.63**	0.46**	0.39**	0.62**	0.50**	-0.11	0.33**	0.37**	0.04	0.31**	0.31**	0.12	-0.23**	0.28**	0.34**
Baseline TR aBMD	0.17*	0.49**	0.38**	0.26**	0.46**	0.35**	-0.13#	0.24**	0.25**	-0.06	0.19*	0.25**	0.16*	0.21**	0.23**	0.20*
Baseline LS Area	0.27**	0.53**	0.39**	0.22**	0.48**	0.38**	-0.001	0.29**	0.27**	0.05	0.25**	0.22**	0.08	-0.10	0.25**	0.27**
Baseline LS BMC	0.23**	0.54**	0.42**	0.28**	0.53**	0.40**	-0.11	0.25**	0.29**	0.01	0.23**	0.24**	0.11	-0.16*	0.23**	0.25**
Baseline LS aBMD	0.17*	0.49**	0.39**	0.30**	0.52**	0.36**	-0.13#	0.22**	0.28**	-0.02	0.20*	0.24**	0.13#	-0.15*	0.22**	0.24**
PA Score	0.13#	0.07	-0.02	0.09	-0.02	-0.06	0.06	0.09	0.07	0.05	0.08	0.07	-0.01	0.14	0.13#	0.08
Load Time	0.09	0.13#	0.07	0.06	0.03	-0.01	-0.04	0.10	0.12	0.01	0.11	0.14#	0.07	0.05	0.14#	0.12
Sport Nights	0.11	0.19*	0.12	0.06	0.06	0.01	0.06	0.20*	0.18*	0.01	0.22**	0.32**	0.18*	0.10	0.25**	0.22**
Calcium Intake	0.07	0.04	-0.04	-0.15#	-0.12	-0.13#	-0.04	0.01	0.00	0.03	0.08	0.06	0.00	0.01	0.03	0.02

**P<0.01; *P<0.05; #P<0.1. TB=total body, LS=lumbar spine, PF=proximal femur, FN=femoral neck, TR=trochanter, vBMD=(calculated) volumetric bone mineral density, PA score=physical activity score, load time=time in loaded physical activity, sport nights=number of extracurricular sport nights per week.

Table 11: Pearson Product Moment Correlations of age, Tanner stage, body size and body composition (and their changes), and lifestyle factors with 8-month change in bone area, bone mineral content (BMC) and areal bone mineral density (aBMD) for boys at Time 2 (N = 180, 5 exclusions)

Change in:	TB AREA	TB BMC	TB BMD	LS AREA	LS BMC	LS BMD	PF AREA	PF BMC	PF BMD	FN AREA	FN BMC	FN BMD	FN vBMD	TR AREA	TR BMC	TR aBMD
Age	0.14#	0.28**	0.17*	0.22**	0.31**	0.20*	0.17*	0.27**	0.21**	0.06	0.11	0.04	-0.01	0.14#	0.29**	0.30**
Baseline Tanner Stage	0.17*	0.26**	0.09	0.17*	0.27**	0.19*	0.23**	0.29**	0.20*	0.04	0.12	0.09	0.02	0.25**	0.32**	0.22**
Final Tanner Stage	0.21**	0.27**	0.07	0.20*	0.31**	0.25**	0.31**	0.38**	0.29**	0.09	0.19*	0.14#	0.01	0.21*	0.32**	0.29**
Baseline Height	0.37**	0.55**	0.28**	0.30**	0.44**	0.27**	0.34**	0.48**	0.31**	0.14#	0.28**	0.17*	-0.01	0.36**	0.51**	0.34**
Height Change	0.50**	0.57**	0.22**	0.52**	0.62**	0.43**	0.54**	0.57**	0.42**	0.15*	0.34**	0.32**	0.06	0.62**	0.65**	0.43**
Baseline Sit Height	0.35**	0.55**	0.31**	0.36**	0.49**	0.29**	0.36**	0.50**	0.36**	0.13#	0.30**	0.20*	0.004	0.36**	0.52**	0.38**
Sit Height Change	0.30**	0.31**	0.06	0.35**	0.41**	0.23**	0.28**	0.30**	0.18*	0.12	0.21*	0.17*	0.02	0.33**	0.35**	0.21*
Baseline Leg Length	0.33**	0.49**	0.22**	0.21**	0.34**	0.22**	0.28**	0.39**	0.23**	0.12	0.23**	0.13#	-0.02	0.32**	0.44**	0.27**
Leg Length Change	0.19*	0.24**	0.16*	0.15*	0.19*	0.18*	0.27**	0.26**	0.24**	0.03	0.13	0.14#	0.04	0.29**	0.29**	0.22**
Baseline Weight	0.35**	0.55**	0.32**	0.26**	0.41**	0.31**	0.32**	0.43**	0.30**	0.13#	0.30**	0.22**	0.00	0.34**	0.48**	0.37**
Weight Change	0.41**	0.34**	-0.01	0.08	0.15*	0.13#	0.25**	0.32**	0.26**	0.11	0.22**	0.18*	0.02	0.20*	0.25**	0.21**
Baseline BMI	0.27**	0.42**	0.26**	0.18*	0.30**	0.24**	0.24**	0.31**	0.22**	0.10	0.24**	0.19*	0.01	0.25**	0.35**	0.30**
BMI Change	0.19*	0.04	-0.17*	-0.14#	-0.15*	-0.09	-0.02	0.01	0.04	0.02	0.03	0.03	0.00	-0.08	-0.08	-0.03
Baseline Fat Mass	0.25**	0.40**	0.26**	0.17*	0.28**	0.25**	0.24**	0.29**	0.20*	0.10	0.23**	0.17*	0.00	0.26**	0.33**	0.27**
Fat Mass Change	0.17*	-0.07	-0.28**	-0.24**	-0.31**	-0.21**	-0.07	-0.13#	-0.12	-0.04	-0.13#	-0.13#	-0.05	-0.12	0.21**	-0.22**
Baseline Lean Mass	0.42**	0.63**	0.32**	0.33**	0.49**	0.33**	0.35**	0.52**	0.37**	0.14#	0.33**	0.24**	0.02	0.38**	0.57**	0.44**
Lean Mass Change	0.46**	0.60**	0.31**	0.39**	0.54**	0.40**	0.45**	0.60**	0.52**	0.21**	0.46**	0.41**	0.07	0.41**	0.57**	0.52**
Baseline TB Area	0.35**	0.60**	0.37**	0.33**	0.48**	0.30**	0.34**	0.51**	0.37**	0.13#	0.33**	0.25**	0.03	0.38**	0.58**	0.45**

TB=total body, LS=lumbar spine, PF=proximal femur, FN=femoral neck, TR=trochanter, vBMD=(calculated) volumetric bone mineral density, PA score =physical activity score, load time = time in loaded physical activity, sport nights= number of extracurricular sport nights per week.

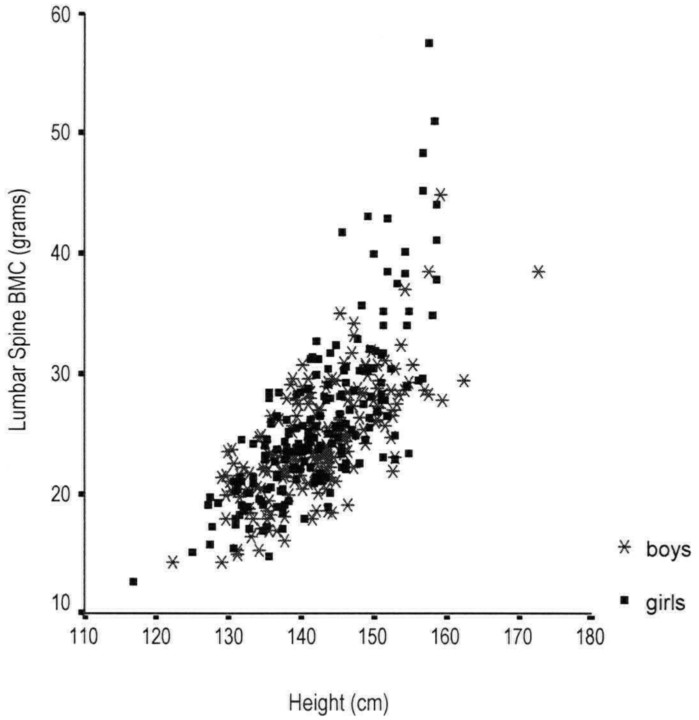
(Table 11 continued)

Change in:	TB AREA	TB BMC	TB BMD	LS AREA	LS BMC	LS BMD	PF AREA	PF BMC	PF BMD	FN AREA	FN BMC	FN BMD	FN vBMD	TR AREA	TR BMC	TR aBMD
Baseline TB BMC	0.31**	0.57**	0.32**	0.30**	0.44**	0.25**	0.31**	0.51**	0.38**	0.11	0.32**	0.25**	0.04	0.33**	0.56**	0.47**
Baseline TB aBMD	0.06	0.21**	0.08	0.09	0.14#	0.01	0.10	0.27**	0.24**	0.02	0.14#	0.13#	0.04	0.05	0.25**	0.30**
Baseline LS Area	0.29**	0.42**	0.16*	0.27**	0.37**	0.16*	0.25**	0.41**	0.30**	0.07	0.24**	0.21**	0.08	0.25**	0.42**	0.36**
Baseline LS BMC	0.28**	0.49**	0.26**	0.29**	0.41**	0.15#	0.24**	0.43**	0.32**	0.08	0.29**	0.25**	0.07	0.19*	0.44**	0.40**
Baseline LS aBMD	0.17*	0.40**	0.30**	0.21*	0.29**	0.06	0.13#	0.29**	0.21*	0.07	0.23**	0.18*	0.04	0.08	0.31**	0.31**
Baseline PF Area	0.33**	0.54**	0.30**	0.36**	0.47**	0.26**	0.26**	0.44**	0.31**	0.08	0.23**	0.15#	0.03	0.36**	0.55**	0.41**
Baseline PF BMC	0.32**	0.55**	0.31**	0.31**	0.43**	0.23**	0.24**	0.46**	0.32**	0.06	0.25**	0.19*	0.05	0.29**	0.53**	0.41**
Baseline PF aBMD	0.21*	0.40**	0.24**	0.16*	0.24**	0.10	0.14#	0.33**	0.22**	0.01	0.20*	0.19*	0.05	0.14#	0.35**	0.28**
Baseline FN Area	0.28**	0.43**	0.19*	0.21**	0.33**	0.19*	0.26**	0.39**	0.25**	-0.13#	0.05	0.13#	0.21*	0.25**	0.41**	0.35**
Baseline FN BMC	0.31**	0.49**	0.22**	0.19*	0.32**	0.16*	0.23**	0.41**	0.26**	-0.07	0.13#	0.15#	0.12	0.19*	0.42**	0.34**
Baseline FN aBMD	0.24**	0.40**	0.18*	0.13	0.22**	0.09	0.13#	0.30**	0.19*	-0.01	0.15#	0.12	0.02	0.11	0.31**	0.24**
Baseline vBMD	0.04	0.11	0.06	-0.01	0.00	-0.04	-0.05	0.04	0.03	0.09	0.12	0.03	-0.12	-0.07	0.03	0.00
Baseline TR Area	0.33**	0.53**	0.27**	0.36**	0.50**	0.30**	0.21**	0.44**	0.36**	0.08	0.25**	0.20**	0.05	0.30**	0.55**	0.46**
Baseline TR BMC	0.33**	0.55**	0.29**	0.33**	0.49**	0.30**	0.21*	0.45**	0.36**	0.07	0.27**	0.22**	0.06	0.27**	0.55**	0.44**
Baseline TR aBMD	0.18*	0.36**	0.23**	0.13*	0.26**	0.16*	0.08	0.28**	0.21*	0.04	0.20*	0.17*	0.03	0.10	0.33**	0.23**
PA Score	0.02	0.13#	0.18*	0.07	0.14#	0.16*	0.11	0.19*	0.20*	-0.05	0.01	0.08	0.09	0.16*	0.23**	0.24**
Load time	0.15*	0.21**	0.13#	0.13#	0.13#	0.08	0.09	0.15#	0.14#	-0.01	-0.01	-0.02	-0.01	0.19*	0.25**	0.19*
Sport Nights	0.11	0.11	-0.04	0.04	0.11	0.10	-0.04	0.03	0.05	-0.06	0.02	0.09	0.09	0.11	0.16*	0.18*
Calcium Intake	0.09	0.07	-0.01	0.01	-0.03	-0.06	0.10	0.07	-0.01	-0.05	-0.08	-0.10	-0.03	0.05	0.07	0.03

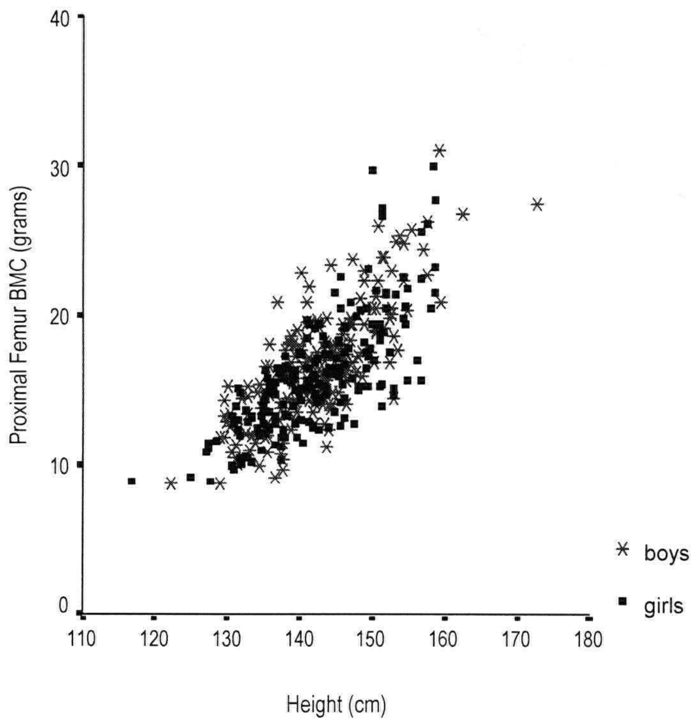
**P<0.01; *P<0.05; #P<0.1; TB=total body, LS=lumbar spine, PF=proximal femur, FN=femoral neck, TR=trochanter, vBMD=(calculated) volumetric bone mineral density, PA score =physical activity score, load time = time in loaded physical activity, sport nights= number of extracurricular sport nights per week.

Table 12: Baseline, 8-month absolute and percent change for height, weight, and total body bone mineral content (TB BMC), and average calcium intake and PAQ-C physical activity score by school (Year 1). Mean (SD).

School	Height – baseline (cm)	Height Change (cm)	% Height Change	Weight – baseline (kg)	Weight Change (kg)	% Weight Change	TB BMC – baseline (g)	TB BMC Change (g)	% TB BMC Change	Calcium Intake (mg)	Physical Activity Score (/5)
CONTROL											
Wowk	143.0 (7.7)	3.7 (1.4)	2.6 (0.9)	39.2 (10.5)	2.1 (1.9)	5.9 (4.5)	1146 (224)	113 (48)	9.8 (3.3)	920.3 (472.7)	3.0 (0.5)
Errington	143.6 (8.1)	4.2 (1.1)	2.9 (0.7)	37.3 (8.4)	2.6 (1.1)	7.0 (2.3)	1111 (142)	124 (44)	11.2 (4.0)	717.7 (344.0)	2.8 (0.3)
Diefenbaker	143.0 (6.7)	4.2 (1.4)	2.9 (1.0)	38.3 (10.7)	3.3 (2.3)	8.8 (4.8)	1135 (267)	122 (62)	10.8 (4.9)	866.0 (264.9)	3.2 (0.4)
Lee	143.6 (6.7)	3.8 (1.0)	2.7 (0.7)	36.5 (7.6)	2.8 (2.3)	7.8 (5.9)	1133 (162)	113 (56)	10.0 (4.9)	789.5 (297.0)	3.0 (0.5)
Brighouse	145.3 (7.7)	4.2 (1.6)	2.9 (1.1)	41.4 (10.7)	3.8 (2.0)	9.0 (4.0)	1180 (245)	125 (52)	10.5 (3.6)	841.6 (490.0)	3.0 (0.5)
Kigour	141.1 (7.2)	4.2 (1.2)	2.9 (0.8)	35.8 (7.0)	3.1 (1.8)	8.4 (4.3)	1096 (211)	107 (50)	9.7 (4.1)	766.3 (351.6)	2.9 (0.5)
Kidd	141.5 (7.9)	4.3 (1.0)	3.0 (0.7)	39.7 (13.3)	4.0 (2.3)	9.6 (3.7)	1145 (284)	124 (67)	10.2 (3.9)	726.9 (387.5)	3.1 (0.5)
INTERVENTION											
Ferris	140.4 (6.7)	3.8 (1.2)	2.7 (0.8)	32.0 (5.6)	2.5 (1.4)	7.5 (3.7)	1041 (144)	95 (40)	9.2 (3.9)	746.8 (264.6)	3.1 (0.7)
Steves	138.4 (5.8)	4.4 (1.0)	3.1 (0.6)	32.9 (9.1)	3.1 (1.8)	9.2 (4.1)	1006 (200)	104 (40)	10.4 (3.5)	759.5 (334.5)	3.1 (0.7)
Garden City	141.4 (7.5)	4.1 (1.0)	2.9 (0.7)	36.3 (9.0)	3.1 (3.4)	9.5 (7.2)	1110 (220)	127 (56)	11.3 (3.8)	712.4 (338.0)	2.9 (0.4)
Tait	137.1 (9.0)	3.9 (1.3)	2.9 (0.9)	36.0 (10.3)	3.1 (2.3)	8.5 (5.6)	1016 (234)	123 (56)	11.9 (4.0)	958.7 (408.3)	3.0 (0.5)
Westwind	143.7 (6.1)	3.9 (1.2)	2.7 (0.8)	38.1 (7.6)	2.9 (1.9)	7.4 (4.1)	1140 (187)	108 (43)	9.5 (3.6)	904.1 (428.9)	3.1 (0.5)
Rideau Park	143.2 (8.3)	3.9 (1.5)	2.7 (1.0)	37.0 (10.8)	3.0 (2.1)	8.2 (5.1)	1104 (239)	122 (63)	10.8 (4.2)	843.3 (353.8)	3.2 (0.5)
Mitchell	142.4 (7.6)	3.7 (0.9)	2.6 (0.6)	35.8 (7.6)	2.7 (2.1)	7.8 (5.3)	1088 (171)	112 (34)	10.4 (3.1)	901.3 (506.1)	2.8 (0.6)
Total	142.4 (7.6)	4.0 (1.2)	2.8 (0.8)	37.4 (9.5)	3.0 (2.1)	8.1 (4.8)	1115 (220)	116 (52)	10.3 (3.9)	836.5 (397.1)	3.0 (0.5)

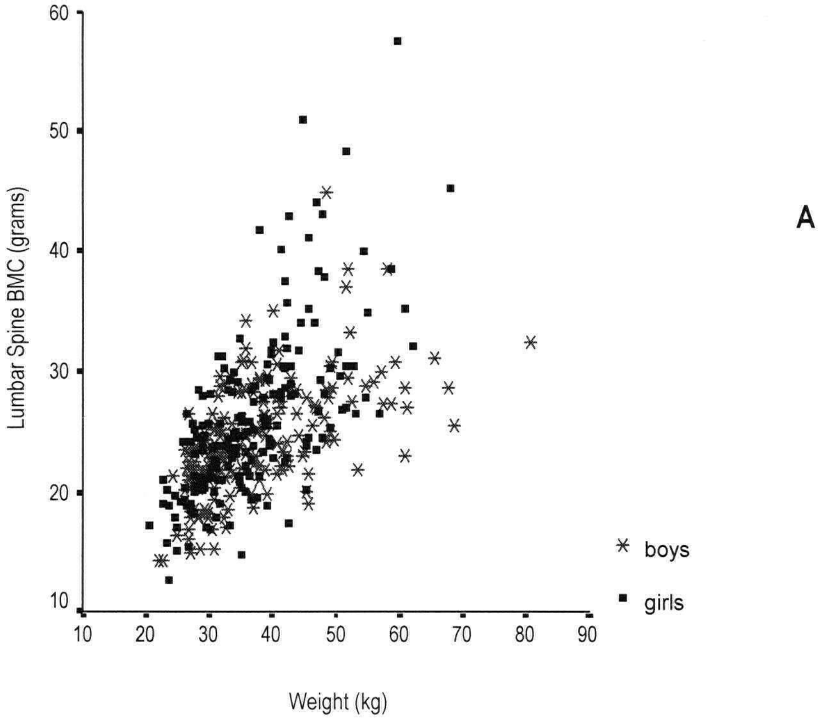


A



B

Figure 6: Height vs lumbar spine BMC (A) and height vs proximal femur BMC (B) in girls (n=189) and boys (n=187) at baseline (Fall 1999). Girls: excluding 1 Down's syndrome and 1 polycystic kidney removal/precocious puberty. Boys: excluding 1 Down's syndrome, 1 cerebral palsy, 1 casted club foot, 1 recent heart surgery (+medications), 1 refusal to do baseline bone measurements.



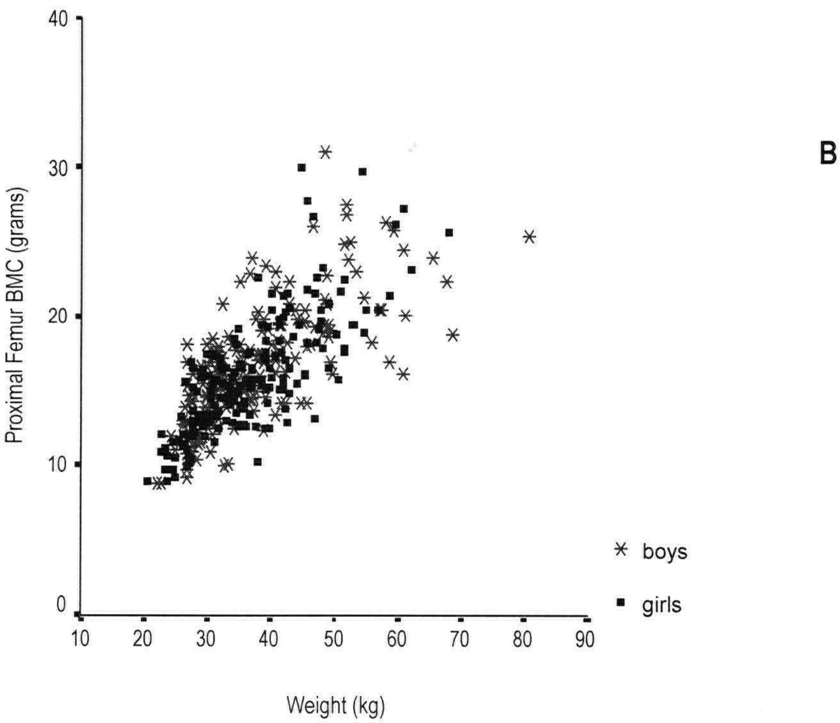


Figure 7: Weight vs lumbar spine BMC (A) and weight vs proximal femur BMC (B) in in girls (n=189) and boys (n=187) at baseline (Fall 1999).

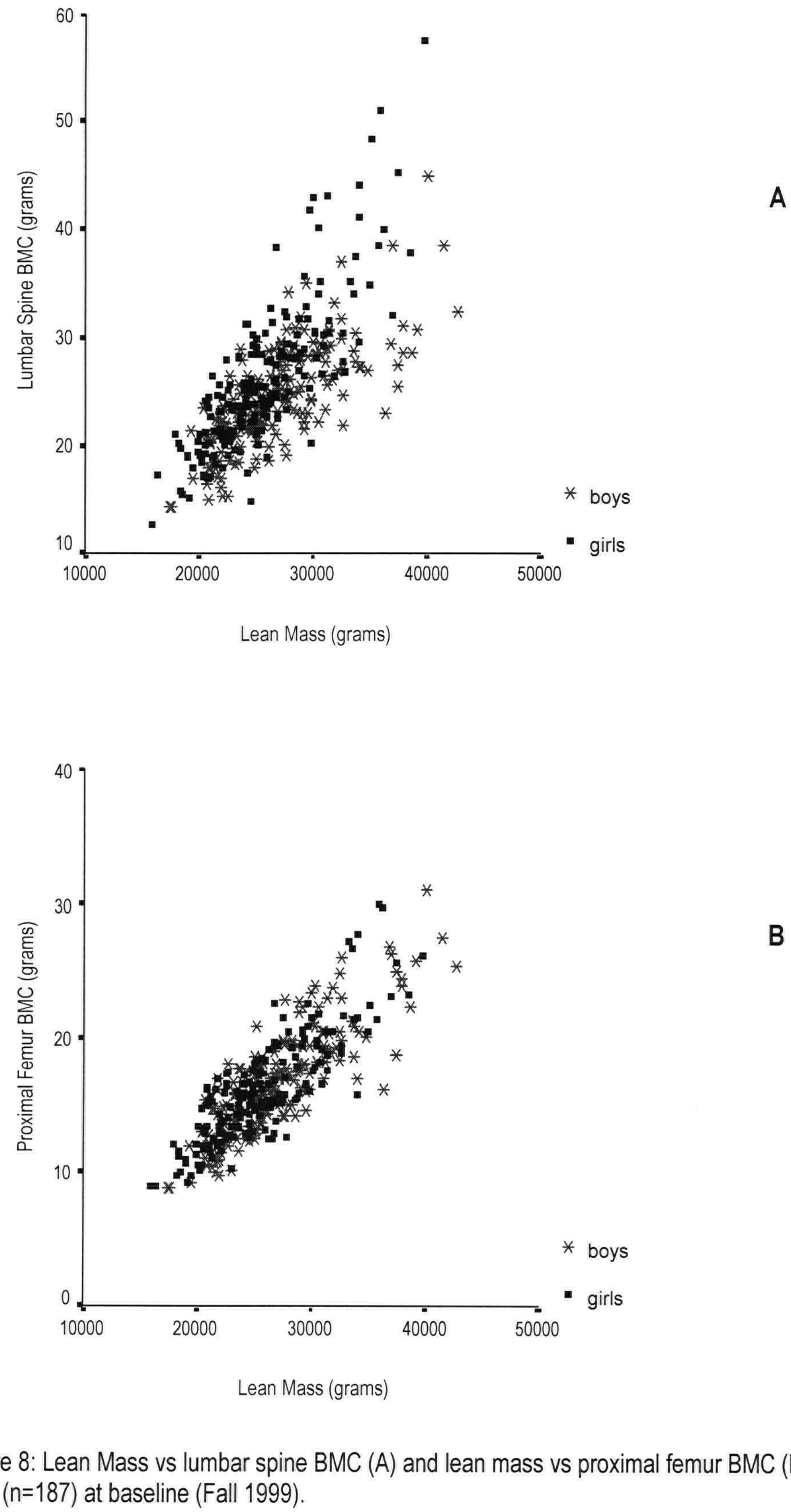
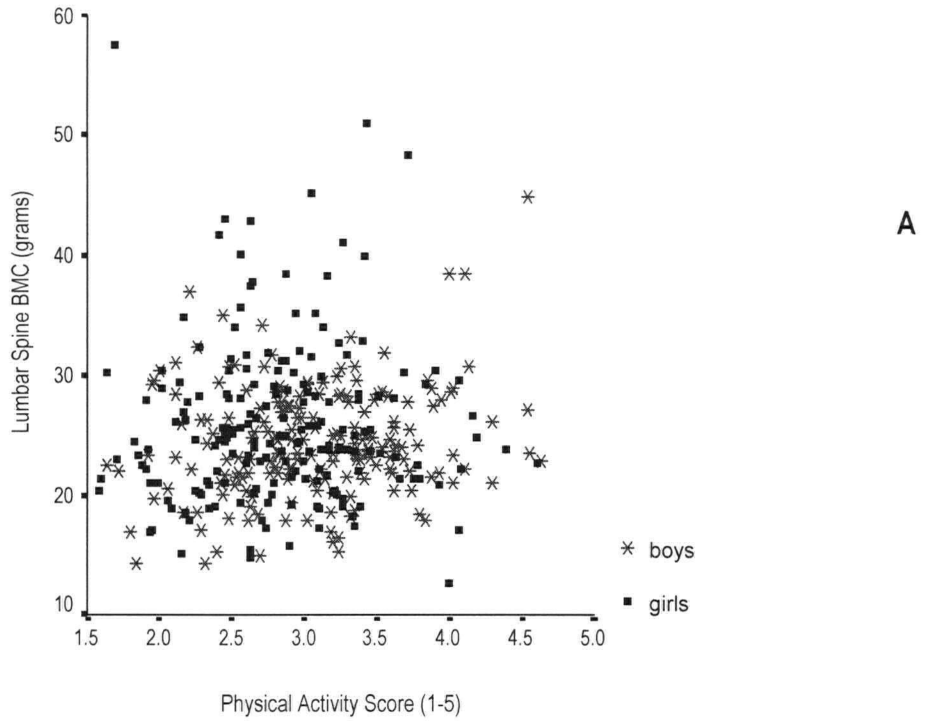


Figure 8: Lean Mass vs lumbar spine BMC (A) and lean mass vs proximal femur BMC (B) in girls (n=189) and boys (n=187) at baseline (Fall 1999).



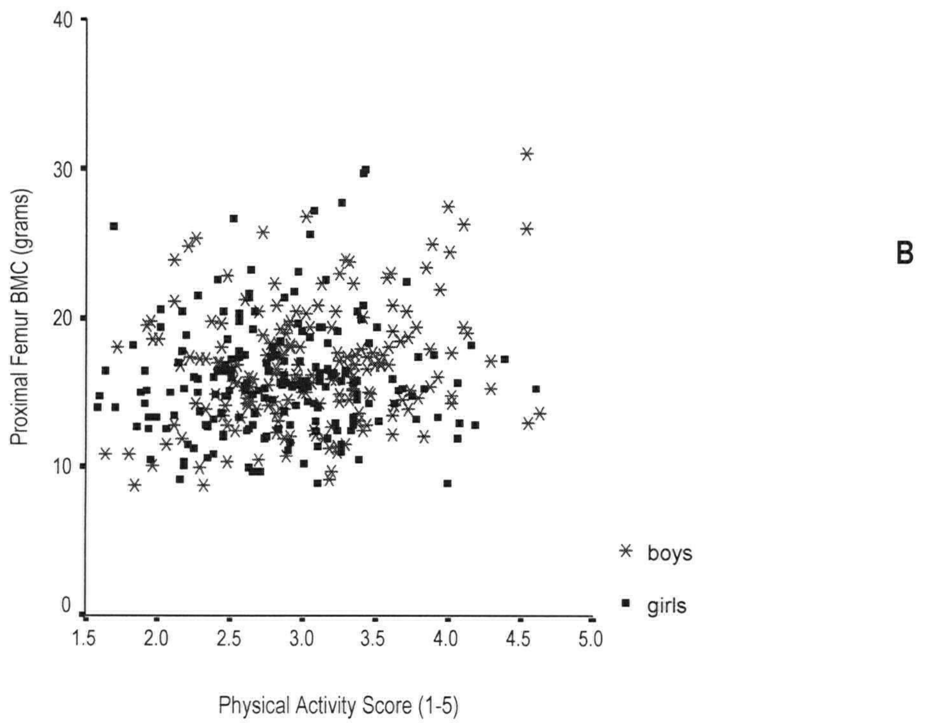
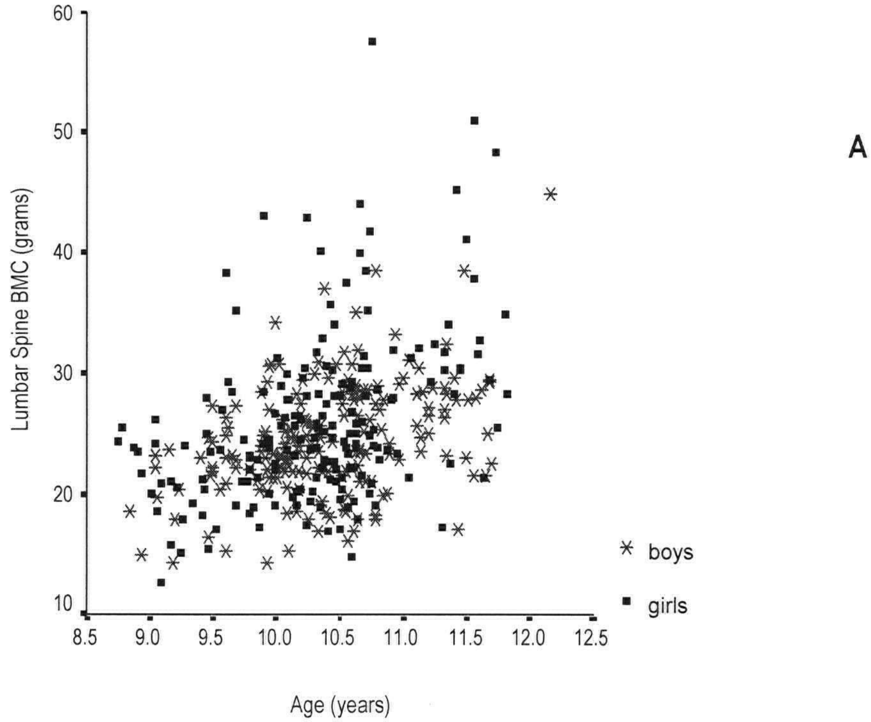


Figure 9: Physical activity score vs lumbar spine BMC (A) and physical activity score vs proximal femur BMC (B) in girls (n=189) and boys (n=187) at baseline (Fall 1999).



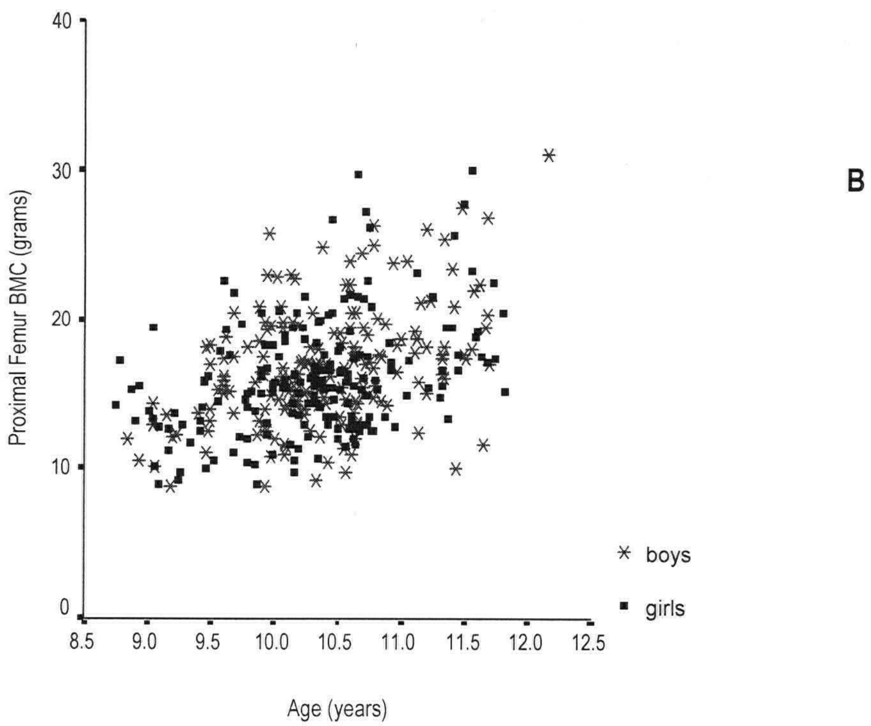


Figure 10: Age vs lumbar spine BMC (A) and age vs proximal femur BMC (B) in girls (n=189) and boys (n=187) at baseline (Fall 1999).

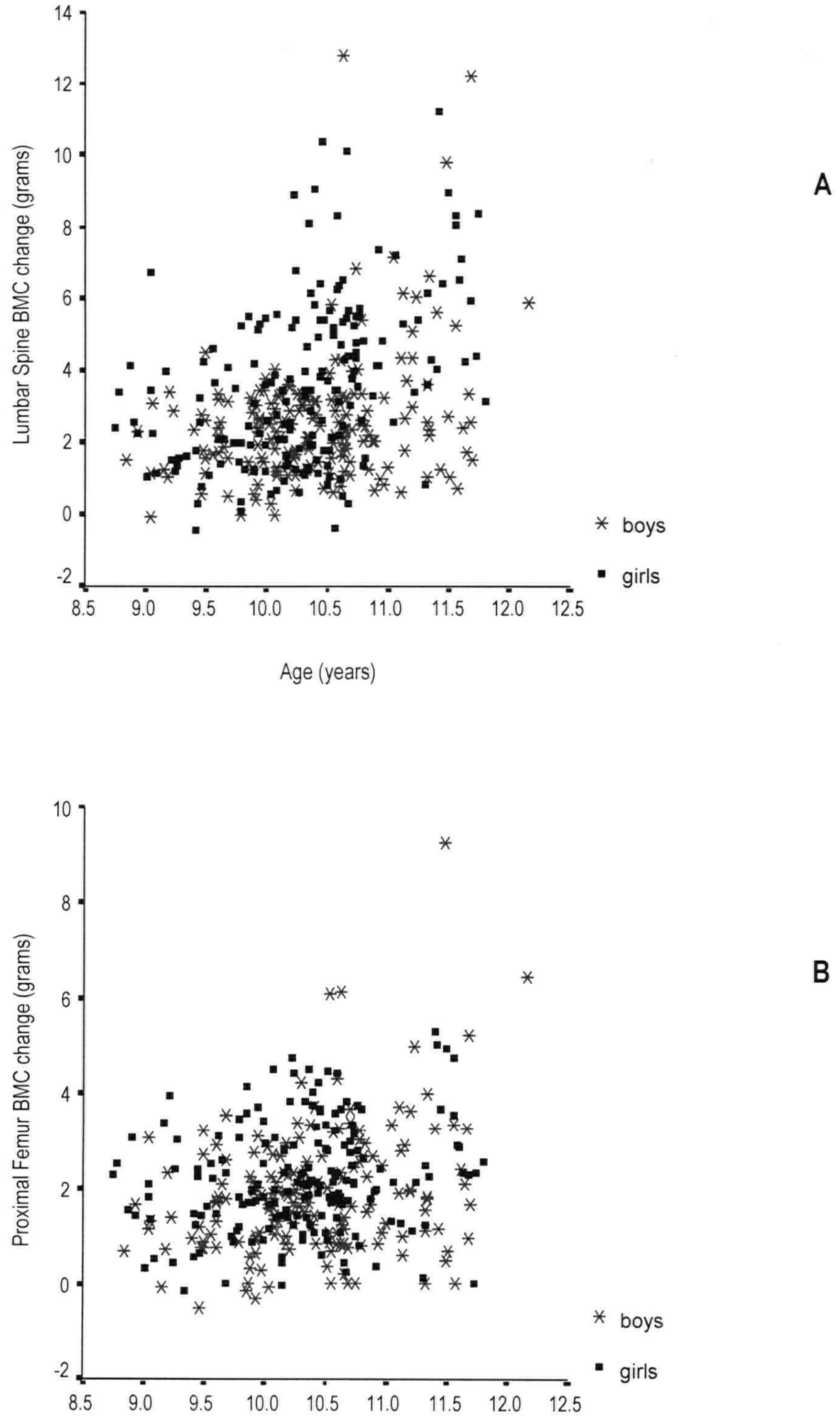
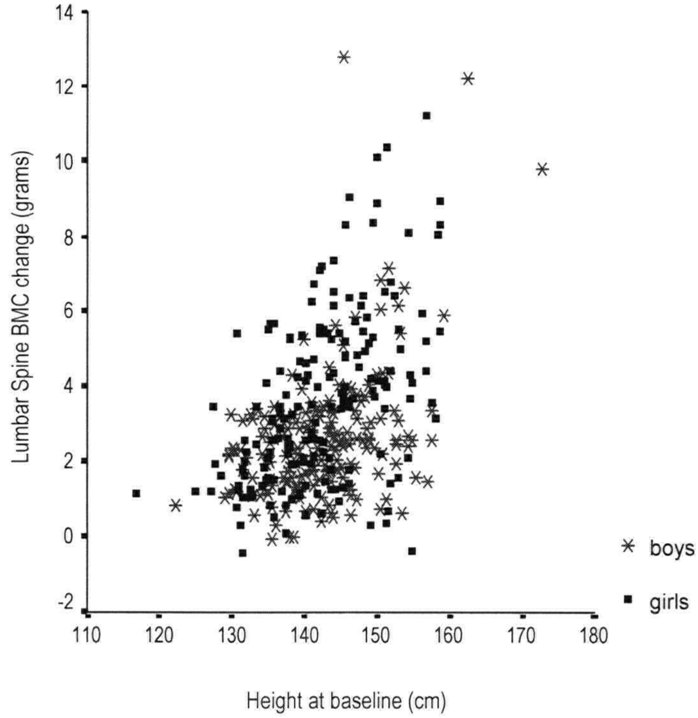
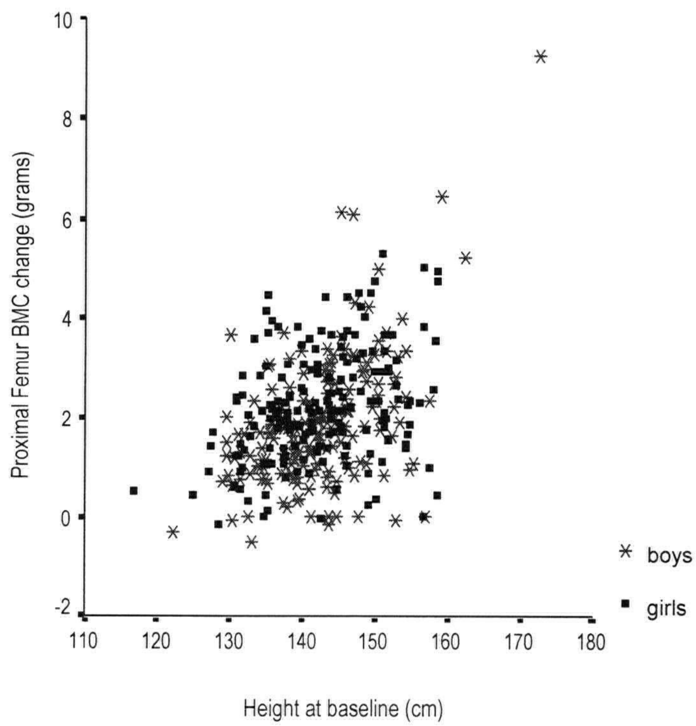


Figure 11: Age vs lumbar spine BMC 8-month change (A) and age vs proximal femur BMC 8-month change (B) in girls (n=179) and boys (n=180) (Fall 1999 – June 2000). Girls: excluding 1 Down's syndrome and 1 polycystic kidney removal/precocious puberty. Boys: excluding 1 Down's syndrome, 1 cerebral palsy, 1 casted club foot, 1 recent heart surgery (+medications), 1 fractured tibia.



A



B

Figure 12: Height vs lumbar spine BMC 8-month change (A) and height vs proximal femur BMC 8-month change (B) in girls (n=179) and boys n=180) (Fall 1999 – June 2000).

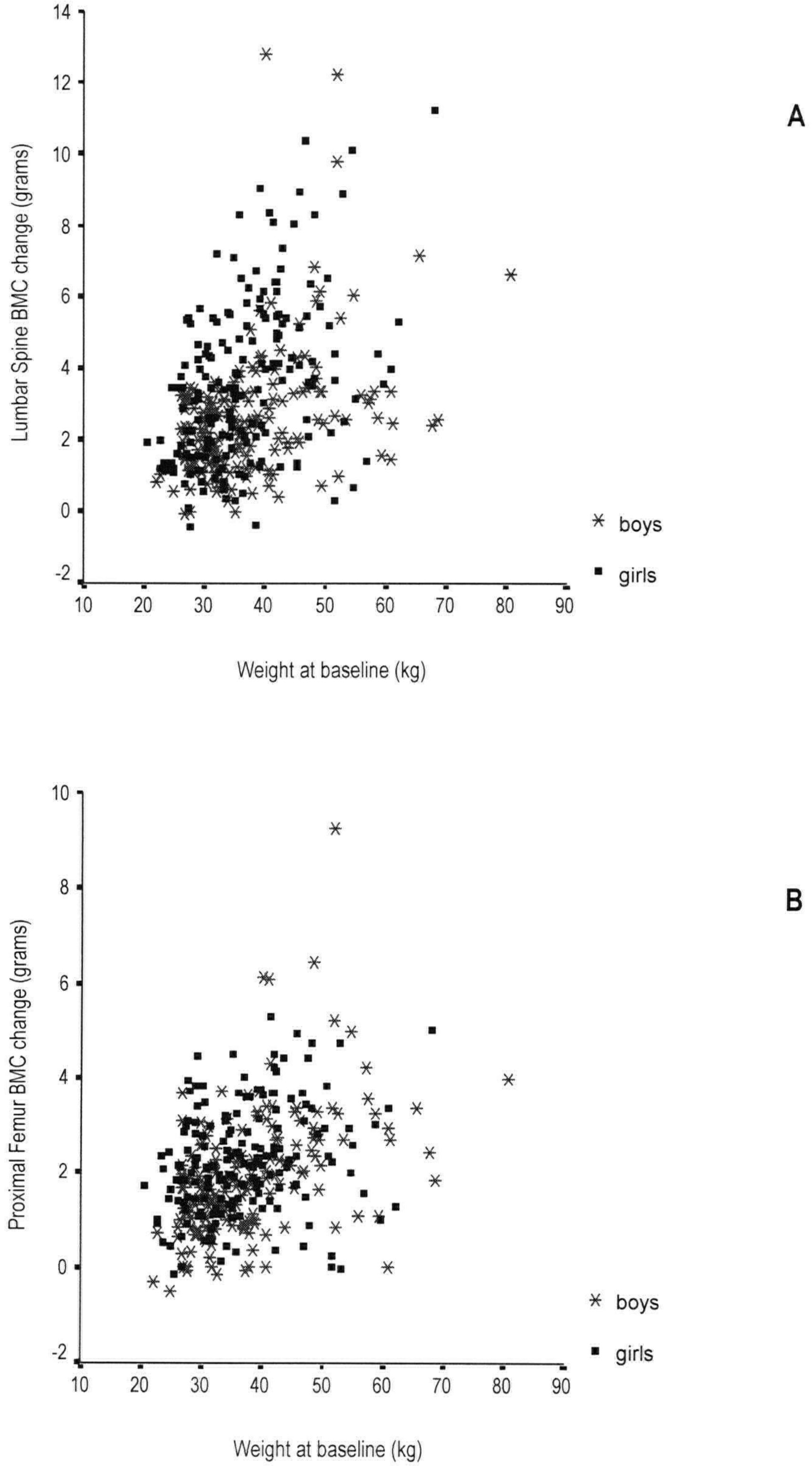
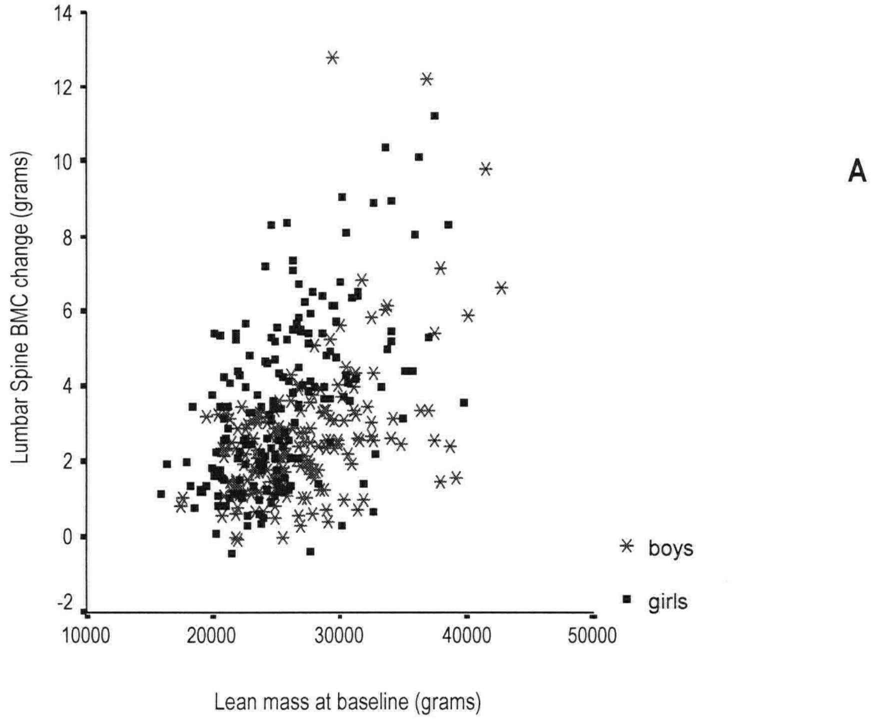


Figure 13: Weight vs lumbar spine BMC 8-month change (A) and weight vs proximal femur BMC 8-month change (B) in girls (n=179) and boys (n=180) (Fall 1999 – June 2000).



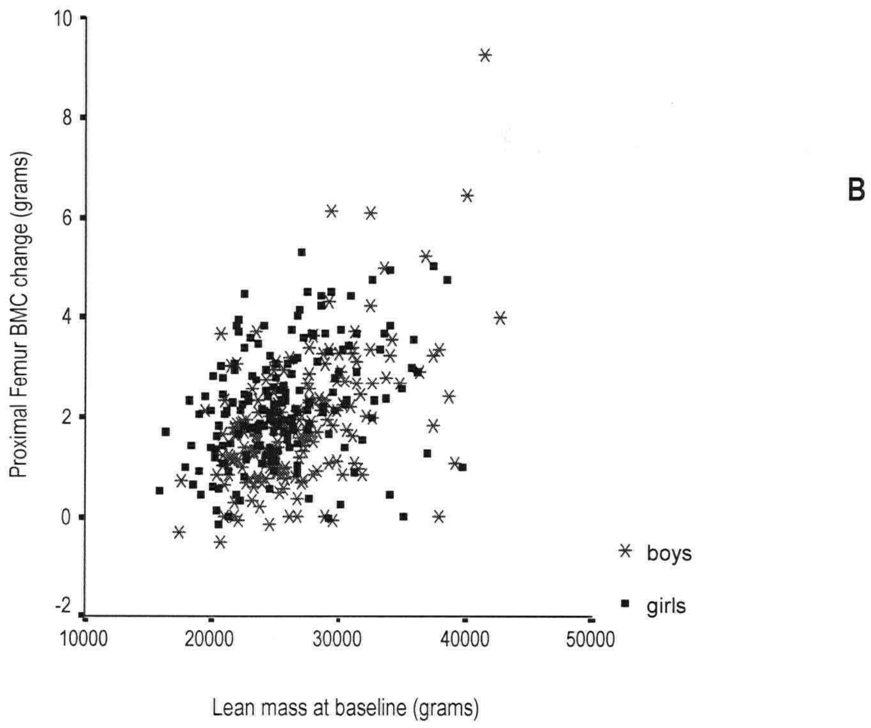
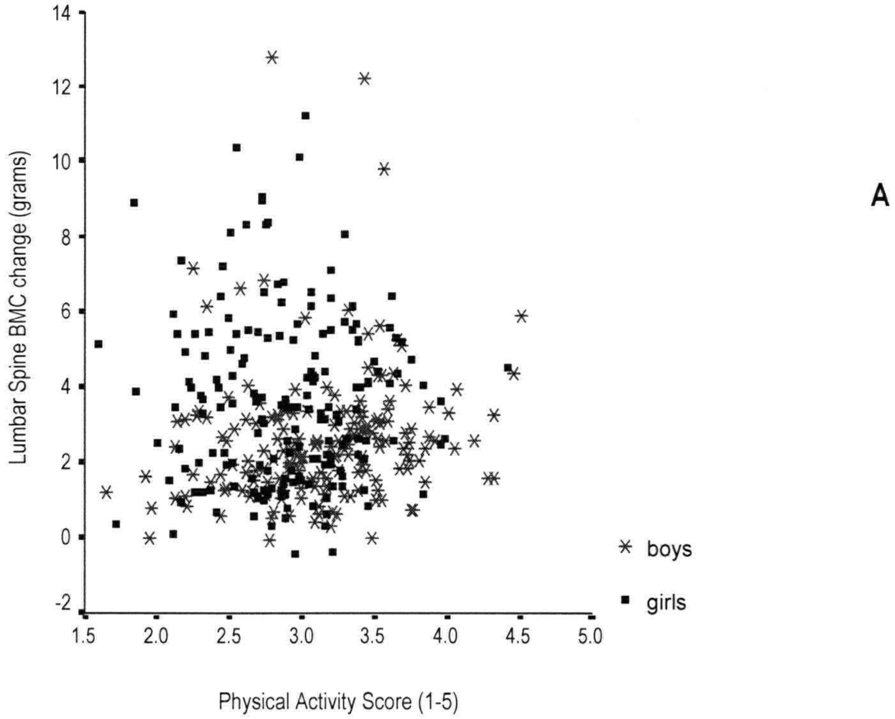


Figure 14: Lean mass at baseline vs lumbar spine BMC 8-month change (A) and lean mass at baseline vs proximal femur BMC 8-month change (B) in girls (n=179) and boys (n=180) (Fall 1999 – June 2000).



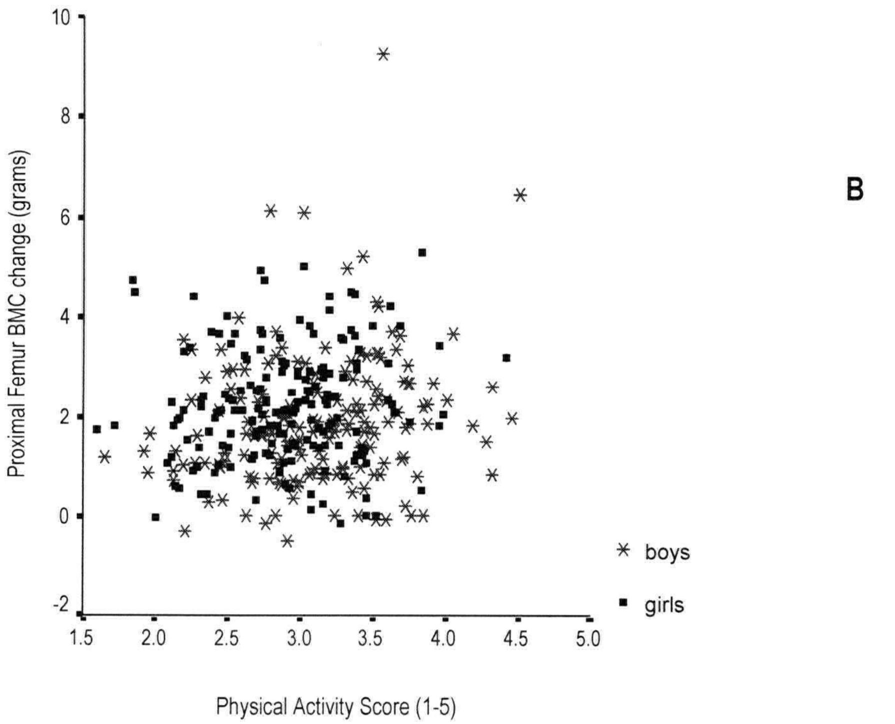
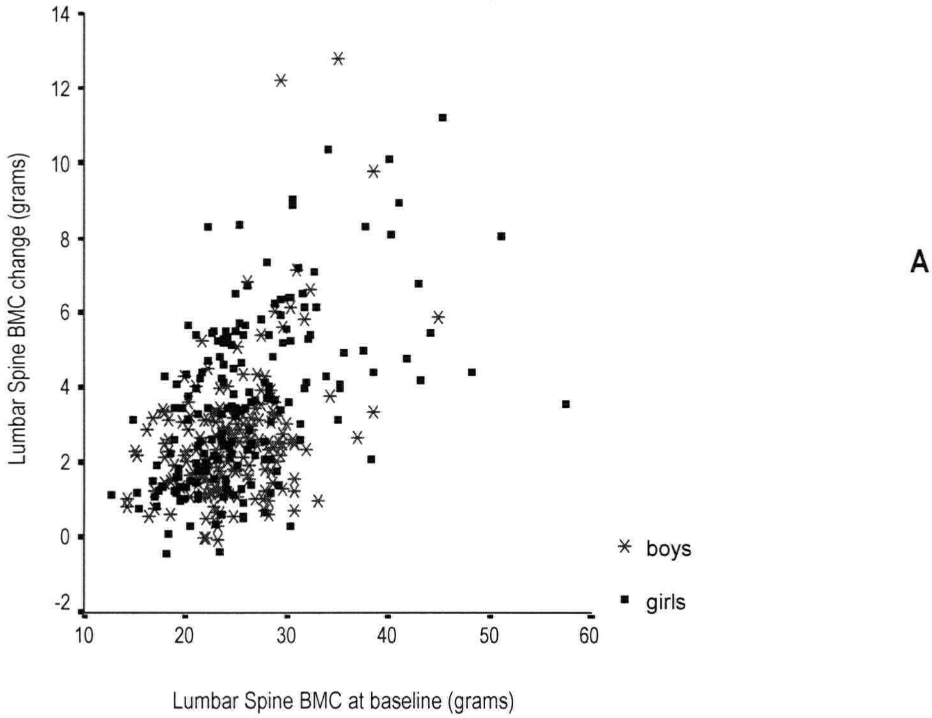


Figure 15: Physical activity score vs lumbar spine BMC 8-month change (A) and physical activity score vs proximal femur BMC 8-month change (B) in girls (n=179) and boys (n=180) (Fall 1999 – June 2000).



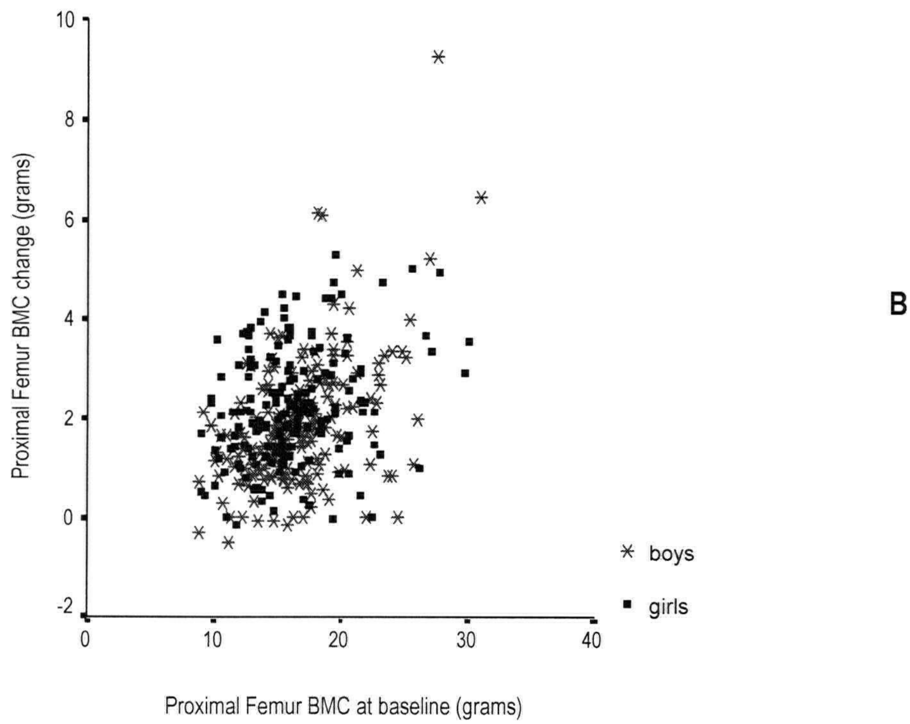
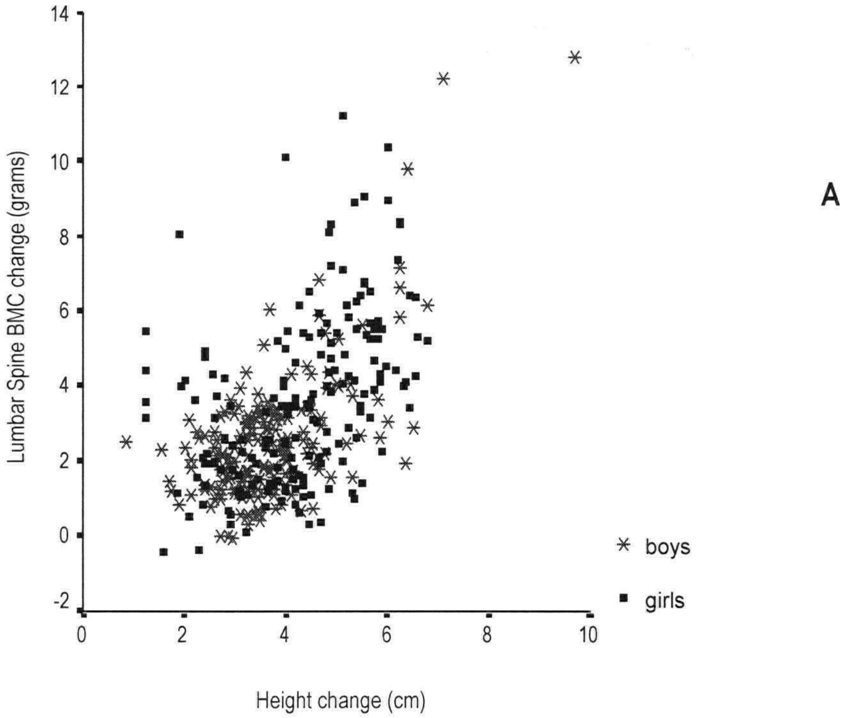


Figure 16: Baseline BMC vs lumbar spine BMC 8-month change (A) and baseline BMC vs proximal femur BMC 8-month change (B) in girls (n=179) and boys (n=180) (Fall 1999 – June 2000).



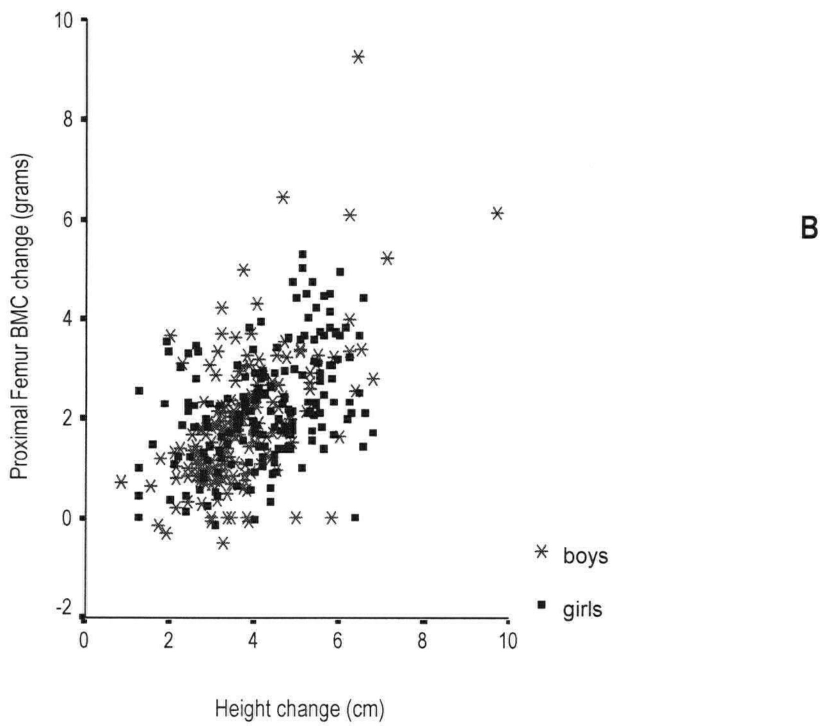
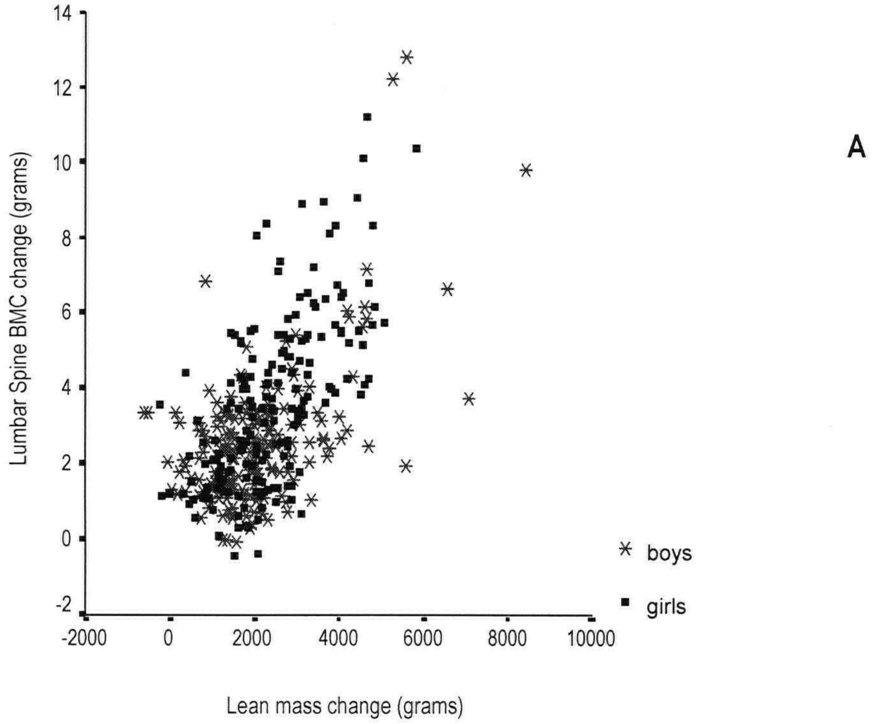


Figure 17: 8-month change in height vs lumbar spine BMC 8-month change (A) and 8-month change in height vs proximal femur BMC 8-month change (B) in girls (n=179) and boys (n=180) (Fall 1999 – June 2000).



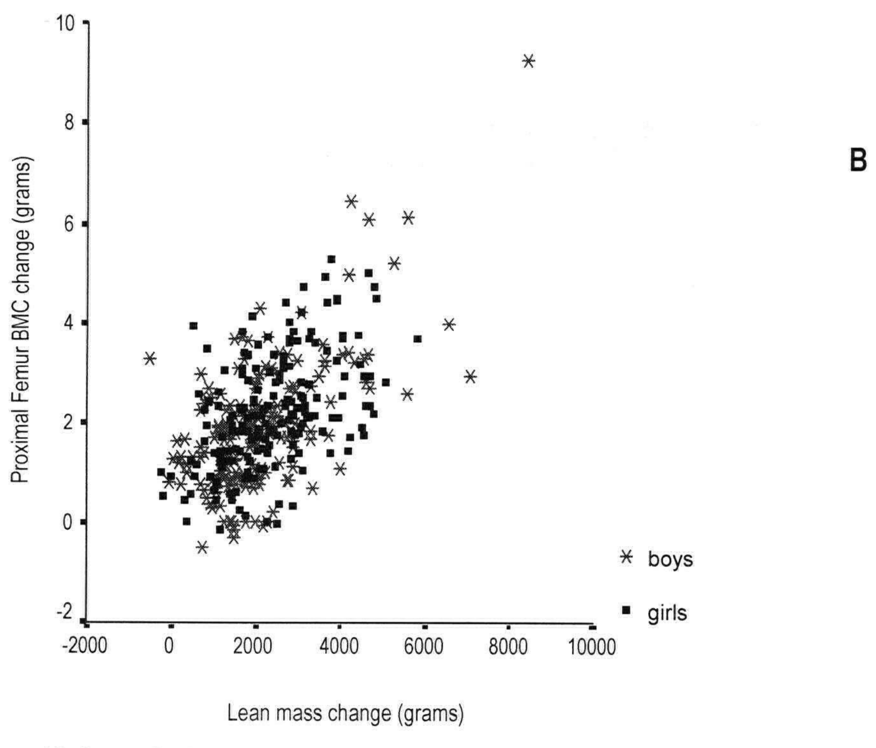


Figure 18: 8-month change in lean mass vs lumbar spine BMC 8-month change (A) and 8-month change in lean mass change vs proximal femur BMC 8-month change (B) in girls (n=179) and boys (n=180) (Fall 1999 - June 2000).

6 - Part I: Lifestyle Risk Factors for Osteoporosis in Asian and Caucasian Girls

6.1 *Introduction*

Osteoporosis-related hip fractures are an increasingly serious health care problem. Although annual fracture rates are escalating in part because of the greater number of elderly people in the community, age-adjusted rates are also rising exponentially (260). Thus, public health policy must address preventive strategies such as optimizing bone health in childhood.

It has been postulated that the predisposition for low bone mass and subsequent osteoporosis may be firmly entrenched in the lifestyle habits assumed during childhood (15). In fact, osteoporosis may be better classified as a disease of inadequate bone accrual than excessive bone loss (15). The childhood years are a crucial time of accelerated bone mineral accrual (261). It has recently been shown that girls will gain as much bone mass in the two years of peak bone mineral accrual as women lose, on average, in 20 years of postmenopausal life (11). Low levels of physical activity (11) and low calcium intake (178), may pose risk for inadequate bone mineral accrual during childhood. However, whether these factors influence bone mass during pre- or early-puberty has received little attention.

Heredity is an important determinant of areal bone mineral density (aBMD) (17), and areal bone mineral density (aBMD) remains the best predictor of osteoporotic fracture (19). It is well established, for example, that Caucasians and Asians have greater fracture rates than Blacks (166).

The relationship between ethnicity and aBMD in children has been less studied. In two studies, black youths had greater bone mass than Caucasian and non-Black youths (89,171). As well, young prepubertal (8.9 years old) Canadian-Caucasian boys had significantly higher femoral neck aBMD (21) when compared to their Asian peers in British Columbia. In this cohort, bone parameters were quite similar between Asian and Caucasian prepubertal girls. Asian boys and girls residing in the United States had similar aBMD at several sites in pre- and early puberty (Tanner Stages 1-2) as compared to Caucasian children of the same sex and maturity (175).

With respect to childhood physical inactivity and bone mass, we have previously reported that Asian children had lower activity levels than Caucasian children living in the same city (21). Physical activity is, clearly, an important determinant of bone accrual during peak growth in children (11), but whether the contribution of loaded physical activity to aBMD varies from pre- to early-puberty has not been evaluated.

Although results have been generally positive, reports from intervention studies of dietary calcium and bone mass in children vary in the magnitude of the reported bone response (262). Some of the many reasons that may contribute to this are: the length of the intervention, the type of calcium supplementation given, and authors reporting results by chronological age rather than maturity status of the child.

Finally, and most importantly, pubertal stage is a crucial variable in pediatric biology. It is the major determinant of change in aBMD during the growing years (11). Thus, pediatric studies must distinguish lifestyle and heritable influences at different maturational periods rather than evaluating children as a homogeneous group, or by chronological age. The relative contribution of ethnicity, physical activity and dietary calcium intake to bone mass in children at different stages of maturity has not been clearly identified.

Therefore, the primary purpose of this cross-sectional study was to compare bone mineral content (BMC) and aBMD in Asian and Caucasian girls at two stages of maturity (Tanner stages 1 and 2). Our secondary purpose was to compare lifestyle factors associated with BMC and aBMD in these Tanner 1 and Tanner 2 girls. Finally, we aimed to identify the relative contributions of body size, lean mass and fat mass, calcium, physical activity and ethnicity to aBMD at the femoral neck.

6.2 Methods

Detailed methods are provided in Chapter 4 of this thesis (Methods), and are briefly summarized here.

6.2.1 ***Subjects***

This study is the baseline report of a bone loading exercise intervention in a multi-ethnic group of 383 children that included 191 girls aged 9 to 12 years. Girls were classified as Asian if both parents were born in: Hong Kong or China (77%), Taiwan (14%), Philippines (7%), or Japan (2%). Girls were considered Caucasian if both parents were born in North America or Europe. Line drawings of Tanner breast ratings (1-5) were used to identify the maturity level of each girl (101). One Caucasian girl was excluded from this analysis because she had Down's Syndrome. We included 56 Asian (26 Tanner stage 1 (T1) and 30 T2), and 75 Caucasian (30 T1 and 45 T2) girls in this study.

6.2.2 ***Measurements***

Bone area (BA, cm^2), BMC (grams) and aBMD (grams/cm^2) were assessed at the total body (TB), lumbar spine (LS) and proximal femur (PF) and its femoral neck (FN) and trochanteric (TR) subregions using a Hologic QDR 4500W bone densitometer (DXA) as previously described (Chapter 4, Methods). Total lean mass and fat mass (grams, both) were obtained from total body DXA scans.

Sitting height and standing height (stretch stature, both) were measured to the nearest mm using a customized wall-mounted stadiometer. Leg length was calculated as the difference between standing height and sitting height. Segmental lengths are defined as sitting height for upper body and leg length for lower body. We assessed body weight with an electronic scale to the nearest 0.1 kg and the mean of two measures was used for analysis.

We administered a food frequency questionnaire (FFQ) to estimate dietary intake of calcium. A bilingual (Chinese-English) trained measurer assisted Chinese children. Questionnaires were analyzed by calculating a daily calcium intake (mg) based on the calcium content of food items.

General physical activity was determined by a modified version of the Physical Activity Questionnaire for Children (PAQ-C) (208), which assesses daily activity in the moderate to vigorous range over the previous seven days. Final *general physical activity* scores were calculated as an average of the PAQ-C items, in a continuous range between 1 (low activity) and 5 (high activity). The questionnaire was modified to include (1) a separate estimate of time (hr/wk) spent in common sports and activities designated as loaded (impact > walking) (*loaded physical activity)*, and (2) an indication of the number of nights per week the child participated in organized sports activities or activity lessons (*sport nights*). Measurers facilitated item-by-item completion of the questionnaire.

6.3 Statistical Approach

To identify differences between Asian and Caucasian girls we utilized independent t-tests and compared independent group means for age, body size/composition (bone area (BA), height, sitting height, leg length, weight, fat mass and lean mass) and lifestyle factors (general physical activity score, loaded physical activity, sport nights, and calcium intake) within Tanner Stage 1 and Tanner Stage 2. We used ANCOVA to compare BMC and aBMD within Tanner Stages.[1] For BMC, lean mass, fat mass, BA (TB, PF, LS) and segment lengths (PF, LS) served as covariates. For aBMD, lean mass, fat mass, and segment lengths (leg length (PF), sitting height (LS), or height (TB)) were entered as covariates.

We used hierarchical regression to estimate the contribution of size and body composition variables, maturity, lifestyle, and ethnicity to absolute values of femoral neck aBMD for the pooled sample (with variables entered in the following order: (1) leg length, (2) lean mass, (3) fat mass, (4) Tanner stage, (5) calcium, (6) loaded physical activity, (7) ethnicity). The order of variable input into the hierarchical model was determined through established relationships between body size and body composition and moderate, significant correlations ($r=0.37–0.54$, $P<0.01$) between leg length, lean mass and fat mass and FN aBMD in this cohort of girls. Maturity was accounted for in the next step. Calcium and physical activity were entered to account for the influence of lifestyle differences between Asian and Caucasian girls. Ethnicity was entered last to examine the unique relationship between this variable and FN aBMD once the influence of all previous variables had been controlled.

Data were analyzed using SPSS statistical software, Windows version 8.0 (SPSS Inc., Chicago, IL, 1993). Descriptive data are presented as mean (SD). Significance was set at $P< 0.05$ for all statistical analyses.

[1] Comparisons were made *within* Tanner stages only due to a tendency for high variability in some variables, and the assumption of homogeneity of regression slopes for covariates on dependent variables was not met (notably for lean mass and bone area on BMC) when multiple maturity groups were considered together.

6.4 *Results*

6.4.1 ***Ethnicity and Residency***

Of the 75 Caucasian girls, 71 were born in Canada, 1 in Finland, 1 in England, and 2 in the United States. Of the 56 Asian girls, 12 were born in Canada, and the remaining 44 had averaged 4.9 (2.4) years in Canada. Table 13 summarizes body composition, lifestyle, and bone parameters for Asian and Caucasian girls in Tanner Stages 1 and 2.

6.4.2 ***Tanner Stage 1 Comparisons***

Within Tanner stage 1 (T1), the Caucasian girls were leaner and had longer legs ($P<0.05$ for both). All other size and body composition variables did not differ significantly between ethnic groups within T1. Tanner 1 Caucasian girls consumed, on average, 35% more calcium than the same maturity Asian girls ($P<0.001$), were generally more active ($P<0.05$), and spent 9% more time in loaded physical activity each week (NS). Forty percent of Asian girls and 66% of Caucasian girls reported being involved in sports or physical activities outside of school. Within T1 girls, adjusted TB and FN BMC were greater in the Caucasian girls ($P<0.05$, eta squared = 0.087 (TB) and 0.094 (FN)). Other mean values for bone parameters did not differ between ethnicities within T1 girls at any site, except that Caucasian girls had higher mean BA for the total proximal femur ($P<0.05$, eta squared=0.087).

6.4.3 ***Tanner Stage 2 Comparisons***

As was the case in T1 girls, Caucasian girls in Tanner stage 2 (T2) had significantly longer legs than Asian girls at the same maturity level ($P<0.05$). Other body size and composition variables did not differ between ethnicities within T2. On average, Caucasian girls consumed 41% more calcium than Asian girls ($P<0.001$). They also participated significantly more often in extracurricular sports nights each week ($P<0.05$) and spent 44% more time in loaded physical activity ($P<0.01$) as compared to the Asian girls. The general physical activity scores were similar between these groups (2.72 for Asians *vs.* 2.88 for Caucasians). Twelve of the Asian T2 girls participated in extracurricular sports and physical activities, whereas thirty-six Caucasian T2 girls reported participation in extracurricular sports and physical activities.

After correcting BMC for BA (TB, PF, LS), lean mass, fat mass, and segment lengths (PF, LS), and aBMD for lean mass, fat mass, and segment lengths, T2 Caucasian girls had significantly higher values for the TB ($P<0.05$, eta squared=0.072 (BMC) and 0.091 (aBMD)), PF ($P<0.01$, eta squared=0.122 (BMC) and 0.120 (aBMD)), and FN ($P<0.005$, eta squared=0.133 (BMC) and 0.139 (aBMD)) than T2 Asian girls. BMC and aBMD at the LS did not differ significantly between ethnicities, nor did bone area at any site. The ethnic difference in FN aBMD at the two maturity stages is provided (Figure 19).

6.4.4 ***Regression Analysis***

To examine the variance in FN aBMD explained by ethnicity, the following independent variables were entered sequentially into hierarchical regression; (1) leg length, (2) lean mass, (3) fat mass, (4) Tanner stage, (5) dietary calcium intake, (6) loaded physical activity, (7) ethnicity. Results are presented (Table 14). The final model explained 40.2% (final R Square) of the variance in FN aBMD. In the second step, lean mass contributed significantly to the model, accounting for 15.9% ($P<0.01$) of the variance in femoral neck aBMD, with fat mass contributing 2.8% ($P<0.05$), and ethnicity accounting for an additional 3.6% ($P<0.01$). Lean mass, fat mass and ethnicity were significant in the final model. All standardized residuals were less than 3 SD from the predicted values (98% of residuals were less than 2 SD.).

Table 13: Body size, body composition, lifestyle factors, and bone parameters for Tanner 1 and 2 Asian and Caucasian girls. Results are mean (SD). TB = total-body, LS = lumbar spine, PF = proximal femur, FN = femoral neck, TR = trochanter.

	Tanner I		**Tanner 2**	
	Asian	Caucasian	Asian	Caucasian
N	26	30	30	45
Age (yr)	10.1(0.5)	10.1 (0.6)	10.5 (0.7)	10.4 (0.6)
Height (cm)	138.0 (5.0)	140.6 (7.3)	142.5 (6.8)	144.4 (7.2)
Weight (kg)[np]	30.0 (4.4)	32.5 (5.8)	37.9 (7.2)	40.2 (9.2)
Leg length (cm)	64.8 (3.3) [a]	67.1 (4.4)	66.1 (3.41)[c]	68.3 (4.1)
Sitting height (cm)	73.2 (2.4)	73.7 (3.4)	76.3 (4.1)	76.1 (3.7)
Lean (g)[np]	22217 (2383) [a]	23927 (3088)	25938 (3562)	27340 (4269)
Fat (g)[np]	6774 (2568)	7544 (3113)	10737 (4219)	11673 (5472)
Calcium (mg/day)*	608 (255)[b]	937 (342)	579 (339)[e]	1030 (483)
General Activity score	2.7 (0.5)[a]	3.0 (0.6)	2.7 (0.6)	2.9 (0.6)
Sport nights /wk	1.0 (1.3)	1.5 (1.7)	1.3 (2.0)[d]	2.5 (1.8)
Loaded Physical Activity time (hr/wk)[np]	4.2 (4.0)	4.6 (3.5)	3.3 (2.7)[d]	5.9 (4.1)
TB area (cm^2)	1140 (124)	1198 (148)	1294 (158)	1359 (184)
TB BMC (g)	957 (135)[a]	1029 (151)	1083 (183)[c]	1190 (209)
TB BMD (g/cm^2)	0.84 (0.05)	0.86 (0.04)	0.83 (0.05)[d]	0.87 (0.05)
LS area (cm^2)	36.4 (2.7)	37.6 (4.3)	40.4 (4.9)	41.1 (5.0)
LS BMC (g)	22.5 (3.5)	23.6 (4.0)	26.6 (6.0)	27.2 (5.9)
LS BMD (g/cm^2)	0.62 (0.07)	0.62 (0.05)	0.66 (0.07)	0.66 (0.08)
PF area (cm^2)	21.1 (2.1)[a]	22.5 (2.6)	23.7 (4.5)	24.3 (3.0)
PF BMC (g)	13.4 (2.1)	15.1 (2.6)	15.3 (2.9)[d]	17.8 (3.6)
PF BMD (g/cm^2)	0.63 (0.07)	0.67 (0.08)	0.66 (0.09)[d]	0.73 (0.08)
FN area (cm^2)	3.9 (0.3)	4.1 (0.4)	4.3 (1.0)	4.3 (0.3)
FN BMC (g)	2.3 (0.3)[a]	2.6 (0.4)	2.6 (0.5)[d]	3.0 (0.5)
FN BMD (g/cm^2)	0.60 (0.06)	0.64 (0.07)	0.62 (0.09)[d]	0.69 (0.07)
TR area (cm^2)	5.1 (1.1)	5.3 (0.9)	5.9 (1.3)	6.3 (1.3)
TR BMC (g)	2.7 (0.7)	2.7 (0.6)	3.2 (1.0)	3.6 (1.1)
TR BMD (g/cm^2)	0.5 (0.07)	0.51 (0.06)	0.54 (0.08)	0.57 (0.07)

[np] Variable tended to be slightly skewed (skewness statistic>2), and I ran both parametric and nonparametric statistics. The significance levels were the same, regardless of the test.
*Equal variances not assumed (separate variance t-test used).
[a] Significantly different from Tanner 1 Caucasian girls ($P < 0.05$) ; [b] Significantly different from Tanner 1 Caucasian girls ($P < 0.001$); [c] Significantly different from Tanner 2 Caucasian girls ($P < 0.05$); [d] Significantly different from Tanner 2 Caucasian girls ($P < 0.01$); [e] Significantly different from Tanner 2 Caucasian girls ($P < 0.001$).
In statistical analyses: TB BMC: covariates = bone area, lean, fat; LS, PF, TR, FN BMC: covariates= segment length, bone area, lean, fat; TB, LS, PF, TR FN, aBMD: covariates = segment length (leg length (PF, FN, TR) or sitting height (LS)) or height, lean, fat.

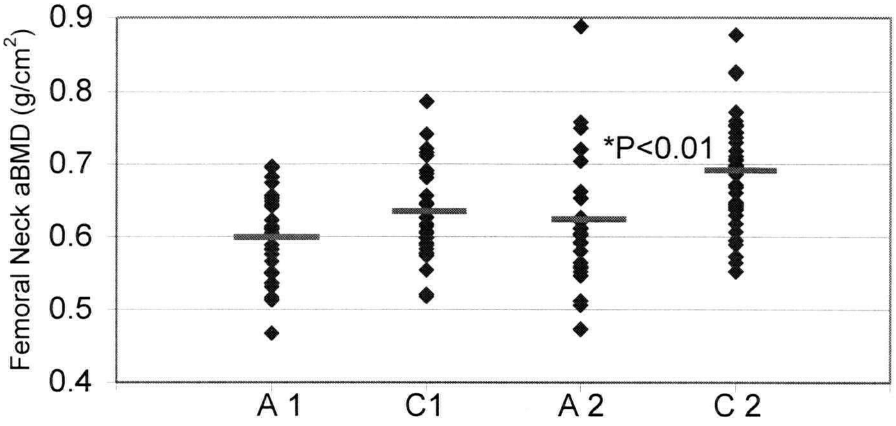


Figure 19: Femoral Neck aBMD values by group (A 1 = Asian Tanner Stage 1, C 1 = Caucasian Tanner Stage I, A 2 = Asian Tanner Stage 2, C 2 = Caucasian Tanner Stage II). ▬ represents mean value for the group. *P<0.01: significant difference between A2 and C2.

Table 14: Results of hierarchical regression model for femoral neck aBMD for Asian and Caucasian girls (n=131). Variables significant in the final model are underlined.

Independent Variable	R Square Change	Significance of F Change	Final Unstandardized Coefficients	Final Standardized Coefficients	Significance in Final Model	Final Model R Square
(Constant)			0.457		<0.001	0.402
Leg Length	0.140	0.01	-0.0004916	-0.025	0.81	
Lean Mass	0.159	0.01	0.00000723	0.363	**0.01**	
Fat Mass	0.028	0.02	0.00000377	0.221	**0.03**	
Tanner Stage	<0.001	0.82	-0.003754	-0.023	0.77	
Calcium Intake	0.027	0.03	0.00001085	0.058	0.47	
Load Time	0.013	0.12	0.001808	0.086	0.24	
Ethnicity*	0.036	0.01	-0.03581	-0.223	**0.01**	

*Ethnicity coded as a dummy variable, where Asian =1 and Caucasian = 0.

6.5 *Discussion*

The skeleton undergoes a critical period of development around puberty, but there have been few studies that examine to what extent lifestyle factors (e.g., physical activity, dietary calcium intake) and ethnicity contribute to bone mass at different stages of maturity (22,175). We examined the specific contribution of these lifestyle factors and of ethnicity to bone mineral in a larger sample of Tanner 1 and Tanner 2 Asian and Caucasian girls than has previously been studied.

Our novel observations were that: (i) aBMD differences between Asian and Caucasian girls were present at Tanner 2, but not Tanner 1; (ii) loaded physical activity differences between ethnicities mirrored the maturity-related aBMD findings, and (iii) the discrepancy in dietary calcium intake differences between ethnicities was greater for Tanner 2 than Tanner 1 girls. Each of these findings is discussed below.

6.5.1 *Maturity-Related Ethnic Differences in Bone Mineral Not Explained By Body Size*

In contrast to the previous study from our research group that examined an independent sample of eight and nine-year-old Asian and Caucasian children in British Columbia (21), we observed significantly greater BMC for the total body and femoral neck in ten-year-old Caucasian Tanner 1 girls as compared to their same age and maturity Asian counterparts. This contrast in results is likely related to characteristics of the independent cohorts. The previous study examined a combined group of boys and girls (34% Asian), while 46% of the Tanner 1 girls in the current study were Asian. Most significant is the difference in the ages between cohorts (8.9 (0.8) yr vs 10.1(0.5) yr), suggesting that the girls in the previous sample were 1-2 years less mature. In the current study, other bone parameters, notably aBMD at all sites, displayed no difference by ethnicity. The observed differences in BMC may indicate a trend towards higher bone mineral in the Caucasian girls, which might be further realized with advancing maturity. In fact, we noted greater ethnic differences in bone parameters across several sites in the Tanner Stage 2 group. The Caucasian girls had, on average, nine percent greater BMC and aBMD (corrected for soft tissue and size) than Asian girls at the total body, total proximal femur, and femoral neck.

In adolescents, reports of interracial variations in bone parameters measured on a projectional instrument such as DXA have largely attributed differences to discrepancies in body or bone size (175). However, in this sample of Asian and Caucasian girls living in close geographic proximity, there was no significant difference in height, although Caucasian girls had significantly longer legs. The T1 Caucasian girls also had significantly greater lean mass; however, aBMD values did not differ significantly from Asian girls when lean mass was controlled. Similarly, we are confident that the highly significant ethnic differences for bone parameters observed in the T2 girls were not a function of body size or composition, as bone differences remained after height, leg length, and tissue mass effects were controlled. The comparable bone areas for all T2 girls supports a real, as opposed to a size-artifact related, difference in bone mass between ethnic groups.

Only one other study has compared BMC, aBMD and calculated bone mineral apparent density (BMAD) at the FN between Asian and Caucasian girls in 2 maturity categories (175). However, due to the small sample size, Tanner Stages 1 and 2 (n=5), and Tanner Stages 3 and 4, respectively, were collapsed for analyses. FN BMC was greater for Caucasian as compared to Asian girls in both the Tanner 1-2 and 3-4 groups. As no difference was observed between ethnic groups for FN aBMD or FN BMAD these findings may indicate a size effect.

We did not observe an ethnic difference in bone mineral at the lumbar spine in either maturity group and this is consistent with other studies of pre- and peri-pubertal children (21,174,175). It has previously been shown that mechanical loading or unloading may contribute to a region-specific response in bone mineral (263). Bone mass at the proximal femur reflects loading patterns to a greater extent, whereas bone mass at the lumbar spine represents the influence of endocrine factors (5). Differences in habitual loading patterns between Asian and Caucasian girls in the present study may contribute to the greater differences between ethnicities at the proximal femur.

6.5.2 *Maturity Related Ethnic Differences in Physical Activity*

While it is well accepted that girls become less active as they approach puberty (249), we observed no difference in general physical activity between girls at Tanner stages 1 and 2. There was a slight ethnic difference in general physical activity in Tanner 1 girls, however no difference was noted between Tanner 2 girls. In contrast, we did note a difference in the amount of *loaded* physical activity, a disparity that reached significance ($P<0.01$) in Tanner 2 girls. Tanner 2 Caucasian girls engaged in almost twice as much loaded physical activity as the same maturity, height and weight Asian girls. Furthermore, the number of times per week the Caucasian T2 girls participated in extracurricular sport or activity lessons exceeded that of the Asian T2 girls (2.5 *vs* 1.3 times per week, $p<0.01$). In general, Caucasian girls participated in extracurricular dance and soccer, whereas Asian girls were active in swimming lessons outside of school. These findings agree with a report that found Asians and Pacific Islanders residing in California were less likely to compete on sports teams and had a lower frequency of participation in vigorous exercise than their Anglo-Saxon peers (180).

The positive relationship between physical activity and bone mineral density during childhood is becoming more clearly established as prospective evidence becomes available (234). Loaded activities that involve new patterns of bending or higher than usual forces elicit a more profound skeletal response than "everyday" childhood activities (264). Bone responds by increasing formation with a subsequent increase in bone mass, size and moment of inertia and thus, bone strength (265). This response was demonstrated with exercise in both young animals (266) and more recently in intervention studies with children (23,234).

There also appears to be a link between the bone response to mechanical loading and age or maturity at participation in loaded activity. In 7-17 year old female racquet sport athletes, heavy training was associated with definitively greater dominant arm-to-nondominant arm differences in aBMD as compared to healthy controls

(~4 - 12%, $p<0.05 - p<0.001$, depending on Tanner Stage). This finding was consistent across measurement sites in girls who had surpassed Tanner Stage 2 (22). Further, only the humeral shaft (the site experiencing the greatest loads in racquet sports) in Tanner Stage 2 athletes showed evidence of greater bone mineral accrual. It is of interest then, that the greatest disparity in TB, PF, and FN BMC and aBMD between ethnic groups was not evident in the present study until T2. T2 Asian girls were loading 3.3 hr/wk as compared to 5.9 hr/wk for the T2 Caucasian girls.

The idea of a period during growth when bone is most responsive to loading activity is controversial, as there is evidence that highlights both the prepubertal years and peri-pubertal years (261). To date, no prospective study has investigated the bone response to exercise simultaneously in pre- and early pubertal girls. However, there are a number of advantages that define early and /or peripuberty as a time for greater osteotrophic response to exercise. During early puberty (Tanner Stage 2 and 3), BMC accrual velocities accelerate (11), bone mineral is laid down on both the periosteal and endosteal surfaces (131), and the amplitude of bone-enhancing systemic hormones is increased (123). We acknowledge that the definitive role of exercise at different stages of maturity can be delineated only with controlled prospective intervention trials. Thus, we are aware of the limitations of the cross-sectional design of the present study. However, it seems plausible that physical loading of the skeleton may have a more pronounced effect in the bone-enhancing environment of puberty, and that inactivity, or under-loading, may have a more pronounced negative effect.

6.5.3 *Maturity-Related Ethnic Differences in Dietary Calcium Intake*

Calcium intakes differed by 35% and 41% between ethnicities for the T1 and T2 girls, respectively. Chinese children have traditionally had intakes below 700 mg/day (202). Whether Asian children require the same amount of calcium in their diets as Caucasian children is unclear. Percent calcium retention differed between North American Caucasian and Chinese children in separate reports (267,268). Prepubertal Chinese children had an average calcium retention of $63 \pm 11\%$ while maintaining diets of approximately 300 mg/day (268), while prepubertal Caucasian girls retained 28 $\pm$8% from a diet containing 900 mg calcium/day (267).

Generally, the early pubertal period is associated with a higher percent dietary calcium absorption than the pre- or late pubertal stages (267). In our study, the T2 Caucasian girls, who had the highest average calcium intake of all the groups (1011 mg), may enjoy a calcium advantage over their T2 Asian peers. In contrast, T2 Asian girls reported mean calcium intakes that were the lowest of all groups. The mean calcium intake for the T1 Caucasian group was 28% under the current dietary reference intake (DRI) of 1300 mg/day (186). Calcium intake for the T1 Asian girls was 53% below the DRI.

There is recent evidence to support the link between genetic polymorphisms on the Vitamin D receptor (VDR) gene and aBMD. The VDR gene may have different frequencies depending on race, and may influence skeletal retention of calcium in children (187). The benefit of increasing dietary calcium intake (+300 mg calcium/day over 18 months) was demonstrated in Chinese children with low calcium intakes (202).

6.5.4 ***Ethnic Contribution to Areal Bone Mineral Density***

The determinants of bone mineral in the present study are similar to those that have been consistently reported for children, with lean mass explaining a significant proportion (16%) of the variance in FN aBMD. Although Tanner stage (1-5) has emerged as a significant predictor of aBMD in previous studies (22,175), the girls in our study were relatively close maturationally. Shared variance between maturity, lean mass and fat mass may undermine the role of maturity as an independent predictor of aBMD. Further, the Asian Tanner 1 and Asian Tanner 2 girls had 6.6% and 3.2% lower mean aBMD, respectively, than the least mature Caucasian girls. This discrepancy would alter the relationship between maturity and FN aBMD. Unique to this study was the additional contribution of ethnicity to FN aBMD. In the pooled group of Tanner Stage 1 and 2 Asian and Caucasian girls, lean mass, fat mass and ethnicity together accounted for 40% of the variance in aBMD at the femoral neck. Ethnicity independently accounted for nearly 4% of the variance in aBMD at this site, after body size/composition and lifestyle influences were accounted for. This suggests that a significant effect for ethnicity is present at the femoral neck.

6.6 Summary

In summary, the present study demonstrated a notable disparity in loaded physical activity and dietary calcium between early pubertal Asian and Caucasian girls. Once body size and composition were controlled for, there was a significant difference for TB, PF, and FN aBMD in the most mature group only. These findings suggest that early pubertal Asian girls in British Columbia may be at a disadvantage for optimal bone health. Further prospective work investigating the role of maturation in the bone mineral response to an exercise intervention is warranted.

7 - Part II: A School-Based Loading Intervention Augments Bone Mineral Accrual in Early-, But Not Pre-Pubertal Girls

7.1 *Introduction*

As osteoporosis is reaching epidemic proportions in North America (1), evidence is accumulating that highlights childhood as an opportune time for positive lifestyle habits to reduce the risk of the disease (126). The amount of bone gained during the two years of peak accrual at adolescence approximates the quantity of bone lost during adulthood (11). Weight-bearing or high impact physical activity during growth increases the rate and magnitude of bone accrual (11), and is related to increases in bone size, bone mineral content (BMC), and areal bone mineral density (aBMD) (14,22). Thus, childhood is a critical time to intervene with lifestyle strategies that may prevent osteoporosis-related fractures in later life.

The few previous exercise interventions performed in prepubertal and peri-pubertal children have shown a positive effect of loading activities (running and jumping) (23,92,226,227,234). The two previous prospective exercise trials within post-menarcheal cohorts showed no bone mineral accrual advantage in the exercise groups (239,240). Retrospective studies also suggest that premenarche is a time of greater osteotrophic response to exercise than postmenarche (223).

However, the proposed narrow 'window of opportunity' before or during puberty when bone is most responsive to exercise is not yet clearly defined. Further definition of this optimal time for exercise intervention is critical so that interventions can target the most physiologically responsive group. Only one exercise study has intervened simultaneously in groups at different levels of maturity (23). This study had a relatively small number of subjects at pre- and early puberty at baseline, and these groups were pooled and classified as premenarcheal for analysis. Premenarcheal (aged 11 yr) girls in the exercise group had significantly greater changes in lumbar spine and femoral neck BMC as compared to premenarcheal controls, while no exercise advantage was evident in postmenarcheal (13 yr) girls. To date, no study has examined the bone response to a single exercise intervention in groups of prepubertal and early pubertal girls.

The objective of this study was to investigate the changes in BMC, aBMD and vBMD in prepubertal and early pubertal girls who participated in a 7-month, school-based bone loading exercise intervention, and to compare changes to those of maturity-matched controls.

7.2 *Methods*

Detailed methods for subject recruitment, classification and measurement are provided in Chapter 4 (Methods), and are briefly summarized here.

7.2.1 ***Design and Exercise Intervention***

This study was a randomized, prospective, school-based intervention, and included 7 intervention and 7 control elementary schools. Schools were randomly assigned to either control or intervention groups. Baseline measurements were made during the fall of the school year (September – October), the intervention was completed from late October to late May (7 months in duration), and final measurements were made at the end of the school year (June). The school-based exercise intervention program is described in detail in Section 4.4.1 (Methods – Exercise Intervention: Year 1 Exercise Intervention Program). A high impact, circuit-training program composed of jumping activities was implemented 3 times per week, for approximately 10 minutes a session.

7.2.2 *Subjects*

We recruited principals, teachers, and students from Richmond, a multi-ethnic school district outside of Vancouver, Canada to participate in this study. A total of 383 girls and boys from grades 4, 5, and 6 (~9 – 12 years of age), and 33 teachers participated in the study. The current analysis focuses on the 191 female participants. Two girls were excluded from this analysis based on conditions that could affect normal physical activity or bone development (Down's syndrome and polycystic kidney removal). Two other girls were excluded from analysis because of advanced maturity (Tanner stage 5). All other girls were healthy and none had medical conditions or took medications known to influence bone mineral or bone metabolism. Line drawings of Tanner breast ratings (1-5) were used to identify the maturity level of each girl (101). In this study, girls who were Tanner breast stage 1 at baseline were classified as prepubertal (PRE), and Tanner breast stage 2 and 3 were classified as early pubertal (EARLY). The girls were interviewed by one female researcher at follow-up to ascertain menarcheal status and approximate date of menarche, if applicable.

7.2.3 *Bone Densitometry*

Bone area (BA, cm^2), BMC (grams) and aBMD (grams/cm^2) were assessed for the total body (TB), lumbar spine (LS), and proximal femur (PF), and its femoral neck (FN) and trochanteric (TR) subregions using a Hologic QDR 4500W bone densitometer (DXA). Volumetric bone mineral density (vBMD) was calculated for the femoral neck, assuming this region approximates a cylinder (vBMD = $(4BMC*(\text{height of FN box}))/(\pi * (\text{FN area})^2)$) (91). Total body lean mass and fat mass (grams, both) were obtained from total body DXA scans. A spine and anthropomorphic phantom were scanned daily to maintain quality assurance of the QDR 4500.

7.2.4 *Height, Weight, and Jump Performance*

We measured sitting height and standing height (stretch stature both) to the nearest mm using a customized wall-mounted stadiometer. The mean of two measures was used for analysis. Leg length was calculated as the difference between standing height and sitting height. We assessed body weight with an electronic scale to the nearest 0.1 kg and used the mean of two measures for analysis. We measured long and vertical jump performances as outlined (Chapter 4 – Methods, Section 4.8 (Physical Performance)).

7.2.5 *Questionnaires*

The girls completed a food frequency questionnaire (FFQ) to estimate dietary intake of calcium (179). Questionnaires were analyzed by calculating a daily calcium intake (mg) based on the calcium content of food items. The questionnaire was administered at baseline (fall), during the winter, and at final measurement (spring), and the average calcium intake is reported.

General physical activity was determined by a modified version of the Physical Activity Questionnaire for Children (PAQ-C) (208), as described in section 4.9.2 (Methods – Questionnaires: Physical Activity). This questionnaire was administered at baseline, during the winter, and at final measurement. Values reported are the average of the 3 administrations.

7.2.6 *Statistical Analyses*

As the rates of linear growth and bone mineral accrual are known to differ between prepubertal and early pubertal girls, we compared C and I groups within each maturity category (PRE or EARLY). Independent t-tests were used to compare baseline values for all variables (age, body size/composition, BMC, aBMD, vBMD) and average yearly values for calcium and physical activity.

To assess absolute change, differences for body size (height, sitting height, leg length and weight), and composition (lean and fat mass) were compared using independent t-tests. ANCOVA was used to evaluate differences in absolute change for BMC (covariates were baseline BMC, baseline age, Δ bone area, Δ in height, and final (PRE) or baseline (EARLY) Tanner stage), aBMD (covariates were baseline aBMD, baseline age, Δ in height, and Tanner stage) and vBMD (covariates were baseline vBMD, baseline age, and Tanner stage) between C and I groups. Rationale for the use of age, change in bone area and change in height as covariates are established (88,138,269). We also used final Tanner stage as a covariate in the PRE group to adjust for differences in onset of puberty in some of the girls (Table 15). We used baseline Tanner stage as a covariate in EARLY girls due to discrepant numbers of girls in Tanner stage 2 and 3 between C and I groups. Tanner stage 3 has been associated with greater changes in height (270) and bone mineral (22,269) than Tanner stage 2. We report % change, calculated and adjusted with the above covariates (with the exception of baseline bone value).

Data were analyzed using SPSS statistical software, Windows version 8.0 (SPSS Inc., Chicago, IL, 1993). Data are presented as mean (SD), except for bone change data, presented as adjusted mean (95% confidence interval). Significance was set at $P < 0.05$ for all statistical analyses.

7.3 Results

7.3.1 *Subjects*

Six control school girls (N=6 changed schools) and 4 intervention school girls did not return for follow-up measures (N=1 lost interest, N=3 changed schools). Thus, prospective data were available for 26 PRE-C and 44 PRE-I girls; 64 EARLY-C and 43 EARLY-I girls (total N=177).

Classes at intervention schools performed the circuit intervention a mean of 57 ± 10 times over the school year. We estimated that classes could perform a maximum of 72 intervention sessions over the school year. Thus, the compliance averaged 80% across schools.

7.3.2 *Maturity, Ethnicity, Anthropometric Characteristics and Lifestyle Factors*

Baseline and final Tanner stages of all participants are summarized (Table 15). A greater percentage of the PRE-C group (62%) than the PRE-I group (41%) advanced to Tanner stage 2. More EARLY-C as compared to EARLY-I girls began the intervention at Tanner stage 3 (22% *vs* 14%). Tanner stage changes were comparable for EARLY-C and EARLY-I groups. Nine girls had reached menarche by follow-up measurement (N=1 PRE-C, N=5 EARLY-C, N=3 EARLY-I). The ethnic distribution within intervention and control groups was similar.

Baseline and change values for body size and anthropometric characteristics, and jump performance are given (Table 16). There were no significant differences at baseline or for change scores between control and intervention groups within maturity categories for height, sitting height, leg length, weight, lean mass, fat mass, or jump performance. Intervention and control groups were also similar within prepuberty and early puberty with respect to physical activity and estimated calcium intake (mg / day) (Table 16).

7.3.3 *Bone Mineral Content, Bone Mineral Density and Volumetric Bone Mineral Density*

Table 17 summarizes baseline (mean (SD)) and adjusted change (mean (95% confidence interval) values for BMC, aBMD, and vBMD across sites. There were no differences in baseline or 8-month change bone values between PRE-I and PRE-C girls at any site. Percent change for the lumbar spine and femoral neck are given (Figure 20 (a and b)).

Baseline BMC, aBMD and vBMD did not differ between EARLY-C and EARLY-I girls (Table 17). Adjusted 8-month change was significantly greater in EARLY-I girls as compared to EARLY-C girls for BMC (11.3 *vs* 9.4%, eta squared=0.048), aBMD (6.7 *vs* 5.1%, eta squared=0.042) and vBMD (3.6 *vs* 0.5%, eta squared=0.053) at the femoral neck, and BMC (16.5 *vs* 14.7%, eta squared=0.043) and aBMD (8.2 *vs* 6.5%, eta squared=0.073) at the lumbar spine (all $P<0.05$). Adjusted 8-month change in BMC at the total proximal femur followed the same trend, but did not reach significance (15.3 *vs* 14.1%, $P=0.055$). Adjusted 8-month percent change for the lumbar spine and femoral neck are shown (Figure 20 (c and d)).

Table 15: Baseline and final Tanner stage for pre- and early pubertal girls in control and intervention groups.

	Prepubertal		Early Pubertal		
Baseline – final Tanner Stage	Control	Intervention	**Baseline – final Tanner Stage**	Control	Intervention
1 –1	10 (38%)	25 (57%)	2 – 2	36 (56%)	23 (53%)
1 – 2	16 (62%)	18 (41%)	2 – 3	14 (22%)	12 (28%)
1 – 3	0	1 (2%)	2 – 4	0	2 (5%)
			3 – 3	11 (17%)	5 (12%)
			3 – 4	3 (5%)	1 (2%)
Total	26 (100%)	44 (100%)	Total	64 (100%)	43 (100%)

Table 16: Baseline and change values for descriptive variables of pre- and early pubertal girls in control and intervention groups. Mean (SD) reported.

	Prepubertal		Early Pubertal	
	Control	Intervention	Control	Intervention
N	26	44	64	43
Age (years)	10.1 (0.5)	10.0 (0.6)	10.5 (0.6)	10.4 (0.7)
Height (cm)	137.3 (6.2)	138.6 (7.6)	145.6 (6.4)	143.8 (7.7)
8-Month Change	4.1 (1.2)	3.9 (1.1)	4.7 (1.2)	4.3 (1.4)
Sitting Height (cm)	72.5 (3.2)	73.3 (3.4)	77.1 (3.5)	76.0 (4.5)
8-Month Change	1.4 (1.6)	1.7 (1.3)	2.3 (1.2)	2.2 (1.1)
Leg Length (cm)	64.8 (3.8)	65.5 (4.6)	68.6 (3.6)	67.6 (4.2)
8-Month Change	2.7 (1.5)	2.2 (1.2)	2.3 (1.2)	2.1 (1.1)
Weight (kg)	31.1 (5.6)	31.2 (6.1)	41.3 (8.3)	39.1 (8.3)
8-Month Change	2.5 (1.1)	2.3 (1.5)	4.2 (1.9)	3.6 (1.8)
Lean mass (g)	22 455 (3022)	22 943 (3160)	27 802 (3980)	26 588 (4478)
8-Month Change	1807 (920)	1807 (931)	2925 (968)	2786 (1247)
Fat mass (g)	7566 (2916)	7289 (3432)	12 046 (5341)	11 107 (4667)
8-Month Change	533 (728)	397 (993)	1038 (1554)	616 (1624)
Long Jump (cm)	125.6 (15.5)	126.0 (15.4)	123.9 (17.2)	122.7 (17.3)
8-Month Change	6.6 (11.0)	7.4 (10.9)	7.2 (10.9)	8.4 (11.8)
Vertical Jump	21.9 (5.1)	22.4 (4.8)	22.7 (4.0)	21.9 (5.9)
8-Month Change	3.1 (3.8)	2.3 (3.2)	3.4 (4.7)	3.3 (3.6)
General Physical Activity	2.80 (0.47)	2.96 (0.52)	2.90 (0.52)	2.90 (0.51)
Loaded Physical Activity	4.5 (3.2)	4.9 (3.0)	5.7 (3.7)	5.7 (4.1)
Extracurricular Sport Nights	1.2 (1.2)	1.2 (1.4)	1.7 (1.5)	1.6 (1.4)
Calcium Intake	713 (270)	854 (400)	773 (374)	797 (392)

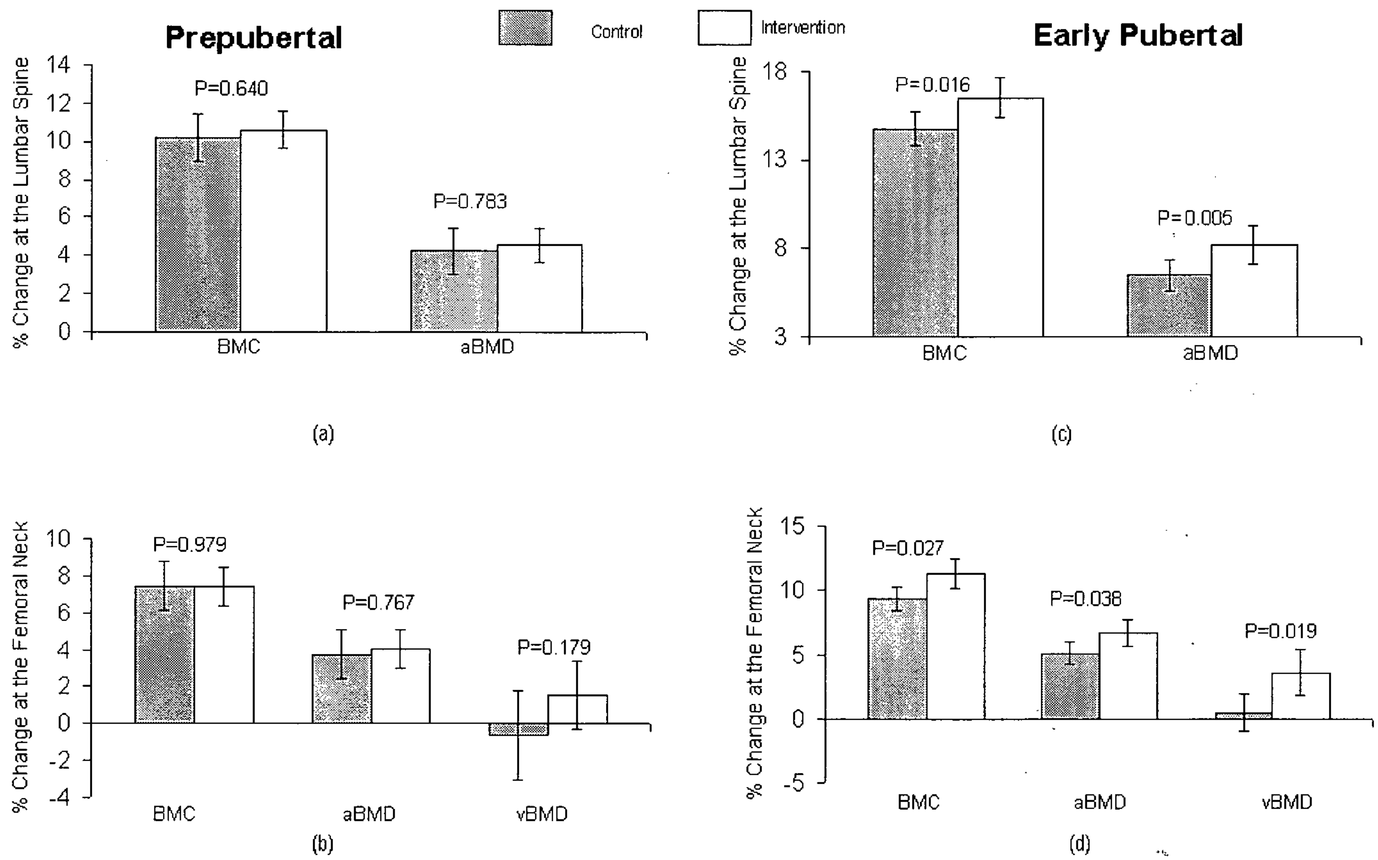
No significant differences between control and intervention groups within prepuberty or early puberty.

Table 17: Baseline and Change (Δ) values for bone mineral content (BMC), areal bone mineral density (aBMD), and volumetric BMD (vBMD, FN only) for the total body (TB), lumbar spine (LS), proximal femur (PF), femoral neck (FN), and trochanter (TR). Mean (SD) reported unless otherwise stated.

	Prepubertal				Early Pubertal			
	Control Baseline	Δ (95% CI)[a]	Intervention Baseline	Δ (95% CI)[a]	Control Baseline	Δ (95% CI)[a]	Intervention Baseline	Δ (95% CI)[a]
TB BMC	1005 (149)	91.9 (85.2–98.6)	974 (174)	92.1(87.0–97.2)	1198 (232)	145.0 (137.4-152.6)	1154 (222)	151.3(142.0-160.7)
TB aBMD	0.86 (0.04)	0.017 (.011-.023)	0.84 (0.06)	0.017(0.013-.021)	0.87 (0.07)	0.030(0.025-0.035)	0.86 (0.06)	0.033(0.026-0.039)
LS BMC	22.76 (3.65)	2.34 (2.03-2.64)	22.61 (4.55)	2.43(2.20-2.66)	28.79 (6.62)	4.18(3.92-4.44)[b]	27.00 (7.47)	4.70(4.38-5.02)
LS aBMD	0.63 (0.06)	0.027(0.019-0.034)	0.61 (0.07)	0.028(0.022-0.034)	0.69 (0.10)	0.044(0.038-0.049)[c]	0.66 (0.09)	0.057(0.050-0.064)
PF BMC	13.82 (2.60)	1.95 (1.79-2.10)	13.98 (2.64)	1.82 (1.70-1.94)	17.97 (4.23)	2.38(2.23-2.53)	16.92 (3.40)	2.62 (2.44-2.80)
PF aBMD	0.66 (0.08)	0.032(0.024-0.039)	0.65 (0.08)	0.028(0.023-0.034)	0.71 (0.10)	0.044(0.038-0.050)	0.70 (0.09)	0.050(0.043-0.057)
FN BMC	2.48 (0.37)	0.18 (0.15-0.21)	2.44 (0.36)	0.18 (0.16-0.21)	2.90 (0.51)	0.26 (0.24-0.29)[b]	2.80 (0.51)	0.31 (0.28-0.34)
FN aBMD	0.63 (0.07)	0.024 (0.016-.0.031)	0.61 (0.07)	0.025(0.019-0.031)	0.67 (0.09)	0.034(0.028-0.039)[b]	0.66 (0.09)	0.043(0.036-0.050)
FN vBMD	0.303 (0.041)	-0.002(-0.010-0.006)	0.300 (0.045)	0.005(-0.001-0.010)	0.299 (0.036)	0.002(-0.003-0.006)[b]	0.297 (0.040)	0.010 (0.004-0.015)
TR BMC	3.47 (0.69)	0.59 (0.54-0.65)	3.39 (0.84)	0.57 (0.53-0.61)	3.71 (1.26)	0.78(0.74-0.82)	3.59 (1.12)	0.79(0.74-0.84)
TR aBMD	0.54 (0.07)	0.026(0.018-0.035)	0.53 (0.07)	0.025(0.019-0.032)	0.56 (0.09)	0.046(0.040-0.052)	0.56 (0.08)	0.045(0.038-0.052)

[a]means adjusted in ANCOVA for baseline age, baseline BMC, Δ height, Δ bone area, Tanner stage* (BMC); for baseline age, baseline aBMD, Δ height, and Tanner stage (aBMD); and for baseline age, baseline vBMD, and Tanner stage (vBMD). CI = Confidence Interval.
*Tanner stage covariate: final (Prepubertal); initial (Early Pubertal)
[b]change is significantly different from intervention group ($P<0.05$).
[c]change is significantly different from intervention group ($P<0.01$).

Figure 20: (a) % Change in bone mineral content (BMC) and areal bone mineral density (aBMD) at the lumbar spine (LS) in prepubertal (PRE) control (C) and intervention (I) girls. (b) % change in BMC, aBMD and volumetric (vBMD) at the femoral neck in PRE C and I girls. (c) % change in BMC and aBMD at the LS in early pubertal (EARLY) C and I girls. (d) % change in BMC, aBMD and vBMD at the FN in EARLY C and I girls. Error bars represent 95% confidence intervals.



7.4 *Discussion*

This study is the first prospective intervention to show a maturity-related, region-specific bone response to weight-bearing exercise in pre- and early pubertal girls. This has important practical implications for school physical education programs. Girls who were early pubertal at the start of the exercise intervention program gained significantly more bone at the femoral neck and lumbar spine than their maturity-matched, non-participating counterparts. In contrast, the exercise intervention did not promote bone gain in girls who were prepubertal at baseline. Therefore, a 10-minute, thrice weekly, school-based intervention program may be an important public health measure for pediatricians to promote for girls around age 10.5 years (grades 5 and 6).

7.4.1 ***Maturity Modulates Bone Response***

Previous intervention studies in girls of similar maturity are consistent with these findings. Australian premenarcheal girls training for 30 minutes, 3 times per week in a variety of weight-bearing exercises gained significantly more bone (up to 5.5%, $P<0.05$) than healthy controls (234). A large proportion of the exercising group were Tanner Stage 2 or 3 at baseline (mean Tanner Stage = 1.6). Imbalances in maturational stages and possibly ethnic distributions between control and intervention groups may have contributed to the extent of their observed group differences in BMC and aBMD. Nevertheless, after corrections for changes in height and weight, many differences between groups remained significant.

Finnish premenarcheal girls (60% Tanner stage 2 or 3 at baseline) gained significantly more FN (4%) and LS (3.3%) BMC than their maturity-matched controls after 9 months of high impact exercise (23). Bone gain in postmenarcheal intervention girls did not differ from that of controls. Taken together with our data, it appears that the 'window of opportunity' for exercise-induced bone gain may exist in early puberty and before menarche.

The time when girls accrue BMC maximally (on average, age 12.5 years, commonly, Tanner stage 3-4) (11), is the only stage of life when bone mineral is laid down on both the periosteal and endosteal surfaces (131). At approximately 10.5 years, the amplitude of the hypothalamic 'gonadostat' changes, causing shifts in bone-enhancing hormones, such as estrogens, androgens, growth hormone, and insulin-like growth factor-I (123). As well, the growth hormone response to acute bouts of vigorous exercise may be more pronounced in Tanner stage 3 girls, than in either less or more mature girls (270). As the chronological age for pubertal onset, and therefore, the occurrence of peaks in hormone amplitudes and BMC velocity may differ by ethnicity (173,175,271), the most osteogenic period for loading exercise may occur at slightly different ages across ethnic groups, but at a similar maturational stage.

In 7 – 17 year old female racquet sport athletes, even strenuous training was not associated with playing-to-nonplaying arm aBMD differences in Tanner Stage 1 girls (22). In more mature girls (Tanner Stages 3-5), however, the dominant arm had clearly greater aBMD regardless of total training time. In contrast, Fuchs and colleagues recently reported 4.5 and 3.1% greater gain in BMC at the FN and LS, respectively, in prepubertal girls and boys (5.9 – 9.8 yr) participating in a very high impact jumping program, as compared to healthy controls (227). These data imply that in prepubertal girls, only such intense training (14) can augment bone mineral beyond that associated with normal growth.

7.4.2 *Type of Intervention Influences Bone Response*

The larger percent differences between intervention and control groups noted in previous studies in girls (23,234) (up to 5.5% *vs* 1.6 – 3.1% in our study) may have resulted from exercise programs that were more intense than our intervention. Our exercise program was brief (10-12 minutes) and easy to implement in the pre-existing physical education curriculum. In contrast, the previous intervention programs had longer sessions (30 - 40 minutes), carried out in addition to the physical education program (23,234). Furthermore, the participants were not randomized to the intervention group (23,234), which may have biased the samples in terms of adherence or predisposition to exercise.

7.4.3 *Bone Response is Site-Specific*

In the early pubertal girls, our intervention promoted bone gain at the femoral neck and lumbar spine but not at the trochanter. This pattern is consistent with results of an intervention with similar exercises (23). Impact forces are greatly attenuated prior to reaching the lumbar spine (272). Therefore, bone gain at that site is more likely mediated via the action of trunk muscles that would benefit from repetitive jumping (especially tuck jumps) and hip stabilizers and flexors that would respond to increase muscular control of landing (273,274). This warrants further investigation, but serves to remind clinicians that muscle-building exercise, not just impact, can effectively promote bone mineral gains at several sites. This has been shown repeatedly in adults (275,276) but remains to be studied in children.

In addition, our findings illustrate that outcomes vary depending on whether we report BMC, aBMD or vBMD. The largest percent difference between early pubertal exercisers and controls was for adjusted (for chronological age and maturity) femoral neck vBMD (>3%), as compared to differences less than 2% for BMC and aBMD. It is likely that vBMD represents the actual change in bone mineral within the FN volume, free of the influence of disparate growth rates between exercisers and controls that inherently bias BMC and aBMD change. The limitation of vBMD is that it provides only an *estimated* volume based on geometric assumptions at the FN.

7.4.4 *Relevance and Persistence of Changes in Bone Mineral*

The 2 to 3 % greater changes in bone mineral observed at the femoral neck and lumbar spine in the early pubertal intervention girls are clinically significant (277). If maintained, this augmentation may substantially reduce fracture risk in old age (278). No study has thus far assessed the bone effects in an intervention longer than 9 months in duration, nor has an intervention follow-up been conducted. The effect of a longer intervention (at least 2 to 3 years) requires study. Until long-term follow up data are available the question of whether gains persist into adulthood can only be addressed from retrospective data. Retrospective studies of adult, former elite dancers (mean age 52 years) (13), racquet sport athletes (mean age 22 years) (245), and gymnasts (mean age 25 years) (14) suggest that the positive bone effects of high impact training in childhood are longstanding.

7.5 Summary

Physical activity during the growing years positively influences bone mineral accrual (11). Despite this, there is a distinct trend in North America to sideline physical education in elementary schools (279,280). The American Academy of Pediatrics Policy Statement on Physical Fitness and Activity in Schools (RE 9907) suggests that "schools are in a uniquely favorable position to increase physical activity and fitness among students" and that pediatricians are "encouraged to assess activity patterns as part of routine health maintenance"(281). The present study extends this thinking to the specific benefits of physical activity for bone health and provides an example of a feasible PE curriculum that can be easily implemented in a mixed ethnic group of Grade 6 (> 10 yr. old) girls. Further work investigating similar parameters in boys is warranted.

8 - Part III: Bone Mineral Response to a 7-Month Randomized Controlled, School-Based Jumping Intervention in 121 Prepubertal Boys: Associations With Ethnicity and Body Mass Index

8.1 *Introduction*

Exercise in childhood promotes bone mineral accrual and consequently, bone strength (11,14). These factors are likely to reduce fracture risk in childhood (282) and, if maintained, may also reduce the risk of osteoporotic fracture in the elderly (283).

Mechanically loading immature bone may influence its future strength profoundly (5). Body mass provides a skeletal load during growth, and is related to both absolute bone mass (88,137,284) and bone mineral change in children (88,138,285), and adult bone mass (286). High impact exercise augments skeletal loading, yet it is unclear to what extent body mass may interact with exercise to influence skeletal change in over- or average weight children.

Exercises that are unusual, with ground reaction forces greater than those typically encountered in walking and running games, likely stimulate greater adaptations in young bones (126,130). In young children, jumping activities that are easily performed irrespective of the level of athletic skill, can provide ground reaction forces as high as 9 times body weight (227).

Several exercise interventions that incorporated jumping, weight training and/or high impact running games have successfully augmented bone mineral accrual at a range of skeletal sites in girls (23,234,287) and in combined groups of boys and girls (92,227). However, to our knowledge, only one exercise intervention has examined the bone response to exercise in an independent group of young boys (226), and all but one study (92) has been limited to Caucasian children.

Hip fracture rates are highest in Caucasians, and worldwide rates in men are rising continuously (166). Interestingly, Asian men sustain a greater number of hip fractures than do Asian women (168). Asian children in North America may be at even greater risk for low bone mass in adulthood, as compared with their North American - Caucasian counterparts (21,288). Early intervention in children's activity habits may provide an important population-health initiative to reduce osteoporotic fractures in the future. Thus, it is critical to characterize the bone response to weight bearing exercise in both boys and girls and in different ethnicities.

The primary purpose of this randomized controlled trial was to compare the changes in bone mineral content (BMC) and areal density (aBMD) between prepubertal boys participating in a 7-month, school-based, jumping intervention and their controls. During these primary analyses, we identified that body mass and ethnicity may have modified the intervention effects on bone mineral. Thus, we completed secondary analyses after categorizing boys as either having high body mass index (hiBMI) or average/low BMI (avBMI). Our secondary analyses in these two groups of boys investigated how the jumping intervention affected bone mineral change in Asian and Caucasian boys of average/low BMI, and in boys with high BMI.

8.2 *Methods*

Detailed methods for subject recruitment, classification and measurement are provided in Chapter 4 (Methods), and are briefly summarized here.

8.2.1 ***Design***

This randomized, school-based intervention included 7 intervention and 7 control elementary schools. Baseline measurements were made during the fall of the school year (September – October 1999). Schools were randomly assigned to intervention and control groups after individual informed consent forms were returned. The intervention ran from late October to late May (7 months in duration) and final measurements were made at the end of the school year (June 2000). Each set of measurements was completed during a 4- to 5-week period. The school-based exercise intervention program is described in detail in Section 4.4.1 (Methods – Exercise Intervention: Year 1 Exercise Intervention Program). A high impact, circuit-training program composed of jumping activities was implemented 3 times per week, for approximately 10 minutes a session.

8.2.2 ***Subjects***

The recruitment process and an overview of the original cohort were previously provided (4.2 – Methods – Recruitment of Schools, Teachers, and Students, and 5.1 – Overview – Participants). This analysis reports on the 192 male participants.

Each boy's health history questionnaire was completed by his parents. Each parent's birthplace was self-reported. All boys reported where they were born and what language was spoken at home. Boys were considered Caucasian if both parents were of North American or European origin. Ninety-two percent of Caucasian boys were born in Canada. Boys were classified as Asian if both parents, or all 4 grandparents were born in Hong Kong (71%), Taiwan (18%) or the Philippines (11%). Of the Asian boys, 20% were born in Canada; 30% had immigrated 6 to 10 years before baseline measurement; and 50% had immigrated 5 years or less before baseline measurement. Boys from mixed-ethnic backgrounds or of other ethnicities were excluded from this analysis.

Three boys were excluded from this analysis because they had medical conditions that could affect normal physical activity or bone development (Down's syndrome, fractured tibia, recent heart surgery). For generalizability of results and to ensure approximately equal variances by group, one other boy (Asian, Control) was excluded from this analysis as his bone mineral change across sites was consistently 3 standard deviations above the mean.[2] All other boys were healthy and none took medications known to influence bone metabolism. The institutional Research Ethics Board approved this study.

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[2] This boy's bone data was checked carefully for scan artefacts and errors in positioning and analysis. There were no discernible errors. His height and weight gains were within the normal range. The decision to remove his data was made as inclusion of his bone change data caused the distribution to positively skew.

After excluding the 4 boys described above, there were 133 prepubertal Asian and Caucasian boys at baseline. Line drawings of Tanner pubic hair ratings (1-5) were used to identify the maturity level of each boy (101). In this study, boys who were Tanner pubic hair stage 1 at baseline were classified as prepubertal. Boys who advanced two Tanner stages over the course of intervention were excluded from this analysis (N=2 Asian, N=6 Caucasian).

8.2.3 *Bone Mineral Assessment*

Methods and precision for the bone mineral assessment are previously described in Chapter 4 – Methods – Section 4.6. Briefly, we assessed the following bone parameters: BMC (grams) for the total body (TB), and BMC and aBMD (grams/cm^2) for the lumbar spine (LS), and proximal femur (PF), and its femoral neck (FN) and greater trochanter (TR) subregions using a Hologic QDR 4500W bone densitometer (DXA). Total body lean mass and fat mass (grams, both) were obtained from total body DXA scans. Due to a technical error in acquiring proximal femur scans from 4 boys at baseline, these data were not available; all other boys had complete TB, LS and PF data. Estimated volumetric bone mineral density (vBMD) was calculated for the femoral neck, assuming this region approximates a cylinder, by the equation: vBMD = (4BMC*(height of FN box))/ Π * (FN area)^2 (91).

8.2.4 *Height and Weight*

We measured standing height (stretch stature) to the nearest mm using a customized wall-mounted stadiometer. We assessed body weight with an electronic scale to the nearest 0.1 kg. For both height and weight, we used the mean of two measures for analysis.

8.2.5 *Questionnaires*

8.2.5.1 Calcium

All boys completed a food frequency questionnaire (FFQ) to estimate dietary intake of calcium (21). The FFQ includes ethnic-specific, calcium-rich foods, and was validated in Asian and Caucasian adolescents living in Vancouver (179). A bilingual (Chinese-English) trained measurer assisted Chinese children. Questionnaires were analyzed by calculating daily calcium intake (mg) based on the calcium content of food items. The questionnaire was administered at baseline (fall), during the winter, and at final measurement (spring), and the average of three calcium intakes is reported.

8.2.5.2 Physical Activity

General physical activity was determined by a modified version of the Physical Activity Questionnaire for Children (PAQ-C), described in section 4.9.2 (Methods – Questionnaires – Physical Activity) (208). The modification included an estimate of time (hr/wk) spent in common sports and activities designated as loaded (impact > walking) physical activity. This questionnaire was administered at baseline, during the winter, and at final measurement. We report the mean of the 3 administrations.

8.2.6 ***Statistical Analyses***

8.2.6.1 Primary Analyses: Intervention Effects

We used independent t-tests to compare baseline values for all variables (age, body size/composition, BMC, aBMD, vBMD) and average yearly values for calcium and physical activity between Control and Intervention Groups.

To compare change in BMC, aBMD and vBMD, we used a one-factor (intervention-control) analysis of covariance (ANCOVA), with baseline body mass (to control for initial size), change in height (to control for change in body size), physical activity loading time (to control for activity outside of the intervention), and age (to control for the range between grade 4, 5 and 6 boys) as covariates. We report main effects of the intervention on 8-month change in bone parameters.

Baseline body mass, change in height and age were used as covariates based on the established relationships between these variables and change in bone mineral (88,137,138,175,289). Furthermore, of the baseline body size variables (ie, baseline height, baseline bone, BMI), baseline body mass was the most highly correlated with change in bone parameters ($r = 0.3 - 0.5$, $P<0.001$) in this cohort of prepubertal boys. We also used loaded physical activity as a covariate for two reasons: 1) mean yearly values were not equivalent between groups (Table 18), and 2) within this cohort, loaded physical activity was the most consistent predictor of bone mineral change across sites in an exploratory stepwise linear regression. We report adjusted mean % change in bone parameters.

8.2.6.2 Secondary Analyses

Primary analyses demonstrated a large range in body mass – 22.0 kg to 68.6 kg. We also identified an imbalance in the Asian:Caucasian ratio within BMI centiles: 3 of the 4 boys below the 5th percentile for baseline BMI were Asian, and 69% of boys above the 75^{th} percentile for baseline BMI were also Asian. This range resulted in unequal variances in baseline body mass and BMI between Asian and Caucasian groups. As change in bone mineral is generally predicted by baseline body size (taller, heavier children have larger unadjusted absolute gains in bone mineral than shorter, lighter children) (88,138,285), change in bone mineral had similar large variance, most notably in the Asian Control group. However, bone mineral gains in boys in the highest quartile for baseline BMI were inconsistent, and these boys accrued some of the highest and lowest amounts of bone mineral over 8 months.

To evaluate the role of ethnicity and intervention on change in bone parameters, in groups with equal variance, we grouped boys according to baseline BMI. Boys who fell below the 75^{th} percentile for baseline BMI (19.4 kg/m^2) formed the average/low BMI group (N = 91, avBMI), while boys whose BMI was above the 75^{th} percentile formed the high BMI group (N = 30, hiBMI). We examined ethnic and intervention effects within the avBMI group, and intervention effects only in the hiBMI group.[3] The number of Asian and Caucasian boys who were categorized as avBMI and hiBMI is tabled (Tables 19 and 20).

8.2.6.2.1 Boys With Average/Low Body Mass Index (avBMI): Intervention and Ethnicity Effects

We used ANOVA to compare baseline values for all variables (age, body size/composition, BMC, aBMD, vBMD) and average yearly values for calcium and physical activity between Asian-Control, Asian-Intervention, Caucasian-Control, and Caucasian-Intervention. To compare change in bone parameters, we used a 2 (Asian, Caucasian) x 2 (Intervention, Control) analysis of covariance (ANCOVA), with baseline weight, change in height, loaded physical activity, and final Tanner stage as covariates. We analyzed main and interaction effects of the intervention and ethnicity on bone mineral change.

8.2.6.2.2 Boys With High BMI (hiBMI): Intervention Effects

We used independent t-tests to compare baseline values for all variables (age, body size/composition, BMC, aBMD, vBMD) and average yearly values for calcium and physical activity between hiBMI-Control and hiBMI-Intervention groups. To evaluate a main effect for change in bone mineral between groups, we used a one factor (Intervention, Control) ANCOVA, with baseline weight, change in height, loaded physical activity and final Tanner stage as covariates.

We report adjusted mean % change in bone parameters. Data were analyzed using SPSS statistical software, Windows version 8.0 (SPSS Inc., Chicago, IL, 1993). Baseline data are presented as mean (SD), and change data are presented as (adjusted, for bone parameters) mean (95% confidence interval). Significance was set at $P < 0.05$ for all statistical analyses.

8.3 Results

8.3.1 *Subjects and Compliance*

Four boys moved during the school year (2 Control, 2 Intervention). Thus, after excluding 8 boys who advanced 2 Tanner stages over the intervention, prospective data were available for 60 Control and 61 Intervention boys. Ninety-one boys were below the 75th percentile for baseline BMI (avBMI=19 Asian Control, 22 Asian Intervention, 25 Caucasian Control, and 25 Caucasian Intervention), and 30 boys above the 75th percentile for BMI (16 hiBMI Controls and 14 hiBMI Intervention) (total N=121).

Classes at intervention schools performed the circuit intervention a mean of 57 (SD 10) times. The maximum possible number of intervention sessions was 72. Thus, compliance averaged 80% across schools.

[3] There were small numbers of Caucasian boys in the hiBMI group (Table 20), therefore, we lacked the power to look at the effect of ethnicity (or an interaction between intervention and ethnicity) in the hiBMI analysis.

8.3.2 *Primary Analyses: Intervention Effects*

The number of participants who advanced to Tanner stage 2 over the course of the intervention was similar between control (N = 22, 37%) and intervention (N = 25, 41%) groups. Baseline and change values for body size and anthropometric characteristics, and average yearly values for physical activity and calcium intakes are reported (Table 18). There were no significant differences at baseline or in 8-month change between control and intervention groups for height, weight, lean mass, or fat mass, or in average physical activity or dietary calcium intake.

Table 18 summarizes baseline (mean (SD)) and adjusted change (mean (95% confidence interval)) values for BMC, aBMD, and vBMD across sites. Baseline bone parameters were similar between intervention and control groups. There was a significant main effect for intervention for adjusted change in TB BMC (9.8 vs. 8.2%, P<0.01, eta squared=0.059), and PF aBMD (3.4 vs. 2.4%, P<0.05, eta squared=0.036). There was a trend for greater gain in LS BMC for the intervention group (10.6 vs. 9.2%, P = 0.10). Change at other skeletal sites and for other bone parameters did not differ significantly between intervention and control groups.

8.3.3 *Secondary Analyses*

8.3.3.1 Boys With Average/Low Body Mass Index (avBMI): Intervention and Ethnicity Effects

Within the avBMI group, the number of boys advancing to Tanner stage 2 in intervention (38%) and control (36%) groups was similar. More Caucasian (52%) than Asian (20%) boys advanced to Tanner stage 2. Baseline and change values for body size and anthropometric characteristics, and average yearly values for physical activity and calcium intakes are reported (Table 19). There were no significant differences at baseline or in 8-month change between control and intervention groups within ethnicities for height, weight, lean mass, fat mass, or in average physical activity or calcium. Caucasian controls were taller than Asian controls at baseline (P<0.05).

Table 19 summarizes baseline (mean (SD)) and adjusted change (mean (95% confidence interval)) values for BMC, aBMD, and vBMD across sites. Baseline bone parameters did not differ between control and intervention groups within ethnicities. Asian controls had significantly lower baseline LS BMC than Caucasian controls, and lower FN BMC as compared to both groups of Caucasian boys, (all P<0.05).

There was a significant main effect of intervention for adjusted change in TB BMC (9.6 vs. 7.5%, P<0.01, eta squared=0.100), LS BMC (10.1 vs. 8.1%, P<0.05, eta squared=0.049), PF aBMD (3.2 vs. 2.1%, P<0.05, eta squared=0.053), and TR aBMD (3.4 vs. 1.8%, P<0.05, eta squared=0.056). Change at the femoral neck was not significantly different between groups. Additionally, adjusting change in LS BMC and FN BMC for baseline BMC, as per baseline differences noted above, did not alter the outcomes. Adjusted individual values for change in LS BMC and TR aBMD for intervention and control groups are shown (Figure 21 (a & b)). There were no significant main effects for ethnicity, and no significant ethnicity by intervention interaction effects on bone mineral change.

8.3.3.2 Boys With High Body Mass Index (hiBMI): Intervention Effects

The number of Asian and Caucasian boys in intervention and control groups was comparable. Control and intervention groups did not differ at baseline or in change in height, weight, lean mass, fat mass, or in average physical activity or dietary calcium intake (Table 20). At baseline, all bone parameters were similar between groups. Adjusted 8-month bone change did not differ significantly between hiBMI control and hiBMI intervention at any site or for any bone parameter (Table 20). Further adjustment for change in fat mass to account for a trend towards greater gain in the control group did not alter this outcome.

Table 18: Average lifestyle factors, and baseline and change (Δ) (if appropriate) for age, body size and composition, bone mineral content (BMC), areal bone mineral density (aBMD), and volumetric BMD (vBMD, FN only) for the total body (TB), lumbar spine (LS), proximal femur (PF), femoral neck (FN), and greater trochanter (TR) in Control and Intervention groups. Mean (SD) unless otherwise indicated.

	Control (N = 60)		Intervention (N = 61)	
	Baseline	Δ (95% CI)*	Baseline	Δ (95% CI)*
Age (years)	10.3 (0.7)		10.2 (0.6)	
#Tanner Stage 2 at final		22		25
Activity Score (1-5)	3.1 (0.5)	--------	3.1 (0.6)	--------
Loaded Physical Activity (hr/week)	6.9 (4.5)	--------	7.8 (5.6)	--------
Average Calcium Intake (mg/day)	853 (379)	--------	840 (416)	--------
Height (cm)	141.8 (7.1)	3.4 (3.2 – 3.7)	140.6 (6.0)	3.6 (3.3 – 3.8)
Weight (kg)	36.6 (10.1)	2.6 (2.0 – 3.2)	35.5 (8.3)	2.7 (2.1 – 3.2)
Body Mass Index (kg/m²)	18.0 (4.0)	0.4 (0.1 – 0.6)	17.8 (3.2)	0.4 (0.2- 0.6)
Lean Mass (kg)	26.29 (4.38)	1.89 (1.59 – 2.19)	25.95 (3.80)	1.96 (1.66 – 2.25)
Fat Mass (kg)	8.97 (6.22)	0.54 (0.16 – 0.93)	8.36 (4.70)	0.49 (0.11 – 0.88)
Long Jump (cm)				
Vertical Jump (cm)				
TB BMC (g)	1083 (199)	90.0 (81.9 – 98.2)	1074 (174)	105.8 (97.7 – 113.9)[a]
LS BMC (g)	24.25 (4.50)	2.19 (1.93 – 2.46)	23.73 (4.20)	2.50 (2.24 – 2.76)
LS aBMD (g/cm²)	0.61 (0.06)	0.020 (0.015 – 0.025)	0.61 (0.06)	0.024 (0.019 – 0.029)
PF BMC (g)	16.20 (3.85)	1.51 (1.28 – 1.74)	15.70 (2.71)	1.71 (1.47 – 1.94)
PF aBMD (g/cm²)	0.71 (0.09)	0.017 (0.013 – 0.022)	0.71 (0.07)	0.025 (0.020 - 0.030)[b]
FN BMC (g)	2.83 (0.49)	0.17 (0.14 – 0.21)	2.81 (0.42)	0.16 (0.12 – 0.20)
FN aBMD (g/cm²)	0.68 (0.08)	0.014 (0.009 – 0.019)	0.68 (0.07)	0.015 (0.010 – 0.021)
FN vBMD (g/cm³)	0.31 (0.04)	-0.006 (-0.011 – (-0.001))	0.32 (0.03)	-0.003 (-0.008 – 0.002)
TR BMC (g)	2.68 (0.89)	0.50 (0.43 – 0.56)	2.46 (0.72)	0.50 (0.44 – 0.57)
TR aBMD (g/cm²)	0.55 (0.07)	0.014 (0.009 – 0.020)	0.54 (0.06)	0.021 (0.015 – 0.026)

Physical activity and calcium intake values are the average of 3 reports. Mean (SD) reported unless otherwise stated.

CI = Confidence Interval.

*Adjusted change for bone parameters (for age and weight at baseline, change in height, and average loaded physical activity, ANCOVA).

[a]Significantly different from Controls, $P<0.01$.

[b]Significantly different from Controls, $P<0.05$.

Table 19: Average lifestyle factors, and baseline and change (if appropriate) for age, body size and composition, bone mineral content (BMC), areal bone mineral density (aBMD), and volumetric BMD (vBMD, FN only) for the total body (TB), lumbar spine (LS), proximal femur (PF), femoral neck (FN), and greater trochanter (TR) in *average BMI Asian and Caucasian Control and Intervention groups*. Mean (SD) reported unless otherwise stated. CI = Confidence Interval.

	Asian				***Caucasian***			
	Control Baseline (N = 19)	Δ (95% CI)*	Intervention Baseline (N = 22)	Δ (95% CI)*	Control Baseline (N = 25)	Δ (95% CI)*	Intervention Baseline (N = 25)	Δ (95% CI)*
Age (years)	10.3 (0.8)		10.2 (0.7)		10.2 (0.7)		10.2 (0.4)	
#Tanner Stage 2 at final		2		6		14		12
Activity Score (1-5)	3.0 (0.4)	--------	3.0 (0.6)	--------	3.0 (0.5)	--------	3.1 (0.6)	--------
Loaded Physical Activity (hr/week)	7.5 (5.1)	--------	8.2 (6.1)	--------	5.8 (4.5)	--------	7.8 (5.5)	--------
Average Calcium Intake (mg/day)	822 (298)	--------	754 (351)	--------	862 (329)	--------	1012 (486)	--------
Height (cm)	137.5 (6.7)	3.1 (2.7–3.6)	138.9 (5.4)	3.4 (3.0–3.7)	143.0 (6.2)	3.4 (3.0–3.8)	139.7 (6.0)	3.2 (2.8-3.6)
Weight (kg)	31.0 (5.1)	1.9 (1.4-2.5)	31.9 (4.4)	2.5 (2.0-3.1)	32.4 (4.1)	2.0 (1.5-2.5)	31.8 (3.8)	2.1 (1.6-2.6)
BMI (kg/m^2)	16.3 (1.7)	0.2 (0.1-0.5)	16.4 (1.5)	0.5 (0.2-0.7)	15.8 (1.3)	0.2 (0.02-0.4)	16.3 (1.5)	0.3 (0.1-0.5)
Lean Mass (kg)	23.36 (2.80)	1.56 (1.21-1.91)	24.42 (3.02)	1.85 (1.52-2.17)	25.56 (3.12)	1.47 (1.17-1.78)	24.73 (2.52)	1.43 (1.13-1.74)
Fat Mass (kg)	6.44 (2.89)	0.32 (-0.03-0.67)	6.32 (1.95)	0.46 (0.13-0.78)	5.55 (1.84)	0.52 (0.21-0.82)	6.06 (1.86)	0.49 (0.19-0.79)

Table 19 (Continued)

	Asian				Caucasian			
	Control Baseline (N = 19)	Δ (95% CI)*	Intervention Baseline (N = 22)	Δ (95% CI)*	Control Baseline (N = 25)	Δ (95% CI)*	Intervention Baseline (N = 25)	Δ (95% CI)*
TB BMC (g)	960 (148)	71.8 (58.1–85.6)	1000 (152)	100.4 (87.9–112.8)[a]	1079 (170)	85.1 (73.1-97.1)	1037 (138)	94.2 (82.5-106.0)[a]
LS BMC (g)	21.50 (3.45)[cc]	1.82 (1.35-2.28)	22.33 (3.51)	2.23 (1.81-2.65)[b]	24.62 (4.01)	1.88 (1.48-2.29)	23.51 (4.00)	2.34 (1.94-2.73)[b]
LS aBMD (g/cm²)	0.58 (0.07)	0.016 (0.007-0.025)	0.58 (0.05)	0.024 (0.016-0.033)	0.62 (0.06)	0.018 (0.010-0.026)	0.61 (0.06)	0.020 (0.012-0.028)
PF BMC (g)	13.62 (3.14)	1.16 (0.75-1.56)	14.60 (3.26)	1.38 (0.99-1.77)	16.28 (3.30)	1.32 (0.96-1.68)	15.51 (2.04)	1.50 (1.14-1.86)
PF aBMD (g/cm²)	0.66 (0.08)	0.015 (0.006-0.024)	0.68 (0.07)	0.022 (0.014-0.031)[b]	0.70 (0.09)	0.012 (0.005-0.020)	0.70 (0.07)	0.023 (0.015-0.030)[b]
FN BMC (g)	2.50 (0.36)[cc,ci]	0.11 (0.05-0.18)	2.62 (0.46)	0.16 (0.10-0.22)	2.88 (0.50)	0.17 (0.12-0.23)[b]	2.81 (0.34)	0.12 (0.07-0.18)
FN aBMD (g/cm²)	0.64 (0.07)	0.009 (-0.0005-0.019)	0.65 (0.07)	0.011 (0.001-0.020)	0.68 (0.09)	0.012 (0.003-0.020)	0.69 (0.06)	0.015 (0.007-0.024)
FN vBMD (g/cm³)	0.315 (0.034)	-0.004 (-0.013-0.005)	0.313 (0.034)	-0.009 (-0.017-(-0.0002))	0.307 (0.042)	-0.008 (-0.016-0.00003)	0.321 (0.029)	0.002 (-0.006-0.009))
TR BMC (g)	2.18 (0.59)	0.37 (0.26-0.49)	2.24 (0.91)	0.44 (0.34-0.55)	2.75 (0.87)	0.45 (0.35-0.55)	2.50 (0.61)	0.45 (0.35-0.55)
TR aBMD (g/cm²)	0.52 (0.06)	0.011 (0.001-0.020)	0.53 (0.06)	0.017 (0.008-0.026)[b]	0.55 (0.08)	0.007 (-0.001-0.016)	0.54 (0.06)	0.020 (0.012-0.028)[b]

*Adjusted change for bone parameters (for final Tanner stage, weight at baseline, change in height, and average loaded physical activity, ANCOVA).
[cc]Significantly different from Caucasian Controls; [ci]Signficantly different from Caucasian Intervention (ANOVA, P<0.05).
[a]Change is significantly greater than controls (intervention main effect, ANCOVA, P<0.01).
[b]Change is significantly greater than controls (intervention main effect, ANCOVA, P<0.05).

Table 20: Average lifestyle factors, and baseline and change (if appropriate) for age, body size and composition, bone mineral content (BMC), areal bone mineral density (aBMD), and volumetric BMD (vBMD, FN only) for the total body (TB), lumbar spine (LS), proximal femur (PF), femoral neck (FN), and greater trochanter (TR) in *High BMI* (top 25th percentile for baseline BMI), mixed ethnicity Control and Intervention groups. Mean (SD) reported unless otherwise stated. CI = Confidence Interval.

	Control Baseline N = 16 (11 Asian + 5 Caucasian)	Δ (95% CI)*	Intervention Baseline N = 14 (10 Asian + 4 Caucasian)	Δ (95% CI)*
Age (years)	10.6 (0.6)		10.4 (0.7)	
#Tanner Stage 2 at final		6		7
Activity Score (1-5)	3.1 (0.6)	--------	3.0 (0.4)	--------
Loaded Physical Activity (hr/week)	8.0 (3.7)	--------	7.2 (5.5)	--------
Average Calcium Intake (mg/day)	876 (532)	--------	667 (253)	--------
Height (cm)	145.0 (6.7)	3.8 (3.2-4.4)	145.0 (5.0)	4.5 (3.9-5.1)
Weight (kg)	49.8 (9.4)	4.2 (2.3-6.1)	47.8 (6.8)	3.8 (1.7-5.8)
BMI (kg/m^2)	23.6 (3.6)	0.7 (-0.08-1.5)	22.7 (2.6)	0.2 (-0.5-1.3)
Lean Mass (kg)	30.92 (3.98)	3.06 (2.27-3.85)	30.54 (3.26)	3.19 (2.34-4.03)
Fat Mass (kg)	17.32 (5.88)	0.85 (-0.58-2.28)	15.66 (3.83)	0.55 (-0.98-2.08)
TB BMC (g)	1237 (195)	129.1 (112.2–146.0)	1256 (139)	123.9 (105.7–142.0)
LS BMC (g)	26.93 (4.72)	3.36 (2.73-3.99)	26.31 (4.62)	2.97 (2.29-3.64)
LS aBMD (g/cm^2)	0.65 (0.05)	0.032 (0.022-0.042)	0.64 (0.06)	0.029 (0.018-0.040)
PF BMC (g)	19.15 (3.34)	2.46 (1.94-2.99)	17.73 (1.71)	2.30 (1.71-2.89)
PF aBMD (g/cm^2)	0.79 (0.08)	0.031 (0.021-0.041)	0.76 (0.06)	0.027 (0.015-0.038)
FN BMC (g)	3.14 (0.38)	0.26 (0.17-0.35)	3.08 (0.34)	0.21 (0.11-0.31)
FN aBMD (g/cm^2)	0.73 (0.07)	0.023 (0.013-0.034)	0.72 (0.06)	0.021 (0.009-0.033)
FN vBMD (g/cm^3)	0.33 (0.038)	-0.006(-0.014-0.002)	0.32 (0.024)	-0.002(-0.012-(-0.007))
TR BMC (g)	3.18 (0.96)	0.75 (0.59-0.91)	2.73 (0.52)	0.64 (0.47-0.83)
TR aBMD (g/cm^2)	0.58 (0.07)	0.033 (0.020-0.046)	0.57 (0.05)	0.022 (0.007-0.037)

*Adjusted change for bone parameters (for final Tanner stage, weight at baseline, change in height, and average loaded physical activity, ANCOVA).
No significant differences at baseline or in 8-Month change.

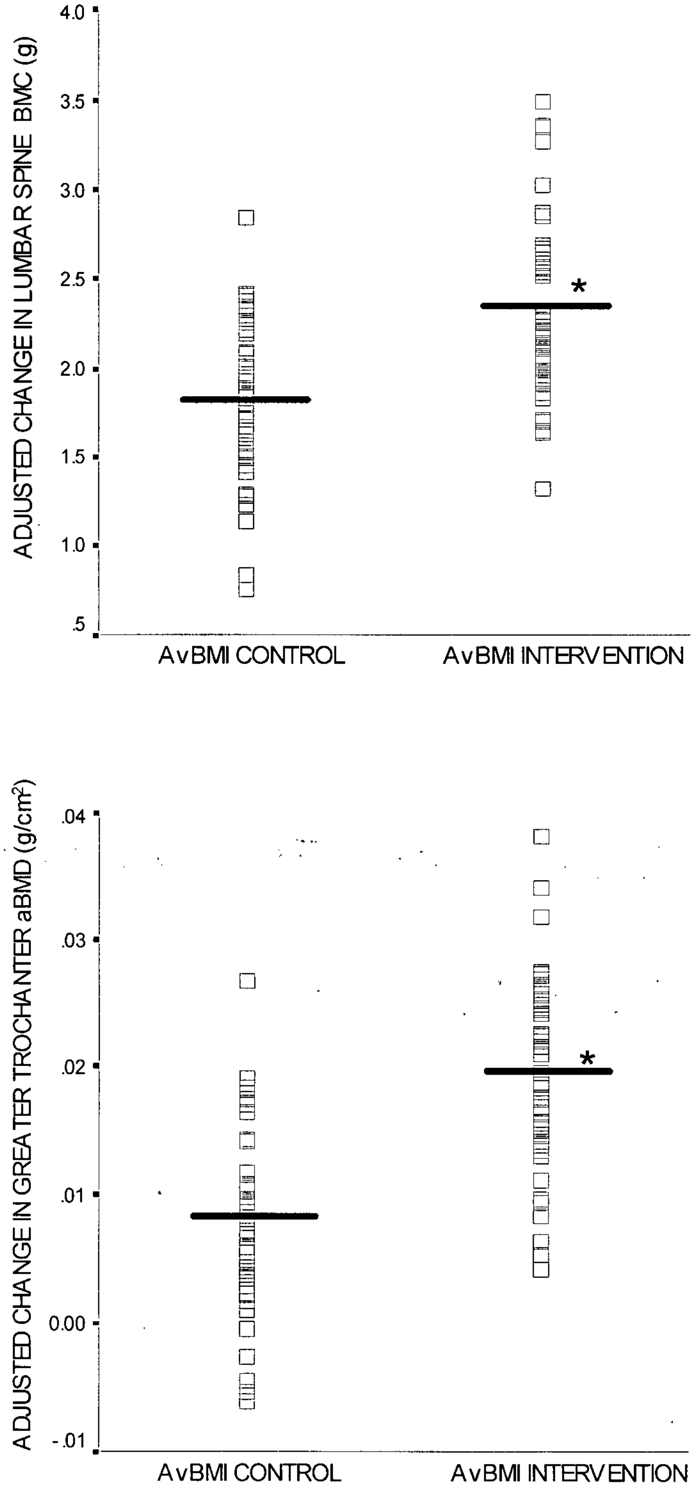

Figure 21: Individual adjusted values for 8-month change in (a) BMC at the lumbar spine and (b) aBMD at the greater trochanter for average BMI (AvBMI Control, N = 44), and Intervention (AvBMI Intervention, N = 47) boys.

*Main effect of Intervention > Control (ANCOVA, P<0.05). ▬ = adjusted mean (adjusted for baseline weight, change in height, final Tanner stage, and loaded physical activity.)

8.4 *Discussion*

This is the first prospective intervention study to demonstrate, in the primary analysis, a significant effect of a school-based jumping program on bone mineral change at the total body and proximal femur in a large cohort of prepubertal Asian and Caucasian boys. Because of the unequal variances between Asian and Caucasian boys, we conducted an independent analysis of Average/Low and High BMI groups. Although this was not our a priori question, it allowed us to examine the question of an ethnic response to loading without violating statistical assumptions for ANCOVA. Secondary analyses revealed that Asian and Caucasian boys in the intervention group who were below the 75th percentile for BMI demonstrated greater gains in total body and lumbar spine BMC, and aBMD at the total proximal femur and greater trochanter, compared with their same-ethnicity, control counterparts. However, intervention boys above the 75th percentile for BMI in this cohort did not gain bone mineral relative to controls of similar BMI.

8.4.1 *Primary Analysis of Intervention Effects*

The relatively small intervention effect we observed in the total sample (1 – 1.6% greater TB and PF bone mineral gain) was consistent with the only other exercise intervention in prepubertal boys (226). Our previous study that utilized a similar, but less structured and less intense exercise intervention (92) also demonstrated significant effects at the proximal femur (trochanteric subregion) in Asian and Caucasian boys and girls. Several factors, relevant to change in bone parameters in both the primary and secondary analyses, will be discussed in the following sections.

8.4.2 *Boys with Average/Low BMI (avBMI): Intervention Effects on Change in Bone Parameters*

The finding of a significant intervention effect (+1.9%) for change in TB BMC in average BMI boys is consistent with results of the single previous intervention in boys (N =40), that reported a 1.2% greater increase in TB aBMD in the intervention group (226). A seven-year prospective observational study also demonstrated that peak absolute values and the magnitude and rate of total body bone mineral accrual were significantly greater in highly active compared with low active children (11). Change in total body bone mineral suggests a significant response to the intervention, not merely redistribution of bone mineral from a relatively unloaded site.

Although small, the change in trochanteric aBMD in the intervention group was double that of controls and it was reflected in the significant increase at the total proximal femur. Change at the femoral neck was not different between intervention and control groups. Site-specificity of the bone response to loading in the present, and previous, studies (23,92,287) highlight the possibility of a combined influence of sex and maturity. Previous work by our group demonstrated a significant 1.2% augmentation of bone mineral at the greater trochanter in younger prepubertal, Asian and Caucasian boys and girls (92). Recently, we demonstrated a lack of response at the trochanter in prepubertal girls (10 yrs old, late Tanner stage 1) (287) involved in the same intervention as boys in the current study. Changes at the proximal femur and its regions were not reported in the only previous study of prepubertal boys alone (226).

We previously (Part II) demonstrated that the physical maturity of subjects contributes substantially to bone mineral accrual as well as the skeletal response to exercise (287). Early pubertal girls (287) following the same intervention as the boys in the current study had significantly greater change at the femoral neck (+3.1% for vBMD) than controls of similar maturity, while change at the trochanter was similar between groups. A jumping intervention in Finnish premenarcheal (mainly Tanner stage 2-3) girls augmented gains at the femoral neck (+4% for BMC), but not the trochanter (23). Taken together, these studies suggest that the proximal femur, and especially the femoral neck, may respond to a greater extent in early puberty as compared to prepuberty. However, further prospective studies of boys' responses to exercise at different maturational stages are needed.

This intervention incorporated 2-foot and 1-foot jumps and multidirectional movements which required contraction of the gluteal and quadriceps muscles that attach to the greater trochanter. Therefore, a change in bone mineral at this site was not unexpected. The incidence of trochanteric fractures in older adults is increasing (166), and there is a higher ratio of trochanteric than femoral neck fractures in both Caucasian (166) and Asian (168) men than women. At the population level, aBMD is a good predictor of osteoporotic fracture in old age (290). Whether site-specific gains in aBMD made in childhood persist into adulthood and old age, and whether these gains reflect increased bone strength (87), are important areas for further investigation.

We also observed increased bone mineral at the lumbar spine in the average BMI intervention boys (+2% greater change in BMC). This result is consistent with investigations in similarly aged, prepubertal boys (+2.8%) (226), and younger prepubertal boys and girls (+3.1%) (227). However, exercise intervention did not affect change in lumbar spine bone mineral in our previous study of a younger, less mature, cohort of Asian and Caucasian boys and girls (92). The 'late Tanner stage 1' status of the boys in the current study may have conferred a readiness for skeletal response to loading (126), as compared to the less mature children in our previous study (92). Furthermore, that study (92) utilized a moderate impact exercise program that was less focussed than either the current study or other interventions that augmented bone mineral gain at the spine (226,227). Thus, results from these studies suggest that a response at the lumbar spine may be elicited in prepubertal children who undertake a greater magnitude increase in impact loading activity.

8.4.3 *Boys with Average/Low BMI (avBMI): Ethnicity Effects on Change in Bone Parameters*

These are the first data comparing ethnic differences in bone mineral response to an intervention in boys alone. After adjusting for body size, maturity and physical activity, there was no ethnic difference in the bone accrual response to exercise over seven months at any measured site. In a previous intervention study in Asian and Caucasian boys and girls (92) after controlling for lean mass, fat mass and sex in hierarchical regression, ethnicity did not significantly predict aBMD change with exercise (92). Thus, the response of bone to exercise may not depend on ethnicity, but further studies are required.

Ethnic differences in bone mineral may not appear until early puberty or later (173,288). In a mixed longitudinal study that compared ethnic differences in bone mineral gain in 193 boys, Asians reached a lower peak spinal areal aBMD than Caucasian boys, and tended to achieve this peak at an earlier age than boys from other ethnicities (89). These data point to the need for studies in boys at different stages of maturity to better evaluate the role of ethnicity in bone mineral change.

8.4.4 *Boys with High BMI (hiBMI): Intervention Effects on Change in Bone Parameters*

There is a strong positive relationship between body mass and absolute values of bone mineral (88,137,284), bone size (136,289), and change in bone mineral (88,138,285) in children. The issue of body mass has received little attention in prospective studies of the bone response to exercise. The high BMI boys in the present study weighed, on average, 45% more than the mean for boys of the same age in Hong Kong (49.1 kg *vs*. 34.0 kg) (291), and 37% more than the mean for 10-year old Caucasian-Canadian males (35.8 kg) (135). The 75th percentile for BMI in our cohort (19.4 kg/ m^2) was slightly higher than that of 10.5-year-old boys in both the United States (18.5 kg/m^2) (292), and China (18.2 kg/m^2) (293). Thus, the present cohort may have included a disproportionate number of overweight boys. Exercise intervention did not appear to further influence the already relatively large gains in bone mineral observed in boys who were above the 75th percentile for baseline BMI.

Body mass exerts stress on growing bone, leading to positive bone adaptations (294). In obese children (defined as 20% above the expected weight for height), bone age maturation is accelerated (295). It is possible that the skeletons of heavy boys may not respond to a loading exercise program when already under substantial adaptive stress due to body mass. Overweight and obese children have greater absolute bone mineral than normal weight children, however, their bone mineral mass is lower than that predicted from their body mass (296). In our cohort, boys in the highest quartile for BMI had a lower mean ratio of total body BMC: body mass (25.8±3.4 grams/kg body mass) than boys in the lower quartiles for BMI (32.2±2.8 grams/kg body mass). This imbalance between bone mass and total body mass may predispose heavy and obese children to childhood fractures (282).

An alternative hypothesis is that the high BMI boys in the intervention group were not motivated to perform the jumping exercises, and may have found the type of intervention difficult to execute. Intervention teachers reported constant participation and effort on behalf of the overweight boys, however, we can not be certain that their jumps were of high quality. Along these lines, previous studies suggest that adults who have higher percent body fat over-estimate activity (297) and have a less accurate recollection of vigorous activity (298). An approximate 50% exaggeration of self-report activity in overweight children has been documented (299). We adjusted change in bone parameters by participation in extracurricular physical activity. An overestimation of physical activity would likely be equivalent between intervention and control groups and should not affect overall findings.

The possibility that the endocrine profile, and its effect on bone, of overweight children differs from normal weight children should be noted. The complex relationships between hormones including growth hormone and IGF-1, maturity, obesity and exercise pose interesting possibilities for explaining the skeletal response to exercise in heavy children (117,130,300,301). However, we did not evaluate hormone levels in the current study. Further endocrinological research is warranted to explore the mechanisms that control bone metabolism in overweight boys.

As a final point, the important influence of body composition on the accuracy (or inaccuracy) of DXA measures is noteworthy. Recent work utilized a simulation model and demonstrated that a sizable overestimation of BMC and aBMD can exist when percent body fat is high (86). In light of this, comparisons of both baseline and change in DXA-measured bone mineral between normal (~ 19% body fat in the current study), and high BMI children (~33% body fat) must be interpreted with caution. Further investigations of the utility of DXA in the accurate assessment of bone mineral accrual in overweight children are necessary.

8.5 Summary

In summary, a 10-minute jumping intervention, implemented three times per week over 7 months, had a small, but significant effect on total body and proximal femur bone mineral accrual in a large cohort of Asian and Caucasian boys, when data were analysed without regard to boys' BMI. However, body mass index at baseline was significantly related to bone mineral accrual and this may play a role in dampening the effect of a jumping intervention, as total body, lumbar spine, proximal femur and trochanteric bone mineral changes were augmented by 1.2 to 2.2% in Asian and Caucasian boys of average or low BMI, as compared to their controls. In contrast, we observed no additional skeletal response to loading intervention in prepubertal boys with high BMI. We conclude that a school-based exercise intervention can positively influence bone parameters in prepubertal boys, and that this response appears to be independent of ethnicity. Further studies exploring the mechanisms whereby BMI influences bone accrual in this age group may be warranted.

9 - Part IV: A School-Based Exercise Intervention Elicits Substantial Bone Health Benefits: A 2-Year Randomized Controlled Trial In Girls

9.1 *Introduction*

The World Health Organization reports that lack of physical activity is a major underlying cause of death, disease, and disability and attributes more than two million deaths annually to physical inactivity (302). The health costs of chronic diseases associated with physical inactivity are nearly 1 trillion dollars annually in the United States (1). There is considerable evidence that strongly links *physical inactivity* to the increasing prevalence of osteoporosis (303). The costs of osteoporosis and related fractures continue to escalate and currently exceed $6 billion per year in the United States (1) and are of considerable concern world-wide (3).

It is increasingly accepted that the roots of adult osteoporosis are cultivated in childhood (11,206,304), and the WHO agrees that the debate around non-communicable diseases must be redirected towards prevention (302). A recent editorial argued strongly that of all modifiable lifestyle factors that influence bone, exercise during growth has the greatest capacity to reduce the public health burden of osteoporosis-related fractures (304). The author called for safe, effective, relatively simple and inexpensive programs of activity in childhood to reduce the burden of adult osteoporosis (304).

We previously reported a 1-3% bone mass advantage at the lumbar spine and proximal femur in children who completed a simple, yet diverse, weight-bearing exercise program for 10-20 minutes, 3 times per week (287,305). This program was instructed by classroom, not PE specialist, teachers (287,305). Others have reported similar findings with more intense after school programs (23,226,234) and repetitive school-based box jumping programs (227). However, all previous prospective trials of exercise interventions have been completed in one academic year (10 months or less). The question of whether reported short-term benefits continue to accrue, or are lost as children mature, has not been addressed. Thus, in this final study, we compared bone mineral change in girls who completed a 20-month, school-based exercise program during elementary school physical education to that of girls at control schools. As a secondary analysis, we examined, through hierarchical regression, the contributions of maturity, body size and composition, and physical activity to bone mineral change at the lumbar spine and femoral neck in the pooled group of girls.

9.2 *Methods*

Detailed procedures are provided in Chapter 4 (Methods) and are briefly summarized here.

9.2.1 ***Design***

To assess school PE as a vehicle for the intervention and to avoid exercise contamination within schools, we randomized schools to either exercise intervention (n=7 schools) or control (n=7 schools) groups. We measured children at the beginning (late September-October) and end (late May-June) of two consecutive school years (4 measures per subject). The intervention was implemented for 7 months each year with a two-month break from the study during summer holiday.

9.2.2 *Subjects*

At baseline, the Year 1 cohort included 191 girls in grades 4, 5, and 6 (8.8 to 11.7 years old) from the Richmond School District. Richmond is home to approximately 34% Hong Kong Chinese, 57% North American / Western European Caucasian, 5% East Indian and 4% other ethnic origin or mixed ethnicity. The fourteen schools participating in Year 1 were stratified by race (<33% Asian or >33% Asian) and number of participants (<20 or >20) prior to randomization to ensure equivalence between groups. Each girl's parents completed a health history questionnaire for their daughter.

All control schools consented to participate in Year 2. Attrition in the schools between years 1 and 2 occurred in 4 ways: 1) In 2 intervention schools *principals* declined participation for the school. This was either because target teachers were in their first year of teaching and principals felt that the intervention would be distracting or there were too few (1 or 2) study children per classroom to warrant participation of new teachers (-29 girls); 2) Three intervention school *teachers* declined to participate, although other classes from these schools continued in the program (-8 girls); 3) Sixteen girls in the intervention group and 29 girls in the control group either moved to a new school or did not respond to the consent form in Year 2 (-45 girls) and 4) Eleven control girls in grade 7 were not sent a consent form as only grades 5 and 6 remained in the intervention schools (-11 girls). (However, 5 control girls in grade 7 had already provided consent and indicated keen interest over the summer between Years 1 and 2, and we measured those 5 grade 7 girls as part of the cohort in Year 2). Thus, of the 87 intervention and 92 control girls who completed Year 1, 34 (39%) and 52 (57%) girls, respectively, participated in Year 2. There was a low rate of attrition *within* each year of the study (4%).

In the intervention group, there was a small difference in age (0.3 years) between girls who dropped out and those who continued, as a result of the attrition (intervention group) and omission (control group) of grade 7 girls. Baseline lumbar spine BMC was higher in those girls who dropped out of the intervention group than those who continued, which reflected the difference in age. Eight-month change in lumbar spine BMC was similar between those girls who dropped out and those who continued. There were no differences in baseline or 8-month change in height, weight, lean mass, fat mass, or femoral neck BMC between intervention or control girls who withdrew after Year 1 and those who continued in Year 2.

The University of British Columbia Clinical and Behavioural Sciences Research Ethics Board approved this study.

9.2.3 *School-Based Exercise Intervention*

The details of the Year 1 and Year 2 exercise intervention are provided (Chapter 4, Section 4.4). In both years, the program was designed to provide a progressive, 10-12 minute program of diverse weight-bearing exercises during regularly scheduled physical education class (2 times per week) and on one other day during the week. Slight modifications were made in Year 2 to accommodate a higher proportion of high impact jumps (250), and ensure that the program was progressive from Year 1 to 2. The exercise stations incorporated plyometric jumps, alternating-foot jumps, and 2-foot obstacle jumps. In-school training of teachers (N=15) was conducted according to a guideline manual (Appendix 2). We visited each class twice during the school year to monitor the intervention and teachers detailed exercise activities and attendance in a manual. On average there were 57 and 46 exercise sessions in Year 1 and Year 2, respectively. Girls at control schools participated in 2, 40-minute regular PE sessions/week as mandated by the Richmond School Board.

9.2.4 *Dependent variables*

The primary outcome was 20-month change in bone mineral content (BMC, grams) for the total body (TB), lumbar spine (LS), and proximal femur (PF), and its femoral neck (FN) and trochanteric (TR) subregions, assessed using a Hologic QDR 4500W bone densitometer (DXA). We also report bone area (BA, cm^2) as a secondary outcome. Scans were analyzed using standardized procedures (252) by one researcher (KJM). Total body lean mass and fat mass (grams, both) were obtained from total body DXA scans. Precision and quality assurance procedures for densitometry are provided in Chapter 4 (Section 4.6). We also assessed vertical and long jump (to the nearest mm) to represent dynamic power at baseline and 20 months (Chapter 4, Section 4.8).

9.2.5 *Independent variables*

Maturity was rated (1-5) against Tanner breast line drawings (101). The girls were either assisted by a member of the research team with each follow-up assessment, or completed it with written instructions (in English or Chinese) under parental supervision at home. Menarcheal status and date of menarche were ascertained at each laboratory visit, as relevant.

We measured sitting height and standing height (stretch stature both) to the nearest mm, calculated leg length and assessed body weight with standard protocol (Chapter 4, Section 4.7)

We used a food frequency questionnaire (FFQ) to estimate dietary intake of calcium (179). A bilingual (Chinese-English) trained measurer assisted Chinese children. The questionnaire was administered 3 times during each school year (fall, winter and spring, 6 times total), and we report average calcium intake across 20 months.

Moderate to vigorous physical activity during the previous seven days was determined by a modified version of the Physical Activity Questionnaire for Children (PAQ-C) (208). Final *general physical activity* scores were calculated as an average of the PAQ-C items, in a continuous range between 1 (low activity) and 5 (high activity) (11,211). We modified the PAQ-C to include an estimate of time spent in *loaded physical activity* and the number of nights of extracurricular sports activities/week (*sport nights*). The questionnaire was administered 3 times (fall, winter and spring) during each school year and the average score was used for analysis.

9.2.6 *Statistical Analyses*

We compared baseline age, height and weight (body size), lean and fat mass (body composition) and bone parameters (BMC and BA) and dynamic power between control and intervention groups using independent t-tests. We similarly compared 20-month *change* in body size and composition, dynamic power, average physical activity and calcium intake between intervention and control groups. We used ANCOVA to evaluate 20-month change in our primary and secondary outcomes (BMC and BA). As there is considerable variability between children in a number of body size, maturity and lifestyle factors known to influence change in bone (21,92,109,287,288), we analyzed, and adjusted for these differences, by subject so as not to bias results by comparing school means. We adjusted for independent variables that were significantly associated with the dependent variable, including baseline height and BMC, change in height, final Tanner stage and extracurricular physical activity. Previous studies by the UBC Bone Health Research group and others (133,234,269,287) have highlighted the relationship between change in height, final Tanner stage and physical activity (average physical activity score) with change in bone mass. Significant correlations between independent and dependent variables were moderate to high and ranged from r=0.3 to r=0.7 for bone change versus physical activity, Tanner stage, baseline height, baseline BMC, and change in lean mass and height (Table 21).

Prospective studies that have examined the relative contributions of maturation, body size and composition (and their changes), and physical activity to regional bone mineral accrual during puberty in girls are scarce (12,88,138,285), and are generally lacking in an estimation of the influence of lean tissue mass. We developed hierarchical regression models to determine the unique contributions of 1) lean mass change; 2) maturity; 3) *change* in body size (height), 4) initial size (height and BMC); 5) general physical activity; and 6) specific physical activity in the intervention (coded as a dummy variable, where intervention =1). There was a theoretical basis for the chosen order of entry in the model. Lean mass may be the strongest predictor of bone mass during growth (21,92,151,234,288), and the muscle-bone relationship is well-established (40). Advancing maturity is a critical factor predicting bone mineral accrual, however, its interrelationship with lean change has not been examined. Previous studies (12,138) demonstrated the relationship between height gain and bone mineral gain, but again, this has not been weighted against the influence of lean mass. Initial status for height and BMC are important to change in bone mineral. Finally, the influence of general physical activity and exercise within the intervention program were evaluated when the effects of lean mass, maturation and initial status were controlled. Correlations between independent variables and the dependent variables (LS and FN BMC change), and intercorrelations between independent variables are tabled (Table 21). Dependent and independent variables were normally distributed. There was a linear relationship between each independent variable and the dependent variables. We examined each independent variable's contribution to the final model, and considered deletion of variables that did not contribute a significant R square change, and/or were not significant in the final model.

Data were analyzed with SPSS version 8.0, and significance was set at P<0.05.

9.3 *Results*

During Year 2, 2 control girls moved away, and 2 intervention girls withdrew following withdrawal of their classroom teacher from the study. Thus, 20-month data were collected for 50 control and 32 intervention girls. To balance differences in maturity between intervention and control groups, we excluded the only 2 girls (controls) who were postmenarcheal at baseline, as well as the 5 control school girls who were in grade 7. Therefore, we included 43 Control and 32 Intervention girls in the current analysis.

Girls at intervention schools participated in 45 ± 13 intervention sessions. To assess the possibility of a school effect we evaluated percent change in BMC across intervention schools (N = 87 girls) during Year 1. Seven-month percent changes (adjusted for change in body size, age, and maturity) were consistent across schools, with a narrow range of 3% at both the lumbar spine (11.1-14.5%) and femoral neck (6.9-9.9%).

Baseline data, and 20-month change in body size, composition and dynamic power are presented (Table 22). Control girls were 0.4 years older ($P<0.01$), and tended to be slightly taller and heavier (NS) than Intervention girls at baseline. A greater percentage of Control (98%; 42/43) than Intervention (84%; 27/32) girls were in Tanner stages 2, 3 and 4 at final measurements. Thirty-four percent (15/43) of controls and 25% (8/32) of intervention girls achieved menarche by final measurement. Intervention girls showed a trend toward improved long jump (7.6 *vs* 4.3%, $P = 0.10$) and vertical jump (35.7 *vs* 25.9%, $P = 0.14$) compared with control girls.

At baseline, girls in the control schools had significantly higher unadjusted TB ($P<0.05$), LS ($P<0.01$), and PF BMC ($P<0.05$) and FN BMC and area ($P<0.05$). Adjusted 20-month change in LS (41.7 *vs* 38.0%, $P<0.05$, eta squared=0.073) and FN BMC (24.8 *vs* 20.2%, $P<0.05$, eta squared=0.065) were significantly greater in the intervention group (means adjusted for baseline BMC, final Tanner stage, baseline height, change in height and physical activity score) (Table 23). The adjusted differences (intervention – control) in LS and FN BMC change by interval within the 20-month study are presented in Figure 22. Adjusted bone area changes were similar between control and intervention groups.

Regression: 20-Month Change in Lumbar Spine BMC. Results of the linear hierarchical regression, including all 7 predictors are presented (Table 24). The final model explained 73.1% of the variance in lumbar spine BMC change (final R Square). Entering lean mass change in the first step caused a significant R square change (0.511, $P<0.01$), however, due to intercorrelation between lean mass change and final Tanner stage ($r=0.63$), lean mass change was not significant in the final model ($P=0.48$). Entering average physical activity (6th step) did not cause a significant R square change, and was not significant in the final model ($P=0.14$). I performed the regression a second time, omitting lean mass change and average physical activity. The results of this simplified model are presented (Table 25). This final model explained 71.7% of the variance in lumbar spine BMC change (final R square), with each independent variable eliciting a significant R square change. Final Tanner stage accounted for 41.3% of the variance in lumbar spine BMC change in the first step. In subsequent steps, height change (11.2%), baseline height (6.3%), baseline lumbar spine BMC (10.9%), and intervention or control group (2.0%) caused significant changes in R square. All independent variables were significant in the final model (Table 25), with the exception of baseline height, which was retained due to a trend for a baseline difference in height between control and intervention groups. In this final model, all residual values were ± 2.5 standard deviations (99% were ± 2 standard deviations), and of random pattern.

Regression: 20-Month Change in Femoral Neck BMC. Results of the linear hierarchical regression, including all 7 predictors are presented (Table 26). The final model explained 58.1% of the variance in femoral neck BMC change (final R Square). Entering lean mass change in the first step caused a significant R square change (0.48, P<0.01). Similar to that observed for the previous model (lumbar spine BMC change), lean mass change was not significant in the final model (P=0.19), due to the intercorrelation between lean mass change and final Tanner stage. Entering height change (3rd step) did not cause a significant R square change (0.3%, P=0.54), and was not significant in the final model (P=0.90). As well, baseline femoral neck BMC did not contribute significantly to the model (R square change 0.1%, final P value = 0.60). The contribution from intervention group membership was not significant in this model (P=0.06). I performed the regression a second time, omitting lean mass change, height change, and baseline femoral neck BMC. The results of this simplified model are presented (Table 27). This final model explained 55.6% of the variance in lumbar spine BMC change (final R square), with each independent variable eliciting a significant R square change, and remaining significant in the final model. Final Tanner stage accounted for 25.6% of the variance in femoral neck BMC change in the first step, with baseline height (12.4%), general physical activity (14.5%), and intervention or control group (3.0%) contributing significantly to the model in subsequent steps. In this final model, all residual values were ± 2.3 standard deviations (97% were ± 2 standard deviatons), and of random pattern.

Table 21: Correlations between independent variables and 20-month change (Δ) in bone mineral content (BMC) in pooled sample of Intervention and Control girls (N = 75).

	Lumbar Spine BMC Δ	Femoral Neck BMC Δ	Final Tanner Stage	Baseline BMC (LS)	Baseline BMC (FN)	Baseline Height	Height Δ	Lean Mass Δ	Physical Activity Score
Final Tanner Stage	0.643*	0.506*	---	0.30**	0.28*	0.455**	0.42**	0.63**	-0.08
Baseline BMC (LS)	0.558*	---	0.30**	----	0.73**	0.76**	0.06	0.38**	-0.05
Baseline BMC (FN)	---	0.408*	0.28*	0.73**	---	0.62**	0.13	0.46**	0.03
Baseline Height	0.532*	0.544*	0.46**	0.76**	0.62**	---	0.24	0.55**	-0.11
Height Δ	0.575*	0.427*	0.42**	0.06	0.13	0.24*	1.000	0.74**	0.24*
Lean Mass Δ	0.699*	0.654*	0.63**	0.38**	0.46**	0.55**	0.74**	---	0.18
Physical Activity Score	0.153	0.313*	-0.08	-0.05	0.03	-0.11	0.24*	0.18	---

*P<0.05; **P<0.01

Table 22: Baseline Characteristics and 20-Month Change (Δ) for Control and Intervention girls.

	Control (N = 43)	% Change	Intervention (N = 32)	% Change
Baseline Age (yr)	10.3 (0.4)**		9.9 (0.6)	
Final Tanner Stage (1/2/3/4/5)	1 / 15 / 21 / 6 / 0		5 / 13 / 12 / 2 / 0	
# Postmenarcheal at Final	15		8	
# Asian/Caucasian/Other	10 / 24 / 9		12 / 14 / 6	
Baseline Height (cm)	142.4 (6.6)		139.6 (8.6)	
Δ in Height (cm)	11.1 (2.2)	7.8 (1.7)	11.1 (2.6)	7.9 (1.6)
Baseline Weight (kg)	38.5 (8.6)		34.6 (8.4)	
Δ in Weight (kg)	9.6 (3.2)	25.4 (7.8)	8.6 (4.0)	24.8 (9.4)
Baseline BMI (kg/m^2)	18.8 (3.3)		17.6 (3.2)	
Δ in BMI (kg/m^2)	1.5 (1.1)	7.8 (5.8)	1.2 (1.2)	7.0 (7.0)
Baseline Sitting Height (cm)	75.3 (3.7)		73.6 (4.2)	
Δ Sitting Height (cm)	5.6 (1.5)	7.3 (2.3)	5.5 (1.9)	7.4 (2.4)
Baseline Leg Length (cm)	67.0 (3.6)		66.1 (5.3)	
Δ Leg Length (cm)	5.6 (1.5)	8.3 (2.3)	5.5 (1.7)	8.3 (2.4)
Baseline Lean Mass (kg)	25.8 (4.1)		24.3 (4.2)	
Δ Lean Mass (kg)	6.6 (1.8)	25.9 (6.5)	6.3 (2.7)	25.6 (9.4)
Baseline Fat Mass (kg)	11.3 (5.7)		9.2 (4.6)	
Δ Fat Mass (kg)	2.5 (2.3)	23.2 (20.2)	1.8 (1.9)	21.9 (17.9)
Baseline Long Jump (cm)	123.7 (16.7)		121.8 (17.8)	
Δ Long Jump (cm)	3.5 (12.4)	3.4 (10.5)	8.3 (12.9)	7.6 (11.4)
Baseline Vertical Jump (cm)	22.3 (4.9)		20.9 (4.7)	
Δ Vertical Jump (cm)	5.4 (5.0)	25.1 (26.2)	6.7 (4.7)	35.7 (32.6)
Average Physical Activity Score (/5)*	2.9 (0.5)		2.9 (0.5)	
Average Loaded Activity (hr/wk)*	5.4 (3.0)		5.8 (3.6)	
Average Sport Nights (#/week)*	1.9 (1.4)		1.7 (1.5)	
Average Calcium Intake (mg/day)*	907 (363)		899 (478)	

*Values are average of 6 assessments (Fall, Winter, Spring x 2 school years).
**Control mean greater than Intervention group, P<0.01, independent t-test.

Table 23: Baseline and adjusted 20-Month change (Δ) in total body, lumbar spine, proximal femur, femoral neck, and trochanter bone mineral content (BMC) and area (for regions) in Intervention and Control girls.

	Control (N = 43)		**Intervention (N = 32)**	
	Baseline (SD)	Δ (95% CI)*	Baseline (SD)	Δ (95% CI)*
Total Body BMC (g)	1121 (220)	333 (310-356)	1008 (192)	359 (332-386)
Lumbar Spine Area (cm^2)	39.7 (4.4)	7.3 (6.7-8.0)	37.4 (5.5)	7.5 (6.7-8.2)
Lumbar Spine BMC (g)	26.5 (5.7)	9.3 (8.4-10.1)	22.5 (4.5)	10.9 (9.9-11.9)[a]
Proximal Femur Area (cm^2)	23.5 (3.1)	5.3 (5.0-5.7)	22.4 (3.3)	5.3 (4.9-5.7)
Proximal Femur BMC (g)	16.5 (4.0)	6.7 (6.3-7.1)	14.8 (3.3)	6.8 (6.3-7.3)
Femoral Neck Area (cm^2)	4.2 (0.3)	0.35 (0.29-0.41)	4.1 (0.4)	0.42 (0.34-0.49)
Femoral Neck BMC (g)	2.8 (0.5)	0.55 (0.48-0.61)	2.5 (0.5)	0.66 (0.58-0.73)[a]
Trochanter Area (cm^2)	5.8 (1.3)	2.1 (1.9-2.2)	5.3 (1.3)	1.9 (1.7-2.1)
Trochanter BMC (g)	3.2 (1.1)	1.9 (1.8-2.1)	2.8 (0.9)	1.8 (1.6-1.9)

*Adjusted for baseline bone value, baseline height, final tanner stage, change in height, average physical activity.

[a] Change is significantly greater in Intervention group, P<0.05, ANCOVA.

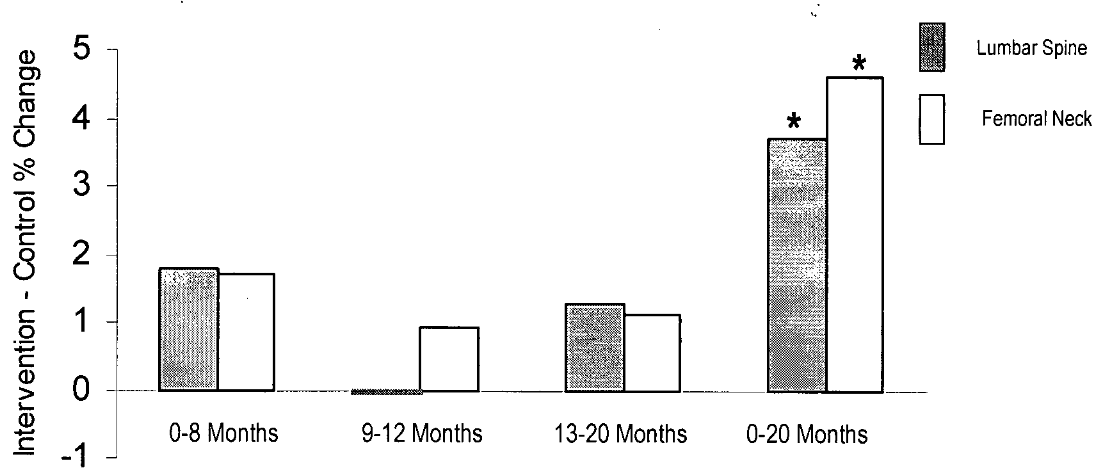

Figure 22: Difference in adjusted lumbar spine and femoral neck bone mineral content (BMC) % change (Intervention - Control group) over 0 – 8 Months (first intervention period), 9 – 12 Months (no intervention), 13 – 20 Months (second intervention period), and 0 – 20 Months (study duration).

*Difference in adjusted 0 – 20 Month changes were significantly greater in the intervention group (P<0.05). Differences at other timepoints NS. (Adjusted for baseline BMC and height, change in height, Tanner stage at end of interval, and average physical activity, ANCOVA).

Table 24: Results of hierarchical regression model (#1, all independent variables included) for lumbar spine bone mineral content 20-month change, N = 43 Control, N = 32 Intervention.

	R Square Change	Significance of F Change	Final Unstandardized Coeffiecients	Final Standardized Coefficients	Significance in Final Model	Final Model R Square
						0.731
Constant			-6.235		0.492	
Lean mass change	0.511	<0.001	2.079E-04	0.093	0.478	
Final Tanner stage	0.060	0.002	2.509	0.400	<0.001	
Height change	0.008	0.244	0.668	0.318	0.003	
Baseline height	0.026	0.033	-0.109	-0.168	0.139	
Baseline BMC	0.099	<0.001	0.509	0.569	<0.001	
General physical activity score	0.007	0.196	1.052	0.103	0.138	
Intervention or control group	0.018	0.036	1.535	0.154	0.036	

*Dummy variables used to designate intervention or control grouping (intervention (1), control (0)).

Table 25: Results of hierarchical regression model (#2, simplified) for lumbar spine bone mineral content 20-month change, N = 43 Control, N = 32 Intervention.

	R Square Change	Significance of F Change	Final Unstandardized Coefficients	Final Standardized Coefficients	Significance in Final Model	Final Model R Square
Constant			-4.104		0.603	0.717
Final Tanner Stage	.413	<0.001	2.562	0.408	<0.001	
Height Change	.112	<0.001	0.855	0.407	<0.001	
Baseline Height	.063	0.002	-0.113	-0.174	0.116	
Baseline BMC	.109	<0.001	0.534	0.597	<0.001	
Intervention or control group	.020	0.030	1.574	0.158	0.030	

*Dummy variables used to designate intervention or control grouping (intervention (1), control (0)).

Table 26: Results of hierarchical regression model (#1, all independent variables included) for femoral neck bone mineral content 20-month change, N = 43 Control, N = 32 Intervention.

	R Square Change	Significance of F Change	Final Unstandardized Coefficients	Final Standardized Coefficients	Significance in Final Model	Final Model R Square
Constant			-2.345		<0.001	0.581
Lean mass change	0.408	<0.001	2.970E-05	0.225	0.190	
Final Tanner stage	0.017	0.147	9.946E-02	0.269	0.019	
Height change	0.003	0.536	-1.983E-03	-0.016	0.901	
Baseline height	0.043	0.020	1.267E-02	0.330	0.005	
Baseline BMC	0.001	0.757	3.554E-02	0.060	0.589	
General physical activity score	0.087	0.001	0.200	0.332	0.000	
Intervention or control group*	0.022	0.062	9.564E-02	0.163	0.062	

*Dummy variables used to designate intervention or control grouping (intervention (1), control (0)).

Table 27: Results of hierarchical regression model (#2, simplified) for femoral neck bone mineral content 20-month change, N = 43 Control, N = 32 Intervention.

	R Square Change	Significance of F Change	Final Unstandardized Coefficients	Final Standardized Coefficients	Significance in Final Model	Final Model R Square
Constant			-2.918		<0.001	0.556
Final Tanner stage	0.256	<0.001	0.140	0.378	<0.001	
Baseline height	0.124	<.001	1.712E-02	0.446	<0.001	
General physical activity score	0.145	<0.001	0.235	0.390	<0.001	
Intervention or control group*	0.030	<0.032	0.106	0.181	0.032	

*Dummy variables used to designate intervention or control grouping (intervention (1), control (0)).

9.4 *Discussion*

This school-based intervention is a safe, effective, relatively simple and inexpensive program of diverse activities that can be implemented in elementary school physical education to enhance bone mineral accrual during childhood. The nearly 5% benefit we observed at 20 months in girls at the exercising schools was approximately double that seen in our 7-month intervention in these girls. This is the first study to demonstrate that a targeted exercise program during early- and peri-puberty has a cumulative (approximately 2% per school year) bone benefit (287) (Figure 22) compared with accrual rates for same-age children who are normally active.

9.4.1 *The Exercise Intervention*

The magnitude of BMC change over two years in the present study approximated the reported gains over 9-10 months in two studies that examined girls at a similar maturational stage (Tanner stages 1 – 3 at baseline) (23,234). These after-school, weight-bearing exercise programs were implemented for 30 – 35 minutes, 3 times/week (23,234) compared to our 10 –15 minute, 3X/week school-based session.

The girls in the present study were already active and performed, on average, 5.6 hours of recreational loaded physical activity per week. Thus, these brief sessions of high impact activity were designed to impose diverse patterns of movement that elicited ground reaction forces and rates of force beyond those associated with daily activities or running (229). This strategy may elicit an optimal osteogenic adaptation in growing bone (58,306). Further, the short duration program imposed minimal demands on the large team of teachers who delivered the program.

9.4.2 *Skeletal Site-Specificity of the Intervention Response*

The skeletal response we observed was region and site-specific. We previously reported significantly greater gains for LS and FN BMC after 7 months of exercise in our initial cohort (287) (Chapter 7, Part II). An augmented response to exercise may also be maturity, sex- and surface-specific. The present findings agreed with a similar site-specific response in Finnish premenarcheal, but not postmenarcheal, girls (23). Similarly, a 7-month intervention elicited significantly greater changes at the proximal femur in early but not pre-pubertal girls (287). Either decreased endosteal expansion or increased endosteal apposition (as measured by the hip structural analysis (94)) underpinned these differences in mass and structure at the proximal femur at 8 months in these girls (133). We have also reported greater gains for TB BMC, and PF and TR aBMD in prepubertal boys (305) and for trochanteric aBMD in a combined group of younger (8.9 years) prepubertal boys and girls following a more moderate intervention (92).

9.4.3 *Determinants of Change in BMC*

The regression models for both lumbar spine and femoral neck BMC change generally agree with previous short-term longitudinal studies of early pubertal girls (234,269,307), and uphold a central role for lean mass and maturity in BMC gain over 20 months. However, the strong relationship between lean mass and maturity was also highlighted, and including both of these predictors in the models explained little more variance than including maturity alone (simplified models). Initial size and change in size appeared to be more strongly associated with gain in lumbar spine than femoral neck BMC.

General physical activity did not account for variance in lumbar spine BMC change. However, membership in the exercise intervention group positively influenced bone mineral accrual at this region, accounting for a small, but significant portion of the variance (2.0%) after body size, body composition and maturity were controlled.

In contrast, general physical activity accounted for a large portion of the variance in femoral neck BMC gain (8.7% when lean mass was included in the model, 14.5% when lean mass was excluded). Membership in the intervention group contributed significantly (3.0%) to the femoral neck model only when lean mass was excluded. The modification of the variance attributable to physical activity by the inclusion of lean mass in the regression model agrees with previous literature suggesting that the effect of exercise on bone is mediated by muscle (95,308).

9.4.4 *Effectiveness of the School-Based Exercise Intervention*

Although attrition *within* school years of the study was low (only 4% of the cohort did not return for measurements at the end of the school year), attrition *between* Year 1 and Year 2 was high (61% of the original intervention cohort). However, 70% of dropouts arose when teachers chose not to participate in the study. Thus, success of school-based intervention depends on recruitment at all levels: School Board, principals, teachers, students and parents. However, to put the nature of school-based participation in perspective, a total of approximately 470 Richmond students, who *were not* enrolled for measurement in our study, performed the intervention exercises on a regular basis with their classes during Years 1 and 2. Thus, school physical education provides a feasible means to promote a strategy of exercise known to enhance bone mass and bone strength (133) gains in early puberty. We suggest that an emphasis on physical education training of teachers within the school systems might increase the effectiveness and long-term incorporation of physical activity initiatives within the curriculum (309).

9.5 Summary

In summary, the exercise intervention, implemented over 2 school years, augmented 20-month bone mass gain at the LS and FN by 3 to 5% in initially, 10-year- old girls. These results suggest that an exercise program begun in early puberty may result in a significantly greater peak bone mass and a reduced risk of fragility fractures in later life, compared with no involvement during these formative years. Investigations in retired, elite female dancers (13) and gymnasts (14) suggest that a bone mineral advantage, attributable to exercise training in childhood, may be maintained in adulthood. Should this 5% bone mass accrual advantage persist into adulthood, this may translate into a considerable reduction in osteoporotic fracture risk (278). Follow up of the girls in our study at full maturity is necessary to ascertain whether this maturational period represents a critical period when environmental stimuli evoke persistent adaptations in structure or function.

10 - Integrated Discussion

This chapter integrates the findings of the four major parts of this thesis. I discuss the effects of (i) ethnicity, (ii) maturity, and (iii) exercise on bone mineral status and bone mineral accrual in this cohort of pre and peri-pubertal boys and girls. Limitations of the studies are discussed in each of these sections, and these provide avenues for future pediatric bone research. The chapter closes with conclusions for each of the four studies.

10.1 Asian and Caucasian Ethnicity and Bone Mineral Accrual

10.1.1 *Ethnic Differences in Bone Mineral in Prepubertal and Early Pubertal Asian and Caucasian Children*

The results from Parts I and III highlight some unique aspects of this cohort and the interrelationships between ethnicity, lifestyle and bone mineral status (Parts I and III) or accrual (Part III). The cross-sectional comparison of girls in Tanner stage 1 and 2 suggested a fairly strong association between ethnicity and bone mineral status in early puberty, while this relationship was lacking in prepuberty. I found large differences (~+10%, adjusted for body size and composition) across several skeletal sites and bone parameters in favour of the Tanner stage 2 Caucasian girls compared to the Tanner stage 2 Asian girls. In girls, ethnicity contributed significantly to variance in femoral neck aBMD, after controlling for body size and composition, and lifestyle factors in hierarchical linear regression (Part I). No previous study has evaluated bone mineral content or density in a cohort of exclusively Tanner stage 2 Asian and Caucasian girls. However, earlier cross-sectional studies suggested that ethnic differences in bone mineral in Asian, Caucasian and Black girls may become more apparent with increasing maturity (172-174).

Few differences in bone mineral were noted between Asian and Caucasian *prepubertal* girls (Part I) or boys (Part III). The only significant differences were in bone mineral content, a bone parameter strongly influenced by bone size. This finding is supported by previous work in *prepubertal* children, where all bone parameters by DXA (BMC and aBMD) were remarkably similar in Asian and Caucasian prepubertal girls (21). Although there was a trend for aBMD at the proximal femur to differ in the prepubertal boys (21), this difference disappeared when data were adjusted for body size and composition. Horlick and colleagues (171) found no significant differences between total body BMC in *prepubertal* Asian and Caucasian girls and boys. However, in this study, there was a trend for prepubertal Caucasian girls to have higher total body BMC than Asian girls (1240 *vs* 1156 grams), which is consistent with the results of the study of prepubertal girls in Part I. There were no significant ethnic differences in 8-month bone mineral *gain* in the prepubertal boys (Part III). This largely agrees with the previous study from our research group (21), in which young prepubertal Asian and Caucasian girls and boys accrued similar amounts of bone over 8 months. Thus, my thesis provides very little evidence to support an ethnic difference in bone mineral status or gain in *prepubertal* boys or girls, after adjusting for body size. However, the few observed differences in BMC may indicate ethnic differences in bone mineral that might emerge with increasing maturity.

10.1.2 *Mechanisms Underpinning Ethnic Differences in Bone Mineral*

Due to the inherent limitations of our cross-sectional study (Part I), and including *only* prepubertal boys in Part III, I can only speculate as to mechanisms behind an emerging difference in bone mineral with advancing maturity. It is interesting to note that the physical activity levels of prepubertal girls (Part I) and boys (Part III) did not differ greatly with ethnicity. However, there were notable ethnic discrepancies between hours of loaded physical activity and extracurricular sports participation in *early pubertal* girls (both nearly double in Caucasian girls, (Part 1). It is possible that there was a synergy between increasing levels of physical activity in the Tanner stage 2 Caucasian girls and their higher bone mass. Tanner stage 2 Asian girls, on the other hand, had *lower* levels of loaded physical activity than both Asian and Caucasian *prepubertal* girls. The connection between advancing maturity and lower levels of physical activity has been documented previously (249,310). It appears that, in this cohort, with the onset of puberty, Asian girls may be reducing their level out of physical activity more than Caucasian girls. Thus, they may not be taking full advantage of this critical time for bone gain. However, definitive investigation of the links between ethnic-specific lifestyle and bone health requires longitudinal analysis.

Calcium intake is another lifestyle factor that differed between ethnicities, but large discrepancies between Asians and Caucasians were limited to the *girls* in this cohort (Part I, Asian ~600 mg/day; Caucasian ~980 mg/day). Although there was a trend for greater calcium intakes in the prepubertal Caucasian than Asian *boys* (Part III, Asian ~750 mg/day; Caucasian ~950 mg/day), these differences did not compare with the 40% differences that existed between Caucasian and Asian girls in both Tanner stage 1 and 2 (Part I). These intakes are higher than those observed in previous studies of Asian children living in Asian countries (178,268). There is evidence that the percent calcium retention may be higher when calcium intake is lower (268). These results coupled with a generally weak (girls) or nonexistent (boys) relationship between calcium intake and bone mineral *status* (Tables 8 and 9), and lack of association between calcium intake and bone mineral *gain* (Tables 10 and 11), suggest that nutritional intakes may have been adequate for bone mineral accrual in this cohort. However, the limitations of our assessment tool cannot be ignored. The food frequency questionnaire, although tailored to include popular Asian foods that are high in calcium, is a recall assessment, that has limited reliability in this young population.

Finally, there may be genetic differences between Asians and Caucasians that set the blueprint for bone mass and structure differences between these ethnic groups. The limited research in this area suggests differing frequencies of various polymorphisms, such as those related to the Vitamin D receptor (VDR) gene (187,311), may exist between races. One study analyzed the VDR gene Fok1 polymorphism in a mixed-ethnic group of 72 children (64 girls, Tanner Stage 1 to 3) (187). Calcium absorption was measured by the relative 24-

hour urinary recovery of a calcium stable isotope (187). There were fairly large (>8%) differences in total body aBMD by genotype, which might be explained by great differences in calcium absorption: children with the FF genotype had 41.5% and 17% greater calcium absorption than those with Ff and ff genotypes, respectively (187). When the analysis was limited to prepubertal children, the aBMD differences between genotypes were not significant. It is possible then, that the *FokI* polymorphism accounts for more variation in aBMD during puberty, and it can be speculated that this relates to a greater need, and capacity (312), for maximal calcium absorption during this time. Furthermore, polymorphisms that predict bone mass may be genetically linked to maturation or body size (164). The role of maturation in the emergence of genetic differences in bone parameters is supported by our observation of large, significant differences in BMC and aBMD between Asian and Caucasian girls in Tanner stage 2 *only* (Part I), and previous studies that demonstrated greater disparity between Black and Caucasian girls' spinal cancellous bone density (by QCT) with increasing Tanner stage (172,173). Identifying genetic variations that predict bone mass and structure offers the potential to contribute to explaining inter-racial differences in bone health. Further, identification of 'at-risk' children could provide focus for intervention programs targeting children susceptible to inadequate bone gain during growth.

10.2 Maturation, Body Composition and Bone Mineral Accrual

The role of maturation in bone mineral status and accrual has long been recognized (140). This thesis has made a novel contribution to this area by highlighting the ethnic differences in bone mineral status between Tanner stages 1 and 2 (Part I), describing the variance in bone mineral status and 20-month change that is accounted for by maturational influences, body size and composition (Part I and Part IV), and providing initial evidence of a time during early puberty when bone might be more responsive to exercise (Part II).

10.2.1 *Tanner Stages and Bone Mineral Accrual*

As evidenced by the results of Part I, advancement into early puberty does not necessarily guarantee a large increase in bone mineral. Figure 19 illustrates the differential effect of maturational stage on bone mineral status between Asian and Caucasian girls. Caucasian girls in Tanner stage 2 had 8% greater aBMD at the femoral neck as compared to Tanner stage 1 girls of the same ethnicity. There was only a 3% difference between Asian Tanner stage 1 and 2 girls. As previously indicated, this ethnic variation may be mediated by lifestyle factors or genetics, or both. Whether Asian girls have a later time of maximal increase in bone mineral content, a shorter accrual period, or a lower peak bone gain velocity than Caucasian girls can only be addressed with a longitudinal study. However, there is little doubt that pubertal advancement generally confers a bone mineral advantage, the magnitude of which varies among children and between ethnicities. This is supported by evidence from the regression analysis in Part IV, in which each higher Tanner stage was associated with a 2.6 and 0.14 gram bone mineral content advantage (unstandardized β) at the lumbar spine and femoral neck, respectively.

10.2.2 *Lean Mass, Fat Mass and Bone Mineral Accrual*

Maturation represents increases in body size and changes in body composition. The strong relationship between lean mass and bone mineral is substantiated with data from both our cross-sectional (Part I) and 20 month longitudinal (Part IV) studies, and is consistent with previous work (152,313). Muscle imposes the largest mechanical loads on bone, and the resultant bone strains influence the biological mechanisms that promote gains in bone strength (314). Gain in lean mass and bone mass is a natural part of the maturation process in girls (102); increased physical activity is not. (181) Thus, exercise during puberty could only enhance a natural progression towards increased muscle mass and peak bone mass.

During maturation, fat mass generally increases more in girls than boys (315). Fat mass was a significant predictor of femoral neck aBMD in Asian and Caucasian girls, explaining 2.8% of the variance at this site, after accounting for lean mass (Part I). This means that, in this cohort, having extra body weight in the form of fat mass was positively associated with aBMD. Currently, this is a controversial area of study. Studies that examined the relationship between high body weight or obesity and bone mass or structure in children are scarce. Goulding and colleagues (296) reported that although overweight children had higher absolute bone mass than normal weight children, their bone mass was less than that predicted based on body weight. This may be an inappropriate way to examine bone mass in obese children, if their skeletons can only compensate for body weight in small ways. We would not then expect their bone mass and strength to be incrementally greater than that of normal weight children (M. Leonard, Pediatric Nephrologist, personal communication, October 2001). More recently, speed of sound by quantitative ultrasound (an indicator of bone strength) was *lower* in obese than age- and gender-matched non-obese children (316), and was not influenced by the degree that the child was overweight. In Part III, prepubertal boys who were classified as High BMI (above the 75th percentile for BMI in this cohort) had higher percent body fat (33% vs 19%), BMC and aBMD at a range of sites than boys below the 75th percentile for BMI. How greater body weight and fat influences bone mineral accrual with maturation over the long term is an important area for further investigation, especially in light of the current concern over DXA inaccuracy with high percent body fat (86).

10.2.3 *Maturation, Exercise and Bone Mineral Accrual*

The idea of a 'critical window' during the maturational process when bone might respond optimally to mechanical loading through exercise is the subject of recent debate (126,130,304,317). Part II in this thesis was the first to examine how exercise influences bone mineral changes in pre- and early pubertal girls simultaneously. Our companion paper (133) recently highlighted the structural changes underpinning the observed gains in femoral neck bone mineral content and areal density in the early pubertal girls. As noted earlier in this thesis, early puberty (as distinct from prepuberty) offers a unique physiological environment that might especially suit promotion of bone adaptation with exercise. While recognizing the limitations of its cross-sectional design, Part I offers limited support for this theory, as the high levels of loaded physical activity in the early pubertal Caucasian girls may have contributed to their higher proximal femur and femoral neck BMC and aBMD as compared to Asian girls. Perhaps their physical activity participation occurred at a most advantageous time. As our investigation was limited to *prepubertal* boys in Part III, this thesis offers no insight into whether or not there exists a skeletally advantageous time for boys to exercise during maturation. The effects of exercise on the skeleton across the four studies are discussed in more detail in the following section.

10.3 The Effect of Exercise on Bone Mineral Accrual

Mechanical loading is a dominant force shaping the immature skeleton. The loads to which bones must adapt during growth largely dictate, in conjunction with genetics and nutrition, the amount and arrangement of bone tissue that will be available for structural support (75). The four determinants of whole-bone strength (and presumably, its likelihood of fracturing) relate to its material properties, the amount and kind of tissue present, its shape, size and distribution of tissue in space, and the fatigue damage present (318). Due to the limitations of dual-energy x-ray absorptiometry, we focused on the evaluation of the effect of exercise on the *amount of bone tissue* present, and generally omitted analyses of the material properties, architecture, and fatigue damage. Once the Hip Structural Analysis Program (94) became available, it was possible for researchers to quantify the magnitude of the structural response to exercise in children (133). It appears that, at the femoral neck, exercise-induced gains in cross-sectional area that promote bone strength are underpinned by a reduction in endosteal expansion in early pubertal girls (133). However, full discussion of these results are outside the scope of this thesis.

10.3.1 ***Factors Specific to the School-Based Intervention***

We implemented a program of exercise designed to provide high impact, progressive, and varied stimuli to the participating children's skeletons. This intervention program was described as *'moderate'* in a recent editorial (304), but represented a substantially more structured, progressive and challenging format than the previous intervention in Richmond (92). Whether our design considerations were achieved is an important issue.

- *Was the intervention 'high impact'?*

A persuasive, and ever-growing, body of literature suggests that extremely high impact exercise, such as gymnastics (14), racquet sports (22), Olympic lifting (221), and triple jumping (319), are more osteogenically advantageous than activities that confer a less pronounced impact to the young skeleton. However, the children in our study were not elite athletes, and therefore we had the task of designing an intervention composed of activities that children could perform, irrespective of skill level. Our program included various jumping activities with ground reaction forces (GRF) between 3.5 and 5.5 times body weight, which exceeds the GRFs associated with running activities in children (229). Notably, the GRFs of our circuit intervention were substantially *lower* than those of Fuchs and colleagues (8.8 times body weight) (227). This discrepancy is critical when comparing the results of their smaller cohort study with ours. Over 8 months, the prepubertal children in their study gained 3–4.5% more BMC at the lumbar spine and femoral neck, compared with a maximum between-groups difference of 3% in femoral neck vBMD in early pubertal girls (Part II) in our 8-month studies. Thus, relative to the intervention by Fuchs and colleagues (227), and one other similar intervention (23), our program may be more appropriately classified as 'moderate' impact. I believe it would be helpful if all authors quantified the ground reaction forces in future pediatric bone interventions.

- *Did the intervention present varied, novel, and unusual stimuli to the skeleton?*

The creative nature of the circuit intervention was one of its strengths. The strongest evidence to support the theory that our intervention did present unusual stimuli was that the children had to *learn* all the exercises – they had not previously performed the activities in their physical education classes. Learning took place at each of the three levels in Year 1, after demonstrations from our research team. The unusual distributions of strains encountered in the circuit exercises may have allowed for an osteogenic response (Parts II – IV), even in conjunction with a 'moderate' level of impact (58). In both birds (63) and sheep (38), strains typical of daily activities coupled with unusual loading patterns elicited a profound osteogenic response.

Strain *rate* is also an important modulator of bone adaptation. In Year 2 of our study, we increased the proportion of drop jumps that the children performed during intervention sessions. This modification was based on biomechanical assessment of the exercises (250) which indicated that drop jumps with a plyometric component had the highest strain rate of the jumps within the circuit. Interestingly, Judex and colleagues (10) recently demonstrated a 740% higher tarsometatarsal strain rate in roosters performing drop jumps than in roosters walking. Although strain distribution and strain magnitude differed marginally between the two activities, periosteal and endosteal bone formation rates increased dramatically in the jumpers (10). Thus, the inclusion of activities with high strain rate in the intervention circuit was likely a factor that promoted the bone response.

As yet, no controlled study has evaluated the effects of *modes* of exercise on bone mass or structural change in children at a single maturational stage. Thus, at this time, it remains difficult to recommend *one format* of exercise over another to promote the attainment of healthy peak bone mass and optimal bone structure. However, from this series of analyses (Parts II – IV), it is apparent that the circuit intervention offers bone benefits not incurred by the standardized physical education program in Richmond.

- *Design Limitations*

A design limitation of this school-based intervention is that children were randomized to control and intervention group *by school*. This represents a quasi-experimental design, which may result in the erroneous attribution of between-school differences to intervention outcomes. This design was the only feasible plan to test the effects of this large-scale intervention. Randomization by child would have resulted in control and intervention children sharing classrooms. As the intervention program took place within the physical education classes, it would have been impossible for teachers to limit participation by control children, and thus, the control group would most likely have been contaminated by exposure to the intervention.

There is no evidence to suggest that between-school differences contributed to the intervention results. First, to reduce the influence of the potential confound of ethnicity, we stratified by ethnic representation before randomizing schools. Second, to balance numbers between control and intervention groups, we also stratified by the number of children within schools who consented to participate. Number of children consenting to participate may have been a surrogate for teacher enthusiasm or dedication to the Healthy Bones program, and thus, this factor was balanced between intervention and control groups as well. Third, the between-school differences in baseline body size and total body BMC and their changes (Table 12) were slight, and merely reflected the percentage of grade 6 vs grade 4 participants at each school. The similarities in body size and total body BMC changes over 8 months suggest that children were growing at similar rates, regardless of school. Fourth, across schools, mean calcium intakes fell within a narrow range (712–959 mg, with large standard deviations) and mean physical activity scores showed little variation (Table 12). Thus, the children at schools in the Richmond School District that participated in our study were similar with respect to many potential confounding variables. Although randomization by school does present some important considerations, I feel it remains the optimum strategy when performing a truly school-based intervention.

10.3.2 *Regions of Skeletal Response*

Between Parts II and IV, there were some differences and similarities in the skeletal sites that responded to the intervention. These differences may be a function of sex or maturity of the focus group, or discrepancies in exercise execution.

First, there was a lack of skeletal response in the prepubertal intervention girls in Part II, which contrasts with the significant response in the prepubertal boys (Part III). The reasons for this discrepancy can only be speculated, and may be related to differences in maturity between 10-year-old prepubertal girls and boys. Also, one of the inherent limitations of a school-based intervention is that the effort and proper execution of the jumps could not be monitored across children, as would be done in a laboratory-based program. Thus, it is possible that the girls participated with less vigour, or with different landing or take-off strategies than boys (320,321).

Second, it is intriguing that we observed greater change in *trochanteric* aBMD in the intervention boys in both the primary (NS) and secondary (average BMI boys only, $P<0.05$) analyses of Part III. The only other study to show a significant effect of exercise at the greater trochanter in children (notably – a combined group of boys and girls) was from our Bone Health Research Group (92). Other studies in *girls* at various maturational stages (23,234), and Parts II and IV in this thesis, have consistently shown effects at the *femoral neck*. Further, observations from our cross-sectional study of Asian and Caucasian girls (Part I) offer an interesting complement to these findings. Loaded physical activity differed greatly between Asian and Caucasian girls in

Tanner stage 2, as did BMC and aBMD at the proximal femur and femoral neck. However, bone parameters at the trochanter remained similar between ethnicities, suggesting a lesser effect of physical activity at the trochanter than the femoral neck in these girls. The studies within this thesis provide some indication that the regional specificity, within the proximal femur, of the skeletal response to exercise may be related to sex and/or maturity, although this speculation is premature and requires further exploration.

Third, the response we observed at the lumbar spine in Parts II and IV and in the secondary analysis of Part III is consistent with previous work (23,226,227,234). However, it was not entirely expected, due to the moderate impact of our intervention program. We would expect the GRFs generated in the legs to be greatly dissipated by the time they reached the spine (272). Thus, the influence of trunk muscle pull may have been substantial to elicit the observed osteogenic response.

10.3.3 *Exercise Intervention in Elementary Schools: Benefits and Maintenance of a Bone Response*

Overall, Parts II – IV provide evidence of a region-specific positive effect of a school-based loading intervention on bone mineral accrual in early pubertal girls and prepubertal boys. Results from the 20-month analysis in Part IV suggest that similar gains (+~2%) are made in each school year of intervention, resulting in a widening gap in the bone mineral advantage in favour of the intervention children. To date, we have no follow-up study of an exercise intervention in children, thus, we do not know whether or not any bone mineral advantage remains once the exercise stimulus is withdrawn. Retrospective studies in adults suggest that the bone benefits incurred with high level training during growth may linger (13,14). Although the limited research suggests a weak relationship between childhood physical activity and that which is sustained in adulthood (322), the health benefits of participation during youth and over a lifetime, in relation to incidence of chronic disease, are substantial (1). Furthermore, contrary to a popular focus in health care research and fund allocation, the burden of osteoporotic fracture in our society is unlikely to be solved cost-effectively with drug therapy. To prevent one fracture with the drug therapy approach, "large numbers need to be exposed to the costs {of drug treatment}, inconveniences, and side effects ... at no benefit whatsoever" (304). Over the long term, physical activity represents a more appropriate, cost-effective solution, with multiple, positive health implications.

Schools are our best avenue for promoting and encouraging active lifestyles for children, and given the low level of activity of many British Columbia children (323), exercise interventions within this system are warranted. Our intervention was simple and feasible, and adopted and completed by 15 of 16 intervention teachers (1 chose not to participate) approached during Year 1, and 15 of 22 intervention teachers (6 chose not to participate and 1 did not complete the year) approached in Year 2. Furthermore, by the end of Year 2, in excess of 800 Richmond children had participated in the 10-minute circuit program as part of their P.E. class, regardless of their participation in the study. Thus far, the long-term impact of school-based exercise interventions, with respect to adult physical activity and incidence of chronic disease, can only be speculated upon. However, the alternative to promoting physical activity during childhood is bleak - picture a Canadian society of overweight, diabetic, weak young adults, who will suffer hip fractures in the millions, upon reaching 60 years of age. We mustn't delay intervention efforts in the school systems: ... "anyone who remains a 'couch potato' throughout the adolescent growth spurt will grow bones only of the strength needed to sustain that sedentary condition, and if much stronger bones are subsequently needed, it could be too late." (5).

10.4 Summary and Conclusions

10.4.1 ***Part I: Lifestyle Risk Factors for Osteoporosis in Asian and Caucasian Girls***

Summary (Primary Objectives):

(a) Adjusted total body and femoral neck bone mineral content were significantly greater in Caucasian than Asian prepubertal girls (Tanner stage 1). Bone mineral content (BMC) at other regions and areal bone mineral density (aBMD) at all regions were similar between prepubertal Asian and Caucasian girls.

(b) Adjusted total body, proximal femur and femoral neck BMC and aBMD were significantly greater in Caucasian than Asian early pubertal (Tanner stage 2) girls. Bone parameters at other sites were similar between ethnicities, although there was a consistent trend in favour of the Caucasian girls.

Summary (Secondary Objectives):

(a) General physical activity, but not loaded physical activity or extracurricular sport nights, was significantly greater in Caucasian than Asian *prepubertal* girls.

(b) Loaded physical activity and extracurricular sport nights, but not general physical activity, were significantly greater in Caucasian than Asian *early pubertal* girls.

(c) Calcium intakes were significantly greater in Caucasian than Asian girls in both prepubertal and early pubertal groups. On average, girls of both ethnicities had calcium intakes below the recommended nutrient intake of 1300mg.

Conclusions

(a) In prepubertal girls, there are minor ethnic differences in bone mass for the total body and proximal femur, in favour of Caucasian girls. There is no association between ethnicity and adjusted aBMD in prepubertal Asian and Caucasian girls.

(b) In early pubertal girls, adjusted bone mineral content and aBMD for the total body, total proximal femur, and femoral neck are associated with ethnicity. Tanner stage 2 Caucasian girls appear to have a bone mineral advantage over their Asian counterparts.

(c) Prepubertal Asian and Caucasian girls appear to have fairly similar physical activity patterns. Early pubertal Caucasian girls are more active than their Asian counterparts.

(d) Calcium intake is associated with ethnicity, regardless of pubertal status. Asian girls consume less calcium than Caucasian girls, and explaining this risk factor for osteoporosis to children and parents may be warranted.

10.4.2 *Part II: A School-Based Loading Intervention Augments Bone Mineral Accrual in Early-, But Not Pre-Pubertal Girls*

Summary:

(a) Eight-month changes in BMC, aBMD, and vBMD did not differ significantly at any skeletal region between *prepubertal* intervention and control girls.

(b) Eight-month changes in lumbar spine BMC and aBMD, and femoral neck BMC, aBMD, and vBMD were significantly greater in *early pubertal* intervention than control girls. Eight-month changes in bone parameters for the total body, proximal femur and greater trochanter did not differ significantly between early pubertal intervention and control girls.

Conclusions:

(a) A 10 minute, 3 times/week jumping intervention appears to be an insufficient stimulus to elicit an osteogenic response in prepubertal girls.

(b) The simple school-based exercise intervention is a feasible strategy to elicit bone mineral advantages at the lumbar spine and femoral neck in early pubertal (Tanner stage 2 and 3) girls.

10.4.3 *Part III: Bone Mineral Response to a 7-Month Randomized Controlled, School-Based Jumping Intervention in 121 Prepubertal Boys: Associations With Ethnicity and Body Mass Index*

Summary (Primary Objective):

(a) Eight-month changes in total body BMC and proximal femur aBMD were significantly greater in boys at intervention schools than boys at control schools. Changes at other regions and for other parameters were similar between groups.

Summary (Secondary Objectives):

(a) Eight-month changes in total body and lumbar spine BMC, and proximal femur and trochanteric aBMD were significantly greater in exercise intervention boys of low or average BMI than controls.

(b) Eight-month changes in bone parameters at all sites were similar between Asian and Caucasian boys.

(c) Eight-month changes in bone parameters at all sites were similar between intervention boys with high BMI and their controls.

Conclusions:

(a) A 10-minute, 3 times per week jumping intervention implemented over 7 months in physical education classes elicited an osteogenic response for the total body and total proximal femur in prepubertal, 10-year-old Asian and Caucasian boys.

(b) Additional benefits of the intervention are observed in boys of low or average BMI, such that bone mineral gain at the lumbar spine and greater trochanter are positively affected.

(c) Ethnicity and 8-month bone mineral accrual are not associated (when maturity, body size and physical activity are adjusted) in prepubertal Asian and Caucasian boys.

(d) A 10-minute, 3 times per week jumping intervention may not be a suitable program of exercise to elicit osteogenic benefits in prepubertal boys with high BMI.

10.4.4 *Part IV: A School-Based Exercise Intervention Elicits Substantial Bone Health Benefits: A 2-Year Randomized Controlled Trial In Girls*

Summary (Primary Objective):

(a) Twenty-month changes in lumbar spine and femoral neck BMC were significantly greater in intervention than control girls. Changes in BMC at other regions were similar between groups.

Summary (Secondary Objective):

(a) Maturity accounted for the greatest amount of variance in 20-month change in lumbar spine and femoral neck BMC in this cohort of pubertal girls. General physical activity accounted for a significant amount of variance in 20-month change in femoral neck, but not lumbar spine, BMC. Membership in the intervention or control group accounted for a small, but significant portion of the variance in BMC change at both regions, most notable when lean mass change was excluded from the model.

Conclusions:

(a) A 10-minute, 3 times per week exercise intervention implemented over 2 school years confers bone mineral gains in the range of 3-5% at the lumbar spine and femoral neck in pre- and early pubertal girls. As this 20-month gain was double that observed in Part II, it appears that equivalent gains are made during each year of intervention participation.

(b) Maturity is a strong predictor of change in bone mineral in pre- and early pubertal girls. Change in lean mass has a strong association with maturity, and inclusion of both factors in a regression model does not improve the prediction of 20-month change in BMC. Physical activity has greater influence over bone mineral accrual at the femoral neck than the lumbar spine in pubertal girls. This highlights the need to consider controlling for these variables in pediatric bone research.

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11 - Appendices

Appendix 1: Information Letters and Consent Forms

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Healthy Bones Study

Heather McKay PhD, Kerry MacKelvie MSc, Moira Petit MSc

Information to Families:
At the University of British Columbia, we are beginning a study involving Grade 4, 5 and 6 students that will look at whether a program of physical activity, and specifically, jumping exercises benefit the bones of growing children. We will also look at the role of calcium and other nutrients on bone development. The results will provide important information on the role of physical activity and proper nutrition during the childhood years for the prevention of osteoporosis and bone fractures in later life. This is the second part of a study that began with grade 3 and 4 students during the 1997-98 school year. The first project was extremely successful and the Richmond Board of Education has granted approval for this next step in the study and your child's school has been selected. The principal and staff have endorsed this project and are making time available for the children to participate.

Half of the elementary schools that will be participating will complete a 10-15 minute circuit training program 3 times per week, while the other schools will participate in a 10 minute stretching program 3 times per week. Children who are involved in the study will have their bone status and growth and development measured two times over the year. The total time your child will be away from school for measurements will be approximately 2 hours in October and 2 hours in June. Bone measurements will be taken by a trained operator at the University of British Columbia Bone and Mineral Measurement Lab. Questionnaires (see pg. 3 for details) and a brief analysis of the forces involved in the circuit training program will also be completed during this visit. Children will be transported to the university in small groups and supervised en route by a study staff person in addition to the driver of the mini-van. Alternative, equally fun and educational activities will be made available for children who choose not to participate in the measurements. Questionnaires regarding physical activity, maturity status and nutritional patterns of the children will be administered three times over the year in the school classroom. Results of the study will be provided to you and your child at the completion of the study and "bone health" will be addressed within classroom lessons.

In addition to the bone density measurements, we are also interested in collecting information about your child that may help us explain the changes in growing bone that occur with the circuit training program. You may consent to your child's participation in the central Healthy Bones Study as outlined above without consenting to this additional component. We are exploring the contributions of bone-related genes to bone mineral density, which can be investigated from the analysis of DNA. This is a simple procedure where we run a Q-tip like swab over the inner cheek to collect cells from which DNA is extracted and analyzed.

An interesting addition to the current study is the evaluation of changes in bone shape by magnetic resonance imaging (MRI). This is an exciting aspect of the study, as the technique produces clear, cross-sectional pictures of bone and muscle tissue that cannot be obtained in bone density measurements. We will be selecting a very small group of girls to participate (with your consent) in this portion of the study. We will contact the families of those girls selected within the next 2 weeks by mail, and follow up with a phone call to explain the MRI procedures and answer any questions.

The Healthy Bones Study is an important investigation that will potentially offer new information aimed at the prevention of osteoporosis. If you agree to have your child participate in this study and undergo the

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Healthy Bones Study Consent Form for Families

Procedures:

Central Components:

> Your child's participation in the central part of the project will involve two testing sessions (approximately two hours each, including transportation time), one at the beginning and one at the end of the school year at the University of British Columbia in Vancouver. Each session will include the following procedures:

1. Measures of height and weight will be taken. In addition your child will be asked to complete questionnaires that will assess their physical activity, calcium intake and physical maturity. A trained study staff person will discuss the importance of these assessments with the children. A brief health history questionnaire will be sent home to be completed by a parent or guardian.

2. Characteristics of the bone in the heel area will be assessed by quantitative ultrasound. This is a very simple procedure that only requires that your child remain still for 2 minutes with his or her heel resting on the measurement device. There are neither risks or discomfort associated with this procedure.

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

4. At the October measurement time only, we will do a brief analysis of the biomechanical forces involved in the circuit exercises. This will take place at the UBC Biomechanics Laboratory, and simply requires that your child jump six times while the impact is measured. This will take approximately 10 minutes to complete.

Secondary Component:

> If you agree to have your child participate in the central study (as above), we ask you to consider your child's participation in this secondary component, as outlined below:

1. Research on the genetics of osteoporosis has recently identified specific genes that may be linked to high or low bone mineral density and which might, consequently, predispose individuals to the development of osteoporosis. We would like to collect a single sample of cells from your child's inner cheek which would allow us to identify these genes. This painless procedure takes about 2 minutes and simply involves passing a cotton swab over the inside of the cheek. The DNA extracted from the tissue sample will be identified by a number and analyzed for the presence of genes related to bone mineral density ONLY.

Rights and Welfare of the Individual:

You have the right to refuse your child's participation in this study. It is understood that you are free to withdraw your child from any or all parts of the study at any time without penalty. Your child's identity will remain confidential as all individual records and results will be analyzed and referred to by number code only and kept in the University of British Columbia Bone and Mineral Measurement Lab. The lab remains locked and only those directly involved in the study (namely, Dr. Heather McKay, Kerry MacKelvie and Moira Petit) will have access to your child's records and results. Your child will not be referred to by name in any study reports or research papers. Your child's individual results will remain confidential as they will not be discussed with anyone outside the research team (Dr. McKay, Kerry MacKelvie, and Moira Petit).

Please be assured that you may ask questions at any time. We will be glad to discuss your child's results with you and your child 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 (822-3120) or Kerry MacKelvie (822-0021)at the University of British Columbia. If you have any concerns about your child's rights or treatment as a research subject, please contact Dr. R. D. Spratley at the office of Research Services and Administration at UBC (822-8598).

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HEALTHY BONES STUDY CONSENT FORM

Parent's Consent Statement:

I, ____
(please print the name of one or both parents)
understand the purpose and procedures of this study as described and I voluntarily agree to allow my child, ______________________________, to participate in (please ✓):

____The central components of the Healthy Bones Study (height, weight, questionnaires, bone mineral density, ultrasound, and biomechanical (jumping force) measurements).

____I **do not agree** to have my child participate in the central components of the Healthy Bones Study.

Please check (✓) the secondary component below if you agree to the central components of the Healthy Bones Study and wish your child to participate in this part of the study.

____Bone-related gene analysis from a cheek smear.

____**I do not agree** to have my child to participate in the bone-related gene analysis.

I understand that at any time during the study we will be free to withdraw without jeopardizing any medical management, employment or educational opportunities. I understand the contents of all three 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 study.

______________________ Signature of Parent/Guardian	______________________ Date
______________________ Signature of Witness	______________________ Date
______________________ Signature of Investigator	______________________ Date

Child's Statement:
I understand the purpose and procedures of this study as described and I voluntarily agree to participate. I understand that at any time during the study, I will be free to withdraw without jeopardizing any medical management, employment or educational opportunities. I understand the contents of the consent 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 study.

______________________ Signature of Child	______________________ Date

健康骨骼研究計畫

親愛的家長們:

首先我們感謝您與您的孩子對於1997/98學年度[健康骨骼研究]計畫的成功與貢獻.此項[健康骨骼研究]計畫將於今年十月份在UBC進行第二階段的測驗.此項[健康骨骼研究]計畫將針對4,5,6年級國小學生的體育活動特別是跳躍運動加以進行測試進而瞭解是否運動有助於兒童在幼年時期的骨骼發育.再者我們也將針對鈣質以及其他養分的攝取加以進行測試進而瞭解是否鈣質以及其他養分的攝取有助於兒童在幼年時期的骨骼發育.此項[健康骨骼研究]計畫的結果將有助於我們對骨骼發育有更進一步的了解,甚至更可藉由此項研究計畫了解將來某些疾病如骨骼疏鬆症的預防.因此參與此項[健康骨骼研究]計畫的相關學校教師以及研究人員將適度的安排與調配時間以順利配合您的孩子參與此項 [健康骨骼研究]計畫.

此項[健康骨骼研究]計畫將在UBC進行,一路上我們將會有專門的研究人員在專車上照顧您的孩子.參與此項[健康骨骼研究]計畫的國小學童將會平均分配於兩組以便同時進行此項[健康骨骼研究]計畫.其中一組的國小學童將於每週進行3次的體能訓練.而此項體能訓練每次維時10至15分鐘.而另一組的國小學童將於每週進行3次的伸展運動訓練,而此項伸展運動訓練每次維時10分鐘.此項[健康骨骼研究]計畫將於今年十月份(所需的時間大約總共兩個小時)以及明年六月(所需的時間大約總共兩個半小時)分別進行所有關於您的孩子的體育活動,生長情形及養分的攝取問卷調查也會在未來的一年裏陸續完成.

因此在明年六月份,我們將會有專門的研究人員在 **UBC Biomechanics Laboratory** 爲此項[健康骨骼研究]計畫中做簡短的分析報告此外我們將會的有專門的研究人員也會在 **UBC Bone And Mineral Measurement Lab** 在未來的一年裏分別於兩次爲您的孩子針對您的孩子的骨骼特質以及生長情形加以詳細記錄做爲您對您的孩子未來生長情形之參考.

在此項[健康骨骼研究]計畫中,我們也由衷希望可以獲得您的同意進一步取得您的

健康骨骼研究計畫

家長同意書

[健康骨骼研究]計畫實驗步驟:

您的孩子所參與的此項[健康骨骼研究]計畫共包含兩部份.此項[健康骨骼研究]計畫將於今年十月份(所需的時間大約總共兩個小時包含接送時間)以及明年六月份(所需的時間大約總共兩個小時包含接送時間)分別在 **UBC** 進行.

[健康骨骼研究]計畫實驗步驟如下:

1.每次進行實驗時我們會有專門人員爲您的孩子解釋所有的實驗及測試的步驟.之後測量身高與體重.在進行實驗的同時,我們的專門人員會需要您的孩子爲[健康骨骼研究]計畫填寫有關您的孩子體育活動生長情形及鈣質的攝取之問卷調查.再者爲了確認您的孩子在測驗時所給我們有關日常活動及飲食習慣的資料,我們附上一份問卷給您,請您儘快完成.您的回答將有助於我們了解體育活動生長情形及鈣質的攝取等因素對於您的孩子骨骼發育影響.

2.每次進行實驗時我們會有專門人員爲您的孩子進行全身骨骼(包含臀部及脊椎)所有的測試.此項[健康骨骼研究]計畫中所有的實驗及測試都是無痛,而且所有的實驗及測試步驟以及所有的專門人員都經由加拿大政府審查及檢測合格.您的孩子在此項[健康骨骼研究]計畫中將會平躺於一張平台上,以便於我們的專門人員爲您的孩子進行全身骨骼X光的測試.此項X光的測試將會進行大約十五分鐘.

3.在明年六月份體能訓練測試將在 **UBC Biomechanics Laboratory** 進行.屆時我們會有專門人員爲您的孩子解釋及示範所有的體能訓練的動作.在此項[健康骨骼研究]計畫中您的孩子將會需要跳躍六次,而此項跳躍動作將維時10分鐘以便於我們的專門人員爲您的孩子進行測試及記錄.

在此項[健康骨骼研究]計畫中我們也由衷希望可以獲得您的同意進一步取得您的孩子的DNA以及超音波骨骼掃瞄資料.這些有關您的孩子DNA以及超音波骨骼掃瞄資料將有助於我們對於您的孩子的骨骼成長與發育有深一步的瞭解.此項[健康骨骼研究]計畫的目的在於研究鈣質的攝取量,日常生活的活動量,和遺傳之間的關係.父母親和小孩的

參與 健康骨骼研究計畫 同意書

家長同意書:

我本人_______________________________瞭解此項[健康骨骼研究]計畫的
(請清楚填寫家長或監護人姓名)

實驗目的以及各項步驟,因此我同意讓我的孩子_________________________
(請清楚填寫孩子的姓名)

參與此項[健康骨骼研究]計畫._請於下列項目中打勾

_______我同意讓我的孩子參與此項[健康骨骼研究]計畫(包含身高與體重骨骼密度以及跳躍訓練之測量).

_______我不同意讓我的孩子參與此項[健康骨骼研究]計畫.

如果您同意讓您的孩子參與此項[健康骨骼研究]計畫,並同意讓我們獲得您的孩子的DNA以及超音波骨骼掃瞄資料.請於下列項目中打勾

_______我們將會藉由棉花棒在您的孩子的口腔中取得口腔黏膜藉.

_______利用超音波掃瞄機測量您的孩子的後腳跟的骨骼特質.

我瞭解此項[健康骨骼研究]計畫實驗目的以及各項步驟.我的孩子亦可以隨時退出此項[健康骨骼研究]計畫,而不會侵害我及我的孩子的任何權益.如果我有任何問題,會有相關的專門人員爲我解釋並給我滿意的答覆.

_______________________________　　　　_________________________
家長或監護人簽名　　　　日期

_______________________________　　　　_________________________
見證人簽名　　　　日期

_______________________________　　　　_________________________
實驗人員簽名　　　　日期

您的孩子同意書

我瞭解此項[健康骨骼研究]計畫實驗目的以及各項步驟.我亦可以隨時退出此項[健康骨骼研究]計畫,而不會侵害我的任何權益如果我有任何問題,會有相關的專門人員爲我解釋並給我滿意的答覆.

____
您的孩子的簽名

日期 ________________________

THE UNIVERSITY OF BRITISH COLUMBIA

School of Human Kinetics
210, War Memorial Gym
6081 University Boulevard
Vancouver, B.C. Canada V6T 1Z1
Tel: (604) 822-3838 Fax: (604) 822-6842

The Healthy Bones Study

Heather McKay PhD, Kerry MacKelvie MSc, Moira Petit PhD
School of Human Kinetics, University of British Columbia

Information to Families: Summer 2000

We wish to express our sincere thanks to all of the children who have participated in the Healthy Bones Study during the 1999-2000 school year, and to their parents and guardians for their contributions thus far. We appreciate your interest in the project and the support you have given our research at the University of British Columbia. Enclosed you will find some of your child's results from the first year of the study. Results regarding the effects of the exercise program that some schools have been participating in will be made available to you and the Richmond elementary schools as the analyses are completed.

The Healthy Bones Study is an important investigation focused on the discovery of new information aimed at osteoporosis prevention. The results will provide important information on the role of physical activity and proper nutrition during the childhood years for optimal bone health throughout the lifetime. We have received further government funding to support this unique health research initiative, and our group is keen to continue the program during the next school year. Ideally, we would like to measure as many of the original participants (including your child) as possible. This would be the first study in the world to examine the effects of a 2-year exercise program on bone in an elementary school setting.

At this time, we would like to invite your child to participate in our study during the 2000-2001 school year. Should you choose to participate, we must receive parental/guardian consent. As all participants will likely have new classroom teachers next year, the schools that will be participating will not be determined until September when classes are finalized. Measurement procedures will be identical to those your children were familiarized with in the 1999-2000 school year: bone density measures, height and weight, long jump, vertical jump, and questionnaires. Your child will be measured at UBC in the fall of 2000, and the spring of 2001. Detailed information for all measurements is provided in the attached consent form. We ask that you please consider your child's participation for a second year, complete and sign the consent form and return it to us in the stamped envelope provided.

Again, thank you for your contribution thus far. We have enjoyed working with families and with the children in our laboratory and in their classrooms. Feel free to contact us at the numbers below if you have questions regarding the enclosed results or about your child's continued participation in The Healthy Bones Study.

Sincerely,

Kerry MacKelvie (822-0021)

Dr. Heather McKay (822-3120)

Healthy Bones Study Consent Form for Families

Procedures:

- Your child's participation in the second year of the Healthy Bones Study will involve two testing sessions (approximately 2 1/2 hours each, including transportation time), one at the beginning and one at the end of the school year at the University of British Columbia in Vancouver. Each session will include the following procedures:

1. Measures of height and weight will be taken. In addition your child will be asked to complete questionnaires that will assess their physical activity, calcium intake and physical maturity. A trained study staff person will discuss the importance of these assessments with the children. A brief health history questionnaire will be sent home to be completed by a parent or guardian.

2. Characteristics of the bone in the heel area will be assessed by quantitative ultrasound. This is a very simple procedure that only requires that your child remain still for 2 minutes with his or her heel resting on the measurement device. There are neither risks or discomfort associated with this procedure.

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

Rights and Welfare of the Individual:
You have the right to refuse your child's participation in this study. It is understood that you are free to withdraw your child from any or all parts of the study at any time without penalty. Your child's identity will remain confidential as all individual records and results will be analyzed and referred to by number code only and kept in the University of British Columbia Bone and Mineral Measurement Lab. The lab remains locked and only those directly involved in the study (namely, Dr. Heather McKay, Kerry MacKelvie and Moira Petit) will have access to your child's records and results. Your child will not be referred to by name in any study reports or research papers. Your child's individual results will remain confidential as they will not be discussed with anyone outside the research team (Dr. McKay, Kerry MacKelvie, and Moira Petit).

Please be assured that you may ask questions at any time. We will be glad to discuss your child's results with you and your child 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 (822-3120) or Kerry MacKelvie (822-0021)at the University of British Columbia. If you have any concerns about your child's rights or treatment as a research subject, please contact Dr. R. D. Spratley at the office of Research Services and Administration at UBC (822-8598).

THE UNIVERSITY OF BRITISH COLUMBIA

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6081 University Boulevard
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HEALTHY BONES STUDY YEAR II CONSENT FORM

Parent's Consent Statement:

I, ____

(please print the name of one or both parents)

understand the purpose and procedures of this study as described and I voluntarily agree to allow my child, ______________________, to participate in (please ✓):

____The Healthy Bones Study Year II (height, weight, physical activity / nutrition / maturity questionnaires, bone mineral density, and ultrasound)

____I do **not agree** to have my child participate in the Healthy Bones Study Year II.

I understand that at any time during the study we will be free to withdraw without jeopardizing any medical management, employment or educational opportunities. I understand the contents of all three 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 study.

______________________ ______________________

Signature of Parent/Guardian Date

______________________ ______________________

Signature of Witness Date

______________________ ______________________

Signature of Investigator Date

Child's Statement:

I understand the purpose and procedures of this study as described and I voluntarily agree to participate. I understand that at any time during the study, I will be free to withdraw without jeopardizing any medical management, employment or educational opportunities. I understand the contents of the consent 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 study.

______________________ ______________________

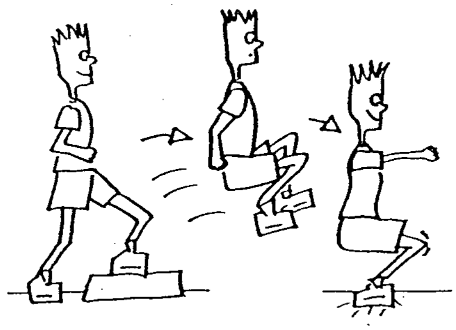
Signature of Child Date

Appendix 2: Excerpts from Intervention Manuals

RIDEAU PARK

STEVES

MITCHELL



BRIGHOUSE

GARDEN CITY

THE HEALTHY BONES STUDY 1999-2000

WESTWIND

FERRIS

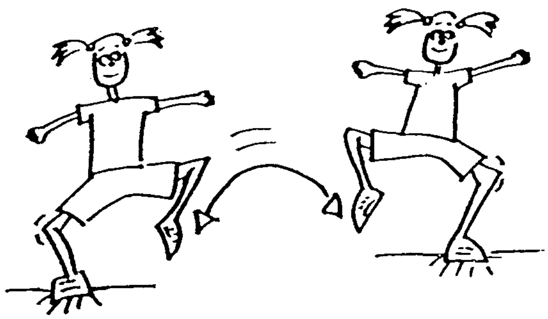
KILGOUR

KIDD

DIEFENBAKER

ERRINGTON

TAIT



LEE

WOWK

THE UNIVERSITY OF BRITISH COLUMBIA

The Healthy Bones Study

Lifestyle habits contribute significantly to the health of our bones throughout life. Although we cannot control the influence of genes on our bones, we can make educated decisions about physical activity and nutrition that may play important roles in osteoporosis prevention. Current research suggests that the positive effects of physical activity on the skeleton are greatest when the activities are implemented in the prepubertal years. It is at this time that our bones appear to be the most responsive to loading through weight-bearing exercise. Increases in bone strength and density in the growing years may have important implications for peak bone mass and the risk of osteoporotic fracture in later life.

The primary purpose of this study is to determine if jumping activities such as those presented in the circuits in this manual will benefit the bones of growing children. We will also examine the contribution of dietary calcium to bone health. Children, whose parents have consented for them to be measured, will have their bone status, growth, and development evaluated twice during the school year. We believe that the results of this unique study will provide important information regarding the roles of physical activity and calcium nutrition in the prevention of osteoporosis.

The teachers, principals and students within the Richmond elementary schools are key players in the success of this research. We thank you for your interest and participation, and hope that this program is educational and exciting for both teachers and students!

The UBC Bone and Mineral Research Team

The Healthy Bones Resource Manual

For your convenience, we have laid out this manual in several colour-coded sections. All games and movements conform to the existing guidelines for Physical Education in the Instructional Resource Package (IRP).

Part I (orange): Warm up activities and stretches to do prior to the high impact circuit. These games are ideal for a quick and fun warm up, and require very little equipment or organization. The activities could also be used on a day when the circuit set-up is just not possible, or as great, 'bone-friendly' P.E. activities upon completion of the Healthy Bones Study.

Part 2 (yellow): Level 1 Circuit Stations, to be used from the beginning of the study until Christmas holidays.

Part 3 (red): Level 2 Circuit Stations, to be used from January to Mid-March.

Part 4 (blue): Level 3 Circuit Stations, to be used from Mid-March until the end of the school year.

Part 5 (white): Ideas for teaching 'Healthy Bones' units in Science and Health. Pictures, diagrams, and worksheets can be easily photocopied to make overheads or hand-outs.

Healthy Bones Circuit Training

Circuit training is...

- ✓ fun
- ✓ interactive
- ✓ fast-paced
- ✓ time efficient
- ✓ adaptable
- ✓ progressive

... and we feel that for all these reasons, it provides a great format for the high impact program that is the key to Healthy Bones. It is essential that the students complete the 10-minute circuit 3 times per week. Stations within the appropriate level can be combined in any way, but due to the progressive nature of the program, stations from different levels are not combined within one circuit. Here are some general guidelines:

- ✓ Complete a brief warm-up prior to high impact circuit.
- ✓ Complete a circuit 3 times per week.
- ✓ Choose 5 stations from the appropriate level and mount the corresponding posters in the circuit space.
- ✓ Explain and demonstrate each station, and monitor correct techniques as laid out in the manual and on posters.
- ✓ Allow 1 to 2 minutes for the completion of the station activity and 1 minute total to travel between stations and become acquainted with a new station.
- ✓ Use a whistle or command of your choice to signal the beginning and end of stations.
- ✓ Have a set direction of movement through circuit.
- ✓ Use the musical tapes provided or other selections of your choice.

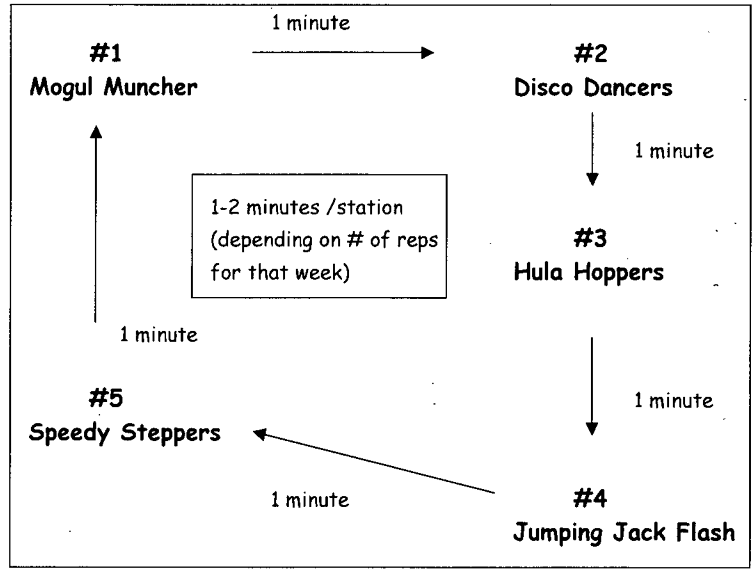
Circuit Format (see diagram)

Level 1 Circuits: October - December
Level 2 Circuits: January - Mid-March
Level 3 Circuits: Mid-March - May

Each circuit is made up of 5 loading stations (your choice) within the appropriate level. It is a good idea to create circuit-travelling teams of 5 - 6 students per team and then assign teams to a starting station. The students will spend approximately 1 to 2 minutes at each station, and rotate on your command (set a time for rotations, i.e., 1 minute is recommended to incorporate a rest and time to become familiar with the next station). During the first week at a new level, have the students do 10 repetitions at each station (if the jump alternates legs, have them do 10 on each leg), and increase the number of repetitions by 1 each week. For example, week 1: 10 jumps, week 2: 11 jumps, week 3: 12 jumps, etc. When a new level starts, go back to 10 repetitions and increase from there. We will provide color-coded posters that should be set up at each station, to remind students of the actions.

A demonstration from you or a capable student will help familiarize the students with the actions prior to starting the circuits. Ask students to practice new actions a couple of times before the circuit starts. It is important that the students adopt the proper techniques to ensure safety and avoid injury. It will also have a direct impact on the amount of skeletal loading students will get from the circuit.

Sample Circuit (remember to use station posters!)



- **#6 Water break**
- **#7 Continue with planned P.E. program or return to classroom.**

ACTIVITY: red light, green light

Activity Time: 10-15 minutes

Equipment: none

Activity Description:

- All children in the class line up at one end of the gym, behind a designated 'start' line.
- upon starting the game, the students objective is to get to the opposite end of the gym as quickly as possible using a particular hopping skill.
- the instructor or a designated leader starts the game by calling out 'green light' at which point students begin to hop across the gym. the instructor continues to repeat 'green light.....green light', and may change this to 'red light' when they choose. upon hearing the words 'red light' students must freeze on the spot. anyone caught moving after 'red light' will go back to the start line. when the instructor repeats the 'green light' command, students continue hopping toward the finish line.
- After all students have reached the far gym wall, a new student leader is chosen to call out the next game.
- this activity can be repeated with a number of small variations

Impact Emphasis:

⇒ each game should focus on a different technique. game 1: students must hop on both feet together. game 2: right foot only. game 3: left foot only. game 4: 2 r foot hops, 2 l foot hops etc.

⇒ technique is of utmost importance. students who repeatedly cheat on their technique (shuffling, skipping, or running) must go back to the start line and start over.

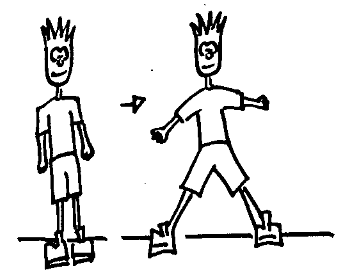
Level One

JUMPING JACK FLASH

EQUIPMENT: None

STARTING POSITION:
- Legs together
- Hands at side

ACTION:
- Jump and spread legs (like a jumping jack)
- Bring arms up over head
- Clap
- Land with "soft knees"



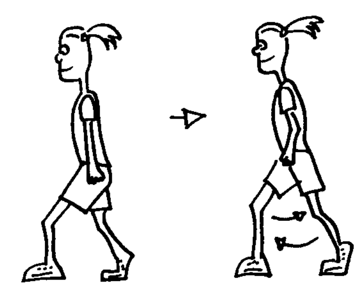
Level One

LEAPING LIZARDS

EQUIPMENT: None

STARTING POSITION:
- Stand with feet together

ACTION:
- Small hop to a lunge position
- Bounce
- Return to starting position



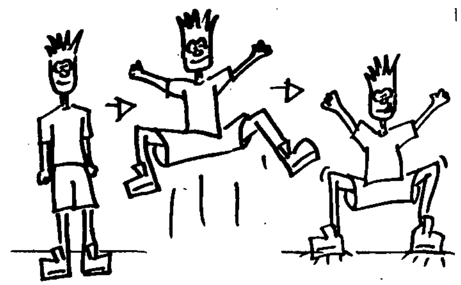
Level Three

JUMPING JACK FLASH

EQUIPMENT: None

STARTING POSITION:
- Legs together
- Hands at side

ACTION:
- Jump with knees together, bringing them up as high as possible
- Bring arms over head and clap



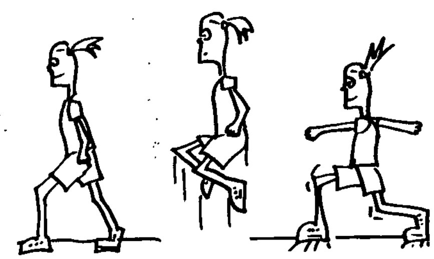
Level Three

LEAPING LIZARDS

EQUIPMENT: None

STARTING POSITION:
- Legs together
- Hands at side

ACTION:
- Jump into tuck position
- Land in scissor step with legs bent



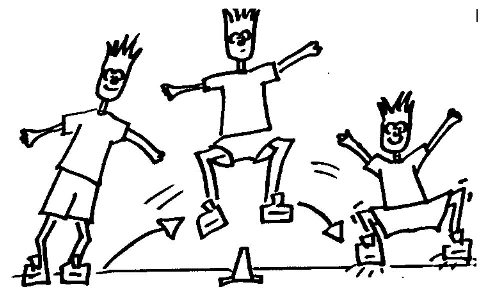
Level Three

MOGUL MUNCHERS

EQUIPMENT: One pylon per student

STARTING POSITION:
- Stand beside pylon with feet together

ACTION:
- Tuck jump from side to side over pylon



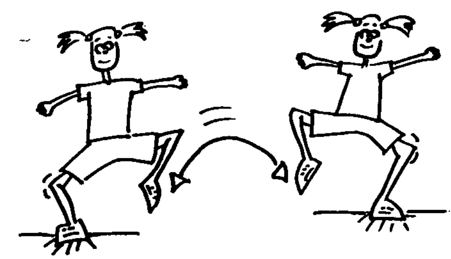
Level Three

DISCO DANCERS

EQUIPMENT: None

STARTING POSITION:
- Stand with one leg up and bent

ACTION:
- Jump from leg on floor to other leg
- Repeat on initial standing leg
- Get "air"!!



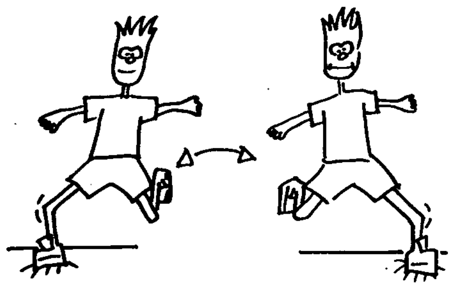
Level Three

TERRIFIC TRIATHLETES

EQUIPMENT: None

STARTING POSITION:
- Stand with feet shoulder width apart

ACTION:
- Jump from side to side with full power
- Swing arms in rollerblading style



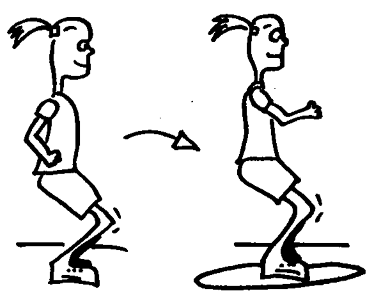
Level Three

HULA HOPPERS

EQUIPMENT: One hula hoop per student

STARTING POSITION:
- Stand on one side of hoop

ACTION:
- Tuck jump into hoop and out to the other side



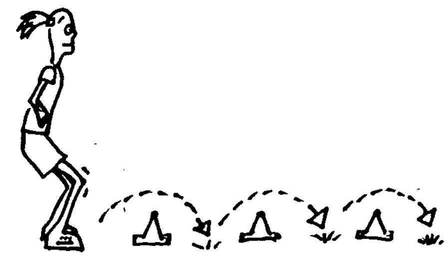
Level Three

RAPID RELAY RACERS

EQUIPMENT: Four pylons per student arranged in a straight line

STARTING POSITION:
- Stand facing first pylon

ACTION:
- Tuck jump over pylons
- Run back to start
- Repeat until whistle blows



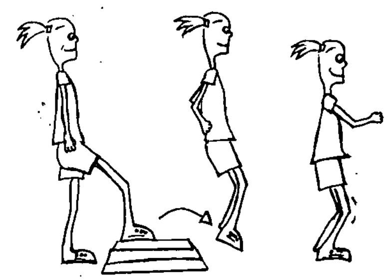
Level Three

SPEEDY STEPPERS

EQUIPMENT: One step and four risers per student (two risers under each side of step)

STARTING POSITION:
- Stand facing platform

ACTION:
- Step onto platform
- Jump off



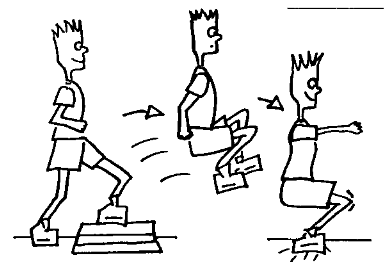
Level Three

SUPER STUNT STARS

EQUIPMENT: One step and four risers per student (two risers under each side of step)

STARTING POSITION:
- Stand on platform

ACTION:
- Stunt jump of students' choice off of platform



WATCHING FOR THE SCIENTIFIC PROCESS

Watch the Scientist carefully! What part of the scientific process are they completing in the lab? Here is a hint: predicting, observing, recording, verifying, hypothesizing, concluding and maybe they do more! Write about your findings.

Teacher____________________

School____________________

Activity Journal

Date	Activity Time	Stations (initials)	Comments
October 5	10:00 am	LL, DD, TT, RRR, JJF	Kids had difficulty with rhythm

Tait

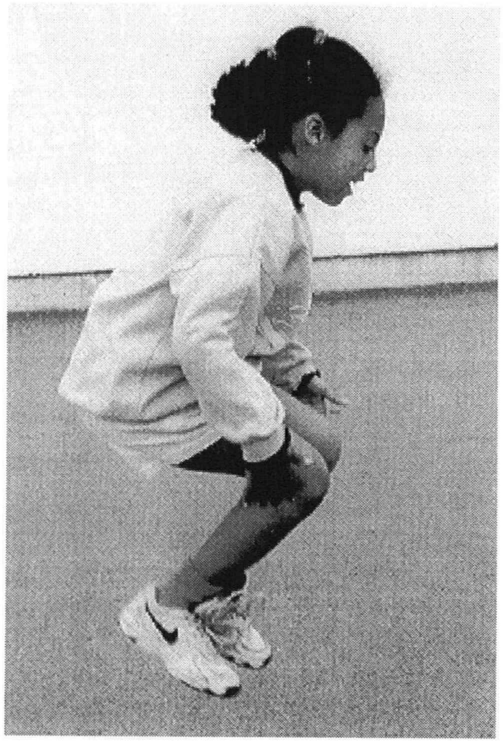
Jesse Wowk

Garden City

Brighouse

Diefenbaker

Kilgour



Mitchell

Ferris

Errington

Westwind

Thomas Kidd

Walter Lee

The Healthy Bones Study
2000-2001

School of Human Kinetics, University of British Columbia

The Healthy Bones Study: 1997-2001

The Healthy Bones Study began in the Richmond School District as a pilot project in 1997. In the early stages, 10 elementary schools were involved, with almost 200 Grade 3 and 4 students participating. In 1999, we began a larger study in Richmond, with nearly 400 Grade 4, 5, and 6 students involved from 14 elementary schools. During the 2000-2001 school year, we are attempting to follow a subset of these students for one more year. By doing so, we will be able to study the effects of a 2-year physical activity intervention on growing bone. After year two, this will be the longest study of this nature in the world. The contribution of the teachers, students and principals at the 'Healthy Bones' elementary schools is critical and of tremendous importance to bone health research in Canada. We consider you our partners in research, so thank you so very much for your participation.

Lifestyle habits such as physical activity and dietary calcium contribute significantly to the health of our bones throughout life. Although we cannot control the influence of genes on our bones, we can make well-informed decisions about physical activity and nutrition that may play an important role in preventing bone loss and subsequent osteoporosis in later life. Our previous research and that of others suggests that the greatest effects of physical activity on the skeleton are observed when activities are undertaken during the active growing years. During the years from 10 through puberty our bones may be as responsive to loading through weight-bearing exercise as they will ever be. That's not to say that adults don't benefit from weight-bearing

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Lifestyle habits such as physical activity and dietary calcium contribute significantly to the health of our bones throughout life. Although we cannot control the influence of genes on our bones, we can make well-informed decisions about physical activity and nutrition that may play an important role in preventing bone loss and subsequent osteoporosis in later life. Our previous research and that of others suggests that the greatest effects of physical activity on the skeleton are observed when activities are undertaken during the active growing years. During the years from 10 through puberty our bones may be as responsive to loading through weight-bearing exercise as they will ever be. That's not to say that adults don't benefit from weight-bearing physical activity - they do. As we age the primary task of our skeleton becomes one of conserving, rather than increasing, bone mass.

Children often get a fair amount of exercise through running, but less frequently experience extra loads on their skeletons' through jumping. This is not true for those children involved in sports such as figure skating or gymnastics or dance who experience loads on their skeletons that approximate 10-15 times their body weight! Higher impacts from jumping result in positive adaptations in the growing skeleton and - not surprisingly - stronger bones. Our goal as individuals (and as researchers) is to design a program that is easy to do and that will reap the maximum rewards for the skeleton during the growing years and thus, reduce the risk for osteoporotic fracture in later life.

Initial results from our project in 1999-2000, as well as results from the 1997-1998 study in younger Richmond children (Appendix 1), have shown us that there is, indeed, a positive benefit from an 8-month, school based jumping program. The reason we are working with these same children in the 2000-2001 school year is to answer three as yet unanswered questions. (1) Do the positive benefits we observed in the jumping schools after one year persist over time? (2) Will there be further gains in bone strength from jumping after a second year in the program? And finally, as there is still considerable discussion around the 'optimal' time (in relation to the onset of puberty) to load the skeleton through exercise we would like to know (3) What are the effects of physical activity on bone in children at different stages of maturity?

Students who participate in the study will be measured at UBC twice during the 2000-2001 school year. At these times, we will assess bone status, growth and physical performance in jumping tasks by direct measurement. We will also assess physical development, calcium intake, and general levels of physical activity by questionnaire. We sincerely believe that the results of this unique study will provide important information regarding the roles of physical activity and calcium nutrition in the prevention of osteoporosis.

Again, thank you for your contribution to our research program. We look forward to working with you during this school year.

Dr. Heather McKay Kerry MacKelvie M.Sc Dr. Moira Petit Meghan Donaldson BSc.

The 'Healthy Bones' Research Team

The Healthy Bones Study Intervention: 2000-2001

I. Format:

The entire intervention is meant to comprise a **5-station, continuous warm-up** at the beginning of your scheduled physical education classes (~10 minutes, 2 x / week). A third jumping session should be undertaken in the classroom or outside (~3 minutes, 1 x /week).

During the warm-up students will pause from continuous jogging or skipping to complete the specified jump at each station, and then continue with their lap around the gym. **The number of jumps is critical, however, the amount of running time is not. If you find that it is taking too long to complete the designated number of laps of your gym, modify the warm-up so that the loop is set up in a smaller space.**

Five Stations: 3 step stations (we will provide the steps), one alternating foot jump station, and one 2-foot jump station.

The same format is followed during all 3 levels. *Levels are only differentiated by an increase in the step height* (which is simpler than last year). **Within levels, the number of laps of the circuit must increase each week, as will the number of in-class jumps (see 'Specifics').** It is expected that teachers will continue with the regular P.E. curriculum after completing the 'Healthy Bones' session.

II. Timeline:

Level I: October - end of November (platform base only for step stations)

Level II: December - end of January (platform base + 2 risers on each side of base)

Level III: February - June (platform base + 4 risers on each side of base)

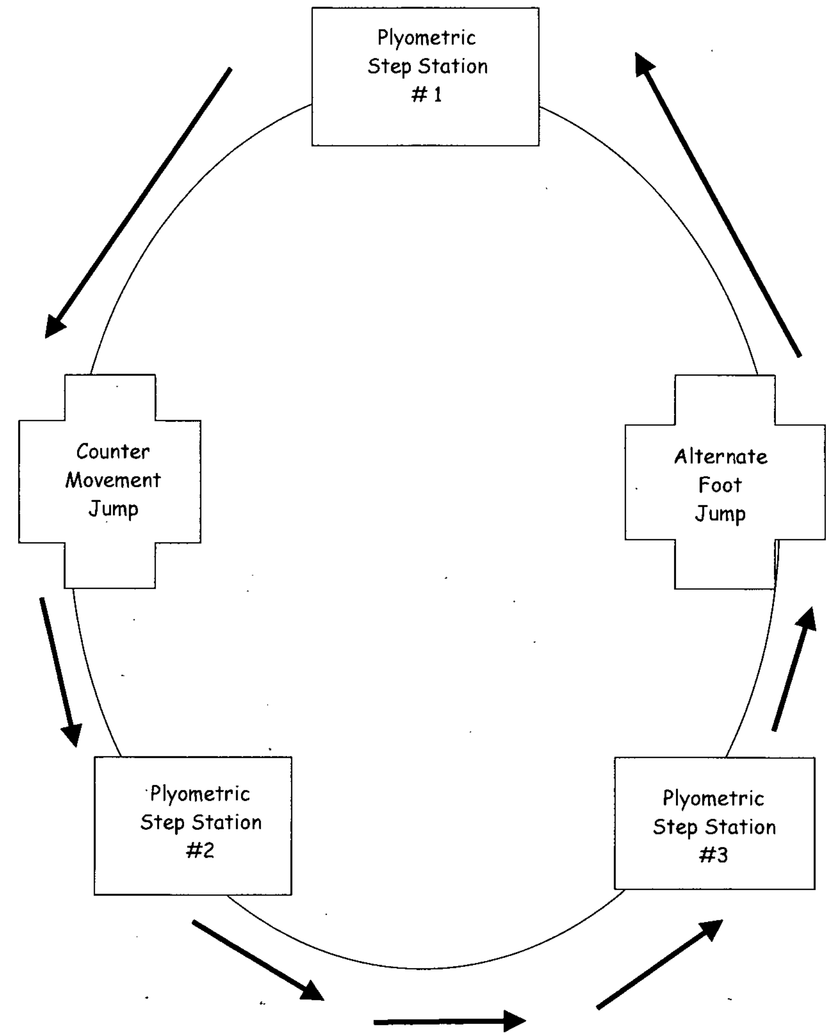
III. The Specifics: P.E.

❖ CONTINUOUS WARM - UP

Level I. The circuit starts at Level I, week one with 6 laps of the gym with 5 jumps during each lap (see circuit diagram on following page). We suggest that you alternate between jogging and skipping between jump stations at each P.E. class for variety. At the 3 step stations, **only the green platform** should be used at Level I. The children will simply jump down from the platform landing with knees slightly bent to avoid injury. Upon landing they should immediately jump as high into the air as they can with arms stretched overhead. This second (plyometric) part of the jump is key so encourage your students to put their best effort into it. The alternating foot and counter movement jumps can be performed with or without equipment. Please view the illustrations in the next section. When you begin the program the children will be performing 30 jumps (6 laps). **Add an additional lap (5 jumps) each week.** You will likely complete ~5 weeks at Level I, so you will have achieved 10 laps (50 jumps).

Level II. When you move up to Level II, return to 6 laps (30 jumps), and **add 2 purple risers to each side of the step platform.** Add a lap each week, until the end of January; you'll probably reach 11 laps (55 jumps).

Level III. At the start of February (Level III), return to 6 laps, and **add 2 more purple risers to each side of the platform** (there should now be 4 risers on each side for Level III). This level continues for a longer timeframe than the previous 2, so add a lap every 2 weeks. You should reach 13 laps (65 jumps) by the end of the study.



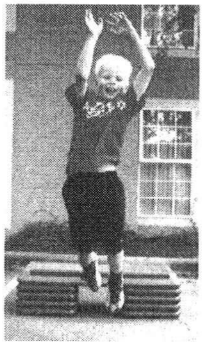
Continuous Warm - Up Set Up

❖ JUMP STATIONS FOR CONTINUOUS WARM - UP IN P.E.

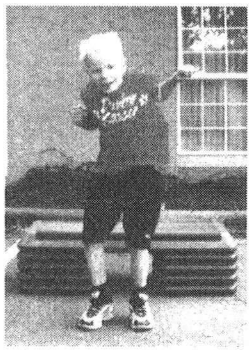
Plyometric Step Stations #1, 2, and 3:

A plyometric jump is simply one that involves a pre-stretch of the muscle. A pre-stretch can occur when the child performs a preliminary load or jump, and then follows this with a maximal jump. From our research during the 1999-2000 Healthy Bones Study, we found that this type of jumping causes the highest forces to be transmitted to the bones. Bones will respond maximally to these higher forces and become stronger to sustain the loads.

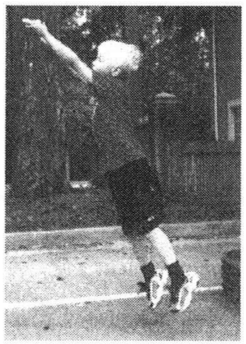
At these stations, the students should step (not jump or run) onto the platform and jump forward (not upwards) off. Immediately after landing, students take a maximal jump off two feet, reach for the sky, and land on two feet.



1. Jump forward



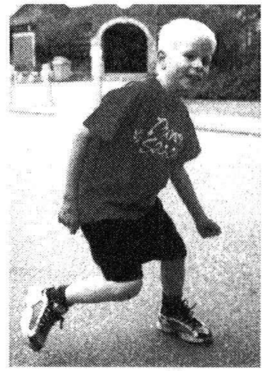
2. Landing



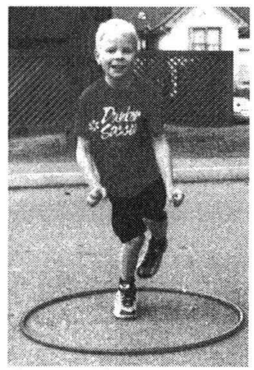
3. Jump up immediately following landing

Alternating Foot Station

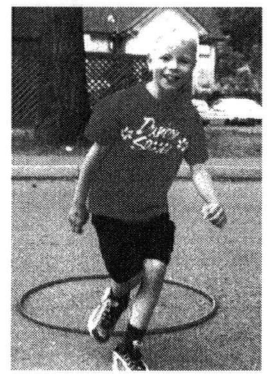
This can be as simple as a hop from one foot to the other (make sure students hop **once on each foot** each time they are at the station). For variety, students can hop over obstacles, hop through a hoop, hop forwards, backwards, or side-to-side. The only requirement is that this station be a one-footed jump.



Hop from side-to-side.



Hop on one foot into the hoop, and onto the other foot out of hoop.

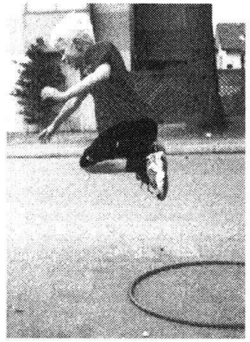


Counter Movement Jump S

A counter movement jump is simply one that **starts in a standing position**, and is completed when the student jumps forwards, backwards, to the side, or over an obstacle with a **two-foot landing**. Students can do a tuck jump (getting the knees up towards the chest) in any direction. Alternatively, you may want to use a set of obstacles, which are progressively higher from Level I to Level III (as diagrammed). Loud landings are encouraged at this station!

2-Foot jump into a hoop, or out of a hoop.

This is a good example of a tuck jump. →



Level I

Level II

Level III

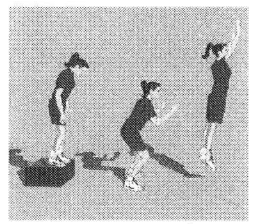
Example of progression or obstacles for 2-foot jump.

III. **The Specifics: Classroom**

❖ **CLASSROOM JUMP SESSION**

Each week, the 3rd jumping session should take place in the classroom or outside if the weather and time permits. Simply indicate the date that the classroom session was completed, and check off the 'Healthy Bones' participants on the attendance sheet provided.

During **Level I**, week one, have the students do 15 **plyometric jumps** on the spot. The children will simply jump on the spot, landing with knees slightly bent to avoid injury. Upon landing they should immediately jump as high into the air as they can with arms stretched overhead (see picture below … the students do not need to jump off a box, they can do the jumps on the spot). This second (plyometric) part of the jump is key so encourage your students to put their best effort into it. With each additional week, add 5 jumps. The children can pause between sets of jumps if necessary. Remember that although we suspect that these children will have improved cardiovascular health at the end of the program - that is not our goal. We are in the bone business - so rest periods are fine.



Plyometric jumps for Level I – classroom session.

During **Level II**, students should emphasise lifting their knees towards their chests, as they perform a **half-tuck jump** (on the spot). Level II begins with 20 jumps at week one and 2 jumps are added per week.

During **Level III**, the students should perform **full tuck jumps** (knees to chest). Level III begins with 20 jumps 1 jump is added each week. Remember, children should be encouraged to pause between jumps if they get tired. The emphasis should be on maintaining the quality of the jumps at all time, rather than completing them in world-record time!

Appendix 3: Questionnaires

____
____
____

What are these medication(s) for? ____
____

3.0 Bone History

3.1 Has your child ever been hospitalized, confined to bed or had a limb immobilized (i.e., arm in a cast)?
______ yes ______ no
If **yes**, list condition, approximate date and time involved

(Example:	wrist fracture	summer, 1990	10 weeks)
	Reason	**Date**	**Time Involved**

3.2 Is there a history of wrist, hip, or spine fractures in your family? ______ yes ______ no
If **yes**, indicate who was affected
______ mother ______ father
______ maternal grandmother ______ paternal grandmother
______ maternal grandfather ______ paternal grandfather

3.3 Is there a history of osteoporosis in your family? ______ yes ______ no
If **yes**, indicate who was affected
______ mother ______ father
______ maternal grandmother ______ paternal grandmother
______ maternal grandfather ______ paternal grandfather

3.5 Is there a history of any other bone disease in your family?
______ yes ______ no
If **yes**, please indicate the family member(s) affected
1. ________________________________
2. ________________________________

What is the name of the condition(s) affecting this family member?
1. ________________________________
2. ________________________________

4.0 Physical Acitivity

4.1 How would you rate the physical activity level of your child? (physical activity is defined as vigorous activity that makes them sweat and/or breathe hard)
______ Inactive
______ Sometimes active
______ Moderately active
______ Often active
______ Very active

THANK YOU FOR YOUR PARTICIPATION

HEALTHY BONES STUDY: PERSONAL DATA FORM, FALL 1999

NAME:____________________ **TODAY'S DATE:**________________

AGE:_______ **GENDER:**_____________ **BIRTHDATE:**______________

SCHOOL:_______________ **GRADE**____ **GROUP NUMBER: 1 / 2 / 3 (circle one)**

Have you participated in the Healthy Bones Study before? yes / no (circle one)

What language do your parent(s) speak at home?____________________

What country were you born in?____________________

What country was your mother born in?____________________

What country was your father born in?____________________

How long have you lived in Canada?____________________

If you have not lived in Canada all your life, where did you live before coming to Canada?____________________

ATHROPOMETRY AND FITNESS TESTING

Measurer: ________________

HEIGHT:	______	______	______
WEIGHT	______	______	______
SITTING HEIGHT	______	______	______
CALF GIRTH	______	______	______
VERTICAL JUMP standing______	jump 1______	jump 2______	jump 3______
LONG JUMP	______	______	______
ULTRASOUND: BUA	______	______	______
SOS	______	______	______
QUI	______	______	______

HEALTHY BONES STUDY: PERSONAL DATA FORM, SPRING 2001

NAME:________________________ TODAY'S DATE:__________

AGE:_______ GENDER:____________ BIRTHDATE:________________

SCHOOL:_______________ GRADE_____ TEACHER ________________

ADDRESS: ____________________CITY: __________POSTAL CODE:__________

PHONE NUMBER: ______________MOTHER'S NAME __________FATHER'S NAME __________

<u>DURING THIS SCHOOL YEAR:</u>

DID YOU BREAK ANY BONES? (YES / NO) WHICH BONE(S)? __________ HOW LONG WAS IT IN A CAST? __________.

WERE YOU SICK FOR GREATER THAN A MONTH? (YES / NO) WHAT DID YOU HAVE?_______.

WERE YOU IN THE HOSPITAL? (YES / NO) FOR HOW LONG? __________________

WHY? __________________________.

ANTHROPOMETRY, JUMPS AND ULTRASOUND

Measurers: Anthro:________ Jumps: ________ Ultrasound: L ____ H ____

HEIGHT:	______	______	______
WEIGHT:	______	______	______
SITTING HEIGHT:	______	______	______
CALF GIRTH:	______	______	______
VERTICAL JUMP: (jump-standing difference) jump 1______	jump 2______	jump 3______	
LONG JUMP:	______	______	______
ULTRASOUND (HOLOGIC): BUA	______	______	______
SOS	______	______	______
QUI	______	______	______
ULTRASOUND (LUNAR): BUA	______	______	______
SOS	______	______	______
QUI	______	______	______

Healthy Bones Activity Questionnaire: Spring 2001

Name:______________________________ Age:________
Sex: M___ F___ Grade:______

We would like to know about the physical activity you have done in the last 7 days. This includes sports or dance that make you sweat or make your legs feel tired, or games that make you huff and puff, like tag, skipping, running, and climbing.

Remember:

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.

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1. PHYSICAL ACTIVITY IN YOUR **SPARE TIME (this <u>does not</u> include P.E classes)**.
Have you done any of the following activities in the **past 7 days**? If yes, how many times and for how long? (Remember, **recess is 15 minutes long, and lunch is usually ½ an hour** (30 minutes)).

Tick only one circle per row	No	1-2	3-4	5-6	7 or more times	time per session
Skipping	O	O	O	O	O	______
Four Square	O	O	O	O	O	______
Creative Playground	O	O	O	O	O	______
Tag	O	O	O	O	O	______
Walking for exercise	O	O	O	O	O	______
Bicycling	O	O	O	O	O	______
Jogging or running	O	O	O	O	O	______
Aerobics	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	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	______
Other:__________	O	O	O	O	O	______
______________	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 do most of the time at **RECESS?** Check only one.

O Sat down (talking, reading, doing school work)
O Stood around or walked around.
O Ran or played a little bit.
O Ran around and played quite a bit.
O Ran and played hard most of the time.

4. In the last 7 days, what did you normally do **AT LUNCH** (besides eating lunch)? Check only one.

O Sat down (talking, reading, doing school work)
O Stood around or walked around.
O Ran or played a little bit.
O Ran around and played quite a bit.
O Ran and played hard most of the time.

5. In the last 7 days, on how many days **RIGHT AFTER SCHOOL**, did you do sports, dance, or play games in which you were very active? Check only one.

O None.
O 1 time last week.
O 2 or 3 times.
O 4 times last week.
O 5 times last week.

6. In the last 7 days, on how many **EVENINGS** did you do sports, dance, or play games in which you were very active? Check only one.

O None.
O 1 time last week.
O 2 - 3 times.
O 4 - 5 times last week.
O 6 - 7 times last week.

7. How many times did you do sports, dance, or play games in which you were very active **LAST WEEKEND?** Check only one.

O None.
O 1 time.
O 2 - 3 times.
O 4 - 5 times.
O 6 or more times.

8. 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.

9. How many hours per day did you watch television or play Nintendo last week? (each show is usually a half hour or 30 minutes). Check only one.

O I watched less than 1 hour or have no TV.
O I watched more than 1 hour but less than 2.
O I watched more than 2 hours but less than 3.
O I watched more than 3 hours but less than 4.
O I watched more than 4 hours.

10. 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?____________________________

11. 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

12. Do you participate in **organized sport** or **activities** (music lessons, Chinese school, tutoring, girl guides, boy scouts) outside of school?

O Yes
O No

If yes, what sport(s) or activities do you do?

____

____

How many nights during the week do you do these activities? (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: ____________________

THANK YOU!

Healthy Bones Study Food Frequency Questionnaire: Spring 2001

Name/Grade:______________________________ **Date:**____________________

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 one 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 an 'X' 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.

	Per day	Per week	Per month	Don't eat
Orange Juice, 1 cup	1			
French fries, regular serving		3		
Cauliflower, ½ cup (125 ml)				X
Bread or toast, 1 slice	6			

NUMBER OF TIMES I EAT THE FOOD

	Per day	Per week	Per month	Don't eat
Bread or toast, 1 slice or 1 roll				
Muffin, 1 large				
Pizza, 1 medium slice				
Cheeseburger or veggieburger with cheese				
Cheese: 1 slice processed OR 1 piece hard cheese (plain or in sandwich)				
Broccoli, ½ cup (125 ml)				
Gai-lan (Chinese broccoli), ½ cup				
Bok- choi (Chinese cabbage), ½ cup				
Ice cream (large scoop)				
Frozen yogurt (large scoop)				
Fast food milkshake				
Cottage cheese, ½ cup				
Yogurt, small (174 ml) carton or equivalent				
Canned salmon or sardines with bones, ½ small can				
Soft drink, 1 can or large glass				
Tofu, 2 oz (60 gm)				
Milk on cereal				
Orange juice, 1 cup				
Milk (any type including chocolate), 1 cup				
Macaroni & cheese, 1 cup (250 ml)				

I usually drink (choose one only)

_____milk OR
_____chocolate milk OR
_____soy milk OR
_____rice milk

Are you allergic to any foods?

_____NO
_____YES: (what foods?____)

Do you use any **vitamin and/or mineral** supplements? **(This question is <u>not</u> about medications)**

	Daily	>3x/week	1-3x/week	<1/week
Multivitamin	_____	_____	_____	_____
Multivitamin/mineral	_____	_____	_____	_____
Iron	_____	_____	_____	_____
Vitamin C	_____	_____	_____	_____
Calcium	_____	_____	_____	_____
Other	_____	_____	_____	_____

What is the brand/name of the supplement?____

THANK YOU!

Self Assessment of Maturity Status: Boys

As you keep growing over the next few years, you will see changes in your body. These changes happen at different ages for different children, and you may already be seeing some changes, others may have already gone through some changes. Sometimes it is important to know how a person is growing without having a doctor examine them. It can be hard for a person to describe themselves in words, so doctors have drawings of stages that all children go through.

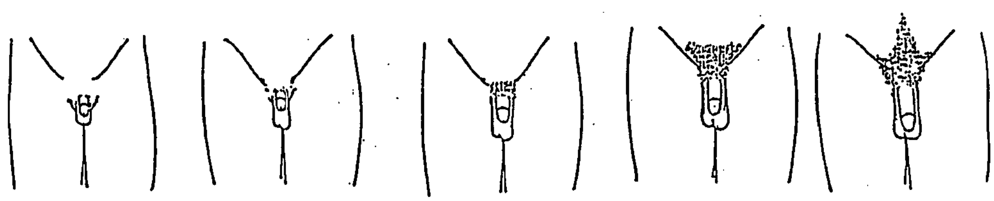
There are 5 drawings of pubic hair growth which are attached for you to look at. All you need to do is pick the drawing that looks like you do now. Put a check mark above the drawing that is closest to you stage of development for pubic hair. Put the sheet in the envelope and seal it so your answer will be kept private.

SPRING 2001

NAME: ______________________________

BOYS: After reading the descriptions under each drawing, please place a check mark above the drawing that looks most like your stage of pubic hair development. Seal your response in the envelope provided. Thank you!

1 ________ **2** ________ **3** ________ **4** ________ **5** ________



1	2	3	4	5
There is no pubic hair at all.	**There is a small amount of long, lightly coloured hair. This hair may be straight or a little curly.**	**There is hair that is darker, curlier and thinly spread out to cover a somewhat larger area than in stage 2.**	**The hair is thicker and more spread *out, covering a larger* area than in stage 3.**	**The hair now is widely spread and covering a large area, like that of an adult *male*.**

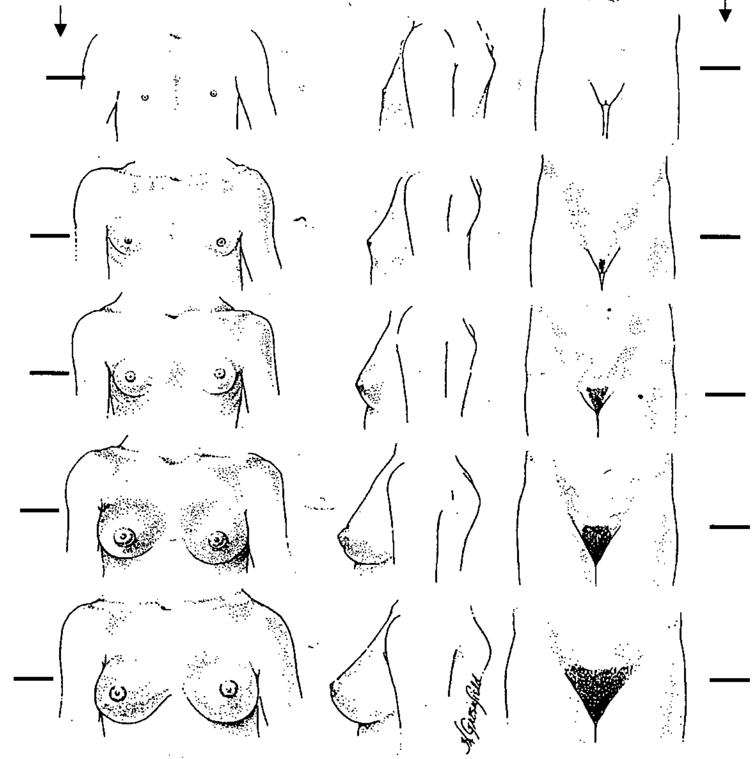
Healthy Bones Study Self Assessment of Maturity Status: Girls

As you keep growing over the next few years, you will see changes in your body. These changes happen at different ages for different children. You may already be seeing some changes, and some of your friends may have already gone through some changes. Sometimes it is important to know how a person is growing without having a doctor examine them. It can be hard for a person to describe themselves in words, so doctors have drawings of stages that all children go through. There are 5 drawings of breast growth, and 5 drawings of pubic hair growth on the next page. All you need to do is pick the drawings that look like you now. Put one check mark on the line at the drawing that is closest to your stage of development for breast growth, and one check mark at the drawing that is closest to your stage of pubic hair growth. Put the sheet in the envelope and seal it so that your answer will be kept private.

Spring 2001 **Name** ____________________

Please put a check mark on the drawing that looks most like (1) your stage of breast development, and (2) your stage of pubic hair development. Seal your response in the envelope provided. Thank you!

Choose one: **(1) BREAST** **(2) PUBIC HAIR** Choose one:



Have you had your 1st period? Yes _____ No _______

If yes, do you remember when? Month _______ Year ________

THANK YOU.

Appendix 4: Results for Study Participants

The Healthy Bones Study 1999-2000

Mitchell

Height and Weight

In the fall of 1999, you were **136.5** centimetres (cm) tall and weighed **26.4** kilograms (kg). In the spring of 2000, you were **140.4** cm tall and weighed **29.6** kg. Our study included 200 boys and 200 girls aged 9 to 12 years. The average height and weight for boys and girls in our study are shown in the table below. It is not unusual to see differences in height and weight in children of the same age. The reason for this is that some children mature at an earlier age and at a faster rate than their classmates. Also, you could have a gene from your Mom or Dad that might make you a basketball player (tall) or a distance runner (light and lean). These differences are a normal part of growth and development.

	Time 1 (Fall 1999)		Time 2 (Spring 2000)	
	Girls	Boys	Girls	Boys
Height (cm)	142.2	142.3	146.5	146.1
Weight (kg)	37.0	37.6	40.0	40.3

Jumps

Your long jump score was **143.9** cm in the fall, and **149.1** cm in the spring. Your vertical jump score (the difference between your standing height and jumping height) was **29.4** cm in the fall and **35.9** cm in the spring.

Physical Activity

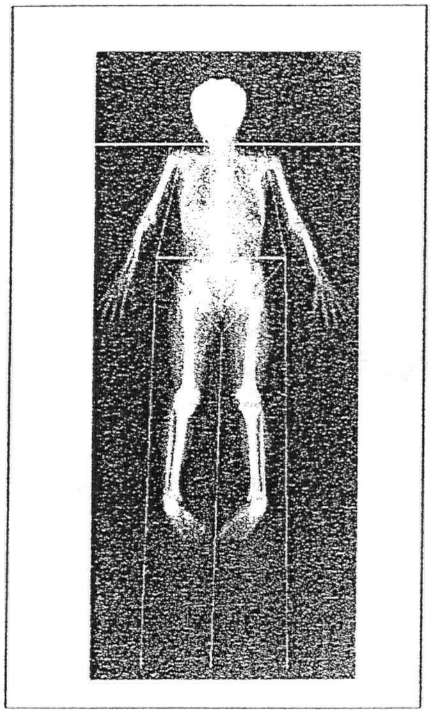
Your average score on the physical activity questionnaires was **2.66** . This is scored on a scale where 1 represents the lowest level of weekly physical activity and 5 represents the highest. Relative to the other participants in the study, your score was: low / average / high. Physical activity is always encouraged as it plays an important role in bone gain during childhood and adolescence.

Calcium

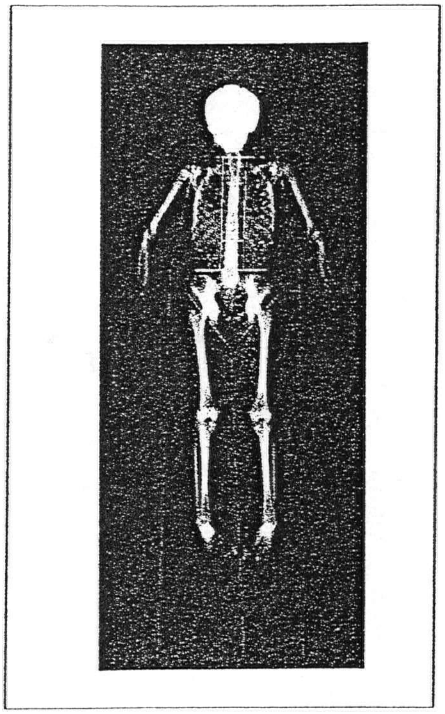
Your average calcium intake from food items was **762** mg/day. The daily recommended intake of calcium for children your age is 1300 mg/day. Consuming low fat dairy products is a good way to increase your calcium intake -- there is approximately 300 mg of calcium in one glass of milk.

The Healthy Bones Study 1999-2000

Fall 1999



Spring 2000



In the fall of 1999, your total body bone mineral content was **857** grams. Over the 1999-2000 school year, your bone content increased to **986** grams. The skeleton of a fully mature female is made up of approximately 2200 grams of bone mineral, while the bone mass of a fully mature male is typically about 2900 grams. You're well on your way!

THE UNIVERSITY OF BRITISH COLUMBIA

School of Human Kinetics
210, War Memorial Gym
6081 University Boulevard
Vancouver, B.C. Canada V6T 1Z1
Tel: (604) 822-3838 Fax: (604) 822-6842

August 20, 2001

To all Healthy Bones Study Participants, Parents and Guardians:

Thank you for your continued interest and participation in our on-going study. Your contribution has been invaluable in the investigation of bone mineral gain, exercise and nutrition in young people. Papers produced from this study have been accepted for publication in *Medicine and Science in Sports and Exercise* (November 2001) and *The Journal of Pediatrics* (October 2001). As well, we have presented this work and acknowledged Richmond's contribution at several major international scientific conferences in Canada, the United States, Australia, Norway, Sweden, and Finland.

Results from our study demonstrate the important role of vigorous, weight-bearing physical activity in healthy bone mineral gain during the growing years. On average, students at 'intervention' schools (ie, the 'jumping' schools) gained more bone at the hip and spine than students at 'control' schools. And, regardless of how much jumping occurred in gym class, children who were more active in sports outside of school gained more bone than less active children.

We are currently in discussion with Richmond schools about the possibility of measuring the children again next year (once in Sept-Oct and once in May-June). We would not intervene in physical education as we have in the past, but merely monitor the growth of their skeletons for one more year. It will also help to see if the benefits the children in the jumping schools have gained are maintained. We will be sending consent forms home in the second week of school in the fall with those children whose schools agree to continue their involvement with the project. If your and your child agree to participate for this final **measurement only** year – please return the forms to the school promptly with your signature.

It has been a pleasure to work with the students, teachers and principals in the Richmond School District over the past two years. If you have questions about the enclosed results please phone Kerry MacKelvie at UBC, at 822-0021.

Thank you for your time and interest – we genuinely appreciate your contribution.

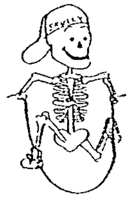
Sincerely,

Dr. Heather McKay Kerry MacKelvie, MSc.

2349

The Healthy Bones Study 2000-2001

Mitchell



Your Results: Growth and Physical Performance

	Age at Test	Height (cm)	Weight (kg)	Long jump (cm)	Vertical Jump (cm)
Fall 1999	10.6	136.5	26.4	143.9	29.4
Spring 2000	11.2	140.4	29.6	149.1	35.9
Fall 2000	11.5	143.1	30.8	163.6	32.3
Spring 2001	12.2	148.9	37.0	132.1	34.3

Averages for Healthy Bones Study Participants

	# Participants, Age*	Height (cm)	Weight (kg)	Long Jump (cm)	Vertical Jump (cm)
Fall 1999	191 Girls: 10.3	142.2	37.1	123.5	21.4
	192 Boys: 10.4	142.3	37.5	134.3	24.1
Spring 2000	181 Girls: 10.9	146.5	40.2	131.1	23.8
	185 Boys: 11.0	146.1	40.4	144.2	26.4
Fall 2000	86 Girls: 11.3	148.8	43.0	129.7	24.9
	95 Boys: 11.4	147.9	42.1	143.1	27.3
Spring 2001	82 Girls: 11.9	153.0	46.7	128.9	26.8
	91 Boys: 12.0	152.1	45.0	141.6	30.0

*This represents the average age of participants - our study included children between 8 and 12 years of age in 1999. Children grow at different rates depending on their age and physical maturity - there was quite a bit of variability in all growth measures.

Bone Health Research Group, School of Human Kinetics, University of British Columbia

Your Results: Nutrition and Physical Activity

	Estimated Calcium Intake From Food (mg / day)*	Time spent in moderate to vigourous physical activity (hours /week)**	Physical Activity Questionnaire Score (1 is low, 5 is high)
Fall 1999	753	6.5	3.1
Spring 2000	373	4.3	3.1
Fall 2000	183	6.0	2.4
Spring 2001	289	1.5	2.4

*This estimate includes only food sources (not vitamin supplements) , and was calculated from a questionnaire that includes calcium-rich foods. The recommended daily intake for children aged 9 -13 years is 1300 mg / day.

**We recognize that many young people have difficulty in estimating time spent in physical activity - please keep this in mind when interpreting the physical activity results.

Averages for Healthy Bones Study Participants

	# Participants, Age	Calcium (mg/day)	Physical Activity Time (hours / week)	Physical Activity Score (out of 5)
Fall 1999	191 Girls: 10.3	818	8.9	2.8
	192 Boys: 10.4	851	12.1	3.2
Spring 2000	181 Girls: 10.9	757	10.9	3.0
	1885Boys: 11.0	801	14.7	3.2
Fall 2000	86 Girls: 11.3	898	9.3	2.9
	95 Boys: 11.4	801	12.5	3.1
Spring 2001	82 Girls: 11.9	938	10.6	3.0
	91 Boys: 12.0	920	14.5	3.2

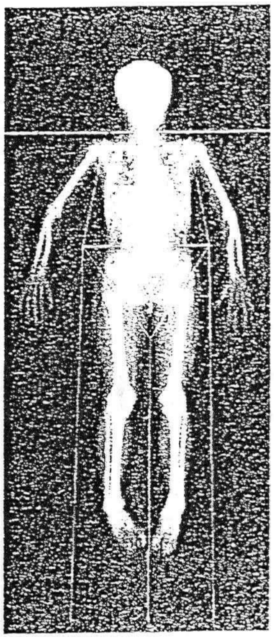
Thank you for participating this year! Have a great summer!

Bone Health Research Group, School of Human Kinetics, University of British Columbia

The Healthy Bones Study 2000-2001: Total Body Bone Mineral Results

Fall 2000

Name:	Sex: Female	Height: 143.1 cm
Patient ID: 2349	Ethnicity: White	Weight: 30.8 kg
DOB: 14 April 1989		Age: 11



Scan Information:

Scan Date: 13 October 2000
Scan Type: a Whole Body
Analysis: 04 January 2001 11:29 Version 8.26
Whole Body
Operator: MP
Model: QDR 4500W (S/N 48346)

DXA Results Summary:

Region	Area (cm^2)	BMC (g)	BMD (g/cm^2)
L Arm	80.68	47.95	0.594
R Arm	78.71	45.68	0.580
L Ribs	79.89	39.14	0.490
R Ribs	65.26	30.98	0.475
T Spine	83.85	46.46	0.554
L Spine	29.27	19.89	0.679
Pelvis	134.87	114.75	0.851
L Leg	213.58	184.30	0.863
R Leg	223.46	201.20	0.900
Subtotal	989.57	730.35	0.738
Head	203.69	299.92	1.472
Total	**1193.26**	**1030.28**	**0.863**

These are the bone mineral results from the total body scan we performed at the UBC Bone Densitometry Lab in the Fall of 2000. Typically, as you grow, the **area (cm^2)** of each body part becomes larger, and the amount of bone, or bone mineral content (**BMC in grams**), increases as well. On this report, bone mineral density (**BMD in grams/cm^2**) equals the bone mineral content in a specific area of bone.

By comparing the results from this scan with the ones from the Spring of 2001, you can see how much bone mineral you gained in each part of your body over this school year. It is normal to see differences in the amount of bone mineral between the right and left sides of the body. Some of you may have grown quite a bit, and you will have gained a large amount of bone mineral. Others of you may not yet have started your growth spurt, and the amount of change in your bones may have been relatively small. Either way, getting plenty of exercise and eating well will help you to develop a strong skeleton during this important time in your life!

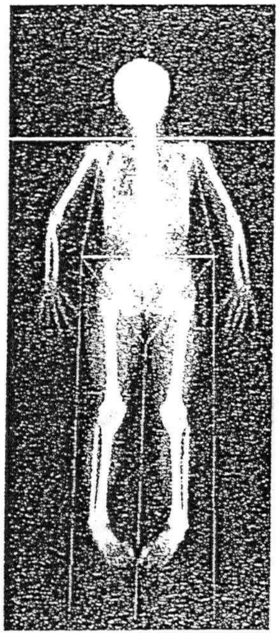
The Healthy Bones Study 2000-2001: Total Body Bone Mineral Results

Spring 2001

Name:
itient ID: 2349
OB: 14 April 1989

Sex: Female
Ethnicity: White

Height: 148.9 cm
Weight: 37.0 kg
Age: 12



Scan Information:

Scan Date: 14 June 2001
Scan Type: a Whole Body
Analysis: 15 June 2001 14:53 Version 8.26
Whole Body
Operator: MP
Model: QDR 4500W (S/N 48346)

DXA Results Summary:

Region	Area (cm²)	BMC (g)	BMD (g/cm²)
L Arm	102.83	60.52	0.589
R Arm	103.23	60.64	0.587
L Ribs	95.71	45.56	0.476
R Ribs	76.33	36.39	0.477
T Spine	81.08	52.96	0.653
L Spine	38.36	31.27	0.815
Pelvis	139.62	129.07	0.924
L Leg	236.52	217.36	0.919
R Leg	229.79	219.07	0.953
Subtotal	1103.48	852.84	0.773
Head	213.58	306.72	1.436
Total	**1317.06**	**1159.56**	**0.880**

These are the bone mineral results from the total body scan we performed at the UBC Bone Densitometry Lab in the Spring of 2001. Typically, as you grow, the **area (cm²)** of each body part becomes larger, and the amount of bone, or bone mineral content **(BMC in grams)**, increases as well. On this report, bone mineral density **(BMD in grams/cm²)** equals the bone mineral content in a specific area of bone. You can compare the results under **BMC** to see how much bone mineral you gained over 8 months.

On average, the skeleton of a fully mature female is made up of approximately 2200 grams of bone mineral, while the bone mass of a fully mature male is typically about 2900 grams.