The Role of Exercise in the Development of Bone Strength during Growth

Submitted in total fulfilment of the requirements of the Degree of

DOCTOR OF PHILOSOPHY

By

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September 2002
I certify that the thesis entitled ‘The Role of Exercise in the Development of Bone Strength During Growth’

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is the result of my own work and that where reference is made to the work of others, due acknowledgment is given.

I also certify that any material in the thesis which has been accepted for a degree or diploma by any other university or institution is identified in the text.

Leanne Kaye Saxon

Signed ............................................................

9th September, 2002
“In the domestic duck the bones of the wing weigh less and bones of the leg more, in proportion to the whole skeleton, than do the same bones in the wild duck; and this change may be safely attributed to the domestic duck flying less and walking more than its wild parents.”

*Darwin, 1859*
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ACKNOWLEDGEMENTS

I wish to thank my family, friends and colleagues who have contributed to this accomplishment and significant time in my life. In particular I would like to offer my sincere appreciation to the following people:

- Dr Shona Bass for her supervision and commitment to support, guide and challenge me to strive for excellence.
- Dr Rob Daly for his constant support and willingness to assist in any way possible.
- Dr Sandy Iuliano-Burns for her friendship and assistance in study one of my PhD.
- Professor Damian Jolly for his statistical advice throughout the four years of my candidature.
- Dr Geraldine Naughton for her expertise in designing the exercise intervention.
- Milgate Primary School Principal Christine Lister for her support and commitment to the exercise/calcium intervention study that was conducted at the school.
- Teacher Deb Oliver for her enthusiasm and interest in taking the exercise classes and for her innovative suggestions in designing the classes.
- The girls from Milgate Primary School who participated enthusiastically in the exercise/calcium intervention study
- Tennis coaches at the Victorian Institute of Sport for their assistance in recruiting tennis players
- Dr Steve Study, the director of Nuclear medicine at the Alfred Hospital, and radiographers Amanda Hunt and Glenn Rush for their technical assistance
- The tennis players and their mothers for their time and willingness to participate in the tennis study
- Alison and Krista Larwood for proof reading my thesis
- Clare Hume and Lisa Hodge for their friendship and assistance in the final phase of my candidature.
ABBREVIATIONS

BMC = Bone mineral content
BMD = Bone mineral density
GH = Growth hormone
IGF = Insulin-like growth factor
g = Grams
cm = Centimetre
BW = Body weight
I_{\text{min}} = \text{Polar moment of area, minimum value}
I_{\text{max}} = \text{Polar moment of area, maximum value}
I_p = \text{Polar moment of area}
DXA = Dual energy X-ray absorptiometry
MRI = Magnetic Resonance Imaging
QCT = Quantitative Computed Tomography
pQCT = peripheral quantitative computed tomography
\mu\text{QCT} = \text{micro quantitative computerised tomography}
LS = Leanne Saxon
AR = Alex Robling
SI = Sandy Iuliano
DO = Deb Oliver
GN = Geraldine Naughton

NB. Bone density refers to areal bone density (g.cm$^2$) throughout the thesis.
ABSTRACT

Exercise during growth may increase peak bone mass; if the benefits are maintained it may reduce the risk of fracture later in life (1). It is hypothesised that exercise will preferentially enhance bone formation on the surface of cortical bone that is undergoing bone modeling at the time (2). Therefore, exercise may increase bone mass accrual on the outer periosteal surface during the pre- and peri-pubertal years, and on the inner endocortical surface during puberty (3). An increase in bone formation on the periosteal surface is, however, more effective for increasing bone strength than medullary contraction (4).

While exercise may have a role in osteoporosis prevention, there is little evidential basis to support this notion. It is generally accepted that weight-bearing exercise is important, but it is not known how much, how often, what magnitude or how long children need to exercise before a clinically important increase in bone density is obtained.

In this thesis, the effect of exercise on the growing skeleton is investigated in two projects. The first quantifies the magnitude and number of loads associated with and in a moderate and low impact exercise program and non-structured play. The second project examines how exercise affects bone size and shape during different stages of growth.

Study One: The Assessment of the Magnitude of Exercise Loading and the Skeletal Response in Girls

Questions: 1) Does moderate impact exercise lead to a greater increase in BMC than low impact exercise? 2) Does loading history influence the osteogenic response to moderate impact exercise? 3) What is the magnitude and number of loads that are associated with a moderate and low impact exercise program?

Methods: Sixty-eight pre-and early-pubertal girls (aged 8.9±0.2 years) were randomised to either a moderate or low impact exercise regime for 8.5-months. In each exercise
group the girls received either calcium fortified (~2000 mg/week) or non-fortified foods for the duration of the study. The magnitude and number of loads associated with the exercise programs and non-structured play were assessed using a Pedar in-sole mobile system and video footage, respectively.

Findings: After adjusting for baseline BMC, change in length and calcium intake, the girls in the moderate exercise intervention showed greater increases in BMC at the tibia (2.7%) and total body (1.3%) (p<0.05). Girl’s who participated in moderate impact sports outside of school, showed greater gains in BMC in response to the moderate impact exercise program compared to the low impact exercise program (2.5 to 4.5%, p<0.06 to 0.01). The moderate exercise program included ~400 impacts per class, that were applied in a dynamic manner and the magnitude of impact was up to 4 times body weight.

Conclusion: Moderate-impact exercise may be sufficient to enhance BMC accrual during the pre-pubertal years. However, loading history is likely to influence the osteogenic response to additional moderate impact exercise. These findings contribute towards the development of school-based exercise programs aimed at improving bone health of children.

Study Two: Exercise Effect on Cortical Bone Morphology During Different Stages of Maturation in Tennis Players

Questions: 1) How does exercise affect bone mass (BMC) bone geometry and bone strength during different stages of growth? 2) Is there an optimal stage during growth when exercise has the greatest affect on bone strength?

Methods: MRI was used to measure average total bone, cortical and medullary areas at the mid- and distal-regions of the playing and non-playing humerii in 47 pre-, peri- and post-pubertal competitive female tennis players aged 8 to 17 years. To assess bone rigidity, each image was imported into Scion Image 4.0.2 and the maximum, minimum
and polar second moments of area were calculated using a custom macro. DXA was used to measure BMC of the whole humerus. Longitudinal data was collected on 37 of the original cohort.

Findings: Analysis of the entire cohort showed that exercise was associated with increased BMC and cortical area (8 to 14%), and bone rigidity (11 to 23%) (all p<0.05). The increase in cortical bone area was associated with periosteal expansion in the pre-pubertal years and endocortical contraction in the post-pubertal years (p<0.05). The exercise-related gains in bone mass that were accrued at the periosteum during the pre-pubertal years, did not increase with advanced maturation and/or additional training.

Conclusion: Exercise increased cortical BMC by enhancing bone formation on the periosteal surface during the pre-pubertal years and on the endocortical surface in the post-pubertal years. However, bone strength only increased in response to bone acquisition on the periosteal surface. Therefore the pre-pubertal years appear to be the most opportune time for exercise to enhance BMC accrual and bone strength.
CHAPTER I

Introduction
INTRODUCTION

Definition and the Epidemiology of Osteoporosis

Osteoporosis is a disease characterised by a low bone density and deterioration of the structural quality of bone, leading to weakness and fragility, which results in increased risk of fracture (5). Common sites for fracture are the thoracic and lumbar spine, femoral neck, distal radius, pelvis and humerus (5). The morbidity and mortality, and associated costs have made osteoporosis a major public health problem.

In 2001, nearly two million Australians (10% of the population) had osteoporosis-related conditions – three-quarters of which were women (6). Due to the demographic trend towards an ageing population, this incidence is projected to rise to three million people by 2021, with a fracture occurring every 3½ minutes. In Australia, it is estimated that 1 in 4 women and 1 in 6 men will suffer an osteoporotic fracture. This incidence is higher in those over the age of 60: 1 in 2 women and 1 in 3 men (5). Of the diagnosed fractures, 46% are vertebral, 16% are hip and 16% are radial. Approximately 20% of those who sustain a hip fracture die within 6 months due to complications, and many of the remainder lead significantly altered lives due to chronic pain, disability and depression (7). It is estimated that around 50% of people who suffer a hip fracture never regain their pre-fracture mobility.

In Australia, osteoporosis costs $1.9 billion per annum in health costs and a further $5.6 billion in indirect costs (ie. lost earnings). In total, this represents 1.2% of Gross Domestic Product (GDP) (8). Statistics from Europe and America are comparable to the prevalence and cost estimates in Australia, showing diagnosed osteoporosis affects 5% and 10% of the population respectively. Fractures occur in about 0.5% of the European and American populations per annum. Approximately 0.3 and 0.2% of the GDP is spent on the costs of osteoporosis in both Europe and America respectively (9,10).
The Assessment of Bone Strength and Risk of Fracture

Osteoporotic fractures are measurable events. However, bone strength and the risk of fracture are not easily determined. Bone strength is influenced by bone geometry, trabecular architecture, accumulation of micro fractures and bone density. Of these characteristics, bone density is the most easily measured and accounts for 60 to 80% of the variance in the ultimate strength of bone (11,12). Therefore, bone density is used as a surrogate measure of the bone’s resistance to bending and to assess bone fragility, efficacy of treatment, fracture risk and rate of bone loss (13). The World Health Organisation Panel has used bone density to categorise osteoporosis. Normal bone density is defined as 1 standard deviation (SD) of the young adult reference mean (age 20 to 45 years); low bone density (osteopenia) is defined as -1 to -2.5 SDs below the young adult reference mean; and osteoporosis is below -2.5 SD. Severe or established osteoporosis is defined as bone density below -2.5 SD in the presence of a fragility fracture (14,15). It is estimated that a decrease in bone density of –1 SD is associated with a 1.5 to 2.5 increased risk of fracture (16).

Although bone density predicts the risk of fracture, it is only a prediction, not a measure of the certainty of fracture (13). Numerous studies have shown considerable overlap in bone density between those with and without fractures (17-19). This is because other factors contribute to fracture risk, such as the person’s propensity to fall, the degree of trauma, and bone geometry (20-22). By combining bone density and geometric factors, such as bone cross-sectional area and moment of inertia, it is possible to identify more accurately (up to 90%) those who will sustain a fracture (23). Although the technology exists to measure bone geometry, no clinical trials have been conducted that support its need and use as a tool to predict fractures.

Several techniques, including dual energy X-ray absorptiometry (DXA) (24-26), quantitative computed tomography (QCT) peripheral quantitative computed tomography (pQCT) (27-29), micro quantitative computerised tomography (µQCT), and magnetic resonance imaging (MRI) (30) have been used to assess bone geometry in
humans and/or animals. DXA has been used to estimate bone geometry by researchers measuring total and cortical bone width from the images of bone, and assuming a certain bone shape (ie. that the vertebrae and femoral shaft are cylindrical). However, this incorrectly assumes that the bone is symmetrical and is the same for every individual (31). In fact, DXA has been reported to have low accuracy in measuring bone width, ranging from 7-16% (30). Peripheral QCT has the advantage of being able to measure both cross-sectional area and cortical and cancellous bone volume, with acceptable repeatability of 1.8-8% (32) and accuracy of 2% (11). However, the ionising radiation exposure is greater in pQCT compared to DXA, limiting its use in research, particularly on children.

MRI can be used in preference to pQCT because bone geometry data can be collected without the use of radiation. However unlike pQCT, MRI cannot measure BMC. The co-efficient of variation of MRI to measure bone geometry is low, ranging from 0.5 to 3.6% (30). The accuracy is high for measuring bone volume and area (1.6 to 3.5%) but not for total or cortical bone width (ranging from 4 to 16%) (30). These findings suggest that pQCT and MRI are ideal techniques for measuring bone geometry, with reasonable accuracy and repeatability.

The Importance of Peak Bone Mass

Osteoporosis is considered to be a disease of the elderly because fractures occur late in life. However, the pathogenesis of osteoporosis may have its origins in the first two decades of life. It was first thought that excessive bone loss during ageing was responsible for osteoporosis, but low BMC in old age may be the result of low peak BMC gained during the first two decades of life, an excessive bone loss during ageing, or both (33).

Low peak BMC may be a more important contributor to osteoporosis than rapid loss of bone during ageing (33). There are a number of findings that support this notion. First, pre-menopausal daughters of women with spine fractures have reduced spine bone
density and daughters of women with hip fractures have reduced femoral neck bone density (34). Second, there is greater population variability in peak BMC than there is in BMC among individuals with and without fractures. Lumbar spine bone density of a person in the 5th percentile is 33% less than a person’s bone density in the 95th percentile. This difference in peak bone density is greater than the difference in bone density found in individuals with and without fractures (34). Finally, the amount of calcium gained during growth is greater and more rapid than the amount lost during ageing. At birth, an individual is born with approximately 25g of bone, this increases to ~2300g to 3000g by 20 years of age (35-37). This magnitude and rate of bone mass accrual is around four times greater during growth than the amount lost (500g in women) during aging. These findings suggest that peak BMC may be a more important contributor than bone loss, to the risk of osteoporosis later in life.

Environmental Factors Affecting Peak Bone Mass

Genetic factors have been reported to account for approximately 80% of the total variance in peak BMC (38,39). Environmental factors such as exercise, dietary calcium and excessive smoking may account for 40% of the variance (40). The findings from a number of studies suggest that environmental factors may interact with each other, and their summed effects determine the extent to which BMC genotype is expressed (41). For instance, exercise may only enhance BMC accrual in the presence of adequate dietary calcium intake (42,43). Furthermore, the cumulative contributions of genetic and environmental factors explain more than 100% of the variance in peak BMC, therefore these factors may interact to some extent, to allow or inhibit expression of peak BMC genotype (44).

The Role of Exercise in Increasing Peak Bone Strength

Exercise during growth is often recommended as a means of increasing peak BMC (45,46). This notion is largely based on the results of cross-sectional studies that show a higher BMC in athletic populations compared to non-athletic controls (up to 35%) (1,47-50). In contrast, exercise during adulthood appears to have minimal or no benefit
on BMC. The results from intervention studies in adults show that additional weight-bearing exercise, weight training and high impact exercise may only maintain or lead to small increases in BMC (1 to 5%) (51-53). In addition, exercise in the elderly may have a minimal benefit on BMC by decreasing the rate of bone loss (54,55). Exercise in the elderly may be more effective in indirectly preventing fractures by increasing muscle strength, balance and joint flexibility and decreasing the risk of falls (56). Thus, the first two decades of life may be the only time when exercise may result in clinically important increases in bone density, and if maintained, it may reduce the future risk of osteoporosis.

Although there is data that shows exercise increases bone density during growth, few studies have investigated whether exercise also increases bone size. The distribution of bone may influence bone’s strength with or without corresponding changes in bone density (4,57). For instance, bone formation on the periosteal surface places bone further from the neutral axis, resulting in a greater increase in bone strength compared to the same amount of bone formed on the endocortical surface (58). Thus, measuring changes in BMC alone do not take into account bone distribution or whether these changes necessarily make the bone stronger (59). It is hypothesised that exercise may enhance bone formation on the surface that is predominately undergoing bone formation at the time ie. on the periosteal surface during the pre-pubertal years, and the endocortical surface during the post-pubertal years (3). However, this hypothesis has not been rigorously tested in humans. This information may determine if there is an optimal time during growth for exercise to result in the greatest gains in bone strength.

Further research is needed before exercise during growth can be recommended as a means of preventing osteoporosis. Firstly, randomised controlled exercise trials in normally active children are needed to ascertain cause and affect relationships between exercise and BMC. Secondly, the type, magnitude, duration, and the frequency of the physical activities that lead to an increase in BMC, needs to be assessed. Thirdly, exercise intervention studies with long follow-up periods are needed to determine if the
benefits are maintained later in life when the risk of fracture is high. Finally, the effect of exercise on BMC and bone geometry needs to be determined to understand how exercise influences bone strength.

In this thesis, the effect of exercise on the growing skeleton is investigated in two projects. The first project discusses the effect moderate and low impact exercise has on BMC during the pre-menarcheal years. In addition, the type, magnitude and number of loads associated with the exercise programs and non-structured play are presented. To assess these parameters, pre- and early-pubertal girls were randomised to take part in a moderate or low impact exercise program for 8.5-months. The type, magnitude and number of loads associated with the exercise classes and during non-structured play (ie. recess) were assessed from video footage and using a Pedar in-sole mobile system. The second study examines how exercise affects bone size and strength during different stages of growth. Cortical, total and medullary area, and bone rigidity were assessed in the playing and non-playing arms of pre-, peri- and post-pubertal tennis players. MRI was used to assess bone morphology and bone rigidity; DXA was used to measure BMC. The side-to-side differences in the arms of tennis players highlighted the effect exercise has on cortical bone during each stage of growth.

In summary, study one provides information relating to what type and how much exercise is needed to increase BMC in normally active children. While study two provides a model of how cortical bone changes in size and strength in response to exercise, during different stages of growth. The findings from these studies stand-alone and are interrelated. For instance, based on the findings from study two, we can assume in study one how cortical bone changes in size and strength, in response to exercise during the pre-pubertal years. Furthermore, the results from study two support the rational for introducing exercise to pre-pubertal children in study one; the results from study two and others in the literature indicate that the pre-pubertal years may be the most opportune time for exercise to initiate an osteogenic response.
In this thesis, the review of literature focuses on how exercise during growth may influence cortical bone strength. Chapter II provides a review on the temporal patterns of growth in size, mass and structure of the skeleton during different phases of maturation in both sexes. This growth chapter provides the platform for chapter III, which reviews how exercise affects the skeleton during different stages of maturation.
CHAPTER II

Growth Related Changes in Bone Mass, Bone Structure and Cortical Bone Density
INTRODUCTION

Bone Histology

Bone first appeared more than 500 million years ago in primitive fish. The internal skeleton of these fish was comprised of cartilage. In the evolutionary branch that led to humans, most of the cartilage was replaced by bone (60). The human skeleton is comprised of 206 bones that function together to provide support against gravity, protection for vital organs, and leverage for muscle action. Bone is essential for maintaining calcium and phosphate homeostasis, immune function, and hematopoiesis. Bone matrix makes up more than 90% of the volume of bone (61). It is a composite material consisting of water (10%), an organic (20%) and inorganic (65%) component. The organic component is mostly made up of collagen fibers and the inorganic component is a mixture of calcium salts (calcium phosphate, calcium carbonate) (61). The calcium salts are organised around the collagen fibers and account for 80-90% of the variance in bone strength (61). The collagen fibers provide flexibility that allows bone to respond to forces by bending rather than breaking (61). Therefore, the composition of bone results in a strong yet flexible structure, that provides some resistance to breaking.

In bone, the lacunae or pockets within the matrix contain bone cells. There are five different types of cells 1) osteoprogenitor, 2) osteoblasts, 3) osteocytes, 4) osteoclasts and 5) bone lining cells. *Osteoprogenitor* cells are stem cells that can divide to produce osteoblasts. *Osteoblasts* are responsible for the production of new bone. These cells synthesise and release the proteins and calcium salts that make-up the bone matrix. Osteoblasts are found on the surfaces of bone and differentiate into osteocytes and bone lining cells. More than 90% of the bone cells in the mature human skeleton are *osteocytes* (61). Together with periosteal and endosteal cells, they line the bone matrix. Osteocytes may be responsible for the detection of stresses on bone and control the movement of ions in and out of the matrix. The role of *osteoclasts* is to remove bone matrix. These cells release acids and enzymes that dissolve the matrix and release calcium salts into circulation. This process is known as *bone resorption* and is
important for maintaining plasma calcium and phosphate concentrations (61, 62). Bone lining cells lie directly against the bone matrix and play a role in attracting osteoclasts to the surface and stimulating them to resorb bone.

In summary, the large network of cells covering the internal and external surfaces of bone may be extremely sensitive to stresses on the bone and may be able to control the movement of ions in and out of the bone matrix. The interconnections between osteocytes, osteoblasts and bone lining cells are likely to sense deformation of bone and streaming potentials occurring within the bone matrix, and coordinate the formation and resorption of bone (61).

Cortical and Cancellous Bone

Bone is mineralised into two basic structural compartments: cortical (or compact) and cancellous (or trabecular) bone. Of the total skeleton, 80% is made up of cortical bone and 20% is cancellous bone (61). Cortical bone is densely compacted tissue found on the outer surface of bone, providing a strong protective layer. In long bones, cortical bone forms the diaphysis and there is little or no cancellous bone in this region. Cortical bone is made up of functional units called osteons. Layers of bone matrix, or lamellae, form osteons that surround central canals called Haversion canals. These canals contain blood vessels and generally run parallel to the surface of the bone (60). The longitudinal orientation of osteons may explain why cortical bone is resistant to tensile and compressive forces that are applied parallel rather than perpendicular to the long axis of the bone (63).

The superficial cellular layer of cortical bone is called the periosteum and the inner cellular layer is the endosteum. The periosteum consists of two layers: an outer layer that is dense and fibrous and an inner later that is looser, more vascular and cellular (61). During bone growth, the cells of the inner layer secrete an organic matrix that enlarges the size of the bone. The outer layer has fewer cells and more collagen, and some tendons and ligaments insert primarily into this outer layer of the periosteum (61).
The endosteum lines the marrow cavity, covers the trabeculae of cancellous bone and lines the inner surfaces of central canals. The endosteum consists of a single layer of osteoprogenitor cells that covers the bone matrix. However this cellular layer is not contiguous, and at sites where the matrix is exposed, osteoclasts and osteoblasts remove or deposit bone matrix (60).

Cancellous bone is found within the vertebrae and at the end of long bones inside the cortical shell ie. proximal femur and distal radius (61). Cancellous bone is formed during the process of endochondral ossification and in the modeling process that alters the shape of bones such as the ilium and the vertebral bodies during growth (147,302,303,304). The composition of the bone matrix is the same for cortical and cancellous bone. However, in cancellous bone there are no osteons or blood vessels, and the matrix forms interconnecting struts and plates called trabeculae. Trabeculae are typically orientated in the direction that strains are applied, but also have extensive cross branching. Cancellous bone has approximately twenty times more surface area to volume ratio compared to cortical bone, and thus has a higher rate of bone remodeling. Therefore, cancellous bone is considered more susceptible to changes in mechanical loading and bone loss with ageing (61).

The Dynamic Nature of Bone, Bone Modeling and Bone Remodeling

The shape and mass of bone is determined by bone modeling and bone remodeling (64). In general, modeling refers to alterations in the shape of the bone, whereas remodeling refers to the turnover of bone that does not alter bone shape. In the first year of life, the removal and replacement of bone proceeds at a rapid rate. Later in childhood, the rate of bone turnover slows down to 10% of the amount in the first year, and either stays the same rate or decreases throughout life (61). During childhood and adolescence, bone formation mostly results from bone modeling, although bone remodeling also occurs. In adulthood, bone turnover is a primarily due to bone remodeling.
In bone modeling, resorption and formation occur simultaneously on different surfaces without local coupling. Bone modeling can result in bone formation on the periosteal or on the endocortical surface without any prior resorption (65). This process can also result in bone resorption on the endocortical surface without additional bone formation (65). Thus, bone modeling generally modifies the shape of the bone by increasing the periosteal diameter of the bone (due to periosteal bone formation), increase the size of the medullary cavity (due to endocortical bone resorption) or decrease the size of the medullary cavity (due to endocortical bone formation) (66). It is the basis for large increases in bone size and much smaller changes in bone shape.

Throughout life, bone is continually being replaced through the process of bone remodeling. In this process, resorption and formation occur sequentially at the same location because of local coupling between osteoclasts and osteoblasts. During adulthood, an equal amount of bone is resorbed and replaced, so BMC is neither increasing nor decreasing, and bone shape remains the same. Bone remodeling sites can occupy 4 to 10% of a bone’s surface, and result in approximately one-fifth of the skeleton being replaced in one year (67). Bone remodeling mainly occurs on the endosteal surface and has a greater affect on cancellous bone because of its greater surface area compared to cortical bone (65). During old age, the rate of remodeling increases and results in bone loss because bone resorption is faster than bone formation; ie osteoclasts remove bone faster than osteoblasts deposit it and as a consequence bone loss occurs (65). There is also some evidence that the ability of the osteoblasts to refill the excavated bone is impaired with age (301). This bone loss produces cortical thinning and intracortical porosity, especially near the medullary cavity, trabecular thinning, complete loss of trabecular plates and loss of connectivity (13).

**Hormonal Regulators of Growth**

Bone metabolism is regulated by polypeptide, steroid, and thyroid hormones, as well as local factors. During growth the major systemic hormones involved in skeletal development are: GH, IGF-I, estrogen and androgens (68,69).
Pre-Pubertal Growth

The pre-pubertal years constitute the period of growth from birth to the end of Tanner stage 1 (approximately age 10 in girls and 12 in boys). This period of growth results in 85% of adult height and 50% of peak BMC accrual (70,71). After the first 6-9 months of life, growth is mediated by the production of growth hormone (GH) and insulin-like growth factors I and II (IGF-I and IGF-II) (72,73).

Growth hormone is released in a pulsatile manner and the amplitude of the pulses increases during puberty (74). Thus, GH levels are relatively low during the pre-compared with the peri-pubertal years (75). Similarly, IGF-I levels gradually increase during the pre-pubertal years and peak during puberty (76). In contrast, IGF-II levels remain constant throughout growth (76). There is a close relationship between IGF-I and GH levels during the pre-pubertal years, as shown in clinical studies that report high levels of IGF-I in patients with acromegaly and low levels in dwarfs with GH deficiency (77,78).

Growth hormone has two main actions on bone growth: one direct, through the division of chondrocytes on the growth plate, and one indirect, through the stimulation of osteoblast formation and activity via the production of IGF-I and IGF-II (78,79). A number of studies report an association between GH and IGF-I levels and BMC accrual during growth. The analysis of children who are GH deficient shows they have a low bone density and a reduced bone size, compared to healthy age-matched controls (80). Furthermore, increases in IGF-I levels parallel increases in BMC during growth, suggesting a relationship between IGF-I and BMC accrual (81,82). Findings from animal and human studies show that IGF-I increases BMC accrual by increasing bone size (ie periosteal bone formation) (83-85). Bachrach et al (1988) found normal bone density, but smaller bones in adults with childhood-onset GH deficiency and low IGF-I levels (85). Furthermore, Mora et al (1999) reported that IGF-I levels are the best predictors of cross-sectional bone area at the femoral mid-shaft (84).
Growth during infancy is not only dependent on the production of GH, but also sex hormones, testosterone and estrogen. The analysis of infants shows that GH and sex hormones levels are higher in males compared to females during the first few months of life. In males, testosterone levels remain elevated until 2 months of age and then decline. In females, however, testosterone reduces to childhood levels by 2 weeks of age, and estrogen levels reach pre-pubertal values during early infancy (86). Little is known about the interaction between GH and sex hormones during childhood, mostly because the sex hormone levels are too low to detect. However, there are indications of a small effect of androgens on growth before adolescence. At about 7 to 8 years of age, the secretion of androgens increases, at the same time a transient increase in height occurs, known as the mid-growth spurt (87,88).

Pubertal Growth

Puberty is associated with a rapid increase in height and BMC accrual (70,71). This period of growth is associated with a 2 to 3 fold increase in GH levels that peak during Tanner stages 3-4 (89). IGF-I levels also rise over time, whereas IGF-II levels remain constant (76). There is a strong relationship between GH, IGF-I and sex hormones during puberty. This relationship is verified by the results of clinical studies that investigated the effect that a disturbance in one of these hormones may have on bone growth (87,90-92). These data provide ample evidence that sex hormones increase the production of GH and IGF-I, and that this interaction is essential to achieve normal pubertal growth.

Estrogen and androgens are secreted during childhood. However, the levels are too low to stimulate bone growth and the development of secondary sex characteristics (93). During early puberty (Tanner stage 2 to 3), androgen and estrogen levels begin to rise in boys and girls respectively. Both androgen and estrogen are produced by each sex, however androgen levels are higher in boys and estrogen levels are higher in girls (94). During puberty, sex hormones act in synergy with GH and IGF-I to induce a growth
spurt in height and BMC (94,95). Sex hormones are responsible for approximately half of the height gained during puberty, as patients with GH deficiency have a 50 to 60% lower growth spurt (96). Androgens and estrogens are also important for normal BMC accrual, as subjects who are sex hormone deficient and undergo hormone treatment, show significant increases in BMC (97-99). Similarly, Dhuper et al (1990) related BMC of adolescent girls with an estrogen score based on physiological events known to reflect estrogen levels (100). They found that girls who had low estrogen exposure during the pubertal years, had the lowest cortical BMC.

The importance of estrogen in regulating BMC accrual in girls has been shown in a number of studies. First, a longitudinal study reported that a rise in estrogen and IGF-I levels correlated with an increase in BMC in girls during puberty ($r = 0.4$ to $0.6$, $p<0.0001$) (101). Second, estrogen levels are 8 times higher in pre-pubertal girls than in boys (102). Third, biochemical measures of bone turnover and estrogen peak at approximately 12 years of age in girls (Tanner stage 3) and decrease at menarche when bone formation begins to slow down (103). Finally, girls with anorexia nervosa have low estrogen levels, and may have a lower BMC and bone size compared to eumenorrheic girls (104,105).

Estrogen is also important in boys, as androgens are converted to estrogen by the enzyme cytochrome P-450 aromatase (106). Estrogen then stimulates a response from the bone via the estrogen receptor and indirectly through the production of GH and IGF-I (78). The importance of estrogen on height and BMC accrual in boys has been reported in numerous growth studies. First, GH levels correlate with estrogen levels in boys but not with androgen levels (107). Second, administering an estrogen blocker to pre-pubertal boys decreases the production of GH (92). Third, the rapid increase in androgens occurs late during puberty, after peak height velocity. At the time of peak height velocity, androgen levels are 18% and 44% of peak levels in girls and boys respectively, while estrogen levels are 75% and 72% of peak levels (107). Fourth, the administration of estrogen in pre-pubertal boys results in a tripling of the ulna growth
rate (108). Fifth, patients with androgen insensitivity, show normal pubertal growth and estrogen levels correlate with IGF-I levels (109). Finally, a case study on a 28-year old man showed that he had osteopenia and continued linear growth, despite having high estrogen levels and normal testosterone levels. Further work led to the discovery that this man had a mutation in the genes that code for the estrogen receptor (110). Similar symptoms have been reported in patients with a mutation of the aromatase gene that converts androgens to estrogen (111,308). Males with either of these gene mutations show deficits in bone density and because epiphyseal closure is not stimulated by estrogen, they maintain slow pre-pubertal growth into adult life. Collectively these studies implicate estrogen, not androgens, as the sex hormone influencing GH and IGF-I levels. Therefore, estrogen appears to regulate the increases in linear growth and BMC in both sexes during puberty. Androgens however, indirectly contribute to BMC accrual by increasing muscle mass, and by being aromatised to estrogen (112). Unfortunately more detailed data on the effects of estrogen and androgen in men is limited by the rarity of the defects.

Growth hormone and sex hormones have surface and site-specific effects on cortical bone. Bone modeling appears to be regulated by androgens and/or GH and IGF-I on the periosteal surface, and by estrogens on the endocortical surface (84,85,113-115). Estrogen also inhibits periosteal bone formation (by inhibiting preosteoblast proliferation and decreasing osteoblast activity) and bone resorption at the endocortical surface (by reducing osteoclast number) (309). As a consequence, bone formation is facilitated more at the endocortical surface resulting in an increase in BMC and a smaller medullary area (114). Furthermore, sex hormones predominantly regulate pubertal growth at the axial skeleton, and GH and IGF-I regulate growth at the appendicular skeleton (116). However, longitudinal growth at both the axial and appendicular skeleton eventually ceases due to a surge in the production of estrogen (110,115). In summary, estrogen reduces proliferation of cartilage cells in the proliferative zone of the growth plate. The hormone may also decrease chondrocyte hypertrophy. The net result of these changes is a decrease in linear growth rate. On the
periosteal bone surface, estrogen inhibits preosteoblast proliferation and decreases osteoblast activity. On the endocortical surface, estrogen reduces osteoclast number and decreases bone resorption (307).

In summary, the role of hormones in BMC accrual is not completely understood. Growth hormone and IGF-I are the main hormones contributing to bone modeling during the pre-pubertal years. During puberty, an increase in the production of sex hormones is associated with a rapid increase in height and BMC accrual. Sex hormones influence bone directly or indirectly by enhancing the production of GH and IGF-I. However, the estrogen-related increase in GH and IGF-I appears to be the main mediator of the increases in linear growth and BMC during puberty in boys and girls (112).

**BONE MASS ACCRUAL**

**Introduction**

Bone mass increases throughout childhood, reaching peak levels by late adolescence or early adulthood. Thereafter, with increasing age and a decrease in the production of sex steroids, bone loss occurs. Bone mass increases during growth due to an enlargement in bone size (length, width and depth) and an increase of bone within the periosteal envelope (ie medullary contraction or an increase in trabecular thickness). Changes in BMC can be described in absolute terms (g), or adjusted for bone area and expressed as areal bone density (aBMD, g.cm\(^{-2}\)), or adjusted for overall bone size and expressed as volumetric bone density (vBMD, g.cm\(^{-3}\)). Cortical bone density is the amount of bone per unit volume of its own bulk (g.cm\(^{-3}\)) (117).

Areal bone density increases during the pre- and peri-pubertal years because long bones increase in size. The densitometer detects a bigger bone and reports a higher bone density, suggesting that as children grow their bone density increases (118). Similarly in puberty, periosteal diameter is greater in boys than in girls, so the densitometer detects a bigger bone and reports a higher bone density in boys, giving the impression
that boys have denser bones than girls. This is not the case. The amount of bone within
the periosteal and endocortical envelopes (eg cortical bone density) remains relatively
constant during life and there is no difference between men and women (84,119-121).
The failure to adjust BMC for bone size will lead to the erroneous view that bone
density increases during growth.

**Changes in Bone Length During Growth**

Growth in bone length can be categorised into three additive and partially superimposed
components: infancy, childhood and puberty (Figure 2.1). Growth during infancy is
considered to be a continuation of fetal growth that begins before or at mid-gestation
and continues until about 4 years of age (70). After birth, infants often change
percentiles on the growth chart by changing their rate of growth to reach their
genetically programmed growth pattern. This process is usually complete by 2 years of
age (122). Once children establish their position on the growth chart, they generally
grow at predictable rates throughout childhood. The infancy contribution to final height
is approximately 44% in males and 46% in females (Table 2.1) (70).

Childhood growth beings at the end of the first year of life and continues until skeletal
maturity. During the third year of life, the infancy component virtually disappears and
growth is mainly attributable to the childhood component. The commencement of
childhood growth is marked by an abrupt increase in growth rate. The peak velocity
during this growth spurt is 17 cm.year\(^{-1}\) and is characterised by an acceleration of leg
length growth, while growth of the trunk remains constant (123). This growth spurt is
likely to correspond to the age at which GH begins to have a significant effect on linear
growth. From 3 years of age to the onset of peak height velocity, growth is nearly
identical in both boys and girls. Growth velocity slows during the pre-pubertal years to
reach a minimum velocity of about 5 cm.year\(^{-1}\) in both sexes (123). It is a period of
more stable growth compared to infancy and puberty. However, a mid-childhood
growth spurt at 7 to 8 years has been observed in two thirds of healthy children. This
transient acceleration in height may be due to the production of androgens; involve
growth in lower and upper limbs; and may be similar in both sexes (70,124). The contribution of childhood growth to final height is the greatest of all three phases of growth, approximately 47% in boys and girls (70).

Pubertal growth is considered a bi-phasic period of growth that includes both growth-promoting and final height limiting processes (123). The onset of the pubertal growth spurt is defined as a clear increase in growth rate (~7cm.year\(^{-1}\)) and occurs two years before peak height velocity (123). This acceleration in growth is due to a combination of pubertal growth and a deceleration of childhood growth. Peak height velocity occurs at approximately 12 years of age in girls (Tanner breast stage 3) (124-126). By the time girls reach menarche (~12.7 years of age), growth is slowing down; the skeleton is within 97% of its final height; and adult height is usually attained within 2.8 years (103). Peak height velocity occurs two years later in boys compared to girls, resulting in an additional 10 cm in height during the childhood period of growth (5 cm.year\(^{-1}\) for 2 years). Pubertal growth also continues for 4 years in boys compared to 3 in girls, resulting in an additional 2 to 3 cm in height. The magnitude of peak height velocity is also greater in boys than in girls (8 versus 10 cm.year\(^{-1}\), respectively) (123,127). Due to these sex-related differences in growth, men are ~13.5 cm taller compared to women in adulthood (123,126).
Growth in stature does not occur at a constant rate each year from birth to maturity. Longitudinal growth in the appendicular skeleton is completed sooner than the axial skeleton, and distal segments (radius-ulna, tibia-fibula) complete their growth before proximal segments (humerus, femur) (127). In addition, there are sex-related differences in growth due to temporal patterns in the duration and magnitude of pre- and pubertal growth. During the three phases of growth, there is asynchrony in the growth velocity of the upper and lower body (Table 2.1). During infancy, the increase in trunk height contributes more to the change in height than leg length (62% versus 48%). During childhood, growth is predominately due to a greater increase in leg length compared to trunk length (57% versus 43%) (70,125,128). In the early stages of puberty, growth is predominately due to an increase in leg length, but as puberty progresses the acceleration in the trunk becomes more apparent. The increase in trunk height is more than double pre-pubertal values (103). The pubertal contribution to leg growth is similar in men and women (8% and 6% of final limb length, respectively), but it is greater at the trunk in men compared to women (9% and 6% of final trunk height, respectively) (70).
Table 2.1 Average total height, sitting height and leg length for each component of growth (70).

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Sex</th>
<th>Infancy Component</th>
<th>Childhood Component</th>
<th>Puberty Component</th>
<th>Combined Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitting Height</td>
<td>Boys</td>
<td>48.9</td>
<td>51.8</td>
<td>36.7</td>
<td>38.9</td>
</tr>
<tr>
<td>Leg Length</td>
<td>Boys</td>
<td>30.1</td>
<td>35.3</td>
<td>48.5</td>
<td>57.0</td>
</tr>
<tr>
<td>Total Height</td>
<td>Boys</td>
<td>79.0</td>
<td>44.0</td>
<td>85.2</td>
<td>47.2</td>
</tr>
<tr>
<td>Sitting Height</td>
<td>Girls</td>
<td>48.0</td>
<td>54.2</td>
<td>34.7</td>
<td>39.2</td>
</tr>
<tr>
<td>Leg Length</td>
<td>Girls</td>
<td>28.8</td>
<td>37.2</td>
<td>43.7</td>
<td>56.4</td>
</tr>
<tr>
<td>Total Height</td>
<td>Girls</td>
<td>76.8</td>
<td>46.2</td>
<td>78.4</td>
<td>47.3</td>
</tr>
</tbody>
</table>

In summary, 85% of adult height is accrued during infancy and childhood. Thus, exposure to risk factors during this time may have a greater affect on final height, compared to a similar exposure during puberty. The difference in final height between men and women is mostly due to longer pre-pubertal growth in males, resulting in a longer leg length (70). The vertebrae of men are only slightly taller than in women (120). This difference in final height contributes to the greater peak BMC in men compared to women.

Change in Bone Mass during Growth

*Pre-Pubertal Years*

At birth the skeleton contains approximately 25g of bone and this increases to 200g within the first 9 months and 1100g by 10 years of age. (35,126). Bone mass increases because bone length and periosteal diameter increases. However, the rate of BMC accrual varies according to sex and skeletal site. For example, a longitudinal study on Canadian children reported that at 10 years of age, BMC at the femur was 65% of adult values but only 40% at the lumbar spine (71). However, data reported in the literature is not consistent. A mixed cross-sectional longitudinal study by Bass et al (1999) found 40% of peak BMC had been accrued at the axial and appendicular sites in girls by 10 years of age but reported no regional differences (103). A cross-sectional study by
Fournier et al (1997) reported girls and boys had accrued a similar percentage of their peak BMC values at the lumbar spine and femoral neck (25% and 32% in girls and 51% and 45% in boys, respectively) at the age of peak height velocity, (126). The girls had also accrued less of their peak BMC than the boys. Further research is needed to clarify the discrepancies in BMC gains at the axial and appendicular skeleton.

Differences in the experimental design may explain the variability of the findings. Longitudinal studies enable data from the same individual to be compared at their different time points as they move through the study. This reduces the variability that occurs in cross-sectional, or mixed-longitudinal study designs. The increased variability in the latter designs can reduce the ability to detect differences between the different age and sex cohorts and between different skeletal sites.

Peak BMC is approximately 10% higher in males. However, it is not certain at what age sex-related differences become apparent. Rupich et al (1996) reported that differences may be apparent during infancy; total body BMC was 7% greater in males compared to females (aged 1-18 months), who were matched for age, weight and height (35). The results of studies during childhood are conflicting. Bailey et al (1996) conducted a 4-year prospective study on more than 100 boys and girls aged 8 to 18 years (71). They found total body, lumbar spine and femoral neck BMC was similar in boys and girls until age 13 to 15, thereafter it was greater in boys compared to girls. These findings are consistent with other studies that have reported BMC was similar at the total body in boys and girls during the pre-pubertal years (37,129,130) but not with others (131,132).

It has been reported there are no sex-related differences in lumbar spine BMC during the pre-pubertal years (37,133,134) while others have found a higher BMC in girls (130,131,135). Moreover, several studies have shown males have a higher femoral neck BMC than females prior to puberty (129,136,137), while others have not (37). There are a few reasons why the data is conflicting. First, small sample sizes were sometimes
used, resulting in low statistical power. Second most studies compared sex differences by chronological age, thus it is likely that there will be some differences due to the sexual dimorphism in maturation rates. Hence, adjustment for maturity by use of skeletal age, or with respect to peak velocity provides comparisons between sexes at the same relative stage of development. Bailey’s studies in Canada are good examples of adjustment for maturation (71, 322,140,323,344,47).

Another source of inconsistency is that some researchers presented BMC, bone density, or both. Areal bone density is a two-dimensional adjustment of the amount of bone, in a three-dimensional structure. The length and width of the scanned bone is known, but not its depth. Because the depth of the bone is not measured, a bone with a greater depth will be reported as being denser. The bone is not denser, it does not have a greater amount of bone within the periosteal envelope, the bone is just larger (118). Thus, adjusting BMC for bone volume can provide insight into the effect bone size has on BMC accrual during growth.

A number of studies have measured the changes in BMC during the pre-pubertal years, after adjusting for bone size (129,138,139). These studies have shown that as the bone grows in length and depth, BMC inside the periosteum increases in proportion to the enlarging volume of bone. For example, after adjusting BMC at the femur for bone volume, it is no longer greater in boys than in girls, nor does it increase with age (129,138). Therefore, BMC may be greater in boys because of enhanced bone expansion, not because of greater increases in BMC per unit volume of bone. Similarly, vertebral bone area is 17% greater in boys than in girls, however after adjusting for bone volume, there is no difference in BMC between the sexes and it is independent of age, at least until puberty (139). These findings suggest that sex-related differences in bone size may explain any differences in BMC during the pre-pubertal years.

In summary, the pre-pubertal years represent a time of large increases in BMC, contributing to approximately 50% of total peak BMC. Therefore, these years are a
critical time for BMC accrual and any negative or positive influence during this time could reduce or enhance peak BMC. There are discrepancies in the data regarding whether boys and girls accrue a similar amount of bone during the pre-pubertal years. A number of studies report no BMC difference between boys and girls at any site, while others report that BMC may be higher in girls at the lumbar spine and lower at the femoral neck. Differences in BMC, however, may be explained by differences in bone size.

Pubertal Bone Mass Accrual
During the pubertal growth spurt (Tanner stages 2 to 4) there is an acceleration of growth resulting in increased skeletal length and BMC accrual. Although there is a minimal contribution to overall height during puberty (15%), it is a significant time for BMC accrual. The percentage of peak BMC accrued during the pubertal growth spurt was calculated in the Saskatchewan Pediatric Bone Mineral Growth Study on 228 boys and girls over a 6 year period (140). Growth curves were developed using mean height and BMC values calculated from all the data collected throughout the study. To adjust for maturational differences, comparisons in BMC were made 2 years before and after peak height accrual (age 10-14 in females and 12-16 in boys). Thirty-five percent of peak total body and lumbar spine BMC, and 27% of peak femoral neck BMC was accrued during this time. Similar findings are reported elsewhere for gains in lumbar spine BMC in girls (141-143).

Pubertal BMC accrual differs between the sexes in two ways. First, it occurs 2 years later in boys; as boys have a longer period of pre-pubertal growth. Second, the growth spurt in BMC accrual is larger in magnitude and continues at least one year longer in boys (71,133,322). Bailey et al (1996) reported that by the time children had reached peak height velocity (age 12 in females and 14 in males, Tanner stages 2 to 3), both sexes had achieved a similar percentage of their peak BMC. However, males had a 20 to 36% higher absolute BMC compared to females (total body 1072g versus 772g, lumbar spine 27g versus 19g, femoral neck 2g versus 1g respectively) (71). The same
research team found 3 years before peak BMC accrual, BMC values in girls are 69% of those in boys; whereas 3 years after peak BMC accrual this value drops to 51% (322). This same group later reported that at peak BMC accrual, boys gain 407± 92 g. year\(^{-1}\), whereas girls accrue 322 ± 66 g.year\(^{-1}\) (344). Therefore from the early stages of growth, males appear to have a higher BMC than females and this difference is increased during the adolescent growth spurt due to a higher amount of bone gained per year.

The greater BMC accrual in males is likely to be due to a greater increase in bone size. Gilsanz et al (1994) measured the size and density of the lumbar vertebrae of children using pQCT and found that neither cancellous nor cortical bone density (g.cm\(^{-3}\)) differed between males and females during Tanner stages 1 to 5 (144). However, cross-sectional area of the vertebrae was 17% larger in boys and was evident during Tanner stage 1. The disparity in vertebral size between the sexes increased during growth. However, the number of subjects in Tanner stages 2 to 5 (n = 10 to 19 per group) may have been too small to draw any valid conclusions. To show the effect bone length has on BMC accrual, Theintz et al (1992) compared changes in lumbar spine BMC with statural height (133). The results showed the increase in lumbar spine BMC was primarily due to linear growth during the pre- and early pubertal years (Tanner stages 1 to 2) and due to both an increase in height and bone accrual within the periosteal envelope during puberty (Tanner stages 3 to 5). The findings were similar for boys and girls, although the magnitude of the increase in BMC was greater in males than in females. A limitation with this analysis is that statural height was used instead of vertebral height or area. (Vertebral area has been found to be a stronger predictor of lumbar spine BMC than vertebral height in boys and girls (139)).

Adjusting BMC for bone volume provides insight into the effect overall size has on BMC. Katzman et al (1991) measured BMC, bone density, and volumetric bone density at the total body, lumbar spine, mid radius and femur using DXA, in 45 girls aged 9 to 21 years (145). From age 9, BMC and bone density increased at all sites, and the increases were the most rapid around the time of menarche, and plateaued after age 16.
After adjusting BMC for bone volume, BMC increased at the spine and mid-radius but not at the femoral neck or total body. Ninety percent of the increase in BMC at the femoral neck and total body was due to periosteal expansion. In contrast, 50% of the increase in BMC at the spine and mid-radius was due to periosteal expansion and 50% was due to BMC accrual within the periosteal envelope. Similar findings are reported elsewhere and are the same in boys (129,138,139,146).

Katzman et al (1991) hypothesised that at predominantly cortical sites, BMC inside the periosteum increases in proportion to the enlarging volume of the whole bone, thus volumetric bone density will not change at the total body and femur during growth (145). In contrast, sites containing a high proportion of trabecular bone, such as the lumbar spine, apparent density of the vertebral body increases during growth because trabeculae thickness increases while growth in the external size of the vertebral body ceases (139,147).

The size of an individual’s skeleton in adulthood may be determined during early stages of growth. Loro et al (2000) used QCT scans to measure bone area and bone density at the femur and vertebrae from Tanner stages 2 to 5, in 20 boys and 20 girls (121). A strong correlation was found between each bone trait and Tanner stage in all subjects. For instance, a child whose bone size and bone density was low compared to the normal distribution (ie 5\textsuperscript{th} percentile) was also low in early adulthood. Volumetric bone density was not calculated. Consequently, a person’s bone size, bone density and possibly volumetric bone density at the axial and appendicular skeleton may track through growth maintaining the same position in the normal distribution at maturity, as was present during childhood. This suggests that an individual’s bone density and size may be determined before birth (118). To date it is not known what genetic, hormonal and environmental factors may account for this variation in bone density and size.

During the pre-pubertal years, gains in BMC and height follow a similar pattern, although during puberty it is not longer synchronous. There is a one-year delay
between peak height velocity and BMC accrual. This delay occurs between Tanner stages 2 to 3 and 3 to 4, or between the ages of ~12 and 13 in girls, and ~14 and 15 in boys (126,133). The dissociation between linear growth and BMC accrual during puberty was demonstrated in a study by Bass et al (1999) on 109 pre, peri- and post pubertal girls (103). By 10 years of age, 80% of adult height had been attained and only 40% of peak total body BMC. At the time of peak height velocity, 90% of adult height had been achieved, while only 60% of peak BMC. Similar results have been reported at the femur and spine, and the delay in BMC accrual may be longer and more pronounced in males at the femur (126) (Figure 2.2). This delay may result in a transient state of low bone density and a decreased resistance to bending. A low bone density has been found to be a risk factor for forearm fractures during growth (148) and it may explain the increased incidence of fractures detected during puberty (149,150).

![Figure 2.2](image)

**Figure 2.2** The percent difference in the increase in standing height and bone density at the lumbar spine (left) and femur (right) at each pubertal stage (126).

In summary, approximately 40% of peak BMC is accrued during puberty and is equivalent to the amount of bone most people lose throughout their adult life (151). The timing of peak BMC accrual is approximately one year after peak height velocity. After peak BMC velocity, BMC is slowly accrued and eventually reaches peak values. The pubertal gains in BMC at predominately cortical sites appear to due to periosteal expansion, whereas at predominately trabecular sites, it is mostly due to an increase in BMC within the periosteal envelope. Therefore, volumetric bone density is not likely to
change at the femur and total body, but increase at the lumbar spine. The constant rate or tracking of BMC accrual and bone size from early childhood to late maturity suggests that children could be screened to detect whether they are at risk of low peak BMC.

**Peak Bone Mass**

By the end of puberty, boys and girls have accrued approximately 85 to 90% of their peak BMC. According to Preece and Baines (1978), the age of peak height velocity provides an accurate benchmark to reflect the timing of maturation; occurring when linear growth is approximately 90% of final adult size (324). Bone mass slowly increases after pubertal growth to reach peak values of approximately 3000g in males and 2300g in females by 30 years of age (71,131). There is conflicting data regarding the age at which peak BMC or bone density is achieved. Some studies report bone density reaches a peak before 20 years of age (129,133,136,142,145,152,153) while others suggest the mid-30’s or 40’s (141,154).

The findings from cross-sectional studies suggest that peak BMC occurs at a relatively early age, sometime between years 14 to 26 (138,142,145,153,155). In contrast, longitudinal data suggests that BMC continues to increase during adulthood and reaches a peak sometime between 17 and 47 years of age (133,136,141,154). Over a two-year period, Sabatier et al (1999) found small but significant increases of 0.3% per year in lumbar spine bone density and 0.7% per year in BMC between 27 and 47 years of age (141). Similarly, Recker et al (1992) reported a 13% gain in total body BMC and a 7% gain in lumbar spine BMC during the third decade of life (154). Women in this study were followed for on average 3.4 years. The data was expressed as a percent change over the decade, so changes in BMC accrual during the third decade were difficult to assess. In contrast, a one-year longitudinal study by Theintz et al (1992) reported no significant gain in BMC or bone density at the lumbar spine, femoral neck or mid-femoral shaft after age 17 years in females and 20 years in males (133). Similarly, a longitudinal study by Lu et al (1994) found peak BMC and bone density values were achieved 1 to 2 years after peak height and weight was reached in males and females
Differences in the reported age of peak BMC could be explained by different study designs and equipment being used, different skeletal sites being studied, changes being expressed as BMC or bone density, or data not being expressed relative to pubertal stage or years since menarche.

Males have ~10% higher peak BMC compared to females because they have a larger bone size (157). When BMC is adjusted for bone size, there is no difference between men and women (144,156). It is likely that men have a larger bone size and larger peak BMC because their skeleton needs to withstand the greater loads imposed on it by a higher body weight and lean mass compared to females. This notion is supported by Lu et al (1994), who found after adjusting peak bone density for weight and lean mass there was no difference between males and females (156).

In summary, it is not certain at what age peak BMC occurs. A difference in peak BMC between males and females may be because males need a larger skeleton to withstand the greater loads imposed on it (due to having a higher body weight and lean mass (136). Conversely, it is possible that the larger skeleton permits a greater body weight and lean body mass. The attainment of peak BMC has important implications on the future risk of osteoporosis. Fifty percent of the variability in BMC in old age has been attributed to the peak BMC gained during growth (158). Therefore, the risk of bone fragility in old age is largely determined by the amount of bone gained during childhood and adolescence.

**BONE STRUCTURE**

**Surface Specific Changes in Cortical Bone during Growth**

Bone modeling and remodeling occur on the periosteal and endosteal surfaces of cortical bone throughout life and subsequently result in changes in bone geometry. How the bone is distributed around the medullary cavity partly determines how resistant the bone is to fracture (58). Until recently, bone strength has been estimated in human studies from measurements of BMC. However, changes in bone geometry may affect
bone strength, with or without changes in BMC (4,159,160). Growth-related changes in bone geometry have been investigated in only a few studies (3,103,120). The most detailed of these is published by Garn (1970), who measured changes in bone diameter of the second metacarpal from more than 25,000 radiographs of men and women (3). The following section describes the changes in bone formation at the periosteal and endocortical surfaces during growth and the resultant change in cortical thickness and bone strength. Differences between girls and boys are also highlighted.

**Periosteal Surface Related Changes**

The cellular layer surrounding the outside of cortical bone is called the periosteum. Bone formation on this surface results in a larger bone diameter and bone being distributed further away from the neutral or central axis. Periosteal bone apposition occurs throughout life, although the rate of accrual varies and it differs at various sites, and between the sexes.

During infancy, periosteal apposition increases rapidly and continues to increase during childhood, albeit at a slower rate. Garn et al (1970) measured bone geometry at the second metacarpal and reported that there are two phases of growth, and both are similar in both boys and girls (3). The first is a continuation of pre-natal growth, and is associated with a rapid increase in periosteal diameter (2.0 mm.year\(^{-1}\)) that ceases after 6 months and declines to approximately 0.5 mm.year\(^{-1}\) by the second year of life. The second phase constitutes childhood growth, and is associated with a more moderate increase in periosteal diameter (0.25 mm.year\(^{-1}\)) that continues until puberty (3) (Figure 2.3). These changes follow the same temporal pattern as linear growth; therefore by 12 years of age, bone has increased in both length and width.

During the pre-pubertal years, GH and IGF-I mediates bone apposition on the periosteal surface in both sexes (84). However, the magnitude of the increase may be slightly greater in boys at the second metacarpal (4%) and lumbar spine (11%) compared to girls, but there appears to be no sex-related differences at the femoral mid-shaft (3,120).
It is not known however, why boys show greater increases in periosteal bone apposition compared to girls at some, but not at all skeletal sites.

During puberty, there is a growth spurt in periosteal bone apposition in both boys and girls (3,103,144). This growth spurt begins earlier in females compared to males (10 versus 14 years of age, respectively). However, the increase is larger in magnitude in boys and continues for 4 years in boys compared to 2 in girls (Figure 2.3) (3). The peak growth spurt results in a 0.4 mm.year$^{-1}$ increase in periosteal diameter at the second metacarpal in males, and a 0.3 mm.year$^{-1}$ increase in females (3). After puberty, periosteal apposition continues in males and is likely to be regulated by testosterone, GH, and IGF-I (84,113,115). In contrast, periosteal apposition is inhibited in females after puberty, most likely due to the inhibitory effect of estrogen on periosteal bone formation (114). As a result of these changes, periosteal diameter increases 85% in males and 50% at the second metacarpal in females during growth (3).

Gilsanz et al (1997) also reported on the disparity in the growth-related changes at the periosteal surface between boys and girls (120). No difference in vertebral height was detected between boys and girls at any stage of puberty, but vertebral width was 17% greater in boys during the pre-pubertal years. The disparity in vertebral width between boys and girls increased up to 44% during puberty but only 22% difference after puberty. Differences in vertebral volume suggest that periosteal diameter was not different in boys compared to girls until puberty (Tanner stage 3). After puberty, periosteal diameter at the vertebrae was 30% greater in boys compared to girls. By comparison, Garn (1970) reported periosteal diameter was 15% larger at the second metacarpal in adult males compared to females (3). Further research is needed to detect growth-related differences in size at other skeletal sites and to investigate the mechanism behind these differences.
In summary, periosteal apposition occurs at a slow, constant rate during the pre-pubertal years, rapidly increases during puberty and continues to increase after puberty in males, but not in females. Throughout growth, periosteal apposition is greater in males, resulting in a larger bone relative to females at all ages; 4 to 11% during the pre-pubertal years and 15% to 30% after puberty (3,120). Thus, factors relating to sex are likely to be playing a role in the regulation of bone size during the pre-pubertal years. Further research is needed to determine what these factors are. An increase in the secretion of testosterone in males and estrogen in females may explain why males have a larger bone size after puberty (113,115). Estrogen has been found to suppress periosteal expansion and this may explain why after menopause, with the reduction in estrogen, periosteal bone formation may occur. Growth-related changes on the endocortical surface further explain sex-related differences in bone size.

**Endosteal Surface Related Changes**

The endosteal cellular layer is located on the inner surface of cortical bone and on the surface of trabecular bone. The following section describes the changes on the endosteal surface of cortical bone (endocortical surface) during growth. The

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**Figure 2.3** The increase in periosteal diameter at the second metacarpal with age (upper plots) and the growth velocity of change in periosteal diameter (lower plots) in boys and girls. Data from six Central American countries (n=800) (3).
endocortical surface is responsive to the production of estrogen and in its presence, bone formation may occur resulting in a smaller medullary cavity (114). Alternatively, in its absence, bone resorption may occur at the endocortical surface, resulting in a wider medullary cavity (114). The sensitivity of the endocortical surface to estrogen is likely to explain the sex-related differences in the size of the medullary cavity throughout life.

Similar to the periosteal surface, there are two phases of growth on the endocortical surface. The first is considered a continuation of post-natal growth and is associated with a rapid increase in bone resorption, resulting in a larger medullary cavity in boys and girls. The second phase is considered childhood growth and begins after the first year of life. During childhood the rate of endocortical resorption is less, but is greater in magnitude in males compared to females. Thus, by the end of the first decade of life the medullary diameter is 6% larger in males (Figure 2.4) (3). These findings are based on the changes in bone morphology of the second metacarpal from men and women in six Central American countries; however, the data was not adjusted for sex differences in body size (ie height and weight).

Depending on the site measured, endocortical bone resorption continues into the peri-or post-pubertal years. After puberty, bone apposition occurs on the endocortical surface in girls and may occur in boys but to a much lesser extent (3). A mixed cross-sectional, longitudinal study on 109 pre-, peri- and post-pubertal girls showed endocortical apposition occurred during the peri-pubertal years at the second metacarpal, and began one year after menarche at the femoral shaft (103). In contrast, a cross-sectional study showed endocortical apposition occurs after the onset of menarche at the second metacarpal in females (3). Despite the discrepancies in the timing of endocortical apposition, the reversal from resorption to formation after puberty is likely to be due to the secretion of estrogen (113,114).

The production of estrogen may also explain the differences in the size of the medullary cavity between boys and girls (114). Endocortical bone apposition may occur in both
sexes, although the onset is earlier and the magnitude is greater in females (3). In females, bone apposition reduces the size of the medullary cavity to the same size that it was by the end of the first year of life and results in it being 30% narrower than the male medullary cavity. Endocortical bone apposition may also be region-specific, occurring less at weight bearing sites such as the femur, than at non-weight bearing sites such as the metacarpals or radius (3,103,134,161).

In summary, the endocortical surface undergoes numerous changes throughout growth. Bone resorption occurs during infancy and childhood, and bone apposition occurs during puberty in females. (It may also occur in boys but to a much lesser extent). The changes on the endocortical surface are likely to be due to alterations in the production of estrogen: when estrogen levels are low, bone resorption occurs and when are estrogen levels are high, bone apposition occurs (114). Thus, the higher estrogen levels in females may explain why they have a greater magnitude of change detected at the endocortical surface compared to males during puberty (114).

**Gain and Loss of Cortical Thickness**

Cortical bone thickness changes in response to the bone modeling and remodeling occurring on the periosteal and endocortical surface. Cortical thickness will remain
unchanged if both periosteal and endocortical surfaces expand to a similar extent. If endocortical expansion is greater than periosteal apposition, cortical thickness will be reduced. However, if periosteal expansion occurs with little or no change on the endocortical surface, cortical thickness will increase.

During infancy and childhood, both periosteal and endocortical surfaces expand in size in both males and females (3). During the first 8 months, expansion is greater on the endocortical compared to the periosteal surface, resulting in a decrease in cortical thickness in both male and females (3). Whereas during childhood, cortical thickness begins to increase because periosteal expansion is greater than endocortical expansion. In males, the changes on each surface are greater than in females. Subsequently, cortical thickness is 12% greater in males compared to females by the end of the pre-pubertal years (3).

During puberty, there is a rapid increase in periosteal apposition and continued endocortical resorption in both sexes. After puberty, both periosteal apposition and endocortical resorption continues in males, whereas periosteal apposition decreases in females and endocortical apposition occurs (3). Although the surface-specific changes in bone formation differ between the sexes after puberty, there is a similar increase in cortical thickness in both males and females (45%) (3). Because there is a greater increase in cortical thickness in boys during the pre-pubertal years (12%), and a similar increase in boys and girls after puberty, cortical thickness is 12% greater in adult males compared to females.

In support of the findings reported by Garn (1970), Bass et al (1999) found cortical thickness increased at the metacarpal (30%) and femur (37%) from Tanner stages 1 to 5 in girls (3,103). At the femur, the growth-related changes at each surface were similar to those reported by Garn (1970); that is, greater periosteal expansion compared to endocortical resorption during the pre-pubertal years and endocortical apposition after
puberty (3). However, at the second metacarpal, endocortical contraction occurred two years earlier during puberty (103).

In summary, cortical thickness progressively increases during the pre-pubertal years due to a greater increase in bone formation on the periosteal surface compared to bone resorption on the endocortical surface. During puberty, cortical thickness continues to increase due to additional bone formation on the periosteal surface in males and on the endocortical surface in females. Because periosteal apposition is greater in males than in females during the pre- and post-pubertal years, cortical thickness is approximately 12% greater in males at the end of the growth. There are however, a number of limitations with the growth-related studies that are published to date. For instance, they all are cross-sectional; cortical thickness is mostly measured from two-dimensional images provided by DXA or X-ray (3,103); small sample sizes are used (134); adjustments were not always made for differences in height or weight (3); and in one study pubertal stage was not assessed (3). Prospective studies that measure changes in bone geometry from transverse images of bone (ie from MRI or QCT) during growth are needed to confirm these cross-sectional findings.

**CHANGES IN THE DENSITY OF THE SKELETON DURING GROWTH**

**Structural Changes in Cancellous Bone During Growth**

Cancellous bone is found within the vertebrae and at the end of bones inside the cortical shell. The matrix in cancellous bone forms struts and plates called trabeculae. These trabeculae have a larger surface area to volume ratio compared to cortical bone. Therefore they may be more susceptible to changes in the environment, such as mechanical loading (61). Cancellous bone density (g.cm$^3$) can be measured in vivo using QCT. QCT can be used to quantify the amount of mineral in spatial resolution, but it does not account for the volume of marrow in trabecular bone (162). MRI has higher spatial resolution compared to QCT, so it is able to measure trabecular size and number with greater accuracy, however it is not able to quantify the amount of bone.
Because of the reluctance to expose children to radiation using QCT, there are limited growth-related data on the changes in cancellous bone density.

Cancellous bone density increases during growth and reaches a peak when longitudinal growth ceases (119,139,144,163,164). From 4 to 20 years of age, cancellous bone density of the vertebrae increases from 150 to 170 mg.cm$^{-3}$ (144). Parfitt et al (2000) reported that the number of trabecular established at the growth plates do not increase with age (147). Thus, the increase in cancellous bone density during growth is likely to be due to an increase in bone volume and trabecular thickness, not trabecular number (147).

The exact age peak cancellous bone density occurs at is difficult to determine because only cross-sectional data is available. Gilsanz et al (1988) reported that cancellous bone density (g.cm$^{-3}$) at the vertebrae was 9% higher in adolescents compared to young adults (163). Similarly Fujita et al (1999) measured cancellous bone density of the radius using QCT, and found it remained constant from 5 to 25 years, and then gradually declined during adulthood (165). These findings corroborate with studies that show cancellous bone loss begins to occur during the third decade of life (166,167).

The person’s sex does not appear to influence cancellous bone density at any stage of puberty (121,134,144,165). Gilsanz et al (1997) reported that cancellous bone density at the vertebrae increased from 4 to 20 years of age in both sexes (boys 16%, girls 8%), and no difference in cancellous bone density was detected between the sexes (120). In addition, cortical (or true) bone density (g.cm$^{-3}$) was no different between the sexes, but vertebral width and depth (not length) were approximately 20% greater in males. Therefore, if the strength of the vertebral body is greater in males than females, it is due to differences in bone size, not cancellous or cortical bone density or the amount of bone within the periosteal envelope.
In summary, cancellous bone density increases during growth due to an increase in trabecular thickness, not trabecular number or true bone density. In addition, males and females have the same cancellous bone density (number and thickness of trabeculae) within the vertebral body; what differs is vertebral body size. Thus, growth does not build a denser skeleton in males and females, it builds a larger skeleton.

**Does Cortical Bone Density Increase during Growth?**

Cortical bone is the densely compacted tissue found within the periosteal and endosteal surfaces. Cortical bone density represents the material density of the bone (ie. the amount of collagen and mineral in a given volume of bone, or ‘true’ bone density), that is, cortical BMC adjusted for bone volume (excluding the medullary cavity, Haversion canals, lacunae, and canaliculi). It correlates well with the ultimate strength of the femur in animals and at the radius in humans (168,169). Cortical bone density can be assessed directly by measuring the ash mineral weight of dried bones, or indirectly using QCT. However, the spatial resolution of the QCT images cannot differentiate between cortical bone and non-cortical bone tissue (ie blood vessels and intracortical canals) (162). In addition, QCT has limited use in children because it involves high doses of radiation (27,170).

Cortical bone is densely packed tissue, and it is hypothesised that the amount of bone within a cubic centimeter (g.cm\(^{-3}\)) does not change throughout life. However, during a typical 6-month remodeling sequence, the new bone (primary bone) is not as “dense” as already matured cortical bone (secondary bone), because it is not yet completely mineralised (61). Therefore, when remodeling is high, there is more un-mineralised bone and cortical bone density may decrease. In contrast, when osteoblasts are depositing bone on the periosteal surface and when there is little intra-cortical remodeling, cortical bone density may increase (171). Frost (1960) measured the number of un-mineralised osteoid’s on the bone’s surface and found the numbers decrease with age: 10% at age 5, 3% at age 15, 0.8% at age 30 and 70 (171). Therefore, there are fewer sites of un-mineralised bone as the body gets older, thus cortical bone
density is likely to increase with age. The change in proportions of un-mineralised bone may lead to a transient increase or decrease in cortical bone density (103). Bass et al (1999) reported that cortical bone density increased 12% one year after menarche, and decreased 4 years later to values similar in pre-pubertal girls. The decrease in cortical bone density may have been due to a delay in the mineralisation of the matrix.

The findings relating to changes in cortical bone density throughout life are, however, conflicting. The results of indirect measures of cortical bone density at the lumbar spine and femoral mid-shaft, show that cortical bone density remains constant during growth ($2.00 \pm 0.07 \text{ g.cm}^{-3}$), and appear not to be influenced by age, height, weight, puberty, sex or race (84,119-121). In contrast, others have reported that cortical bone density increases during growth (1.2 to 12%) and reach peak values in adulthood (27 or 50 years of age) (155,163,170). These findings are supported by Currey et al (1975) who measured cortical bone density from cadavers and found it increased 4% from 2 to 48 years of age (172).

In summary, the data on the growth-related changes in cortical bone density are limited and conflicting. There are findings that suggest cortical bone density does not increase with age, while others suggest that cortical bone density does increase with age because there are fewer un-mineralised sites on the surface of cortical bone with age (171). Furthermore, cortical bone density has been closely associated with bone strength (168,169). Therefore, further research is needed to determine whether cortical bone density increases with age and if it is responsive to environmental factors such as therapeutic drugs and exercise.

**Limitations with Measuring Bone Mass**

There are two main reasons for measuring BMC in children. First to diagnose and quantify bone loss in children with disorders known to affect bone density. Second, to improve our understanding of bone growth and bone strength; as low BMC later in life may be attributed to a low peak BMC achieved during growth (34). To date, our
understanding of how bone is accrued during growth has been obtained by measuring BMC using DXA. DXA is often used because of its ability to measure BMC with a high degree of precision and accuracy, and the associated low dose of radiation. DXA determines the mass of mineral present in the total body or in a selected region. It does not provide a separate measure of cortical and cancellous BMC, but rather a combination of both. Changes in BMC only indicate the result of the metabolic BMC balance between bone formation and resorption (173).

Bone mass can be expressed in absolute terms (g of mineral) or adjusted by the projected area and expressed as bone mineral density (aBMD, in g.cm\(^{-2}\)). Expressing BMC per unit area provides a partial correction for bone size. The length and width of the scanned bone is known, but not its depth. Because the depth of the bone is not measured, a bone with greater depth will be reported as being denser. The bone is not necessarily denser, it does not have a greater amount of bone within the periosteal envelope, the bone is just larger (174). DXA does not provide a three-dimensional measure of volumetric bone density (vBMD), that adjusts BMC for bone volume or provide a measure of the amount of mineral within a cubic square of bone (cortical or true BMD, g.cm\(^{-3}\)).

In prospective studies, measuring bone density is useful in adults because serial measurements in the same individual will reflect changes in BMC, since size and shape of the bone are unlikely to change significantly over time (175). However, the size and shape of bone varies considerably in children and adolescents, making it difficult to interpret the changes to an increase in size, mass and density. It has been reported that bone volume (ie length, width and depth of bone) increases more than bone area (length and width) during growth; therefore, densitometry may underestimate the bone density of small bones and overestimate the bone density of large bones (Figure 2.5) (176).
Figure 2.5. Schematic illustration showing the effect of bone size on BMD (aBMD). BMD of the larger bone cube (as measured from DXA) is double the value in the smaller bone, despite having the same vBMD (112).

Volumetric bone density provides a measure of the amount of bone within a volume of bone, not all of which is mineral. Therefore, volumetric bone density is useful for comparing children of different sizes because it adjusts BMC for overall bone size. However, it does not provide information on the morphological basis of any increase in bone density. For instance, volumetric bone density could increase if there were no change in external bone size but a decrease in diameter of the medullary cavity; or if periosteal diameter were to expand more than the medullary cavity. Therefore, measuring periosteal and endocortical diameter would help to explain any changes in volumetric bone density (177,178). Furthermore, bone morphology can be used to calculate the bone’s resistance to breaking (ie. moment of inertia) which, compared to BMC alone, provides a more accurate assessment of bone strength (4).

Several investigators have attempted to estimate volumetric bone density (g.cm$^{-3}$) by using bone area measured from DXA and assuming a certain bone shape (103,179). For instance, the lumbar spine and femoral neck are often defined as cylindrical. This method is based on a number of limitations: 1) it assumes an average bone shape and does not account for inter-individual variations; and 2) bone shape changes during puberty and may lead to erroneous estimates of volumetric bone density.

The amount of bone within a cubic square of bone is called cortical or true bone density (g.cm$^{-3}$). True bone density can be estimated by using QCT. This equipment provides
transverse images of bone and can quantify trabecular and cortical bone density. True bone density can be used as an indicator of the bone’s structural stiffness/strength (173). A limitation with QCT, however, is that it involves high radiation exposure and is not widely used in children.

In summary, the size and shape of bone varies considerably in children and adolescents. Therefore, when comparing children of different ages and sex, BMC needs to be adjusted for overall bone size (bone volume). However, measuring volumetric bone density alone does not provide information on the morphological basis of an increase in BMC. Bone geometry needs to be measured to explain any increase in BMC at different skeletal sites and between individuals, and to improve the estimation of bone strength.

DEVELOPMENT OF BONE STRENGTH DURING GROWTH

Introduction to Biomechanics of Mechanical Loading

Bone is not only stiff and strong, but is also flexible and able to absorb energy. When a load is applied to bone, deformation will occur, resulting in the generation of internal resistance to the applied force. This internal resistance is known as stress. Stress is defined as the amount of force applied to the area over which it acts. (58). Stress may be classified as compressive, tensile or shear, depending on how the load is applied. Compressive stresses are developed if loads are applied so that the bone becomes shorter; tensile stresses are developed when the bone becomes stretched; shear stresses occur when a region of bone slides relative to an adjacent region (Figure 2.6) (180). Most loading patterns are a combination of each of these stresses. For example, bending produces tensile forces on the convex side and compression on the concave side. The measurement of deformation, normalized by the original length is called strain. Strain is defined as percentage change in length, or relative deformation. For example, if a material is stretched to 101% of its original length, it has a strain of 10,000 micro strain, or 1% (or 0.01). Stresses applied to normal, well-mineralized bone tissue
will cause small strains, whereas the same stresses applied to poorly mineralized bone will produce large strains (58).

![Diagram of Tension, Compression, and Shear](image)

**Figure 2.6.** Schematic representation of the three basic types of stress: tension, compression and shear.

When bone is subjected to mechanical loading, its behavior depends on both its mass and geometry. Most fractures occur as a result of axial compression, bending or torsional (twisting load that creates shear stresses on bone) loads. If a bending or torsional load is applied to bone, the distribution of bone will determine how resistant the bone is to fracture (58). Ideally for greatest strength, the bone should be distributed as far away from the neutral axis as possible (58) (Figure 2.7). The term used to describe the bone’s resistance to bending is the “areal moment of inertia” and to torsion is the “polar moment of inertia” (58,180). Moment of inertia increases linearly with BMC and is proportional to the squared distance of the bone to the neutral axis (58). Therefore, the further the bone is placed from the neutral axis, the higher the moment of inertia and the more resistant it is to bending. This concept can be demonstrated by observing how resistant a ruler is to bending when it is turned on its edge compared to when it is held flat (63). Section modulus is another measure that reflects the cross-sectional strength of bone. It is calculated by dividing the bending or torsional moment of inertia by the periosteal diameter.
Figure 2.7 Moment of inertia properties of cortical bone (181). Although the cortical cross-sectional area of the three bones is nearly similar, the bending strengths differ because of the relative distribution of bone from the neutral axis (182).

Bone Mass, Bone Geometry and Bone Strength

The bone’s resistance to fracture is dependent on both its bone density and its structural properties (183). At least 60 to 80% of the variability in bone strength is explained by differences in BMC, while other characteristics of bone, such as periosteal diameter, cancellous architecture and distribution of bone explain the remaining 20 to 40% (4,184). The influence of bone geometry on bone strength can be estimated using biomechanical calculations (ie. moment of inertia and section modulus) that incorporate bone geometry data and estimate the bone’s resistance to bending. These calculations are able to discriminate better between patients with or without hip fractures, compared to bone density alone (185). The following section describes how bone strength differs during growth and between the sexes.

Bone geometry is the result of bone modeling and remodeling occurring on the surfaces of cortical and trabecular bone. During the pre- to post-pubertal years, the external size of bone increases due to greater periosteal apposition than endocortical resorption, and the increase in size is greater in males than females. In females, estrogen inhibits periosteal apposition late in puberty but stimulates the acquisition of bone on the endocortical surface (3,114). To determine how changes in geometric structure affect bone strength, Schoenau et al (2001) estimated polar moment of inertia, section

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modulus and cortical BMC of the radius in 469 males and females aged 6 to 40 years of age (186). From Tanner stages 1 to 5, cortical BMC and bone strength respectively increased in males (104 to 192%) and in females (105 to 146%). After adjusting bone strength for cortical BMC, bone strength increased during growth in males but not in females (Figure 2.8). Moreover, for a given BMC, males had a stronger bone compared to females after Tanner stage 2. These findings suggest that during puberty there is a commensurate increase in bone strength and BMC in females. In contrast, males show a proportionally greater increase in bone strength compared to increases in BMC. These findings reflect the positive influence periosteal apposition is likely to have on bone strength in males, and how endocortical apposition may confer no benefit to bone strength in females (3,186).

![Section Modulus/Cortical Bone](image)

**Figure 2.8** Section modulus adjusted for bone size (cortical bone mass) is significantly greater in boys (circle) compared to girls (square) in the peri- to post pubertal years (186). *p<0.01 versus girls.

Greater periosteal bone formation and bone strength in males may be due to a number of factors. Firstly, males secrete a greater amount of androgen and this is associated with an increase in periosteal bone formation (113,115). In turn, bone distributed further away from the neutral axis is associated with a higher moment of inertia (182). Secondly, greater strain may be placed on bone in males compared to females because they have a higher body weight. This higher strain may stimulate the bone to adapt by increasing in mass and strength (187). In support of this notion, Van Der Meulen et al
(1996) found bone strength and BMC were similar in pre- and peri-pubertal boys and girls (25). However, during puberty, there was a greater increase in BMC and strength in boys (30 to 40%); both were associated with a greater increase in body mass.

The effect bone size has on the resistance to fracture was highlighted in a study by Skaggs et al (2001) who compared radial bone size and density in girls (aged 4 to 14) with a history of forearm fractures, to those with no fracture history (57). The controls were matched for age, height, weight and Tanner stage. The girls with a fracture history had an 8% smaller cross-sectional bone at the distal radius compared to controls, but similar cancellous and cortical bone density (Figure 2.9). Neither length, fat or muscle mass of the radius differed between girls with and without forearm fractures. The authors concluded that although a person may have normal bone density, a small bone size might increase their risk of fracture. A small bone size may also be a risk factor for stress fractures in athletes. Crossley et al (1999) reported that tibia cross-sectional area was 7% smaller in long-distance runners who had a history of tibia stress fractures compared to controls, but there was no difference in BMC or bone density (188).

**Figure 2.9** Radial cross sectional area of 50 fractures (open circle) and 50 non-fractured (filled circle) girls matched for age, height, weight and Tanner stage (57).

The association between bone size and fracture risk in old age was reported in a study by Seeman et al (2001) who compared bone size and density of patients with a history
of fracture to healthy controls (174). Seeman et al (2001) reported that men with a history of spine fracture have reduced vertebral body width (-0.5 ± 0.1SD) but not femoral neck width. Whereas men with a history of hip fracture have reduced femoral neck width (-0.5 ± 0.1) and vertebral body width (-0.3 ± 0.1SD). By not accounting for bone volume, the measure of BMC alone exaggerated the deficit at the hip by 9% in those with a hip fracture and at the spine by 40% in those with spine fractures. A limitation of this study was that the controls were 10 years younger than the study group. Therefore, it is not known what the difference in bone size is between older adults with and without a history of fracture. Thus, these data provide preliminary evidence that small bone size may be a risk factor for fracture, but this needs further investigation.

In conclusion, bone strength increases during the pre to post-pubertal years. However, an increase in bone strength is in proportion to an increase in BMC in females. In contrast, in males an increase in bone strength is greater than the gains in BMC. This means that males have a stronger bone for a given amount of BMC. This difference is attributed to greater periosteal bone expansion in males compared to females, and hence more bone being distributed further from the neutral axis and the bone being more resistance to breaking (182). The clinical implications of differences in bone size have also been demonstrated. Small bone size was demonstrated to be a risk factor for fracture in the young and old and a risk factor for stress fractures in athletes.
CHAPTER III

The Effects of Exercise During Growth on Cortical Bone Mass, Size and Strength
The Adaptive Response of Bone to Mechanical Loading

Anatomist Julius Wolff is often given credit for first articulating the law that explains the effect of mechanical loading on bone (189). In 1892 Wolff proposed that mechanical stress was responsible for determining the architecture of bone. Recent interpretations of Wolff’s law have proposed that changes in bone structure are brought about by a feedback system, in which changes in local mechanical strains drive bone cells to change bone structure. The most biologically relevant of these theories is the “mechanostat” hypothesis put forth by Frost (191). Frost’s mechanostat theory is unique in its distinction between modeling and remodeling processes, thresholds for activating lamellar or woven bone formation, and its application to the aetiology of osteoporosis. Frost’s theory describes a window of mechanical usage that should be considered normal or physiological. When local mechanical strains in bone exceed the upper boundary of the physiological window, called the minimum effective strain (MES), bone will undergo modeling and change its structure to reduce the local strains to below the MES. If the mechanical loads on the skeleton are very large, the bone strains will be pushed into the pathological overload zone causing woven bone formation on bone surfaces (Figure 3.1). This theory also incorporates a lower MES, below which bone tissue will be resorbed until the local strains are increased.

![Figure 3.1](image-url) The four mechanical usage windows defined by Frosts mechanostat theory. Each window is separated by a minimum effective strain (MES) threshold for which values are given in micro strain.

Turner (1999) challenged a number of assumptions in Frosts mechanostat theory: 1) that bone loss, due to disuse declines rapidly to zero; 2) the “physiological window” is the same for every bone including non-weight-bearing sites such as the skull; 3) bone
Chapter III: Literature Review

resorption occurs at the neutral axis; and 4) the loss of estrogen is considered similar to a state of disuse (192). To address these issues Turner (1999) developed a new theory called the “principle of cellular accommodation”. His theory is based on the notion that the bone will respond to a change in mechanical loading, but the response will eventually phase out as bone cells “accommodate” to the new load. The point at which the bone’s response phases out varies from site to site, ie. weight-bearing versus non-weight-bearing sites.

Physical Activity Studies of Children and Adolescents

According to Frost’s mechanostat theory, strains greater than that needed for steady state remodeling will cause a modeling response that increases BMC to meet the increasing load requirement (191). Since bone modeling occurs during childhood, exercise is likely to have a greater effect on bone density during growth than in adulthood. The results of exercise intervention studies have indicated that exercise in adulthood may result in small increases in bone density (1 to 5.5%), no change, or bone loss (51-53). Exercise during childhood and adolescence may have no effect, or moderate to large gains in bone density (2 to 35%) (1,47-50). However, it is not known at what stage during growth exercise has the greatest osteogenic effect (am increase in the activity and number of osteoblasts), and what is the optimal mode, duration and intensity of exercise that needs to be prescribed for an osteogenic response to occur. Moreover, most studies that have investigated the effects of exercise have focused on changes in BMC or bone density; measures that do not account for bone size. Bone strength is however influenced by bone density and bone geometry, and changes in strength may occur with or without changes in bone density (50,57,193). The following section reviews data on the effects of physical activity during growth, from cross-sectional, uni-lateral and prospective studies. The changes in bone density and bone geometry are discussed separately.
Cross-Sectional Studies

High-Impact Exercise

The comparison of young athletes participating in high-impact activities with non-active controls clearly shows the benefit of physical activity on bone density during growth. Dyson et al (1997) used DXA and peripheral QCT to compare bone density and cross-sectional area of bone in elite pre-pubertal gymnasts (aged 7 to 11) who were training on average 18 hours per week, to normally active age-matched controls (50). Bone mass adjusted for bone volume (estimated using DXA and assuming a certain bone shape), was greater in the gymnasts at the femoral neck (20%) and lumbar spine (8%). At the distal radius, bone density was greater in the gymnasts due to an increase in cortical (16%) and trabecular (27%) bone density. There was a trend for the gymnasts to have a larger total cross-sectional area at the distal radius (unadjusted for the gymnast’s smaller stature). Furthermore, there was no difference in bone density between the mothers of gymnasts and controls. Therefore, bone density of the gymnasts was unlikely to be high prior to entry into the sport.

Bass et al (1998) also reported pre-pubertal female gymnasts have a higher bone density, adjusted for bone volume, at the femur (16%) and lumbar spine (12%) compared to age-matched controls (despite the gymnasts having a small stature) (1). A higher bone density was also reported at the arms (11%), a site that is uniquely loaded in gymnastic training. Thus, similar to Dyson et al, the findings were site-specific, as the gymnasts had a higher bone density at the sites typically loaded during training. A dose-response relationship was also detected between years of training and gains in BMD (r =0.3 and 0.4). Over a 12-month period the increase in bone density (g.cm\(^{-2}\)/year) at the total body, spine and legs was 30 to 85% more rapid in the gymnasts compared to controls. Volumetric bone density also increased in the gymnasts, but remained the same in the controls. This was due to a greater increase in BMC than size in the gymnasts (ie endocortical contraction), and a commensurate increase in BMC and size in the controls.
Grimston et al (1993) found no significant difference between girls participating in weight-bearing exercise and non-weight-bearing exercise (194). They compared bone density of 9 girls participating in weight-bearing exercise (running, gymnastics, tumbling and dance) to 9 swimmers aged 10 to 16 years. No differences in bone density were reported at the lumbar spine and femoral neck between the two groups, which remained after adjusting for age and years training (weight bearing group 7.1 ± 1.1 years training, swimmers 6.3 ± 0.7 years). There are a number of limitations with this data, for instance, no controls were studied, there were low subject numbers, there was a large variation in loading in the weight-bearing activities, a greater gain in bone density was detected in males participating in weight-bearing exercise, and puberty status was not assessed.

These findings suggest that high impact exercise may significantly increase bone density (up to 30%) at sites that are typically loaded during training. In addition, bone density is likely to increase with an increase in duration of training during growth. Moreover, both cortical and trabecular bone are likely to respond to additional weight-bearing exercise. A limitation with cross-sectional studies however, is that athletes may have a higher bone density prior to entry into the sport.

**Unilateral Studies**

Comparing the side-to-side differences in the arms of squash and tennis players is ideal for showing the effects of exercise on BMC because genetic, endocrine and nutritional factors are controlled for, and any side-to-side difference in BMC can be attributed to the altered loading pattern of the sport. This study design eliminates the possibility of selection bias: that is, a higher BMC may have existed prior to entry into the sport.

Jones et al (1977) was the first to compare the unilateral differences in the cortex of the humerus in playing compared to non-playing arm of adult tennis players (31). Radiographs (X-rays) were used to measure bone geometry and BMC in the arms of 48
male and 30 female tennis players (aged 18 to 50) who had been training on average 14 and 18 years, respectively. Cortical thickness was greater in the playing arm of the male (35%) and female (28%) tennis players. Similarly, Dalen et al (1985) compared the side-to-side differences in the arms of 7 professional male tennis players (aged 17 to 35) and found BMC and bone area was greater in the playing arm compared to the non-playing arm (40% and 27% respectively) (196). Calbert et al (1998) compared the side-to-side differences in 9 male tennis players (aged 25 ± 8) who started playing during the pre-pubertal years and found exercise increased BMC (21%) and cortical area (14%) (197).

Due to the cross-sectional nature of these studies, it is difficult to determine what stage during growth the greatest benefits were achieved. To address this question, Haapasalo et al (1996) compared the side-to-side differences in the arms of tennis players who started during adulthood (~age 29 years) to those who started tennis early in childhood (~age 10 years) (198). The differences in BMC, bone density, cortical thickness and bone strength were greater in the younger starters (12 to 31%) compared to the older starters (3 to 12%) (Figure 3.2). Moreover, the exercise effect in the older starters was comparable to the side-to-side differences in controls.

Similar findings were reported by Kannus et al (1995) who compared the side-to-side differences in the arms of national level female tennis and squash players (aged 16 to 50), who started before or during menarche (n = 62) to those who started more than 15 years after menarche (n = 43) (199). After adjusting for height and age, the bilateral differences in BMC were 2 to 4 times higher in those who started before or during menarche (10% to 24%) compared to those who started after menarche (2% to 9%). This finding was evident at the humerus, but not at the radius. There is no data available to explain this site-specific finding. It is possible that smaller strains may be applied to the head of the radius because less bending may occur at this site compared to the humerus. Alternatively, the main muscles involved in the tennis stroke are attached to the distal portion of the humerus, not the radius (278). Because muscle contraction
exerts a force on bone, this may explain why the humerus showed an osteogenic response but not the radius. In summary, the results of this study showed that the growing bone may be more responsive to mechanical loading than older, more mature bone.

![Bone Mass and Strength](image)

**Figure 3.2** The side-to-side differences in bone density, bone size and bone strength in the arms of tennis players who started during childhood compared to those who started in adulthood. (198). * p<0.05 versus older women.

To determine exactly what pubertal stage the bone is most responsive to exercise, Haapasalo et al (1998) compared the side-to-side differences in the arms of tennis players and controls from Tanner stages 1 to 5 (200). The side-to-side differences in bone density at the humeral shaft were significantly different in the tennis players than the controls at Tanner stage 2 (10.8 years). However, differences at all three measures sites (humeral shaft, proximal humerus and distal radius) were higher in the tennis players than controls at Tanner stage 3 (12.6 years) (Figure 3.3). The distal radius was not assessed in girls in Tanner stage 1. Within the player’s group, the total number of training hours, years training and sessions per week increased with Tanner stages. The total training hours and sessions per week did not correlate with the player’s side-to-side differences in bone density at the three measured sites for players in Tanner stage 1 or 2, but it did for players in Tanner stages 3 to 5.
Although the authors concluded from their data that the osteogenic effect of exercise may not become apparent until Tanner stage 3, and that exercise during Tanner stage 1 does not result in gains in bone density, their conclusion needs to be interpreted with caution. There was a significant difference in hours of training within each Tanner stage. For example, the mean hours of tennis for the girls in Tanner stage 1, 3 and 5 were 271, 744 and 1131 hours respectively. Although this has the potential to bias the results, the authors commented that players in Tanner stages 3 to 5 had a similar training history during Tanner stage 1 to 2 as those players assessed in this study. However, it is also possible that exercise may have increased bone density in Tanner stage 2, but the difference was not apparent until Tanner stage 3. Moreover, the side-to-side differences in bone density in controls were 3 to 5% in Tanner stage 1 and 2 and 0% in Tanner 3. Therefore, greater differences would be required in Tanner stage 1 and 2 for tennis players to be significantly different to controls (Figure 3.3).

**Figure 3.3.** The side-to-side bone density differences in the proximal humerus in controls (black bar) and players (white bar). At the proximal humerus the percent difference in the players was significantly different to controls at Tanner stage 3 to 5 (200). *p< 0.001 players versus controls.

In summary, the results of uni-lateral studies provide compelling evidence for positive effects of exercise on bone during growth. These findings show that additional loading during growth may increase bone density, size and strength up to 40%. The optimal
time to increase loading appears to be during Tanner stages 2 to 3, as greater increases in bone density appear to occur during this time compared to post-menarche or adulthood. To confirm these cross-sectional findings, prospective studies are needed to compare the side-to-side differences in bone density in the arms of tennis players who started training at different stages of puberty. Another limitation with these findings is that the “apparent” exercise effect detected in normally non-weight bearing bones (ie humerus) may not occur in weight-bearing bones, therefore the clinical relevance of these findings are questionable.

The Effect of Physical Activity on Bone Mass in Normally Active Children

Childhood and puberty have been identified as critical times for BMC accrual (71). There is however, limited data available on the effect recreational physical activity has on BMC accrual during growth and if any increases are maintained into adulthood and later in life. This information is needed before exercise can be recommended to children as means of reducing the risk of fragility related fractures later in life.

Bailey et al (1999) investigated whether physical activity influences BMC accrual during the adolescent period (47). Fifty-three girls and 60 boys were followed over 6 years. Growth velocity curves were fitted for each child and age at peak BMC accrual was determined for each child. Exercise was a significant predictor of BMC accrual during the 2 years surrounding peak BMC accrual (age 12-14 in girls and 13-15 in boys) at the total body, femoral neck and lumbar spine. Exercise was also a predictor of BMC at the total body and femoral neck, but not the lumbar spine, one-year post peak BMC accrual. Controlling for maturation and differences in size, there was a 10 to 18% greater gain in BMC in active boys and girls compared to those classified as inactive, 1 year after peak BMC (Figure 3.4). Therefore, those who were more active during growth accumulated more bone and had a higher peak BMC. There are a number of limitations with this study: 1) there was a small sample size representing high and low activity levels (n = 13 to 15); 2) it was not clear if the questionnaire excluded time spent engaged in non-weight bearing activities that may not be osteogenic, such as swimming.
(201); and 3) the estimation of peak BMC was 1 year after peak total body BMC accrual. However, peak BMC varies from site to site, and may not reach peak until 30 years of age (141); 4) more than 1 year may be needed to confirm the long-term benefits of physical activity; 5) sampling bias may have affected the results because more active children may have a larger muscle mass (and hence BMC) and are more likely to continue playing sport than less active children (202); 6) there is no data on the changes in BMC among the groups at the beginning of the study; 7) this sample is highly selected as it represents about 50% of the sample originally included in this longitudinal study; and 8) no definition was provided on what constitutes an inactive, average and active child.

Figure 3.4 Bone mass accrual velocity (g.year⁻¹) by inactive, active and average physical activity groups for boys and girls (47). *p<0.005, **p<0.001 compared to inactive.

Slemenda et al (1991) reported a positive association between bone density at the radius, spine and hip and hours of weight-bearing activity in younger children aged 5 to 14. Time spent watching television and hours of physical activity at school were not related to bone density (203). In a 3-year prospective study, the same research group found an association between reported hours of weight-bearing activity and change in femoral bone density in pre-pubertal children (aged 7.4 ± 1.5 years) (143). However, there was no relationship between physical activity levels and change in femoral neck BMD in peri-pubertal girls.
Ruiz et al (1995) found weekly hours of physical activity was related to lumbar spine and femoral neck bone density in girls, but not boys (aged 7 to 15 years) (49). Physical activity was also a stronger predictor of bone density than calcium intake. These findings suggest that physical activity may have a beneficial effect during puberty. However, there was no explanation why physical activity may be a stronger predictor of bone density in girls compared to boys. The questionnaire included hours of non-weight bearing activity (ie swimming), but it is not known if more boys than girls participated in non-weight bearing exercise. This may explain why no relationship was detected between physical activity and bone density in boys (201). Alternatively, boys may have had a smaller range of reported hours of physical activity, therefore making it statistically difficult to detect a relationship.

To determine if higher physical activity levels during growth confer a benefit to bone density in adulthood, a number of retrospective studies have measured bone density in adults and related these to estimated lifetime physical activity levels (204-207). These studies reported that hours of physical activity during adolescence were associated with a greater bone density in adulthood, independent of current physical activity. In support of these findings, Groothausen et al (1997) reported that the ground reaction force associated with the activities a person engaged in from 13 to 27 years explained 25% of the variance in lumbar spine bone density in adulthood (age 27 years) (208). Taken together, these findings support the notion that weight-bearing, high impact exercise during growth has long-term benefits on bone density. However, it is not known if there was a relationship between BMC and physical activity levels during childhood. It is also possible that selection bias confounded the findings, as those who participated in sport may have had a higher BMC prior to entry into the sport. This bias cannot be eliminated in retrospective studies, so the results need to be interpreted with caution.

In summary, the findings from these studies suggest that physical activity in normally active children may increase BMC accrual and that the benefits may be maintained into adulthood. There are, however, two limitations with the data relating to the relationship
between physical activity and BMC: 1) the ability of the parents to assess their child’s activity levels; and 2) the ability of the subjects to accurately recall their physical activity levels during growth. Stronger evidence for the effects of exercise on BMC is provided from randomised control trials or uni-lateral studies on tennis players.

**Prospective Exercise Studies**

A number of prospective randomised controlled studies investigating the effects of exercise in children and adolescents have recently been published (45,209-214,320). These studies are needed to validate cross-sectional and cohort studies that may be confounded by sampling bias. Randomised control trials provide the strongest level of evidence to detect a cause and affect relationship between exercise and BMC accrual (202).

One of the first exercise intervention studies to be completed in children was conducted in primary schools over a 10-month period (209). Four schools from the same ethnic, socio-economic and geographic area were assigned to either an exercise or control school. Girls from grades 4 to 5 (age 9 to 10 years) were invited to participate in the study. The exercise regime consisted of 30 minutes of weight-bearing activities, including jumping and a small amount of weight training 3 times a week. There was a greater increase in BMC at the femur (4.5%) and lumbar spine (5.5%) in the exercise group compared to controls. Girls were pre-menarcheal at the initial phase of the study, but their exact Tanner stage was not reported. After adjusting BMC at the lumbar spine and femoral neck for increases in height and weight, and size at the femoral neck, the changes were no longer significant. Therefore growth and maturation may have influenced the results at the lumbar spine and femoral neck, not exercise.

Smaller gains in BMC were reported in an 8-month exercise intervention in boys and girls (age 6 to 10 years) (210). Third and forth grade male and female students at different schools were randomly assigned to their usual physical education programs (controls group n = 81) or to an intervention program (n = 63). The exercise classes
consisted of 10 or more tuck jumps (3 to 5 times body weight), 3 times a week and jumping activities were conducted twice a week. Bone density increased 1.2% at the trochanter. This difference remained significant after controlling for baseline bone density, change in height and lean body mass, calcium, physical activity, sex and ethnicity. However, this difference is quite small and confined to one region of the skeleton. Moreover, the exercise regime was not progressively overloaded throughout the intervention. All the boys remained Tanner stage 1 throughout the study. Twenty-eight girls progressed from Tanner stage 1 to 2, and 8 girls were in Tanner stage 2 at baseline, but it is not known if they advanced in maturation, or what study group they were in. A more mature child is likely to be going through their adolescent growth spurt, and is likely to show large increases in BMC compared to a less mature child (215,216). This may lead to a type I error as the changes in BMC may be to growth and maturation, and not exercise.

Bradney et al (1998) examined the effect of moderate exercise on bone density in pre-pubertal boys over an 8-month period (26). Twenty boys (aged 8 to 11) were allocated to an exercise program consisting of an additional 30-minute physical education lesson (basketball, weight training, aerobics, soccer, gymnastics, line dancing), 3 times per week, and were compared to 30 controls matched for age, anthropometry measures and baseline bone density. There was a greater increase in bone density at the lumbar spine (L2-4) (2.2%) and legs (3.3%) in the exercise group compared to controls. Volumetric bone density of the femoral shaft (estimated from DXA) increased 5.5% more in the exercise group, due to endocortical contraction. Bone strength did not increase at the femoral shaft, despite the increase in BMC. There was no difference in the gain in BMC, area and volumetric bone density at the third lumbar vertebrae (L3) between the two groups. This is in contrast to the finding that the exercise group showed a greater increase in bone density at L2-4. The authors offered no explanation for this. Furthermore, the exercise program was not designed to be progressively overloaded. Although there is no experimental data to support the need for this, this notion is based on Frosts mechanostat theory that the load must be greater than what bone is normally
exposed to, in order to attain an osteogenic response. The findings however, did show
that weight-bearing exercise may increase bone density in pre-pubertal boys by
increasing cortical thickness.

A randomised-controlled trial investigated the effects of high impact exercise on bone
density in boys and girls over a 7-month period (211). Eighty-nine pre-pubertal
children were randomised into a jumping or control group. The jumping group
performed 100 jumps in a uni-lateral direction off a 61cm box (8.6 ± 1.1 times body
weight), 3 times a week. Gains in BMC were adjusted for baseline BMC, age, changes
in height and weight. The exercise group (boys and girls) showed a 3.1% greater
increase in BMC at the femoral neck compared to controls. However, after adjusting
for bone area, there was no difference in BMC gains between the groups (Figure 3.5).
There was a 4.5% greater increase in BMC at the lumbar spine, but not in bone area,
therefore bone density increased more in the exercise group. In summary, an increase in
bone size explains the gains in BMC at the femoral neck in the exercise group, but not
at the lumbar spine. It is not known if there were differences in the gains in BMC or
size between the sexes. Boys have been reported to show greater gains in bone size at
the vertebrae compared to girls during the pre-pubertal years (120). The findings
however, indicate that jumping at ground reaction forces of 8 times body weight is a
safe and effective method of improving bone density at the spine in children. It is not
known if the program is as effective in adolescent children.
Witzke et al (2000) investigated the effect of additional high impact training on BMC accrual after puberty (212). They introduced plyometric training to 25 post-menarcheal girls (aged 14.6 ± 0.5 years) over a 9-month period and compared the change in bone density to 28 age and maturity-matched controls. Girls in the exercise group performed exercises 3 times a week, using weighted vests (squats, lunges, calf raises) for the first 3-months and then plyometric exercises for 6 months (hopping, bounding, box depth jumps) (4-7 times body weight). The plyometric training was progressively increased throughout the intervention by increasing the number of moderate impacts from 100 to 1000, and the number of high impact jumps from 40 to 100 per session. There was no difference in the gains in BMC at any site between the exercise and control groups. The controls were however more active outside of school compared to the exercised girls (5.6 versus 2.6 hrs.week\(^{-1}\) respectively). Randomising the girls to each group may have prevented this. The authors also reported that the sample size was too small to provide statistical power. Moreover, the length of the high impact plyometric training may have been too short.

The limited effect of exercise during adolescence was also shown in a 9-month randomised controlled exercise trial in 58 pre- and 61 post-menarcheal girls (aged 11.4

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**Figure 3.5** Pictorial representation of jumping exercises (left). Seven month percent change in femoral neck bone mass and area and lumbar spine bone mass in jumpers (black bar) and controls (white bar) (211). *p<0.05, **p< 0.001 versus controls.
± 1.2 and 13.7 ± 1.0 years, respectively) (213). The exercise program consisted of 2
classes per week that involved jumping off a 30 cm box and landing in various
directions. The number of jumps increased from 100 to 150 and one-legged jumps were
incorporated late in the program. The exercise intervention had no benefit on BMC in
the post-menarcheal girls but BMC increased at the femur (3.3%) and lumbar spine
(4.5%) in pre-menarcheal girls. Bone strength was assessed by QCT at the tibia, but no
exercise effect was detected in the pre- or post-menarcheal girls (BMC was not
measured at this site). Blimkie et al (1996) also reported that additional weight training
during adolescence has no real benefit on bone density (45). Therefore, the pre-
menarcheal years appear to be an opportune time for exercise to increase BMC. Further
work is needed to determine if the greatest osteogenic response to exercise occurs
during the pre- or peri-pubertal years.

Sundberg et al (2001) also investigated the effects of exercise during different stages of
growth (214). They assessed the benefit of a 3 and 4-year school-based exercise
intervention on BMC accrual in boys and girls (aged 12 to 13). The exercise group
increased their physical education classes at school from 100 to 160 minutes per week
(4 x 40 minute sessions, 3 sessions included weight-bearing activities). The exercise
group was compared to a control group from another school that continued exercising
100 minutes per week. After the intervention, the boys in the exercise group had a
higher BMC at the femur (8%) and lumbar spine (9%) compared to controls. The results
remained significant after controlling for weight, height, milk intake and physical
activity levels. Additional exercise had no benefit on BMC in girls. There was an 8 to
13% greater gain in bone density in boys who started the intervention 1-year earlier than
other boys (4 versus 3 years intervention). The authors suggested that the boys, who
started the intervention earlier, might have been exercising during an earlier stage of
growth when the bone is more responsive to exercise. However, the boy’s pubertal
stage was not reported. Moreover, BMC was only measured at the end of the
intervention. The results also showed that additional exercise had no benefit on BMC in
girls. This may be due to a number of reasons: 1) the female controls were more active
outside of school; 2) the controls had an earlier onset of menarche; and 3) girls begin puberty at an earlier age compared to boys, therefore the intervention may have started too late in the girls (19% had achieved menarche at baseline).

MacKelvie et al (2002) recently published a 7-month controlled exercise intervention in 133 pre-pubertal boys (320). They randomised 14 schools to either control or intervention groups. The exercise program consisted of 10-12 minutes of high-impact, weight bearing activities that were progressively overloaded by increasing the height (from 10 to 50 cm) and the number of jumps (from 50 to 100 per session) throughout the study. Ground reaction forces for these activities typically were 3.5 to 5 times body weight (210). At the end, the intervention group gained more total body BMC (1.6%) and proximal femur bone density (1%) than the control group. However, when comparing boys in both groups whose body mass index were ~23 (kg/m²), there was no exercise effect. This finding suggests that the skeleton of heavy boys may not respond to additional exercise because it is already under substantial stress due to their body weight. Furthermore, the changes in BMC and bone density are small and are unlikely to reduce the future risk of osteoporosis.

In summary, exercise intervention studies provide the strongest evidence that exercise has a positive effect on BMC during growth. However, it is unclear at what stage during puberty exercise results in the greatest gains in BMC. The findings from the exercise interventions suggest that additional moderate to high impact exercise, 2 to 3 times a week, for 7 to 10 months, may result in small to clinically important gains in bone density (1 to 9%). However, it is not known if the benefits are maintained later in life when the risk of fracture is high. Nor is not known what the optimal type, intensity and duration of exercise is required for an osteogenic response in children. Further work is also needed to assess the effect of exercise on bone morphology as bone strength may increase due to periosteal expansion, with or without corresponding changes in bone density.
Long Term Benefits of Exercise During Growth

For exercise to be considered important in the prevention of osteoporosis, the benefits must be maintained later in life when the risk of fracture is high. To accurately address this issue, long-term intervention or cohort studies following active and non-active children into adulthood are required. The long time interval between exposure and outcome makes these types of studies difficult to conduct. In the interim, researchers have analysed the bone density of retired athletes who began their training in childhood. Any site-specific difference in bone density between retired athletes and controls is presumed to be to the exercise that they undertook during growth.

Duppe et al (1996) measured bone density of active female soccer players (aged 13 to 28) and former soccer players (aged 34-48) who retired on average 9.7 years previously (217). The results showed bone density was higher at the femur (11%) in active players compared to controls. The high bone density appeared to be maintained into adulthood as the retired players had a similar difference in bone density at the femur (11%) compared to controls. No differences in physical activity levels were detected between retired players and controls. Similarly, Bass et al (1998) measured bone density in active elite pre-pubertal gymnasts (aged 10 ± 0.3 years) and retired gymnasts (aged 25.0 ± 0.9 years) who had been retired for 8 years (2 to 20 years) (1). The retired gymnasts were 7.5 ± 0.4 years of age when they started training, trained for 9.5 ± 0.5 years and retired at 16.7 ± 0.4 years of age. Bone density at weight-bearing sites was higher in active gymnasts compared to controls (femoral neck 15%, lumbar spine 24% and arms 12%). The benefits appeared to be maintained into adulthood as the retired gymnasts had a higher bone density at the same sites compared to controls (femoral neck 16%, lumbar spine 6% and arms 9%, p < 0.06 to < 0.01). Nor was there a difference in bone density at the skull (a non-weight-bearing site) between the gymnasts and controls. Furthermore, age-adjusted bone density z scores, relative to controls, showed bone density did not diminish with increasing years since retirement. Thus residual benefits of intense exercise on bone density during growth appear to be maintained into
adulthood, and may be enough to reduce the future risk of fracture; a 10% increase in bone density is associated with a halving of the fracture risk (218).

To determine whether the benefits of exercise are maintained later in life when the risk of fracture is high, Karlsson et al (1996) compared the bone density of retired male weight lifters (age 35 to 79 years) to age-matched controls (219). They found a higher total body bone density (6%) in 24 retired lifters over 50 years of age, who had been retired for 24 ± 13 years. However, the effects of exercise appeared to be attenuated with time because bone density in weight lifters over 65 year old, was no different than controls. However, only 9 subjects were over 70 years of age. Nor is it known if the older lifters trained less during growth compared to the more recently retired lifters.

More recently, Karlsson et al (2000) reported that the residual benefit of playing soccer on bone density during growth may diminish later in life (220). They compared active (aged 17 to 34 years) and retired soccer players (aged 19 to 85 years) to age matched controls. Relative to controls, leg bone density was 12% higher in the active players, 10% higher in soccer players retired for 5 years, 5% higher in those retired for 25 years, but no different in the players retired for over 35 years. The diminution in bone density was ~50% greater in the soccer players compared to the controls (0.33% versus 0.21% per year). There was no difference in the proportion of soccer players with fragility fractures compared to controls (2.1% vs. 3.7%). Thus, three to five decade after cessation of an active career most of the benefits on bone density appeared to be lost.

Although the findings by Karlsson et al (2000) suggest that the benefits of exercise diminish with age, there is a possibility that a type II error may have been reported. Firstly, there was only a small sample of subjects over the age of 65. Secondly, training may have been less vigorous in older retired soccer players, so they may have attained a lower peak BMC. Finally, the soccer players over the age of 70 had a 6.5% higher leg bone density than controls after adjusting for current activity and body composition. Thus, the findings by Karlsson et al (2000) are not conclusive but they do suggest that
the benefits of exercise during growth may be eroded in retirement and have little to no benefit when fractures usually occur.

These retrospective cross-sectional studies provide preliminary evidence that residual benefits are maintained in adulthood, whether they are maintained into old age when fractures occur is unknown. However, there are some limitations to the data. First, it is not known how much bone was gained from exercise before the cessation of the training. Second, it is difficult to assess retrospectively the quantity and quality of exercise and lifestyle factors known to influence bone health in both athletes and controls. Third, although the athletes have ceased training, it is possible that they were more active than the controls. Fourth, it is possible that the subjects are self-selected athletes: those who had a larger muscle mass may have been more inclined to take up and continue playing sport. A larger muscle mass is associated with a higher BMC, so genetic factors rather than exercise may account for the high BMC found in athletes (220).

To eliminate the likelihood of selection bias, Kontulainen et al (1999) compared the side-to-side differences in the arms of semi-retired tennis players (221). This was a 5-year follow-up study on female tennis players who had started training either before or at puberty (mean starting age 10.5 ± 2.2 years, n = 36), or post-puberty (age 26.4 ± 8.0 years, n = 28) and had reduced their training load 50 to 70%. Semi-retirement resulted in a –2 to 0% change in the side-to-side difference of BMC in the young starters, and 0 to 2% increase in the older starters. Therefore, the benefits of exercise may slightly decrease or not change after 5 years of reduced training. The same research group reported similar findings in male tennis players who had been retired for 1 to 3 years (221). However, the data in this study are limited for a number of reasons: 1) the precision of the DXA to measure long-term changes in BMC may have been similar to the magnitude of the change in BMC in the arms, increasing the chance of a type II error (ie. no change reported when bone loss had occurred); 2) the players may have been active enough to maintain an exercise effect; 3) more than 5 years may be needed
to confirm these findings (losses of 0.5 to 1 SD are documented 5 to 15 years after retirement) (202); 4) only 64% of the original cohort of tennis players were studied; and 5) the change in BMC in each arm was not addressed. It is not known if bone loss or bone accrual occurred on the playing or non-playing arm over the 5 years of retirement.

In summary, exercise-induced bone accrual during growth may be maintained for at least 5 to 25 years, but it is not certain if the benefits are maintained into old age when the risk of fracture is high. Further research involving prospective randomised exercise trials is needed to determine whether skeletal benefits from exercise during growth are maintained with reduced levels of exercise. Further research is also needed to determine how bone structure changes (ie bone shape) as a result of exercise during growth and if the benefits are maintained later in life when the exercise has ceased.

**Effects of Exercise on Bone Surfaces During Growth**

It has been hypothesised that exercise preferentially affects the surface of bone that is undergoing bone modeling at the time (2). Periosteal apposition occurs during the pre-pubertal years in boys and girls. During puberty, periosteal expansion continues in boys but ceases in girls as estrogen inhibits periosteal apposition (114). Estrogen however stimulates endocortical bone apposition in girls but not in boys (114). Therefore, exercise may enhance bone size in pre-pubertal boys and girls whereas during puberty, exercise may enhance bone apposition on the periosteal surface in boys and endocortical surface in girls (Figure 3.6). This hypothesis is based on a retrospective data based on a small number of subjects (n = 3) (2). There is limited other data available and the findings are conflicting (26,31,50,198,222-225).
In 1977, Jones et al reported the results of a radiographic study on 78 professional tennis players in which they demonstrated increases in cortical bone area in the playing arm compared to the non-playing arm (31). In 1994, Ruff et al further analysed this data to investigate the differential response of cortical bone during different periods of growth (2). They re-analysed the data on only 46 tennis players (34 males and 12 females) whose entire morphological data was available. The mean age of the players was $25.1 \pm 4.6$ years (range 14 to 39 years), the mean starting age of playing tennis was $9.6 \pm 2.8$ years (range 5 to 19 years) and all had been playing $15.5 \pm 4.8$ years (range 8 to 28 years). The percent side-to-side differences are reported in Table 3.1. Both sexes showed similar responses to exercise leading to large increases in cortical bone area and bone strength.
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Table 3.1  Uni-lateral differences in cross-sectional area of the mid-humerus in the playing compared to non-playing arm of male and female tennis players (mean ± SE) (2).

<table>
<thead>
<tr>
<th>Sample</th>
<th>number</th>
<th>Periosteal</th>
<th>Endocortical</th>
<th>Cortical</th>
<th>Polar Second Moment of Inertia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>34</td>
<td>25.4 ± 1.9</td>
<td>-19.8 ± 2.8</td>
<td>40.6 ± 2.5</td>
<td>68.5 ± 5.2</td>
</tr>
<tr>
<td>Females</td>
<td>12</td>
<td>18.7 ± 2.6</td>
<td>-17.3 ± 3.1</td>
<td>32.7 ± 3.3</td>
<td>43.5 ± 7.3</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>23.7 ± 1.6</td>
<td>19.2 ± 2.2</td>
<td>38.5 ± 2.1</td>
<td>62.0 ± 4.6</td>
</tr>
</tbody>
</table>

All differences are p<0.01

The age at which the players started training was negatively correlated with periosteal diameter (r = -0.3 p <0.05) and cortical area (r = -0.4, p < 0.01) but not medullary area. Therefore, players who started training at an early age had greater periosteal expansion in the playing arm, but did not show signs of endocortical contraction. The results indicate that mechanical loading may have a greater effect on periosteal expansion during the early stages of growth compared to the post-pubertal years. It is difficult however, to make definite conclusions because the playing periods of most subjects overlapped with different developmental periods. For example, a subject who began playing at 9 and measured at 25 would have increased mechanical loading on their arm during late childhood, adolescence and young adulthood. It is therefore difficult to distinguish the effect of playing during different developmental periods.

To determine how bone geometry changes in response to exercise during different stages of growth, Ruff et al (1994) analysed three tennis players; one female who started playing during the pre-pubertal years, and a male and female who started during puberty. The young starter showed large side-to-side differences in cortical area (54%), due to periosteal expansion (34%) and very little endocortical contraction (6%). In contrast, the two players who started during puberty showed smaller side-to-side differences in cortical area (23%), due to greater endocortical contraction (28% and 19%) compared to periosteal expansion (10% and 13%). These findings support the notion that the periosteal surface may be more responsive to exercise during childhood.
and the endocortical surface is more responsive after puberty. However, this study is limited by its small sample size.

Other data on the surface-specific effects of exercise during growth are limited and conflicting. Additional weight bearing exercise over a 10-month, had no effect on the periosteal diameter at the femoral mid-shaft in pre-pubertal boys, but increased bone apposition on the endocortical surface (26). Similar changes in response to exercise were reported in a study on pre-pubertal female gymnasts (1). In contrast, gymnastics training was reported to increase bone formation on the periosteal surface during pre-pubertal years, not on the endocortical surface as previously reported (50). A comparison of the side-to-side differences in the arms of seven professional male tennis players showed that exercise enhanced bone formation on the periosteal surface, not the endocortical surface (196). However in this study training history was not reported and there was a large variability in the exercise response at the endocortical surface (–44% to +64%). Finally, Haapasalo et al (1996) compared the side-to-side differences in the arms of female tennis players, who started playing early in childhood (age 5 to 14 years) (198). The difference in cortical thickness was greater than bone width (20% versus 2%), which suggests that exercise increased bone formation on the endocortical surface during the pre- to peri-pubertal years.

During the post-pubertal years, exercise appears to increase bone apposition on the endocortical surface. Margulies et al (1986) reported that 14 weeks of military training in 18 to 21 year old males increased bone density at the tibia without a change in bone width (223). Similarly, Ashizawa et al (1999) found three tennis players who started tennis training after age 16, had greater trabecular bone density at the distal radius (13 to 17%), but no change in bone size (224). Similarly Haapasalo et al (1996) compared the side-to-side differences in the arms of female tennis players who started training after puberty (19 to 40 years of age) to controls (198). They found the percent difference in cortical thickness was greater in the tennis players compared to controls (~9% versus
~2%) but bone width was similar. These results indicate that exercise increased bone formation on the endocortical surface during the post-pubertal years.

The response to exercise also appears to vary along the length of the humerus. Haapasalo et al (2000) used QCT to compare the side-to-side differences in the arms of male tennis players (age 31 ± 5 years) who started training during childhood (age 10 ± 3 years) (225). Exercise had a greater effect on BMC, periosteal area, cortical area and bone strength of the playing arm at the distal humerus compared to the proximal humerus (27 to 67% versus 16 to 33% respectively). In addition, exercise enhanced periosteal bone apposition (16%) and endocortical expansion (19%) in the playing arm at the proximal humerus, but increased periosteal (21%) and endocortical bone apposition (3%) at the distal humerus (Figure 3.7). However, the players had been retired for 1 to 3 years so it is not known how much of the geometric differences occurred after training. Another limitation of this study was that the proximal versus distal response to exercise was not tested statistically. Finally, the side-to-side comparisons in the arms of tennis players were significantly different to those in controls, at most measured sites (Figure 3.7).

Similar findings were reported earlier by Happasalo et al (1996) who measured bone geometry using DXA in female tennis players (aged 19 ± 3 years) who had started training during childhood (age 9 ± 2 years) (226). Exercise had a greater effect on cortical area at the distal humerus compared to the proximal humerus (31% versus 18%). Furthermore, the BMC and bone size data suggest that exercise preferentially enhanced bone formation on the periosteal surface at the proximal humerus, and on the endocortical surface at the distal humerus. In contrast to the findings from male tennis players (225), bone strength was greater at the proximal (24%) compared to the distal humerus (14%). In the female players, the increase in bone strength at the proximal humerus is likely to be due to bone being distributed further away from the medullary cavity. Whereas in the male players, the increase in bone strength at the distal humerus is likely to be due to an increase in periosteal and endocortical bone apposition, hence
an increase in cortical area at this site. These findings highlight that bone strength is dependent on bone geometry, BMC and cortical thickness (4).

**Figure 3.7** Comparison of the side-to-side differences in bone variables in the arms of female tennis players versus controls at the proximal, mid and distal humerus (225). *p<0.05 vs. non-playing arm. † p<0.05 versus controls.

The surface-specific effect of exercise also appears to vary across the surface of cortical bone in anteroposterior versus mediolateral directions (195). X-rays on the humerus of male and female tennis players showed exercise had a greater effect on the periosteal than the endocortical surface in the mediolateral direction (80% versus 20%) but a similar effect on each surface in the anteroposterior direction (55% versus 45%, respectively). This finding highlights a limitation in all of the studies reported in this section, with the exception of Haapasalo et al (2000) and Aschwitz et al (1999) (224,225). That is, X-ray or DXA provide two-dimensional images of bone in the coronal plane that represent periosteal and endocortical changes in the mediolateral direction only. Furthermore, inferences from a single site cannot be made as changes vary along the length of the bone and changes at one or two points will not capture the
effect of exercise along the length of the bone. Thus, cross-sectional images of bone at a number of sites (by QCT or MRI) are needed to provide more accurate conclusions on the effects of exercise on bone geometry.

In summary, the results on the effect mechanical loading has on bone surfaces during growth are conflicting. It appears as though mechanical loading may increase periosteal bone formation during the pre-pubertal years and the endocortical surface during puberty. These findings however, are not consistent in the literature. In addition, there appears to be heterogeneity in the magnitude of the exercise response along the length of the bone. Further research is warranted that prospectively measures the exercise-related changes in bone morphology at different sites along the length of cortical bone, and during different stages of growth.

**Exercises Programs Aimed at Increasing Bone Mass During Growth**

Exercise during growth is often recommended as a public health measure for osteoporosis prevention. This premise is based upon the notion that exercise during growth will increase peak BMC and, if this is maintained, the future risk of fracture will be reduced. However, there is little information on the type and intensity of exercise that needs to be prescribed to result in clinically important increases in BMC. Approximately 20 years ago, researchers began investigating the effect exercise has on BMC by comparing the side-to-side differences in BMC in the arms of tennis players (195,227). These studies found that additional loading may significantly increase BMC. Further research over the following 10 to 15 years led to the discovery that high impact weight-bearing exercise is more beneficial than low impact weight-bearing exercise (48,228). These findings are based on the cross-sectional comparison of elite athletes to sedentary age-matched controls, however they may be influenced by selection bias – that is, a larger muscle mass may influence a person’s choice to take up and continue sport. And a larger muscle mass is associated with a higher BMC, so genetic factors rather than exercise may account for the high BMC found in athletes (220). Not until the early 1990’s did exercise intervention trials begin to appear, in which the allocation
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of participants was randomized, thus eliminating the likelihood of selection bias. Results of these studies confirmed the notion that additional weight-bearing exercise may significantly increase bone density in normally active children. However, there is little detail provided on the prescribed exercise program, thus making it difficult to ascertain what component of the program resulted in the osteogenic response. To date, the most detailed information on the effect different exercise parameters have on BMC are from animal studies.

Different Modes of Mechanical Loading in Animal Studies

To investigate the effect of exercise on animal bone, mechanical loads are generally applied to bone using a device that does not require surgical intervention and allows normal activity between loading sessions (229). For instance, a four-point bending device applies force to the leg of a rat, generating a strain in the bone. The loading can be varied to apply a different number of impacts, strain magnitude (extent of deformation), strain frequency (number of strains applied within a specified time period), strain rate (changes in magnitude per second, or acceleration or deceleration of deformation) and type of loading (compressive, tensile or shear) (230). More invasive experiments have involved the removal of one supporting limb, however they introduce possible confounding effects of physiological trauma. The following section reviews the effect of different components of mechanical loading in a variety of animal species.

Strain Magnitude

It is proposed that activities that place a large strain on the bone will significantly increase BMC. To investigate this in animals, researchers have applied loads of varying magnitude to bone over a number of months (231,232). The magnitude of the load is measured according to how much strain is applied to the bone *in vivo*. For instance, a high load may deform the bone 3% of its original length and is equivalent to applying a strain of -0.03. Strain has no units and is either reported as relative deformation (a strain of -0.01 = 1%) or in terms of microstrain (10 000 µs = 0.01 strain = 1% deformation) (180). The results of animal studies suggest that a dose-response
relationship exists between strain magnitude and BMC (231,232). Thus, greater gains in BMC will occur in response to loads high in magnitude compared to those low in magnitude.

Rubin and Lanyon (1985) were the first to investigate the effect of strain magnitude on bone formation in vivo (231). They applied 100 loads of varying magnitude to the ulna of skeletally mature birds for 8 weeks. The results showed a dose-response relationship for gains in BMC and strain magnitude (r=0.8). Moderate-low strains (-0.0005) were associated with bone loss, while moderate strains (-0.001) had no additional effect. Strains greater than -0.001 were associated with an increase in BMC, predominantly on the periosteal surface. However, the strains that increased BMC were within the range that the bone would normally be exposed to (ie. during vigorous wing flapping). The authors concluded that the increase in BMC might have been due to a difference in the distribution of strain between everyday and applied loading.

Mosely et al (1997) observed the effect strain magnitude has on bone by applying 1200 loads of varying magnitude for 10 minutes a day, on days 1-5 and 8-12 to the experimental limb of 43 day old rats (232). The loads were either similar (-0.0005), slightly above (-0.001 to -0.002) or well above (-0.003, –0.004) the loads rats would experience during everyday activities. Loads slightly above those normally encountered, suppressed periosteal growth (-9% to –17%), whereas loads well above those normally encountered (3 to 4 times greater than walking), increased periosteal bone formation at the distal ulna (up to 37%) and decreased formation at the proximal ulna (up to –25%) (Figure 3.8). The majority of the new bone was deposited on the medial and lateral surfaces of the experimental limb: sites where the greatest strains were recorded.
There were two conflicting findings in these studies in birds and rats. First, BMC of the birds increased in response to loads that were within the range they would normally be exposed to (231). In contrast, BMC of rats only increased in response to loads that were three to four times the magnitude of those encountered during walking (232). Second, bone formation in the rats corresponded with the sites of peak strain magnitude, but it was not found in the birds. There were a number of differences in these studies that may explain these findings. First, the birds were skeletally mature whereas the rats were still growing. Second, because the loads applied to the birds were different to what they normally encounter, an unusual strain distribution, not magnitude, may have increased bone formation. Third, the birds were allowed to use their wings in between loading episodes: therefore, normal cage activity may have attenuated the osteogenic effect of additional loading in the birds.

In summary, increasing the magnitude of load and producing unusual strain distributions during exercise may provide a positive effect on BMC. However, the findings are limited because the effect strain magnitude has on BMC cannot be uncoupled from the effects of strain rate and strain frequency. When strain frequency is

Figure 3.8. Difference in periosteal new bone formation between ulna loaded at strain of either \(-0.002\) (triangle) or \(-0.004\) (square) and their respective unloaded controls, over the length of the diaphysis. Sections are taken at 1mm intervals such that section 1 is 6 mm proximal to the mid-shaft and section 6 is 12 mm distal to the mid-shaft (232). (Mean ± SE)
held constant, increasing the strain magnitude increases the strain rate; when strain rate is held constant, increasing the strain magnitude changes the strain frequency (233).

**Number and Type of Impacts**

It is hypothesised that increasing the number of impacts in an exercise session will stimulate bone formation. However, excessive loading can have detrimental effects on bone by increasing the risk of stress fracture (234). Fortunately, the results of animal studies suggest that a high number of impacts do not necessarily result in a greater increase in BMC. This phenomenon was demonstrated in a study by Rubin and Lanyon (1984) who applied a different number of impacts at a high magnitude and low frequency to the ulna of roosters, and found that the increase in BMC was not proportional to the number of impacts (235). They found that more than 36 impacts per day did not result in a significantly greater increase in BMC (Figure 3.9). Umenura et al (1997) reported similar findings. They found five jumps per day was sufficient to increase BMC in rats, but increasing the number of jumps to 10 or 100 per day had no additional benefit (344).

![Figure 3.9 Percent change in bone mineral content at the mid-shaft of the ulna in response to a different number of loads applied over a 6-week period (235).](image)

These findings suggest that only a small number of impacts need to be prescribed in an exercise session to have an osteogenic effect. In both studies, the magnitude of these loads were not above those that the animals would normally encounter, therefore the response may have been due the direction that the loads were applied, not the number of
impacts. It has been proposed that bone is more responsive to loads that distribute a
strain in a different direction compared to what the bone is normally accustomed to
(236,237). For instance, tensile or compressive loads are considered to have a greater
effect on BMC accrual than torsional loads (63). (However, the response to torsional
loads may not be necessarily interpreted as a less effective response to loading; it may
just be that the change of shape to adapt to torsional loads, in this case to become more
circular, requires less bone formation). Further research is needed to determine if short
periods of diverse loading can have an osteogenic effect in humans.

\textit{Strain Rates and Strain Frequency}

Bone formation also appears to be responsive to high strain rates and strain frequencies.
It is hypothesised that a faster strain rate and strain frequency increase fluid flow
through the bone matrix and this increases fluid shear forces on bone cells and generates
stress generated potentials that may activate resting bone cells (238,239). However,
strain rate and strain frequencies are interrelated with strain magnitude so it is difficult
to control one variable without increasing another.

Mosely et al (1997) investigated whether strain rate affects bone modeling of the ulna in
growing rats. They applied the same load (-0.004) at different rates (low 0.018 sec$^{-1}$,
moderate 0.03 sec$^{-1}$, high 0.10 sec$^{-1}$) (232). A high strain rate means a shorter period of
time to apply the load and vise versa. The resting period in between loads was adjusted
to ensure the same frequency (Figure 3.10). Twelve hundred impacts were applied to
the ulna on days 4-8 and 11-15. After 18 days, a high strain rate increased bone
formation 54% more than a moderate strain rate, which in turn showed a 13% greater
response than a low strain rate. Changes on the periosteal surface were similar for all
groups; increased bone formation distally and reduced formation proximally. There was
a significant decrease in endocortical bone formation along the diaphysis in the low
strain rate group, however no difference was found in other groups. Although the high
strain rate resulted in large increases in bone formation, the combined strain magnitude
and strain rate (-0.004 at 0.10 sec\(^{-1}\)) were above the loads that the rats would normally encounter.

![Figure 3.10. Schematic description of the low (0.018 sec\(^{-1}\)), moderate (0.030 sec\(^{-1}\)) and high (0.10 sec\(^{-1}\)) strain rates applied to the rat ulna. High strain rates are a shorter time to peak strain and vice versa. The same peak strain was applied in each incidence and strain frequency was held constant at 2 Hz (232).](image)

Similar findings on the benefit of high strain rate were reported by Turner et al (1995) (240). They applied 36 impacts of the same strain frequency and strain magnitude to the rat ulna, but varied the strain rate between groups (0.0, 0.013, 0.026 and 0.039 sec\(^{-1}\)). Comparing the loaded to the unloaded leg the results showed that high strain rates (0.026 and 0.039 sec\(^{-1}\)) increased the rate of endocortical bone formation (94% and 134% respectively), but low strain rates (0.013) had no effect on bone formation. In this study the method of modifying strain rate was different to Mosely et al (232). Strain rate was adjusted by varying the strain range. The range in the magnitude of the load (strain gradient) was 0 to 54 N for the high strain rate group, and 36 to 56 N in the low strain rate group. Thus, the range of strain applied for each impact was 4 times larger in the high strain group compared to the low strain group. Therefore, the increase in bone formation may have been due to a combined effect of strain rate and the range in strain magnitude, rather than strain rate alone.

Strain frequency is considered one of the functionally important mechanical variables that affect bone strength. Rubin and McLeod (1994) indicated that low magnitude...
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mechanical stimuli are incapable of maintaining avian bone mass at 1 Hz but can induce significant bone formation when loading is applied at 20 Hz (319). Turner et al (1994) found that osteogenesis can only be induced in the rat tibia, if loading frequency exceeds 0.2 Hz (318). Moreover, with frequencies ranging from 0.2 to 2 Hz bone formation was approximately proportional to the rate of strain applied to the bone.

A recent study by Hsieh and Turner (2001) found that high strain frequencies result in greater increases in bone formation on the periosteal surface compared to low strain frequencies (317). Loading was applied to the ulna of rats for 360 cycles/day with peak loads ranging from 4.3 to 18 N at frequencies of 1, 5 and 10 Hz. After 2 weeks of loading, periosteal bone formation increased in a dose-response manner with peak load at each of the three loading frequencies tested. An increase in loading frequency resulted in a decrease in the strain threshold required to stimulate an osteogenic response. Furthermore, an increase in loading frequency resulted in enhanced bone formation on the periosteal surface. There was no osteogenic effect detected on the endocortical surface. This latter finding suggests that the strains at this surface were below the osteogenesis threshold.

In summary, a high strain rate and frequency may provide a greater osteogenic stimulus on cortical bone, than the same strain applied more slowly and less frequently. Future work is needed to investigate whether strain magnitude has a greater effect on bone formation than strain rate or frequency.

Summary of Animal Studies

In summary, the results from animal studies show that bone adapts to an increase in strain magnitude, number of times a load is applied, unusual strain distribution, strain rate and frequency. However, there are a number of limitations with the results of these studies: 1) they are specific to only one bone site. Single sites may be unrepresentative of the overall response of the skeleton to mechanical loading; 2) the strain magnitude was at times an extreme level of loading; 3) the relationship between muscle mass and
BMC was not taken into account (242); 4) the findings were from a wide range of animal species, of different ages and from various skeletal sites and 5) in many of the models, there was no active muscle contraction as in a human skeleton.

To date, it is not known which strain-related variable provides the greatest stimulus to bone cells. It is possible that no single variable predominates, but bone cells respond to a combination of bioelectrical and biomechanical changes that occur when a bone is subjected to mechanical load (243). Furthermore, it is difficult to quantify the individual effects each variable has on bone. For instance, strain rate and strain frequencies are interrelated with strain magnitude, so it is difficult to control one variable without increasing another.

There are however, a number of differences between the rat skeleton and human bone that limit the translation of the findings to humans (244): 1) the loading pattern on the rat skeleton, especially the vertebral body, is different to humans because humans are bipeds and rats are quadrupeds; 2) there is no clear growth spurt or onset of puberty in the rat, and the skeleton continues to grow until relatively late in life; 3) woven bone formation is a primary response to loading detected in animals. Woven bone is only formed in humans during bone repair and rapid growth, it is not a response normally observed in response to loading (245); and 4) trabecular bone remodeling may be less pronounced in rats compared to humans, however the findings are not consistent in the literature (246).

In conclusion, on the basis of the information available it is likely that human exercise regimes designed to increase or conserve bone mass should involve high strains imposed at high strain rates and presented in a range of diverse and unusual strain distributions. The number of impacts need only be few (<50), and thus the duration of each exercise need only be short (315). Furthermore, periods of exercise may only need to be repeated no more than three times per week (316).
**Effect of Mechanical Loading in Humans**

The results of animal studies show that the skeleton is responsive to an increase in strain magnitude, number of times a load is applied, unusual loading, strain rate and frequency (229). However, there are limited data from human studies to show what magnitude of load and number of impacts will result in clinically important increases in BMC. This information is needed before exercise programs can be recommended to children that aim to increase their peak BMC and reduce their future risk of fracture. The following is a review of cross-sectional and exercise intervention studies that have provided detail on what type of exercises are associated with an osteogenic response during growth.

**Magnitude of Load and Number of Impacts Associated with Exercise Studies in Children**

The magnitude of load applied to the skeleton during exercise can be measured using a force plate and recording the ground reaction force associated with each activity. Each activity can then be categorised as being high, moderate or low impact. High impact loading is defined as loads more than four times body weight (BW), moderate impact is two to four times BW, and low impact is ground reaction forces less than two times BW (248).

There is substantial data to show that high-impact exercise (> 4 BW) is the most effective for increasing BMC during growth. For instance, athletes who participate in high impact sports (eg gymnastics, volleyball and basketball) have a 5 to 35% higher BMC at the loaded sites compared to sedentary controls (249-251). Whereas athletes participating in moderate to low impact sports (eg running and cross country) have a 5 to 9% higher BMC compared to controls (48,252). Although high impact exercise may increase BMC, these programs have been developed over many years of training and would be inappropriate for normally active children. Therefore, findings on elite athletes provide an example of what is possible not probable in normally active children.
Table 3.2 summarises the findings from a number of studies that have assessed the ground reaction forces associated with various activities. These loads were measured in adults and are likely to be greater than what would be measured in children (253). That is because children have a larger foot area with respect to body weight; thus ground reaction forces are distributed across a larger contact area (253).

<table>
<thead>
<tr>
<th>Sport</th>
<th>Ground Reaction Force (BW)</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Aerobics</td>
<td>1.3 to 2.6</td>
<td>(254)</td>
</tr>
<tr>
<td>Basketball</td>
<td>4.3 to 8.9</td>
<td>(255)</td>
</tr>
<tr>
<td>Gymnastics: landing, aerial skills</td>
<td>10.0 to 14.0</td>
<td>(256)</td>
</tr>
<tr>
<td>Netball</td>
<td>2.0 to 5.7</td>
<td>(257)</td>
</tr>
<tr>
<td>Plyometrics</td>
<td>4.0 to 7.0</td>
<td>(212)</td>
</tr>
<tr>
<td>Soccer: shooting</td>
<td>2.1 to 2.4</td>
<td>(258)</td>
</tr>
<tr>
<td>Sprinting</td>
<td>1.2 to 3.6</td>
<td>(259)</td>
</tr>
<tr>
<td>Volleyball</td>
<td>4.8</td>
<td>(260)</td>
</tr>
<tr>
<td>Walking</td>
<td>1.6</td>
<td>(261)</td>
</tr>
</tbody>
</table>

Few exercise interventions have examined the magnitude of load and number of impacts associated with the prescribed exercise program. McKay et al (2000) provided details on the magnitude of load associated with the activities that were introduced to pre- and peri-pubertal children (210). The exercise program involved 10 or more additional jumps per day, 3 to 5 BW, 3 times a week for 8 months. Bone mass accrual at the trochanter was 1.2% greater in the exercise group compared to the control group. These findings suggest that moderate to high loads may increase BMC by small amounts in normally active children. There were a few limitations with this study: the exercise classes were not progressively increased (in magnitude); the number of impacts performed each session was not recorded; the ground reaction forces associated with the exercise program were determined from only a sample of the jumps; finally the loads were assessed in a laboratory, not in the field.
There are only two exercise intervention studies in children that have quantified both the magnitude and number of impacts associated with the exercise program (211,320). MacKelvie et al (2002) found that 50-100 jumps per session, 3 times a week for 7 months, with a load 3.5 to 5 BW, increased total body BMC 1.6% and proximal femur bone density 1%. Fuchs et al (2001) showed that 100 jumps per day, with an average load of 8.6 BW, 3 times a week for 7 months, resulted in a greater increase in BMC at the femoral neck (3.1%) and lumbar spine (4.5%) compared to a control group (262). These findings suggest that high impact loads may be required to result in clinically important gains in normally active children. However, in the study by Fuchs et al (2001), no significant correlation was detected between average ground reaction force and change in BMC in a sub sample of 13 children (r = 0.3) (262). The lack of correlation may have been due to a small range in the ground reaction forces associated with jumping, a small sample size, or the number of impacts not the magnitude of load may have been responsible for the change in BMC. Further work is needed to determine if fewer impacts at the same magnitude would provide a similar bone response.

The skeleton may also respond differently to weight-bearing exercise, at different stages of growth, maturation and ageing (199,212,213,263). Exercise may have the greatest effect on the skeleton during growth because it may enhance the bone modeling that is occurring on the bone’s surface at the time (64). Kannus et al (1995) showed in a cross-sectional study of female tennis and squash players that the benefit of exercise on BMC of the playing arm was about 2 times greater in women who started playing at or before menarche rather than after it (199). Furthermore, Witzke et al (2000) found plyometric activities, 4 to 7 times body weight, did not increase BMC in post-pubertal girls over a 9-month period (212). In addition, Heinenon et al (2000) introduced a high impact program, involving 100 multi-directional jumps, 2 times a week for 9 months to pre- and post-menarcheal girls (213). Only the pre-menarcheal girls responded positively to the program. Therefore, to obtain an osteogenic response, not only are the magnitude and number of impacts important, but also the maturity status of the individuals.
Chapter III: Literature Review

The responsiveness of bone to weight-bearing exercise may depend on whether the bone perceives it has already adapted to the new load (187). During growth, bone is continually changing its size and shape to withstand the mechanical loads that are imposed on it, i.e. from body weight and exercise. Therefore, additional exercise needs to apply a load that is greater than what the skeleton is normally exposed to for the bone to adapt and increase in size and shape. To date, animal experiments provide the most detailed information on what type of mechanical loading may have an osteogenic effect. The results indicate that: 1) dynamic rather than static loading is more effective; 2) only short duration of mechanical loading is necessary to initiate an adaptive response and the capacity of bone tissue to respond to the stimulus at the one time is saturated after a few loading cycles; and 3) bone cells accommodate to a customary loading, making them less responsive to routine loading signals (180). Only a few exercise intervention studies have integrated these findings into the prescribed exercise program. For instance, few have introduced: 1) dynamic, multi-directional movements; 2) only a few impacts in a short period of time; and 3) loads that are above the normal physiological range that the skeleton is exposed to during everyday activities.

In summary, the limited data from human studies show that athletes who participate in high impact sports have high BMC at loaded sites. Second, an exercise regime that includes 50 to 100 high impacts per day (3.5 to 8 BW), 3 times a week for 7 months, may increase BMC 1% to 4.5% at the loaded sites. Third, that the skeleton may be more responsive to additional high impact exercise during the pre- compared to the post-menarcheal years. There have been no interventions that have prospectively measured the magnitude and number of loads associated with the exercise program. Nor have the programs incorporated all of the findings from animal studies that indicate what components of loading may result in an osteogenic response.
Chapter III: Literature Review

Magnitude of Strain Applied to the Bone in vivo, in Response to a Mechanical Load

For an activity to be considered osteogenic, it must place a strain on the bone that is greater than the minimum threshold required to initiate bone modeling (187). To date, most of the research on how much strain a mechanical load applies to the bone in vivo has been in animals. It is difficult to measure in vivo strains in humans (especially in children) because it requires the implantation of transducers onto bone (264,265).

To determine how much strain is applied to bone during different activities, Milgrom et al (2000) applied 3 strain gauges to the medial tibia of 4 subjects and measured in vivo strains during running and jumping from various heights (265). The results showed there was no difference in the compression, tension or shear strains associated with running or jumping from different heights (26, 39 and 52 cm) (Figure 3.11). In addition, jumping 52 cm in height produced shear strains that were 30% less compared to jumping 26 cm in height (Figure 3.11). These data are contrary to the belief that high jumps place a greater strain on the bone compared to low jumps. Moreover, there was no difference in the amount of strain applied to the tibia when running compared to jumping 52 cm in height (Figure 3.11). There are some limitations with this data: 1) the strain gauges were only attached to one site; 2) strain gauges were stapled not directly bonded to the bone; and 3) a small sample size was used and 4) ground reaction forces were not measured. Despite these limitations, there appears to be no difference in the strain produced in vivo from running and high drops jumps. The authors suggest that the muscles attenuate the forces applied to the body when jumping from various heights to keep the strain levels within a normal physiological range and minimise damage to the bone.
Figure 3.11 Strains applied to the tibia during drop jumps from different heights (left) and when running compared to drop jumping from 52 cm in height (right). No differences in strain were detected during jumping from different heights, nor running and drop jumping from 52 cm in height (265). * p<0.05 versus 26 cm jump.

The same research group however, also reported higher strains on the bone during running compared to walking and cycling (266). Three strain gauges were attached to the medial tibia of 6 subjects and in vivo loads were measured during running at 17 km/hour, walking at 5 km/hour, cycling, performing a leg press and using a step-master. There were no differences in the magnitude of tensile, compressive or sheer strains between walking, performing a leg press, or using the stair master. However, the strains were higher during running compared to walking (2 to 4 times higher) and riding a bike (5 to 8 times higher). Running also had a higher strain rate than all the other exercises. Although only 6 subjects were measured and ground reaction forces were not assessed, these findings suggest that a greater strain is applied to the bone during moderate impact activities (ie running) compared to low impact activities (ie walking and cycling). These findings are conflicting with those reported earlier by Milgrom et al (2000) who did not report a relationship between the height of the jumps and in vivo strain. Therefore further investigation into in vivo strains in humans is required.

A study by Burr et al (1996) supported the finding from animal studies that bone is responsive to loads that are outside its normal physiological range (264). Burr et al (1996) applied a strain gauge to the tibia of one man and measured the in vivo strains
during loaded and unloaded walking, regular running and zig-zag running on a flat surface and running up and down hill. Peak compressive and sheer strains were 2 to 3 times higher during vigorous zig-zag running up and downhill, and sprinting on a level surface, compared to walking on a flat surface with or without a 17 kg weight on the back. In addition, strains from zig-zag running were 1.2 to 2.2 times greater compared to regular running. Thus, although these data were from one subject, the results showed that moderate impact activities placed a greater strain on the bone compared to low-impact activities. Moreover, multi-directional activities place a greater strain on the skeleton compared to uni-directional movements.

There has been little research on the strain produced at different skeletal sites (ie hip versus ankle) during mechanical loading. The bone-on-bone forces at the ankle, knee and hip joints during unloaded and loaded walking were compared in 7 men (267). Loaded walking was examined using 10 and 20 kg loads. Bone-on-bone forces are an alternative to using transducers to measure in vivo strains. The loads acting about the joints can be estimated using ground reaction forces from force plates, the analysis of movement of the body from videotaping, and the muscle forces acting on the joint from electro-myography. Peak bone-on-bone forces were 60% greater at the hip compared to the ankle and knee during unloaded and loaded walking. The lowest bone-on-bone forces were found at the ankle joint (4.2 ± 0.5 BW) during unloaded walking and the highest at the hip (8.0 ± 1.8 BW) during 20 kg walking. There was, however, variability in the data: one subject showed a greater load at the ankle, and two subjects showed loads twice as great at the hip compared to the remaining five. Thus it is difficult to conclude from this data whether greater strains are applied to the hip compared to the ankle and knee, both of which are closer to the point of impact when walking.

In contrast, a higher bone-on-bone force was found more distally at the ankle compared to the knee when running (268). Bone-on-bone forces were not calculated at the hip, but loads of 7 to 11 BW were found at the knee and 10 to 14 BW at the ankle and lower
leg during running. The highest bone-on-bone forces were measured during some of the slowest running sequences. Therefore, peak forces were not necessarily due to the velocity of running or the magnitude of the ground reaction force. The findings do, however, indicate that slightly greater loads may be detected at the ankle than the knee. These findings are conflicting with those reported by Simonsen et al (1995), therefore further research is needed to determine if greater loads are applied at the skeletal sites that are distal or proximal to the point of impact (267).

To determine how much of the load is transferred from the feet to the hip at the point of impact, Bauer et al (2001) estimated the forces applied to the hip during a 61 cm drop jump from 100 trials in 13 children (262). The ground reaction force associated with the jump was $8.5 \pm 2.3$ BW and the force at the hip was $5.7 \pm 1.9$ BW. Thus, attenuation of the ground reaction force to the hip was approximately 49%. However, there was large variation in the ground reaction forces detected during the jump (range 5.5 to 12.5 BW) and the ground reaction forces varied in 5 of the children across trials. Furthermore, the model used to calculate the loads at the hip did not include muscle force data; hence the estimated forces at the hip would have been more accurate if forces from muscular contraction were included. The findings do however; show that approximately 50% of the load applied to the feet during exercise is attenuated by the time it reaches the hip.

In summary, a greater strain appears to be applied to the bone during multi-directional movements compared to unilateral movements, or loads that the bone is normally accustomed to. However, there appears to be some variability in the data regarding the peak forces on bone under varying loading conditions (ie running versus jumping). This raises the question of how accurate and valid the claims are on the relationship between load magnitude and change in BMC in human studies. To address this, randomised control trials are needed where subjects are randomised to different exercise groups that increase one variable known to influence BMC ie. number of impacts, magnitude of
load, strain rate (fast versus slow rate of loading) and direction of movement. This information may provide insight as to what type of load the bone is most responsive to.

The findings from studies during growth also indicate that exercise may only increase BMC accrual during the pre-menarcheal years. To date, it is not known what skeletal sites high impact loads place the greatest strain at (ie. sites proximal or distal to the load). Only 50% of the ground reaction forces from jumping may be delivered to the hip. Knowing how much strain is applied at different skeletal sites will assist in prescribing exercises aimed at increasing BMC at sites associated with a high risk of fracture (ie lumbar spine).

**Limitations in Interpreting Data from Exercise Studies in Children**

Credible evidence that exercise during growth reduces fracture risk is lacking. Although randomised, double-blinded, placebo-controlled trials provide the highest level of evidence, they will never be conducted since neither the investigator or participant can be blinded to the exercise. The alternative is to conduct randomised (unblinded) controlled trials using fracture rates as an end-point. However, the long time interval between exposure and outcome makes these types of studies difficult to conduct. In the interim, researchers have conducted randomised (and non-randomised) controlled trials in children to determine if exercise increases BMC by amounts that are likely to reduce the future risk of fracture (>5% considered clinically important) (218).

It is difficult to study the effects of exercise on BMC during growth for a number of reasons. First, there is variability in the rate of maturation between individuals. Even when children are matched for chronological age, they may differ in their skeletal age or stage of maturation. Therefore, children of the same age may differ in their response to exercise. For instance, if a child is going through their adolescent growth spurt, they are likely to show large increases in BMC in their axial skeleton compared to age-matched children who are less mature (215,216). This may lead to a type I error as the changes in BMC may be to growth and maturation, and not exercise. This was demonstrated in
an exercise intervention in pre-menarchal girls (209). Bone mass increased 5% at the lumbar spine in the exercise group, however after adjusting for changes in height and weight there was no longer an exercise effect. It is therefore important that researchers adjust gains in BMC for maturation, or changes in height and weight. The problem is that a biologically important difference in height or weight between the groups may not be detected because of small sample sizes or large confidence intervals (220). In addition, potentially confounding factors may be considered accountable if a contradictory result is observed or they may be ignored if the data supports the hypothesis.

Second, it is not known if the magnitude of the load applied to the skeleton during exercise is relative to the size of the skeleton. It is possible that the same exercise will place a greater strain on a smaller skeleton, and a smaller strain on a larger skeleton. Therefore, it is not known if the magnitude of load needs to be adjusted according to the size of the child’s skeleton. There is evidence to suggest that more mature children show smaller gains in BMC in response to exercise than children who are still growing (143,212,213). For instance, a 3-year prospective observational study reported gains in BMC were related to weight-bearing activity in pre-pubertal, but not post-pubertal children (143). Similarly, both pre- and post-menarchal girls participated in a high-impact exercise program, but only the pre-menarchal girls showed an osteogenic response (213). It may be that higher loads need to be applied to a more mature skeleton (ie. a larger skeleton) to stimulate a significant increase in BMC.

Third, the osteogenic response to an exercise program may depend on the child’s loading history; that is, the characteristics of loading the child typically experiences throughout the day (269). For instance, children who participate in high impact recreational activities may not respond to a moderate impact exercise intervention because their skeleton has already adapted to high loads. Conversely, children who are less active may be more responsive to the program because their skeleton is not accustomed to high loads. Therefore, to interpret the effectiveness of the exercise
program it is important that recreational activity patterns of children are assessed. To date, there is no validated physical activity questionnaire that assesses a child’s level of participation in activities that are classified by their osteogenic potential (ie. high versus low impact weight-bearing activities). The results of previous research have generally used questionnaires that have been validated by comparing the hours of activity (including non-weight-bearing exercise) with aerobic fitness, degrees of movement using accelerometers or compared answers from children with their parents’ (47,203,270).

Fourth, there is the possibility with an exercise intervention that the results may be biased. For instance, even though a child is assigned to a particular group, a more active child may stay in the program if assigned to the exercise group but withdraw if in the control group. Moreover, a less active child may volunteer at the school randomised to be the source of controls, but may decline if attending the school randomised to undertake the intervention.

Finally, it is difficult to know how to interpret the results of an exercise intervention in children. For instance, it is not known if changes in BMC need to be expressed in absolute terms, percentage terms or as standard deviations. The same absolute increase in BMC in a young child with a small skeleton, will result in a greater percentage increase compared to an older child with a larger skeleton. Moreover, the size and shape of bone varies considerably in children and adolescence, making it difficult to interpret the DXA results. For instance, if changes in BMC are reported as bone density, then the change in BMC may be the result of either an increase in BMC, bone area or both. This may lead to erroneous interpretations on the effectiveness of an exercise program. For instance, Fuchs et al (2001) found exercise increased BMC 3.1% at the femoral neck, however after adjusting for bone area there was no difference between the exercise and control groups (211). It is therefore important that changes in BMC are adjusted for bone size because it may negate the exercise effect.
In summary, when researchers conduct an exercise intervention in children the following needs to taken into consideration: 1) ensure children are matched for maturation, bone age, growth velocity of the axial and appendicular skeleton; 2) ensure large sample sizes are used; 3) consider the loading history, by assessing the recreational activity patterns of children; and 4) adjust changes in BMC for changes in bone size.
SUMMARY

Osteoporosis is a chronic musculoskeletal disease affecting a high proportion of the population over the age of 60. The numbers are likely to increase into epidemic proportions over the next 20 years due to the ageing population (5). It was first thought that excessive bone loss during ageing was responsible for osteoporosis, but low BMC in old age may be the result of low peak BMC gained during the first two decades of life, an excessive bone loss during ageing, or both (13). Considering the amount of bone accrued during growth is more than four times the amount lost during aging, the most effective approach of preventing osteoporosis may be to enhance bone acquisition during growth and potentially increase peak BMC.

Bone mass increases rapidly during the pre-pubertal years, resulting in the gain of approximately 50% of peak BMC and 85% of adult height (70,71). The increase in bone formation during the pre-pubertal years predominantly occurs on the periosteal surface, resulting in a larger bone. During puberty, BMC continues to increase, albeit in a relatively short period of time. This rapid increase in BMC contributes to approximately 40% of peak BMC and the remaining 15% of adult height (70,71). Bone formation continues on the periosteal surface during puberty in males but it is reduced in females (3). In contrast, bone formation occurs on the endocortical surface in females, resulting in a narrower medullary cavity (3). The surface-specific changes are likely to be regulated by the production of GH and IGF-I during the pre-pubertal years, and the production of androgens and estrogen during the pubertal years (84,114,271). Moreover, there may be heterogeneity in the surface-specific changes along the length of the bone and at different skeletal sites (198,213). To date, our knowledge of growth-related changes in bone geometry has been based on the results of cross-sectional studies. Prospective measures taken at different sites within the skeleton and along the length of bone are needed to confirm these findings.

Since peak BMC is influenced by genetic and environmental factors, exercise may play a role in the prevention of osteoporosis. There is compelling evidence that shows
weight-bearing exercise during growth increases BMC accrual but its effectiveness may depend on the type, intensity and duration of exercise, and the age at which the individual starts training. For instance, the findings from elite athletes show high impact training during growth is more effective for increasing BMC than low impact exercise (48,272), while moderate to high impact exercise over a 7 to 10 month period may result in only moderate gains in BMC (209-211). The age at which an individual starts exercising may also influence the osteogenic effect; the skeleton during the pre- to peri- pubertal years appears to be more responsive to exercise than during puberty (143,213). Thus, it is generally accepted that weight-bearing exercise is important, but it is not known how much, how often, what magnitude or how long children need to exercise to obtain a clinically important increase in bone density. Nor is it known what effect loading history has on the osteogenic response to additional weight-bearing exercise.

Exercise is hypothesised to enhance bone formation at the surface that is undergoing bone formation at the time. This is based on one small retrospective study (n = 3) and there is little data available from human studies to support this notion. There is limited data from tennis players that show that increased mechanical loading may increase endocortical bone formation during the pre-to post-pubertal years (226). These findings are based on the retrospective analysis of adult tennis players, making it difficult to ascertain at what stage during growth these changes occurred. Furthermore, the measures were made using DXA that provides images of bone in the medio-lateral plane, not antero-posterior plane. Cross-sectional measures of bone geometry in the coronal plane, using MRI or pQCT, are needed to confirm these findings. In addition, there appears to be heterogeneity in the magnitude of the exercise response along the length of the bone (ie proximal versus distal). However, most studies have measured changes in bone morphology at one site on bone (225, 226). The following chapters address the following questions: What is the magnitude and number of impacts that need to be prescribed for an osteogenic response to occur in children? Does the osteogenic response to a moderate and low impact program differ in the girls who are
active outside of the prescribed exercise program (ie participate in organised sport involving moderate and high impact activities? What effect does exercise have on cortical bone morphology and bone strength during different stages of growth? Is there an optimal time during growth when exercise has the greatest osteogenic effect?
CHAPTER IV

The Impact of Exercise and the Skeletal Response in Children
Abstract

Background

Increasing peak BMC is recommended as a strategy to prevent osteoporosis later in life. A number of exercise intervention studies in children report that exercise may increase BMC accrual, however insufficient detail has been provided about specific components of an exercise program, such as the magnitude, number and direction of the loads needed to elicit an osteogenic response. Therefore, before exercise during growth can be recommended as a means for preventing osteoporosis, exercise interventions are needed where the skeletal response and exercise regime are both quantified. Therefore, we asked: 1) what are the loading characteristics associated with a moderate and low impact exercise regime?; 2) does a 20 minute, moderate-impact exercise program lead to a greater increase in BMC than a low-impact exercise program over a 8.5-month period?; 3) how do the loads in low and moderate impact exercise regimes compare with non-structured play?; 4) does a parental-assessed physical activity questionnaire reflect the time children spend engaged in osteogenic activities during school?; and 5) does the osteogenic response to a low and moderate impact exercise program differ in the girls who participate in organised sport outside of school involving moderate and high impact movements?

Methods

Sixty-eight pre- and early-pubertal girls (aged 8.9 ± 0.2 years) were randomised to one of four study groups; moderate impact exercise with or without calcium, or low impact exercise with or without calcium. All participants exercised for 20 minutes, three times a week for 8.5-months and received calcium fortified (434 ± 19 mg.day\(^{-1}\)) or non-fortified foods. The number and type of loads associated with the exercise classes and non-structured play were assessed from video footage, and the magnitude of loads associated with the exercise classes and non-structured play were assessed using a Pedar in-sole mobile system. Hours engaged in moderate and high impact organised sport was assessed from a parental assessed physical activity questionnaire. Analysis of
covariance (ANCOVA) was used to detect differences in BMC gains between the
groups, adjusting for baseline BMC, change in bone length and calcium intake.
Unpaired t-tests were used to compare the differences in the number and magnitude of
loads between the moderate and low impact exercise regimes and non-structured play.

Findings
The main findings from this study are that: 1) the moderate and low impact exercise
regimes included ~400 impacts per class, however the magnitude of the impacts was 2
to 4 times greater in the moderate impact regime and were applied as multi-directional
movements; 2) the 8.5-month moderate impact exercise program resulted in a 2.7%
greater gain in BMC at the tibia and 1.3% gain at the total body, compared to the low
impact exercise regime; 3) the number and magnitude of impacts were greater in the
moderate impact exercise program compared to non-structured play; 4) the number of
hours spent engaged in osteogenic activities outside of school, as reported by parental
assessed physical activity questionnaire, positively correlated with greater gains in
BMC (r=0.3, p<0.05); and 5) girls who participated in moderate impact sports outside
of school showed greater gains in BMC (2.5% to 4.5%, p <0.01 to 0.06) in response to
the moderate impact exercise regime than the low impact regime.

Summary and Conclusion
The findings from this study indicate that a moderate exercise program consisting of
~400 impacts per session ranging from 2 to 4 BW, 3 times a week for 8.5-months, were
enough to stimulate an osteogenic response in normally active children. The moderate
impact exercise regime consisted of fewer low impacts (<2 BW) and a higher number of
moderate impact (2 to 4 BW) movements compared to those typically performed during
recess. This finding indicates that the exercise regime overloaded the skeleton
compared to what it was typically exposed to during non-structured play. Moreover, a
moderate impact exercise program is beneficial for increasing BMC accrual in children
who already participate in moderate impact sport outside of school. However, it is not
known; 1) what type, number and magnitude of load would be required to further
increase BMC?; 2) if the 1.3 to 2.7% increase in BMC in response to the moderate impact exercise, will be sustained later in life when the risk of osteoporotic fracture increases?; or 3) how long the benefit to BMC will be maintained once the training stimulus has ceased? Further exercise intervention studies, with long-term follow-ups are needed to address these questions. Until these studies are conducted, programs that include additional moderate impact exercise during the pre- to early pubertal years appear to be beneficial for increasing BMC.
**Introduction**

Exercise during growth is often recommended as a public health measure for osteoporosis prevention. This premise is based upon the notion that exercise during growth will increase peak BMC and if this is maintained into adulthood, the future risk of fracture may be reduced. This notion is largely based on the results of cross-sectional studies in athletes and cohort studies comparing active versus non-active children, which indicate that athletes who participate in high impact activities, have a higher bone density (up to 35%) at loaded sites compared to non-active controls (1,48,50). However, with a cross-sectional study design it is not possible to establish cause and affect relationships between physical activity and BMC. The higher BMC in athletes could be attributed to other factors such as sampling bias; that is, a person with an ideal body size and larger muscle mass may be more inclined to participate and continue playing sport. A larger muscle mass is genetically linked with a higher BMC, so genes regulating size rather than exercise may account for the high BMC found in athletes (242,202).

The problem of sampling bias can be partly overcome by within-subject comparisons, such as comparing the side-to-side differences in the arms of tennis players. By comparing the playing and non-playing arms of tennis players, the influence of genetic, endocrine and nutritional factors are eliminated, and any difference between the arms can be attributed to the effects of mechanical loading (exercise). The results of studies in young female tennis players suggest that mechanical loading during growth is associated with large increases (up to 18%) in bone density, size and strength (198,200).

Epidemiological cohort studies that compare active versus inactive groups of children also show that exercise may have an osteogenic effect during growth (47,49,143). Greater gains in BMC (up to 18%) have been reported in active compared to inactive children during the pre- to post-pubertal years (47). Cohort study designs generally have the advantage of analysing large samples, however there are a number of limitations associated with these studies: 1) the data rely on the ability of the child or the
parent to accurately assess the levels of physical activity and 2) sampling bias may have influenced the results (220).

Randomised controlled trials that measure the effect of exercise on BMC over a period of time provide the strongest evidence for cause and effect relationships. The findings from a limited number of exercise intervention trials in non-athletic children show relatively small increases in BMC (1-5%) in response to additional weight-bearing exercise over a 7 to 10 month period (26,209-211,320). However, the majority of these studies lacked sufficient detail on the components of loading prescribed in the exercise intervention, such as magnitude, number of impacts and direction of the loads. Thus, while the results show a positive benefit to BMC with additional exercise, little information has been gained regarding what component of an exercise program may prove to be osteogenic in normally active children.

The findings from studies in elite athletes suggest that high impact weight-bearing exercise (ie gymnastics) is more beneficial than low impact exercise (ie running) (1,48,272). Gymnasts are reported to have up to 35% greater bone density at the hip compared to runners (48). This has been attributed to the larger impact loads that gymnasts are exposed to during training (10 to 18 BW) compared to running (2 to 3 BW) (256,273). However, there is no consensus on the optimal magnitude of load, or the number of impacts that need to be prescribed to normally active children to elicit an osteogenic response. Although high impact exercise appears to increase BMC in athletes, loads of this magnitude are too high to introduce to normally active children due to the potential risk of injury. Therefore, findings in elite athletes provide an example of what is possible and not probable in normally active children.

Two recent exercise intervention studies quantified both the number and the magnitude of loads associated with an exercise regime that was designed to increase BMC in normally active children (211, 320). Fuchs et al (2001) reported that 100 jumps per day, at a magnitude of 8 times body weight, 3 times a week for 7-months, stimulated an
increase in BMC at the femur (3.1%) and lumbar spine (4.5%) (211). MacKelvie et al (2002) found jumping 50 to 100 times per session, from a 10 to 50 cm height (equivalent to 3.5 to 5 BW), 2 times a week for 7-months, enhanced total body BMC (1.6%) and proximal femur bone density (1%) (320). These jumps resulted in loads applied in a uni-directional plane. However, the findings from a human in vivo study showed that the distribution of load is also important because a greater strain is applied to the bone during multi-directional compared to uni-directional movements (264).

To date, animal experiments provide the most detailed information on the effect different mechanical loading stimuli have on change in BMC. The results of these studies indicate that: 1) loads that are high in magnitude are more osteogenic than loads that are low in magnitude; 2) only few loads are necessary to initiate an adaptive response; and the capacity of bone tissue to respond to the stimulus at the one time is saturated by few loading cycles; 3) loads applied at a fast rate are more osteogenic than loads applied at a slow rate; 4) bone cells accommodate to a customary loading, making them less responsive to routine loading signals; and 5) allowing a rest period between loading bouts (on the same day) will increase the bone cells sensitivity to mechanical loading (314, 237, 269).

It is hypothesised that bone formation will occur if the mechanical load is above a certain minimum effective strain threshold or set point to stimulate bone modeling (MESm) (187). This mechanostat theory has two important implications for understanding how the bone may respond to an exercise intervention. Firstly, the exercise program may initially place a load on the bone that is greater than the MESm, resulting in an adaptive response and increased bone formation. However, an equilibrium point may be established such that no further skeletal gains are achieved unless the intensity of the exercise program is increased to provide bone with the necessary stimulus to exceed the new MESm threshold. Given that bone remodeling at one site takes about 6 months to complete, it is likely that a new stimulus needs to be applied at least every 6 months (300). Furthermore, it is not known what component of
loading (strain magnitude, rate, frequency and/or distribution) needs to be increased throughout the exercise program to result in the greatest osteogenic response. Secondly, an individual’s response to an exercise intervention may depend on their loading history (275). If the skeleton perceives it has not adapted to a new load, it will increase bone formation in an attempt to lower the stress applied to the bone (187). To date, there are no human or animal studies to support or disprove the implications of the mechanostat theory on the bones response to exercise.

The purpose of this study was to address the following questions: 1) what are the loading characteristics associated with a moderate and low impact exercise regime?; 2) does a 20 minute, moderate-impact exercise program lead to a greater increase in BMC than a low-impact exercise program over a 7.5-month period?; 3) how do the loads in a moderate and low impact exercise regimes compare with non-structured play?; 4) does the osteogenic response to a moderate and low impact program differ in girls who participate in organised sports outside of school involving moderate or high impact activities?; 5) does a parental-assessed physical activity questionnaire reflect the time children spend engaged in osteogenic activities outside of school?

Given the data reported from animal and human studies, we hypothesis that children involved in a moderate impact exercise program will show greater gains in BMC compared to those involved in the low impact exercise program (272). The gains in BMC are likely to be due to the loads being multi-directional and higher in magnitude compared to the low impact exercise program. Furthermore, the moderate impact exercise program is likely to induce a greater strain on the skeleton compared to non-structured play (due to a higher number of loads that are moderate to high in magnitude). This notion is based on the findings from quantitative and qualitative studies that show children are relatively sedentary during non-structured play: children rarely participate in continuous 20 minute bouts of physical activity, children participate in less than 2 hours per week of physical activity outside of school and the incidence of obesity is rising in children (305-307). Also, children who regularly participate in low
impact sports outside of school are likely to show a greater response to the moderate impact exercise intervention, than those involved in high impact sports. This is based on the Frost’s “mechanostat theory” that if the skeleton perceives it has not adapted to a new load, it will increase bone formation in an attempt to lower the stress applied to the bone (191). Finally, the number of hours children spend in low, moderate and high impact activities outside of school will correlate with changes in BMC over the 8.5-month intervention. This notion is based on the finding by Bailey et al (1999), of a relationship between high and low physical activity levels and gains in BMC during adolescence (47).
Methods

Study design

This was an 8.5-month randomised exercise-calcium intervention that was incorporated into the curriculum of an elementary school from Melbourne, Australia. Parents of girls eligible for inclusion (female students in years 2-5) were invited to attend an information session describing the program and its requirements. Following the information sessions, plain language statements and consent forms were mailed to all of the 120-eligible participants. Informed consent was obtained from 75 subjects and their parents. At baseline three subjects were excluded from the study because they were either obese (body weight > 4SD above the mean, n=2) or involved in high intensity weight bearing exercise (> 8 hours a week, n=1).

Subjects were stratified on the basis of age (7-8 or 8-9 years), ethnicity (Asian or Caucasian) and total body scan mode (Adult or Pediatric), then randomly assigned (using computer generated random numbers) to either a moderate impact exercise (Mod Ex) or low impact exercise (Low Ex) group. The subjects were also taking part in a calcium intervention program (276). Within each exercise group, subjects received food products that were either fortified with an additional 400 mg of calcium per day or had no additional calcium. All measurements were taken at the Bone Metabolism Research Laboratory at Deakin University over a 3-week period at baseline and at the completion of the intervention. The Deakin University Human Research Ethics Committee, and the Directorate of School Education approved the study.

Power Calculations

This is the first study to address the effects of an interaction between exercise and calcium using a randomised control trial, thus we based our power calculations on data reported from similar exercise and calcium interventions (209,345). From these data a sample size of 64 (four groups of 16 subjects) was required to detect a 6% difference with 80% power at the p < 0.05 level. We recruited a total of 75 subjects (18-19 subjects per group) to account for a 15% drop out. Three subjects were excluded at
baseline due to weight and a high number of exercise training hours and four subjects were excluded due to advance maturity (Tanner stages 3 to 5). The remaining number of participants was still adequate to detect the estimated difference. The study was well powered to detect the main effects of exercise because this was a two-group analysis with 33 and 35 subjects per group. We were slightly underpowered to detect the affect of mechanical loading history on BMC, as one group consisted of only 14 subjects (16 per group was needed).

Body Composition and Bone Mass

Body composition and bone mineral content were assessed using dual energy x-ray absorptiometry (DXA) (Lunar Radiation Corporation, Madison, WI, DPX-L). DXA is based on the differential attenuation of two photon energies of 38 and 70 keV traversing a medium consisting predominately of bone and soft tissue. The incident photon energies undergo exponential attenuation. The ratio of fat to fat-free mass (Rst) is determined by measuring the attenuation of each photon energy in an area of soft tissue only. From the total body scan, the Rst values determined at each point in soft tissue are averaged, giving an average Rst value. Using a standard curve with known properties of fat mass and fat-free mass (y axis) and Rst values (x axis), the percentage of body fat can be determined. The derived percentage fat is then multiplied by the total soft tissue mass in the subject to provide the total fat mass in grams. Fat-free mass is then determined from the difference between the soft tissue and the fat mass.

Total body and anterior-posterior lumbar spine (L2-L4) scans were conducted at the beginning and end of the intervention. These scans measured BMC (g) of the total body and lumbar spine, and muscle and fat mass of the total body. Bone mass of the tibia-fibula, femur, radius-ulna and humerus were determined using region of interest boxes on the total body scans. To provide the most accurate measure of total body BMC, Pediatric or Adult protocols (Version 4.6d) were used depending on the participant’s body weight. Compared to the Adult protocol, the Pediatric protocol involves lower radiation exposure, a smaller pixel size and is a slower scan. The Pediatric software
utilises 0.03 mRem, the pixel size is 3.66 x 7.22 mm, and it takes approximately 15 minutes to scan the total body. The upper weight range for Pediatric software is 40 kg. The Adult software (fast mode 150µA) utilises 0.02 mRem, the pixel size is 4.8 x 9.6 mm and it takes approximately 10 minutes to scan the total body. The lower weight range for the Adult software is 30 kg. To maintain consistency, all participants below 30 kg were scanned using the Pediatric software and all participants above 30 kg were scanned using the Adult software. The same scan mode was used at baseline and follow-up.

Bone mass of the lumbar spine (L2-L4) was assessed using Adult software. The scan mode selected was dependent on the thickness of the subjects abdominal region assessed using a Harpenden anthropometer. The medium scan mode (750µA) was used for subjects whose abdominal thickness ranged from 12 to 15 cm. This mode utilises 1.2 mRem, the pixel size is 1.2 x 1.2 mm and takes approximately 6 minutes to scan the lumbar spine. The fast scan mode (3000 µA) was used for subjects whose abdominal thickness ranged from 15 to 26 cm. This mode utilises 2.4 mRem, the pixel size is 1.2 x 1.2 and the scan takes approximately 2 minutes. For subjects whose abdominal thickness ranged from 26 to 28 cm, the slow scan (750µA) was used. This mode utilises 2.4 mRem, the pixel size is 1.2 x 1.2 mm and it takes approximately 6 minutes to complete the scan. The same scan mode was used at baseline and follow-up.

All metal objects (eg jewellery) were removed prior to each scan. Participants were required to wear light clothing such as bathing suits, T-shirt and shorts, or a provided gown. The same investigator performed all scans (LS). Total body scans were performed with the child lying in a supine position, with their hands placed prone on either side of their body. The centerline running the length of the scan table, evenly divided the subject in half. The legs were straight and approximately 10 cm apart, and the feet were relaxed and internally rotated. For the lumbar spine scan, the child was positioned in a supine position, with their knees at 90° of flexion, elevated by a semi soft box provided by the manufacturer.
The precision, based on 3 measurements in 8 healthy individuals, was 0.8% for total body and regional BMC, 1.1% for total body and regional bone mineral density, 0.7% for total body soft tissue, 2.9% for total body fat mass and 1.6% for total body fat-free mass. The repeatability of the operator for measuring regional BMC was 0.8 ± 0.7%. The participants were scanned using the DXA machine at Deakin University, Melbourne Campus.

**Anthropometry**

Height and sitting height were measured using a Holtain wall stadiometer. Subjects were required to stand barefooted, with hands by their side and heels touching the wall. The head was tilted forward so the eyes were in line with the ears. Subjects were required to breath in when the measurement was taken. Sitting height was measured using the same stadiometer while sitting on a stool of a known height, with knees at 90° and hands placed on their lap. Height and sitting height were recorded to the nearest 0.1cm. Body mass was measured in lightweight clothing without shoes using a SECA electronic scale and measured to the nearest 0.1kg. Humerus, ulna, femur and tibia limb lengths on the left-hand side of the body were measured from the total body DXA scans, using the ruler function that is accurate to 1mm. The same investigator performed all measurements (S.I). The repeatability for anthropometric measures was 0.1 ± 0.1%.

**Sexual Maturity**

Pubertal status was determined using a parental-assisted self-assessed questionnaire, based on Tanner staging for breast development (96). Participants were mailed illustrations and a description of the 5 pubic hair and breast development stages, and instructions on completing the self-assessment sheet. Breast development was used for staging as it has been reported to be the best representation of estrogen levels (277). Pubic hair is reported to be the best indicator of androgen levels (277). Pre-pubertal participants were defined as those classified with Tanner stage 1 for breast development. Participants reporting Tanner breast stage 2 to 4 were classified as peri-pubertal. Early puberty was defined as participants in Tanner stage 2. Participants who
had reached menarche were classified as post-pubertal. The accuracy of this method of self-reported pubertal status has been previously described (278).

**Physical Activity Questionnaire**

Physical activity was assessed after each school term, using a parental-assisted questionnaire that was a modified version of the questionnaire developed by Slemenda et al (1991) (203). The questionnaires were mailed out to the parents of each participant at the end of school terms 2, 3 and 4 (Figure 4.1). The questionnaire asked the parent to provide detail on the number of hours their child participated in formally organised activities during the week, and the time their child spent engaged in recreational activity after school and on weekends. Hours spent watching television or in front of the computer throughout the week, were also recorded.

The organised sports the children engaged in outside of school were categorised as being either moderate (2 to 4 body weight, BW) or high impact (>4 BW). The categorisation was based on the findings from previous studies, where ground reaction forces were determined for each sport (279). The average time spent per week, engaged in either moderate and high impact sports was calculated for each child. To ensure the questionnaire was reliable, 10 participants completed the same questionnaire twice within 3 weeks. There was a significant correlation between the two measures for time spent engaged in organised and recreational weight bearing activities, for each child (r=0.9, p<0.001).

**Dietary Assessment**

Dietary intake pre-, mid-, and post- intervention was assessed using a three-day diet diary. Parents of the participants were provided with a set of weighing scales, household cups, spoons, a diet diary and a set of instructions. All foods and drinks consumed were recorded for two weekdays and one weekend day. To assist with the analysis of diet entries, the parents were encouraged to include food wrappers and packaging. Diet diaries were analysed by the same qualified nutritionist (SI). If discrepancies were noted in the entries, or information was missing, a phone interview
was conducted with a parent of the participant to clarify the entries. Total energy and nutrient intake were analysed using FoodWorks Version 2 (Xyris Software Pty Ltd, Australia). Weighted food records were used to assess dietary intake for this age group of girls as this method of dietary assessment is reported to be more accurate than 24-hour recalls and food frequency questionnaires in this age group. Dietary data were obtained from 65 girls at baseline, 63 girls mid-intervention, and 60 girls post-intervention. Daily calcium intake was calculated for the participants using the average of their recorded intake from their diet diaries.

**Exercise intervention**

The exercise intervention was conducted for 34 weeks from April 1999 to November 1999 (terms 2 to 4), excluding 4 weeks of term breaks. All girls were involved in regularly scheduled physical education classes for 30 minutes, 3 times a week. The exercise program was incorporated into these classes for 20 minutes. The exercise sessions consisted of either moderate impact (Mod Ex) or low impact (Low Ex) activities. A paediatric exercise scientist (G.N) and physical education teacher (D.O) developed the curriculum. The physical education teacher conducted the exercise sessions.

The movements incorporated into the sessions were appropriate for the age and development level of the participants. The Mod Ex sessions consisted of jumping, hurdling, hopping, skipping and multidirectional running based games and activities. To increase the training load, the magnitude of the impacts was progressively increased, not the number of the impacts. For instance, in term 2 the girls were skipping 10 cm in height and jumping over hurdles 15 cm in height (2.4 BW). In term 3, the height of the jump was increased to 25 cm, and aerobic step routines were performed on steps 25 cm in height (2.9 BW). In term 4, the height of the aerobic steps was increased to 40 cm, and the girls jumped off benches 50 cm in height (3.9 BW). In addition, 2 small hand weights (0.5 kg each) were introduced in term 4 to hold during the step routine. Multi-directional movements were also included in the program, and raising the height of the
multi-directional movements increased the magnitude of the impacts. For instance, in term 2 girls ran in zig-zag directions around witches hats (2.6 BW); in term 3 they leaped over hurdles placed in a circle, and in term 4 they bunny hopped off a beam 25 cm in height (3.1 BW). Table 4.1 shows an example of the activities prescribed in the Mod Ex and Low Ex classes during each school term.

Table 4.1 Example of exercise sessions prescribed for one week during terms 2, 3 and 4, and the average number and type of impacts in each session.*

<table>
<thead>
<tr>
<th>Term</th>
<th>Session 1 (Monday)</th>
<th>Session 2 (Wednesday)</th>
<th>Session 3 (Friday)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Leaping, jumping, then hopping over rivers (2 ropes) of increasing widths</td>
<td>Run, jump and hop aerobics to music</td>
<td>Obstacle course of shapes drawn on ground - incorporating double foot jumps, hopping and hurdles</td>
</tr>
<tr>
<td></td>
<td>Number of Impacts</td>
<td></td>
<td>Number of Impacts</td>
</tr>
<tr>
<td></td>
<td>Run = 170</td>
<td>Run = 170</td>
<td>Hop = 30</td>
</tr>
<tr>
<td></td>
<td>Jump = 85</td>
<td>Jump = 85</td>
<td>Double foot jump = 30</td>
</tr>
<tr>
<td></td>
<td>Hop = 30</td>
<td>Hop = 30</td>
<td>Hurdle 15 cm = 5</td>
</tr>
<tr>
<td></td>
<td>Total impacts = 285</td>
<td>Total impacts = 285</td>
<td>Total impacts = 310</td>
</tr>
<tr>
<td>3</td>
<td>In pairs, play balloon soccer progressing from running to jumping and hopping in between kicks.</td>
<td>Bench aerobics (25cm) provided by teacher</td>
<td>Circuit with at least 20 to 30 repeats at each station which involve hurdles, skipping and jumping heights</td>
</tr>
<tr>
<td></td>
<td>Number of Impacts</td>
<td></td>
<td>Number of Impacts</td>
</tr>
<tr>
<td></td>
<td>Run = 290</td>
<td>Run = 290</td>
<td>Jump hoop 20cm = 15</td>
</tr>
<tr>
<td></td>
<td>Jump = 30</td>
<td>Jump = 30</td>
<td>Hurdle 15 cm = 50</td>
</tr>
<tr>
<td></td>
<td>Hop = 25</td>
<td>Step up and down = 290</td>
<td>Jump height 25cm = 15</td>
</tr>
<tr>
<td></td>
<td>Total impacts = 345</td>
<td>Total impacts = 320</td>
<td>Total impacts = 410</td>
</tr>
<tr>
<td>4</td>
<td>Skipping activities - individually, in pairs and as a group. Including regular skipping, forward and back and side-to-side movements. Jumping off heights.</td>
<td>Bench aerobics (40cm) with hand weights.</td>
<td>Relays using progressively higher obstacles to jump over</td>
</tr>
<tr>
<td></td>
<td>Number of Impacts</td>
<td></td>
<td>Number of Impacts</td>
</tr>
<tr>
<td></td>
<td>Skip = 200</td>
<td>Step up and down = 300</td>
<td>Double foot jump = 120</td>
</tr>
<tr>
<td></td>
<td>Jump height 51 cm = 25</td>
<td>Jump = 15</td>
<td>Hurdle 15 cm = 20</td>
</tr>
<tr>
<td></td>
<td>Total impacts = 225</td>
<td>Total impacts = 315</td>
<td>Jump height 51 cm = 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total impacts = 450</td>
</tr>
</tbody>
</table>

* the number of impacts represent one foot strike, except jumping represents both feet.
The Low Ex sessions consisted of stretching and low impact dance routines (eg stepping with no jumping). The sessions were progressed over the year by increasing the complexity of the movements (ie. more intricate dance routines) without changing the magnitude and number of impacts per session (~465 impacts per class).

**Figure 4.1** Time-line showing when the assessments were made throughout the 8.5-month intervention. The number of subjects used in the assessment is displayed.

**Biomechanics Assessment- Pilot Study**

A pilot study was conducted prior to the intervention to assess the osteogenic behaviour of children during non-structured play. Two girls (aged 7 and 8 years) from an elementary school in Melbourne were invited to participate in this study. These subjects were filmed during recess on two occasions, one week apart. A Panasonic (VM-VBM10E) camera was used to film the subjects and was positioned in one corner of the playground. During recess, a researcher recorded the activities observed every 5 seconds on a Sony (M-527V) Dictaphone. The types of activities recorded into the Dictaphone were based on a list of activities that were used in an observation study by Klegges et al (1984) (281). The authors considered these activities to be descriptive of children’s behaviour during non-structured play. The video footage was analysed to record the activity the child performed every 5 seconds (the same list of activities and frequency of recording was used when the child was being observed). The similarity

<table>
<thead>
<tr>
<th>Baseline Data</th>
<th>Follow-up Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-months</td>
<td>8 months</td>
</tr>
<tr>
<td>Anthropometry</td>
<td>Filmed Ex classes</td>
</tr>
<tr>
<td>Tanner staging</td>
<td></td>
</tr>
<tr>
<td>Bone density</td>
<td>Filmed Ex classes</td>
</tr>
<tr>
<td>n = 68</td>
<td></td>
</tr>
<tr>
<td>Dietary assessment</td>
<td>Filmed recess</td>
</tr>
<tr>
<td>n = 20</td>
<td></td>
</tr>
<tr>
<td>PA questionnaire</td>
<td>Dietary assessment</td>
</tr>
<tr>
<td>n = 63</td>
<td></td>
</tr>
<tr>
<td>Term 2</td>
<td>Term 3</td>
</tr>
<tr>
<td>1.5-months</td>
<td></td>
</tr>
<tr>
<td>Term 4</td>
<td></td>
</tr>
<tr>
<td>4.5-months</td>
<td></td>
</tr>
<tr>
<td>6-months</td>
<td></td>
</tr>
<tr>
<td>7.5-months</td>
<td></td>
</tr>
</tbody>
</table>

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115
between the observed activities by the researcher and recorded into the Dictaphone and those identified from the video footage was 64%. The pilot study identified the difficulty of filming and observing activities from a static position, and at a long distance from the subject. Therefore a hand held camera with zoom lens, was used in the intervention study to monitor the recreational activities of children.

*Biomechanics Assessment- Exercise Intervention*

The type and number of impacts associated with the Mod Ex and Low Ex regimes were determined by filming a random sample of subjects (n = 5, from grades 2 to 5) during their exercise sessions on 5 occasions, at 6-week intervals throughout the year. Different subjects were filmed each time. To assess the type and number of impacts associated with non-structured play, a random sample of 20 girls were filmed during recess, on two occasions (term 2 and 4) (Figure 4.1). The same girls were filmed each time and may or may not have been filmed during their exercise classes. A hand-held camera with zoom lens (Panasonic VX30) was used to film the exercise sessions and to follow the subjects during recess (20 minutes each). During recess, the participant was videoed undertaking their normal activities. The researcher was as inconspicuous as possible so that the subjects would not alter their normal recess time activity. Where possible, the subjects were video taped from a distance using the zoom lens on the video camera. The temperature was mild on both occasions the subjects were filmed at recess (17 to 22°C). A television (Loewe-Planus) and video player (Panasonic) on slow frame speed, was used to tabulate the number and type of impacts performed during the exercise sessions and at recess.

The magnitude of load associated with each activity performed in the exercise sessions and during recess (as identified from the video footage), was assessed using a Pedar in-shoe mobile system (Novel, Munich Germany, 1999). Eight girls (4 Mod Ex and 4 Low Ex) were randomly selected to take part. The Pedar in-shoe mobile system includes left and right insoles of various sizes, with 99 sensors inside the insole that measure the forces under the foot whilst performing various activities (Figure 4.2). Each insole was
approximately 2 mm thick and the force (pressure) output signal was sampled at 50 Hz. The insoles connect to a Pedar Synchronised Box that is used to control data collection. This Pedar Synchronised Box was placed inside a small backpack located on the participants back (Figure 4.2). The Pedar Synchronised Box was either connected to a power source or run off a battery supply. The Pedar in-sole mobile system allows data to be stored either on a Flash Card located inside the Pedar Synchronised Box, or directly on-line into a computer. A Flash Card was used in this study as it allows more than one series (or trials) of data to be recorded without having to save each series separately (as is the case when storing the data directly on-line using a computer). The Flash Card was capable of recording data for 6 minutes.

![Figure 4.2](image)

**Figure 4.2** Left and right in-soles of the Pedar in-shoe system. Each in-sole has 99 sensors impregnated inside, that record vertical loads of the foot inside the shoe. In the background is a Pedar Synchronised Box that is used to control data collection.

Before data collection, the insoles were calibrated using the Novel calibration device. This device consists of an air bladder that is inflated manually by using compressed air to load the insoles to the various pressures throughout the measurement range. Prior to testing, the participants were asked to remove their runners. Insoles were placed inside their shoes and the backpack was placed securely on their back, with the Pedar Synchronised Box inside. Subjects then put their runners back on. Velcro straps were used to affix the leads to the participants’ lower leg. Subjects were asked to perform the different activities that were identified from the video analysis as typical movements
performed during recess and from either the Low Ex or Mod Ex class. These activities included walking, running, hopping, agility running, skipping, step-routines, skipping with a rope, hurdling, vertical jumps, bunny hopping, jumping 51 cm heights, straddling a beam 25 cm height, and low impact exercise dance routines. The girls were asked to perform the typical movements from their respective exercise classes (ie only girls in Mod Ex classes performed typical Mod Ex movements). Data was collected for two sequential trials for 20 seconds. All data collected on the Flash Card was stored on a Toshiba notebook computer (Satellite 4060XCDT) for further analysis (Figure 4.3). Peak vertical force for the entire foot, expressed as a percentage of body weight, was calculated for each step using the Pedar Emed-sf software (Novel-win version 4.9).

The average peak force for each activity was calculated from the data collected on the 8 girls who participated in this component of the study. For double leg activities, the average peak force for both feet was recorded. The activities were categorised as being either low impact (<2 BW), moderate (2-4 BW) or high impact (>4 BW) (248). The Pedar In-Sole Mobile system is reported to provide a reliable measure of peak force ($r = 0.98$, $p< 0.05$) (282). Ground reaction forces from the Pedar in-shoe system have been compared to those measured on a Kistler force plate (325). A significant positive correlation was reported between the two measures of peak force ($p <0.01$). The Pedar in-sole mobile system recorded a lower first and mid peak force compared to the Kistler platform (15 to 16% $p<0.001$). However, the magnitude of the second peak was higher on the Pedar than the Kistler platform (3%, $p<0.05$).

The impact loads for the intervention group were determined on a sub-sample of girls during a simulated rather than an actual exercise class. This was conducted because the girls take a number of steps and perform a range of movements during each class; therefore it was difficult to distinguish what activity related to what load when analyzing the results. To ensure accurate results, the girls were asked to perform the same movements from an actual class during a simulated class. To ensure this was the case, the teacher who took the exercise classes was there to instruct the girls.
limitation of the study as motion is more contiguous during exercise classes and ground reaction forces may be enhanced or attenuated in a simulated setting.

Figure 4.3 A typical image provided by the Pedar software showing the magnitude of forces applied across the surface of the foot during physical activity.

**Calcium Supplementation and Placebo**

Food products were fortified with 2 grams of milk minerals (400 mg of calcium) (Murray Goulburn Co-operative Ltd, Australia). Sample analysis was performed on each batch of milk mineral using standard methods (346); and consisted of 20% calcium, 10% protein, 2% fat, 10% lactose, 7% free moisture, 39% phosphate, with the remaining 12% containing citrate, lactate, and trace minerals such as sodium and magnesium. Subjects were required to consume ten food products each week; choosing from 25 varieties of muffins, cookies and muesli bars. The placebo group received the same foods, without added calcium. Therefore the placebo food products had the equivalent of 2 grams more basic mixture than the calcium enriched foods. This represents a negligible difference in nutrient and energy intakes between the groups other than the milk minerals. To assist compliance, milk minerals or a placebo equivalent (Poly Joule, Sharp Labs, Australia) were offered in powdered form to include in regular foods and drinks. Calcium enriched milk (or regular milk) was also offered in place of regular milk intake. Compliance was assessed via the weekly return of uneaten products.
Statistical analysis

Data are presented in absolute terms, adjusted values or as percentage change. All data were checked for normality. Comparisons between groups at baseline were made using univariate analysis of variance (ANOVA). Repeated measures ANOVA was used to determine if BMC and growth parameters increased within each group over the intervention period. Group differences in absolute gains in BMC were evaluated using analysis of covariance (ANCOVA) using the LSD test post-hoc (least significant difference), adjusting for baseline BMC, change in bone length and dietary calcium intake. A regression analysis was conducted entering in factors known to be confounders. Baseline BMC, change in bone length and dietary calcium intake proved to be the strongest predictors for gains in BMC. Adjusting for these variables helps to isolate the intervention effects by accounting in part, for growth related changes in bone size and mass.

ANOVA was used to determine if the number and magnitude of impacts progressively increased in each exercise regime over the year. An un-paired t-test was used to test the differences in the number and magnitude of impacts between Mod versus Low Ex sessions, and Mod or Low Ex sessions versus recess. (There was no significant difference in the number and type of impacts performed by each child during recess in assessment 1 and 2, thus the average of the two was used for data analysis). A Kruskal-Wallis non-parametric test was used to compare the number and type of impacts engaged in during recess between girls who engaged in moderate impact and high impact sports outside of school. ANCOVA (adjusting for baseline BMC, change in bone length and dietary calcium intake) was used to assess whether absolute changes in BMC varied between participants who engaged in moderate impact and high impact sports outside of school. Our a priori hypotheses included all of the above analyses; therefore a Bonferroni-Dunn correction was not required. All data are expressed as mean ± SEM. Percentage changes in BMC are shown for ease of understanding. No statistical analysis was performed on the percent changes, only absolute changes. Data were analysed using the statistical package SPSS for Windows, Version 10 (SPSS Inc,
Chicago, Ill). A significance level of $p < 0.05$ and a trend of $p < 0.09$ was used for all comparisons.

The results are presented in three sections. In the first section, the influence of the Mod and Low Ex regimes on BMC accrual is presented. In the second section, the loads in the Mod and Low Ex regimes were compared to those measured during recess. In the third section, the influence of the girl’s history of physical activity (outside of school) on their skeleton’s response to the exercise program is investigated.
Results

The Effect of Moderate and Low Impact Exercise on Bone Mass Accrual.

Cohort Characteristics and Compliance

A total of 68 subjects completed the 8.5-month exercise intervention. Two subjects changed schools and two girls were excluded due to advanced maturation (Tanner stage 3). Sixteen percent of the remaining participants (n = 11) were of Asian decent. Fifty-five girls were classified as either Tanner stage 1 at baseline and either Tanner stage 1 or 2 at follow-up. Thirteen subjects were classified as Tanner stage 2 at baseline and follow-up (Table 4.2). There were no differences in age, pubertal stage or ethnicity between the Mod Ex and Low Ex groups at baseline or follow-up. Nor were there any differences in the weekly hours of participation in weight-bearing sporting activities, or daily calcium intake between the two exercise groups (or between Asian and Caucasian girls) (Table 4.2).

<table>
<thead>
<tr>
<th></th>
<th>Moderate Impact Exercise (n = 35)</th>
<th>Low Impact Exercise (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>8.9 ± 0.2</td>
<td>8.8 ± 0.2</td>
</tr>
<tr>
<td>Weight-bearing exercise (hrs.wk) †</td>
<td>2.1 ± 0.2</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>Calcium ^ (mg.day⁻¹) †</td>
<td>944 ± 62</td>
<td>906 ± 62</td>
</tr>
<tr>
<td>Tanner stage 1 – 1 (number, %)</td>
<td>18 (51%)</td>
<td>22 (66%)</td>
</tr>
<tr>
<td>Tanner stage 1 – 2</td>
<td>8 (23%)</td>
<td>7 (22%)</td>
</tr>
<tr>
<td>Tanner stage 2 – 2</td>
<td>9 (25%)</td>
<td>4 (12%)</td>
</tr>
</tbody>
</table>

^ = Dietary calcium + supplemented calcium
† = Average of three measures taken throughout the intervention (baseline, mid and post intervention)

A total of 81 formal classes were conducted at the school during the exercise intervention. Mean attendance for the exercise sessions was 93% (range 44% to 100%). There was no difference in attendance between the Mod and Low Ex groups. The reasons for missing sessions included sickness, other school commitments (ie class room activities) and out of school excursions. No major injuries occurred during the
exercise intervention in either the Mod Ex or Low Ex class. Nor were there any reports of pain or discomfort in the lower back region, hips or legs.

**Out of School Activity Levels**

Subjects in both the Mod and Low Ex groups spent a similar amount of time engaged in moderate (1.0 ± 0.2 and 1.0 ± 0.2 hrs.wk⁻¹, respectively) and high impacts sports outside of school (1.3 ± 0.2 and 1.0 ± 0.2 hrs.wk⁻¹, respectively). The subjects participated in the following organised high impact sports (>4 BW): basketball, gymnastics, football, netball (27 Mod Ex and 27 Low Ex); and moderate impact sports: tennis, dancing, running (23 Mod Ex and 33 Low Ex). The two groups engaged in a similar amount of unorganised weight bearing activities outside of school (Mod Ex 7.3 ± 0.5 and 7.1 ± 0.4 hrs.wk⁻¹), for instance playing in the park and walking the dog.

**Loading Characteristics of the Mod and Low Impact Exercise Programs**

There were a similar number of impacts per class in the Mod Ex and Low Ex program (412 ± 31 versus 465 ± 34, respectively), except at 4.5 months when there was a greater number of impacts in the Low Ex compared to the Mod Ex regime (p<0.05) (Table 4.3). The number of impacts per class did not significantly change over the 8.5-month intervention in either the Mod Ex or Low Ex regimes. All of the exercises represented activities that were categorised as moderate in magnitude (2 to 4 BW) in the Mod Ex regime, and low (<2 BW) in the Low Ex regime.

**Table 4.3.** The total number of impacts in typical low and moderate impact exercise sessions throughout the intervention (means ± SE).

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Moderate Impact Exercise (n = 35)</th>
<th>Low Impact Exercise (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (1.5 months)</td>
<td>312 ± 46</td>
<td>450 ± 26</td>
</tr>
<tr>
<td>2 (3 months)</td>
<td>317 ± 43</td>
<td>411 ± 63</td>
</tr>
<tr>
<td>3 (4.5 months)</td>
<td>477 ± 74</td>
<td>766 ± 55*</td>
</tr>
<tr>
<td>4 (6 months)</td>
<td>512 ± 20</td>
<td>383 ± 71</td>
</tr>
<tr>
<td>5 (7.5 months)</td>
<td>488 ± 69</td>
<td>414 ± 59</td>
</tr>
</tbody>
</table>

* p <0.05 versus the moderate impact exercise regime.
The average ground reaction forces associated with the activities in the Mod and Low Ex regimes are shown in Table 4.4. The average magnitude of impact was moderate in the Mod Ex regime 2.5 ± 0.1 BW (range 2.0 to 3.9 BW) and low in the Low Ex regime 1.2 ± 0.0 BW (range 1.0 to 2.0 BW). The average magnitude of load was greater in the Mod Ex compared to the Low Ex regime (p<0.05). There were no activities that involved high magnitude loads (>4 BW) in either exercise program. In the Mod Ex program, the magnitude of the loads progressively increased throughout the intervention by increasing the height of jumps or the movements.

Table 4.4 Loads associated with the activities in the moderate and low impact exercise regimes, and during recreational activities (means ± SE).

<table>
<thead>
<tr>
<th>Activity</th>
<th>Ground Reaction Force (Nm.kg⁻¹)</th>
<th>Mean ± SE</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Moderate Impact Exercise</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skip with Rope</td>
<td>2.0 ± 0.2</td>
<td>1.4 - 2.5</td>
<td></td>
</tr>
<tr>
<td>Step Routine</td>
<td>2.1 ± 0.2</td>
<td>1.6 - 3.4</td>
<td></td>
</tr>
<tr>
<td>Hopping</td>
<td>2.4 ± 0.2</td>
<td>1.6 - 3.4</td>
<td></td>
</tr>
<tr>
<td>Hurdle</td>
<td>2.4 ± 0.3</td>
<td>1.4 - 3.1</td>
<td></td>
</tr>
<tr>
<td>Agility Run</td>
<td>2.6 ± 0.3</td>
<td>1.6 - 3.4</td>
<td></td>
</tr>
<tr>
<td>Vertical Jump</td>
<td>3.6 ± 0.2</td>
<td>2.1 - 3.2</td>
<td></td>
</tr>
<tr>
<td>Forward-Back Skip</td>
<td>2.8 ± 0.3</td>
<td>2.0 - 3.6</td>
<td></td>
</tr>
<tr>
<td>Double Foot Jump (20cm)</td>
<td>2.9 ± 0.2</td>
<td>2.4 - 3.4</td>
<td></td>
</tr>
<tr>
<td>Bunny Hop Beam (25cm)</td>
<td>2.9 ± 0.2</td>
<td>2.7 - 3.6</td>
<td></td>
</tr>
<tr>
<td>Side-to-Side Skip</td>
<td>3.1 ± 0.4</td>
<td>2.5 - 3.8</td>
<td></td>
</tr>
<tr>
<td>Straddle Beam (25cm)</td>
<td>3.3 ± 0.3</td>
<td>2.4 - 5.1</td>
<td></td>
</tr>
<tr>
<td>Jump off Sleeper (50cm)</td>
<td>3.9 ± 0.7</td>
<td>3.2 - 5.3</td>
<td></td>
</tr>
<tr>
<td><strong>Low Impact Exercise</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Ex Dance Routine</td>
<td>1.1 ± 0.1</td>
<td>0.9 - 1.5</td>
<td></td>
</tr>
<tr>
<td>Recreational Activities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking</td>
<td>1.3 ± 0.1</td>
<td>0.9 - 1.5</td>
<td></td>
</tr>
<tr>
<td>Skipping</td>
<td>1.9 ± 0.1</td>
<td>1.3 - 2.3</td>
<td></td>
</tr>
<tr>
<td>Running</td>
<td>2.0 ± 0.1</td>
<td>1.4 - 2.2</td>
<td></td>
</tr>
</tbody>
</table>

**The Effect of Mod Ex and Low Ex on Bone Mass**

No differences were detected between the Mod Ex and Low Ex groups for height, bone lengths or body composition at baseline, or change in the variables over 8.5-months (Table 4.5). Repeated measures ANCOVA (covariates; baseline BMC, change in length, calcium intake) showed the increase in BMC was greater at the tibia (2.7%) and
In each exercise regime the girls were supplemented with calcium or received a placebo food product. An exercise-calcium interaction was detected only at the femur; in the Mod Ex group BMC increased 3% more in the girls who were taking calcium compared to those taking the placebo (p < 0.06) and increased 4.2% more than the girls in the Low Ex group who were supplemented with calcium (p < 0.01).

Table 4.5. Body composition, anthropometry, total body and regional BMC for pre- and peri-pubertal girls before and after 8.5-months of moderate and low impact exercise (means ± SE).

<table>
<thead>
<tr>
<th></th>
<th>Moderate Impact Exercise (n = 35)</th>
<th>Low Impact Exercise (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Changes</td>
<td>Changes</td>
</tr>
<tr>
<td></td>
<td>Baseline Absolute Percent</td>
<td>Baseline Absolute Percent</td>
</tr>
<tr>
<td><strong>Body composition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>29.5 ± 1.1 2.7 ± 0.3</td>
<td>1043.6 ± 34.5 112.1 ± 7.8*</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>20.2 ± 0.4 1.6 ± 0.9 7.8 ± 0.4</td>
<td>1035.9 ± 30.8 99.1 ± 5.4</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>7.6 ± 0.7 1.2 ± 0.2 17.2 ± 2.7</td>
<td>162.2 ± 6.8 24.7 ± 1.5</td>
</tr>
<tr>
<td>Percent fat</td>
<td>24.8 ± 1.5 1.3 ± 0.4</td>
<td>23.9 ± 1.3 1.3 ± 0.5</td>
</tr>
<tr>
<td><strong>Anthropometry (cm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>131.4 ± 1.3 3.9 ± 0.2 3.0 ± 0.1</td>
<td>131.5 ± 1.2 4.1 ± 0.2 3.1 ± 0.1</td>
</tr>
<tr>
<td>Sitting height</td>
<td>69.4 ± 0.6 1.6 ± 0.0 2.3 ± 0.1</td>
<td>68.9 ± 0.5 1.7 ± 0.1 2.4 ± 0.2</td>
</tr>
<tr>
<td>Leg</td>
<td>62.0 ± 0.8 2.3 ± 0.1 3.7 ± 0.2</td>
<td>62.4 ± 0.8 2.4 ± 0.1 3.8 ± 0.2</td>
</tr>
<tr>
<td>Femur</td>
<td>32.1 ± 4.4 1.4 ± 0.1 4.4 ± 0.3</td>
<td>32.4 ± 0.3 1.4 ± 0.0 4.4 ± 0.3</td>
</tr>
<tr>
<td>Tibia-fibula</td>
<td>27.6 ± 4.4 1.2 ± 0.1 4.2 ± 0.3</td>
<td>28.1 ± 0.4 1.4 ± 0.1 4.9 ± 0.3</td>
</tr>
<tr>
<td>Arm</td>
<td>46.6 ± 0.6 1.3 ± 0.2 2.8 ± 0.2</td>
<td>46.6 ± 0.5 1.2 ± 0.1 2.7 ± 0.2</td>
</tr>
<tr>
<td>Humerus</td>
<td>23.2 ± 0.3 0.8 ± 0.1 2.8 ± 0.9</td>
<td>23.2 ± 0.3 0.7 ± 0.1 1.4 ± 1.5</td>
</tr>
<tr>
<td>Ulna-radius</td>
<td>17.8 ± 0.3 0.7 ± 0.1 3.9 ± 0.3</td>
<td>18.0 ± 0.3 0.7 ± 0.1 4.1 ± 0.4</td>
</tr>
<tr>
<td><strong>Bone Mineral Content (g)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total body</td>
<td>1043.6 ± 34.5 112.1 ± 7.8* 10.8 ± 0.6*</td>
<td>1035.9 ± 30.8 99.1 ± 5.4 9.7 ± 0.6</td>
</tr>
<tr>
<td>Lumbar spine</td>
<td>16.4 ± 0.6 1.9 ± 0.2 11.5 ± 1.1</td>
<td>15.9 ± 0.5 2.0 ± 0.2 12.7 ± 1.4</td>
</tr>
<tr>
<td>Femur</td>
<td>160.5 ± 7.6 27.3 ± 2.1 17.1 ± 0.9</td>
<td>162.2 ± 6.8 24.7 ± 1.5 15.3 ± 1.0</td>
</tr>
<tr>
<td>Tibia-fibula</td>
<td>133.2 ± 6.3 20.4 ± 1.3** 15.6 ± 0.8**</td>
<td>136.0 ± 5.6 17.4 ± 1.1 13.3 ± 0.9</td>
</tr>
<tr>
<td>Humerus</td>
<td>55.2 ± 2.3 6.2 ± 0.5 11.4 ± 0.8</td>
<td>55.6 ± 2.0 5.8 ± 0.5 10.7 ± 1.0</td>
</tr>
<tr>
<td>Ulna-radius</td>
<td>36.9 ± 1.5 3.8 ± 0.3 10.4 ± 0.9</td>
<td>36.7 ± 1.4 3.9 ± 0.3 11.0 ± 1.0</td>
</tr>
</tbody>
</table>

*p < 0.08 ** p < 0.05 versus low impact exercise group
Loading Characteristics Associated with the Low and Moderate Impact Exercise Regimes and Non-Structured Play

Comparing the Loading Characteristics of the Mod and Low Ex Regimes with Non-structured Play at School (Recess)

During recess the subjects engaged in a mean of 803 ± 59 impacts; the magnitude of these impacts were mostly low (<2BW) (551 ± 39, 70%) and moderate (2-4 BW) (241 ± 37, 28%), with few high impact activities (>4BW) (12 ± 5, 2%). (Because the duration of recess was similar to the Mod and Low Ex regimes (20 minutes), direct comparisons could be made). The subjects engaged in approximately double the number of impacts during recess compared to a typical Mod and Low Ex classes (803 ± 59 versus 412 ± 31 and 465 ± 34, respectively p<0.01). However, the Mod Ex regime had a fewer number of low impacts and a higher number of moderate impacts compared to recess (1% versus 68%, and 99% versus 30%, respectively; p<0.01). In contrast, the Low Ex regime had a similar number of low impacts and a fewer number of moderate impacts compared to recess (98% versus 68% and 2% versus 30%, respectively; p<0.01). Both the Low and Mod Ex regimes had a lower number of high impacts compared to recess (0% versus 2%, p<0.01).

Comparing Degree of Organised Sport Outside of School with Non-Structured Play during Recess

Of the 20 subjects whose non-structured play was analysed during recess, 5 subjects participated in moderate impact sports outside of school (1.4 ± 0.7 hrs.wk\(^{-1}\)) and 15 were involved in high impact sports outside of school (1.7 ± 0.3 hrs.wk\(^{-1}\)) according to the physical activity questionnaire. There was no difference in the total number of impacts performed during recess between the subjects who engaged in moderate or high impact sports outside of school (764 ± 65 versus 817 ± 76). However, subjects who
participated in moderate impact sports outside of school engaged in a higher number of moderate magnitude, and fewer impacts low in magnitude compared to subjects who participated in high impact sports (435 ± 32 versus 588 ± 185, and 309 ± 24 versus 218 ± 176 impacts, respectively: p<0.08 to <0.05). There was no difference in the number of high, moderate and low magnitude impacts performed during recess between those who engaged in moderate and low impact sports outside of school.

The Effect Loading History has on the Osteogenic Response to Moderate Impact Exercise

Does the osteogenic response to the Mod and Low Ex program differ in the girls who were participating in organised sport involving moderate and high impact activities outside of school?

Gains in BMC were positively associated with reported hours of weight-bearing exercise outside of school (based on the questionnaire) (r = 0.3, p <0.05). Within the Mod Ex and Low Ex groups, no differences were detected at baseline or over the 7.5-month study between the subjects who engaged in either moderate or high impact sports outside of school for age, pubertal stage, ethnicity, height, bone lengths, body weight and composition or calcium intake (Table 4.6).

An analysis of covariance revealed that subjects who engaged in either high or moderate impact sports outside of school, showed similar gains in BMC in response to the Mod Ex program. In the Low Ex program, subjects who engaged in high impacts sports outside of school, showed a greater gain in BMC at the femur, lumbar spine, humerus and ulna compared to those who were involved in moderate impact sports (percent gain difference: 3.4%, 6.2%, 5.2%, 3.7%, respectively p<0.07 to <0.01) (Table 4.6 and Figure 4). In addition, subjects in the Mod Ex program who engaged in moderate impact sports outside of school showed greater gains in BMC at the femur, tibia, humerus and total body, compared to subjects in the Low Ex program who also participated in moderate impact sports (percent gain difference: 4.5%, 2.8%, 4.2%, 2.5%, respectively p<0.06 to <0.001) (Figure 4.4). No differences in BMC gains were
detected between subjects in the Mod Ex and Low Ex program who participated in high impact sports outside of school (Figure 4.4).
Table 4.6. Pubertal status, changes in anthropometry, BMC, exercise and calcium intakes in girls participating in moderate and low impact exercise regimes. Subjects have been classified according to their participation in organised weight bearing sports.

<table>
<thead>
<tr>
<th></th>
<th>Moderate Impact Exercise Regime</th>
<th>Low Impact Exercise Regime</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Category of Sport</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate Impact</td>
<td>High Impact</td>
</tr>
<tr>
<td></td>
<td>n = 14</td>
<td>n = 21</td>
</tr>
<tr>
<td><strong>Tanner Stage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-1</td>
<td>8 (57%)</td>
<td>10 (48%)</td>
</tr>
<tr>
<td>1-2</td>
<td>3 (21.5%)</td>
<td>5 (24%)</td>
</tr>
<tr>
<td>2-2</td>
<td>3 (21.5%)</td>
<td>6 (29%)</td>
</tr>
<tr>
<td><strong>Changes in Anthropometry</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>3.9 ± 0.3</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>Sitting Height (cm)</td>
<td>1.6 ± 0.2</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Leg Length (cm)</td>
<td>2.2 ± 0.2</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>3.1 ± 0.5</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>Muscle Mass (kg)</td>
<td>1.4 ± 0.1</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>1.7 ± 0.4†</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>**Changes in Bone Mineral Content (g) **</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total body</td>
<td>109.3 ± 10.7††</td>
<td>107.3 ± 8.7</td>
</tr>
<tr>
<td>Lumbar spine</td>
<td>1.7 ± 0.3</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Femur</td>
<td>28.9 ± 2.7†</td>
<td>26.3 ± 2.2</td>
</tr>
<tr>
<td>Tibia-fibula</td>
<td>21.3 ± 1.8††</td>
<td>19.8 ± 1.4</td>
</tr>
<tr>
<td>Humerus</td>
<td>6.6 ± 0.8††</td>
<td>6.0 ± 0.6</td>
</tr>
<tr>
<td>Ulna-radius</td>
<td>3.7 ± 0.5</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td><strong>Organised Sport (hrs.wk⁻¹) and Dietary Calcium Intake (mg.day⁻¹)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate Impact</td>
<td>1.6 ± 0.4</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>High Impact</td>
<td>0.2 ± 0.1</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>Calcium Intake</td>
<td>696 ± 53</td>
<td>676 ± 36</td>
</tr>
</tbody>
</table>

† Adjusted means ± SE
p<0.09, ** p<0.05, *** p<0.01 vs. moderate impact sports within the same exercise regime
† p< 0.06 †† p<0.05 vs. those participants in the low exercise regime who participated in moderate impact sports
Figure 4.4 Changes in BMC of the girls in the Mod and Low Ex regimes who participated in moderate and high impact sports outside of school. *p<0.05 versus Low Ex group, †p<0.08 ‡p<0.05 versus girls engaged in high impact sports outside of school.
Discussion

The aim of this school-based intervention study was to: 1) quantify the number and magnitude of loads associated with moderate and low impact exercise regime and during non-structured play (recess); and 2) assess the skeletal response associated with the moderate and low impact exercise programs. We found that a moderate impact exercise regime with approximately 400 impacts per session, of jumping, hopping, dynamic activities that produced ground reaction forces up to 4 BW, 3 times a week, resulted in a greater increase in BMC of the total body (1.3%) and tibia (2.7%) over 8.5-months, compared to a low impact exercise regime with the same number of impacts per session that were less than 2 BW. During recess, the girls performed double the number of impacts compared to the moderate and low impact exercise regimes. However, the number of impacts that were moderate in magnitude was higher in the moderate impact exercise regime compared to recess. In addition, loading history was associated with BMC accrual in response to the exercise intervention. For instance, in the low impact exercise group, the increase in BMC was greater in subjects who participated in high impact sports outside of school compared to moderate impact sports. Furthermore, the moderate impact exercise regime resulted in greater gains in BMC in those who participated in moderate impact sports outside of school compared to high impact sports.

This is only the third exercise intervention study that has quantified the magnitude and number of impacts associated with the exercise program and the skeletal response in children (211,320). Regular filming and the use of a Pedar in-sole mobile system provided information on the number, type and magnitude of loads typically associated with the moderate and low impact exercise classes. In the moderate impact exercise program there were approximately 400 impacts per class that ranged from 2.0 to 3.9 times body weight. The number of impacts was similar in the low impact class, but the magnitude of load was 2 to 4 times greater in the moderate impact exercise program. In the moderate exercise program, the magnitude of the impact progressively increased
200% (not the number of impacts) throughout the intervention. This was achieved by increasing the height of the jumps from 15 to 50 cm and the height of the multidirectional movements. Jumping from 50 cm heights were introduced in the last 8 weeks of the program, and produced the highest ground reaction forces of 3.9 times body weight.

The results of animal studies indicate that not only magnitude of load is important, but also the number, rate, frequency and distribution of loading (232,231,235,317). In this study, the number of impacts was similar in both exercise regimes. However, the magnitude of load was greater in the moderate exercise regime and multidirectional movements (ie side-to-side jumping and zig-zag running) were incorporated in the program. Therefore, the greater increase in BMC detected in the moderate impact exercise group may be attributed to both the magnitude of load and the unusual strain applied to the bone during the multi-directional movements (not a greater number of impacts). However, we were unable to determine the relative contribution each parameter of loading had on BMC (ie strain magnitude versus distribution of strain). To estimate this we need to attach a strain gauge to the bone and measure how much strain is applied to the bone during each activity performed in the exercise program. From this data, we could then assume that the activity that applied the highest strain to the bone, contributed the most to the change in BMC. However, it is difficult to measure in vivo strains in humans (especially in children) because it requires the implantation of strain gauges on the bone (264,265).

The benefit high magnitude loads on BMC are well documented, however, little is known about the effect of unusual loads (or the distribution of strain applied to the bone) on BMC (1,211,232). The findings from animal studies suggest that unusual loads may be more osteogenic than equivalent loads presented during normal locomotion (235,237). Rubin and Lanyon applied loads to the ulna of birds that were similar in magnitude, rate and number as those they were exposed to during everyday activity (ie wing-flapping). Although typical loads were applied to bone, BMC
increased at the ulna after 8 weeks of loading. The authors suggested that the ulna responded to an unusual strain distribution, not to an increase in strain magnitude or frequency, or number of impacts. In support of this finding, an in vivo human study reported greater strains (8 to 61%) are applied to the bone during multi-directional running compared to regular running (264). The responsiveness of bone to unusual loads may explain why walking and running exercises have a minimal effect on the skeleton (272,283).

Few exercise interventions have examined the magnitude of load and number of impacts associated with the exercise regime (26,209-211). McKay et al (2000) reported a higher BMC at the hip (1.2%) in children after participating in 8-months of additional exercise that incorporated jumping, hopping and skipping activities. This group measured ground reaction forces associated with some of the jumps prescribed in the program in a small sample of children, and found that ground reaction forces ranged from 3 to 5 BW. However, the ground reaction forces associated with all of the activities are not known, nor are the number of impacts performed in each session. There are only two intervention studies that have quantified both the magnitude and number of loads associated with the exercise program and the skeletal response in children (211,320). In a study by Fuchs et al (2001), the children jumped off boxes 61 cm in height, 300 times a week, with an average load of 8.6 BW. After 7-months of exercise, the exercise group showed a greater increase in BMC at the femoral neck (3.1%) and lumbar spine (4.5%) compared to a control group (211). In this study, the loads were not increased in magnitude, frequency, rate or number, and were only applied in one direction. McKelvie (2002) had children performing 10-12 minutes of high-impact (3.5 to 5 BW), weight bearing jumping activities throughout the study. After 7-months, the exercise group gained more total body BMC (1.6%) and proximal femur bone density (1%) than the control group. Unlike the study by Fuchs et al (2001) the activities were progressively overloaded by increasing the height (from 10 to 50 cm) and the number of jumps throughout the program (from 50 to 100 jumps per session). However dynamic movements were not incorporated into the program.
Compared to the findings by Fuchs et al (2001), we reported a similar increase in BMC (up to 2.7%, p<0.05 to <0.09), despite the loads being only moderate in magnitude (2 to 4 BW) and about one third of their maximum load. However, we did not detect a benefit of exercise at the lumbar spine. This may have been due to the lower magnitude of loads prescribed in our program. We hypothesize that the progressive nature of the exercise program combined with multi-directional loading patterns contributed to the osteogenic response detected at the tibia-fibula. Several other exercise intervention studies in children and adolescents, involving games and jumping type activities, have not detected an osteogenic effect at the spine (210,212). Similarly, a cross-sectional study by Grimston (1993) detected a higher bone density at the femoral neck (8.3%), but not at the lumbar spine in children participating in sports involving impact loads greater than or equal to 3 BW, compared to swimmers (194).

The greatest response to the moderate impact exercise was detected at the tibia-fibula. The tibia-fibula is a weight-bearing site and located close to the point of impact, therefore it is likely to be exposed to the greatest strain during exercise. However, there was no detectable increase in BMC at the femur or lumbar spine; perhaps the strains generated during moderate mechanical loading are attenuated before reaching these sites. Bauer et al (2001) reported that ground reaction forces associated with jumping are attenuated up to 50% by the time it reaches the hip (262). There are little data on the differences in strain produced at various sites during mechanical loading, and the results are conflicting. A small study in 7 men showed that the loads at the joints were 60% greater at the hip compared to the ankle and knee during unloaded and loaded walking (267). In contrast, 45% higher loads were found more distally at the ankle (~12 BW) compared to the knee (~8 BW) when running (268).

Similar findings have been reported in animal studies: the greatest response to loading occurs at the site closest to the point of impact. Mosely et al (1997) applied loads in a distal to proximal direction to the ulna of rats and found periosteal bone formation
increased at the distal end (up to 37%), but was reduced at the proximal end (-25%). In addition, the majority of the new bone was deposited on the medial and lateral surfaces where the highest strains were recorded (232). Iwamoto et al (1999) found 8 weeks of treadmill running resulted in greater gains in BMC at the distal compared to the proximal tibia metaphysis in rats (43 versus 24%, respectively) (326). These findings support the notion that exercise has site and surface-specific effects on bone and emphasize the need to ensure high loads are applied to the sites at the greatest risk of fracture later in life (ie femur, lumbar spine and radius) (5). In this study, exercise increased BMC at the tibia-fibula but not at the sites associated with the highest risk of fracture. Further work is needed to establish whether a longer exercise intervention (with loads being continuously increased) may result in gains at other sites further from the point of impact.

The mechanism underlying the site-specific skeletal changes in response to exercise, has not been identified. One putative explanation is that the most distal aspects of the limb are in close contact with the ground and are thus loaded more directly. A second compelling explanation is that pressurization of the medullary cavity, due to the effects of gravity, enhances bone growth (327). Because the distal aspects of the weight-bearing limbs are the furthest points below the heart, they are subjected to higher interstitial fluid pressures and understandably show the greatest changes in BMC. (The gravitational pull creates a fluid pressure gradient from the top to the bottom of the body). The effect of interstitial fluid pressure on BMC is demonstrated in subjects exposed to prolonged periods of bed rest (328). After 17 weeks of bed rest volunteers showed significant bone loss in their lower extremities, but an increase in BMC in their head. This gain in BMC is associated with a change in interstitial fluid pressure; the head is typically exposed to low fluid pressures but during bed rest it becomes exposed to high fluid pressures (328).

It is not clear why interstitial fluid pressure should affect bone cells. It is possible that osteoclasts are suppressed by fluid pressure, whereas osteoblast activity is enhanced.
Higher intra-medullary fluid pressure increases transcortical fluid flow, which generates shear stresses on osteocytes and bone lining cells. These cells are believed to take part in the response of bone to a change in mechanical loading and signal new bone formation or resorption. The results of cell culture experiments show that osteocytes respond to fluid shear stresses by producing paracrine factors (329). Therefore, fluid shear stresses on osteocytes could trigger cell-to-cell signals that activate new bone formation. Furthermore, mechanically enhanced perfusion may be required to maintain the integrity of osteocytes. Perfusion of osteocytes in long bones is driven by a transcortical pressure gradient that pushes fluid from the marrow to the periosteum. The pressure gradient is enhanced when the bone is exposed to mechanical loading. A recent study demonstrated that osteocytes within a long bone become hypoxic after a few days of disuse (330). Thus, it is likely that a lack of mechanical loading causes osteocyte hypoxia and apoptosis, which in turn signals osteoclasts to resorb bone.

It is generally accepted that a 10% increase in bone density is needed to reduce the risk of fracture by one half (218). Large increases in BMC have been reported in elite athletes involved in long-term, high impact training during growth (30-40%) (1,284). The findings from exercise interventions in normally active children are however less impressive. Prospective studies over 7-10 months, have only reported 1.1 to 5.5% gains in BMC (26,209-211,213,320). There are a number of differences between cross-sectional studies in athletes and prospective studies in normally active children that could explain these findings: 1) the high BMC reported in elite athletes may be the result of training over a long period of time. The exercise interventions were only 7-10 months long; 2) cross-sectional studies may be confounded by self-selection bias – those with a larger muscle mass may be more inclined to take up and continue sport. A larger muscle mass is associated with a higher BMC, so genetic factors rather than exercise may account for the high BMC found in athletes (202); and 3) elite athletes may be exposed to high magnitude impacts, up to 14 times body weight (256). These loads are approximately three times higher than those introduced in this study (<4 BW). Many years of training are required before the skeleton can tolerate loads of this
magnitude. Thus, data derived from elite athletes provide a model of what is possible, but not probable in normally active children. Loads required to elicit an osteogenic response in normally active children that are safe and effective have not been defined.

In this study, the moderate impact exercise program was sufficient to overload the skeleton, and stimulate an osteogenic response in subjects involved in moderate impact exercise outside of school. However, the osteogenic response to the moderate impact exercise program may not have only been due to the magnitude of load but other components of mechanical loading that may stimulate bone formation: strain rate, strain frequency, and the distribution of the load across the bone surface (285). Further research is needed to determine which of these components result in the greatest osteogenic response in normally active children. The prevailing view from animal studies is that the osteogenic response is determined by the interaction of the magnitude and the frequency of the applied loads; only short bouts are necessary; and segregating the load is preferable to applying a continuous load (286,287,314).

When considering what activities will induce an osteogenic response in children, not only does the magnitude of the load (ie ground reaction forces) need to be considered, but also the amount of strain placed on the bone as a result of muscle contractions. In fact, it is proposed that the greatest strain on bones during physical activity comes from the forces produced by muscle contractions (331,333). However, the degree of muscle pull on bone is difficult to measure in vivo in humans. Nevertheless, the prevailing view is that if a significant strain is placed on the bone, as a result of magnitude of load and muscle contractions, then the bone will increase in size and mass. The osteogenic effect muscle pull has on bone, is highlighted in a number of studies that report a close association between muscle strength and bone density in athletes and exercising children (321,209). It is reported that as muscles become stronger, bone will adapt by adding size and mass.
Another factor that needs to be considered (when considering what activities may increase bone density in children), is that the same activity may place a greater strain on a smaller skeleton compared to a larger skeleton. This is theory is supported by numerous studies that report more mature children show smaller gains in BMC in response to exercise than children who are still growing (143,212,213). For instance, both pre- and post-menarcheal girls participated in a high-impact exercise program, but only the pre-menarcheal girls showed an osteogenic response (213). Therefore, it is not necessarily the magnitude of the load that’s important, but rather the strain that the activity imposes on the bone. It may be that higher loads need to be applied to a more mature skeleton (ie. a larger skeleton) to stimulate a significant increase in BMC.

The history of mechanical loading will also affect the skeletal response to additional exercise (275). In this study, the osteogenic response to the exercise program appeared to be related to the subjects loading history, that is, the characteristics of loading the girls typically experienced during leisure and sporting pursuits. In the moderate impact exercise program there was no difference detected in the BMC gains between the subjects who participated in moderate versus high impact sports outside of school. However, the moderate impact exercise regime had a greater effect on BMC accrual than the low impact program in girls who engaged in moderate impact sports outside of school (Figure 4.4). Secondly, subjects in the moderate impact exercise group who participated in moderate impact sports outside of school showed a 7 to 10% (NS) greater gain in BMC at the femur and tibia-fibula compared to those who played high impact sports. Finally, there was no difference in BMC gains between the subjects who participated in high impact sports outside of school in the moderate versus low impact exercise program.

These data pertaining to loading history also provide an illustration of the “principle of cellular accommodation” theory that the bone will respond to a change in mechanical loading, but the response will eventually phase out as the bone cells “accommodate” to the new load. The data show that the bones, whose thresholds are likely to be
accommodated to high impact activities, are relatively insensitive to the moderate impact intervention. Based on these findings exercise interventions are perhaps better targeted towards children who are not participating in high impact activities outside of school. Furthermore, if they are already engaged in high impact activities, perhaps multi-directional activities are required to stimulate an increase in BMC so that the bone can accommodate the load.

Subjects who participated in moderate impact (not high impact) sports outside of school and engaged in the moderate impact exercise program, showed a 2.5 to 4.5% greater gain in BMC at the femur, humerus, ulna-radius, and lumbar spine compared to the low impact exercise program. The greater gains at the loaded sites (femur and lumbar spine) are likely to be due to large strains being applied to these sites when performing the jumping and hopping based activities in the program. The humerus and ulna-radius are typically unloaded sites, however they were loaded in the moderate impact exercise program when they used skipping ropes and hand weights. Therefore, these activities may account for the greater gains in BMC detected at the humerus and ulna-radius. This notion is supported by Pettersson et al (2000) who reported that rope skipping was associated with a higher BMC at the humerus (10%) and distal radius (22%) in adolescent females (288).

Subjects who engaged in high impact sports outside of school showed similar gains in BMC in response to the moderate impact exercise program as those who participated in the low impact exercise program. This finding suggests that the subjects who engaged in high impact sports outside of school may have been less responsive to the moderate impact exercise program. This may be explained by the notion that the skeleton had already adapted to high loads associated with the high impact sports. According to Frost’s “mechanostat” theory, a minimum effective strain (MES_m) threshold (or set points) needs to be exceeded to initiate bone modeling and result in an increase in BMC (191). Thus it is possible that these subjects may have had a high MES_m threshold, and thus very high loads may have been needed to stimulate an osteogenic response.
Further work is needed to measure the strain rate, strain frequency and direction of load associated with prescribed exercise programs and various sporting activities. Although studies have reported the ground reaction forces associated with various sports (279), it is not known how many moderate and high impacts children are exposed to during a typical sporting game (ie basketball, tennis). Therefore, it is difficult to make comparisons on the types of loading associated with the moderate impact exercise program in this study, to those sustained during organised sports.

To determine whether the moderate impact exercise regime overloaded the skeleton compared to non-structured play, the number and type of impacts prescribed in the exercise program were compared to those typically performed during recess. There were fewer overall impacts in the moderate exercise program compared to recess, however the number of moderate impacts (2-4 BW) was 95% higher in the exercise program. Therefore, the moderate exercise program placed a greater strain on the subject’s skeleton compared to those sustained during typical leisure play at recess. In addition, there were no differences in the number and type of impacts performed during recess between the subjects who participated in the moderate versus low impact exercise program. These data support our findings that the moderate impact exercise program was sufficient to facilitate an osteogenic response: above what may be gained in response to the loads during non-structured play.

Filming the subjects to assess their osteogenic behaviour during non-structured play was a unique component of this study. A pilot study identified the difficulty of filming and observing activities from a static position, so a small hand held camera with a zoom lens was used to monitor the subjects during recess. Although this was a time-consuming component of the study, it provided valuable data on the number and type of impacts subjects typically engage in during non-structured play. A limitation of this study was that recess was only used to quantify the subject’s behaviour; further research is needed to determine if their behaviour varies throughout the day (ie. at lunchtime or after
school). A number of other studies have used direct observation and filming techniques to assess physical activity. However, none have assessed the osteogenic behaviour of children during non-structured or organised play (281,289). Instead, these studies measured the general behavioural patterns of children and the time spent engaged in aerobic activities throughout the day.

In this study, a modified parental assessed physical activity questionnaire assessed the number of hours girls spent engaged in organised weight-bearing activities. A positive relationship was detected between reported hours of both moderate and high impact sports participation (according to the questionnaire) and gains in BMC (r = 0.3). Thus, girls who participated in high impact sports outside of school showed greater gains in BMC compared to those who engaged in moderate impact sports. To date, there are no validated physical activity questionnaires that assess time spent engaged in osteogenic activities (ie basketball, gymnastics). A number of studies have reported a correlation between parental or self-reported levels of physical activity and BMC in girls and boys (47,49,143,290). Others have found no relation between reported hours of physical activity and BMC or bone size in children (291). These findings suggest that the instrument used was not sensitive to activities that are osteogenic in nature. These studies validated the questionnaires by using a Caltrac motion sensor, parental report, aerobic fitness or 7-day recall. These methods of validation may not be suitable because they estimate how aerobically fit the children are, not how much time they spend engaged in osteogenic type activities.

There were a number of limitations associated with this study. Firstly, the exercise intervention was prescribed for only 8.5-months. Perhaps if the intervention were progressively overloaded over a longer period of time, greater gains at other sites (ie femur and lumbar spine) would have been achieved. Second, there was no control group that did not participate in any exercise program or receive any food products. Therefore, it is not certain if the changes in BMC were due to growth or due to the additional exercise or calcium. The non-supplemented and low impact exercise group
was the closest to a typical control group. Third, the Pedar in-sole mobile system collects data for 6 minutes. Therefore, the ground reaction forces that children were exposed to throughout the day were not measured. To account for this, in part, the girls were filmed during recess, and the activities they typically engaged in during non-structured play were identified. The ground reaction forces associated with these activities were then measured using the Pedar in-sole mobile system. Fourth, the number and magnitude of impacts associated with playing various sports, such as basketball, netball and tennis, were not measured, only ground reaction forces data were available from previous studies (279). Therefore, comparisons of the number and magnitude of impacts could not be made between the exercise program and organised sport. Fifth, 8 girls were used to measure the ground reaction forces associated with each activity. A larger sample would have enabled the comparison of ground reaction forces performed by girls who engage in moderate versus high impact sports outside of school. Sixth, to validate the questionnaire, reported hours of organised moderate and high impact sport were compared with the gains in BMC. It is possible, however, that children may be more active during unorganised activities at lunchtime or after school. Therefore, the questionnaire needs to consider the number and magnitude of loads children engage in throughout the entire day before it can be considered a valid tool for estimating the osteogenic behaviour of children. Seventh, there were more mature girls in the moderate exercise group (Table 4.2) and in the moderate exercise and high impact group (Table 4.6) (although not statistically different). Therefore, it is possible that a calcium, exercise and maturity interaction may have influenced the results (347). However, adjusting the changes in BMC for baseline BMC, change in length and calcium intake should eliminate this possibility. Eighth, due to the small numbers we cannot determine if the exercise was more effective on BMC in the pre- or peri-pubertal girls. Ninth, a type-II error may have been reported when comparing the four groups (based on exercise history) because multiple statistical comparisons were made without correction. Finally, structural changes in bone were not measured. This would have provided insight into the benefits of exercise on bone size, which is closely associated with bone strength. A number of studies have shown exercise may enhance bone
strength by altering bone geometry but not BMC. Thus, it is possible that the moderate exercise program enhanced bones strength at other skeletal sites (ie lumbar spine) but we were unable to detect it.

Furthermore it is possible that calcium intake may have influenced the results. Half the girls in each exercise group were supplemented with an additional 400mg of calcium per week. However, to eliminate the potential confounding effect calcium intake may have had on BMC, it was used as a covariate in the statistical analysis. Furthermore, there was a similar number of girls supplemented with calcium in each group, therefore it did not bias the results for one group. There was however, a trend detected (p<0.06) for an exercise and calcium intake interaction at the femur. Although, no exercise effect was detected at the femur in the moderate impact exercise group (except in those who were undertaking moderate impact, not high impact, activities outside of school), thus it is unlikely to have influenced the results showing an exercise effect on BMC at the tibia-fibula or total body.

Follow-up measures in these girls are needed to determine whether the changes in BMC are maintained later in life when the risk of fracture is high. To date no one has assessed whether the benefits of additional exercise in normally active children are maintained after the intervention has been withdrawn. Data from retired athletes suggest that the benefits are maintained. Kontulainen et al (1999) showed that the side-to-side difference in BMC in the playing versus non-playing arm are maintained in retired female tennis players (212). Positive findings have also been reported in retired soccer players (219), although the benefits appear to diminish as the number of years from retirement increase (220). However, retrospective studies show that high levels of physical activity during adolescence are associated with a higher BMC in adulthood. There are a number of limitations with these studies, such as the potential for selection bias and the precision of the instruments to measure long-term changes in BMC. Nor is it known, how long and at what intensity children need to exercise to attain long-term
benefits. Despite these limitations, these data suggest that gains in BMC are maintained after exercise has ceased.

We hypothesize that if changes in BMC were accrued on the periosteal surface than the benefits to bone strength may be maintained later in life. If bone is accrued on the periosteal surface, even small changes in BMC can result in significant increases in bone strength (314). Gains on this surface are unlikely to be lost as the periosteal surface mostly undergoes bone modeling (not bone resorption) during growth and bone remodeling during adulthood. If the moderate impact exercise enhanced bone formation on this surface, than the benefits to bone strength are likely to be maintained later in life. Unfortunately, bone geometry was not measured in this study. We can only presume (according to Raff’s hypothesis) that exercise may have enhanced bone formation on the surface undergoing bone modeling at the time; which is the periosteal surface during the pre- to peri-pubertal years (2).

In summary, approximately 400 impacts per session, 3 times a week, with multidirectional movements that produce ground reaction forces up to 4 times body weight, may stimulate an osteogenic response in normally active children. In addition, loading history may influence BMC accrual; the osteogenic response to the moderate exercise program was greater in the children participating in moderate impact sports compared with those participating in high impact sports outside of school. Further research is needed in populations with different levels of physical activity (ie loading history) to establish whether the effectiveness of an intervention differs according to their baseline levels of physical activity.

In conclusion, the exercise program prescribed in this study may be ideal for implementing into schools’ curriculum because the classes include only moderate impact loads and yet showed significant increases in BMC accrual. However, before this program can be recommended as a means of preventing osteoporosis, the girls need
to be followed up to determine whether the skeletal benefits are maintained after the intervention has ceased.
CHAPTER V

The Effect of Exercise and Growth on Cortical Bone Morphology During Different Stages of Maturation in Female Tennis Players
Abstract

Background
The effect of exercise and growth on cortical bone is often described in terms of changes in BMC and the accumulation of peak BMC. However, measuring BMC alone fails to consider whether these changes make the bone stronger (59,186). A given amount of bone appears to influence a bone’s resistance to bending depending on where it is located. For instance, bone formation on the periosteal surface, further from the neutral axis, will result in a greater increase in bone strength compared to bone formation on the endocortical surface (182).

It is hypothesised that exercise enhances bone mineral accrual on the surface undergoing bone formation at the time, ie. on the periosteal surface during the pre-menarcheal years and the endocortical surface during the post-menarcheal years (3). However, this hypothesis has not been rigorously tested in humans. Moreover, the growth and exercise-related changes in cortical bone morphology may be site specific ie. the changes may differ at the proximal versus the distal end of bone (225). Thus we asked; i) does exercise lead to periosteal expansion before puberty and endocortical contraction after puberty?; ii) does the osteogenic response vary along the length of the bone?; iii) is there an optimal time during growth when exercise has the greatest osteogenic effect?

Methods
Average total bone, cortical and medullary areas, and structural rigidity were determined at mid and distal regions of the playing and non-playing humerii (30-40% and 50-60% of humerus from condyles, respectively), and BMC of the whole humerus in 47 pre-, peri- and post- pubertal competitive female tennis players aged 8 to 17 years. Paired t-tests were used to compare bone morphometry and strength between the playing and non-playing arm, and the mid to the distal third of the humerus. Analysis of variance (ANOVA) was used to detect differences between pubertal groups for the
variables in the playing arm, non-playing arm and the side-to-side differences. Longitudinal data over a 12-month period was collected on 37 tennis players to confirm the cross-sectional findings.

Findings

The main findings from this study were: i) That exercise during growth resulted in a bone that is 6 to 9% larger and 11 to 23% more rigid. The cross-sectional area of the bone cortex was 8 to 17% greater due to periosteal expansion in the pre-pubertal years and endocortical contraction in the post-pubertal years. ii) The surface specific effect of growth and exercise varied along the length of the humeral diaphysis and, iii) The pre-pubertal years appear to be the most opportune time for exercise to enhance BMC accrual and bone strength. The longitudinal data confirmed most of the cross-sectional findings.

Interpretation

These results contribute towards understanding of the structural basis behind the growth and exercise-related changes in BMC and bone strength during the pre- to post-pubertal years. The results supported the hypothesis, that exercise may enhance the surface specific, growth-related changes in bone mineral accrual. In the pre-pubertal players, exercise enhanced bone formation on the periosteal surface and to a lesser degree bone resorption on the endocortical surface, resulting in an increase in BMC (12%), overall bone size (6%) and bone strength (11%). This initial adaptation to exercise did not increase during the peri- or post-pubertal years, despite further training and advancement in maturation. Thus, the pre-pubertal years appear to be the most opportune time for exercise to enhance BMC accrual and bone strength. Investigating players, who started playing tennis at different stages of puberty, are needed to confirm this finding. Heterogeneity in the bone’s surface-specific response to exercise highlights the need of future studies to measure changes in bone geometry at different sites along the bone. This may not only be important for measuring the effect of
mechanical loading on cortical bone, but also on disease, immobility and pharmacological agents.
Introduction

It is well established that exercise during growth has the potential to result in biologically important increases in bone mineral density, with residual benefits likely to be maintained later in life (1). While bone density can account for much of the variance in bone strength, there are additional characteristics such as bone size and structure that affect bone strength, with or without changes in bone density (160,193). Most studies investigating the effects of exercise on bone strength have focused on changes in BMC and bone density: measures that do not account for changes in bone size or structure. The importance of bone size on bone strength is demonstrated in a study by Skaggs et al (2001) who reported that children who had sustained a forearm fracture had a smaller bone size, but bone density was normal (57). Similarly, older adults who had a history of fracture showed deficits in bone size and volumetric bone density at the site of fracture (118). Therefore, increasing bone density and bone size during growth may play an important role in the prevention of osteoporosis.

Little is known about how bone size changes during growth, or the effect exercise has on bone size. Growth-related changes in cortical bone morphology are reported in a study that measured bone diameter of the second metacarpal from the radiographs of women aged 1 to 80 years old (3). The results showed that periosteal expansion occurs during the pre-pubertal years, enhancing overall bone size. In contrast, periosteal bone formation is reduced during the post-pubertal years, but endocortical apposition occurs resulting in a narrower medullary cavity. As a result of these changes, cortical thickness increases during growth (3). The surface specific changes are likely to be modulated by the production of growth hormone during the pre-pubertal years, and estrogen during the post-pubertal years (84,114).

It has been hypothesised that exercise during growth preferentially affects the surface that is undergoing bone formation at the time (2). Therefore in girls, exercise may enhance periosteal expansion before puberty and endocortical contraction after puberty.
This hypothesis has not been rigorously tested in humans. A study on three tennis players supports this notion (2). Ruff et al (1994) used radiographs to compare the side-to-side differences in cortical bone geometry in the arms of one female tennis player who started playing early in childhood, and one male and female who started during puberty. The younger started showed large side-to-side differences in cortical area (54%) due to periosteal expansion (34%) and very little endocortical contraction (6%). In contrast, the two players who started during puberty showed smaller side-to-side differences in cortical area (23%) due to greater endocortical contraction (28% and 19%) compared to periosteal expansion (10% and 13%). These findings support the notion that the periosteal surface may be more responsive to exercise during childhood and the endocortical surface is more responsive after puberty. However, this study is limited by its small sample size.

There are a number of other studies that have measured the surface-specific, maturity-dependent response to exercise. The findings are however, limited and conflicting (223,292,198,103,50,196). There are several reasons why these inconsistencies exist. These include, small sample sizes, diverse training histories, different skeletal sites being studied and different types of mechanical loading. A number of studies were retrospective, thus making it difficult to determine the exact time during growth when the surface-specific changes occurred. Furthermore, radiographs and DXA were used to measure changes in bone geometry. These images however, only provide a two-dimensional projection of bone in the coronal plane, which captures periosteal and endocortical changes in the mediolateral, not anteroposterior direction. To accurately measure changes on the surfaces of cortical bone, in all directions, measurements need to be made from cross-sectional images provided by QCT or MRI.

The results of animal studies support the findings from human studies. Estrogen appears to suppress periosteal bone formation and reduce endocortical resorption or enhance endocortical bone formation (339-342). In addition, the effect of exercise and
estrogen combined has a minimal effect on periosteal diameter but may enhance suppression of endocortical bone resorption (339,343,340).

Bone formation on the periosteal surface is more beneficial compared with apposition on the endocortical surface because biomechanical measures of bone strength are linked to an increase in bone diameter. Periosteal bone formation results in bone being distributed further away from the neutral axis and this increases the bone’s resistance to bending (58). This was highlighted in a study by Kardinaal et al (2000) who used QCT to measure bone geometry of the radius in females aged 11 to 23 years (293). From Tanner stage 1 to 5, the resistance to bending increased 53% and was closely related to periosteal diameter and BMC, not endocortical apposition. The limited effect of endocortical apposition on bone strength has been reported in a number of exercise-related studies. For instance, an additional 30 minutes of physical education classes, 3 times a week over 8-months, increased bone apposition on the endocortical surface in pre-pubertal boys, but did not confer a benefit to bone strength (26). Similarly, exercise enhanced BMC accrual on the endocortical surface of the playing arm in adult female tennis players, but did not increase bone strength (198). It is not known how exercise influences bone’s resistance to bending during different stages of growth, nor is it not known if there is an optimal time during growth for exercise to result in the greatest increases in bone strength.

To add to the complexity of the cortical bone’s response to exercise, the magnitude of the response appears to vary along the length of bone (198,199,224,225,326). Differences in the magnitude of the exercise response are likely to reflect varying loading patterns along the length of the bone. Distal sites closer to the point of impact are likely to experience greater strains compared to proximal sites. Few human studies have compared the exercise response at proximal, mid and distal sites on the loaded bone. Haapasalo et al (2000) used QCT to measure the side-to-side differences in the arms of recently retired male tennis players who started training during childhood (225).
They detected a greater osteogenic response at the distal compared to the proximal end of the humerus, and endocortical contraction at the distal site alone. The magnitude of the exercise response may also vary along the length of the radius in tennis players (224,294,295). Although the findings are not consistent in showing which site shows the greatest osteogenic response, they do highlight the importance of measuring the exercise response at more than one site along the length of the bone.

The aim of this study was to examine whether exercise enhances the growth related changes in cortical bone during growth. More specifically we asked i) does exercise lead to periosteal expansion before puberty and endocortical contraction after puberty?; ii) is there an optimal time during growth when exercise has the greatest osteogenic effect?; and iii) is the osteogenic response consistent along the length of the bone? To address these questions we compared the side-to-side differences in the arms of pre-, peri- and post-pubertal female tennis players. Tennis players provide an ideal model to study changes in bone because genetic, endocrine and nutritional factors are controlled for and any side-to-side difference in BMC and geometry can be attributed to the altered loading pattern of the sport.

On the basis of previous findings we hypothesize that exercise will enhance periosteal bone formation during the pre-pubertal years, and endocortical bone formation during the post-pubertal years (2). The exercise-induced changes to BMC and bone strength are likely to be greater at the distal humerus; a site closer to the point of impact and where the forearm muscles attach to the bone (225,326). Finally, the osteogenic response will be greater during the pre-pubertal years when the girls started playing tennis. This is based on the notion that the bone will increase BMC in order to adapt to the new mechanical load or stress placed on the bone when they first start playing tennis (191).
Chapter V: Study Two

Methods

Subjects
Forty-seven pre-, peri- and post-pubertal competitive female tennis players aged 8 to 17 years were recruited from tennis clubs located within metropolitan Melbourne. Players were included if they had been playing competitive tennis for a minimum of two years, and were currently playing at least 3 hours per week. Thirty-four players were competing at a national, state and regional level, and 13 at a high standard club level. Forty girls were right-handed, and 41 girls used a double-handed backhand. All girls were clinically healthy, were not receiving medication known to affect bone metabolism, or had any upper extremity injury. Deakin University and Alfred Hospital Ethics Committee approved the study, and written consent was obtained from all participants and their parents.

Twelve-month longitudinal data was collected on 37 of the original sample. Ten girls did not take part because they were either no longer playing (n = 2), not willing to participate (n = 4), or had relocated (n = 4).

Questionnaires
Sexual maturation was self-assessed with parental guidance using the standard five scale Tanner stages for breast development (96). Subjects were classified as pre-pubertal (Tanner stage 1), peri-pubertal (Tanner stage 2-4), or post-pubertal (post menarche).

A detailed training and medical history of each subject was obtained by a questionnaire developed for this study. The results were completed by each subject and confirmed by a parent of the child. The questions provided detail on the subjects starting age, years of active playing, the number and duration of playing sessions per week for every 2 year period from the time they started playing, other physical activities, possible injuries, medication and known diseases.
Chapter V: Study Two

**Anthropometric Measurements**

Standing and sitting height were measured using a Holtain wall stadiometer. Weight was measured in normal indoor clothing without shoes using a SECA electronic scale. Limb lengths were measured using a Holtain anthropometer, accurate to one millimetre. The same qualified investigator took anthropometric measures. The CV for anthropometric measures was 0.1 ± 0.1%.

**Strength Measurements**

Grip strength was measured with a standard grip strength dynamometer. The grip was adjusted for hand size. One warm-up trial was made with each hand. Three maximal effort trials were performed alternatively with right and left arms. Each trial was followed by a 30-second resting period. The mean value of the three readings is included in this study.

**Bone Geometry and Bone Mass**

Bone geometric variables of the playing and non-playing humerus in the coronal plane were determined by Magnetic Resonance Imaging (MRI) using a 1.5 Tesla whole-body unit (Signa Advantage GE Medical Systems, Milwaukee, WI) with the use of a commercial transit-receive torso coil. T1 weighted spin-echo images at a repetition time (TR) of 600 ms and echo time (TE) of 14 ms were acquired in the axial plane. Field of view was 200 mm and the matrix size was 512 x 192. Players were scanned lying in the anatomical position (arm externally rotated). Humeral length was determined in the sagittal plane using the ruler function from the lateral epicondyle to the superior border of the head of the humerus.

The region of interest (ROI) was 30% to 60% (~90 mm) from the distal end of the humerus. The ROI was divided into thirds and variables of the proximal third of the ROI (representing the mid-portion of the humerus) were compared to the distal third (Figure 5.1). A series of 5 mm slices (with 5 mm gaps between slices) were scanned along the length of the ROI. Each axial image was analysed using the OSIRIS imaging software program (Digital Imaging Unit, Centre of Medical Informatics, University
Hospital of Geneva, 1995). For each image, total bone and medullary areas (mm²) were isolated by manually drawing contours around the periosteal and endocortical boundaries. Cortical area was calculated as total bone area less the medullary area. Bone area for the 5 mm gap was determined as the mean of the two slices on either side of the gap.

![Typical MRI transverse slices of cortical bone (black) and medullary area (white) of the playing and non-playing humerus of a post-pubertal female tennis player.](image)

**Figure 5.1** Typical MRI transverse slices of cortical bone (black) and medullary area (white) of the playing and non-playing humerus of a post-pubertal female tennis player. Regions of interest were analysed at the mid and distal humerus, each representing 10% of the total arm length (30 to 40% and 50 to 60% measured from the distal condyles).

Average total bone, cortical and medullary areas were determined by summing the cross-sectional areas of each slice in the ROI and dividing this value by the total number of slices in the ROI. The short-term precision (co-efficient of variation, CV) of MRI to measure total and cortical bone areas is 1.02% and 0.21%, respectively. MRI has also been reported to provide an accurate measure of bone volume and area (1.6 to 3.5%)
(30). (This is true for a symmetrical bone where changes in contour have little influence on edge detection). The same operator analysed all the MRI scans (LS) and was blind as to the dominance of the arm of the tennis players.

Bone mineral content of the playing and non-playing humerus was measured using dual x-ray absorptiometry (DXA) (Lunar DPX-L, Madison, WI). DXA is based on the differential attenuation of two photon energies of 38 and 70 keV traversing a medium consisting predominately of bone and soft tissue. The incident photon energies undergo exponential attenuation. The ratio of fat to fat-free mass ($R_{st}$) is determined by measuring the attenuation of each photon energy in an area of soft tissue only. From the total body scan, the $R_{st}$ values determined at each point in soft tissue are averaged, giving an average $R_{st}$ value. Using a standard curve with known properties of fat mass and fat-free mass (y axis) and $R_{st}$ values (x axis), the percentage of body fat can be determined. The derived percentage fat is then multiplied by the total soft tissue mass in the subject to provide the total fat mass in grams. Fat-free mass is then determined from the difference between the soft tissue and the fat mass.

The densitometer was calibrated daily by a dual material standard according to manufacturer’s recommendations. All metal objects eg jewellery, were removed prior to each scan. Participants were required to wear light clothing such as bathers, T-shirt and shorts, or a gown that was provided. Total body scans were performed with the child lying in a supine position, with their hands placed prone on either side of their body. The centerline running the length of the scan table evenly divided the subject in half. The legs were straight and approximately 10 cm apart, with the feet relaxed and rotated in. Subjects were scanned using the adult total body software (version 4.3d). This software (fast mode 150µA) utilises 0.02 mRem, the pixel size is 4.8 x 9.6 mm and it takes approximately 10 minutes to scan the whole body. The same scan mode was used for each subject at baseline and follow-up. The same experienced operator (LS) made all of the measurements and was blind as to the dominance of the arm of the tennis
player. Humeral BMC was measured by using the ROI box to outline the humerus from the head of the humerus to the lateral and medial condyles. The coefficient of variation (CV) of the investigator (LS) to measure humeral BMC was $1.7 \pm 0.4\%$. The CV of this DXA machine to measure total body BMC is $1.3\% \pm 0.4\%$.

**Bone Strength**

More than 80% of the strain produced in the long bone cortices are caused by bending moments (311). Thus, the humerii are likely to be predominately exposed to bending loads during tennis. The bending (and torsional) rigidity of a long bone can be estimated using beam theory- a principle used in mechanical engineering to estimate a beam’s capacity to resist bending and torsion. The structural geometry of a long bone diaphysis enables it to be modeled as a hollow beam and its resistance to bending and torsion can be estimated from cross-sectional images of the bone relative to its long axis. The most relevant measures to estimate the bones resistance to bending and torsion are the second moments of area. A bone with a greater second moment of area have a greater resistance to bending than bones with less second moments of area (all other things being equal eg bone mass).

The second moment of area considers two measures – cross-sectional area and the distribution of that area. Bone with its material distributed farther away from the neutral plane (the plane at which there is neither torsional or compressive strains), will exhibit a greater resistance to bending than a bone with the same amount of bone distributed closer to the neutral plane. Furthermore, a bone with less material but is distributed more advantageously will have a higher second moment of area, than a bone with more material but distributed less advantageously. The second moment of area ($I$) is derived by dividing the section into a small series of small areas, and multiplying each small area ($dA$) by its squared distance from the neutral axis ($y^2$) (Figure 5.2). This is calculated over the entire cross section.
The plane of greatest bone rigidity is known as the major axis, and the second moment of area is known as $I_{\text{MAX}}$. The plane of least bending rigidity is known as the minor axis, and the second moment of area along that axis is denoted $I_{\text{MIN}}$. The resistance to twisting, or torsion, is another measure of bone strength. The polar second moment of area ($I_p$) reflects the bone's ability to resist torsional forces applied about the neutral axis, passing through the center of mass of the section. It is equal to the sum of the maximum and minimum moments of area ($I_p = I_{\text{MAX}} + I_{\text{MIN}}$).

To assess bone rigidity, each MRI slice was imported into Scion Image 4.0.2 (Scion Corporation, Frederick, MD, U.S.A.), and the maximum ($I_{\text{MAX}}$, mm$^4$), minimum ($I_{\text{MIN}}$, mm$^4$), and polar ($I_p$, mm$^4$) second moments of area were calculated using a custom macro. The macros calculate $I$ about all possible neutral planes and reports the largest value as $I_{\text{MAX}}$ and the smallest value as $I_{\text{MIN}}$, and they are always perpendicular to one another. The same experienced operator (AR) made all of the measurements. Only $I_p$ was presented in the results because it represents a generic measure of the bone’s ability to resist bending in a number of planes (or directions). Therefore, this measure more closely reflects the type of loads imposed on the humerii during tennis.

**Figure 5.2** For irregular cross-sectional bone, the second moment of area is calculated using the Parallel-Axis theorem. The sum of the contribution of each pixel provides the second moment of area. $\Sigma$ is sum, $w$ is width, $h$ is height, $A$ is area and $d$ is distance from neutral axis (180).
Bone length can significantly effect the second moment of area (312,313). For instance, the amount of force generated at the shoulder joint increases as the moment arm (humerus length) increases. To avoid interpreting a structurally rigid humerus from a tall individual as evidence for a growth or exercise effect, bone length needs to be adjusted for. Other factors that affect second moment of area include body mass (25). However, body mass is unlikely to have an effect at a non-weight bearing site, so it does not need to be controlled for. Muscle size and force also have a significant effect on second moment of area (28). The cross-sectional area of a muscle is proportional to the force it is capable of generating during contraction. Therefore, individuals with stronger forearm muscles are likely to exhibit more structurally rigid humeral cortices than individuals with weaker muscles. However, the effect of muscular activity (~physical activity) on the cross-section of bone is the variable of interest, therefore, it does not need to be controlled for.

Statistical Analysis
Average bone areas were used for all comparisons within and between pubertal groups. Paired $t$-tests were used to compare bone morphometry and strength between the playing and non-playing arm within each group, and the mid-third to the distal-third of the humerus. (There was no difference in segment length between playing and non-playing arm). Data was expressed adjusted for bone size (ie. as a percentage of the non-playing arm) by dividing the difference between the playing versus the non-playing arm, by the non-playing side, and then multiplying the outcome by 100. Analysis of variance (ANOVA, with Tukey post-hoc comparisons) and ANCOVA (with least significant difference comparisons) using bone length as a covariate were used to detect cross-sectional and longitudinal differences between the pubertal groups, for the variables in the non-playing arm (indicating growth-related changes) and the side-to-side differences (indicating exercise-related changes). Bone length was used as a covariate when comparing differences in bone rigidity ($Ip$) between groups, as it has a significant effect on second moment of area (313). Longitudinal data was adjusted to represent exactly 12-month data for all players. A repeated measure ANOVA was used
to determine if there were changes in the non-playing arm and any difference in the side-to-side comparisons within each pubertal group. Our a priori hypotheses included all of the above analyses; therefore a Bonferroni-Dunn correction was not required. All data are reported as mean ± SE unless otherwise stated. Absolute change in bone area and mass were compared. Percentage values are provided for ease of understanding. Significance is reported as p<0.05, marginally significant trends are reported at p<0.1. Reporting p values less than 0.1 reports the data objectively and allows the reader to make a judgement of the clinical relevance of these findings.
Chapter V: Study Two

Results

Subject Characteristics

Age, pubertal status, body composition, and training history of the tennis players are reported in Table 5.1. All players started playing competitive tennis during childhood (mean age 6.4 ± 0.3 years, range 3 to 10 years) and were at the time of the study training on average 8.3 ± 0.6 hrs.wk⁻¹ (range: 3 to 23 hrs.wk⁻¹). Post-pubertal players started training 1.4 years later than pre-pubertal players (p<0.05). Years training and current training volume (hours per week) were greater in the post-pubertal compared with pre-pubertal players (p< 0.01 to 0.001), but there was no difference in the years training prior to menarche between the groups (Table 5.1). None of the post-pubertal players reported a history of menstrual disturbances, nor were they taking the oral contraceptive pill. All girls participated in school physical education classes, and 6 girls were currently participating in additional weight bearing exercise for 2.3 ± 0.6 hrs.wk⁻¹ (range: 1 to 4 hrs.wk⁻¹) (netball, ballet, athletics and aerobics). Thirty-eight girls participated in weight-bearing exercise during the pre-menarcheal years for on average 2.3 ± 0.3 years (range: 1 to 10 years). These girls trained for 2.4 ± 0.4 hrs.wk⁻¹ (range: 1 to 6 hrs.wk⁻¹) and participated in sports such as netball, basketball, dancing, athletics, and gymnastics.

Longitudinal data on the body composition and training history of the 37 tennis players, who remained in the same pubertal groups, are shown in Table 5.4. All of the pubertal groups showed gains in body composition and arm length (p<0.05 to <0.001), except fat mass and lean mass did not increase in those who remained pre- and post-pubertal, respectively.
Table 5.1 Age, age of menarche, body composition and training history of pre-, peri- and post-pubertal female tennis players at baseline (Mean ± SE).

<table>
<thead>
<tr>
<th>Pubertal Status</th>
<th>Pre- (n=17)</th>
<th>Peri- (n=11)</th>
<th>Post- (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>10.4 ± 0.3</td>
<td>12.2 ± 0.3†</td>
<td>14.3 ± 0.4‡</td>
</tr>
<tr>
<td>Age of menarche (yrs)</td>
<td>-</td>
<td>-</td>
<td>12.5 ± 0.3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>141.6 ± 2.0</td>
<td>151.4 ± 2.2†</td>
<td>162.5 ± 1.4‡</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>34.8 ± 1.4</td>
<td>41.9 ± 1.8†</td>
<td>54.9 ± 1.4‡</td>
</tr>
<tr>
<td>Lean Mass (kg)</td>
<td>26.6 ± 0.9</td>
<td>30.3 ± 1.3†</td>
<td>36.8 ± 0.8‡</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>7.6 ± 0.6</td>
<td>9.4 ± 0.9</td>
<td>15.2 ± 1.1‡</td>
</tr>
<tr>
<td>Total Body BMC (g)</td>
<td>1332 ± 61</td>
<td>1554 ± 93</td>
<td>2341 ± 67‡</td>
</tr>
<tr>
<td>Arm length (mm)</td>
<td>259 ± 5</td>
<td>283 ± 5††</td>
<td>306 ± 3‡</td>
</tr>
<tr>
<td>Side-to-side Difference Grip Strength %</td>
<td>19.9 ± 2.4</td>
<td>15.2 ± 3.2</td>
<td>18.0 ± 2.2</td>
</tr>
</tbody>
</table>

| Humerus BMC (g) |
|-----------------|------------|-------------|
| Playing Arm     | 36.4 ± 1.7 | 51.3 ± 2.7††| 71.6 ± 2.1‡ |
| Non Playing Arm | 32.4 ± 1.7 | 43.5 ± 2.4††| 61.1 ± 1.7‡ |
| Absolute Difference | 4.0 ± 0.6 | 7.8 ± 1.5† | 10.8 ± 1.1††|
| Percent Difference | 13.0 ± 0.5% | 18.7 ± 1.2% | 17.4 ± 0.4% |

| Training history |
|------------------|------------|
| Starting age of playing (yrs) | 5.7 ± 0.4 | 6.5 ± 0.6 | 7.1 ± 0.4 |
| Current training intensity (hrs/wk) | 6.0 ± 0.5 | 7.4 ± 0.9 | 10.8 ± 1.0††|
| Years of playing (yrs) | 4.9 ± 0.3 | 5.6 ± 0.8 | 7.0 ± 0.4† |
| Training pre-menarche (yrs) | 4.9 ± 0.3 | 5.6 ± 0.8 | 5.4 ± 0.4 |

* p<0.05 vs. peri-pubertal players
† p<0.05, †† p<0.001 vs. pre-pubertal players
‡ p<0.01 vs. pre- and peri-pubertal players

Changes Produced by Growth - Studies of the Non-Playing Arm

At baseline, growth-related measurements showed humeral BMC and cortical area were greater in the peri- compared to the pre-pubertal players (30% and 14% respectively, p<0.07 to 0.001) (Table 5.2 and Figure 5.3). This was the net result of greater periosteal than medullary expansion at both the mid (16% and 21% respectively) and distal sites (18% and 25% respectively) (Table 5.2). These changes were confirmed in the 12 months follow-up study; cortical area of the non-playing arm increased in the pre- (18 to 26%) and peri- (22 to 30%) pubertal players; the result of periosteal expansion at both
sites (13 to 20%) (all p<0.01). Medullary expansion was detected at both the mid and distal sites in the pre-pubertal players (4%, p<0.05 to 0.08) and at the mid site in peri-pubertal players (3%, p<0.1) (Table 5.5 and Figure 5.4).

Table 5.2 Cross-sectional data showing the differences in cortical, total and medullary bone areas in the non-playing humerus with advanced maturation. Percent increases with advanced maturation are shown in parenthesis. (Mean ± SE).

<table>
<thead>
<tr>
<th>Pubertal Status</th>
<th>Non Playing Humerus</th>
<th>Pre- to Peri-</th>
<th>Peri- to Post-</th>
<th>Pre- to Post-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mid Region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortical Area (mm²)</td>
<td>18.1 ± 6.1†† (14%)</td>
<td>31.0 ± 6.0 ‡ (21%)</td>
<td>49.1 ± 5.3§ (38%)</td>
<td></td>
</tr>
<tr>
<td>Total Area (mm²)</td>
<td>36.3 ± 12.1 †† (18%)</td>
<td>26.3 ± 11.9 ‡ (11%)</td>
<td>62.6 ± 10.5 (31%)</td>
<td></td>
</tr>
<tr>
<td>Medullary Area (mm²)</td>
<td>18.2 ± 7.5 †† (25%)</td>
<td>-4.7 ± 7.3 (-5%)</td>
<td>13.5 ± 6.5 † (19%)</td>
<td></td>
</tr>
<tr>
<td>Ip (mm⁴)</td>
<td>323.9 ± 820.3††(39%)</td>
<td>2441.2 ± 803.1 ‡(30%)</td>
<td>4765.1 ± 707.7‡ (81%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Distal Region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortical Area (mm²)</td>
<td>15.9 ± 5.7 †† (13%)</td>
<td>26.5 ± 5.6 ‡ (20%)</td>
<td>42.4 ± 4.9 ‡ (36%)</td>
<td></td>
</tr>
<tr>
<td>Total Area (mm²)</td>
<td>29.9 ± 10.6 ‡ (16%)</td>
<td>13.6 ± 10.4 (6%)</td>
<td>43.5 ± 9.1 ‡ (24%)</td>
<td></td>
</tr>
<tr>
<td>Medullary Area (mm²)</td>
<td>14.0 ± 6.3 †† (21%)</td>
<td>-12.9 ± 6.1 (-16%)</td>
<td>1.1 ± 5.4 (2%)</td>
<td></td>
</tr>
<tr>
<td>Ip (mm⁴)</td>
<td>748.5 ± 644.8 (36%)</td>
<td>1264.0 ± 631.3‡(15%)</td>
<td>3012.5 ± 556.3‡(63%)</td>
<td></td>
</tr>
</tbody>
</table>

† p<0.1, †† p<0.05, ‡ p<0.01 vs. pre-pubertal players; ¶ p <0.1  || p<0.05, § p<0.01 vs. peri-pubertal players.

The growth effect differed in magnitude and at each surface, when the peri-pubertal players were compared with the post-pubertal players. In the cross-sectional analyses, cortical area was about 20% greater in the post- than peri-pubertal players, at each site (p<0.001) (Table 5.2). The larger cortical area in the post-pubertal players was associated with a relatively larger periosteal diameter at the mid region (11%, p<0.08) and a smaller medullary at the distal region (16%, p<0.1) (Table 5.2 and Figure 5.3). Over 12-months, cortical area increased at the mid (9%) and distal sites (3%) during the post-pubertal years, due to mostly periosteal expansion at the mid (6%) and distal sites (3%) and medullary contraction at the mid humerus (2.4%) (all p<0.05 to 0.001) (Table 5.5 and Figure 5.5).
I$_{P}$ increased with advanced maturation before and after adjusting for bone length at the mid site (pre- versus post-puberty 30%, p<0.05). In contrast, at the distal site, I$_{P}$ increased with advanced maturation before but not after adjusting for bone length. The polar second moment of area (I$_{P}$) was 25% greater at the mid compared to the distal humerus in the pre- and peri-pubertal players and 35% greater in the post-pubertal players (p<0.001). Over a 12-month period, I$_{P}$ increased in all pubertal groups. The increase was greater in the pre- and peri- compared to the post-pubertal players (p<0.1 to <0.001). After adjusting for bone length, the increase in I$_{P}$ was only greater in the peri- compared to the post-pubertal players (p<0.001).

| Maturity Related Site-Specific Differences on the Surfaces of Cortical Bone |
|-----------------------------|-----------------------------|-----------------------------|
| **Pre-**                    | **Peri-**                   | **Post-**                   |
| Mid                         | Mid                         | Mid                         |
| Periosteal Bone Formation   | Periosteal Bone Formation   | Periosteal Bone Formation   |
| Distal                      | Distal                      | Distal                      |
| Endocortical Bone Resorption| Endocortical Bone Resorption| Endocortical Bone Resorption|

**Figure 5.3**: Schematic scaled representation of cross-sectional data showing the changes at the surfaces of cortical bone during advanced maturation. Cortical area increased by ~14% from the pre- to peri- pubertal years due to greater periosteal than medullary expansion at both sites. From the peri- to post- pubertal years cortical area increased by ~20% due to periosteal expansion at the mid humerus, and medullary contraction at the distal humerus.

**Effects of loading- comparisons of the playing versus the non-playing humerus**

There was no difference between the groups for the number of years training prior to the onset of menarche. Effects of loading were determined by comparing the bone traits in the playing versus non-playing arms. The cross-sectional analyses showed BMC and
cortical area were greater by 11-12% in the playing versus non-playing arm in the pre-pubertal players (Table 5.4 and Figure 5.4). This was the result of periosteal expansion at the mid and distal sites, resulting in a bone that was 6% larger in overall size (p<0.01) (Table 5.4 and Figure 5.4). The medullary cavity was greater at the mid humerus in the playing compared to the non-playing arm (4%, p<0.05), but there was no difference at the distal humerus. There was no additional benefit of exercise on BMC or cortical area with advanced maturation (cross-sectional analysis). However, exercise was associated with medullary contraction (9%) at the distal humerus (p<0.001), but not at the mid humerus (Table 5.3 and Figure 5.4).

**Figure 5.4.** The side-to-side difference in total, medullary and cortical area and bone strength in pre-, peri and post-pubertal players at the mid and distal humerus. † p<0.1, †† p<0.01, ‡ p<0.001 vs. zero; * p<0.1, ** p<0.05 vs. pubertal group.
Over a 12-month period, there was no additional increase in the side-to-side differences in bone area in the pre- or peri-pubertal players (Figure 5.5). In contrast, in the post-pubertal players, the side-to-side difference in cortical area increased a further 4% at the distal site (p<0.05). This was the result of medullary contraction in the playing arm (2%, p<0.05) but no change in periosteal area (Table 5.5 and Figure 5.5).

At baseline, the side-to-side differences in I_p at the mid and distal sites were 11% and 15% respectively in the pre-pubertal players and were no different in the peri- or post-pubertal players (p<0.01) (Table 5.3, Figure 5.4). The side-to-side differences in I_p were greater at the mid compared to distal humerus in the post-pubertal players (23% versus 16%, p<0.05).

The longitudinal data showed no additional increase in the side-to-side differences for I_p at the mid or distal humerus or at any stage of puberty. The muscle strength variables showed no significant correlation with side-to-side differences in BMC, bone size or bone strength parameters at baseline or follow-up. However, within each arm, muscle strength was related to BMC, total area, cortical area and I_p in the playing and non-playing arm in pre-, peri- and post-pubertal players at baseline (r = 0.44 to 0.75, p<0.06 to <0.001), except for BMC, total area and I_p in the non-playing arm of post-pubertal players. Similar findings were reported at follow-up.
Figure 5.5. Twelve-month changes in total, medullary and cortical areas in the playing (circle) and non-playing humerus (triangle) at the mid (black) and distal (white) humerus. *p<0.10, **p<0.05 vs. non-playing arm
Table 5.3  Average bone areas at baseline of the mid and distal regions of the humeral shaft in the playing and non-playing arm of pre-, peri- and post-pubertal female tennis players.

<table>
<thead>
<tr>
<th></th>
<th>Humerus Mid Region</th>
<th></th>
<th>Humerus Distal Region</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bone Area’s (mm²)</td>
<td>Bone Strength</td>
<td>Bone Area’s (mm²)</td>
<td>Bone Strength</td>
</tr>
<tr>
<td></td>
<td>Cortical</td>
<td>Total</td>
<td>Medullary</td>
<td>Ip (mm⁴)</td>
</tr>
<tr>
<td>Pre-Pubertal (n=17)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Playing Arm</td>
<td>137.2 ± 4.0 **</td>
<td>211.6 ± 7.0***</td>
<td>74.4 ± 4.1*</td>
<td>6561.7 ± 400.9***</td>
</tr>
<tr>
<td>Non-playing Arm</td>
<td>128.2 ± 3.6</td>
<td>200.1 ± 6.4</td>
<td>71.9 ± 3.9</td>
<td>5901.4 ± 356.7</td>
</tr>
<tr>
<td>Percent Difference</td>
<td>7.7 ± 1.1</td>
<td>6.2 ± 1.0</td>
<td>3.3 ± 1.8</td>
<td>11.3 ± 2.3</td>
</tr>
<tr>
<td>Peri-Pubertal (n=11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Playing Arm</td>
<td>162.6 ± 7.1 **</td>
<td>256.3 ± 15.4**</td>
<td>93.8 ± 8.7*</td>
<td>9670.7 ± 1198.1**</td>
</tr>
<tr>
<td>Non-playing Arm</td>
<td>146.2 ± 6.3 ††</td>
<td>236.4 ± 13.1 ††</td>
<td>90.2 ± 7.3 ††</td>
<td>8225.3 ± 899.9 ††</td>
</tr>
<tr>
<td>Percent Difference</td>
<td>11.9 ± 2.8</td>
<td>8.9 ± 6.1</td>
<td>3.6 ± 1.8</td>
<td>16.9 ± 4.4</td>
</tr>
<tr>
<td>Post-Pubertal (n=19)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Playing Arm</td>
<td>197.4 ± 3.7 ***</td>
<td>281.2 ± 6.1 ***</td>
<td>83.8 ± 4.1</td>
<td>12946.6 ± 498.5 ***</td>
</tr>
<tr>
<td>Non-playing Arm</td>
<td>177.2 ± 3.1 †§</td>
<td>262.7 ± 6.3 †§</td>
<td>85.5 ± 4.3</td>
<td>10666.5 ± 465.21 †§</td>
</tr>
<tr>
<td>Percent Difference</td>
<td>12.1 ± 1.9</td>
<td>7.5 ± 5.0</td>
<td>-1.9 ± 1.1</td>
<td>17.0 ± 2.6</td>
</tr>
</tbody>
</table>

*p<0.1, **p<0.01, ***p<0.001 vs. non-playing arm; † p<0.1, †† p<0.05, ‡ p<0.01 vs. pre-pubertal players; ¶ p<0.1 || p<0.05, § p<0.01 vs. peri-pubertal players.
Table 5.4 Longitudinal data showing the age, age of menarche, body composition, and training history of those who remained in the same pubertal groups at follow-up (mean ± SE).

<table>
<thead>
<tr>
<th></th>
<th>Pubertal Status</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Pre (n=6)</td>
<td>Peri-Peri (n=15)</td>
<td>Post-Post (n=16)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>Absolute Difference</td>
<td>Baseline</td>
<td>Absolute Difference</td>
</tr>
<tr>
<td>Age (years)</td>
<td>9.9 ± 0.4</td>
<td>1.1 ± 0.1</td>
<td>11.8 ± 0.3</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Age of menarche (yrs)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12.6 ± 0.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>138.9 ± 3.8</td>
<td>5.6 ± 1.0**</td>
<td>149.1 ± 1.8</td>
<td>7.2 ± 0.6**</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>34.2 ± 1.9</td>
<td>3.9 ± 0.9*</td>
<td>40.3 ± 1.7</td>
<td>7.3 ± 1.0**</td>
</tr>
<tr>
<td>Lean Mass (kg)</td>
<td>24.1 ± 1.1</td>
<td>2.7 ± 0.5*</td>
<td>29.2 ± 1.1</td>
<td>5.4 ± 0.5**††</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>8.0 ± 0.9</td>
<td>1.8 ± 1.1</td>
<td>8.6 ± 0.7</td>
<td>2.5 ± 0.9*</td>
</tr>
<tr>
<td>Total Body BMC (g)</td>
<td>1205 ± 56</td>
<td>194 ± 25*</td>
<td>1537 ± 74</td>
<td>403 ± 48**</td>
</tr>
<tr>
<td>Arm length (mm)</td>
<td>251 ± 7</td>
<td>12 ± 4*</td>
<td>274 ± 5</td>
<td>16 ± 1**</td>
</tr>
<tr>
<td>Grip Strength Percent Difference</td>
<td>23.6 ± 6.1</td>
<td>6.7 ± 13.3</td>
<td>16.1 ± 2.7</td>
<td>2.8 ± 4.1</td>
</tr>
</tbody>
</table>

**Humerus BMC (g)**

- Playing Arm
  - 33.5 ± 1.9 7.1 ± 2.3*§ 47.5 ± 2.7 10.6 ± 1.2**§ 71.4 ± 2.4 3.9 ± 1.2**†††
- Non Playing Arm
  - 29.4 ± 1.7 4.1 ± 1.5* 41.8 ± 2.1 8.5 ± 0.9** 60.1 ± 1.7 3.9 ± 1.1**†††
- Absolute Difference
  - 4.1 ± 1.2 3.1 ± 0.7* 5.7 ± 1.1 2.5 ± 1.1* 11.2 ± 1.1 0.0 ± 0.9

* p<0.05, **p<0.01 vs. baseline, † p<0.05, †† p<0.01 vs. pre-pubertal players, ††† p<0.01 vs. peri-pubertal players, ‡ p<0.05 vs. pre- and peri-pubertal players; § p<0.05 vs. non-playing arm
Table 5.5 Longitudinal data showing the average bone areas at baseline and follow-up of the mid and distal regions of the humeral shaft in the playing and non-playing arm of female tennis players who had remained in the same pubertal group at follow-up (mean ± SE).

<table>
<thead>
<tr>
<th></th>
<th>Mid Humerus</th>
<th>Distal Humerus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cortical Area</td>
<td>Cortical Area</td>
</tr>
<tr>
<td></td>
<td>Playing Arm</td>
<td>Non-Playing Arm</td>
</tr>
<tr>
<td></td>
<td>Total Area</td>
<td>Playing Arm</td>
</tr>
<tr>
<td></td>
<td>Medullary Area</td>
<td>Non-Playing Arm</td>
</tr>
<tr>
<td>Peri-Peri n=15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>160.0 ± 5.2</td>
<td>145.1 ± 4.7</td>
</tr>
<tr>
<td>Follow up</td>
<td>206.3 ± 5.4</td>
<td>193.6 ± 4.6</td>
</tr>
<tr>
<td>Absolute Difference</td>
<td>41.4 ± 4.1***</td>
<td>44.9 ± 2.8***</td>
</tr>
<tr>
<td>% Difference</td>
<td>25.8 ± 3.0***</td>
<td>29.8 ± 2.3 ***</td>
</tr>
<tr>
<td>Post-Post n=16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>198.4 ± 4.2</td>
<td>175.1 ± 2.8</td>
</tr>
<tr>
<td>Follow up</td>
<td>213.7 ± 4.0</td>
<td>192.5 ± 3.5</td>
</tr>
<tr>
<td>Absolute Difference</td>
<td>15.0 ± 3.1***</td>
<td>16.4 ± 1.9***</td>
</tr>
<tr>
<td>% Difference</td>
<td>7.9 ± 1.8***</td>
<td>9.4 ± 1.1***</td>
</tr>
</tbody>
</table>

* p<0.1, ** p<0.05, *** p<0.001 vs. baseline, † p<0.1, †† p<0.05 vs. change non playing arm.
Discussion

In this study, we found there was heterogeneity in the surface- and site-specific cortical bone response to growth and exercise. We reported that: 1) growth increased BMC and cortical area by forming bone on the periosteal surface during the pre-and peri-pubertal years and on the endocortical surface during the post-pubertal years. As a result of these changes, the bone was 25% larger and 30% stronger in the post- compared to the pre-pubertal years; 2) Exercise during the pre-to post-pubertal years increased BMC and cortical area 12 to 17%, by enhancing the growth-related changes on the periosteal surface during the pre-pubertal years and endocortical surface during the post-pubertal years. As a result of these changes, the bone was 6 to 9% larger and 11 to 23% stronger compared to the growth-related increases in size and strength; 3) the exercise effect on BMC and bone size in the pre-pubertal players was no different to the post-pubertal players; and 4) the growth and exercise-related changes were different along the length of the humerus.

Changes Produced by Growth - Studies of the Non-Playing Arm

Growth related changes at the periosteal and endocortical surface were different during each stage of puberty and at the mid and distal humerus. From the pre- to peri-pubertal years, cortical area increased 14% due to greater periosteal expansion as apposed to endocortical resorption. During the peri- to post-pubertal years, cortical area increased a further 21% due to periosteal expansion at the mid humerus and endocortical contraction at the distal humerus. Over a 12-month period, cortical area increased at both sites on the humerus during the pre-, peri- and post-pubertal years due to additional growth on the periosteal surface during each stage of puberty and endocortical contraction during the post-pubertal years. Periosteal expansion was 3 times greater in the pre- and peri-pubertal years compared to the post-pubertal years.
Similar findings have been reported by Garn et al (1984), who measured bone diameter of the second metacarpal from more than 25,000 radiographs of men and women (aged 1 to 80 years of age) (3). Cortical thickness increased 45% in women, due to bone formation on the periosteal surface during childhood and on the endocortical surface during puberty. A mixed cross-sectional, longitudinal study by Bass et al (1999) also reported periosteal expansion occurred during the pre- to post-pubertal years, whereas endocortical apposition occurred during the peri-pubertal years at the second metacarpal and one year after menarche at the femoral shaft (103). Our findings were similar; we detected endocortical contraction at the distal end of the humerus and periosteal expansion at the mid-humerus during the peri- to post-pubertal years.

Bone formation on the endocortical surface at the distal humerus after menarche compensated for the resorption that occurred during the pre-to peri-pubertal years. As a result, the medullary cavity was a similar size in the post- compared to the pre-pubertal years. In contrast, because endocortical contraction was not detected at the mid-humerus after menarche, the medullary cavity was larger in the post- compared to the pre-pubertal years. Garn et al (1970) also reported that endocortical apposition occurs after menarche reducing the size of the medullary cavity to that of an infant (3). Storage on this surface has two benefits: 1) it serves as a reservoir of calcium for when women are lactating (3). Women may lose a substantial amount of bone when lactating, so additional bone on the endocortical surface may be needed to prevent bone fragility (297); and 2) bone resorption occurs at the endocortical surface later in life, with the cessation of estrogen, therefore additional bone may offset the risk of fracture (3).

The changes in bone morphology appear to be regulated by the production of hormones during different stages of growth. Growth hormone and IGF-I are the main contributors to bone modeling during the pre-pubertal years and appear to enhance overall bone size
During the post-pubertal years, estrogen and androgens are associated with an increase in BMC accrual. More specifically: androgens appear to enhance the bone promoting effects of GH and IGF-I on the periosteal surface; and estrogen inhibits bone formation on the periosteal surface and contributes to endocortical bone apposition. Thus in this study, GH, IGF-I and androgens may have regulated periosteal expansion, and estrogens may have inhibited periosteal expansion but enhanced bone modeling on the endocortical surface.

Changes produced by exercise - studies of the playing arm

During the pre-pubertal years, exercise increased cortical bone area 8 to 11%. This was due to additional bone formation on the periosteal surface at the mid and distal humerus. At the mid humerus, cortical area increased due to the net result of greater periosteal expansion compared to endocortical expansion. There was no additional exercise effect at the periosteal surface in the peri- or the post-pubertal players, despite additional training, an increase in training hours per week and advanced maturation. In contrast, there was no additional exercise effect at the endocortical surface in the peri-pubertal players, however bone formation occurred at the distal humerus (5%) in the post-pubertal players. This suggests that there had been an initial adaptation to the training in the pre-pubertal players that was maintained, but not increased, with further training (except at the endocortical surface).

The longitudinal data showed no additional exercise effect on the periosteal surface during any stage of puberty, or on the endocortical surface during the pre- and peri-pubertal years. However, additional bone formation was detected on the endocortical surface at the distal humerus during the post-pubertal years, resulting in a 4% increase in cortical bone area. The contrasting findings may be because the 12-month follow-up period is not long enough to detect additional periosteal and endocortical expansion in the
pre-pubertal players. The initial side-to-side differences were the result of tennis training over a 5-year period. Secondly, the exercise effect detected in the post-pubertal players may have occurred soon after menarche, with minimal change occurring two years post menarche.

The cross-sectional and longitudinal data at the distal humerus support the theory proposed by Ruff that exercise will enhance either bone formation or bone resorption at the surface undergoing bone modeling at the time (2). However, in the post-pubertal players there was no detectable apposition on the endocortical surface due to growth, but there was apposition due to exercise. Therefore, the theory proposed by Ruff may be limited, as our findings suggest that the exercise may enhance bone accrual at sites showing no signs of bone modeling.

Previous research on the surface-specific, maturity dependent response to weight-bearing exercise is limited and conflicting. A controlled prospective study of pre-pubertal boys showed that after 8 months of moderate exercise there was no significant change in femoral mid-shaft diameter, but a 5.5% increase in bone density due to a decrease in the medullary diameter (26). Exercise induced endocortical contraction has also been reported in pre-pubertal female gymnasts (1). In contrast, Dyson et al showed pre-pubertal gymnasts have a larger cross-sectional area of the forearm compared to controls, despite having a smaller stature (50). Suggesting that periosteal expansion occurred in response to exercise. During the post-pubertal years, exercise has been reported to increase endocortical apposition in 18-year-old military recruits (223).

There are several reasons why there are inconsistencies in the data. In some cases, sample sizes were small (224), there was diversity in training, different skeletal sites were studied, and the different activities may have exposed the skeleton to loads of different
magnitudes. Furthermore, most of these studies used either radiographs or DXA to measure bone geometry. These methods can only provide a two-dimensional projection of bone in the coronal plane and capture changes in bone geometry in the mediolateral direction, not the anteroposterior direction. Predicting changes in all directions relies on the flawed assumption that the bone is cylindrical and that there is a uniform osteogenic response at the periosteal or endocortical surface. Previous work has shown this is not the case (31). Changes at the periosteal and endocortical surface in all directions can be measured from sagittal images of bone provided by pQCT or MRI. Although Dyson et al (1997) used pQCT to measure bone geometry in children, it is not a preferred technique because it exposes the children to radiation. Another limitation with previous studies is that only one site along the length of the bone was analysed. This may not have captured a potentially different exercise effect at another site along the bone (224,225).

The results of previous tennis studies, on the surface-specific, maturity dependent response to exercise are consistent with those reported in this study. Ruff et al (1994) used radiographs to compare the side-to-side differences in cortical bone geometry in the arms of one female tennis player who started playing early in childhood, and one male and female who started during puberty. The younger started showed large side-to-side differences in cortical area (54%) due to periosteal expansion (34%) and very little endocortical contraction (6%). In contrast, the two players who started during puberty showed smaller side-to-side differences in cortical area (23%) due to greater endocortical contraction (28% and 19%) compared to periosteal expansion (10% and 13%). Thus, the periosteal surface may be more responsive to exercise during childhood and the endocortical surface may be more responsive after puberty. However, this study is limited by its small sample size. Similar findings were reported by Aschwitz et al (1999) in a sub-sample of 3 players who started training at 16 years of age. They reported tennis training increased trabecular bone density (17%), but had no effect on bone size during
the post-pubertal years. These data suggest that exercise enhances endocortical or trabecular bone formation during the post-pubertal years but has minimal effect on the periosteal surface. A limitation with these studies is that they used small numbers and they are retrospective study designs, making it difficult to determine the exact time during growth when the surface specific changes occurred.

The importance of measuring multiple slices within a region is highlighted by our finding of heterogeneity in the exercise response along the humerus. For instance, during the post-pubertal years exercise had no effect on the endocortical surface of the mid-humerus but resulted in endocortical contraction at the distal-humerus. The different response may have been due to varying loading patterns along the bone. Playing tennis is likely to produce high bending loads on the bone, and the loads are likely to be transmitted in a distal to proximal direction (241,268,311). Thus greater strains may be produced at the distal end of the humerus. Additionally, the diameter of the bone may contribute to a greater strain being produced at the distal humerus. The total diameter of the distal humerus is smaller than the mid humerus (Table 5.3) therefore the distal humerus would support a greater load per surface area when playing tennis. This concept is supported by the comparison of the distal tibia to the proximal tibia of rats in response to treadmill exercise. Iwamoto et al (1999) reported a greater response at the distal tibia (43%) compared to the proximal tibia (23%) and suggested that this may be due to a greater strain imposed on the smaller diameter of the distal tibia.

Furthermore, the muscles that produce the largest force during a regular tennis backhand stroke are attached to the distal portion of the humerus: the muscle extensor carpi radialis, flexor carpi radialis and extensor carpi ulnaris (298). Alfredson et al (1996) reported that the moment arm acting on the distal humerus is greater during elbow activity than at the proximal humerus during shoulder activity (299). Moreover, muscle strength of the
shoulder does not correlate with BMC at the proximal humerus, but elbow strength correlates with BMC at the distal humerus (299).

Our data did not show a relationship between grip-strength and any of the bone variables measured at the humerus, however grip strength tests strength of the forearm flexor muscles: flexor-carpi radialis, flexor-carpi ulnaris, flexor digitorum superficialis, brachioradialis and palmaris longus muscles. Only the flexor carpi radialis is reported to produce the largest force at the distal humerus (299). Furthermore these muscles (except brachioradialis) attach to the medial epicondyle of the humerus and not at the sites we measured on the humerus. This may explain why no relationship was detected between grip strength and any of the bone variables at the mid or distal humerus. Further analysis of the MRI scans in this study (unpublished data) indicate a relationship between muscle area and BMC, bone area, medullary area, cortical area and $I_p$ in the playing and non-playing arms of the pre-, peri- and post-pubertal players (unpublished data). Although no comparisons have been made between these relationships at the proximal versus distal end of the humerus, they do support the notion that muscular pull may increase bone strength at the site of muscle attachment.

There is however, conflicting data regarding the relationship between muscle force and bone strength. To demonstrate that muscle force dominates skeletal adaptation requires that greater muscle mass/strength is associated with greater bone mass, independent of body size contributions. A multitude of studies have shown such correlations (332,334,335,348) but not always demonstrated their independence from other body size measures (336). Schonaue et al. for example, show that as much as 76% of the variation in bone strength index in the distal radius may be explained by grip strength alone (348). However, high correlations between muscle strength/mass at one site and bone strength/mass at another unrelated site suggests that the relationship may be related to
other confounding variables (349), or to stimuli that have independent effects on both systems (337). Myburgh et al. showed that 67% of the bending rigidity of the ulna may be explained by the biceps strength, but the biceps has no attachment to the ulna and would not directly generate loads on the ulna (332). Likewise, muscle strength of the quadriceps and hamstring muscles independently predicts bone density of the humerus and spine (337). Thus, inferences on the direct effect of muscle strength on bone adaptation based on these correlations are spurious. However, there are just as many studies that demonstrate that muscle strength has effects on BMC and bone density, that are independent of age, weight, height, or years of estrogen use (334,337,338). Moreover, a case study showed that gains and losses in muscle strength precede gains and losses in bone mass (350). Thus these data prove a direct cause and affect association between muscle strength on bone mass. Regarding this tennis study, to truly test a relationship between muscle and bone strength, muscle strength of the muscles dominating movement of the humerus needs to be measured, and prospective data needs to be collected on the changes in bone mass and muscle strength.

In addition, heterogeneity in the exercise response along the length of the bone may be due to a synergism between exercise and growth acting in a distal to proximal direction. Cross-sectional data shows that skeletal maturation at the distal segments precedes proximal segments (127). For instance, the hands and feet reach peak height velocity at an earlier age compared to the forearm and tibia. Similarly, endocortical contraction has been reported to occur at the metacarpal 2 years prior to the femoral shaft (103). Perhaps this same phenomenon occurs within the same bone i.e. the distal end undergoes maturation prior to the proximal end. Our longitudinal data supports this notion, as endocortical contraction occurred at the distal humerus at baseline, and at the proximal humerus 12 months later.
Heterogeneity in the exercise response along the length of the humerus was reported in a retrospective study on retired male tennis players, who started training during growth (225) Peripheral QCT was used to measure the side-to-side differences in bone geometry at the proximal, mid and distal sites on the humerus. Cortical bone increased at the proximal site of the playing arm, due to proportionally greater expansion at the periosteal surface compared to the endocortical surface (75% and 25% respectively). In contrast, cortical area increased at the distal humerus due to expansion at both the periosteal and endocortical surfaces (96% and 4% respectively). Heterogeneity in the exercise response has also been detected along the length of the radius (199,224,294). However, the findings are not consistent regarding what site (mid or distal radius) shows the greatest osteogenic response.

The findings from animal studies support the contention of a site-specific response to mechanical loading. Mosely et al (1997) reported that mechanical loading increased periosteal bone formation at the distal ulna of rats (up to 37%), but decreased formation at the proximal ulna (-25%) (232). Furthermore, the majority of the new bone deposited on the medial and lateral surfaces were the sites where the greatest strains were recorded. Similarly, Robling et al (2002) reported that exercise results in greater periosteal bone formation and bone rigidity at distal sites along the ulna of rats compared to more proximal sites (314).

In this study, exercise increased BMC 12% during the pre-pubertal years. This was the net result of greater periosteal expansion compared to endocortical expansion. Subsequently, the bone was 12 to 24% more resistant to bending. There was no additional exercise effect on the periosteal surface during the peri- or post-pubertal years, only endocortical apposition during the post-pubertal years; but this had no benefit on bending strength. Bone formation is more beneficial on the periosteal surface because it
is distributed further from the medullary cavity, allowing a greater degree of flexion about the neutral axis (58). Bone formation on the endocortical surface confers no biomechanical advantage to bone strength (unless it is resorbed, as in lactation and ageing). This has been demonstrated in a number of exercise-related studies. Bradney et al (1998) found 8-months of moderate exercise resulted in a 5.5% greater increase in volumetric bone density due to endocortical contraction. However, the resistance to bending did not improve (292). Similarly, Haapasalo et al (1996) reported BMC increased 5% in the playing arm of adult female tennis players due to endocortical contraction, but this did not confer a benefit to bone strength (198).

The importance of bone size on the risk of fracture is highlighted in a number of recently published studies. Skaggs et al (2001) compared cross-sectional bone size in girls who had sustained forearm fractures, to girls age, height, weight and maturity matched, with no fracture history (57). Bone size at the distal radius was 8% smaller in girls who fractured compared to controls, but there was no difference in cortical or trabecular bone density. Similarly, bone size was $0.5 \pm 0.1$ SD smaller and volumetric bone density was reduced in older men who had a history of hip and spine fractures compared to healthy men (174).

The exercise effect attained during the pre-pubertal years was maintained later into puberty despite additional training and advanced maturation. This suggests that the pre-pubertal years may be the most opportune time during growth for exercise to have an osteogenic effect. Furthermore, all girls started playing competitive tennis during childhood and there were no differences in the years training prior to menarche between the groups. Hence, all the girls were exposed to a similar loading intervention at that time. Taking this into consideration, the non-significant change in the exercise effect on bone in the pre- versus post-pubertal players, and Turner’s accommodation hypothesis,
we can argue that bone adapts to the new stimulus of tennis playing during the prepubertal years, and remain adapted throughout growth. That is, there is no new increase in loading stimulus in the peri- and post-pubertal girls because the tennis playing stimulus continues to match the increase in skeletal dimensions (and in fact may decrease relative to skeletal growth).

It is possible however, that because 40% of peak BMC is accrued during the peri- to early post-pubertal years, over a 4 year period (47), exercise may have had an effect during this time, but the changes may have been undetectable compared to the growth-related changes. To accurately test the hypothesis that exercise is more beneficial during the precompared to the peri- and post-pubertal years, comparisons need to be made on the side-to-side differences in the arms of tennis players who started training at different Tanner stages.

The results of previous studies support the notion that exercise may have a greater benefit during the early stages of growth. Haapasalo et al (1998) found the side-to-side differences in bone density at the humeral shaft was different in tennis players compared to controls at Tanner stage 2, and at all measured sites at Tanner stage 3 (200). These findings suggest that exercise may begin to have an osteogenic effect during Tanner stage 2 to 3. However, the players in Tanner stage 3 had a training history 2 to 3 times greater than the players in Tanner stage 2 and 1. Moreover, the side-to-side difference in controls was less at Tanner stage 3 compared to 2 and 1 (3-5% versus 0% respectively), therefore differences between players and controls are less likely to be detected during Tanner stage 2.

Kannus et al (1995) also found a 2 to 4 times greater side-to-side differences in tennis players who started playing before menarche compared to those who started after (199).
Moreover, an exercise intervention was introduced to both pre- (Tanner stage 1 to 3) and post-menarcheal children (Tanner stage 3 to 5), however exercise only enhanced BMC accrual in the pre-menarcheal children (3 to 4.5%) (213). These data suggest that additional exercise may result in a greater osteogenic response during the pre-to peri-pubertal years, compared to the post-pubertal years. Future research is needed to determine specifically when exercise has the greatest benefit on BMC and bone strength during growth.

There are a number of limitations with the findings from this study. First, there was no control group to show the side-to-side differences in bone mass and geometry due to everyday use of the dominant arm versus the non-dominant arm. Comparing the side-to-side differences in the players versus controls would add strength to the findings, reducing the assumption of a type I error being reported. Second, the conclusion that the pre-pubertal years may be the most opportune time to exercise needs to be supported by comparing tennis players who started training before versus after menarche. If the side-to-side differences in bone mass and geometry are greater in those who started before menarche, then we can be more confident with our findings. Third, the longitudinal data showed minimal changes over the 12-month period. Thus, a longer follow-up period may be needed to detect changes in bone. Furthermore, there were small numbers in the follow-up group and large variability in the changes. Therefore it is possible that a type II error was reported. Fourth, sex-hormone levels were not measured, nor could we quantify changes in true bone density. Finally, the results are only relevant for the girls and are site specific. Further research is needed to see if similar trends are detected in male tennis players and if playing tennis has an osteogenic effect at other sites at risk for low bone density and osteoporotic fractures (ie hip and spine).
In conclusion, exercise enhances periosteal bone formation during the pre-pubertal years and endocortical contraction during the post-pubertal years. These changes are region-specific and may reflect a variation in the loading conditions at each site, or growth-related changes occurring in a distal to proximal direction along the length of the bone. The results also indicate that the pre-pubertal years may be the most opportune time for exercise to have an osteogenic effect on bone size, mass and strength. Future work is needed to confirm this finding, by comparing the exercise effect in tennis players who started training at different stages of puberty and if similar trends are evident in male tennis players.
CONCLUSION

Osteoporosis is a public health problem that places a social and financial burden on society, because of the mortality and morbidity associated with fractures (5). Low BMC can be the result of age-related bone loss, failure to achieve sufficient peak BMC in early adulthood, or a combination of both. Maximising bone accrual during growth is considered the best protection against age-related bone loss and subsequent fracture risk.

It is widely accepted that physical activity has a beneficial effect on the BMC accrual during growth. This notion is largely based on the findings from elite athletes showing a significantly larger BMC than sedentary controls. To date, there has been limited research investigating the effect of additional exercise in normally active children. Moreover, it is not known what type, magnitude, duration and frequency of exercise is needed to stimulate an osteogenic response. The results of this thesis demonstrates that short bouts of moderate impact exercise, that include unique strain distributions, undertaken three times a week, provides a sufficient stimulus to result in clinically important increases in BMC in normally active children. However, the responsiveness of the skeleton to additional moderate impact exercise appears to depend on the child’s loading history. For instance, the osteogenic response to the moderate exercise program was greater in the children participating in low-moderate impact sports compared with those participating in high impact sports outside of school.

The school-based moderate impact exercise program introduced to pre and early pubertal girls could easily be implemented into a schools curriculum to improve bone health of children. The exercises take 20 minutes to complete and can be included as a warm-up during regular physical education classes. In addition, the variety of activities prescribed in the exercise program may assist in maintaining long-term compliance. However before this program can be recommended as a means of reducing the future risk of
fracture, long-term follow up measures are required to determine if the benefits are maintained after the exercise intervention has ceased. Further investigations are also required to determine if higher magnitude impacts lead to an osteogenic effect at the spine in pre-pubertal children. Furthermore, it is unknown if these data in girls can be applied to boys, or in girls of more advanced maturity.

The risk of fracture is not only dependent on BMC but also bone shape (57,174). To date most studies that have investigated growth related changes in the skeleton have focused on changes in bone length and BMC alone. The results of this thesis show that growth results in a larger bone size due to bone formation on the periosteal surface and expansion on the endocortical surface during the pre- and peri-pubertal years. Whereas after menarche, growth results in small increases in overall bone size and additional bone formation on the endocortical surface, resulting in a smaller medullary cavity.

The growth related changes in bone shape provides a basis for understanding how exercise may influence bone shape and bone strength during different stages of growth. The findings of this thesis support the notion that exercise may enhance bone accrual occurring at the surface undergoing formation at the time (2). The additional bone formation on the periosteal surface resulted in a greater increase in bone strength during the pre-pubertal years compared to bone formation on the endocortical surface after puberty: due to the bone being distributed further away from the neutral axis and increasing the bones resistance to bending.

The benefits of exercise on BMC and strength achieved during the pre-pubertal years appears to be maintained later into puberty. Thus, the pre-pubertal years may be an opportune time for exercise to enhance BMC and bone strength. Further investigation is needed to confirm these findings by comparing the exercise effect in girls who started
participating in sport at various stages of growth. Furthermore, it is also unknown whether the exercise induced increases in bone size, mass and strength are maintained after training has ceased. These findings also highlight the need for further research into changes in bone geometry not only from mechanical loading but also as a result of disease, immobility and pharmacological agents.

In summary, a low BMC and an increased risk of fracture later in life may be the result of a low BMC accrued during the growing years. Thus, the prevention of fractures requires an understanding of the factors that influence bone during growth. Of these, exercise appears to be a modifiable factor that may increase BMC by clinically important amounts and is accessible to a wide range of the community. Thus, public health strategies directed towards improving bone health in children may be an effective means of reducing the future risk of osteoporosis.
REFERENCES


34. Seeman E 1994 Reduced bone density in women with fractures: contribution of low peak bone density and rapid bone loss. Osteoporosis Int Suppl. 1:S15-25.


References


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