Effects of Different Loading Intensities on Skeletal Adaptation to Exercise in Prepubertal Girls

Submitted by

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ABSTRACT

This study involved a 28-week school-based exercise trial of single-leg drop-landing exercise with 42 girls (Tanner stage 1; 6-10 yr old) randomly assigned to control (C), low-drop (LD) or high-drop(HD) exercise groups. The latter two groups performed single-leg drop-landings (3 sessions.wk^{-1} and 50 landings.session^{-1}) from 14cm and 28cm, respectively using the non-dominant leg. Single-leg peak ground-reaction impact forces (PGRIF) in a sub-sample ranged between 2.5 – 4.4 x body-weight (BW). No differences ($p>0.05$) among groups at baseline for age, stature, lean tissue mass (LTM - DXA - Lunar 3.6-DPX), leisure time physical activity or average daily calcium intake were detected. No significant within group changes for between leg differences from baseline to post-training and no significant differences among groups at baseline, or in magnitude of change for any of the dominant or non-dominant (loaded) leg bone mineral content (BMC g) measures determined by DXA – loaded leg total - 19.06, 25.5, 25.46 [p=.156], femoral neck - 0.14, 0.11, 0.15 [p=.959], greater trochanter - 0.37, 0.06, 0.26 [p=.733], mid femoral shaft - 3.87, 3.87, 3.42 [p=.677] for the C, LD and HD groups, respectively, after adjusting for the covariates baseline body and fat mass, and change in LTM (ANCOVA) were observed. Similarly, following ANCOVA adjustments no significant differences for changes in calcaneal speed of sound and broadband ultrasound attenuation (CUBA Clinical), DXA derived changes in femoral neck (-0.009, 0.033, -0.009; p=.189) and total MFS (0.029, 0.041, 0.053; p=.447) volumetric BMD (g.cm^{-3}), or MFS cortical volumetric BMD, the latter derived by a new technique combining MRI and DXA were identified. TBBMC changed by 79.6g-C, 100.2g-LD and 91.9g-HD (p=.339). Combining data from both exercise groups to increase statistical power produced similar results. No significant within group changes for between leg differences from baseline to post-training and no significant differences among groups at baseline, or in magnitude of change for any of the dominant or non-dominant (loaded) leg bone geometrical (area cm^2) determined by MRI using ANALYZE® software of proximal - 22.18, 12.91, 19.86 [p=.248], mid - 19.83, 15.91, 19.64 [p=.233], or distal - 14.78, 16.07, 13.35 [p=.792], slice cortical area for the C, LD and HD groups, respectively, after adjusting for the covariates baseline body and fat mass, and change in LTM (ANCOVA) were detected. Similarly there were no significant biomechanical cross sectional moment of inertia (CSMI cm^4) changes determined by Scion Image® (Frederick, Maryland: Version-Beta 3B) and a custom

macro program of proximal - 896, 815, 649 [p=.415], mid - 1054, 806, 1087 [p=.471], or distal - 1197, 1079, 966 [p=.606], slice CSMI for the C, LD and HD groups, respectively after adjusting for the same covariates. In contrast to some recent reports, our findings suggest that strictly controlled uni-modal; uni-directional single-leg drop-landing exercises involving low-moderate peak ground-reaction impact forces are not osteogenic in the developing prepubertal female skeleton.

Statement of Sources

This thesis contains no material published elsewhere or extracted in whole or in part from a thesis by which I have qualified for or been awarded another degree or diploma.

No other person's work has been used without due acknowledgement in the main text of the thesis.

This thesis has not been submitted for the award of any degree or diploma in any other tertiary institution.

All research procedures reported in the thesis received the approval of the relevant Ethics/Safety Committees (where required).

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CHAPTER 1

INTRODUCTION

It is now fairly well accepted that exercise during growth produces beneficial effects in bone (Heinonen et al., 2000 Parfitt, 1994; Glastre et al., 1990), and childhood appears to be emerging as a most opportune time to modify bone mass and geometry through exercise (Fuchs, Bauer, & Snow, 2001; MacKelvie, McKay, Khan, & Crocker, 2001; Bradney et al 1998; McKay et al., 2000; Morris, Naughton, Gibbs, Carlson, & Wark 1997; Heinonen et al., 2000; Van Langendonck, Claessens, Vlietinck, Derom, & Beunen, 2003; Courteix et al., 1998; Petit et al., 2002; Bauer, Fuchs, Smith, & Snow, 2001). Bone mass and bone geometry are major determinants of bone strength in humans (Bradney et al 1998; Duncan et al., 2002; Frost, 1997, 1998, & 1999; Heinonen, Sievanen, Kyrolainen, Perttunen, & Kannus, 2001; Petit et al., 2002; Schonau 1998). Many cross-sectional studies concur that young athletes exhibit higher bone mass than sedentary controls (Bass et al., 1998; Bradney et al., 1998; Daly, Rich, & Klein, 1997; Duncan et al., 2002). Further, large increments in bone mineral content (BMC) and areal bone mineral density (aBMD) are reported in some prospective studies involving vigorous exercise during growth (Haapasalo et al., 1998; Huddleston, Rockwell, Kulund, & Harrison, 1980; Grimston, Willows, & Hanley, 1993). Recent advances in non-invasive bone assessment techniques have established that exercise-associated increases in bone strength may be attributed largely to changes in bone size and geometry, with little or no change in volumetric bone density (Haapasalo et al., 2000; Schonau 1998; Mackelvie, McKay, Khan, & Crocker, 2001, Petit et al., 2002); however, little is known about these adaptations in the younger growing skeleton.

For almost two decades, Dual-Energy X-ray Absorptiometry (DXA) measures of bone mineral mass and areal density (aBMD) have been used as indicators of skeletal adaptation to exercise in pediatric studies. However, the one- (BMC) or two- (aBMD) dimensional interpretation of these DXA derived measures in an ever-changing three-dimensional space during growth has recognised limitations (Seeman, 1998 and 2002), and these measures have received deserved criticism (Bolotin, 2001; Hoegler et al., 2002; Molgaard, Thomsen, Prentice, Cole, & Michaelsen,

1997; Prentice, Parsons, Cole, 1994; Haapasalo et al., 2000). To establish a more thorough understanding of skeletal adaptations to exercise, researchers in the field must move beyond simply measuring BMC and aBMD, and incorporate newer, non-invasive techniques that also allow the assessment of possible geometric and biomechanical adaptations that may also influence bone strength. Such a multifaceted assessment approach will provide a better understanding of the heterogenic nature of skeletal adaptation to exercise (Seeman, 2002), especially during childhood which is the period of most rapid changes in bone mineralization, size and shape.

1.1 Determinants of Bone Mineral

The processes of growth, modeling and remodeling enable the developing skeleton to alter its mass and architecture in response to increasing functional loads. Compared to circumpubertal and adolescent populations, relatively few studies have investigated the determinants of BMD in prepubertal children. Limited research in the prepubertal époque suggests that body size (De Schepper, Derde, Van den Broeck, Piepsz, & Jonckheer, 1991; Katzman, Bachrach, Carter, & Marcus, 1991; Molgaard, Thomsen, & Michaelsen, 1998), body composition (McKay et al., 2000; Rice et al., 1993), physical activity (Grimston, Willows & Hanley, 1993; Ilich, Skugor, Hangartner, Baoshe, & Matkovic, 1998; Janz et al., 2001; Lehtonen-Veromaa et al., 2000; McKay et al., 2000; Morris, Naughton,. Gibbs, Carlson, & Wark, 1997) and, perhaps also dietary (Barr, Petit, Vigna, & Prior, 2001; Ilich, Skugor, Hangartner, Baoshe, & Matkovic, 1998; Johnston et al., 1992; Rubin et al., 1993; Ruiz, Mandel, & Garabedian, 1995) factors are the major determinants of BMD in prepubertal children. However, the relative influences vary and depend on the number of variables considered in a given analysis and the type of statistical approach used to differentiate their contributions (Blimkie et al., 1993; Gunnes & Lehman 1996; Katzman et al., 1991; Rubin et al., 1993). Due mostly to technological limitations, no study to date has investigated the influence of growth and maturity related changes in sex steroid levels, either independently or in interaction with other lifestyle factors on skeletal development in children. Using a recently developed highly sensitive radioimmunoassay technique, this thesis studied the inter-relationships between estradiol levels, other putative determinants and bone mineralization in prepubertal girls between 6 and 10 years of age.

1.2 Effects of Exercise Training on BMC and BMD

The developing musculoskeletal system is subjected to progressively higher functional loads due to the combined influences of changing body mass and the transport of this mass against gravity in activities of daily living, leisure physical activity, sports and exercise. Studies showing a positive relationship between physical activity level, hours of intensive sports training and increased BMC suggest a dose-response relationship between exercise and development of bone mass in children (Bass et al., 1998; Cassell, Benedict & Specker, 1996; Courteix, Lespessailles, Jaffre, Obert, & Benhamou, 1999; Dyson, Blimkie, Davison, Webber, & Adachi, 1997; Grimston, Willows, & Hanley, 1993; Pettersson, Nordstrom, & Lorentzon, 1999; Slemenda, Miller, Hui, Reister, & Johnston, 1991). Evidence also suggests that the type and magnitude of the exercise performed are important factors modifying the skeletal adaptive response. Weight–bearing exercises (eg gymnastics, running, dancing), where gravitational forces acting on body mass impart relatively high ground reaction forces appears to have a greater osteogenic effect compared to weight-supported exercise (eg swimming, cycling), where gravitational influences on body mass are attenuated (Cassell et al., 1996; Duncan et al., 2002). Higher BMC and aBMD have also been reported in gymnasts and children performing relatively high-impact exercise, suggesting that this type and intensity of loading may also be osteogenic. Further, a number of recent intervention studies (Fuchs et al., 2001; Bradney et al., 1998; Morris, Naughton, Gibbs, Carlson, & Wark, 1997; McKay et al., 2000), have also suggested, although not unequivocally (MacKelvie, McKay, Khan, & Crocker, 2002; Petit et al., 2002), that the osteogenic adaptive response to exercise might be developmentally sensitive and demonstrate greater responsiveness at certain ages and developmental stages than at others. Methodological constraints, however, limit the interpretation and conclusiveness of these studies. The second study of this thesis investigates the effects of differing magnitudes of impact loading in prepubertal girls on both the traditional measures of bone mass and density derived from DXA, as well as the true volumetric density of the cortical shell; the latter derived from combined measures of BMC determined by DXA and cortical bone volume determined by magnetic resonance imaging (MRI).

1.3 Geometric and Biomechanical Adaptation of Bone to Exercise in the Growing Skeleton

Due primarily to technological constraints of available assessment techniques, studies of mechanical loading effects on skeletal geometric and biomechanical adaptations have been limited mostly to animals. Evidence of important functional architectural adaptations to mechanical loading, including changes in endocortical and periosteal dimensions along the length of bones, and changes in bone cross-sectional shape have been observed in animal studies (Forwood & Parker, 1991; Lanyon, 1992b; Mosley, March, Lynch, & Lanyon, 1997; Mosley & Lanyon, 1998). Histomorphologic and geometric adaptations of bone to mechanical loading in animals also appear to be dose-dependent and different in immature compared to mature skeletons (Forwood & Burr 1993). Only a few studies have investigated geometric (Duncan et al., 2002; Faulkner et al., 2003; Petit et al., 2002;) and biomechanical (Bauer, Fuchs, Smith, & Snow, 2001; Duncan et al., 2002; Faulkner et al., 2003; Petit et al., 2002) adaptations to exercise in children. Comparative studies (Duncan et al., 2002; Faulkner et al., 2003) indicate that weight-bearing exercise like running and gymnastics is associated with more favourable geometric and biomechanical characteristics in relation to bone strength, than weight-supported activities like swimming and cycling. The differences reflect the specific mechanical-loading patterns inherent in these activities. Only a few prospective studies have investigated geometric and biomechanical adaptations to exercise in prepubertal children. One study (Bradney et al., 1998), reported increases in cortical thickness with 8 months of mixed weight-bearing exercise in prepubertal boys, whereas another (Fuchs et al 2001) found an increase in femoral neck area in a mixed group of prepubertal boys and girls following 7 months of predominantly drop-jumping exercise. In the only other prospective study involving geometric measures of bone, (Petit et al., 2002), 7 months of jumping (10-minutes, 3 times per week) had no effect on any of the measures of bone structure in prepubertal girls. A group of more mature girls in that study, however, showed significant gains in geometric and biomechanical parameters suggesting that these skeletal adaptive responses may be more sensitive to exercise in the époque between pre-puberty and the onset of menarche (Petit et al., 2002). Noteworthy about these prospective studies however, is that they all used DXA, a technique with questionable accuracy for measuring three-dimensional structures, to estimate geometric adaptations to exercise.

Advances in non-invasive technology have allowed recent examination of exercise related differences in skeletal geometric and structural properties in humans (Duncan et al., 2002; Hong, Hipp, Mulkern, Jaramillo, & Sayder, 2000; Woodhead et al., 2001, Hoegler et al 2002; Kroger, Vainio, Nieminen, & Kotaniemi, 1995). The ability to non-invasively measure bone geometry including medullary cavity diameter, cortical bone thickness and cortical bone volumes and density with these new technologies offers a novel approach for investigating the heterogeneity of the skeleton's adaptive responses in children on multiple fronts, not simply in terms of mineral mass and density changes. Additionally, the ability to assess biomechanical measures related to bone functional strength such as cross-sectional moments of inertia, affords new insight into the integrative and holistic nature of the osteogenic adaptive response to exercise in children. In the third study of this thesis we use a previously validated (Woodhead et al., 2001) magnetic resonance imaging (MRI) technique to assess three dimensional bone geometry and biomechanical adaptations to exercise in prepubertal girls.

1.4 The Muscle-Bone Relationship in Children

Evidence of a close functional relationship between muscle strength and bone adaptation in humans is emerging. Muscular forces acting on the skeleton during exercise appear to cause the largest loads and bone strains, and muscle forces are now generally considered to be the predominant factor modulating skeletal adaptations to exercise (Lanyon, 1996a; Frost, & Schoneau, 2000). It appears that bone and muscle are functionally mutual (Frost, & Schoenau, 2000; Blimkie and Hoegler, 2003). The Mechanostat Theory (Frost, 1987) is the predominant theory describing the putative relationship between mechanical loading, muscle forces and bone adaptation. The theory contends that an individual's typical mechanical usage and average muscle force history direct the integrated control of longitudinal growth, and bone modeling and remodeling during childhood. Physical activity and muscle strength have been found to be independent, significant predictors of BMD for the total body and proximal femur (Nordstrom, Thorsen, Bergstrom, & Lorentzon, 1996a). High bone mass and altered relationships between bone mass, muscle strength and body constitution in adolescent boys with a high level of physical activity have also been observed (Nordstrom et al., 1996a). Increases in BMD have been associated with weight-bearing, forceful muscle contractions of the quadriceps in adolescent hockey players

(Nordstrom, Nordstrom, Thorsen, & Lorentzon, 1996). Little is known about the trainability of strength and power before puberty, however, and the influence of changes in muscle size and force on bone during growth (Schoenau, 1998; Faigenbaum, 2000; Faigenbaum, Westcott, & Long, 1999; Morris et al., 1997). These issues need to be resolved to allow development of effective exercise programs that optimise skeletal health, while minimizing risk of injury in children. Little is known about the muscle-bone relationship in children, and no study to date has examined the relationship between changes in muscle size and strength with exercise, in relation to changes in bone properties in prepubertal children. The final study in this thesis investigates the relationship between muscle size and force, and bone geometric and biomechanical properties in prepubertal girls.

1.5 Investigative Difficulties

Most studies investigating the relationship between exercise and bone in children have been cross-sectional in design. With this approach, the possibility of selection bias cannot be excluded. Accordingly there is a need for simply designed, single gender, prospective, randomised, controlled studies with well-documented pubertal staging to investigate possible cause and effect relationships between exercise and skeletal adaptations during growth (Seeman, 2002). Interpretations of some prospective studies are confounded when participants are allowed to choose their preferred group or are not matched for pubertal status (Morris, Naughton, Gibbs, Carlson, & Wark, 1997). Additional problems emerge when the exercise intervention involves a combination of exercises (Bradney et al., 1998), or studies do not quantify or account for changes in recreational or everyday activities. Other limitations in school-based interventions include poorly defined and poorly quantified loads (Bradney et al., 1998; McKay et al., 2000; Witzke, & Snow, 2000) that preclude identification of the most effective types of exercises or possible dose-response relationships.

Despite the recent interest and substantial body of published research, a number of issues remain unresolved, regarding exercise and skeletal adaptation in children. These include, but are not limited to the following: 1) The evolving role of changing sex hormone levels and their interaction with physical activity and exercise in determining skeletal development, 2) The absolute and

relative importance of the various parameters of exercise conditions, such as the magnitude of loading, on skeletal adaptations to exercise training, 3) The nature of the skeletal adaptive changes to exercise, e.g. the relative changes in bone mineral density, geometry and biomechanical characteristics, and 4) The importance of muscle size and force capacity in modulating the skeletal adaptive response to exercise. This thesis incorporates a multifaceted musculoskeletal assessment approach involving both traditional and newer non-invasive technologies to addresses these issues in a series of related studies.

1.6 Purpose

This thesis focuses on the prepubertal époque, since this may comprise a sensitive developmental stage for skeletal responsiveness to extrinsic modifiable factors such as physical activity and nutritional practices. Girls rather than boys were chosen as the population for these studies, since risk of osteoporosis, which is dependent in part on bone accrual and bone strength development during growth, is higher in females than males. The primary purpose of this thesis is to investigate the relationship between magnitude of impact exercise (drop landing from different heights) and adaptations in bone mineralisation (DXA), geometry (by a recently developed MRI technique developed in our centre), biomechanical (MRI) and material properties (quantitative ultrasound) in prepubertal girls. The secondary purpose addresses 1) investigation of the importance of extrinsic lifestyle (physical activity and diet) and intrinsic biological (endocrine sex steroids) factors as determinants of the aforementioned bone properties, and 2) elucidation of the muscle-bone relationship in prepubertal girls. Information from these investigations will contribute to health promotion guidelines for optimisation of skeletal growth and development of girls prior to puberty.

1.7 Definition of Bone Terms

It has been noted that the terms BMD, BMC and bone mass are often used interchangeably (and at times incorrectly) in the literature being reviewed. For this thesis definitions of key terminology are as follows:

Mass: the property of a body that causes it to have weight in a gravitational field

Bone Mass: bone mass is the combined measure of its constituents: bone mineral, organic matrix (largely collagen), and water that causes bone to have weight in a gravitational field.

Bone Mineral Content (BMC): amount of mineral measured in grams (g) contained in the skeleton or parts of the skeleton.

Bone Mineral Density (BMD): amount of mineral mass of the skeleton per unit volume (see Areal Bone Mineral Density and Volumetric Bone Mineral Density).

Areal Bone Mineral Density (aBMD): determined as the quotient of bone mineral content (BMC) and DEXA-derived cortical bone area to provide a relative value of bone mineral per measured bone area ($gm.cm^{-2}$).

Volumetric Bone Mineral Density (volBMD): determined as the quotient of bone mineral content (BMC) and cortical bone volume ($gm.cm^{-3}$).

CHAPTER 2

REVIEW OF LITERATURE

2.1 Bone Growth in Childhood

2.1.1 Infancy

Healthy children increase about three times in length from birth to the end of the pubertal growth period. Axial and appendicular portions of the skeleton undergo marked changes in size and shape, and large quantities of calcium and phosphate accumulate within the skeleton. Reducing the skeleton to ash, which is primarily mineral, reveals that there is relatively no difference (in percentage terms) among infants, children, adolescents and adults. Bone mineral actually accrues from birth to early adulthood (e.g. from 25g calcium to over 1000g - Saladin, 1998). From approximately three years of age until the beginning of puberty, male and female children grow at a slowly decelerating rate. A small growth spurt in skeletal length may occur at approximately 8 years of age and a distinct deceleration occurs immediately before puberty (Gertner, 1999).

There are two processes by which bones grow. Growth in width occurs when cortical bone increases by periosteal apposition. Growth in length occurs when cancellous (trabecular) bone increases by endochondral ossification (Parfitt, 1994). Bone densitometry has been used widely to assess bone development upper and lower appendicular sites as well as the lumbar spine and femoral neck. However, the use of densitometry is being called into question since true volumetric BMD cannot be determined with current non-invasive techniques because of insufficient spatial resolution. Furthermore, densitometric data in children are rarely interpreted in light of the biological processes they reflect (Rauch & Schoenau, 2001). Expanding on the 1892 treatise of Wolff's Law, Frost (2001) accepted that healthy, postnatal load-bearing bones were designed to have only enough strength to keep voluntary loads from causing fractures.

2.1.2 Early Childhood

Increases in bone mineralisation occur gradually in early childhood (Glastre et al., 1990). Failure to achieve an optimal level of bone mass at maturity may be linked to a more fragile skeleton and osteoporosis in later life. For a comparison of BMC and bone width there is a point below which BMC is less in girls for a given height and body mass while above this point, BMC is greater (DePriester, Cole, & Bishop, 1990). This point corresponds approximately to an age of 8 years in boys and 9.2 years in girls. When compared with girls, body mass correlates more strongly with bone width than in boys - implying that bone width increases more in boys for a given increase in body mass than in girls. However, it is possible that body mass masks other modifiable lifestyle factors such as exercise and the corresponding increase in lean tissue mass that may underlie these gender specific relationships. The most important predictor of total body bone mineral density in both boys and girls appears to be bone-free lean tissue mass (Faulkner et al., 1993; Wiebe et al., 2002).

2.1.3 Late Childhood

Traditionally, estrogen was not thought to have growth-promoting effects. Experimentally it was shown that low doses (15 $pmol.l^{-1}$) of estrogen (in which estradiol is the most abundant), caused more than a 60% increase in stature over the prepubertal growth rate in boys and girls (Cutler, 1997). Low levels of estradiol appeared to stimulate growth and bone maturation which may explain the more rapid epiphyseal maturation of prepubertal girls compared to boys. Prepubertal girls have approximately 8-times higher levels of serum estradiol than prepubertal boys. A low level of estradiol drives the growth spurt in girls. The beginning of the growth spurt is preceded (up to 6 months) by budding of the breasts. In contrast estradiol levels observed in prepubertal boys appear to have no effect on growth rate or bone maturation. Cutler (1997) contended that epiphyseal maturation of prepubertal girls was more rapid than that of boys because of higher estradiol levels present in girls. From infancy to the onset of puberty aBMD increases in the total body (Faulkner et al., 1993; Geusens et al., 1991, Gordon & Webber, 1993; Katzman et al., 1991) and femoral neck (Lu et al., 1994). In females this gain has been reported to range between 1% and 4% (De Schepper, Derde, Van den Broeck, Piepsz, & Jonckheer, 1991; Glastre et al., 1990; Grimston, Morrison, Harder, & Hanley, 1992; Kroger, Kotaniemi Kroger, & Alhava, 1993; Lu et al.,

1994; Rubin et al., 1993). In children, steadily increasing muscle strength increases bone loads and strains above a modeling threshold. As a result, bone strength and bone mass both increase (Bailey et al., 1999; Frost, 1997).

2.1.4 Adolescence

During the circumpubertal years there is a more rapid increase in aBMD for the total body (Faulkner 1993, Geusens et al., 1991, Katzman et al., 1991; Lu et al., 1994) and femoral neck (Bonjour, Theintz, Buchs, Slosman, & Rizzoli, 1991; Grimston et al., 1992; Katzman et al., 1991 Kroger et al., 1992; Slemenda et al., 1994; Theintz et al., 1992) than in the prepubertal years.

Most (Bonjour et al., 1991; Geusens et al., 1991; Grimston et al., 1992; Katzman et al., 1991; Kroger et al., 1992; Lu et al., 1994; Theintz et al., 1992) but not all (Rubin et al., 1993) studies indicate substantial increases in total body and femoral neck aBMD with increasing sexual maturity. The increase in lumbar BMC and BMD at puberty is earlier and more pronounced in girls than boys (Gordon, Halton, Atkinson, & Webber, 1991). Growth during puberty contributed about 51% of peak bone mass in girls while in boys the contribution was only 15%. Bone mineral acquisition accelerates during adolescence (Faulkner et al., 1993). Attainment of peak bone mass appears to be site specific with the rate of increment particularly pronounced over a 3-year period (11-14yrs) in females (Theintz et al., 1992). The peak bone mass accrual period is complete by age 16yrs. However, a more definitive understanding would be possible if researchers had cited biological rather than chronological age. Nevertheless, increasing evidence suggests that peak total body bone mass and peak bone density occur in late adolescence (Hansen et al., 1991; Kroger, Kotaniemi, Kroger, & Alhava, 1993; Welten et al., 1994).

Total body aBMD peaks and plateaus in females (Lu et al., 1994) between the ages of 15-25yrs, then remains stable or decreases slightly between 25 years and menopause at approximately 50yrs of age. Femoral neck aBMD peaks between late adolescence and 40yrs of age. Femoral neck aBMD however, does not vary with age during this time (Bonjour et al., 1991).

Compared to densitometric measures of BMD, there is relatively little known about developmental changes in volumetric BMD during childhood. Nevertheless, emerging evidence implies that volumetric bone density of trabecular and cortical bone is an age-independent parameter, which does not change significantly with increasing age or muscular strength (Schoenau, 1998).

2.1.5 Developmental Changes in Bone Geometry and Biomechanics

The complicated story of osteogenesis does not rest with assessment of areal and volumetric BMD alone. The ultimate test of a bone's health is its mechanical competence. Depending on peak loads to which bones are habitually subjected, bone strength differs. Despite large differences in bone strength between regions of the body (eg ribs and femurs), material properties experience relatively little change with aging or gender during the infancy period. As the skeleton develops, differential pacing and direction of growth occurs at the periosteal and endocortical surfaces (Bass et al., 1999). It has been shown (Schoenau, Neu, Rauch, & Manz, 2001) that in puberty, males add bone mostly on the periosteal surface, where the effect on bone strength is highest, whereas females add bone on the endocortical surface, which has a small effect on bone strength. Both endocortical apposition and periosteal apposition result in increased cortical thickness, however endocortical apposition, which may be linked to a future pregnancy and lactation-related calcium reservoir, is mechanically inefficient when compared with periosteal apposition (Schoenau et al., 2001).

2.2 Bone Cells, Cell Function and Structural Organization of Bone Tissue

2.2.1 Bone Cells

Whole body and regional changes in bone mineralization and geometry during growth are reflective of the integrated activity and functions of specialized bone cells. The four principal types of bone cells: osteogenic (or osteoprogenitor) cells, osteoblasts, osteocytes and osteoclasts can be identified. After undergoing mitosis the osteoprogenitor cells become osteoblasts and these are involved in the building-up phase of the skeleton. Osteocytes are the mature bone cells and the osteoclasts are the large cells that break down or reabsorb the bone matrix (Saladin, 1998).

2.2.2 Osteogenic (Osteoprogenitor) Cells

Osteoprogenitor cells develop from mesenchyme, an embryonic connective tissue from which all other connective tissues arise. Mesenchyme is gelatinous and derived from mesoderm, the middle of the three primary germ layers that gives rise to connective tissues, blood and blood vessels and muscles. Osteoprogenitor cells occur in the endosteum - the inner surface of the periosteum and within the main canal, the bone's medullary cavity. Unlike other bone cells, osteoprogenitor cells remain capable of mitosis and therefore, are the only source of new cells of the osteoblast and osteoclast types.

2.2.3 Osteocytes

Osteocytes are embedded in concentric layers of bone matrix around a central canal. The hard bone matrix is composed of organic components (collagen fibres) and inorganic hydroxyapatite (mainly calcium and phosphorus) crystals. A cement or ground substance binds the fibres and crystals into a compact unit (Malina, & Bouchard, 1991). When the bone-forming osteoblasts deposit bone matrix and becomes trapped within tiny spaces (lacunae) they are then known as osteocytes and stop producing matrix but remain active in matrix maintenance. Osteocytes assist in maintaining proper calcium and phosphate balance between blood and bone. Osteocytes within the mineralised matrix are in direct communication with surface osteoblasts through cellular processes. This structural organisation allows for direct contact of active osteoblast or surface lining cells with osteocytes. Thus, bone cells responding to varying physiological signals can communicate their responses. Osteocytes develop when the mineralising osteoid envelops the surface osteoblasts. Osteoblasts and osteocytes are metabolically and electrically coupled through different gap junction proteins (Lian et al., 1999). As would be expected, a new osteocyte has most of the characteristics of the osteoblast from which it was formed. There is a reduced cell volume and decreased importance of protein synthesis organelles, however osteocytes have been shown to be able to synthesise new bone matrix at the surface of lacunae (Baron, 1999 p4). Osteocytes are fated to be phagocytised and digested by osteoclasts during bone resorption.

2.2.4 Osteoblasts

Osteoblasts are bone-forming cells that differentiate from osteoprogenitor cells and are found on the surfaces of bone. Osteoblasts synthesise the collagen and glycosaminoglycans (GAGs) of the bone matrix and play a role in the mineralisation of bone. The GAGs intertwine and trap water, forming a substance that varies from a fluid to semi-stiff hydrated gel. GAG content is associated with greater stiffness in ground substance. Osteoblasts are found in clusters and always line the layer of bone matrix produced before it is calcified. The calcification process takes about 10 days. The plasma membrane of osteoblasts is rich in alkaline phosphatase and contains receptors for parathormone (but not calcitonin) as well as steroid receptors for estrogen. Vitamin D3 is present in the nucleus as well as several adhesion molecules and receptors for cytokines (Baron, 1999). Osteoblasts are incapable of mitosis but osteogenic cells multiply rapidly under the stress of fractures and differentiate into large numbers of osteoblasts. Osteoblasts often line up in rows on the surface of a bone resembling cuboidal epithelium and here they build new bone matrix.

2.2.5 Osteoclasts

Osteoclasts are bone-dissolving cells that form by fusion of monocytes. Monocytes are one of the five types of white blood cells. Osteoclasts secrete acids and enzymes that break down mineral salts and organic matter of matrix and release minerals into blood plasma. Osteoclasts are very large cells that contain as many as fifty nuclei and often rest in small depressions on bone which they create by dissolving the matrix. The side of the osteoclast facing the matrix has a ruffled border which results from many deep infoldings of cell membrane. Infoldings increase the surface area available for secretion of enzymes or for absorption of bone components. Tiny crystals of matrix are found between the infoldings when bone is being actively resorbed. Osteoclasts also contain many lysosomes near the ruffled border which produce a bone-dissolving enzyme. Lysosomes are released after the attachment of the cell to the matrix in a sealed off compartment. Osteoclasts therefore, play a major role in the acidic process of bone resorption (Baron, 1999).

2.2.6 Anatomical Structure and Composition of Bone

A dominant microscopic feature of bone is the osteon. Osteons are round or oval with a central canal that contains blood vessels to supply nourishment to each mature bone cell (osteocyte). The canal is surrounded by layers of calcified tissue (lamellae) that appear similar to concentric growth rings of a tree trunk.

2.2.7 Material Composition and Function of Bone

The most abundant protein in the body is collagen. Together with chondroitin sulphate, collagen forms about a third of bone composition. The other two thirds consists of mainly calcium salts that are deposited around the collagen fibres (Saladin, 1998). Some collagen fibres are continuous with the tendons that attach muscle to bone while other fibres continue into the calcified matrix of the bone. The skeleton is composed mostly of bone (a specialised connective tissue) but also includes cartilage, ligament and tendon attachments, blood vessels, marrow, fat tissue and water. Bone tissue is the most important component of stature accounting for approximately 15% of body weight in newborn infants and 16-17% of body weight in an adult less than 50 years of age. The composition of bone enables it to perform three unique functions:(a) it can serve as a site of muscle attachment allowing for locomotion and mechanical support; (b) it provides protection for vital organs and bone marrow; and (c) it acts as a storage reserve for ions, especially calcium and phosphate for the maintenance of mineral homeostasis which is essential to life (Baron, 1999).

Bone consists of cells, fibres and ground substance. The composition varies with age, anatomical position, nutrition and health status. The mineral component comprises between 50 - 70% of adult bone, the organic matrix 20-30%, water 5-10% and lipids <3%. The mineral, hydroxyapatite, which also gives load-bearing strength to bone, provides mechanical rigidity. Crystals of bone mineral are very small and contain impurities such as carbonate, magnesium, and acid phosphate. As bone matures there is an increase in the size of the crystals, which is due to the addition of ions to the crystals and to the accumulation of crystals. Crystals also contain fewer impurities. By being soluble these crystals act as a reservoir for calcium, phosphate and magnesium ions. If too few crystals are present or the crystals are too small, mechanical strength of the bone will be compromised. Similarly, if crystals are too numerous or crystals are excessively large, bones may

become brittle, compromising mechanical strength. Thus, in summary, there is an optimal crystal size distribution, as well as an optimal amount of mineral for healthy bone (Lian et al., 1999) with size and distribution of mineral crystals in bone matrix able to influence bone mechanical properties. (Martin 1993).

Flexible and elastic properties of bone are due to collagen in the matrix. Cells that form, repair and remodel bone react to hormonal, mechanical and other signals. Lipids are found in the membranes of these cells. Lipids control the flow of ions and also are involved directly in mineralisation. Water in bone cells is important for maintenance of tissue.

2.3 Types of Bone Tissue

2.3.1 Compact (Cortical) Bone

Structurally the mid-section of long bones consists of a tubular diaphysis or shaft. A thick collar of compact bone surrounds the marrow cavity and, in adults, this cavity contains marrow that is yellowish in colour. The exterior of the ends of long bone (epiphyses) is composed of compact bone.

2.3.1.1 Lamellar and Woven Bone

Lamellar bone has a highly organised collagen structure that becomes fully mineralised. It is formed slowly and precisely on existing bone surfaces. Conversely, woven bone is formed rapidly with a loosely organised collagen structure. The irregular, loose packing of the collagen fibres makes for a less dense porous structure (Khan et al., 2001). In the human skeleton woven bone is most commonly associated with disease states (Turner, 1992).

The joint surface of each epiphysis is covered with a thin layer of hyaline cartilage that cushions the bone ends as the joint moves. Between the diaphysis and epiphysis in an adult bone is the epiphyseal line. This is the remains of the epiphyseal plate (or growth plate) that is the site of bone growth during childhood and adolescence. Growth at this site has allowed the bone to lengthen.

The outer layer of the periosteum covers and nourishes the bone. The inner layer of this membrane consists mainly of osteoblasts and osteoclasts. The periosteum is richly supplied with nerve fibres, lymphatic vessels and blood vessels. It is held to the underlying bone by clumps of collagen fibres that extend from the fibrous layer into the bone matrix. The periosteum provides an insertion point for tendons and ligaments (Marieb, 1998).

Hair-like canals (canaliculi) tie all osteocytes in an osteon together, permitting nutrients and wastes to be easily relayed from one osteocyte to the next throughout the osteon. Although bone matrix is hard and impermeable to nutrients, its canaliculi and cell-to-cell relays allow bone cells to be very well nourished. Osteocytes maintain bone matrix. If osteocytes die the surrounding matrix is resorbed (Marieb, 1998).

2.3.2 Trabecular (Spongy, Cancellous) Bone

The interior of the epiphyses of long bones (and in flat bones) contains bone that is spongy in appearance and is therefore referred to as spongiosa or spongy (trabecular or cancellous) bone. In contrast to compact bone, spongy bone consisting of trabeculae, resembles poorly organised tissue. However trabecular arrangement is not haphazard (Saladin, 1998). Trabeculae align precisely along lines of stress as much as possible. Thus, the tiny bone struts are carefully positioned. Only a few cell layers thick, the trabeculae contain irregularly arranged lamellae and osteocytes interconnected by canaliculi. No osteons are present. Nutrients reach the osteocytes of the spongy bone by diffusing through the canaliculi from marrow spaces between the trabeculae (Saladin, 1998).

2.3.3 Chemical Composition of Bone Tissue

Bone has both organic and inorganic components. Organic components include the cells, osteoblasts, osteoclasts and osteocytes, and the osteoid. Osteoid, which makes up about one third of the matrix, includes proteoglycans, glycoproteins and collagen fibres. All of these components are made and secreted by osteoblasts. Organic substances, particularly collagen, not

only contribute to a bone's structure but are also responsible for flexibility, elasticity and great tensile strength that allow bone to resist excessive stretch and twisting (Saladin, 1998).

Inorganic components of bone (65% of the mass) consist largely of calcium phosphate and mineral salts. Calcium salts are present in the form of minute crystals that lie in and around collagen fibres in extra-cellular matrix and account for the bone's exceptional hardness that allows it to resist compression. Healthy bone is half as strong as steel in resisting compression and fully as strong as steel in resisting tension. Because of bone salts, bones persist long after death (Marieb, 1998).

2.3.4 Ossification

The process by which bone calcifies or mineralises is called calcification. It begins around the sixth or seventh week of embryonic life and continues throughout adulthood. Intramembranous ossification occurs where bone is formed directly on or within fibrous membranes. This is the process, which initiates ossification of most flat bones, (Marieb 1988). As osteoblasts group together in fibrous membranes, they partly secrete collagenous fibres that form a matrix into which calcium salts deposit. This process is termed 'calcification'. When the group of osteoblasts is completely surrounded by the matrix that has calcified, a trabecula is formed. As more and more trabeculae form and connect together, an open latticework of spongy bone appears. When layers of bone are formed osteoblasts are trapped in lacunae, lose their ability to form bone and become trapped osteocytes. Sites where trabeculae form become centres of ossification and spaces between trabeculae are filled with red bone marrow. The periosteum forms from the connective tissue that first surrounded the growing section. Much of the newly formed bone will be remodeled until bone finally reaches an adult shape and size (Marieb 1988).

Bone is also formed using hyaline cartilage bone models as patterns for bone construction. As ossification proceeds, hyaline cartilage must be broken down which means the process is more complex than that of the intramembranous bone formation. This process of endochondral ossification is responsible for ossification and development of most long bones. A cartilage model of future bone is formed and midway along its shaft (in the case of long bones) cells are stimulated to become osteoblasts by a nutrient artery penetrating the original perichondrial cartilage

membrane (Baron, 1999). A collar of compact bone forms around the middle of the diaphysis. The perichondrial membrane is the periosteum. Degenerated cartilage cells are replaced by chondroblasts. Like osteoblasts, the chondroblasts become progressively embedded within their own matrix where they lie within lacunae and become chondrocytes. Unlike osteocytes, chondrocytes continue to proliferate for some time which is partly facilitated by the jelly-like consistency of cartilage. At the periphery (perichondrium) the mesenchymal cells continue to proliferate and differentiate. Bone continues to form peripherally around this centre gradually expanding in length toward the epiphyses. This is called appositional growth (Baron, 1999). A marrow cavity is produced by osteoclasts breaking down bone inside the shaft. Even though the processes of intramembranous and endochondral bone formation are different, no differences in mature bone structure are evident. Both processes involve the replacement of connective tissue with bone.

Two centres of ossification are present in bone. The first occurs in the metaphysis or primary centre of ossification toward the middle of the long bone. The second centre of ossification occurs at the epiphysis, which is made up of trabecular bone. Spaces enclosed by thin trabeculae are filled with hematopoietic bone marrow. An epiphyseal cartilage or growth plate separates ossification centres. Longitudinal growth of bones results from the layer of proliferating cells and expanding cartilage matrix within the epiphyseal growth plate. By the end of the growth period the layer becomes entirely calcified, remodeled and replaced by bone. In the diaphysis, a thick dense layer of calcified tissue forms compact bone of the cortex (cortical bone) that encloses the medullary cavity (Marieb 1988).

2.4 Differences Between and Importance of the Modeling and Remodeling Processes

2.4.1 Bone Growth and Modeling

Bone modeling is a biological process. In response to the demands of functionality, bone cells form and resorb bone, altering mass and architecture to correspond to varying mechanical and hormonal demands throughout life (Frost 1997). Growth requires the process of modeling to alter

bone shape and mass by resorbing and forming bone and varied surfaces for and extended period of time. The diaphysis (shaft) of long bones is narrower than the metaphysis (transitional zone between bone head and primary marrow space). Growth of a long bone progressively destroys the lower part of the metaphysis and transforms it into a diaphysis. Transformation is accomplished by continuous resorption of osteoclasts beneath the periosteum. Growth in diameter and modification of bone shape is called modeling (Baron, 1999). Modeling results from deposition of new membranous bone beneath periosteum that continues throughout life. Osteoblasts are activated when bone is added (formation drifts). In general, the effect of modeling is to increase bone mass and strength (Frost 1997).

2.4.2 Bone Growth and Remodeling

In contrast to bone modeling, which occurs on unrelated surfaces, osteoblasts and osteoclasts are coupled in a process of replacing old with new bone - termed 'remodeling'. Evidence (Dalsky, 1990), suggests that osteoclasts and osteoblasts act as part of a unit of cells called a basic multicellular unit (BMU). This unit responds to regulatory conditions coordinating the resorption and deposition of bone. The term basic multicellular unit (BMU) was first coined by Frost (1963) based on his landmark histomorphometric analysis of iliac bone biopsies. Activity of osteoclasts and osteoblasts determine a balance in bone mineral. If circumstances favour bone resorption, osteoclast activity increases and a negative bone mineral balance results (resorption drifts). Increased osteoblastic activity favours bone deposition. Bone resorption occurs when catalytic enzymes, which have a phagocytic action, are secreted by osteoclasts. A net positive bone mineral balance occurs due to osteoblastic activity when stimuli favour bone deposition. Most BMUs are reported to be in a resting state with approximately 20% active in trabecular bone and less than 5% active in compact bone.

Bone remodeling is regulated by physical stress and a hormone regulation system. When the concentration of calcium in blood is above normal, the thyroid gland releases the hormone calcitonin that inhibits osteoclast activity thus favouring bone deposition. If blood calcium concentration is below normal, the parathyroid gland releases parathyroid hormone (PTH) that

stimulates the osteoclasts, thereby increasing bone resorption. Thus bone is in a continuous cycle of being built up and being broken down (Saladin 1998).

Remodeling also reflects the amount of gravitational force and muscular tension exerted on bones. Bony features as well as the density of bone are directly related to the stresses to which bone has been subjected. Turnover of bone occurs in distinct packets throughout the skeleton (Marieb, 1998).

Remodeling of each packet takes a finite period of about 3-4 months in cortical bone but taking longer in trabecular bone. The remodeling sequence is always the same; activation of osteoclast precursors, osteoclastic bone resorption followed by osteoblastic bone formation to repair the defect (Mundy, 1999).

The body recycles 5-7% of its bone mass every week. Subsequently, up to half a gram of calcium may enter or leave the adult skeleton each day (Marieb, 1998). Bone remodeling occurs progressively at all periosteal and endosteal surfaces as bundles of osteoblasts and osteoclasts perform their tasks. In healthy young adults, even though total bone mass remains constant, remodeling is not uniform. Cortical and trabecular bone do not change with age in exactly the same way (Mundy, 1999). Bone remodeling cells on trabecular bone surfaces are in intimate contact with cells of the marrow cavity that produce a variety of osteotropic cytokines. It is likely that cells in cortical bone, which are more distant from the influences of cytokines, are controlled by more systemic osteotropic hormones such as parathyroid hormone. Some bone areas are very actively remodeled while others are not. The distal part of the femur is fully replaced every six months (Marieb, 1998).

Bone remodeling rate is largely dependent on the activation frequency of osteoclasts. Osteoclast activation, the first event during bone remodeling, is followed by osteoclast formation, polarisation, formation of a ruffled border, resorption, and ultimately cell degeneration (apoptosis) within plasma membranes. The bone resorption phase (lasting approximately ten days) is followed by repair of

the defect. An accumulation of osteoblasts is attracted to the site for the repair process, which takes approximately three months.

Mundy (1999), identified the sequential process of specific cellular events occurring at resorption sites. Initially, osteoclast apoptosis precedes any osteoblastic cell changes. The series of osteoblastic changes that follow includes chemotactic attraction of osteoblasts or their precursors to sites of resorption. Osteoblastic activity is possibly mediated by local factors produced during the resorption process because resorbing bone releases chemotactic factors for cells with osteoblast characteristics. The changes that occur in osteoblasts involve cell proliferation and differentiation. When mineralised bone is formed osteoblastic activity ceases. The following diagram (Diagram 2.1) summarises the activators and inhibitors of osteoclastic activity (derived from Mundy, 1999).

	Activates osteoclasts	Inhibits osteoclasts
Systemic Hormones	• Parathyroid hormone • 1.25 Dihydroxyvitamin D	• Calcitonin
Local Hormones	• Interleukin –1 • Lymphotoxin • Tumor Necrosis Factor (TNF) • Osteoclast genesis differentiation inducing factor (ODIF) • Vitamin A • Thyroid hormones • (Thyroxin, Triiodothyronine) • Estrogen LACK	• Interleukin-18 • Interferon γ • Transforming Growth Factor β (TGFβ) • Natural Phosphate • Calcium • Glucocorticoids • Bisphosphonates
Osteoclast Normal Production Requirement	• Colony Stimulating Factor –1 (CSF) • Interleukin–6(stimulates formation)	

Activators and Inhibitors of Osteoclastic Activity

Diagram 2.1

2.4.3 Remodeling of Cortical Bone

About 85% of total bone in the human body is composed of cortical bone. Cortical bone occurs mostly in long-bone diaphyses of the appendicular skeleton. Cortical bone is removed primarily by endosteal resorption and resorption within Haversian canals. Resorption within Haversian canals leads to porosity of cortical bone. However, periosteal bone continues to increase the diameter of cortical bone throughout life.

2.4.4 Remodeling of Trabecular Bone

Only about 15% of the human skeleton is composed of trabecular bone but changes in trabecular bone that occur after the age of 30 years largely determine whether spinal osteoporotic fractures will occur (Mundy, 1999). Depending on the technique used, the decline in trabecular bone mass begins early in adult life and occurs earlier than cortical bone mass decline.

2.5 Factors Influencing Bone Development and Strength During Growth

2.5.1 Intrinsic Non-Modifiable Determinants of Bone Development

Skeletal development is directed by intrinsic non-modifiable factors including genetics, gender, puberty, endocrine status and ethnicity

2.5.1.1 Genetics

Genetic factors are known to explain a major proportion of peak bone mineral mass variance (Ferrari, Rizzoli, Slosman, & Bonjour, 1998) and the basic morphology of the skeleton is determined genetically (Forwood, 2001). Nevertheless, bone mass heritability, proposing the influence of genetic background as the major determinant of peak bone mass, is still equivocal. Evidence of mothers' BMD being the strongest predictor of bone mass of young women in their third decade (Picard, Imbach, Couturier, Lepage, & Picard, 2001) has been challenged (Francois, Benmalek, Guaydier-Souquieres, Sabatier, & Marcelli, 1999; Mcguian et al., 2002). However, there is strong support for an early genetic influence on lumbar spine bone mass. Other reports suggest that a substantial amount of observed changes in BMD and subsequent attainment of peak bone mass is accounted for by other somatic growth characteristics that themselves may be largely genetically determined (Dequeker et al., 1987; Fassler & Bonjour, 1995; Jones, & Nguyen, 2000; Kroger et al., 1993; Parfitt, 1997; Pollitzer & Anderson, 1989; Riggs & Melton, 1986; Rubin et al., 1993; Seeman, 1998; Slemenda, Christian, Williams, Norton, & Johnston, 1991). More than 60% of peak bone mineral mass is gained during puberty and familial resemblance for most bone traits is already present between daughters and their mothers before puberty. Furthermore, in girls, tracking of bone traits during pubertal growth has been suggested (Ferrari et al., 1998).

Studies involving twins need to be viewed with caution however, because of the similar environmental influences (Dequeker et al., 1987; Slemenda et al., 1991). Heritability of bone mass extends to prepubertal children and is gender- and possibly site-specific as well as under separate genetic control to growth (Jones and Nguyen (2000). Familial influences play an important part in bone development and hence the achievement of peak bone mass. Generation studies have been used to assess the genetic importance in bone development (Danielson et al., 1999). Daughters of mothers with osteoporosis exhibit lower than normal lumbar spine mass compared to daughters of mothers without osteoporosis (Seeman et al., 1989). Equivocally, femoral neck volBMD was reduced in women with hip fractures but was not reduced in their daughters (Tabensky, Duan, Edmonds, & Seeman, 2001). Nevertheless, other studies have demonstrated strong familial resemblance in BMD (Barthe et al., 1998; McKay et al., 1994) that is detectable before puberty (Ferrari, Rizzoli, Slosman, & Bonjour, 1998). Familial influences, therefore, whether they are due to mostly genetic or common environments is an important consideration in the determination of bone development and peak bone mass (Lutz & Tesar, 1990).

2.5.1.2 Gender

Gender is an important consideration when assessing peak bone mass. Gender-specific pubertal hormones have differential effects on the accumulation of bone mineral with estrogen lowering the remodeling threshold for females at puberty (Schiessl, Frost, & Jee, 1998). This permits females to achieve a significant and larger portion of their adult body mass during puberty, compared to males.

No differences in BMC between prepubertal male and female children have been observed. The only increase in BMD after age 10yr in females is associated with puberty (Gordon, Halton, Atkinson, & Webber, 1991). Before puberty BMD is greater in females than in males, however because of a larger cross-sectional area of the lumbar vertebrae in males there are no differences when BMC is measured. This is expected since bone growth is linked to body mass during development and females achieve a significant portion of their adult body mass during puberty compared to males (Gordon et al., 1991). BMC measures depend on both bone mineralisation and bone size. BMD measures, although not independent of bone size are less influenced by it. Thus,

increases in BMC associated with puberty contribute a greater proportion of the total BMC than BMD and the difference between BMC and BMD patterns is probably due to the gender differences in age related pattern of increase in bone size (Gordon et al., 1991). The associated contributions to peak bone mass according to Gordon et al., (1991) are BMD (39% compared to 11%) and BMC (55% compared to 21%) for females and males respectively. The relatively greater increase in bone size than bone mineralisation at the time of puberty reflects the greater contribution bone size makes to peak BMC.

Some studies report greater values in whole body BMC after 14 yrs of age in males (Faulkner et al., 1996) compared to females whereas others report no differences whole body BMC or BMD at any age (Baxter-Jones, 2003). Greater values in whole body BMD after 16 yrs of age in males have been observed (Faulkner et al., 1996) and volumetric BMD measures of the femoral shaft were higher in males between the ages of 5 and 27 yrs (assuming a cylindrical femoral shaft) but no differences were observed in volumetric BMD measures of the femoral neck (Lu, Cowell, LLoyd-Jones, Briody, & Howman-Giles, 1996).

Before puberty there were no sex differences in BMD of the trabecular bone of the vertebral body (Gilsanz et al., 1998). Thus, it could be inferred that before puberty, trabecular number, thickness and their true (or material) BMD do not differ by gender (Seeman, 1998). Additionally, no differences in trabecular number and thickness in white males and females in young adulthood have been observed (Aaron, Makins, & Sagreiya 1987).

Bone density increases markedly in males and females during puberty (Gilsanz et al., 1988) but between the ages of 17 and 21 yrs, no differences in BMC or density at any site were observed in females (Faulkner et al., 1996). Females had greater overall BMD in the pelvis but this difference was only significant at the 15-16 year age group (Faulkner et al., 1993). Females were reported to have greater lumbar spine BMC at ages 12 and 13, but by 17 yrs of age, the male values were greater. As well, males have greater femoral neck BMC and density across all age groups (Faulkner et al., 1996).

The greatest difference between height and BMD gains in males occurs around 13-14 years and is more pronounced in the lumbar spine and femoral neck than in the mid-femoral shaft. In females, the greatest difference between height and BMD gains occurs at a younger age (11-12 years) and is of a lower magnitude than in males. In both genders, the maximal difference occurred during the period of peak height velocity, corresponding to Tanner pubertal stages P2-P3 (Fournier, Rizzoli, Slosman, Theintz, & Bonjour, 1997).

2.5.1.3 Puberty

Puberty is emerging as a crucial period for peak bone mass development. Approximately half of the bone mass of young adults is achieved before puberty (Fassler & Bonjour 1996) and approximately 90% has accrued before 18yrs of age (Magarey et al., 1999). A substantial body of knowledge suggests that pubertal stage has an influential effect on BMD in both genders (Boot, de Ridder, Pols, Krenning, & de Muinck Keizer-Schrama, 1997; Grimston, Morrison, Harder, & Hanley, 1992; Kroger et al., 1993; Lu et al., 1994) females (Bass et al., 1999) and males (Gordon, Halton, Atkinson, & Webber,. 1991). Reproductive hormone secretion rates increase dramatically during this time. The pre-menarcheal (approximately Tanner stage 4) period of puberty is also cited as a critical time for bone mineral accumulation (Haapasalo et al., 1994; Heinonen et al., 2000; Kannas et al., 1995). A steady increase in BMD was observed before puberty followed by accelerated BMD increases during puberty, which generally begins around 10 years of age in girls and 13 years of age in boys (Rubin et al., 1993).

After adjusting for bone size effects no visible change in bone mineral apparent density (BMAD) were reported in females 9-20 years of age (Katzman et al., 1991). However, the use of the proposed formula for BMAD with the prepubertal population is problematic because only 3 of 49 participants were prepubertal in the determining study. Values for aBMD values do not take bone size into account and may therefore, overestimate volumetric bone density changes (see Limitations in Measuring BMD) in the growth years (Katzman et al., 1991; Kroger et al., 1993; Kroger, Kotaniemi, Vainio, & Alhava, 1992).

2.5.1.4 Endocrine Status

Endocrine status is known to have an important influence on bone growth and development. Deficiencies of sex hormones (estrogens, progesterone and testosterone) during the formative years appear to result in decreased peak bone mass. In the past, pubertal growth was thought to be stimulated by testicular androgen in boys and by adrenal androgen in girls (Cutler, 1997). More recent understanding contends that the human pubertal growth spurt doubles prepubertal growth rate, contributes more than 15% to the total adult height and initiates epiphyseal fusion which terminates linear growth (Cutler, 1997).

Just prior to menarche, the hormone estrogen correlates positively with total body BMC and BMD (Lloyd et al., 1992) and estrogen appears to influence bone modeling and remodeling as well as bone response to mechanical loading (Frost, 1999; Lanyon, 1996; Schiessl et al., 1998; Wardlaw, 1996). Estrogen reduces sensitivity of basic multicellular units (BMUs) to parathyroid hormone (PTH) reducing osteoclast activity and bone resorption. Testosterone performs a similar function in men. Additionally, decreased bone mass has been observed in females where there has been interruption in the level of estrogen/progesterone production due to excessive exercise training (Drinkwater et al., 1984) or menopause (Schiessl et al., 1998; Talmage, Stinnett, Landwehr, Vincent, & McCartney, 1986).

Frost (1999) postulated that a number of modes of remodeling were possible on endocortical bone surfaces adjacent to marrow, each of which could be modulated by estrogen. Specifically, Frost (1999) contended that conservation-mode remodeling of endocortical bone would minimise bone loss if estrogen adequacy was detected and subsequently osteopenia would be prevented. Conversely, disuse-mode remodeling of endocortical bone would occur during periods of acute estrogen deficiency. The resulting losses of bone next to marrow would expand marrow cavities, thin cortices, and reduce trabecular bone ‘mass’, but would not reduce outside bone diameters. This mechanism may explain osteopenia following estrogen deficiency in females (natural or experimental), and the constriction in marrow cavity size (due to increased endocortical bone deposition) that occurs in females with increases in estrogen levels from mid-puberty to early

adulthood. This would be in addition to the known effect of estrogen on existing osteoclast and osteoblast function.

2.5.1.4.1 Hormonal Influence on the Remodeling Process

Bone remodeling processes are controlled by hormonal negative feedback organisation that maintains calcium-ion concentration in blood and the bone response to mechanical and gravitational forces that act on the skeleton. Thyroid and parathyroid glands are involved in hormonal remodeling control. The interaction between calcitonin, which is secreted by the thyroid gland, and parathyroid hormone (parathormone) secreted by the parathyroid gland, regulates the remodeling process.

Parathormone (PTH) is released when calcium ion concentration decreases. PTH stimulates osteocytes to dissolve bone matrix surrounding small cavities in bone (lacunae) and release calcium (and other minerals) into the blood. PTH also stimulates a rapid increase in the number of osteoclasts that dissolve bone tissue at bone surfaces. Osteoclasts break down bone matrices of all ages. PTH reduces excretion of calcium by the urinary system and increases excretion of phosphorus. A lower plasma concentration of phosphorus prevents the formation of hydroxyapatite. Vitamin D is needed for calcium absorption by the small intestine; also, by stimulating the production of an enzyme in the kidneys that activates vitamin D, PTH indirectly enhances calcium absorption. A negative feedback loop decreases PTH secretion as the blood calcium concentration rises

Although having almost no effect in adults, calcitonin lowers the calcium concentration by inhibiting osteoclast activity, which releases less calcium from the skeleton (Saladin, 1998). Within 15 minutes of the release of calcitonin, osteoclast activity is reduced by as much as 70% in children (Saladin, 1998). Calcitonin increases the number and stimulates the activity of osteoblasts, which deposit calcium into the skeleton. Blood calcium level reductions are proportionally equal to calcitonin released. Calcium is essential for a variety of physiological processes including muscle contraction and the peristaltic action required for processes involving digestion, nerve impulse transmission and blood coagulation. The hormonal control of calcium is concerned specifically with

maintaining the blood calcium level. If the level of calcium in the blood remains low for an extended period, bones become excessively demineralised developing large holes. In summary, bones are simply a storage medium from which calcium is drawn when needed.

Other hormones affect osseous tissue in ways that are not well understood. Growth hormone, thyroid hormone and insulin stimulate osteoblast activity and growth at epiphyseal plates. Furthermore, sex steroids (estrogen, progesterone and testosterone) responsible for the growth spurt in adolescence; eventually cause epiphyseal plates to close thus stopping growth (Saladin, 1998).

2.5.1.5 Ethnicity

Differences in BMD were observed among different ethnic groups (Ellis, Shypailo, Abrams, & Wong, 2000). In adult females (24 - 65 yrs) bone density at lumbar spine and distal radius bone density was higher in African-Americans (blacks) at all ages than in Caucasians (whites) (Luckey et al., 1989). Based on a review of the literature, Pollitzer and Anderson (1989) point out that black-white differences in bone mass appear to be related to ethnicity because blacks have not only greater skeletal calcium content, but also greater total body potassium and muscle mass. However, before puberty there appears to be no racial or gender differences in volumetric BMD of the trabecular bone of the vertebral body.

In young adults peak vertebral volumetric BMD is higher in black than in white men and women, but not different between men and women of the same race. Blacks have thicker trabeculae (not greater numbers) than whites, and thicker cortices (Han, Palnitkar, Rao, Nelson, & Parfitt, 1996). Other studies do not support this contention probably due to morphological differences in African blacks and American blacks as well as sample size considerations (Seeman, 1998). In children, ethnicity has a significant and differential effect on the bones in the axial and appendicular skeletons. In the axial skeleton, black children had greater cancellous bone density, but similar cross-sectional area of the vertebral bodies. Conversely, in the appendicular skeleton, black children had greater femoral cross-sectional area, but similar cortical bone area and cortical bone density. Vertebral bone density and femoral cross-sectional area at sexual maturity are higher in

black children. Such significant variations may contribute to the racial differences in the prevalence of osteoporosis between black and white adults (Gilsanz et al., 1998). Racial differences appear to emerge at puberty, but within a race, the increases in males and females during puberty were no different (Gilsanz et al., 1988).

Controversially, in long bones (such as the femoral midshaft) with bones of the same external dimensions, BMD may be higher in blacks than in whites in two ways. Firstly, either the cortex will be thicker (and the medullary cavity smaller) or secondly, the cortical BMD might be higher. Differences in dietary calcium intake among ethnic groups have been identified as a confounding variable in these comparative studies. Measures of aBMD at mid radius, lumbar spine, trochanter and femoral neck of black and white children between the ages of 7 and 12 years revealed a significantly greater density in black as opposed to white children at each site (Bell, Stevens, Garza, Gordon, & Edwards, 1991). aBMD varied directly with age and body mass. Controlling for Tanner stage and body mass Southard et al., (1991) found race (ethnicity) to be not significant. Adult studies although limited by racial differences in body weight, socio-economic, health, and nutritional status indicate higher bone density in African-Americans at all ages than in Caucasians (Luckey et al., 1989).

Caucasian and Asian children differ in body size, diet and amount of physical activity undertaken. Both BMC and aBMD have been reported to be lower at lumbar spine, femoral neck and whole body sites in Asian compared to Caucasian children (Bhudhikanok et al., 1996; MacKelvie et al., 2002; MacKelvie et al., 2001; MacKay et al., 2000; Nowack Brizzolara & Lally, 1995). The difference has been attributed in part to the smaller bone size of Asian children (Bhudhikanok et al., 1996) as well as a disparity in lifestyle factors. The lifestyle factors include consumption of calcium, which was significantly less than Caucasian children (MacKay et al., 2000) together with significantly less involvement in loaded physical activities.

2.5.2 Modifiable Determinants of Bone Development

Determinants of bone development can be modified and include: body mass, nutrition (especially dietary calcium intake), as well as other lifestyle factors - the most important of which is exercise.

2.5.2.1 Body Mass

Body mass is closely associated with bone development and peak bone density and is a significant predictor of both total body and lumbar spine BMC in adolescent girls (Boot et al., 1997; Cooper et al., 1995; Rice et al., 1993; Rubin et al., 1993; Valimaki et al., 1994). However, no relationship between height and BMD was found (Boot et al., 1997) when results were adjusted for bone size. On the other hand, with prepubertal girls, lean tissue mass (rather than body mass) has been identified more specifically as the significant predictor of bone mass (Ilich, Skugor, Hangartner, Baoshe, & Matkovic, 1998; Courteix, Lespessailles, Jaffre, Obert, & Benhamou, 1999; Rice et al., 1993; Madsen, Adams, Van Loan, 1998). The use of body weight (mass) as a covariate in studies of BMD may lead to erroneous results in this population (Courteix et al., 1998) since mass includes different tissue types (fat, muscle, bone, fluid).

2.5.2.2 Dietary Calcium Intake (Nutrition)

Traditionally, discussion of the development of strong and healthy bones was dominated by reference to adequate nutrition. Given that serious malnutrition can adversely affect bone strength, bone strain engendering mechanical usage, the primary modulator of skeletal adaptation, needs calcium, vitamins and proteins (Frost, 1986).

Nutrition is considered a lifestyle factor with the potential of influencing bone mineral status and in particular, optimum calcium intake during childhood is seen as a necessary condition for bone health (Bailey, 1996; Heaney, 1991; Kelly et al., 1990). Most children have trouble reliably recalling the quantities of food eaten (Dwyer, Krall, & Coleman, 1987). Problems can be encountered when measuring children's dietary intake (Wynder, 1990). Therefore, obtaining parental assistance may be beneficial for a complete picture of the child's eating habits (Steen, 1996). Recording dairy foods eaten during a normal week may provide an indication of a child's calcium intake, however, this method is yet to be validated and the influence of dietary calcium intake on bone development is conflicting. Positive correlations have been found between dietary calcium intake and BMD in pubertal (Turner et al., 1992), circumpubertal (Gunnes & Lehman, 1996; Boot et al., 1997; Grimston et al., 1992; Ilich et al., 1998; Rubin et al., 1993; Ruiz, Mandel, &

Garabedian 1995) and prepubertal (Ruiz, Mandel, & Garabedian 1995) studies of children. Controversially, other studies (Katzman et al., 1991; Kroger et al., 1993; Turner et al., 1992) have found no association between bone mass and dietary calcium intake.

It is important to note that in most (Johnston et al., 1992; Boot et al., 1997; Grimston et al., 1992; Rubin et al., 1993; Valimaki 1994; Uusi-Rasi et al., 1997), but not all studies (Ilich et al., 1998; Lee et al., 1994; Turner et al., 1992; Welten et al., 1994), the mean calcium intake was around or above the recommended daily intake. In attempts to ensure sufficient calcium intake, a number of calcium supplementation studies have been undertaken (Bonjour et al., 1997; Johnson et al., 1992; Lee et al., 1994; Lloyd et al., 1993). No nutritional supplements can make sedentary people develop the strong bones of weightlifters nor can they normalise whole bone strength in paralysed limbs (Frost, 1986). In prepubertal children whose average dietary intake of calcium approximated the recommended dietary allowance, 3 years of calcium (citrate malate) supplementation on identical twins augmented the rate of increase in bone mineral density (Johnston et al., 1992). After 18 months of supplementation (calcium carbonate) of participants accustomed to a low calcium diet (Chinese children), significantly greater gains in BMC and BMC/bone width than control were observed (Lee et al., 1994). In a randomized 12-month trial, Specker and Binkley (2003) report a significant increase in cortical thickness and area in calcium supplemented participants involved in gross motor (as apposed to fine motor) activity in prepubertal children. Furthermore, calcium enriched foods significantly increased bone mass accrual in prepubertal girls (Bonjour et al., 1997).

Whereas some researchers (Gunnes & Lehmann, 1996; Rubin et al., 1993) have found that the skeleton appears to be more responsive to calcium supplementation in the pre and early stages of puberty compared to the adolescent stage, others (Lloyd et al., 1993) and Wosje & Specker, (2000) indicate that older (pubertal) children appear to have greater annual increases in total body and spinal BMD. Including exercise in addition together with fifteen months of calcium (carbonate) supplementation enhanced bone mineral status in adolescent girls (Stear et al., 2003). Combining short bouts of moderate exercise with calcium supplementation (calcium fortified foods) in pre- and

early-pubertal girls resulted in greater gains in bone mass at loaded sites (Iuliano-Burns, Saxon, Naughton, Gibbons, & Bass, 2003).

Investigations into the retention of bone mineral gain once supplementation is removed reveal that the increased acquisition rate was transitory with no remaining benefit in either total amount or rate of acquisition (Lee et al., 1997). However, with milk-extracted calcium phosphate taken during the prepubertal period, increases in bone mass accrual were sustained beyond (3.5 years) the end of supplementation (Bonjour et al., 2001).

Calcium may play a facilitating role by enabling other factors such as genetic potential and exercise to influence bone adaptations. (Barr, 1998; Pollitzer & Anderson,1990; Kelly, Eisman, & Sambrook, 1990; Ruiz, Mandel, & Garabedian,1995; Krall & Dawson-Hughes, 1993; Parfitt, 1997; Rubin, Hawker, Peltekova, Fielding, Ridout, & Cole, 1999). It is also hypothesised that calcium intake may have its largest influence on those with the greatest bone adaptation potential (Kelly et al., 1990). Therefore, for individuals with the least genetic potential and least influence from exercise, calcium may not be beneficial. Alternatively, those with greater genetic potential and higher levels of exercise may be limited by an inadequate calcium intake (Kelly et al., 1990). It is recommended that children consume at least 800 mg of calcium per day (Australian Recommended Dietary Intakes 2001). Yet, to enhance bone development and decrease fracture risk, athletes such as gymnasts should consume a level of calcium that is greater than the recommended daily allowance (Benardot, 1996; Chestnut, 1991). Heaney (1991) states calcium intake should be as high as 1500 mg in adolescents to ensure achievement of peak bone mass. Controversially, elite gymnasts have been found to consume as little as half the recommended daily allowance for calcium (Crawford, Obarzanek, Morrison, & Sabry, 1994). Adaptation of bone with low calcium intake therefore, does occur, although not sufficiently to compensate for the low intake (Heaney, 1991).

2.6 Summary of Non-Modifiable and Modifiable Non-Mechanical Determinants of Bone Development

Previous studies have demonstrated that skeletal mass accumulation is under strong genetic control with the other determinants identified in varying strengths as contributors to peak bone

mass (Rubin et al., 1999). Variations in BMD attributable to genetic factors vary from 46% (Krall & Dawson-Hughes, 1993) to 80% (Pocock et al., 1987). The wide variability in reported genetic influences may be attributed to methodological concerns associated with population heritability studies. Although genetic factors are important determinants of adult bone mass, the non-heritable variables, including body mass, dietary calcium intake, endocrine status, and environmental factors may also have significant, yet variable influences on bone mineral accrual during childhood and achievement of peak bone mass (Barr, 98; Kahn et al., 1994; Ulrich, Georgiou, Snow-Harter, & Gillis, 1996; Bachrach, 1993). The relative influence of genetic and environmental determinants on bone mass is still unclear (Barthe 1998). Gender differences are evident at different times during pubertal development and appear to be related to hormonal influences and an age-related pattern of changes in bone size. Puberty is thus established as an important period for peak bone mass development. The effect of puberty appears to be race-specific but gender-independent (Gilsanz, Roe, Mora, Costin, & Goodman, 1991) with some studies reporting pubertal stage being the strongest single predictor of BMD (Rubin et al., 1993; Ruiz, Mandel, & Garabedian, 1995) suggesting that about 45-40% of adult peak total body bone mass may be accrued during this period (Sentipal, Wardlaw, Mahan, & Matkovic, 1991). Bailey et al. (1999), report 26% accrual during the 2 years surrounding peak height velocity. Similarly, estrogen status - functionally linked to puberty in females - is influential in the process of bone development.

2.7 Mechanical Determinants of Bone Development

2.7.1 Mechanical Factors

Mechanical factors are clinically relevant because of their ability to influence growth, modeling and remodeling activities that can maximize, or maintain, the determinants of fracture resistance (Forwood, 2001). Mechanical loads, greater than those habitually encountered by the skeleton, effect adaptations in cortical and cancellous bone, reduce the rate of bone turnover, and activate new bone formation on cortical and trabecular surfaces. In doing so, they increase bone strength by beneficial adaptations in the geometric dimensions and material properties of the tissue. Mechanical load places a proportionally predictable strain on bone (Forwood, 2001). Under normal

conditions if strain on bone exceeds the threshold range for modeling, bone strength is slowly increased. As bone keeps increasing in strength, characteristic peak strains will not surpass that threshold. An absence of strain stimuli results in no modeling response. Subsequently, imposition of mechanical loads above threshold strain influences postnatal bone strength and architecture. Large strains dominate modeling and remodeling processes whereas small strains have minimal effect (Lanyon 1996). Bones can therefore become stronger than required for their highest voluntary loads.

2.7.2 Muscle and the Muscle-Bone Unit

Addressing the functional approach to bone strength involves the synergy of interaction of the muscle and bone (muscle-bone mutualism). Less than 25 years ago, development of bone strength in children and adolescents was thought to be dominated by non-mechanical mediators. More recent evidence suggests that development of bone strength is highly dependent on muscle forces, which induce the largest skeletal mechanical loads (Frost, & Schoenau, 2000). Muscle cross-sectional area (MCSA) is accepted as a physiological indicator of muscle strength and muscle force and bone strength (under certain loading conditions) are related to their respective cross-sectional areas (Heinonen et al., 2001b). Bone mass has been found to be closely and linearly associated with muscle mass throughout life (Ferretti, et al., 1998).

The muscle-bone unit has not been extensively described during growth. Recent evidence suggests however, that the muscle-bone relationship is similar between the sexes prior to puberty (Schiessl et al. 1998). At puberty however, these ratios shift and favour increased bone mass and area, respectively in females, per unit of LTM or muscle (Schoenau et al., 2000). Whether similar relationships exist for other functionally related muscle-bone units, and whether they are influenced by growth and exercise training remains unclear. A major part of the axial force in long bones is a response to muscle activity, the strength of which depends on the lever arms available to the external loads (Lu, Taylor, O'Connor, & Walker, 1997). Most of the bending moment along a limb is transmitted by a combination of tensile forces in muscles and compressive forces in bones, resulting in the moments transmitted by the bones being smaller than the limb moments (Lu et al., 1997). Whole-bone strength adapts mainly to peak momentary muscle forces rather than low-force

muscular contractions repeated to exhaustion. The nature of mechanical loads and resultant forces experienced in sports such as soccer or weightlifting involving intense acceleration of the body, increase the momentary muscle strength that puts much larger loads on bones than low-force exercises (Heinonen et al., 1995). Bone mass and bone strength normally increase during growth. Bone development and strength in young adulthood plateaus and then declines. Similarly, muscle strength increases during growth, plateaus in young adults and then declines (Schiessl 1998). Bone strength and bone mass normally adapt to the largest voluntary loads on bones (Frost, 1997). The loads come from muscles, not body weight. During growth, loads on bones from body weight and muscle forces increase, and modeling accordingly, increases bone strength and bone mass. Strains caused by forces muscles place on bones control modeling and remodeling processes.

The varied ways individuals use specific parts of their skeletons cause variance in the strength of different bones, which helps to explain why the strength of some bones does not accurately predict the strength of other bones (Frost 2001). Variations in bone strength within the bones of an individual may reflect increased sites of mechanical loading.

Functional mechanical loading inherent in activities of daily living, sports or exercise are now considered the primary modifiable modulator of skeletal adaptations in bone mass and architecture. The mechanism by which mechanical loading influences skeletal adaptation and interacts with other putative regulators of skeletal development has been conceptualised in the formulation of the Mechanostat Theory.

Frost (1964 and 1987) theorised that remodeling was controlled by an ability of mechanical loading to alter curvature of the surface. With constant stress (or strain magnitude) over time bone will remain in equilibrium and a strain threshold is suggested above which increases in bone growth occur and below which bone loss results. Therefore, there is a minimum stress required to maintain skeletal integrity. Frost (1987) indicated that a response would only be activated if the load was above the minimal effective stress (MES) and later, revised this theory in terms of strain rather than stress. According to Frost (1987), bones are programmed with strain set points

(minimal effective strains) where bone modeling adapts the bone to major overloading while BMU-based remodeling adapts the bone to significant underloading. MES is also the mechanism by which bone makes adaptations to structure. For example, trabeculae of trabecular bone are oriented in the direction of applied pressure and traction (Fleisch, 1997).

2.8 Determinants of Bone Strength

Bone strength is determined by four features (a) stiffness of the material which denotes the true density and resilience (b) type (ie woven, lamella, cortical or trabecular) (c) size and shape which subsequently affects intracellular tissue and space (d) fatigue damage or microdamage (Frost 2001). Bone strength is not simply a matter of bone mass or mineral distribution density alone. It is a function of both bone mass and geometric distribution of mass (Khan et al., 2001; Seeman, 2002). More accurately, rather than mass it is a function of bone mineral properties and geometry (Forwood, 2004 – personal communication). An increase in bone strength (or increased resistance to skeletal load) could just as well be achieved by a change in bone shape as by an increase in bone mass.

When subjected to loading, the ability of bones to resist fracture depends on their mass, material properties, geometry and tissue quality. For example, for the same bone area and density, small increases in the diaphyseal radius have a disproportionate influence on torsional strength of bone. Mechanical loads, greater than those habitually encountered by the skeleton, effect adaptations in cortical and cancellous bone, reduce the rate of bone turnover, and activate new bone formation on cortical and trabecular surfaces (Forwood 2001). By so doing, mechanical loads increase bone strength by beneficial adaptations in the geometric dimensions and material properties of the tissue. There is no direct evidence to demonstrate anti-fracture efficacy for mechanical loading, but the geometric alterations engendered undoubtedly increase the structural properties of bone as an organ, increasing the resistance to fracture (Forwood 2001).

Non-mechanical growth mediators (eg growth hormone, androgens, calcium, vitamin D) may influence bone strength either directly by their action on bone cells, or indirectly by their concurrent influence on muscle size and strength development. However, hormones and other non-

mechanical agents that affect bone strength can either help or hinder the 'bone strength-muscle strength' relationship, but cannot replace it (Frost & Schoenau 2000).

2.9 Mechanostat Theory

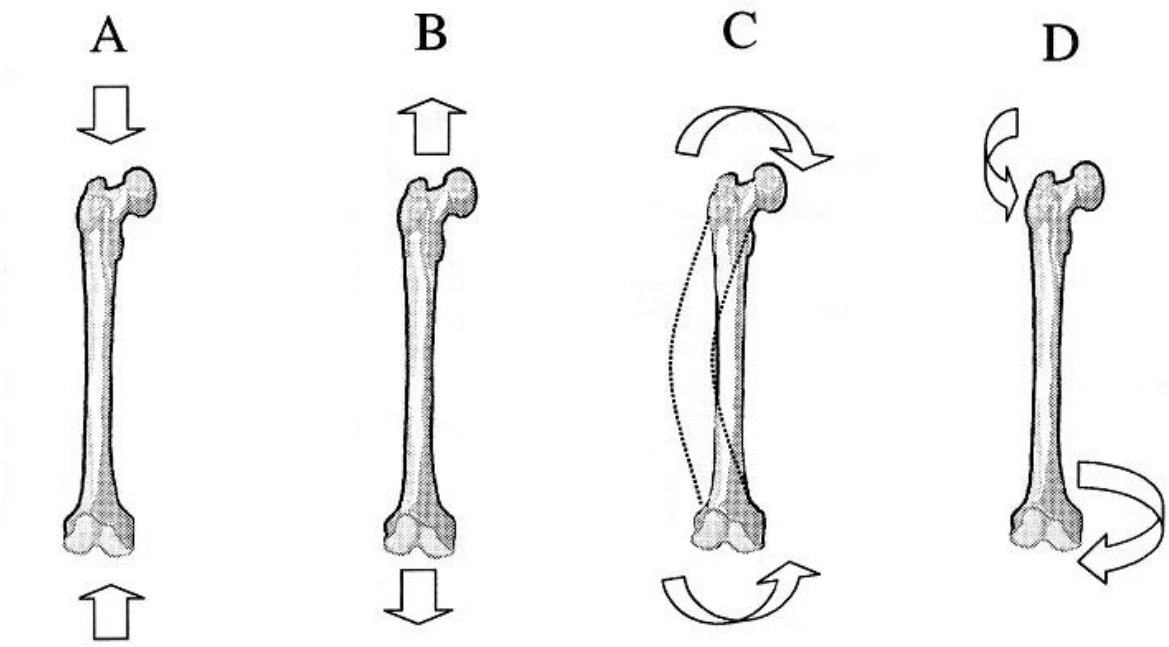
A number of existing theories explain how bone may progressively adapt to mechanical loads (Frost 1964 and 1987; Carter, Fyhrie, & Whalen, 1987; Grimston 1993). The process appears to be controlled by a feedback mechanism. Increased strains applied to bone result in positive bone adaptations enabling force to be spread over a larger area. Less force per unit area across the bone surface results, reducing the risk of fracture with subsequent similar loads (Conroy & Earle, 1994; Frost, 1983). This relationship is referred to as the "Mechanostat Theory" due to its similarities to a thermostat. It suggests that there is a range of strain values that will elicit no adaptive response (no change), but above which there will be a positive adaptive response (increased bone) and below which there will be a negative response (decreased bone). Thus, if a mechanical load is above the MES, bone will be formed to adapt to strains placed upon it; however, if mechanical load is below the MES, bone will decrease. The range is about 0.0008 to 0.002 unit bone surface strain (Frost, 1983). The feedback mechanism makes modeling and remodeling thresholds increase bone strength when stimulated and remove bone where it is not needed mechanically (Frost 2001).

2.9.1 Minimum Effective Strain

The hypothesis of a requirement for a MES as a necessary determinant of bone architecture has been present since 1964 (Frost, 1983). Strains below the MES apparently do not evoke adaptive architectural bone modeling but those above the MES do. Bones are deformed under loading conditions. In mechanics, deformation is described in terms of 'strain', and bending, torsion or compressive loading can induce strain. With reference to bone, strain is deformation or change in dimension and/or shape of bone by externally applied loads. Strain is a measure of the extent to which a bone is deformed when subjected to a stress. Linear strain is the ratio of the change in length to the original length. A strain of 1 (or 100%) represents a doubling of a particular dimension. In vivo studies have shown that bone strains in or above the 1500-3000 microstrain

(με) range cause bone modeling to increase cortical bone mass, while strains below the 100-300 microstrain range release BMU-based remodeling which then removes existing cortical-endosteal and trabecular bone (Frost 1987). Functional strains in bone tissue are normally less than 0.003 (ie 0.03% or 3000 microstrain). Volume strain is the ratio of the change in volume to the original volume. Shear strain is the angular distortion in radians of a body subjected to a shearing force (Isaacs, Daintith, & Martin, 1999). Bone strength is determined and defined by the amount of strain the bone can endure (Snow-Harter & Marcus, 1991). Strains vary across the cross-section of long bones as a result of loading due to normal functional bending and positive or negative adjustments to curvature along with changes in mass and cross-sectional shape (Lanyon, 1996). In humans, muscle forces during locomotion create predominantly bending moments causing strain along the longitudinal axes of weight-bearing long bones. The adaptive bone response depends on the nature of the activity, with larger bone adaptations predicted on the outer (periosteal) rather than on the inner (endosteal) surface due to the tensile and compressive strain reactions of bone to the applied bending moments. Mechanically, the strength of bone is dependent on its material and structural (geometric and biomechanical) properties, their relative importance varying under different loading conditions (Fowood 2001, Martin 1991).

Stress is internal resistance generated in bone to counteract force applied it. Strain can cause different types of stress to a bone (see Diagram 2.2). Stresses include (i) compression, (shortening or pushing - see A) where two forces are directed towards one another (ii) shear, where two forces occur parallel to each other but not along the same line (iii) tension, (stretching or pulling - see B) where two forces are in opposition to one another, and (iv) bending (as a result of pushing – see C) and (v) torsion, (twisting – See D).



Bone Stresses

Diagram 2.2

[from Kontulainen, S. (2002). Training, detraining and bone: Effect of exercise on bone mass and structure with special reference to maintenance of the exercise induced bone gain. PhD Thesis, University of Jyvaskyla, Finland, p19.]

Bones also encounter forces through gravity and muscular contraction, which alter their original dimensions. This in turn creates stress, which results in an internal resistance to counteract the applied force. Resistive stress is equal in magnitude but opposite in direction to the applied load (Einhorn, 1992; Snow-Harter & Marcus, 1991).

2.10 Epigenetic vs Homeostatic Bone Formation Theory

In addition to the Mechanostat Theory, the homeostatic theory of bone formation, in which a system endeavours to reach and maintain a steady state between two extremes, also has its proponents (Carter et al., 1987; Frost 1964, 1983, and 1987). This theory includes elements of negative feedback that provide a structure of minimal mass that is adequate for functional needs.

If the system is disturbed, then a feedback signal sets events in motion that strive to return to a pre-existing steady state level. Conversely, epigenetic regulation is driven by positive feedback loops where steady states occur only at the extreme levels of the system. Once a final state (called an attractor state) at either extreme has been reached, the system will stabilise until disturbed. Woven bone formation observed in loading experiments carried out on animal models (rats, rabbits, roosters, turkeys, dogs sheep and pigs) has been attributed to epigenetic regulation (Turner, 1992). This theory proposed that formation of lamellar bone is homeostatically regulated, whereas formation of woven bone is epigenetically regulated. Initial formation of woven bone is later replaced by lamellar bone.

2.11 Strain Mediated Fluid Flow Influence on the Remodeling Process

Bone adapts more robustly to dynamic compared to static loads (Lanyon & Rubin, 1984; Rubin & Lanyon, 1984). Static loading, although having no effect on endocortical bone formation rate, actually suppresses periosteal bone formation. Dynamic loading increases osteogenesis on both periosteal and endosteal surfaces (Burr, Robling, & Turner, 2002). Dynamic cyclical loads are required to initiate adaptive responses in bone. The strain stimulus that elicits the adaptive response is proportional to the strain magnitude and frequency of the loading stimulus (Turner, Owan, & Takano, 1995).

Strain-mediated fluid flow through canalicular channels allows bone to adapt to its mechanical environment. Cyclic loading may be the predominant stimulus that moves fluid through bone. Thus there is a direct link between loading frequency and the bone adaptive response within the mechanical environment. It is known that a deforming bone produces an electrical current (piezoelectric effect). Since compressed and stretched regions are oppositely charged it has been suggested that electrical signals direct the remodeling process. Nevertheless the effect of strain-induced fluid flow is to cause fluid sheer stress on cell membranes, rather than a piezoelectric effect in calcium crystals (Forwood 2004 – personal communication).

2.11.1 Strain 'Error' Distribution Osteogenesis

Strains produced in bone tissue through functional load bearing induce adaptive changes in bone mass and architecture. Normal functional strains, regarded as homeostatic, cause natural bone modeling and remodeling throughout the skeleton. However, suggestions that functional strains, which are large and not uniformly distributed (strain errors) induce increased osteogenesis has led to some positive results in investigations with children. Exercise sessions have been conducted that included unusual strain distributions, involving high peak strains and strain rates (Heinonen et al., 2001b; Mackelvie et al., 2001; McKay et al., 2000; Petit et al., 2002). Unusual strain distributions, high strains, and high strain rates appear to be particularly osteogenic (Lanyon, 1996).

2.12 Mechanical Influence on the Remodeling Process

The pull of muscles on bone as well as gravitational pull stimulates bone remodeling. The concept that bone mass and structure is dictated by mechanical stress was suggested over a century ago by Julius Wolff. He postulated that any change in function of a bone is followed by changes in internal structure, and external structure agrees with mathematical laws. Wolff's mechanical theory hypothesised that long bones were thickest (in the middle) where the bending stresses were greatest and curved bones were thickest where they were most likely to buckle. Struts are formed in trabeculae of spongy bone along compression lines and large bony projections occur where heavy, active muscles attach. Attachment sites of the most used muscles of weight lifters are enormously thickened and bones in the feet of ballet dancers gradually grow bulkier in response to the intense pressure of dancing (Marieb, 1998)

2.12.1 Mechanisms Regulating Strain Induced Osteogenic Adaptations to Mechanical Loading

The mechanisms by which bone responds to mechanical stimuli are still uncertain however, heavy usage leads to heavy bones or bony areas and non-use leads to bone wasting. Under increasing forces on bone, modeling increases bone strength and mass and remodeling maintains extra bone (Frost 1998). When blood calcium levels need to be increased, the parathormone released

appears to target osteoclasts in least stressed areas. In this way mechanical stress appears to determine where bone remodeling occurs.

2.13 Conditions of Exercise and the Osteogenic Adaptive Response in Children

Mechanical loading in children is inherent in physical activity, sports, exercise, and leisure time activities. The following section summarizes the literature pertaining to the influence of physical activity on skeletal development in children.

2.13.1 Comparative Studies

A number of comparative studies report higher bone mass accumulation when active children are compared with non-active children (Khan et al., 1996 Dyson et al., 1997). Evidence that physical activity is associated with increased aBMD has been observed in prepubertal (Courteix et al., 1998), as well as male and female adolescents (Pettersson et al., 1999; Nordstrom et al., 1996). The impact-loading and weight-bearing features of activities such as gymnastics are associated with more positive bone adaptations when compared with non-impact-loading and non-weight-bearing effects of activities such as swimming (Courteix et al., 1998; Grimston et al., 1993; Duncan et al., 2002). This relationship, although not unequivocal (Valdimarsson, Kristinsson, Stefansson, Valdimarsson, & Sigurdsson, 1999), is consistent across all weight-bearing sites. Cross-sectional comparative studies suggest that high volume impact loading occurring in gymnastic training is associated with higher aBMD in prepubertal girls (Courteix et al., 1999; Dyson et al., 1997). A significant but moderate correlation between total weight-bearing hours and femoral neck aBMD has also been reported (Slemenda et al., 1991). Gymnastics in particular, is a weight-bearing activity that has resulted in significantly greater aBMD compared to controls for total body (Cassell et al., 1993; Dyson et al., 1997), femur (Dyson et al., 1997;), tibia (Padro, Eisenman, Sands, Beveridge, & Chan, 1995) and lumbar spine BMD (Dyson et al., 1997; Padro et al., 1995). Numerous comparative studies demonstrate the differences between athletic groups and non-athletic controls and these studies have indicated differences of up to 30% in aBMD of bones loaded through exercise (Duncan et al., 2002; Fiore et al., 1996; Heinonen et al., 1995; Heinonen et al., 1993). Additionally, both physical activity and muscle strength have been found to be

independent significant predictors of bone mineral density in most (Heinonen, McKay, Whittall, Forster, & Khan, 2001; Nordstrom, Pettersson, & Lorentzon, 1998; Nordstrom, Thorsen, Bergstrom & Lorentzon, 1996; Pettersson, Nordstrom, & Lorentzon, 1999) but not all (Pettersson, Nordstrom, & Lorentzon, 1999) studies of children and adolescents. Weight-bearing exercise appears to have a greater osteogenic effect than non weight-bearing exercise in children (Cassell et al., 1993; Duncan et al., 2002; McCulloch et al., 1992; Risser et al., 1990). The type of weight-bearing activity is also an important determinant of bone density (Nordstrom, Pettersson, & Lorentzon, 1998). The relative weightlessness of swimming may contribute to decreased aBMD similar to the zero gravity experienced by astronauts (Risser et al., 1990). Findings indicating that swimmers have lower aBMD than sedentary controls, however, could be a result of self-selection or as a consequence of low aBMD contributing to buoyancy and thus, successful swimming. Evidence also suggests that weight-bearing activities associated with training and competing in athletic events and games leads to significantly greater bone mineral density when compared to age-matched, control peers (Duncan, 2002; Dyson et al., 1997). Considerable data supports high intensity loading (Robinson, et al., 1995) and forceful muscle contractions (Nordstrom et al., 1996) as optimal activities for bone tissue accrual. It would seem that loading through muscular contractions (e.g. in dancing vs swimming) may actively stimulate bone deposition if the exercise is weight-bearing in nature with muscle tendon attachments providing a deforming load in cortical bone (Young et al., 1994). Recent reports indicate that the earlier the age of commencing physical activity the greater the increase in bone density at weight-bearing sites of the hip and lumbar spine (Khan et al., 1996). Investigations of retired athletes show higher bone mass with a history of childhood weight-bearing physical activity (Cooper, et al., 1995) when compared with normative data or well-matched controls.

2.13.2 Associative/ Predictive Studies

Most, but not all (Southard et al., 1991) associative/predictive studies report that physical activity is positively associated with aBMD in children (Rubin et al., 1993; Ruiz et al., 1995; Lehtonen-Veromaa, Mottonen, Nuotio, Heinonen, & Viikari, 2000) and adolescents (Rubin et al., 1993; Ruiz et al., 1995; Turner et al., 1992; Thorsen, Nordstrom, Lorentzon, & Dahlen, 1999). Weight-bearing physical activity has been associated with higher baseline aBMD (Slemenda et al., 1991) and more

rapid bone mineralisation in prepubertal and preadolescent children aged between 6 and 14 years. Both physical activity and muscle strength have been found to be independent significant predictors of aBMD in a good number of associative studies (Haapasalo, et al 1998; Madsen, Adams, Van Loan, 1998; Rice et al., 1993; Schoenau, Neu, Mokov, Wassmer, & Manz, 2000; Thorsen, Nordstrom, Lorentzon, & Dahlen, 1999; Witzke, & Snow, 1999). Weight-bearing physical activity has also been associated with higher baseline aBMD (Slemenda et al., 1991). Commencing physical activity at an early age affords the greatest chance to increase bone density at weight-bearing sites of the hip and lumbar spine with osteogenic hormonal activity (especially for girls) in pubertal years likely to be the opportune time for exercise induced increases in aBMD (Bass et al., 1995). After correcting for weight and pubertal status, a significant positive effect of physical activity was associated with increased lumbar spine aBMD (Rubin et al., 1993) in children (6-18 years). Weight-bearing exercise as opposed to non weight-bearing exercise has been positively associated with an osteogenic effect in children (Cassell et al., 1996).

Some research suggests a positive correlation between muscle forces acting on bone and aBMD in circumpubertal children and adolescents (Bass et al., 1995; Gunnes & Lehmann, 1996), but relatively fewer studies have directly investigated prepubertal populations. Further, one of the most significant predictors of bone mass in preadolescent females that is constantly reported relates to body mass – more distinctively lean tissue (muscle) mass (Ilich, Skugor, Hangartner, Baoshe, & Matkovic, 1998; Courteix, Lespessailles, Jaffre, Obert, & Benhamou, 1999; Rice et al., 1993; Madsen, Adams, Van Loan, 1998), thus a muscle-bone relationship appears evident.

Mechanical loading emerges as an important component of musculoskeletal adaptations to exercise (Turner 1998, Turner & Pavalko 1998) with bone cells accommodating to a mechanical loading environment, although less responsive to routine or customary loading (Turner & Pavalko 1998). High volume impact loading occurring in gymnastic training is associated with higher aBMD in prepubertal girls (Bass et al., 1995; Cassell et al., 1996). Gymnastics is a weight-bearing activity that has resulted in significantly greater aBMD compared to controls for total body (Bass et al., 1998; Cassell et al., 1996; Davison, Blimkie, Dyson, Webber, & Adachi, unpublished;), femur (Bass et al., 1998) and lumbar spine (Bass et al., 1998). Increases in prepubertal female gymnasts in

particular, compared to bone-age matched controls have been observed (Bass et al., 1998) with similar observations in other studies involving dancers (Khan et al., 1998). Impact exercise, such as jumping, in children is associated with increased bone mass, bone size, bone geometry and bone density (Bass et al., 1995; Cassell et al., 1996).

Investigations of retired athletes associate higher bone mass with a history of childhood weight-bearing physical activity when compared with normative data or well-matched controls (Bass et al., 1998; Kannas et al., 1995; Khan et al., 1998; Teegarden et al., 1996). In prepubertal, as well as retired gymnasts (matched for age height and weight) aBMD at weight-bearing sites has been observed to be consistently higher than the predicted mean for controls at all sites except the skull (Bass et al., 1998). Similar conclusions have also been found in other studies involving dancers and evidence linking exercise training during the early years is increasing. An increase in aBMD of the femoral neck and total hip of retired dancers was independently and positively associated with hours of ballet classes undertaken between 9 and 12 years of age (Khan et al., 1998). However, no difference was found in lumbar spine or upper limb aBMD between the former dancers and controls suggesting a site-specific effect of loading. Nevertheless, it would appear that the earlier the age of commencement of physical activity the greater the increase in bone density at weight-bearing sites of the hip and lumbar spine (Bass et al., 1995).

Physical characteristics such as age, height and weight (De Schepper et al., 1991; Rice et al., 1993) with weight being refined to lean tissue mass (Illich et al., 1998; Nordstrom et al., 1995; Witzke & Snow, 1999; Wiebe et al., 2002) appear to be the most important predictors of BMD. Additionally, studies of circumpubertal children have also associated moderately strong genetic influences (Tao et al., 1998) with increases in aBMD at specific bone sites. Additionally, energy expenditure by adolescent females (estimated by questionnaire) revealed that physical activity contributed significantly to aBMD at all regions of the femur (Turner et al., 1992).

2.13.3 Longitudinal/Observational Studies

The advantages/strengths of longitudinally designed studies vs cross-sectional and/or retrospective studies lie in the potential to assess accurate growth dynamics over time. This advantage offers

the ability to infer cause and effect of an intervention. A cross-sectional design gives growth status at a given age and may not accurately reflect the dynamics of growth. The age group average will have mixed maturity levels, which will attenuate the magnitude of any growth effect.

The positive influence that physical activity has on aBMD has been observed in pre and peripubertal populations over time (Daly, Rich, Klein, & Bass et al., 1999). Over a 12-month observational period, controls as well as the athletes, showed modest but significant increases in total body BMC and femur aBMD (Bennell et al., (1997). Changes in bone density were independent of exercise status except at the lumbar spine suggesting that bone response to mechanical loading depends on the bone site and the mode of exercise. Children participating in the most physical activity over a 6-year period accrued more BMC at the total body, femoral neck and lumbar spine than children participating in the least physical activity (Bailey et al., 1999). Weight-bearing physical activity has been associated with higher baseline aBMD and more rapid bone mineralisation during three years of observation (Slemenda et al., 1994) in prepubertal and preadolescent children aged between 6 and 14 years. Nevertheless, as a result of observation, it has been highlighted that peripubertal studies tend to confuse the question of bone response to increased mechanical loading induced by augmented physical activity due to the mixed pubertal nature of the population (Daly et al., 1999; Katzman et al., 1991). Not all studies have found positive associations between physical activity and bone mineralisation in children. A retrospective study found physical activity during childhood was positively associated with radial aBMD in women but not men (Fehily et al., 1992). Similarly, physical activity was not correlated with aBMD in the lumbar spine, radius or femur of prepubertal and pubertal girls. However, the type of loading experienced during each sessional activity was only defined as high, moderate or low based on the number of exercise sessions per week (Katzman et al., 1991).

Positive effects of high strain rates on bone adaptations have been reported when data from the Amsterdam Growth and Health Study was analysed. Peak strain was considered to be a better predictor of lumbar spine aBMD than duration or energetic intensity levels of weight-bearing physical activities. Additionally, varied short-term periods of weight bearing were more effective

than long, repetitious loading involving lower peak periods (Groothausen, Siemer, Kemper, Twisk, & Welten, 1997).

2.13.4 Prospective Intervention Studies

Prospective interventions with pre- and early-pubertal children involving physical activity have resulted in increases in bone mineral content (BMC) and aBMD dependent on pubertal stage and type of impact exercise (Morris, Naughton, Gibbs, Carlson, Wark, 1997; McKay et al., 2000; Bradney et al., 1998; Scerpella Davenport, Morganti, Kanaley, & Johnson 2002; MacKelvie, McKay, Khan, & Crocker, 2002; Heinonen, Sievanen, Kannus, Oja, Pasanen, & Vuori, 2000; Fuchs et al., 2001; Petit et al., 2002).

2.13.4.1 Prepubertal

After 7 months of impact exercises eliciting a peak ground reaction force of not more than 5 times body weight no significant increases in either BMC or aBMD were detected (MacKelvie et al., 2002; Petit et al., 2002). Similarly, after 9 months of rope skips, hops and jumps from 40cm boxes there were no differences between exercise and control groups. However, in a sub-group of twin pairs who did not participate in high-impact sports during their leisure time, significant differences were observed in BMC and aBMD at the proximal femur (Van Langendonck et al., 2003). In contrast, for impact exercises where the peak ground reaction force was greater than 5 times body weight, (Fuchs et al., 2001) gains in aBMD were significantly ($p<05$) greater than controls at the lumbar spine with BMC significantly greater at both lumbar spine and femoral neck. These gains were maintained for at least 7 months post intervention (Fuchs et al., 2002). Furthermore, after 8 months of 30-minute weight bearing physical education lessons 3 times weekly, participating prepubertal boys realised a two-fold increase in lumbar spine, legs and total body aBMD in comparison to their non-participating peers. Additionally, the femoral midshaft of the exercisers increased significantly in cortical thickness (and consequently section modulus) compared to controls (Bradney et al., 1998).

2.13.4.2 Early-pubertal (Beginning Puberty)

In peripubertal studies efforts have been made to analyse the pre- and early-pubertal data separately. The more mature girls in the study by Petit et al., (2002) showed significantly greater gains in FN and intertrochanteric aBMD. These changes were underpinned by increased bone cross-sectional area and reduced endosteal expansion. Similarly the early pubertal girls gained significantly more bone at the femoral neck and lumbar spine in the study by MacKelvie et al., (2001) however there was no difference in gains at the other (total body, proximal femur and trochanter) sites. Increases in lumbar spine and femoral neck BMC after 9 months of step-aerobics (2 sessions weekly) have been observed for pre-menarcheal (not prepubertal) girls (Heinonen et al., 2000).

Weight-bearing exercise in children leads to increased bone mass, size, geometry and density (Petit et al., 2002; Witzke & Snow 2000). As little as 1½ hours of weight-bearing physical education lessons per week appeared enough to stimulate bone formation in prepubertal and preadolescent males aged 8 to 11 years during an 8-month period (Bradney et al., 1998). Significant increases occurred in aBMD in the exercise group at all sites except the head and arms. Evidence also suggests that weight-bearing loading during training and competition in events such as gymnastics and other athletic events such as soccer and ice-hockey lead to significantly greater bone mineral density in both males and females when compared to non-athletic peers (Dalsky et al., 1988; Slemenda et al., 1991). Gymnastics in particular, where forces at the hip can be as high as 12 times body weight (McNitt-Gray, Yokoi, & Millward, 1993) is a weight-bearing activity that has resulted in significantly greater aBMD compared to controls for, femur tibia and lumbar spine (Nichols et al., 1994). Gymnastics training in prepubertal children (with an average of 102 and 217 impacts per session involving upper and lower extremities respectively) has also been associated with peak ground reaction force magnitudes of 3.6 to 10.4 times body weight (Daly et al., 1999). In contrast, dancing incurs only moderate ground reaction forces between 2 - 5 times body weight which is a ground reaction force similar to running (Groothausen et al., 1997). In prepubertal, as well as retired gymnasts, when matched for age height and weight, aBMD at weight-bearing sites is consistently higher than the predicted mean in controls at all sites except the skull (Nichols et al., 1994). Interestingly, Nichols et al., (1994) reported freshman gymnasts increased their lumbar

spine aBMD more than veteran gymnasts. This could have been due to the freshman gymnasts having a greater potential for increases due to lower levels of aBMD compared to veteran gymnasts who may have been closer to their physiological limit. Greater whole body and regional aBMD in pre-adolescent female gymnasts occurred when the training volume of impact loading was high. Similar conclusions have been found in other studies involving gymnasts (Dyson et al., 1997).

Substantial data suggests that high intensity loading and activities involving high muscle forces may be significant independent predictors of bone mineral density (Heinonen, et al., 1996; Hawkins et al, 1999; Morris, Naughton, Gibbs, Carlson, & Wark, 1997). High intensity loading (Heinonen, et al., 1996; Pruitt, et al., 1995) and forceful muscle contractions are optimal activities for bone tissue accrual with eccentric muscle training possibly being more osteogenic than concentric muscle training (Hawkins et al., 1999). Current investigations reveal exercise (such as jumping) in children leads to increased bone mass, size, geometry and density (Fuchs et al., 2001; McKay et al., 2000; Petit et al., 2002; Witzke & Snow 2000).

Not all recent intervention studies have found positive associations between physical activity and bone mineralisation in children (Mackelvie, McKay, Khan, & Crocker, 2001; Petit et al., 2002). There may, however be a “window of opportunity” for bone response in early puberty corresponding to the interval between onset of puberty and onset of menarche in young girls although the findings are equivocal (Heinonen et al., 2000; Morris et al., 1997; Petit et al., 2002). In girls, early puberty may be a particularly opportune time for exercise interventions to have a positive effect on bone health. Methodological issues however, pose difficulties. With a note of caution it has been emphasized that peripubertal studies can cloud the issue of prepubertal bone response to increased mechanical loading induced by augmented physical activity (Lehtonen-Veromaa et al., 2000; Uusi-Rasi et al., 1997; Daly et al., 1998). In all likelihood the effects of exercise on bone, if imposed around puberty may be confounded by the adolescent growth spurt and result in greater increases in bone mass than if exercise increases are imposed after puberty (Boot et al., 1997; Grimston, Morrison, Harder, & Hanley, 1992; Kroger et al., 1993; Lu et al., 1994; Rubin et al., 1993; Ruiz et al., 1995; Uusi-Rasi et al., 1997). It is possible for training effects to be

masked by large growth effects and asynchronous advancement of participants within study groups. Groups that may be matched at the outset of a study may not be so well matched at the end of the study, therefore biasing the interpretation of the training effects. A small difference in age of 2 or 3 months or pubertal status in baseline characteristics in an exercise and control group can produce large changes. Benefits of exercise in the less mature group will be obscured by the accelerated growth in the controls (Seeman 2002). Alternatively, if the exercise group is more maturationally advanced, changes will be greater than in the controls independently of the benefit the exercise may have produced. Even if loading intensity is measured and is equivalent in two exercising groups, differences occur in the rate of maturational progression of the axial and appendicular skeleton between pre- and peripubertal groups. Growth and maturational development during peripubertal studies thus, clouds the issue of bone response to increased mechanical loading induced by augmented physical activity.

2.14 Summary

Emerging evidence from a variety of research approaches support the strong link between augmented physical activity and increases in BMC and aBMD in children. As well, other skeletal adaptations include bone geometry with bone area changes in children being reported (Fuchs and Snow, 2002; Fuchs et al., 2001; Morris et al., 1997). Physical activity is frequently cited as a strategy to increase peak bone mass. Peak bone mass, thought to accumulate by early adulthood, is the result of the amount of bone achieved during childhood and adolescence. Some investigators suggest that increased exercise prior to puberty has a beneficial effect on bone health later in life (Bass et al., 1998; Courteix, Lespessailles, Jaffre, Obert, & Benhamou, 1999; McKay et al., 2000) but little is known about the relative trainability of bone at different stages of puberty and very few longitudinal prospective studies exist to support this contention. Indications may well be that the earlier the age of commencing physical activity the greater the increase in bone density at weight-bearing sites of the hip and lumbar spine (Bradney et al., 1998). If BMC can be increased during the formative growth years, delay or prevention of osteoporosis may be affected since the bone mineral surfeit will be larger. Prevention of osteoporosis by optimising bone mass accretion during childhood and adolescence may be more effective than treatment later in life and may be most effective before the onset of puberty.

Weight-bearing sports such as running and gymnastics appear to be a good deal more osteogenic than sports such as swimming and cycling, however, excessive exercise duration or intensity may result in inability of bone to recover from loading. High muscle forces during growth also seem to have a beneficial effect on bone adaptations later in life, but little is known about the trainability of strength and power before puberty compared with trainability of bone in relationship to strength.

Physical activity and muscle strength have been found to be independent significant predictors of bone mineral density. Some evidence of the mechanism responsible for the adaptation of bone may be found in the positive relationship between strength and bone adaptation in humans and muscular forces acting on the skeleton during exercise

With a lack of precision and inconsistency of loads in most studies to date, greater specificity of load and more prescriptive interventions are needed (Matkin et al., 1998). Recent evidence from several studies involving children suggests that exercises must incorporate moderate to high impact loading to induce osteogenic benefits (Fuchs et al., 2001; MacKelvie et al., 2002; McKay et al., 2000; Specker and Binkley, 2003; Petit at el., 2002; Bauer et al., 2001). No study design to date involving children has clearly isolated the importance of ground reaction forces associated with impact loading exercise, to justify such an inference. For example, no two studies of children have used the same frequency of loading, the duration and number of training sessions per week have varied between studies, and some studies used a constant loading stimulus while others have increased the loading intensity progressively throughout the intervention program. No conclusive evidence exists to suggest which combination of exercise parameters is optimal for enhancement of bone development in children or whether in fact the osteogenic response in children is dependent on progressive intensity loading. In contrast to acknowledged positive effects on bone, impact exercise, especially if imposed repetitively and chronically has been identified as a risk factor for joint degenerative disease such as osteoarthritis in humans (Turner, 2000; Forwood, 2001). Clearly, better differentiation of the parameters of mechanical loading is required before recommendations can be made confidently regarding the type and intensity of

exercise most conducive to overall musculoskeletal development during growth, not just bone health.

2.15 Technological Limitations Inherent in Assessing the Skeletal Adaptive Response to Exercise in Children.

The assessment of bone accumulation is performed using a number of modalities. There are controversial opinions as to the efficacy of different modalities. Some of the modalities considered as traditional for many years are now having their veracity reassessed. A need exists for valid, reliable, and relatively inexpensive instrumentation (Petit, 2002; Seeman, 2002)

2.15.1 Technique - X-ray

(includes single-(SPA) and dual-(DXA) photon absorptiometry, quantitative computed tomography (QCT), peripheral quantitative computed tomography (pQCT)

2.15.1.1 Technical Description (X-ray)

Single photon methods use a radionuclide source mounted at 180 degrees with respect to the detector. The bone is scanned in a rectilinear fashion and the difference in the count rate between the bone and soft tissue regions allows calculation of the thickness of bone mineral content in the scan path.

Dual photon absorptiometry was initially performed using a radionuclide as a radiation source emitting two suitable energies. This has now largely been replaced by an X-ray tube with a heavily filtered spectrum emitting two narrow photon distributions.

Single and dual CT methods require very careful calibration

2.15.1.2 Sites Typically Measured (X-ray)

Single photon - appendicular skeleton (usually forearm)

DXA – Whole body, lumbar spine, femoral neck, trochanter, femoral shaft

2.15.1.3 Major Outcome Measures Using the X-ray Technique

Single photon – can determine thickness of bone mineral content in the scan path (BMC-g)

Dual photon – determines thickness of bone mineral content in the scan path and allows a two-dimensional volumetric (aBMD) calculation. Allows measurement of spine and hip since this technique eliminates the need for constant soft tissue thickness as in single photon measurements (BMC-g; aBMD-g.cm^{-2})

CT scanner – identification of trabecular component and determination of true BMD in mg hydroxyapatite per unit volume (BMC-g; volBMD-g. cm^{-3})

2.15.1.4 Strength of the X-ray Technique

Both absorptiometry and computed tomography provide measures of bone mineral mass (BMC) as well as estimates of BMD. The absorptiometric technique calculates the mass of the entire skeleton (total body BMD) or parts of the axial skeleton (lumbar vertebrae scan) as well as for a standard scan length (eg femoral neck or mid-femoral shaft). The mass is normalized using the segment length and bone width resulting in an areal BMD in g.cm^{-2}. Computed tomography, on the other hand, normalises the mineral mass for the bone segment volume and results in a true measure of volumetric BMD in units of g.cm^{-3}.

SPA is non-invasive and uses low dose radiation.

DXA is non-invasive and uses low dose radiation. With DXA method it is possible to correct for an even distribution of fat across the scanning path. DXA is the most widely used technique for bone measurements

With the CT scanner the trabecular component can be identified and measurements confined to these parts. The true BMD will be measured only in the bone tissue of interest. Precision of CT methods is high (single 1-2% ; dual 3-5%) and accuracy also high (single 4-7% ; dual 3-5%).

Volumetric density assessment of bone afforded by CT offers an advantage since it is not influenced by either body or skeletal size for bone measurements in children.

2.15.1.5 Limitations of the X-ray Technique (Technical and Health Risks)

Single photon methods cannot separate trabecular and cortical bone compartments. Measurements are restricted to the appendicular skeleton, usually the forearm, since the bone must be encased in a constant thickness of soft tissue or its equivalent (commonly a water box).

With the dual photon (DXA) method an uneven distribution of fat will introduce error into the measurements. Furthermore with the A-P projection of the spine, the posterior elements consisting of cortical bone are included in the result. Precision of dual energy methods is 2-6% and accuracy is about $\pm$ 5%. DXA is unable to account for the large changes in body and skeletal size that occur during growth.

The largest drawback in using CT is increased radiation exposure required for the procedure. Ionising radiation reduces the use of computed tomography as a research tool especially when measuring prepubertal children.

2.15.2 Technique – Ultrasound (includes Velocity of Sound and Broadband Ultrasound Attenuation)

2.15.2.1 Technical Description (Ultrasound)

Ultrasound is a mechanical sound wave consisting of frequencies above the range of human hearing (>20KHz). The ultrasound wave is delivered via a transducer that is used to convert an electrical sound signal into a mechanical vibration. Received sound signals are compared with a standard or reference waveform. Ultrasound waves are then able to provide information regarding (a) velocity and (b) attenuation of sound. Ultrasound characterisation of bone is based on the hypothesis that bone in different biomechanical states of elastic modulus, stiffness and structure create different values for velocity and attenuation of sound (Kaufman & Einhorn, 1993). The most common mode of ultrasound transmission through tissue is longitudinal. This occurs when the

vibrating tissue particles are parallel to the wave. When the particles move perpendicular to the wave, a shear wave is produced. Waves can also move between two surfaces and are known as surface or Rayleigh waves. In bone, the ultrasound waves are usually a mixture of these modes (Kaufman & Einhorn, 1993).

(a) Velocity of sound (VOS), also referred to as speed of sound (SOS), reflects the material properties of bone. Material properties include elastic modulus and compressive strength and are independent of architecture (Einhorn, 1992). VOS has been reported to have a moderate correlation with BMD at the same site in osteoporotic and normal children (r = .67; Jaworski, Lebiedowski, Lorenc, & Trempe, 1995) and a high correlation with elastic modulus (r = .97; Ashman, Corin, & Turner, 1987).

(b) Attenuation of sound is also referred to as broadband ultrasound attenuation (BUA). The attenuation of an ultrasound wave results in a loss of acoustic energy through a reduction in amplitude. This is determined by the degree of scattering and absorption. In scattering, the height of the transmission wave is reduced due to the redistribution of energy in one or more directions. The amount of scattering is dependent on the architectural (acoustic) properties of the bone and the wavelength of the ultrasound signal used. This relates to the trabecular pattern of the bone (Kaufman & Einhorn, 1993). Absorption of the ultrasound wave occurs when a portion of the transmitted sound wave is converted directly into heat. This is associated with the density of the bone and has been used to: (i) determine fracture risk *in vitro* (Gluer, Wu, Jergas, Goldstein, & Genant, 1994); (ii) discriminate between normal and osteoporotic women (Heaney et al., 1989) and children (Jaworski et al., 1995), and; (iii) assess bone characteristics in pregnant adolescent and young women (Sowers, Jamausch, Scholl, & Scholl, 1998).

2.15.2.1.1 Contact Ultrasonic Bone Analyser (CUBA)

The CUBA portable ultrasound device uses a direct contact of two 17 mm 1 MHz transducers positioned on an axial alignment for the measurement of ultrasonic velocity and broadband attenuation in cortical and trabecular bone. The device is linked to an IBM-PC compatible laptop computer and controlled by dedicated menu driven software. Soft-tissue compensation is

performed using an ultrasonic pulse-echo technique. CUBA has been successfully validated using reference materials (Langton,. Ali, Riggs, Evans, & Bonfield 1990).

2.15.2.2 Sites Typically Measured (Ultrasound)

The most common site for ultrasound measurement using CUBA is the calcaneus. Portable ultrasound (Omnisense) equipment is able to measure distal forearm and leg.

2.15.2.3 Major Outcome Measures Using the Ultrasound Technique

Ultrasound assesses bone material and structural properties. Limited studies (Daly, Rich, & Klein, 1997; Lappe, Recker, & Weiddenbusch, 1998; Wu, Gluer, Jergas, Bendavid, & Genant, 1995) have used ultrasound transmission to show the effects of weight-bearing exercise in children. Ultrasound velocity in the calcaneus, distal radius and phalanx of the index finger was significantly higher in prepubertal male gymnasts indicating greater elastic modulus and stronger bones than controls (Daly, Rich, & Klein, 1997). However, there was no difference between groups for ultrasound attenuation in the calcaneus. Ultrasound attenuation has previously been related to trabecular pattern (Wu, et al., 1995) with gymnasts possessing more highly mineralised bone than controls despite a similar trabecular pattern (Daly et al., 1997). These studies suggest that impact loading through gymnastics increased bone density and stiffness before puberty and increased trabecular structural patterns during puberty.

2.15.2.4 Strength of the Ultrasound Technique

Ultrasound is attractive because it is low in cost, portable, easy to use and does not emit ionising radiation. Examination of bone via ultrasound transmission can provide information not only regarding bone material properties but the spatial distribution and structural properties of the trabecular bone as well (Jergas & Genant, 1993). Subsequently, ultrasound has been found to more sensitively predict hip fractures than DXA (Stewart, Reid, & Porter, 1994) in elderly women. In contrast, ultrasound did not predict BMD as effectively as single X-ray absorptiometry in a similar elderly female population (Salamone, Krall, Harris, & Dawson-Hughes, 1994).

2.15.2.5 Limitations of Ultrasound Technique (Technical and Health Risks)

Analysis of calcaneal bone by QUS is considered to be useful in quantifying fracture risk. However, diagnosis of osteoporosis by QUS measurements remains contentious (Naganathan, March, Hunter, Pocock, Markovev, & Sambrook 1999; Phillipov, Holsman, & Phillips, 2000). Problems are due to the limitations of the present T-scores rather than the technique. Additionally, ultrasound values are dependent on so many structural properties not yet fully understood that it is difficult to use the information meaningfully in children (Gilsanz 1998).

2.15.3 Technique - Magnetic Resonance Imaging (MRI)

2.15.3.1 Technical Description (MRI)

Atoms incorporate a nucleus and a shell made up of electrons. Within the nucleus protons (positively charged particles) constantly spin around an axis producing an electrical current and a ensuing magnetic field. As the motion of the protons continues around their axis a cone-shaped path is traced called 'precession'. The strength of the magnetic field determines the rate of and frequency of precession. Within the earth's magnetic field proteins can arrange themselves in two ways. At a lower energy level they prefer to align themselves parallel to the external magnetic field. The magnetic field of a participant placed in the Magnetic Resonance magnet is longitudinal to the external field of the magnet and can't be measured directly. If a short burst of a radio frequency (RF) pulse (an electromagnetic wave), which has the same frequency as the precession frequency of the protons, is delivered into the participant, transverse (as opposed to longitudinal) magnetisation occurs. That is the RF pulse produces a new transverse magnetisation causing a decline the longitudinal magnetisation. If the RF pulse is turned off the whole system returns to the way it was. That is the longitudinal magnetisation increases again and the new transverse magnetisation abates and disappears (Schlid, 1990).

2.15.3.2 Sites Typically Measured (MRI)

Head (brain) Chest (heart) however is able to image all internal body parts

2.15.3.3 Major Outcome Measures Using the MRI Technique

Morphological images identifying tumors, blood flow irregularities as well as images that can be used for biomechanical and geometrical assessment

2.15.3.4 Strength of the MRI Technique

Contiguous transverse images with a slice thickness of 6mm can be made of sections to enable 3D reconstructions and calculations of bone volume. Cross-sectional area measurements can also be made from single transverse sections. These scans do not involve ionising radiation.

2.15.3.5 Limitations of MRI Technique (Technical and Health Risks)

Morphological images do not enable bone mineral content or density assessments to be made. There are no identified health risks.

2.16 Special Interpretative Consideration for Pediatric Populations in the Context of Exercise Studies.

BMC is highly dependent on the size of the bone scanned (Schonau et al., 2000). This is a drawback because short children will have a lower BMC compared to their healthy age-matched peers due to their smaller bones. This misrepresents the fact that their bones, although smaller, are otherwise completely normal. The same reasoning applies to aBMD, which is the most widely used densitometric parameter at present (Genant, 1996). In a similar way aBMD is often difficult to interpret in children with short stature. Covariance for bone size in studies using absorptiometry fails to account for bone thickness ($g.cm^{-2}$) and systematically underestimates the actual density of smaller individuals. Absorptiometry in children is problematic because growth related changes influence interpretation of aBMD differences that may occur between participants of similar age but different skeletal size. Furthermore, bone volume will change proportionally more than the scanned area. aBMD measures will not be a true representation of the volume present (Bolotin, Sievanen, Grashuis, Kuiper, & Jarvinen, 2001; Bolotin, 2001; Haapasalo et al., 2000). Procedures to avert this limitation have been proposed (Katzman et al., 1991; Kroger et al., 1992 and 1993). The Katzman derived measure for total body is bone mineral apparent density (BMAD - $g.cm^{-3}$). Derivation of BMAD proposed by Katzman (1991) ostensibly applies to females between the ages

of 9-20yrs, however only 3 of the 49 participants in the study were prepubertal. Suggested measures of BMC have been covaried for bone width or bone height (for femoral neck and lumbar spine respectively), or stature (for total body).

Methods to determine total volumetric BMD have recently gained in popularity. However, Hoegler (2003), recently presented evidence that derived vBMD of the mid femoral shaft is overestimated when compared to the MRI measured shaft volume. Computed tomography provides equitable comparison per unit volume, regardless of age or skeletal size.

Mechanical strength of bone is correlated with BMD (Currey et al., 1996). Measurements of the amount of bone in a given area without providing information on architecture, mineral content or crystal properties are insufficient. Few bone studies have related mechanical properties to mineral characteristics (Lian et al., 1999).

Most studies examining associations between exercise and bone adaptations in children have used DEXA to analyse bone adaptations (Sundberg et al., 1998). Nevertheless, other factors including stiffness, type of bone, bone size and shape, may contribute to bone strength in addition to those measured by densitometry (Snow-Harter & Marcus, 1991; Tothill, 1989).

The ability to measure muscle cross-sectional area (CSA) offers a valuable insight into the muscle-bone relationship. (Schoenau, Neu, Beck, Manz, & Rauch, 2002).

Ultrasound can be useful for the study of bone adaptations to changing loading patterns (Perre & Lowet, 1996). However, few studies have been conducted using ultrasound to determine the affect of exercise on bone strength in adults and even fewer in children. Only a limited number of studies have used ultrasound techniques to evaluate exercise or physical activity in children (Daly et al., 1997; Lappe et al., 1998).

2.17 Rationale for This Study

The major unresolved issues concerning the trainability of bone in prepubertal children are whether

(a) adaptive responses in the mineral and material properties of bone are dependent upon magnitude of impact loading exercise

(b) geometric (rather than mineral and material) properties of bone are dependent upon magnitude of impact loading exercise

(c) the magnitude of impact loading exercise will have a similar effect on mineral, material, and geometric properties of bone.

(d) the magnitude of geometric effect of impact loading exercise on bone is significant compared to the effect on bone mineral and material properties in this prepubertal population

2.18 Hypotheses:

1.) That bone adaptive responses will be threshold-dependent and then dose-dependent beyond the threshold in the female prepubertal population.

2.) That impact exercise will have a smaller effect on BMD than on BMC, geometric or biomechanical properties of bone in the female prepubertal population

3.) That trained-leg aBMD ($gm.cm^{-2}$) and volBMD ($gm.cm^{-3}$) of prepubertal girls who have been involved in repeated, weight-bearing, unilateral, muscular contractions at ground reaction forces between 4.54 –4.82 times body weight after an eight-month training period will be greater compared to a control group matched for age and pubertal status.

4.) That there will be significant differences in regional and site-specific bone geometry between trained and non-trained legs of prepubertal girls involved in eight months of weight-bearing training.

5.) That both exercise groups will demonstrate muscle hypertrophy and greater muscle size than controls in the trained legs.

2.19 Limitations of the Study

2.19.1 Limitations

Activity engaged in by participants outside of the intervention, including free play, school physical education, and organised physical activity, was not controlled.

Personal and family nutrition was assessed with no attempt to influence consumption.

Motivation during the intervention other than that described was beyond the scope of this study.

2.19.2 Delimitations

This study will only investigate parameters related to prepubertal females aged 6 to 10. Therefore, results will not apply to other genders or other age groups.

Selection of bone parameters is limited to those detectable using DEXA, MRI, and Ultrasound. Ultrasound can only reveal indications of bone structural and material properties and will be confined to the calcaneus.

A number of secondary markers of musculoskeletal health are confined to nutrition, physical activity and stage of pubertal development.

The hopping based intervention was delimited to 50 hops per training day on the non-dominant leg.

Two load-generating step riser height magnitudes (28 and 14cm) were determined for the study.

CHAPTER 3

METHODS

3.1 Research Design

The research design was a randomised, within and between groups comparative prospective study, designed to examine the effects of variable impact loading, unilateral, leg training on muscle and bone adaptation in prepubertal girls.

3.2 Ethical Approval

The study was approved by the ethics committees of the Children's Hospital Westmead and the Australian Catholic University (Appendix P).

3.3 Recruitment of Participants

Participants were recruited from a local school after gaining permission from the University and Hospital Ethics Committees and relevant Educational authorities. An information sheet describing the purpose and general outline of the study (Appendix A) as well as pamphlets for parents and potential participants were distributed after oral presentations at general school information sessions. Parents of girls who met the eligibility criteria for age and who wished to volunteer, were contacted by phone. If the child satisfied all inclusion criteria an initial appointment for interview and assessment was made.

3.3.1 Selection Criteria:

To be included in the study participants had to be prepubertal, aged between 6 and 10 years, clinically healthy and at Tanner Stage 1 for breast and pubic hair development (Tanner, 1981), involved in more than 3 hours of organised competitive sport or physical activity outside of school per week and free from any prior lower limb fractures or medical conditions or medications known

to affect bone metabolism. Participants with age specific height and body mass beyond the 75th and 25th percentiles (NHANES II) were excluded. A total of 45 girls initially volunteered for the study. Three girls were excluded from the study, as they did not satisfy the inclusion criteria.

3.4 Descriptive Measurements

3.4.1 Height Body Mass and Body Mass Index Measurements:

Standing height was measured to the nearest 0.1 cm using a stadiometer (Seca Mod.220.) Body mass was measured to the nearest 0.1kg using an electronic scale (Weddeburn Scales, Tanita BWB-600). Body mass index (BMI) was calculated by dividing body mass (kg) by height (m) squared (kg.m^{-2}).

3.4.2 Body Composition:

Lean tissue mass (LTM), fat mass (FM) and percent body fat (%BF) were determined following analysis by whole body dual energy X- ray absorptiometry (Lunar DPX, Software version 3.6). Coefficient of variation for lean tissue mass in the laboratory used for this study was 4.52% (in house rice and aluminium phantom).

3.4.3 Heel Width

Heel width was measured to the nearest millimeter on left and right foot of each participant using a sliding caliper. The caliper was positioned at the indentation left on the skin at the calcaneus by transducers of the CUBA clinical ultrasonometer.

3.4.4 Dominant Leg

Dominant leg was identified by having the participants kick a ball placed on the floor. The leg elected to kick the ball was accepted as the dominant leg.

3.4.5 Determination of Pubertal Status

Pubertal status was determined in consultation with the parent(s). Photographs of breast and pubic hair development described by Tanner, (1962) were used to assist parents in providing a ranking of their daughter's pubertal status. This method of determining pubertal status is considered reliable (r = .97) and valid (Duke, et al., 1980; Morris & Udry, 1980). If mother and daughter could not reach a decision concerning pubertal status they were referred to a consulting pediatrician to provide a final determination.

3.5 Questionnaires

3.5.1 Personal/Medical History

Parents were required to complete a medical questionnaire for their daughters, during the first hospital visit. The questionnaire was used to determine medication status (Appendix B) and past or current disease and injury (Appendix C).

3.5.2 Retrospective Physical Activity Assessment

A physical activity assessment was provided by a parent and child completing a retrospective 'Past Year Leisure-Time Physical Activity' questionnaire (Appendix D) in which physical activity level for the previous twelve-month period was reported. Together parent and child were asked to identify leisure activities (including organised activities/sports teams) in which the child had participated at least 10 times over the past year. Estimates of frequency (in months over the last year) and duration (average number of days per week and minutes per day) were obtained for each of these activities. A value determination for average hours per week of organized activity was made using the following formula:

past year (mo) x (4.3 $wk.mo^{-1}$) x ($days.wk^{-1}$) x ($min.day^{-1}$) ÷ (60 $min.hr^{-1}$) ÷ (52 $wk.yr^{-1}$).

The questionnaire used has moderate reliability for the age range of children in this study (Aaron, Kriska, Dearwater, Cauley, Metz, & LaPorte,1995). As well, a Modifiable Activity Questionnaire

was used to obtain information pertaining to perceived intensity of effort over the preceding 14 days. Rating of perceived intensity referred to number of times the child had "done at least 20 minutes of exercise hard enough to make you breathe heavily and make your heart beat fast." An additional question relating to sedentary behaviour was included to estimate routine number of hours spent watching television or playing computer/video games before or after school.

3.5.3 Prospective Physical Activity Assessment

The prospective Bouchard Three-Day Physical Activity Record, where ranking (using average energy expenditure categories on a scale of 1-9) was assessed in 15 minute epochs for two normal week days and one normal weekend day (Appendix E) was included. This assessment was completed at home using breakfast, mid day meal and evening meal as recording time points. Where the weekdays were school days, anticipated activity discussed at breakfast on the day of recording was checked when the child returned home at the evening meal. Codes were used to identify the nature and intensity of the activity. The Bouchard Three-Day Physical Activity Record has moderate reliability for the age range in this study (Aaron et al., 1995).

3.6 Dietary Calcium

Dietary calcium (Ca^{++}) intake was determined using a three-day (two week days and one weekend day) diary (with full written instructions enclosed) completed by each girl and a parent(s). A full verbal explanation was provided to both child and attending parent at the time of initial measurement appointment. Girls were requested to complete the diary (Appendix F) in as much detail as possible with parental assistance maintaining normal eating habits during the assessment period. Calcium intakes that were less than 15% of the recommended dietary intakes for Australian children of similar age (Australian Department of Health and Aged Care: National Health & Medical Research Council, 2001) were verified by follow-up telephone calls. Completed diaries were analysed using Foodworks ™ Food Analysis Program (Xyris Software 1999 Version 2.04.104). Calcium intakes were calculated as absolute daily intake and also adjusted for daily energy intake.

3.7 Cybex Dynamometer

3.7.1 Muscle Strength Measurement Procedure

A Cybex Norm^{TM} dynamometer (Lumex, Inc. Ronkonkoma, New York, U.S.A.) was used to measure isokinetic muscle strength (torque) during concentric knee flexion (KF) and extension (KE) of each leg separately. After a standardised warm-up (6 repetitions at 60^{o}. sec^{-1} with progressive effort) the participant was required to perform three maximal contractions at 60^{o}. sec^{-1}. Measures included peak torque and ratio of extension to flexion torque. These variables have moderate to high reliability and reproducibility (Sale, 1991). Test-retest reliability without repositioning was performed on 32 children in the study. Reliability coefficients for single leg right and left knee flexion and extension strength were r = 0.86, 0.93, 0.97 and 0.91, respectively. Coefficient of variation calculations were performed for strength during right (6.02%) and left (3.67%) leg flexion and during right (4.38%) and left (4.66%) leg extension.

3.8 Endocrine Status

Girls were not required to provide blood samples, but were encouraged to do so by their parents. Samples were obtained from 8 controls, and 13 girls each from the exercise intervention groups. Venous blood (20ml) samples were drawn from the cubital fossa after administering topical amethocaine. Samples were collected at school, between 9:30 am and noon on the day of testing and were centrifuged, stored at $-80^{0}C$ and analysed in a batch to eliminate interassay variability. Serum estradiol (E_2) was measured to confirm pubertal status near the end of the study (wk 27) using a highly sensitive (3 pmol/l) modified (delayed addition of tracer) radioimmunoassay (RIA) - (Clinical Assays^{TM} Estradiol-2 [Diasorin s.r.l. 13040 Saluggia (VC) Italy], which is used routinely in our laboratory. With this assay E_2 levels within a range of 8.1 to 146.8 pmol/l (Blades, B. 1999; New Children's Hospital, personal communication), with a total error CV < 20% were able to be detected. Intraassay CVs were 5.2% at 20.0 $rmol.l^{-1}$ and 5.0% at 44.0 $rmol.l^{-1}$. Interassay CVs were 9.8% at 28.9 $rmol.l^{-1}$ and 7.0% at 50.2 $rmol.l^{-1}$. Measurements were performed in triplicate with all CVs less than 10%. This result was considered acceptable when measuring in $pmol.l^{-1}$ quantities.

3.9 BMC and BMD Measurement

3.9.1 Dual-Energy X-ray Absorptiometry (DXA)

BMD (aBMD) measured in $g.cm^{-2}$ was determined by a pencil beam DPX (Lunar Corp, Madison, Wisconsin, USA) total body scanner (Figure 3.1) with adult software (version 3.6). Measurements of total body, hip (femoral neck), greater trochanter and legs combined (average of both legs) as well as bone mass and body composition were made using standard operating procedures (DPX Technical Manual). Analysis was performed with software version 4.7 by a single researcher. The technique and the measurement procedure, including quality-assurance testing, have been described previously (Lu et al., 1996). Total lean tissue mass (LTM) and total fat mass were derived from the total body scan. The regional analysis facility from the total body scan was used to measure areal BMD of both hips and trochanters, separately. All scans were acquired within a three-week period of the pre and post intervention dates. Combined (bilateral) hip (FNaBMD), trochanter (GTaBMD) and leg (LEGaBMD) bone mineral density were used as criterion measures. Areal BMD measurements were converted to estimates of volumetric BMD (g/cm^3) for the femoral neck (Lu et al., 1996). Measurement precision was established using repeat replacement scans on 2 adult males and one male child and two adult females and one female child. The coefficients of variation (for BMD) were 1.00% and 1.17% for femoral neck and greater trochanter. Mid-third femoral (regional) BMC, LTM and estimated total bone volume (TV) of the legs were determined by applying a region of interest (ROI) box over the mid-third section, on the total body scan.

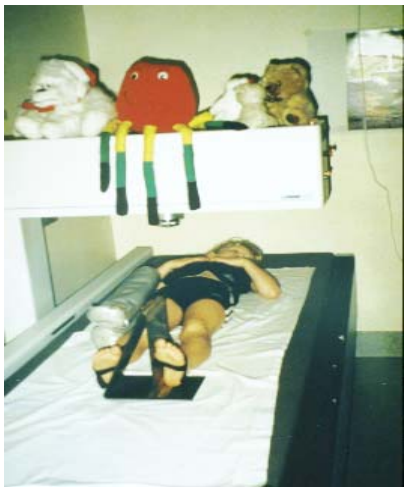


Figure 3.1

Pencil-beam DPX Total Body Scanner

It is not desirable to expose members of the public to risks as great as those considered acceptable for radiation workers. Dose equivalent limits for members of the public should be lower than for radiation workers. For the general public the limit is 1 mSv per year with allowable excursions up to 5 mSv per year provided that their lifetime average is no more than 1mSv per year (National Safety Council of Australia Ltd. 1996). Dual-Energy X-ray Absorptiometry (DXA)

involves exposure to low levels of ionising radiation. The effective radiation dosage for the three scans required is less than 1μSv which is less than that normally received daily from natural sources of radiation. Average daily exposure from natural background sources estimated for residents of a city the size of Sydney (Australia) is 7μSv. Procedures and levels of exposure for this study were similar to other studies approved by Children's Hospital Westmead Ethics committee (Duncan et al., 2000; Farpour-Lambert et al., 1999; Woodhead et al., 2000).

3.9.2 Reproducibility:

Repeated DXA scans of total body and both left and right femoral necks were performed to establish reproducibility for BMC (gm), BA (cm^2) and aBMD ($gm.cm^{-2}$). Six participants had a total body scan and then a scan of either left or right femoral neck. These were repeated with participant repositioning after a short rest interval. The coefficient of variation (calculated as the standard deviation of the repeated measures divided by their mean and expressed as a percentage) was calculated to check measurement precision for each descriptor under analysis headings.

3.9.3 Mid Femoral Shaft Bone Mineral Density

The regional analysis facility from the whole body scan (Lunar DPX, Software Version 3.6) was used to measure aBMD of mid femoral shafts of both dominant and non-dominant legs. aBMD measurements were converted to estimates of volumetric (vol) BMD ($g.cm^{-3}$) for the mid femoral shaft (Lu et al., 1994). Region of interest (ROI) for each leg was defined on the total body scan superiorly, by the oblique line through the femoral neck, medially, by the vertical line separating both legs and laterally, by the vertical line on the lateral aspect of each leg. Average values of these measurements were identified as legs combined (LEGaBMD) value. Measurement for volumetric BMD (volBMD) of the femoral neck (FNvolBMD) was obtained using an approach described by Lu et al., (1994). Trochanteric aBMD was measured using the lower line of the femoral neck region of interest as the upper limit and a line set at 45^0 angle to the actual scan path as the lower limit (DPX Technical Manual). Aforementioned measures of bone are made routinely with high reliability and reproducibility at the Hospital's Department of Nuclear Medicine (e.g.

Coefficient of variation values on the femoral neck were 0.92% and 1.9% for aBMD and volBMD, respectively).

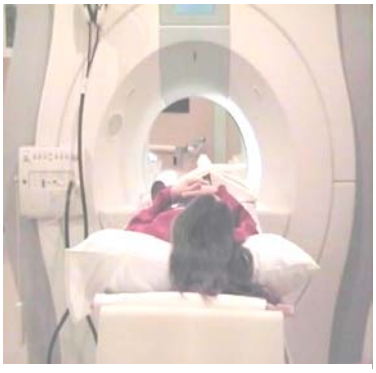
The femur was measured for length by placing a ruler on the proximal tip of the greater trochanter and measuring to the distal most point of the medial condyle of the left femur. A third of this distance was identified and the ruler adjusted to this measure. The default program region of interest (ROI) box was set at this value and positioned directly at the end of the first third of femur measurement. An identical box was created and transferred to the right leg. The relatively low resolution of total body DXA, as compared to MRI, restricts the height of the ROI box to be measured to 9.6-mm units. To correct for the difference in the measured mid-femur lengths, regional BMC, LTM and TV values were multiplied by the quotient of the precise mid-third femur length (as derived from the MRI scout scan) and the DXA- derived mid-femur length.

3.9.4 Increased Accuracy of Cortical Bone Mid Femoral Shaft Bone Mineral Density

Given limitations of absorptiometry and undesirability of using computed tomography, a methodology involving use of magnetic resonance imaging (MRI) has been developed. To more adequately and accurately assess bone mineral density, a measurement using the femoral shaft scan facility to compute an accurate cortical bone volume (cm^3) together with DXA derived femoral shaft BMC (g) was used in this study. A measure of bone volume was obtained from the mid third of the femur using MRI. By combining results from DXA and MRI scans of the mid-third femur, cortical volBMD ($g.cm^{-3}$), defined as the DXA-derived BMC divided by the MRI-derived cortical volume was calculated. BMC and cortical volume values were corrected for the differences in the measured mid-third sections between MRI and DXA, as outlined above. This methodology allows for more acceptable detection of bone volume changes due to periosteal bone accretion accompanying increases in bone diameter. It also appears to be a superior methodology to that of Katzman (1991) and Kroger (1992/1993) since the calculation allows for vagaries of the true mid femoral bone shaft. Subsequently, potential underestimation of true volBMD due to greater proportional change in volume compared to projected scan area will be avoided.

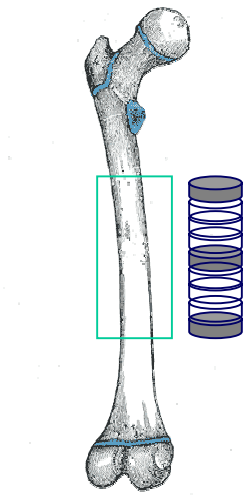
3.10 Bone Geometry

3.10.1 Magnetic Resonance Imaging (MRI)

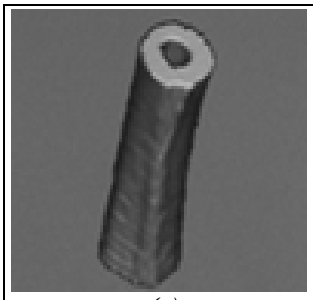


1.5 Tesla MRI Scanner
Figure 3.2

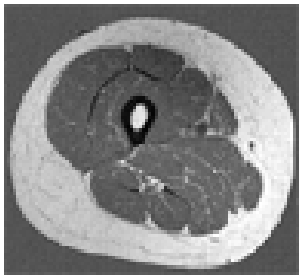
Bone geometry of the mid-third section of the femurs of the dominant and non-dominant legs was measured using a 1.5 Tesla Philips ACS-NT MRI scanner (Best, Netherlands) with a manufacturer supplied body coil at the beginning and end of the study (Figure 3.2). For all MRI procedures, the leg was positioned and stabilised in a custom designed lower-leg splint. Participant positioning and image acquisition procedures have been described previously (Woodhead et al., 2001). The mid-third femur section was identified from an initial scout scan in the coronal plane of the full length of the femur. The mid third section was measured as the distance from the head of the femur to the base of the medial femoral condyle. Contiguous transverse images with a slice thickness of 6mm (pixel resolution = 0.238 mm^2) perpendicular to the long axis of the femur were made of the mid-third section (Figure 3.3), proceeding in a distal to proximal direction of the femur to enable 3D reconstructions and calculations of bone volume (Figure 3.4a). Images were saved in DICOM format. Cross-sectional area measurements were also made from single transverse sections (0.5mm thick) at the proximal and distal borders of the mid-third section and at the mid-point level of the femur (Figure 3.4b). MRI scans did not involve ionising radiation.



Mid-third Contiguous Slices
Figure 3.3



(a)
3-D mid-third Section of Femur



(b)
Cross-section

Figure 3.4

All measurements were made by a single MRI radiographer and all subsequent analyses were performed off-line on a workstation by a single investigator blinded to participant group allocation. Analysis involved the use of Analyse (Mayo Foundation, Rochester:MN, version 7.0), which is a post-processing software package accessible on a dedicated Windows NT computer platform. Participants were encouraged to bring their own CDs and listen to music through headphones to minimize anxiety during the MRI scanning procedure. If requested, a parent was present within the MRI scan area. All MRI scans were completed in the Department of Radiology by a single research assistant.

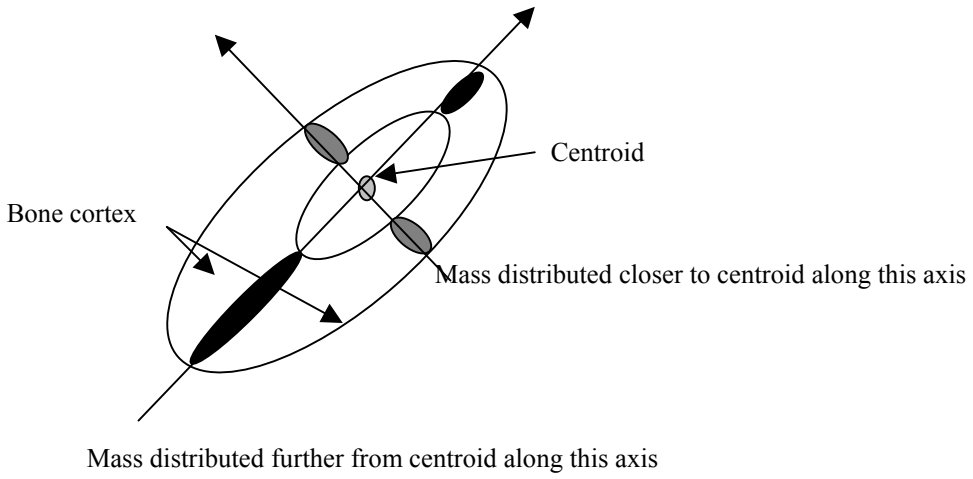
Differences in MRI derived total organ and cortical bone volume measurements compared to water displacement have been established in our centre at less than 2.5% difference, and measurements have excellent intra- and inter-tester reproducibility (coefficients of variation of <1.0%). All MRI measures were made prior to commencement of training and within 1 week of cessation of training at the end of the study.

3.10.2 Biomechanical Variables

Cross-sectional moments of inertia (CSMI), and principal angle of displacement (PAD) were calculated using Scion Image® (Frederick, Maryland: Version-Beta 3B) and a custom macro program. Images were imported from CD-ROM in DICOM format, and converted using MRIConvert (ver. 1.0.2, Lewis Center for Neuroimaging, Univeristy of Oregon) to meta-image format prior to analysis. Analyses were performed on the distal, mid- and proximal slices using a constant grey scale with a threshold range of 230-255. The Scion image macro calculates CSMIs (Ix, Iy) as well as the product of inertia (Ixy) about a set of image-aligned X and Y axes passing through the cortical centroid. Principal CSMIs, the polar moment of inertia, and the orientation of the principal axes are derived from these values using standard transformation equations. Pixel dimensions are accounted for explicitly within the macro thereby abating the need for post-correction factors. A detailed explanation of the procedures is reported previously (Hoegler et al., 2003).

3.10.3 Cross-Sectional Moment of Inertia (CSMI)

Calculating the moment of inertia relating to the cross-section of the bone shaft indicates how bone mass is distributed from centroid to periosteal circumference (Figure 3.5). An algorithm has been developed to estimate strength of the femoral neck from data generated by DXA. The algorithm considers shape of the proximal femur as well as CSMI in the estimate (Yoshikawa et al., 1994). A large CSMI indicates that there is more mass distributed closer to the periosteal perimeter than the centroid. Furthermore, in comparison, a larger CSMI indicates resistance to bending stress (and thus bone strength) has been enhanced. Importantly, a small change in bone cross-sectional area (CSA) created by periosteal bone formation results in a large change in CSMI because it is proportional to the fourth power of the radius. Additionally, large changes in mechanical properties can be brought about by modest changes in aBMD or BMC since mechanical loading tends to add bone to the most structurally relevant location (Robling et al., 2002).



Moment of Inertia
Figure 3.5

The CSMI in this study corresponds to the principal CSMI, obtained by image analysis of the distal, mid and proximal slices of the mid-third femoral shaft region for each participant. The CSMI was obtained by identifying the principal axes for distal, mid and proximal slices of the mid-third femoral shaft region for each participant.

3.10.3.1 CSMI Correction

Correction for CSMI using MRI is the following:

Correction **F**actor = $(4\sqrt{}$ csmi uncorrected x 0.488281$)^4$ where 0.488281 is MRI pixel size

3.10.4 Bone Strength Index (BSI)

Initially, to ascertain the orientation of the bone the principal axis is determined. The principal axis characterises the maximum engineering property of the bone. In order to bend bone in such a way that it would be more resistant to failure, a load would be placed along the principal axis (e.g. a paddle-pop stick bent flat-wise snaps more easily than one bent edge-wise). Comparison of the change in principal axes may indicate how the femur develops over time (or authenticate exercise related adaptations) to accommodate the rigors of functional mechanical load with associated muscle attachment torques.

Bone strength index (BSI) of the mid-femoral shaft mid-slice was determined by using the equation: Bone strength index (BSI) = CSMI x Volumetric Cortical BMD (Ferretti, Capozza, & Zanchetta, 1996).

3.10.5 Correction Factors

Use of different technologies for measurement requires that comparisons between modalities are corrected for any measurement disparities. When using BMC values from DXA-derived mid-femoral shaft length and cortical volume values from MRI-derived mid-femoral shaft (MFS) length, it is important to guarantee that the mid shaft position and length remain the same. The DXA resolution is 4.8 mm x 9.6 mm which is large in comparison with the 0.238 mm^2 pixel size resolution of the MRI scan. A correction factor is necessary therefore, in order to ensure that the MFS length measured by MRI and the MFS segment identified by the DXA scan correspond. The correction factor was derived by first dividing the total MRI measured scout scan femoral length by three (to calculate MRI mid-third femur length). The MRI mid-third length was divided by the DXA calculated mid-third length to yield the correction factor. The correction factor was applied to adjust

the DXA measurement for both dominant and non-dominant MFS BMC measures. Correction factors used in this study were derived the following way:

3.10.5.1 BMC Correction Factor

Correction **F**actor (BMC) = $\frac{\text{MRI scout scan length / 3}}{\text{ROI box height}}$ (then BMC x **CF** = corr BMC)

3.10.5.2 Volume Correction Factor

Correction **F**actor (Vol) = $\frac{\text{MRI scout scan length / 3}}{\text{Number of slices x slice thickness}}$ (then Volume x **CF** = corr Volume)

3.10.5.3 Cortical Volume Bone Mineral Density

Cortical volBMD (g/cm^3) was defined as the DXA-derived BMC divided by the MRI-derived cortical volume. BMC and cortical volume values were corrected for the differences in the measured mid-third sections between MRI and DXA, as outlined above.

3.11 Health Risk Considerations

DXA involved exposure to low levels of ionising radiation. Total cumulative effective dose equivalent of radiation during the course of the study of approximately 1.3 μSv was well below average annual exposure from natural background sources of 2.4 mSv. An information sheet and accompanying consent form was given to parents to inform them of the process (Appendix G).

Drop heights selected in this study represent ground reaction impact forces ranging from approximately 3 to 5 times body weight (McNitt-Gray et al., 1993; Valiant, & Cavanagh, 1985), and are typical of ground reaction forces in popular leisure and recreational sporting activities such as walking, running, soccer, field hockey and net ball (Dufek, & Bates, 1990; Nilsson, & Thorstensson, 1989). A unique feature of this study is that the ground reaction forces involve single leg impacts. Drop-heights of similar or substantially greater heights (depths) have been used in other studies involving children (Fuchs et al, 2001; Petit et al., 2002; MacKelvie et al., 2001) and young adults (Dufek, & Bates, 1990) without any reported incidence of injury. These loads ensured a margin of safety against injury and are representative of the range of load magnitudes likely to be

experienced and tolerated by non-athletes in most prescribed activity programs for promotion of bone development during childhood.

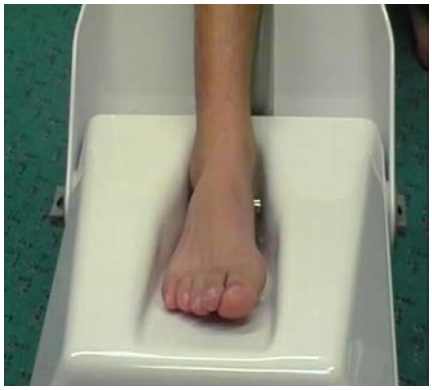
Training took place at school and all training sessions were supervised by exercise trainers and teachers. In the event of injury occurring provision was made for the child to be referred to a Sports Medicine specialist at the Children's Hospital Institute of Sports Medicine (CHISM) for treatment.

Magnetic resonance imaging (MRI) involved exposure to high magnetic fields and radio waves. This technique involved exposure to non-ionising radiation involving no known clinical risks. Parents completed a standard questionnaire for themselves and their daughters before entering the room containing the magnet. The questionnaire was used to determine present or past conditions (eg heart pacemaker) that could be affected by strong magnetic fields (Appendix H). To minimize potential feelings of claustrophobia during the MRI procedure, participants were encouraged to bring reading material or CDs to listen to their own music through headphones. Parents were present if requested by participants.

The calcaneum ultrasound procedure that also involves exposure to non-ionizing radiation posed little clinical risk to participants and had received Ethics Committee clearance for use with children.

3.12 Bone Material Properties

3.12.1 Broadband Ultrasound Attenuation (BUA)



Contact Ultrasound Bone Analyser (CUBA)
Figure 3.6

Bone material properties were determined bilaterally for calcaneus using quantitative ultrasound measurements and recommended procedures at the beginning, mid-point and end of the study. Broadband Ultrasound Attenuation (BUA; $dB.MHz^{-1}$) which purportedly reflects the density and trabecular orientation in bone (Gluer et al., 1993; Langton et al., 1990; Wu, Gluer, Jergas, Bendavid, & Gernant, 1995) was measured by a single investigator. Simultaneously, velocity of sound transmission (VOS; $m.sec^{-1}$), which correlates strongly with elastic modulus (Ashman et al., 1987) and breaking force of bone (Wright, Glade, & Gopal, 1987) was measured for each participant using a Contact Ultrasound Bone Analyser (CUBA) Clinical Pediatric densitometer (Figure 3.6) version 2.5 (McCue Ultrasonics, Winchester, U.K.). This ultrasound device used two 1 MHz transducers positioned on axial alignment linked to an IBM-PC compatible laptop computer controlled by dedicated menu driven software. VOS and BUA have an in vivo short-term (within 2 weeks) reproducibility of 0.13% and 2.8 %, respectively in children and an in-vitro precision of 0.6 % and 2.8% (Daly, Rich, & Klein, 1997).

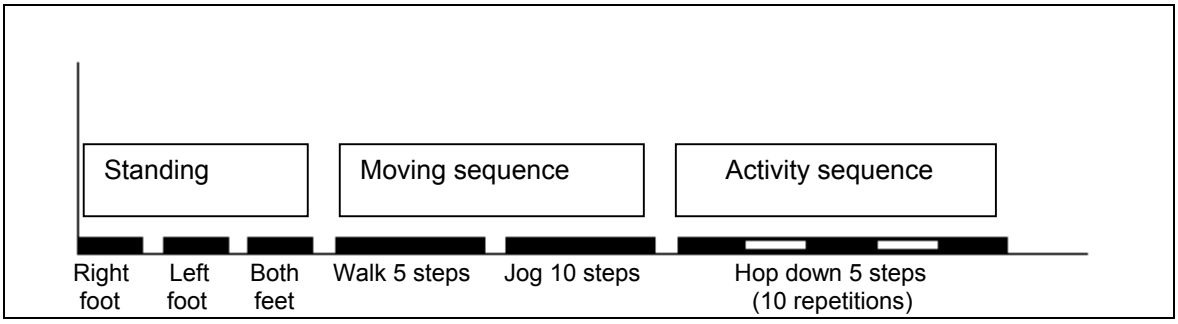
3.13 Magnitude of Training Loads

3.13.1 Pedar Mobile

Peak ground reaction forces from participant's jumping action were measured near the mid-point (week 14) and end (week 28) of the study during actual training sessions for subsets of participants in both training groups (3 participants per group). Similar measurements were made for 3 participants from the control group for level walking and running to account for growth effects. Participants from training groups were subjectively selected to give examples of efficient, average and below average hopping efficiency. Control participants were selected at random. Ground

reaction forces were measured using the Pedar Mobile system, which measures foot forces and pressures during locomotion using a combination of sensors (capacitive transducers) arranged in columns and rows forming a sensor matrix. Participant's feet were placed in runners on top of left and right 'Pedar soles'. Soles were connected to the Pedar (mobile) box and cables fastened to legs of the standing participant by means of velcro straps, one at the ankle and one above the knee of each leg. Each participant was given two or three practice trials of data collection procedure to become familiar with the situation and requirements. Zero measurement was performed by unloading each insole in turn and was carried out immediately before data collection.

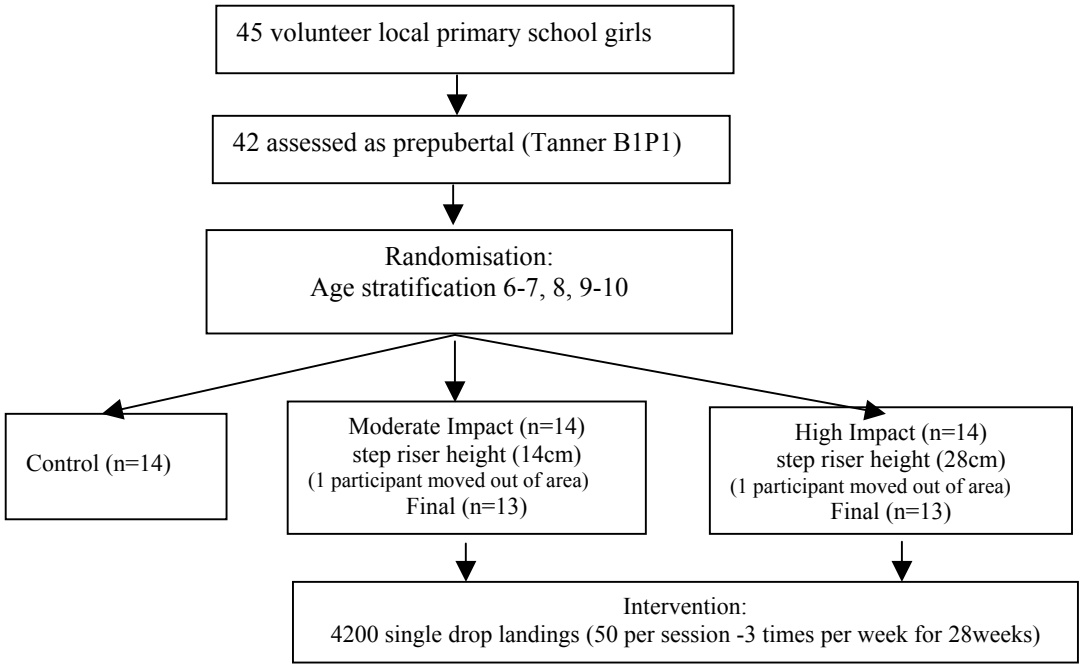
Data for training group participants was collected using the following data collection protocol (Diagram 3.1).



Ground Reaction Force Data Collection Protocol
Diagram 3.1

Data for control participants were collected using standing and moving sequences (Diagram 3.1). The activity sequence consisted of running 30 meters at maximum speed twice with a standardised rest interval between runs. Measurement of Peak Ground Reaction force (step in the sequence exhibiting maximum force) was recorded for each participant.

3.14 Research Design Flow Chart:



3.15 Study Population

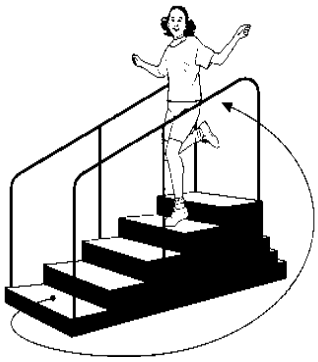
Experimental groups consisted of 28 pre-pubescent girls aged between 6 and 10 years. The control group consisted of 14 girls matched for age and pubertal status. Initially 45 participants were recruited however; three were confirmed to be pubertal on examination by a pediatrician or parental report and were excluded. During the study two participants (one each from the two training groups) withdrew due to family relocation.

3.16 Randomisation

After recruitment, and initial assessment for inclusion or exclusion, participants were stratified by age (6-7yrs, 8yrs and 9-10yrs) and randomly assigned into the control (group #1), moderate impact (group #2 -14cm step) or high impact (group #3 - 28cm step) training groups.

3.17 Intervention

(Note: During the course of the study one participant from Training group #3 was diagnosed with diabetes, and treated).



Training Equipment
Figure 3.7

The training intervention comprised single leg drop-landing exercises from different step heights (Figure 3.7). Training involved the non-dominant leg. The contra-lateral leg served as a within-participant untrained control. Participants trained at school for 8 months (28 weeks excluding holidays) under close supervision of exercise trainers and teachers. Differences in training load magnitude (representing low and high kinetic energy) were established by varying the height of drop-landings for each group. Drop heights for the low drop (LD) and high drop (HD) groups were 14 cm (approximate standard step height in a home), and 28 cm vertical to the landing surface respectively. The drop heights selected in this study were chosen to elicit ground reaction impact forces between 2-4 times body mass (McNitt-Gray et al. 1993; Valiant & Cavanagh, 1985), and are typical of the ground reaction forces in popular leisure and recreational sporting activities such as walking, running, soccer, field hockey and netball (Dufek & Bates, 1990; Nilsson & Thorstensson, 1989). These loads ensured a margin of safety against injury and are representative of the range of load magnitudes likely to be experienced and tolerated by non-athletes in most prescribed activity programs for the promotion of bone development during childhood. Each training group had its own set of benches of specified drop-heights, and each bench had 5 steps. Participants were reminded to wear running shoes for training sessions and were instructed to bend slightly at the knee (eccentric contraction of knee extensors) and hip on contact to decelerate the body's centre of gravity. The contra-lateral control leg remained flexed and non-weight-bearing while traversing the steps. On the last step of each 5 step cycle, participants were instructed to land initially on the single leg, and then to bring the contra-lateral leg to weight-bearing before walking to the back of the steps to initiate another set. A training session comprised 10 repeat sets (or a bout) of the aforementioned 5 step drop-landings, with less than 30 – 60 seconds between sets. With this pacing, the complete training session including stretching at

the beginning and end of each session was completed within 15 minutes. With exception of the first week of training, participants trained 3 times.week^{-1} (5 steps.set^{-1} 50 steps.session^{-1} and 3 sessions.week^{-1} or 150 drop-landings.week^{-1}) totalling 4,200 drop-landings over duration of the study. During the pre-training week (Appendix I), first, second and third training sessions comprised of 3, 6 and 9 sets of the above loading cycle respectively. To ensure full conformity to the exercise program, participants formed separate lines at the top of the stairs at the beginning of each training session and sequentially followed the leader of each group for all exercise sets. The leader of each group picked up a colour coded table-tennis ball at the beginning of each set at the top of the stairs and deposited it into an empty egg carton at the bottom of the stairs at the end of each set. Participants were encouraged to make up missed exercise sessions on non-training days.

Exercise Training
Figure 3.8

The LD and HD groups trained concurrently, side by side (Figure 3.8). Groups began each session of exercise at the same time and similar pacing was ensured, by partnering individuals across groups. Initially a typical training session lasted 22 minutes; at the end of the study training sessions were completed within 8 minutes. The decreased training time was attributed to improved balance and familiarisation with the exercise protocol over the course of the study, but the change in loading rate was similar between LD and HD groups. With this design, number of loading bouts, frequency, pace and duration of loading were constant. The amount of work required by the HD training group to be positioned at the top of the bench for the next set would be twice that required for the LD training group due to the difference in step riser heights (14cm compared to 28cm). To control for this potential additional loading influence (additional ground, muscle and joint reaction forces), a special ramp was constructed, fronted with two steps, that equalled half of the total height difference climbed between groups. The ramp was placed at

the bottom of the stairs (see Appendix J) for the LD group and participants were required to traverse the ramp twice before initiating the next set of exercises. Absolute load magnitude varied by individual body weight, and total work performed during training varied by necessity with drop-landing height across training groups, as did peak mechanical strain rate. These were practical and uncontrollable design limitations of the study. Thus, load magnitude was the sole characteristic of exercise training to vary among training groups.

Participant compliance to training was determined as the proportion of attended sessions (Appendix K), and adherence as the proportion of participants who completed all training and testing sessions. Motivational charts included the names of all training group participants. Total number of training sessions with calculated geographical icon height measures (as a goal for hopping distances – Appendix L) were compiled using popular stickers to indicate completed sessions every 2-3 weeks by control group participants under teacher/trainer supervision. As well, prizes, funny-hat competitions, 'hop down' music and participant supplied music CD's were provided to encourage compliance and adherence to training. Several social functions e.g. a pizza party, an all food party, compilation of a video of initial explanations and relegation to groups as well as training performances were organised during the course of the study to encourage camaraderie and compliance to testing for the control group. A culminating 'magic show' held at school in the evening was organised as a gesture of appreciation to families of participants, teachers and school administrative staff who assisted in the study.

Initially, after each training session and subsequently, at the end of each week of training, a weekly training report sheet was completed. Attendance was checked and participating children were questioned about pain or discomfort associated with or resulting from training in an effort to monitor the effects of the loads being used. Results were recorded (Appendix M).

3.18. Kinetic Energy

Kinetic energy produced by LD and HD participants by dropping perpendicularly was calculated in the following manner using the variable identification:

gravitational acceleration	$a = -9.81$
time of drop	t = *unknown*

initial velocity (standing on step)	*vo = 0*
final velocity (on landing)	*v = -9.81t + vo*
initial displacement (LD standing on step)	s_1 *= 14cm*
initial displacement (HD standing on step)	s_2 *= 28cm*
final displacement (LD andHD)	*s = 0cm*

Solving for time (in seconds) at impact knowing that final displacement is 0 (using the displacement equation $s = ½ at^2$) :

(LD) Final displacement 0 = 0.5 x 9.81$\mathbf{t^2}$ + initial velocity (0) + initial displacement (0.14)

t = 0.1689 secs

(HD) Final displacement (y) 0 = 0.5 x 9.81$\mathbf{t^2}$ + initial velocity (0) + initial displacement (0.28)

t = 0.2389 secs

Solving for velocity (in metres per second) using t and the velocity equation (v = at + vo) knowing that initial velocity (vo) = 0

Velocity at time of impact: (LD)v = -9.81(0.1689) + 0 = 1.657 m/s

(HD)v = -9.81(0.2389) + 0 = 2.344 m/s

Kinetic energy can then be solved as:

$KE_{(LD)} = 0.5\ mv^2$ (for 50kg participant)

$= 0.5(50)(1.657^2) = 68.64J$

and

$KE_{(HD)} = 0.5\ mv^2$ (for 50kg participant)

$= 0.5(50)(2.344^2) = 137.36J$

Durkin, J.L. (2003) (Personal communication)

3.19 Care of Participants

The utmost care was taken not to place participants in any situation of undue stress. Participants and parents were informed and reminded that participation was voluntary and that they were free to withdraw from the study at any time.

3.20 Statistical Analysis

Descriptive data was initially tested for normal distribution. Regression assumptions of independence and constant variance were checked by P-P plots and were accepted as normal if residual values were within a band of ± 2 SDs. Data were also checked for normality by calculating differences between mean and median values, skewness and kurtosis. Values between ± 2 SD were considered representative of a normal population. Non-normal data was logged (base 10) and re-analysed. For descriptive purposes, means and standard deviations were calculated for the key independent and dependent variables using SPSS versions 10 - 11.5. Pearson's correlation (r) analysis was used initially to examine bivariate relationships between all dependent and independent variables. Independent variables were grouped into categories, based on biological plausibility and empirically (published literature) demonstrated relationships, for the purpose of multiple linear regression analysis (MLRA). Only the highest correlated independent variable from each category (based on its bivariate relationship with the dependent variable) was included in the regression model, using stepwise selection. Variables were categorized as follows: age as a separate category; body size which included height (Ht) and body mass (BM); body composition including lean tissue mass (LTM), fat mass (FM) and percent body fat (%BF); physical activity (LTPA); dietary calcium intake (Ca^{++}); leg strength which included knee flexor (KF) and extensor (KE) torques and their (KE/KF) ratio (Ratio); and estradiol (E_2) status. Correlations and regression coefficients were accepted as significant if $P<0.05$. Additionally, factor analysis was undertaken in order to detect structure in relationships between independent variables and to identify collinearity.

For descriptive purposes, means and standard deviations were calculated for variables at baseline and change data over the course of the study. Simple analysis of variance (ANOVA) was used to determine differences among groups for physical and lifestyle characteristics at baseline and for pre-post changes over time. T-tests for dependent samples were performed to determine within group differences for changes in primary bone mineral and ultrasound outcomes between dominant and non-dominant legs. Baseline primary bone mineral and ultrasound measures were analysed using ANCOVA, with adjustments for the covariates baseline body and fat mass. Between group differences for changes in bone mineral and ultrasound outcomes were analysed using ANCOVA, with adjustments for baseline body and fat mass and the change in lean tissue

mass. Bonferroni adjustments were made for all statistical comparisons. Sample size was established a-priori using the procedure described by Day and Graham (1988), based on estimates of the population variance in BMC for pre-pubertal girls of a similar age (Dyson et al., 1997) and an estimated average effect of training of +7% - equivalent to a moderate effect size of 0.5 (Cohen, 1988) reported in comparative studies of young athletes involved with impact loading exercise (Bass et al., 1998; Dyson et al., 1997) and the few prospective training studies at the time, involving prepubertal children (Bradney et al., 1998; Morris et al., 1997). Since there were no differences among groups for any of the key bone outcome measures at baseline, and to increase statistical power, a secondary level ANCOVA analysis with the same covariates used in the initial analysis was performed with both training groups combined. Post-hoc estimates of effect size and statistical power were determined for the primary bone outcome measures using the procedure described by Speed and Andersen (2000). Differences among groups were considered significant if $p<0.05$.

3.20.1 Power Analysis:

Differences in total body bone density of 1.2 - 1.8 SD have been reported in cross-sectional studies between pre-pubescent (Dyson et al., 1997.) and young adult athletes (Kirchner, et al., 1995.) involved in the high impact loading sport of gymnastics, and non-athletic controls. Given that the magnitude of prescribed loading in this study was less than that experienced by high impact loaded athletes, a smaller effect size of 0.75 SD was accepted as statistically significant. On this basis, and accounting for the possibility of drop-outs, a sample size of 14 participants per group was estimated as sufficient to detect significant effects in bone density at $p < 0.05$ with a statistical power of 80%. The total sample initially consisted of 42 participants.

3.20.2 Effect Size

Differences between baseline and post-intervention measures were transformed into estimates of effect sizes expressing the differences between the group mean scores for each variable relative to population variance. SD of the control group was used as an estimate of within-population

variance. Values of 0.2, and larger than 0.5 and 0.8 were taken to represent small, moderate and large group differences respectively (Dyson et al., 1997).

Main and interaction effects of changes in key outcome variables of bone adaptation (BMC, aBMD and volBMD), femur volumes and areas, and calcaneal ultrasound measures over time, were determined by analysis of variance with repeated measures (ANOVA).

Baseline and post-intervention group measures were submitted to paired t-test analysis to detect within-group differences.

Control, LD and HD groups were investigated and analysed for the following dominant and non-dominant leg outcomes; isokinetic muscle strength and power variables (leg flexors and extensors) developed at 60 degrees.sec $^{-1}$; areal estimated and true BMD measures; mid third femur regional and site-specific bone (proximal, mid and distal slices) measures of gross bone morphology; mid femur regional and site-specific bone biomechanical variables (CSMI); muscle cross-sectional area and volumes using magnetic resonance imaging (MRI); velocity of transmission of sound and broadband ultrasound attenuation.

3.20.3 Muscle Strength (Torque)

As part of the design of the study the non-dominant leg was chosen as the training leg for participants in both training groups. When analysing differences that may have occurred in leg flexor and extensor strength between dominant and non-dominant legs of participants, it was imperative that a derived measure be used to identify true relative changes. In some cases it was anticipated that difference in strength between dominant and non-dominant legs would diminish. This would be due to non-dominant leg increasing in strength relative to dominant leg thus showing a decrease in leg strength difference would indicate an increase in non-dominant leg strength. A relative difference expressed as a percentage of the dominant leg strength was therefore calculated and analysed as a more accurate representative variable.

Bivariate correlation and multiple regression analyses were used to describe relationships among the various key dependent (as described above) and independent (physical activity, diet, body composition and growth) variables.

3.20.4 Principal Components and Factor Analysis

Data from the correlation matrix was submitted for factor analysis. From the principal component analysis components with eigenvalues greater than one were identified as factors. Most appropriate interpretation of relationships was selected for the varimax, quartimax, equamax or direct oblimin rotations.

3.21 Winding-up Procedures

Each participant received a personal feedback report at completion of the study after analysis of data (Appendix N). Included in the report was a brief explanation of individual outcome variables with interpretation as well as comparison of individual results to control group average results. Letters of thanks were sent to all participants and certificates of appreciation were awarded to teacher participants and the cooperating school at completion of the study.

CHAPTER 4

4.1 Correlates and Determinants of Bone Mineral Density in Prepubertal Girls

The study was designed to advance understanding of the factors that promote skeletal health in the prepubertal population. Identification of the correlates and determinants of BMD in prepubertal girls is considered to be an important fist step towards the promotion of optimal musculoskeletal health during childhood. In this chapter BMD was the dependent variable in keeping with the majority of prepubertal intervention reports. However, the limitation of BMD is addressed in subsequent chapters.

Peak bone mass is a major determinant of fracture risk in later life (Gunnes, &. Lehmann, 1996; Hansen, Overgaard, Riis, & Christiansen, 1991) and more than 90% of peak bone mass is present by age 18 years (Theinz et al., 1992). Thus, the opportune time to maximise bone mass accrual would may occur during childhood and adolescence. Recent studies have identified a number of correlates of bone mineral density (BMD) in young populations. Chronological age and general physical characteristics such as height and weight (body mass) appear to be the most important (Boot et al., 1997; De Schepper, Derde,. Van den Broeck, Piepsz, & Jonckheer, 1991; Lu, Cowell, LLoyd-Jones, Briody, & Howman-Giles, 1996; Rice et al., 1993; Ruiz, Mandel, & Garabedian, 1995) correlates. However, in circumpubertal children, correlates of BMD at specific bone sites also included moderately strong genetic (Tao et al., 1998), and maturational (Boot et al., 1997; Ruiz et al., 1995; Theinz et al., 1992) factors and variable influences of behavioural or lifestyle factors such as physical activity and nutrition (Bailey, McKay, Mirwald, Crocker, & Faulkner, 1999; Boot et al., 1997; Gunnes, &. Lehmann, 1996; Katzman, Bachrach, Carter, & Marcus, 1991; Matkin, Bachrach, Wang, & Kelsey, 1998; Ruiz et al., 1995). Furthermore, a positive correlation between muscle forces acting on bone and BMD in circumpubertal children and adolescents (Gunnes, &. Lehmann, 1996) is available but limited research has been conducted specifically with prepubertal populations. A concept largely under-explored is the earliest age at which additional physical activity can be linked to increases in bone density at weight bearing sites of the hip and

lumbar spine (Fuchs, Bauer, & Snow, 2001; Katzman, et al., 1991). The skeleton appears to be more responsive to calcium supplementation in the pre and early stages of puberty compared to the adolescent stage (Bonjour et al., 1997; Gunnes, &. Lehmann, 1996; Johnston et al., 1992; Lee et al., 1994).

Compared to the circumpubertal and adolescent populations, there is relatively little information about the determinants of BMD in prepubertal children. A few studies have examined relationships between BMD and body size (De Schepper et al., 1991; Katzman et al., 1991; Molgaard, Thomsen, & Michaelsen, 1998) body composition (McKay et al., 2000), physical activity (Gunnes, &. Lehmann, 1996; Katzman et al., 1991; Matkin et al., 1998) and dietary factors (Bonjour et al., 1997; Gunnes, &. Lehmann, 1996; Johnston et al., 1992; Katzman et al., 1991; Lee et al., 1994) either separately or in combination with other variables in prepubertal children. The uniqueness of the study in this chapter however, is that these variables were investigated concurrently with other hitherto untested putative determinants of BMD, such as muscle strength and serum estradiol levels. The paucity of information in prepubertal children is surprising since childhood might comprise a sensitive developmental period for skeletal responsiveness to extrinsic factors like physical activity and nutritional status. If the importance of these factors can be established, then this information can be used in strategies to promote and optimise bone mineral accrual during the formative growth years. Increased bone mineral accrual may contribute to increased peak bone mass and reduced risk of osteoporosis in later life (Bailey et al., 1999). Therefore, the purpose of this study is to identify and describe the correlates and determinants of bone mineral density (BMD) in prepubertal girls. The further uniqueness of this study is the simultaneous consideration of novel combinations of putative BMD predictor variables, hitherto not investigated in this population.

4.2 Methods

Details of the methods used in this chapter have been presented in Chapter 3. Primary outcome measures were total body aBMD and regional aBMD and volBMD. Specific statistical treatment of the data were tests for normality, descriptive statistics, bivariate correlates of primary measures. To quantify the contribution of high correlates of independent factors with the dependent bone measures in the study, a series of multiple linear regression models were calculated.

4.3 Results

Descriptive characteristics and a summary of the physical activity, nutrition and leg strength results are summarised in Tables 4.1 and 4.2 respectively. Descriptive data were normally distributed and there were no outliers. Participants ranged between 6 and 10 years of age (mean of 7.8 +/- 0.9) and all were prepubertal based on parental reports of the Tanner stages of development criteria for breast and pubic hair development. Serum estradiol levels corresponded to the stage of development ranging between 11 and 61 pmol/l. Areal BMD values (g/cm^2) for the TB, FN, LEG and GT were 0.835 ± 0.045, 0.716 ± 0.054, 0.736 ± 0.059 and 0.621 ± 0.061, respectively (mean ±. SD). FNvolBMD was 0.612 ± 0.057 g/cm^3. The mean values were within ± 2SD of previously published values for each measurement site, for comparably aged girls (De Schepper et al., 1991; Dyson, Blimkie, Davison, Webber, & Adachi, 1997).

Table 4.1 Descriptive Characteristics

Variables (N=42)		**Mean ±SD**	**Range**
Chronological Age (yr)	Age	7.82 ± 0.91	6.64 - 10.37
Height (cm)	Ht	127.00 ± 5.05	116.5 - 140.5
Body Mass (kg)	BM	26.48 ± 4.38	19.9 - 38.3
Lean tissue mass (kg)	LTM	18.56 ± 2.35	14.00 - 22.73
Fat mass (kg)	FM	6.34 ± 2.82	2.75 - 15.62
% Body Fat	%BF	23.85 ± 6.53	13.65 - 45.82
Estradiol Level (pmol/Litre)	E_2	18.74 ± 10.51	11 - 61

Table 4.2 Physical Activity, Nutrition and Leg Strength Characteristics

Variables (N=42)		**Mean ±SD**	**Range**
Past Year Physical Activity (hr/wk)	LTPA	2.55 ± 1.78	0 - 7.5
Dietary Calcium (mg/day)	Ca	566 ± 253	182 - 1354
Knee Flexor Strength (Nm)	KF	21.92 ± 5.82	8.0 - 34.5
Knee Extensor Strength (Nm)	KE	41.26 ± 9.62	17.5 - 64.5
Leg Strength Ratio (KE/KF)	Ratio	1.94 ± 0.40	1.17 - 3.25

Table 4.3 Bivariate Correlations (Pearson's r)

Dependent Variables	**Age (yrs)**	**Ht (cm)**	**BM (kg)**	**LTM (gm)**	**FM (kg)**	**BF%**	**LTPA (hr)**	**Ca (mg)**	**KF (Nm)**	**KE (Nm)**	**Ratio**	**Estradiol (pmol/L)**
Total Body aBMD(g/cm^2)	**.45****	.29	**.54****	**.62****	**.36***	.16	-.03	.09	.25	**.40****	.23	.24
Femoral Neck aBMD (g/cm^2)	.14	.14	.15	**.35***	.03	-.06	.05	-.05	.10	.19	.15	.15
Femoral Neck volBMD (g/cm^3)	.25	.14	-.01	.19	-.17	-.27	.03	-.04	.05	.14	.11	.02
LEG aBMD(g/cm^2)	**.56****	**.46****	**.63****	**.77****	**.40****	.17	-.14	.02	**.33***	**.47****	.17	.20
Trochanter aBMD (g/cm^2)	.13	.09	.11	.15	-.02	-.06	-.02	-.17	.02	.16	.17	.09

* p<.05 **p<.01
Note: Dark vertical lines indicate independent variables grouped by category

A summary of the bivariate relationships between the independent and dependent variables is provided in Table 4.3 Age, body mass (BM), lean tissue mass (LTM), fat mass (FM) and knee extensor torque (KE) were significantly correlated with TBaBMD ($0.36 \leq r \geq 0.62$). LTM was the only independent variable that correlated with FNaBMD ($r = 0.35$ P<0. 05). None of the independent variables correlated with FNvolBMD or with GTaBMD. Age, Ht, BM, LTM and FM were correlated with LEGaBMD, with correlations ranging between 0.33 and 0.77. Neither physical activity, daily calcium intake nor estradiol levels correlated significantly with any of the dependent measures.

Results for the multiple linear regression analysis (MLRA) are summarised in Table 4.4. LTM was the only predictor of TBaBMD and FNaBMD, accounting for 46.7% and 15.9 % of the total explained variation, respectively. LTM was the strongest predictor of LEGaBMD, accounting for 57.9% of the total explained variation, with age accounting for an additional 5.6%. Age was the

only significant predictor of FNvolBMD, accounting for 11.7% of the explained variation. Physical activity, dietary calcium intake and level of estradiol failed to enter as significant predictors for any of the key outcome variables.

Table 4.4 Summary of Stepwise Multiple Linear Regression Analysis For Dependent Variables

Dependent Variables	Predictor	Constant	SE	Adjusted R^2	Explained Variance	Cumulative Explained Variance	p
TBaBMD	LTM	.590	.046	.467	46.7%	46.7%	.000**
FNaBMD	LTM	.521	.071	.159	15.9%	15.9%	.005**
FNvolBMD	Age	.440	.081	.117	11.7%	11.7%	.005**
LEGaBMD	LTM		.000	.579	57.9%	57.9%	.000**
	Age	.275	.008	.635	5.6%	63.5%	.004**
GTaBMD	Nil						

* p<.05 **p<.01

4.4 Discussion

A novel finding of the present study was that serum estradiol level, ranging between 11-61 pmol/litre was neither significantly correlated with, nor predictive of any of the BMD measures in this population of prepubertal girls. There is scant information about the relationship between estradiol and BMD in this population; due mostly to limited sensitivity of previous serum estradiol assays techniques. Whereas the role of estrogen in the promotion of linear skeletal growth and skeletal maturation is now clearly established (Cutler, 1997; Juul, 2001) its influence on the development of bone mineralization remains equivocal. Serum estradiol was not correlated with total body BMD in a recent study (Klein et al., 1998) incorporating relatively small samples of non-obese and obese pre- and early pubertal girls or boys, but was significantly correlated with arm BMD in the combined sample, in the boys alone and in the combined non-obese group. The data are consistent with this earlier report for total body BMD, but cannot be compared directly to their other findings, given the differences in measurement sites, maturity status, gender and group composition between studies. The data suggest, however, that estradiol has only a minor influence (perhaps in a permissive role) on the development of total body BMD and BMD at appendicular weight-bearing sites in prepubertal girls.

Participants in this study ranged between 6-11 years of age, and not surprisingly, age was both significantly correlated with, and was a significant independent predictor of aBMD for several sites including FNvolBMD. These findings are consistent with previous studies (De Schepper et al., 1991; Katzman et al., 1991; Molgaard, Thomsen, & Michaelsen, 1998) which have also reported significant positive associations between age and aBMD, or age in combination with various measures of body composition, gender and race at similar sites in prepubertal children. To the author's knowledge, no other study has investigated the relationship between age and FNvolBMD exclusively in prepubertal children. However, in a recent study (Lu et al., 1996) incorporating a broader age range (5.6 - 27 yrs), chronological age was unrelated to FNvolBMD. The weak and non-significant correlation (r=0.13) between age and FNvolBMD in the study in part supports this previous observation; nevertheless, age remained a significant independent determinant of FNvolBMD after adjusting for the influence of other independent variables in the MLRA. This latter association may reflect the underlying influence of increased skeletal maturity, which was not assessed directly in this study. Associations between skeletal maturity and bone mineral density have been observed previously (Gordon, & Webber, 1993; Lu et al., 1996).

LTM was the highest and most consistent correlate of the body size variables with all the dependent variables except FNvolBMD and GTaBMD. Additionally, LTM accounted for a substantial proportion of the total explained variation for TBaBMD (69.5%) and LegaBMD (57.9 %), with a lesser but still significant contribution to FNaBMD (15.9%). The results point to the importance of LTM in optimising bone mineral accrual at select skeletal sites even during the formative growth years preceding puberty. These results are consistent with previous reports of a positive association between LTM and BMD in prepubertal female gymnasts (Courteix, Lespessailles, Jaffre, Obert, & Benhamou, 1999; Courteix et al., 1998) and circum- and post-pubertal girls (Gordon, & Webber, 1993; Lu et al., 1996) but are unique in demonstrating this relationship in normal healthy prepubertal girls. In a recent multi-ethnic study (Ellis et al., 2000), LTM, in combination with either age and gender, or fat mass and gender accounted for a large proportion (94-95%) of the explained variation in TB bone mineral content in 5-18 year old children. The reciprocal relationship of LTM with FNaBMD and volBMD suggests that lean mass influences mostly the size or volume of the FN rather than its intrinsic mineral composition.

Few studies have investigated the association between LTM and aBMD at the GT in normal healthy children. The GT is a predominant site for muscle attachments, and increased muscle mass and muscle contraction forces, secondary to increased LTM might be expected to be positively associated with aBMD at this site. The lack of association between LTM and BMD at the GT suggests either that there was insufficient variability in muscle size and strength among children in this study to detect this association or that muscle size and force per se are not important determinants of aBMD at this site in prepubertal girls. The latter argument is supported by findings from a recent exercise intervention study with pre- and early pubertal children (McKay et al., 2000), in which the change in bone mineral free lean mass accounted for only 5% of the explained variation in change in GTaBMD over an 8 month period. The lack of association of LTM with BMD at the GT as a site of expected importance suggests that the association of LTM with aBMD at other sites may be mediated predominantly by a more systemic contribution to body mass and the influence of increased body mass on skeletal ground reaction forces during habitual weight-bearing activity. The failure of any of the strength measures to enter into the regression models, despite weak to moderately strong univariate correlations, also suggests that muscle strength is a relatively weak determinant of BMD within this age range.

Both body mass and fat mass were positively correlated with TB and leg areal BMD, but not with either of the FN BMD measures. Body mass has been reported as a significant independent or multivariate determinant of BMD in pre- and post-pubertal children (De Schepper et al., 1991; Ellis, Shypailo, Wong, & Abrams, 2000; Glastre et al., 1990; Katzman et al., 1991). Similarly, fat mass, in combination with LTM and gender was recently reported to account for approximately 95% of the explained variation in TB bone mineral content in a multi-ethnic population of children of adolescents (Ellis et al., 2000). None of these studies, however, differentiated fat mass from body mass, and therefore precluded the establishment of an independent relationship between fat mass and BMD. Despite their significant univariate correlations, neither body mass nor fat mass entered as a significant determinant of any of the BMD outcome measures in this study, after adjustment for LTM. These latter findings may reflect a strong degree of collinearity among the body size variables and suggest that a general body size factor incorporating all three variables (LTM, body

mass, fat mass) may be operating as the key determinant of BMD in this study. This general body size and bone relationship may be explained by the mechanism of increased mechanical loading imposed by the combined mass of the different components of body mass during weight-bearing activities (Rice et al., 1993; Turner et al., 1992). Alternatively, fat mass might contribute to this relationship through endocrine mechanisms, most notably by enhancing increased peripheral conversion of androgen precursors to estrogen (Lanyon, 1996). This seems an unlikely explanation, however, for young girls of the age range in this study, as serum estrogen levels were neither significantly correlated with, nor predictive of the variation in BMD for any of the BMD measures. Whether this putative endocrine mechanism takes on more importance in the peripubertal years when body fatness and estrogen levels increase dramatically in females remains to be determined.

Physical activity level was not a correlate of, nor a significant contributor to, the explained variation in any of the BMD variables in this study. These findings are consistent with some (Glastre et al., 1990; Katzman et al., 1991) but not all (Bailey et al., 1999; Gunnes, & Lehmann, 1996; Janz et al., 2001) studies, investigating the association between physical activity and bone measures in children and adolescents. Discrepancies among results of studies may be explained by differences in age groups studied, approaches used to measure physical activity (e.g. questionnaires vs accelerometry), time course for activity assessment (e.g. past year vs past few weeks), the nature of physical activity assessed (e.g. leisure time vs organized sport) and techniques and sites used for measurement of BMD. Within the narrow age range of this study, past year physical activity did not appear to be an important determinant of BMD in prepubertal girls. These findings suggest a fairly homogenous physical activity level among prepubertal girls, after exclusion of competitive sports participation. An optimal level for promotion of bone mineral accrual however, cannot be determined from the results of the present study.

Average daily dietary calcium intake neither correlated with, nor entered the multiple linear regression models as an important determinant of any of the BMD measures in this study, despite wide individual variability in calcium intakes. The finding of a lack of association between calcium intake and bone agrees with previous results from studies of children of similar (Kroger, Kotaniemi,

Kroger, & Alhava, 1993; McKay et al., 2000) or peri-pubertal (Glastre et al., 1990; Katzman et al., 1991) ages, and for girls of a broader age range (Boot et al., 1997), after adjustment for age. However, other studies (Bonjour et al., 1997; Gunnes, & Lehmann, 1996; Johnston et al., 1992; Lee et al., 1994; Ruiz et al., 1995) have reported significant relationships and positive effects of increased dietary calcium intake on BMD in prepubertal children including girls. Differences in dietary assessment techniques, assessment periods, dietary calcium composition and BMD measurement sites may account for discrepancies among studies. Controversy surrounds the issue of whether increased dietary calcium can influence bone mineral accrual generally throughout the skeleton or preferentially at axial (Ruiz et al., 1995) or appendicular (Bonjour et al., 1997; Lee et al., 1994) sites in children. Although the variability was large, the average calcium intake in this study was lower than that reported in studies encompassing a similar age range (Bonjour et al., 1997; Johnston et al., 1992; Ruiz et al., 1995). The lower than normal level of average dietary calcium intake may also account, in part, for the lack of significant relationships with BMD in this study.

4.5 Conclusion

Identification of the determinants of BMD and the utility of MLRA models to accurately predict BMD in any population depend ultimately on the inclusiveness of the considered predictor variables. This investigation included multiple variables representing body size and composition, behaviour (activity and diet), fitness (strength/torque) and biological (estrogen status) influences, each of which are considered plausible determinants of BMD in humans. Results indicated that neither past year physical activity, muscle strength, dietary calcium intake nor estradiol status are independent predictors of BMD in prepubertal girls aged between 6 and 10 years. Body mass, however, and especially the lean tissue component which may be strongly influenced by genetics, is an important determinant of BMD at most sites in this population. The results therefore would suggest the value of attaining and maintaining as high a lean tissue mass as possible for the promotion of bone health.

CHAPTER 5

5.1 Effects of Single-Leg Drop-Landing Exercise from Different Heights On Skeletal Adaptations in Prepubertal Girls

The following randomised, controlled study was undertaken to investigate whether rigorously controlled uni-modal, uni-directional single-leg drop-landing exercises involving low-moderate peak ground-reaction impact forces are osteogenic in the developing prepubertal female skeleton.

Hypotheses to be tested:

1.) That bone adaptive responses will be threshold-dependent and then dose-dependent beyond the threshold in the female prepubertal population.

2.) That impact exercise will have a smaller effect on BMD than on BMC, geometric or biomechanical properties of bone in the female prepubertal population

3.) That trained-leg aBMD ($gm.cm^{-2}$) and volBMD ($gm.cm^{-3}$) of prepubertal girls who have been involved in repeated, weight-bearing, unilateral, muscular contractions at ground reaction forces between 4.54 –4.82 times body weight after an eight-month training period will be greater compared to a control group matched for age and pubertal status.

Bone mass predicts fracture risk (Slemenda et al., 1997) and peak bone mass (PBM), 70-95% of which is achieved by 18 years of age (Magarey et al., 1999) is considered a major determinant of risk for osteoporosis in later life (Bachrach, 2001). Strategies that impact on optimisation and preservation of bone strength, including increasing PBM are therefore required to counter this anticipated global epidemic in osteoporosis.

Although differing by sex and skeletal site, approximately 50% of young adult peak bone mass is achieved before puberty (Magarey et al., 1999; Sabatier et al., 1996). Continued rapid and

substantial gains are made in BMC and areal BMD (aBMD) during the peri-pubertal years (Magarey et al., 1999; Sabatier et al., 1996), followed by a deceleration during late adolescence and early adulthood (Sabatier et al., 1996; Hui et al., 1999). Growth and maturity related changes in volumetric BMD (volBMD) are less clearly established. Studies using dual energy x-ray absorptiometry (DXA) have reported little or no apparent change in appendicular volBMD from childhood to adulthood (Lu et al., 1994), whereas significant gains have been reported for lumbar spine (Gilsanz et al., 1988) and distal radius (Neu, Manz, Rauch, Merkel, & Schoenau, 2001) and mid-femur volBMD (Hoegler et al., 2003) when measured by quantitative computed tomography (QCT), peripheral QCT (pQCT), and magnetic resonance imaging (MRI) respectively, in the transition from pre-puberty to adolescence. Since bone mineral accrual during early and mid-childhood years serves as the platform for PBM, the pre-pubertal years may be considered a sensitive period for initiating potentially synergistic lifestyle interventions such as physical activity for augmentation of bone mineral accrual and optimisation of bone strength (Khan et al., 1998).

An evolving consensus (Bachrach, 2001; Khan et al., 2000) contends that certain weight-bearing exercises are synergistic with growth in augmenting bone mineral accrual during the prepubertal years. Positive associations have been reported between weight-bearing physical activity and bone density in some (Fuchs, Bauer, & Snow, 2001; Scerpell, Davenport, Morganti, Kanaley, & Johnson, 2002) but not all (MacKelvie, McKay, Khan, & Crocke, 2002) studies of prepubertal children. Furthermore, prepubertal children engaged in elite sport involving weight-bearing and high impact types of exercises have higher bone mass and density than non-athletes or athletes involved with weight-supported activity (Bass et al., 1998; Dyson, Blimkie, Davison, Webber, & Adachi, 1997; Faulkner net al., 2003). The best evidence to date, however, in support of an osteogenic effect of exercise in prepubertal children derives from prospective exercise intervention studies. With this design, weight-bearing exercise has resulted in mixed results, with significant increases in bone density or bone mineral content reported in some (Fuchs et al., 2001; Bradney et al., 1998; McKay et al., 2000; Morris, Naughton, Gibbs, Carlson, & Wark, 1997), but not all (MacKelvie et al., 2002; Specker, & Binkley, 2003; Van Langendonck, Claessens, Vlietinck, Derom, & Beunen, 2003) studies. Exercise prescription and loading however, have been varied and largely unquantifiable.

Without exception, the physical activity programs incorporated in the aforementioned prospective training studies were mixed in nature, incorporating either combinations of aerobic and anaerobic weight-bearing activity (sometimes in combination with weightlifting or climbing), or variable levels of impact loading with uncontrolled pacing, variable directional application of loads and uncertain individual compliance. From a public health perspective, these variably controlled studies may be warranted as an initial step in addressing the efficacy and general applicability of exercise to affect skeletal health. Nevertheless, it is difficult with mixed designs to differentiate the important parameters of the exercise program responsible for the significant skeletal adaptive responses reported in these studies. Differentiation of the osteogenic parameters of exercise through more strictly controlled research designs are required to advance understanding of the efficiency, specificity and safety of exercise prescription for promotion of general musculoskeletal and site-specific skeletal health enhancement in children.

This study reports the effects of an investigator-supervised, controlled, uni-modal and uni-directional single-leg drop-landing exercise program from two different heights (variable magnitude loading) on bone mineral accrual in normal, non-athletic, prepubertal females. It was hypothesized that skeletal adaptations, whether in mineral mass, volumetric density or material properties would be larger following 28 weeks of intervention in: (i) the loaded versus unloaded leg in both exercise intervention groups, (ii) the loaded leg in the intervention groups versus the comparison leg in the controls, and (iii) the loaded leg in the exercise group exposed to the higher compared to the lower drop-landing height.

5.2 Primary Bone Mineral Outcome Measures

Bone mineral content (BMC; g) was determined by DXA. Separate scans were taken for the total body and hip. From the whole body scan, the regional analysis facility was used to measure whole limb and mid-femur BMC of each leg separately. The whole leg region of interest (ROI) was defined on the total body scan superiorly, by the oblique line through the femoral neck, medially, by the vertical line separating both legs and laterally, by the vertical line on the lateral aspect of each leg. The mid-femur region was identified by measuring femur length of the left leg (proximal tip of

the greater trochanter to the distal most point of the medial condyle) using the ruler function, calculating 1/3 of this length and placing a ROI box of identical length over this region, anchored at the distal end of the upper third of the femur. An identical box was created and transferred to the right leg. Femoral neck and greater trochanter BMC were determined using separate hip scans. Measurements of the greater trochanter were made using the lower line of the femoral neck region of interest as the upper limit and a line set at a 45^0 angle to the actual scan path as the lower limit (DPX Technical Manual). Estimates of volumetric BMD (g/cm^3) for the femoral neck (FNvolBMD) and mid-femoral shaft (MFSvolBMD) were made using the approach described by Lu et al., (1994). MFS cortical volumetric BMD was determined using BMC from DXA and MRI measures of bone volume, using a previously validated technique (Woodhead et al., 2001). To minimize experimenter bias, all post-test bone mineral outcome measures were analysed without knowing participant group assignment. An independent observer assigned codes to all post-test data, which were subsequently decoded following completion of data analyses. These measures of bone mineral are made routinely with high reliability and reproducibility in our centre (coefficient of variation ranging between 0.97-1.98 %). More detailed descriptions of methods are available in Chapter 3.

5.3 Safety

Initially, after each training session and then subsequently, at the end of each week of training, every child in the exercise groups was questioned regarding pain or discomfort associated with, or resulting from the exercise intervention.

5.4 Results

5.4.1 Anthropometry

The baseline and change data for the physical and descriptive characteristics are shown in Table 5.1 for the 3-group design. Data not normally distributed were log transformed prior to analysis. Girls ranged between 6 and 10 years and all were prepubertal at baseline and follow-up. Pubertal assessment was based on Tanner's criteria for breast and pubic hair development and estradiol levels (Cutler, 1997; Klein et al., 1998). Stature and LTM were not different among groups at baseline, but FM and percent body fat %BF were greater in the HD group compared to the

controls, and body mass approached significance with higher values in the HD and LD groups compared to controls. Age, stature, body mass and LTM increased ($p<0.05$) over the intervention period (main effect for time), but no differences among groups for changes in any of these variables were observed. The change in LTM approached significance ($p=0.069$), with a tendency towards larger gains for both exercise groups compared to controls.

5.4.2 Physical Activity, Calcium and Estradiol

No differences ($p>0.05$) among the 3 groups at baseline and no differences among groups in magnitude of change in leisure time physical activity level or dietary calcium intake (Table 5.1). Serum E_2 levels were similar among groups at the end of the study (Table 4.1).

5.4.3 Training Load Quantification

Single-leg relative (to body weight-BW) PGRIFs (Pedar Mobile system, Novell, Munich, 1999) for sub-samples (n=3 each) from LD and HD groups were 2.5 - 3.5 and 2.9 – 3.8 x BW, respectively, at the mid-point of the study (wk 14) and 2.7 – 3.6 and 3.6 – 4.4 x BW during a week approaching the completion of the study. Drop-landing kinetic energy ($KE = ½\ m \cdot v^2$; m=mass, v = velocity) estimated for the mid-point of training varied by exercise group; 39.42 ± 6.99 J vs 79.84 ± 12.59 J; mean $\pm$ SD for LD and HD groups, respectively; $p>0.000$. Force platform data collected post hoc from a subset of 3 participants not involved in the study to assess peak rate of force development varied by drop height (1708 ± 670 N/s – LD group and 1498 ± 161 N/s- HD group).

5.4.4 Primary Bone Mineral Outcome Measures

No differences ($p>0.05$) among groups or group x leg interaction effects were observed for any of the unadjusted (Table 5.2 and Table 5.3) or ANCOVA adjusted (Figures 5.1 – 5.10; Appendix O1 and Figures 5.11 – 5.16; Appendix O2) baseline and change data for the primary BMC or BMD measures. As well, no changes ($p> 0.05$) in the magnitude of differences between the dominant and non-dominant limbs from baseline to the end of the study within any of the 3 groups were detected (Table 5.4). All bone mineral content ($p< .01$), but none of the volBMD measures increased over the course of the study (main time effect).

Table 5.1 Physical and lifestyle characteristics. Baseline Means±SD: Change Means±SD

	Baseline				***Change following intervention***			
	Controls	***Low Drop (LD)***	***High Drop (HD)***		***Controls***	***Low Drop (LD)***	***High Drop (HD)***	
N	14	13	13	***p***	14	13	13	***p***
Age (yr)	7.87 ± 0.77	7.79± 0.94	7.89± 1.12	.957	0.77± 0.05	0.79± 0.03	0.77± 0.03	.241
Height (cm)	125.24 ± 6.00	128.82± 5.95	127.13± 2.89	.213	3.41± 2.49	2.95± 2.24	4.45± 2.69	.294
Body Mass (kg)	24.35 ± 3.15	27.51± 5.17	27.99± 4.30	.065	1.37± 2.45	2.41± 0.68	2.14± 1.28	.257
Lean Tissue (kg)	17.93 ± 2.33	19.09± 2.53	18.99± 2.27	.379	1.59± 0.87	2.42± 0.83	2.42± 1.36	.069
Fat Mass (kg)	4.80 ± 1.39	6.89± 3.22	7.38± 3.20•	**.042**	0.30± 1.00	-0.24± 1.41	-0.16± 0.91	.410
% Body Fat	20.21 ± 4.14	24.72± 6.14	26.25± 7.93•	**.042**	-0.26± 3.66	-2.49± 3.94	-2.71± 3.37	.169
Leisure Time Physical Activity ($h \cdot w^{-1}$)	3.03± 1.97	2.39± 1.96	2.18 ± 1.62	.474	0.99± 1.77	-0.17± 1.77	0.38± 3.04	.422
Calcium Intake ($mg.\ d^{-1}$)	537± 245	604± 291	582 ± 251	.798	-132± 254	-131± 243	-51± 335	.698

• High Drop group mean greater than Control ($p<0.05$)

Table 5.2 Unadjusted Data – BMC. Baseline Means ± SD: Change Means ± SD

	Baseline							***Change following intervention***						
	Controls		***Low Drop (LD)***		***High Drop (HD)***			***Controls***		***Low Drop (LD)***		***High Drop (HD)***		
N	14		13		13		**p**	14		13		13		**p**
Total BodyBMC (g)	800.07	± 133.30	899.01	± 194.39	912.34	± 140.36	.142	79.59	± 34.89	100.22	± 24.10	91.89	± 46.33	.339
ND LegLEGBMC (g)	115.17	± 28.67	135.60	± 40.16	133.09	± 22.07	.189	19.06	± 9.31	25.50	± 9.91	25.46	± 10.18	.156
ND Leg FNBMC (g)	1.69	± 0.20	1.75	± 0.24	1.78	± 0.21	.576	0.14	± 0.34	0.11	± 0.33	0.15	± 0.38	.959[#]
ND Leg GTBMC (g)	2.59	± 0.56	3.22	± 1.25	3.00	± 0.76	.198	0.37	± 0.68	0.06	± 1.33	0.26	± 0.99	.672[#]
* ND Leg MFSBMC (g)	19.28	± 4.22	21.99	± 5.74	22.05	± 2.95	.207	3.87	± 1.45	3.87	± 0.99	3.42	± 1.88	.207
D LegLEGBMC (g)	114.35	±27.77	129.50	±40.29	126.68	±19.80	.293	17.60	± 9.26	24.31	± 9.35	24.75	± 10.67	.092[#]
D Leg FNBMC (g)	1.73	±0.23	1.64	±0.21	1.68	±0.21	.652	0.16	± 0.43	0.16	± 0.27	0.25	± 0.30	.746[#]
D Leg GTBMC (g)	2.77	±0.84	3.01	±1.25	2.82	±0.73	.727	0.40	± 1.21	0.27	± 0.75	0.65	± 0.84	.589[#]
D Leg MFSBMC (g)	19.28	±3.97	21.58	±5.55	21.58	±3.15	.339	4.08	± 1.42	3.69	± 1.73	3.50	± 2.16	.956[#]

*N=13 for control group. 1 non-dominant baseline scan unable to be analysed due to movement artefact.

[#] log transformed data

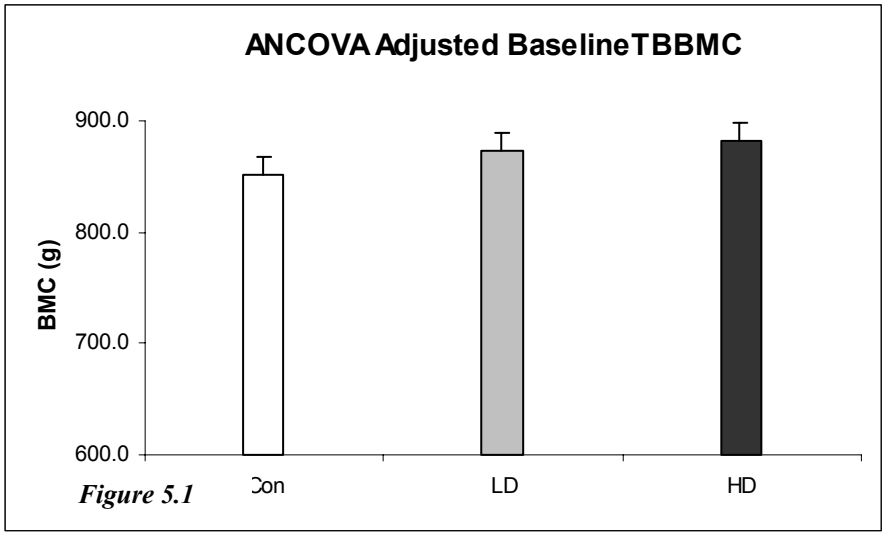


Figure 5.1

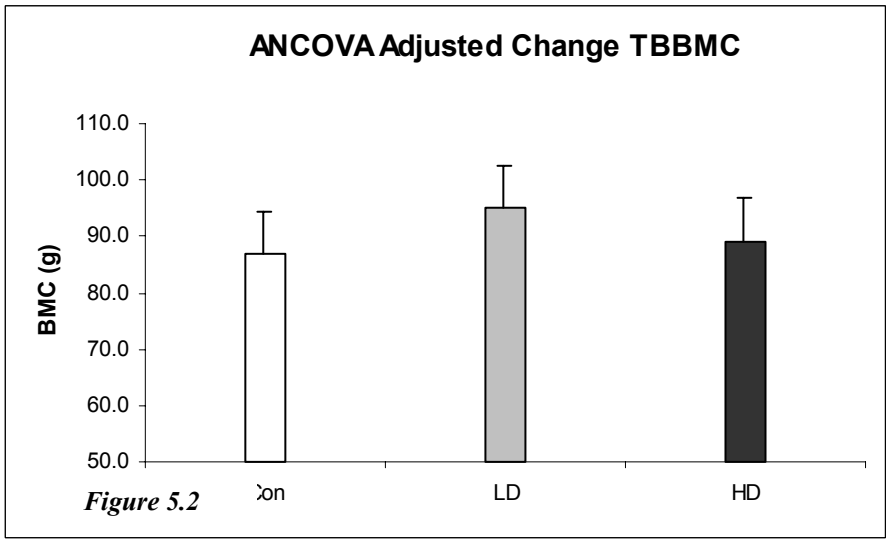


Figure 5.2

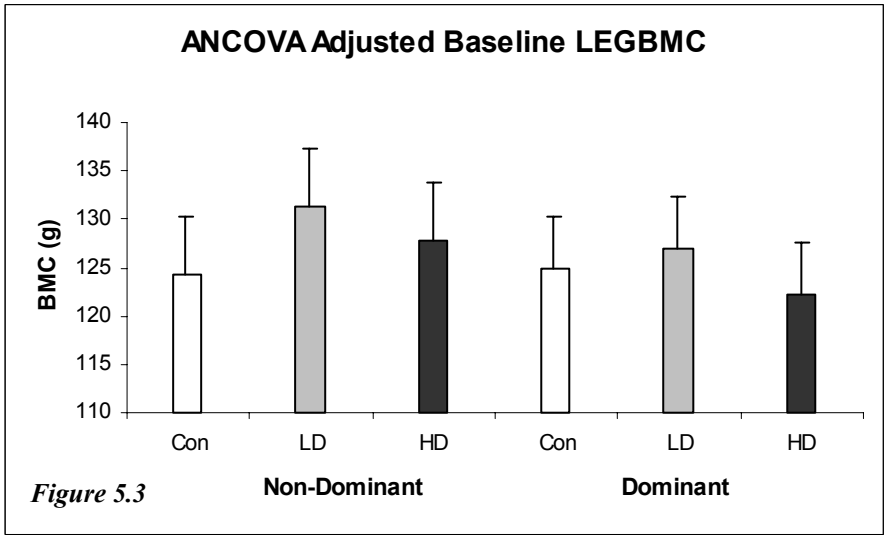


Figure 5.3

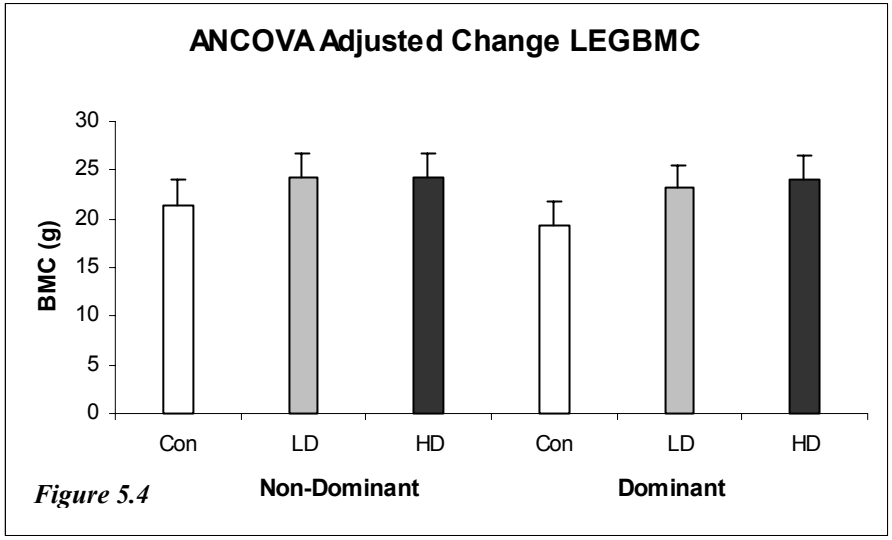


Figure 5.4

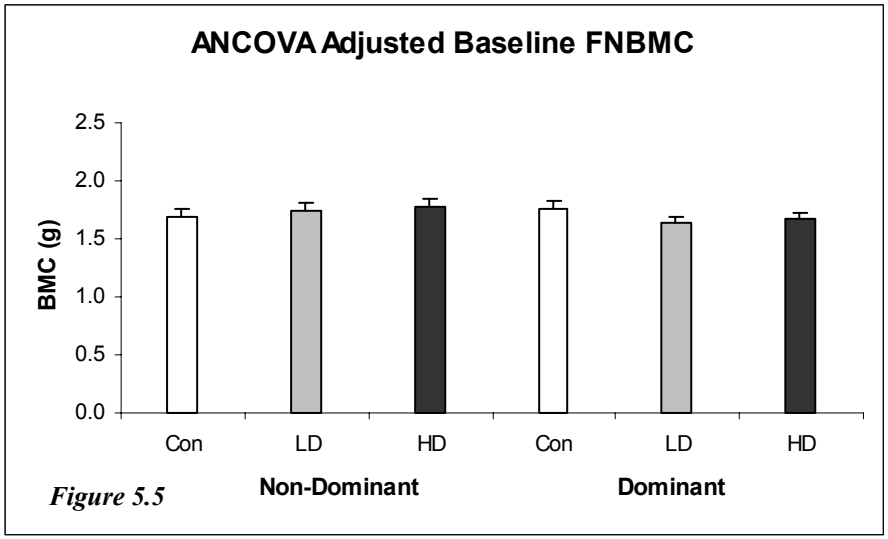
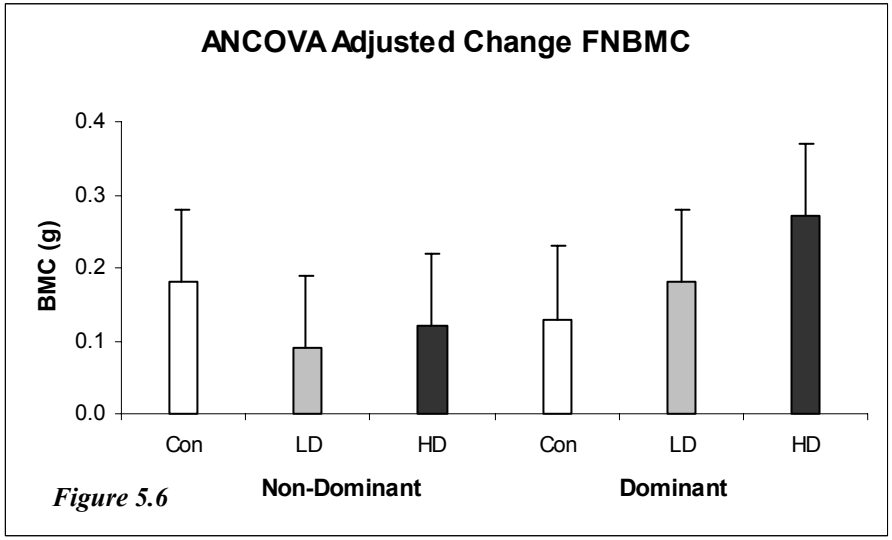


Figure 5.5



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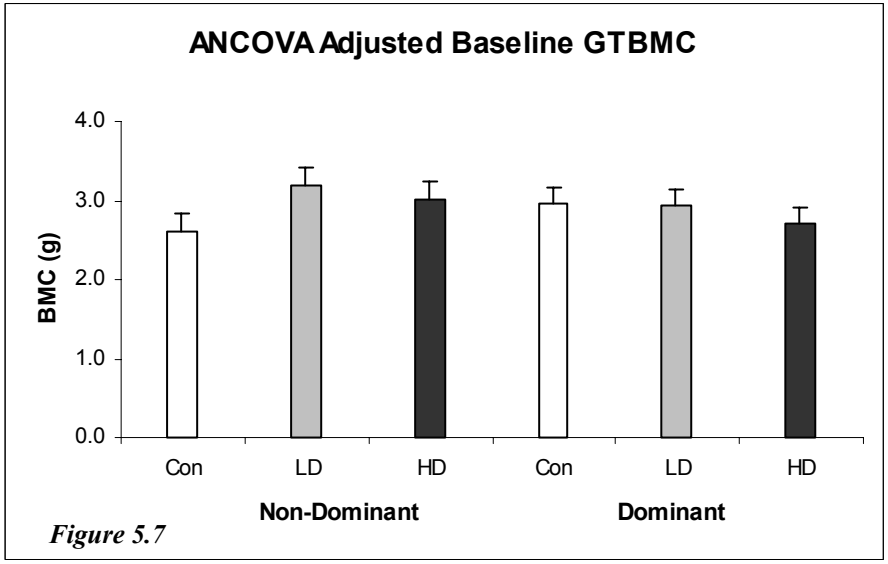

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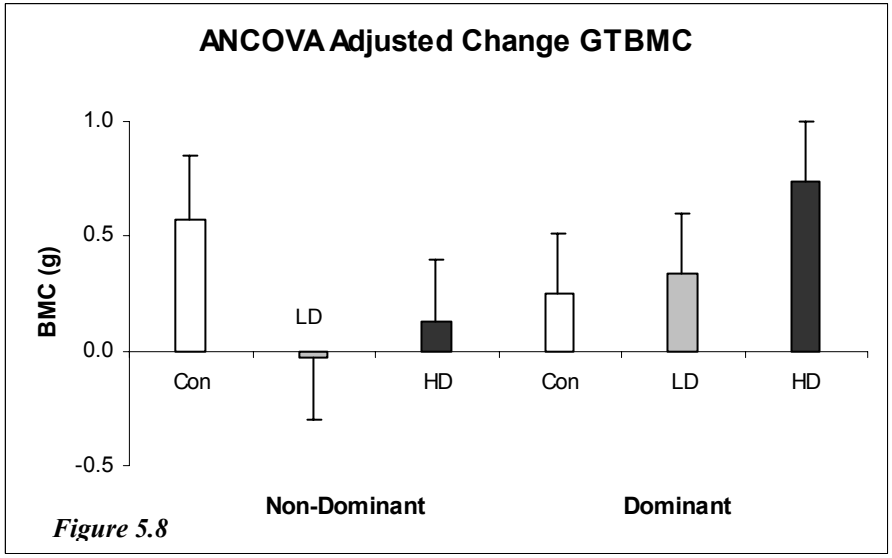

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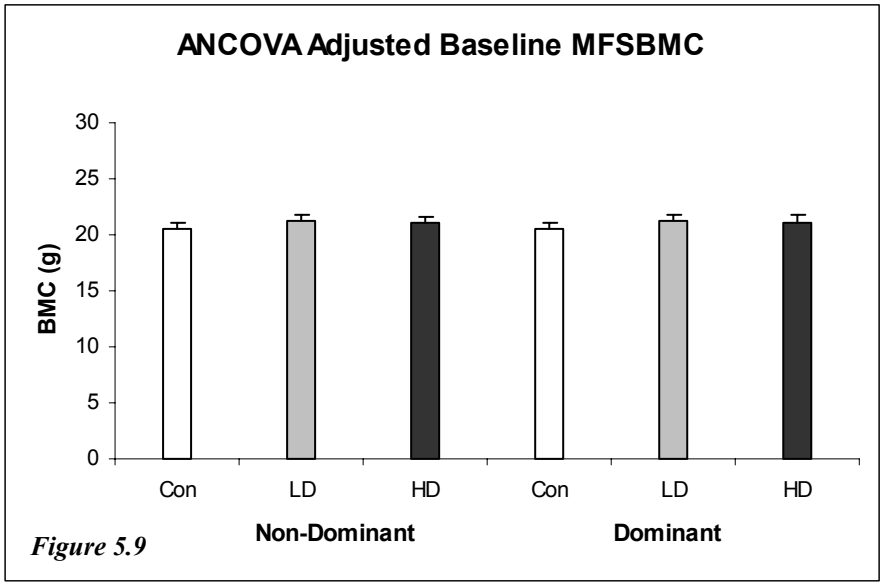


Figure 5.9

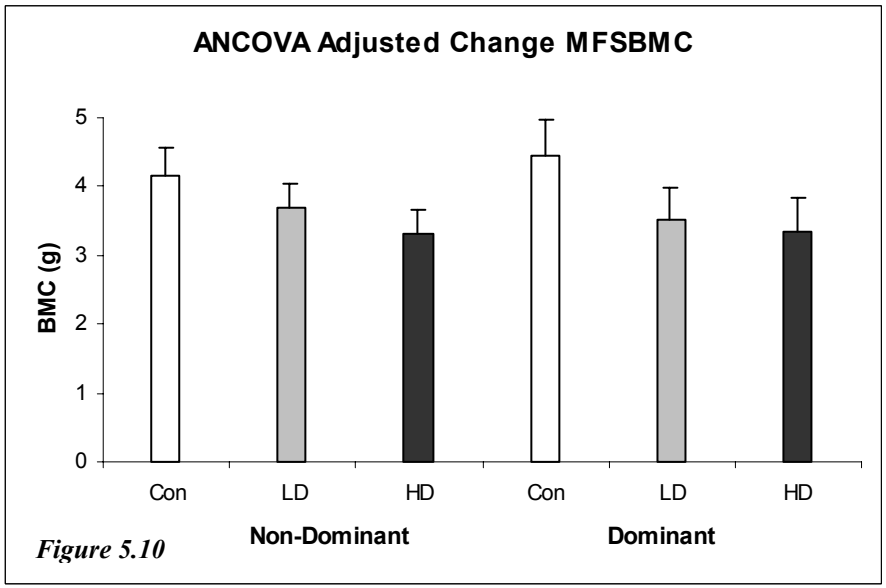


Figure 5.10

Table 5.3 Unadjusted Data - Volumetric BMD for femoral neck, total mid femoral shaft and mid femur shaft cortex. Baseline Means±SD: Change Means±SD

	Baseline				***Change following intervention***			
	Controls	***Low Drop (LD)***	***High Drop (HD)***		***Controls***	***Low Drop (LD)***	***High Drop (HD)***	
N	14	13	13	**p**	14	13	13	**p**
ND Leg femoral neck vol BMD (g/cm^3)	0.648 ± 0.006	0.611 ± 0.007	0.623 ± 0.006	.318	-0.009 ± 0.057	0.033 ± 0.054	-0.009 ± 0.085	.143[#]
ND Leg mid fem shaft vol BMD (g/cm^3)	0.737 ± 0.009	0.731 ± 0.009	0.715 ± 0.006	.774	0.029 ± 0.036	0.041 ± 0.058	0.053 ± 0.053	.539[#]
[δ]ND Leg mid femoral shaft cortical volBMD(g/cm^3)	1.112 ± 0.078	1.132 ± 0.134	1.182 ± 0.121	.312[#]	-0.008 ± 0.099	-0.015 ± 0.092	-0.029 ± 0.082	.229[#]
D Leg femoral neck vol BMD (g/cm^3)	0.627 ± 0.008	0.576 ± 0.007	0.587 ± 0.005	.106	0.017 ± 0.071	0.040 ± 0.074	0.022 ± 0.047	.550[#]
D Leg mid fem shaft vol BMD (g/cm^3)	0.771 ± 0.010	0.755 ± 0.008	0.745 ± 0.006	.718	-0.003 ± 0.060	0.006 ± 0.064	-0.012 ± 0.070	.744[#]
[δ] D Leg mid fem shaft cortical volBMD(g/cm^3)	1.100 ± 0.067	1.111 ± 0.101	1.155 ± 0.092	.271[#]	0.004 ± 0.075	0.006 ± 0.107	0.016 ± 0.081	.210[#]

[δ] N= 11 for Control group. 1 participant refused to participate in the MRI procedure, 1participant DXA and 1 participant's MRI images were unusable due to movement artifact.
[#]log transformed data

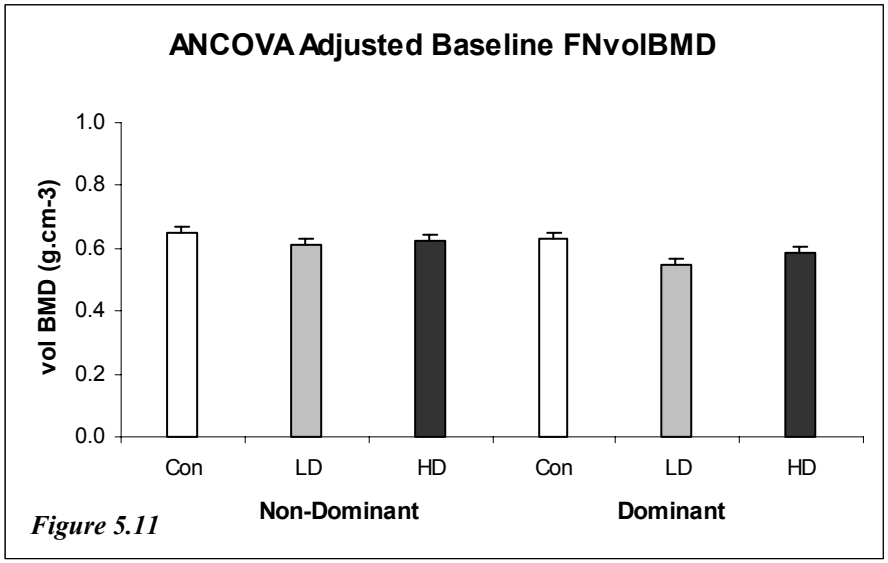


Figure 5.11

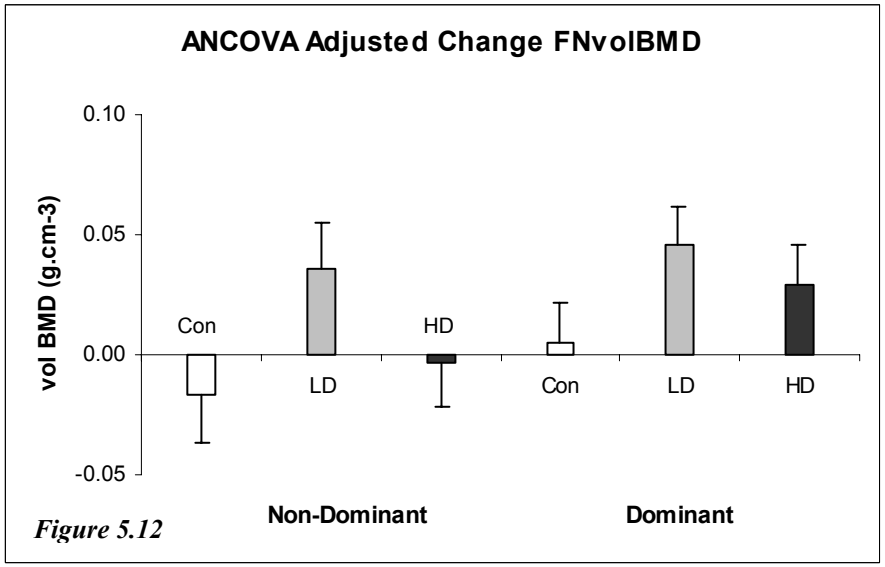


Figure 5.12

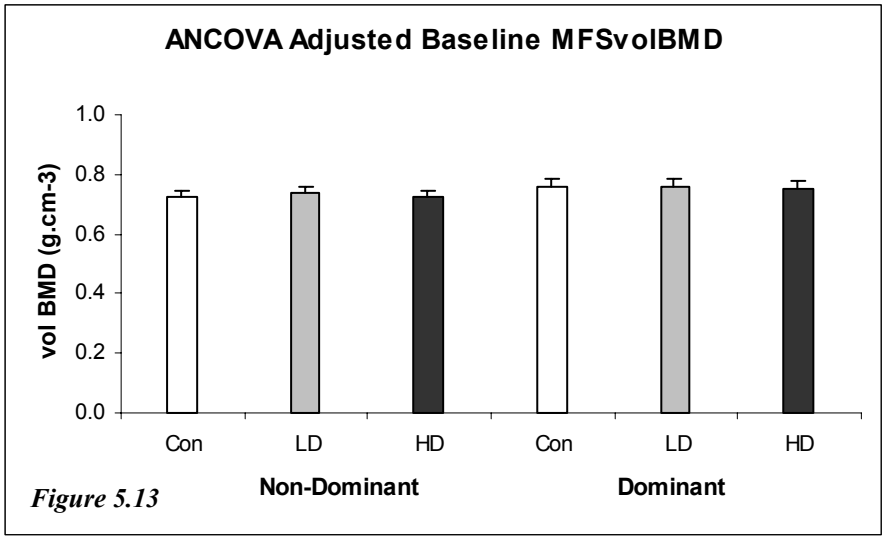


Figure 5.13

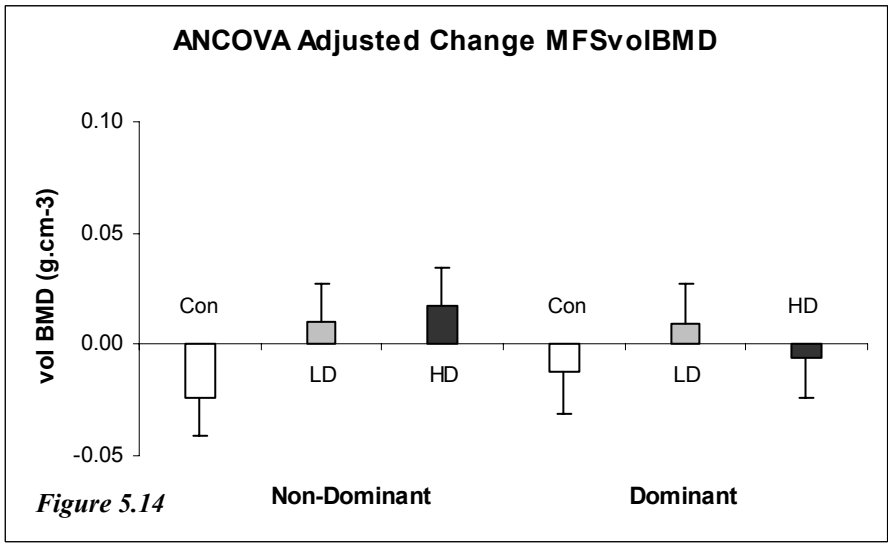


Figure 5.14

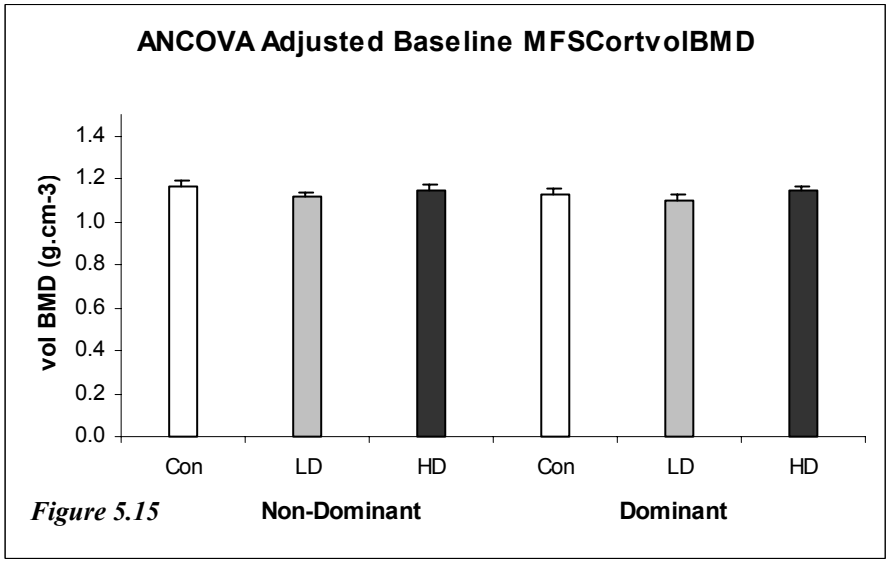


Figure 5.15

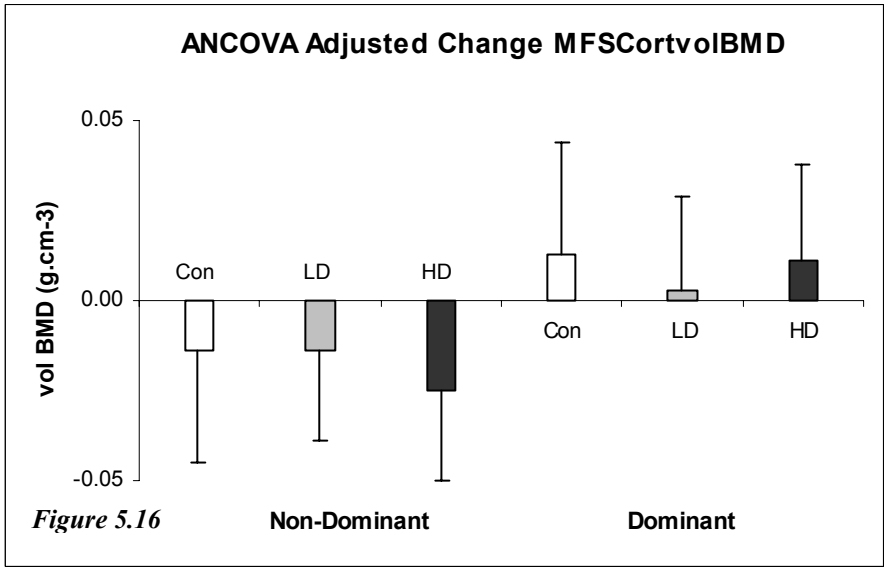


Figure 5.16

Table 5.4 Within-group baseline and post-intervention comparison of differences between dominant and non-dominant limbs for BMC, vol BMD and ultrasound measures Means±SD

	Baseline			***Post Intervention***		
	Non-Dominant Leg	***Dominant Leg***		***Non-Dominant Leg***	***Dominant Leg***	
CONTROL (n=14)			**p**			**p**
Leg BMC(g)	115.17±28.67	114.35±27.77	.690	134.23±31.21	131.95±29.64	.569
FN BMC(g)	1.69±0.20	1.73±0.23	.629	1.83±0.27	1.89±0.28	.518
GT BMC(g)	2.59±0.56	2.77±0.84	.299	2.96±0.94	3.16±0.85	.417
ɜMFS BMC(g)	19.28±4.22	19.63±3.90	.396	23.03±4.40	23.44±4.60	.310
FN vol BMD($g.cm^{-3}$)	0.648±0.064	0.627±0.075	.209	0.640±0.064	0.645±0.054	.738
MFS vol BMD($g.cm^{-3}$)	0.737±0.090	0.771±0.098	.060	0.724±0.082	0.768±0.076	.005
ϑMFS cortical vol BMD($g.cm^{-3}$)	1.112±0.078	1.100±0.067	.738	1.098±0.074	1.114±0.060	.609
Calcaneal BUA ($dB.MHz^{-1}$)	48.4±10.4	48.6±10.4	.904	46.9±9.4	45.0±8.2	.241
Calcaneal VOS ($m.sec^{-1}$)	1662±23	1660±25	.648	1659±24	1658±24	.850
LOW DROP (n=13)						
Leg BMC(g)	135.599±40.161	131.905±40.870	.090	161.099±43.558	156.215±44.126	.204
FN BMC(g)	1.752±0.238	1.649±0.215	.226	1.861±0.262	1.810±0.255	.587
GT BMC(g)	3.217±1.253	3.060±1.292	.718	3.275±1.076	3.333±1.083	.803
MFS BMC(g)	21.987±5.740	21.958±5.580	.945	25.852±5.787	25.648±5.953	.738
FN vol BMD($g.cm^{-3}$)	0.611±0.068	0.576±0.066	.020	0.644±0.069	0.616±0.076	.083
MFS vol BMD($g.cm^{-3}$)	0.731±0.087	0.755±0.083	.428	0.736±0.086	0.761±0.075	.280
MFS cortical vol BMD($g.cm^{-3}$)	1.132±0.134	1.111±0.100	.477	1.117±0.094	1.117±0.072	1.000
Calcaneal BUA ($dB.MHz^{-1}$)	50.1±9.8	48.2±9.2	.244	52.2±8.9	49.7±8.7	.134
Calcaneal VOS ($m.sec^{-1}$)	1658±28	1652±24		1660±23	1662±23	.592
HIGH DROP (n=13)						
Leg BMC(g)	133.089±22.070	128.567±19.254	.082	158.547±29.191	153.319±23.982	.078
FN BMC(g)	1.777±0.213	1.687±0.221	.146	1.924±0.315	1.934±0.210	.937
GT BMC(g)	3.000±0.763	2.830±0.755	.423	3.261±0.747	3.480±0.701	.520
MFS BMC(g)	22.052±2.952	21.883±3.047	.574	25.467±3.764	25.387±3.997	.776
FN vol BMD($g.cm^{-3}$)	0.623±0.060	0.587±0.048	.076	0.615±0.076	0.608±0.068	.542
MFS vol BMD($g.cm^{-3}$)	0.715±0.063	0.745±0.064	.070	0.725±0.064	0.733±0.085	.702
MFS cortical vol BMD($g.cm^{-3}$)	1.182±0.121	1.155±0.092	.303	1.152±0.112	1.169±0.120	.431
Calcaneal BUA ($dB.MHz^{-1}$)	48.1±12.4	46.9±12.0	.638	48.1±12.7	47.7±12.6	.862
Calcaneal VOS ($m.sec^{-1}$)	1652±26	1655±32	.524	1655±32	1657±33	.586

ɜ N=13 for control group. 1participant DXA was unusable due to movement artifact.

ϑN=11 for control group 1 participant refused to participate in the MRI procedure, 1participant DXA and 1 participant's MRI images were unusable due to movement artifact.

Table 5.5 Unadjusted Data - Dominant and non-dominant ultrasound. Baseline Means±SD: Change Means±SD

	Baseline				***Change following intervention***			
	Controls	***Low Drop (LD)***	***High Drop (HD)***		***Controls***	***Low Drop (LD)***	***High Drop (HD)***	
N	14	13	13	**p**	14	13	13	**p**
ND BUA ($dB.MHz^{-1}$)	48.39 ± 10.38	50.13 ± 9.81	48.08 ± 12.43	.875	-1.46 ± 4.52	2.06 ± 5.72	-0.02 ± 5.44	.158[#]
Dom BUA ($dB.MHz^{-1}$)	48.63 ± 10.45	48.16 ± 9.87	46.86 ± 11.95	.905	-3.67 ± 4.87	1.57 ± 5.15	0.87 ± 7.57	.069[#]
ND VOS ($m.sec^{-1}$)	1662 ± 23	1658 ± 28	1652 ± 26	.557	-3.50 ± 8.86	2.62 ± 15.14	3.23 ± 18.24	.444[#]
Dom VOS ($m.sec^{-1}$) [#]	1660 ± 25	1652 ± 24	1655 ± 32	.728	-1.93 ± 11.21	7.58 ± 10.47	2.00 ± 10.37	.052[#]

[#] N=12 in Change data for LD group where adjustment made for 1 outlier greater than 2 SD

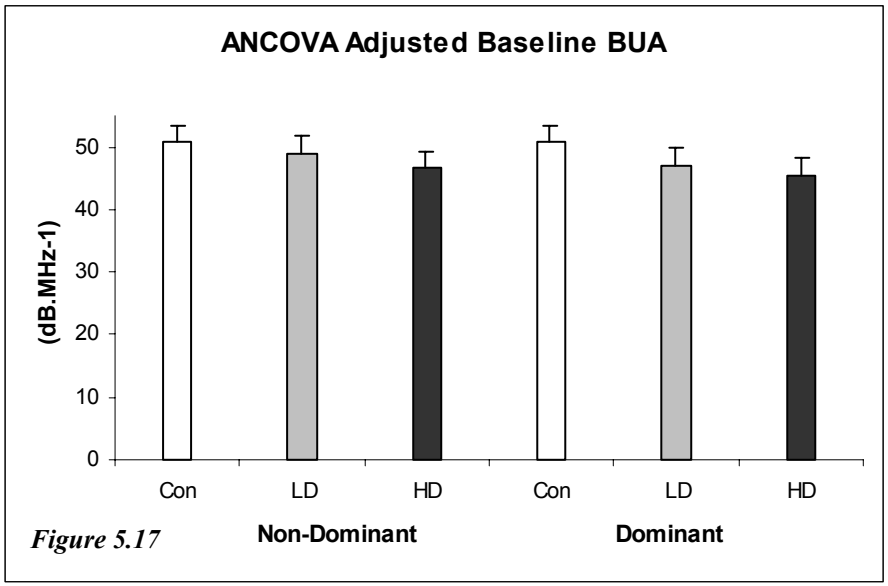


Figure 5.17

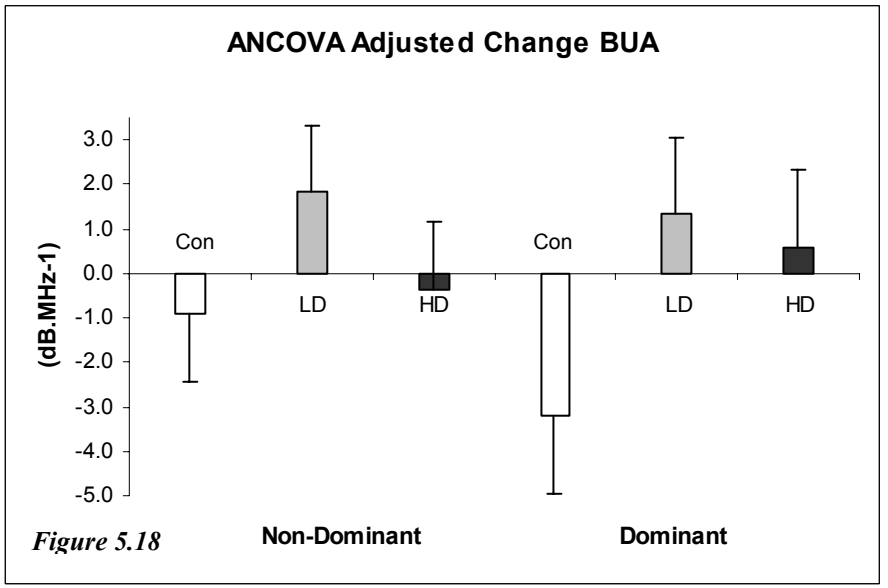


Figure 5.18

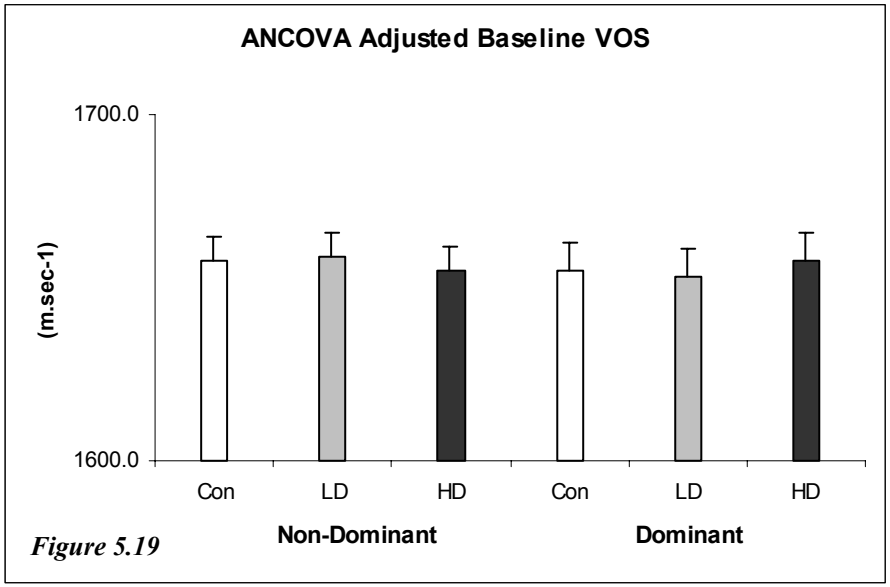


Figure 5.19

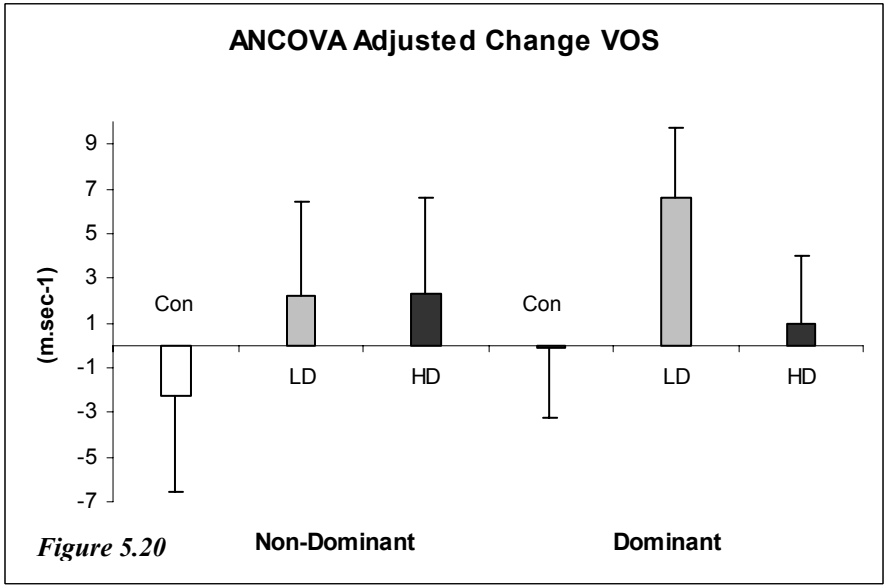


Figure 5.20

5.4.5 Bone Material Properties

No differences (p> 0.05) among groups or group x leg interaction effects for any of the unadjusted (Table 5.5) or adjusted (Figures 5.17 – 5.20; Appendix O3) baseline and change measures for calcaneal BUA or VOS were observed. Similarly, no changes (p> 0.05) in the magnitude of differences between the dominant and non-dominant limbs from baseline to the end of the study within any of the 3 groups were detected (Table 5.4). Neither BUA nor VOS changed over time (p values between .134 and .862).

No within group, between group, or group x leg interactions for any of the primary bone mineral outcome or ultrasound variables were identified when the exercise groups were combined (p> 0.05).

5.5 Discussion

The key findings in the study indicate that single-leg drop-jumping exercises from heights up to 28 cm do not provide a sufficient loading stimulus to elicit an osteogenic response in bone mineral accrual or bone material properties in normally active, non-calcium supplemented prepubertal girls. These findings also suggest that moderate loading magnitude (2.9-4.4 x BW - HD landing group) PGRIFs may not be sufficient, in and of themselves, to induce osteogenic adaptations in the immature human skeleton. These findings suggest that the significant increases in bone mineral accrual reported in previous studies may be coincidental with, but not exclusively explained by the magnitude and nature of the ground reaction impact forces, inherent in their loading regimens.

Results at the outset appear to conflict with findings of significant positive increases in bone mineral accrual at weight-bearing sites, attributable to impact loading exercise in prepubertal girls, reported in some studies (Fuchs et al., 2001; McKay et al., 2000; Morris et al., 1997; Courteix et al., 1998). Several recent studies however, support these findings (MacKelvie et al., 2002; Petit et al., 2002). With few exceptions (Fuchs et al., 2001), the application and quantification of the magnitude of impact loads have been poorly controlled and usually confounded by other potential osteogenic influences (e.g. individual variability in rate of loading, number of loading cycles, and

distribution or direction of applied strains) in the majority of these studies. These concerns are perhaps even more relevant for the recent spate of multi-school, class-based intervention trials (Fuchs et al., 2001; MacKelvie et al., 2002; Bradney et al., 1998; McKay et al., 2000; Petit et al., 2002) with school based, rather than individual randomisation. These trials rely on multiple trainers and supervision at the group rather than individual level, resulting in poorer control over the nature and quantification of loads, and individual participation rates. Given the variability and aforementioned limitations in study design, conclusions regarding the osteogenic importance of the magnitude of impact forces themselves, in exercise studies of children must be interpreted cautiously.

The recent study by Fuchs et al. (2001) is closest to this study with respect to the children's maturity status, and the type and application of the loading stimulus, which involved drop jumps. Assuming an equal bilateral distribution of forces, the peak single-leg GRIFs in this study was mid-way between the LD and HD loading conditions at approximately 4.25 x BW. However, the reported GRIF's in the Fuchs et al., (2001) study represent loading due to the drop phase of the exercise protocol only, and do not account for muscle force loads elicited in executing the subsequent countermovement jump at the end of each drop landing, or the positive work in climbing to the top of the boxes to initiate the exercise cycle. Like this study, all jumps were performed in a uni-planar direction, but children also performed multi-directional movements between boxes, at variable pace, in the execution of supplementary exercise sets. Additionally, differences in bone strain rate (Mosley, & Lanyon, 1998) may partially explain the discrepant findings between this study and the study by Fuchs et al. (2001). The relationship between drop jump height and bone strain rate may not be linear and appears to be influenced by individual landing strategies (Milgrom et al., 2000). Strain rates were surprisingly higher in the LD compared to HD group, but not reported in the study by Fuchs et al., (2001).

While the increase in BMC in the Fuchs' study (2001) can rightly be attributed to the exercise intervention program, it is difficult with their design to separate the relative contributions of the ground impact or hip-joint reaction forces from the potential additional influences of increased muscle forces acting on the skeleton and the varied strain distribution patterns experienced by

participants between jumping sequences. The present study also used uni-planar drop-landings but controlled for group differences in both muscle activity during the non-jumping phases of the exercise protocol and extraneous strain distribution patterns between exercise sets. Differences between these findings and those of Fuchs et al., (2001) therefore, may be explained in part by these subtle, but nonetheless significant differences in exercise protocol.

This study is also similar in terms of the number of impacts and the relative (to body weight) magnitude of loads to the studies on adult females conducted by Bassey, & Ramsdale, (1994) and Bassey, Rothwell, Littlewood, & Pye, (1998). The latter study, however, demonstrated significant increases in various measures of BMD in young adult and older pre-menopausal women with as few as 6 months of exercise intervention. This, despite the supposed lower mechano-sensitivity of the more mature, compared to the still developing pediatric skeleton (Forwood, & Burr, 1993; Seeman, 2002). The major difference compared to the findings of this study (besides age) was that the women in these studies exercised daily rather than 3 times per week, therefore performing substantially more work than the girls in the present study.

Direct comparisons of the present study with those of Fuchs et al. (2001) and other recent studies of pre- and peri-pubertal children (MacKelvie et al., 2002; Bradney et al., 1998; McKay et al., 2000; Morris et al., 1997; Specker, & Binkley, 2003; Petit et al., 2002) are problematic, given differences in other exercise or environmental parameters besides load magnitude, which have been shown in animal studies to elicit an osteogenic response. For example, no two studies of children have used the same frequency of loading, the duration and number of training sessions per week have varied between studies, and some studies used a constant loading stimulus while others have increased the loading intensity progressively throughout the intervention program. There is no conclusive evidence to suggest which combination of exercise parameters is optimal for enhancement of bone development in children or whether in fact the osteogenic response in children is dependent on progressive intensity loading.

This study as well as others (Fuchs et al., 2001; MacKelvie et al., 2002; McKay et al., 2000; Petit et al., 2002) has relied on quantification of the GRIFs as a surrogate measure of intrinsic strain in

loaded bones during exercise intervention studies of children. Competitive gymnastics is characterized by high impact loads reaching peaks of 10-18 times body weight (McNitt-Gray, 1993), and pre- and peri-pubertal female gymnasts have been reported to substantially increase BMD at weight-bearing sites compared to maturity matched controls (Bass et al., 1998; Dyson et al., 1997; Faulkner et al., 2003). Similarly, significant increases in BMD were reported in prepubertal children exposed to double-leg exercise GRI forces ranging between 3.5 – 8.5 times body weight (1.75-4.25 x BW for single-leg equivalent loading), with perhaps slightly larger gains with higher loads (Fuchs et al., 2001; MacKelvie et al., 2002; McKay et al., 2000; Van Langendonck et al., 2003; Petit et al., 2002; Bauer, Fuchs, Smith, & Snow, 2001). Joint reaction forces more proximal to the foot e.g. at the hip, appear attenuated (Bauer et al., 2001), but may be an underestimate of true hip joint forces for lack of consideration of muscle force data in model generation. The assumption underlying these studies, although never tested experimentally in children, is that bone strain varies in relation to the magnitude of the ground reaction force, which itself varies with jump or drop height or direction. The inter-relationships between jump or drop height, PGRIFs and bone strain, however, are complex, not necessarily linear, and participant to individual movement strategy regarding neuromuscular control mechanisms (Duncan et al., 2002). In this study, a two-fold increase in drop height (from 14 - 28 cm) only increased the PGRIF by approximately 30%, despite an estimated doubling in drop height kinetic energy. Furthermore, in a recent study, peak tibial in-vivo tension, compression and shear strains did not differ in a small sample of adults across drop heights of 26, 39 and 52 cm (Milgrom et al., 2000). It is a misconception that drop height will be proportionally related to bone strain. is a misconception. Evidence from motor control literature (eg equilibrium point hypothesis Biryukova et al, 1999; Feldman and Levin, 1995; Gomi and Kawato, 1996), indicated that muscle activity can alter the stiffness of the system by modulating muscle forces, in anticipation of the impending movement which will alter the strain in bone. Likewise, Hsieh and Turner (2001) revealed that the relationship between loading magnitude and bone formation is different for different loading rates, suggesting that loading rates alter the viscoelastic response of the tissue, and hence strain will be different.

It is difficult with the uncertainty regarding these biomechanical relationships to ascribe a direct cause–effect relationship between PGRIFs and the osteogenic adaptive response reported in

exercise intervention protocols involving children. In this regard, the correlation between average ground reaction forces and percent BMC change in the drop jump study of Fuchs et al (2001) was non-significant (Bauer et al., 2001), suggesting that other unaccounted mechanical parameters were perhaps more important determinants of the skeletal adaptive response than load magnitude and resultant GRIFs. It appears highly unlikely that the difference in bone adaptation among these studies, including the present study, can be ascribed solely to differences in bone strain inherent with different levels of PGRIF loading. In other words, the focus on PGRIFs and its importance as a key mechanical loading parameter in eliciting osteogenic adaptations in children may be overstated and unwarranted.

Recent evidence from several studies involving children suggests that exercises must incorporate moderate to high impact loading to induce osteogenic benefits (Fuchs et al., 2001; MacKelvie et al., 2002; McKay et al., 2000; Van Langendonck et al., 2003; Petit et al., 2002; Bauer et al., 2001). As discussed above, no study design to date involving children has clearly isolated the importance of ground reaction forces associated with impact loading exercise, to justify such an inference. In contrast to its putative positive effects on bone, impact exercise, especially if applied repetitively and chronically has been identified as a risk factor for joint degenerative disease such as osteoarthritis in humans (Turner, 2000). Clearly, improved differentiation of the parameters of mechanical loading is required before recommendations can be made confidently regarding the type and intensity of exercise most conducive to overall musculoskeletal development during growth, not just bone health. The findings of this study and those of Bauer et al. (2001) suggest that PGRIFs may not be the differentiating factor regulating osteogenic adaptation in prepubertal children. Consideration must be given to reports that very few loading cycles are required to initiate an adaptive response (Forwood, Owan, Takano & Turner, 1996; Pead, Kerry & Lanyon, 1988). The activity background of some children may include episodes of loading that could easily equal that of the study. Increasing the duration of those episodes would not necessarily have a greater osteogenic effect (Robling, Burr, & Turner, 2000) although increasing the number of bouts of a novel loading history might. Animal research suggests that exercise programs aimed at maintaining or improving bone mass in humans might achieve greater success if the daily exercise

regime is broken down into smaller sessions separated by recovery periods (Robling, Burr, & Turner, 2001; Robling et al., 2002).

Increased muscle forces during exercise have also been proposed as a prominent, and perhaps even the dominant putative modulator of the skeletal adaptive response in humans (Frost, & Schoenau, 2000). The generally unfavourable biomechanical leverage of the human musculo-skeletal system evokes muscle moments several times larger than the PGRFs from even the most strenuous of high-impact exercises e.g. gymnastics (Frost, & Schoenau, 2000). Empirically, however, the relationship between increased muscle strength and bone mass/density in humans remains equivocal (Duncan et al., 2002). The situation is complicated even further with participant variability in the contribution of leg muscle activation to PGRFs during landing manoeuvres (Caster, & Bates, 1995); individuals appear to use different strategies and demonstrate variable neuromuscular activation patterns to identical drop-landing exercises. Additionally, in the recent study by Bauer et al (2001), neither muscle forces, nor (understandably) in vivo bone strains were measured, unfortunately precluding further elucidation of the relationship between ground, muscle and joint reaction forces, bone strain levels and osteogenic adaptation in drop-jumping type exercises.

With the exercise protocol used in this study control for differences between groups in the magnitude of the predominantly eccentric muscle forces involved in decelerating and stabilizing the body mass upon impact could not be established. These muscle forces were not measured directly, but based on a lack of change in the primary bone outcome measures, it appears that the muscle forces during deceleration were similar between the LD and HD exercise groups, and of insufficient magnitude to affect skeletal adaptation. Whereas jumping and drop-landing exercises probably evoke eccentric muscle forces, the transmission of these forces to bone may be largely attenuated by muscular and kinetic shock absorbing mechanisms (Milgrom et al., 2000). The precise role of muscle, joint and ground reaction forces in mediating the osteogenic response to dropping and jumping exercises still remains to be determined.

How then can the null effects of this study with findings from several recent studies of prepubertal children (Fuchs et al., 2001; Bradney et al., 1998; McKay et al., 2000; Morris et al., 1997) that have reported variable magnitude, but nonetheless statistically significant increases in BMC or BMD following intervention programs incorporating impact loading exercise and ground reaction forces comparable to those elicited during the single-leg training regimen be reconciled? Firstly, the results of the study by Morris et al., (1997) may not be directly comparable to the present study, since girls in that study were older than the participants in this study and perhaps encroaching upon, if not already in the early stages of puberty (Seeman, 2002). Secondly, the study by McKay et al. (2000), although reporting a significant increase in trochanteric BMD in prepubertal girls following a training program that incorporated jumping activities, later reported no effect of an identical exercise program on any measure of BMC in a similar sample of prepubertal girls (MacKelvie et al., 2002). The study by Bradney et al., (1998) on prepubertal boys, included multi-modal exercises, and did not asses the magnitude of the GRF's during training, therefore rendering it difficult to compare directly with the findings of the present study. The study by Fuchs et al., (2001) provides the best comparison to this study, but because of the subtle differences in exercise program design discussed above, is also difficult to compare it directly.

5.6 Conclusion

This study controlled for extraneous factors that might otherwise influence the bone adaptive response to loading, and the within-participant contra-lateral leg design minimized the effect of genetic and non-exercise environmental influences, thus reducing the error term in the statistical analysis and increasing the statistical power of this study. Most importantly, the controlled uni-modal drop-landing exercise in this study minimized the effect of variable strain distribution patterns that characterized previous children's exercise intervention programs. Directional variability in skeletal loading during exercise has been shown to cause substantial and significant changes in bone strains in adult humans (Burr, Milgrom, Fyhrie, & Nyska, 1996), and perhaps differences in this, rather than the magnitude of the GRIFs, is the loading parameter that best can explain the differences in the bone adaptive response in these studies.

CHAPTER 6

6.1 Influence of Drop-Landing Exercises From Different Heights on Bone Geometry and Biomechanical Properties In Prepubertal Girls

A positive effect of drop-landing training on BMC, or bone material properties was not demonstrated in the preceding chapter. However, this does not preclude exercise-related changes in bone material, geometry or biomechanical properties that are undetectable by dual energy absorptiometry. The following chapter explores whether variable height drop-landing exercise results in differential adaptive responses in bone material, geometry or biomechanical properties in prepubertal girls.

Hypotheses to be tested:

4.) That there will be significant differences in regional and site-specific bone geometry between trained and non-trained legs of prepubertal girls involved in eight months of weight-bearing training.

5.) That both exercise groups will demonstrate muscle hypertrophy and greater muscle size than controls in the trained legs.

Research into the skeletal adaptation of children to exercise has increased exponentially in the past decade (Bauer et al., 2001; Courteix et al., 1998; Daly, Rich, Klein, & Bass, 1999; Dyson, et al., 1997). Exercise in youth may have a significant influence on peak bone mass and ultimately on fracture risk in later life (Goulding et al., 1998; Bass et al., 1998; Forwood, 2001; Gilsanz, 1998). Previous studies have predominantly focused on adaptation in bone mineral content (BMC) and areal bone mineral density (aBMD), due mostly to the ready accessibility and non-invasiveness of absorptiometric (single and dual photon absorptiometry) bone mineral assessment techniques. Bone strength, however, depending on loading conditions, is determined by factors in addition to mineral mass, including geometrical and biomechanical properties of bone (Duncan et al., 2002; Faulkner et al., 2003; Forwood, 2001; Martin, 1991; Schoenau, 1998b; Bobbert et al., 1986).

Based on studies where BMC and aBMD have been the main outcome measures, the growing consensus is that weight bearing and high-impact exercises are osteogenic for the developing human skeleton (Bass et al. 1998; Bradney et al. 1998; Dalsky et al. 1988; Duncan et al. 2002; Grimston, Willows, & Hanley, 1993; Lehtonen-Veromaa et al 2000; Madsen, Adams, & Van Loan, 1998; Nordstrom, Pettersson, & Lorentzon, 1998; Ulrich, Georgiou, Snow-Harter, & Gillis, 1996; Welten et al. 1994) and that the immature skeleton might be more responsive to exercise than the adult or more mature skeleton (Cassell, et al. 1996; Daly, et al. 1999; Fuchs, et al. 2001; Dyson, et al.1997; Courteix, et al. 1998; Morris, et al. 1997). More recently, however, a number of studies have reported no significant, or only small changes in BMC or aBMD with exercise in children (Specker & Binkley, 2003; Fuchs, Bauer, & Snow, 2001; Scerpella et al., 2002; Bradney et al. 1998; McKay et al. 2000; Morris, et al., 1997; Petit et al. 2002; MacKelvie et al., 2002). The recent studies challenge the acceptance of prepuberty as a sensitive period for exercise-induced osteogenesis.

The skeleton is capable of multiple adaptive strategies to loading (Forwood 2001; Bauer et al., 2001) and the negative results of more recent trials in the immature skeleton may simply reflect the fact that alternative adaptive responses (such as changes in bone geometry and biomechanics) were not concurrently assessed. Initial attempts at assessment of geometric adaptations to exercise relied on planar x-ray (Jones, 1977) or photon absorptiometry techniques (Martin, 1991; Katzman et al., 1991). These techniques are incapable of accurately measuring the three dimensional shape, and compartmental geometry of long bones bounded by their periosteal and endosteal surfaces (Carter, Bouxsein, & Marcus, 1992; Woodhead et al., 2001). Studies using DXA to investigate the influence of exercise on bone size measures in children include outcomes such as derived bone area (BA) or cortical thickness/area (Ferrari, Rizzoli, Slosman, & Bonjour, 1998; Fuchs, et al., 2001; Jones, & Nguyen, 2000; Lehtonen-Veromaa et al. 2000; Mckay et al. 2000; Molgaard, Thomsen, & Michaelsen, 1998; Nordstrom, Pettersson, & Lorentzon, 1998; Petit et al. 2002; Sievanen et al. 1996; Blimkie et al. 1993; Bradney et al. 1998). DXA-dervied reports of bone geometry must therefore, be interpreted cautiously, since the underlying assumption of cylindrical bone shape may not apply to all investigated regions of the skeleton. Nevertheless,

results from several comparative cross-sectional studies of adolescents and young adults (Kontulainen et al., 2002; Duncan et al., 2002; Bass et al., 1999) involving 3-D imaging techniques suggest that exercise associated increases in BMC may be explained in large part by expansion of bone dimensions and geometry.

Recent technological advances have made it possible to assess bone geometry and biomechanical adaptations accurately, simply and non-invasively even in the immature developing human skeleton (Ferrari et al., 1998; Jones, & Nguyen, 2000; Neu, Manz, Rauch, Merkel, & Schoenau, 2001; Dyson et al., 1997). Although more than 60% of peak bone mineral mass is gained by an increase in bone size during puberty, familial resemblance for most bone traits between daughters and their mothers exist prior to puberty (Ferrari et al., 1998; Jones, & Nguyen, 2000). As well, bone area has been found to be one of the significant predictors of bone mass (Ilich, Skugor, Hangartner, Baoshe, & Matkovic, 1998). Using pQCT to scan the distal radius, an estimated two-fold increase in CSA was reported roughly doubled in girls between the ages of 6 and 15yrs (Neu et al., 2001). High-impact activity such as gymnastics appears to influence both bone geometry and biomechanics. Increases in femoral cortical bone CSA can occur either by endocortical or periosteal apposition, or both. Biomechanically, however, if periosteal apposition is accompanied by endocortical resorption the resultant biomechanical advantage (CSMI) is greater than if there were only endocortical apposition with no periosteal apposition. An intervention study of prepubertal males reported that the femoral midshaft cortical thickness increased due to a decrease in the endocortical diameter (ie endocortical apposition - Bradney et al., 1998). A more recent intervention study in prepubertal children attributed changes in femoral neck and the inter-trochanteric region to increased CSA and reduced endosteal expansion (ie endocortical apposition - Petit et al., 2002). Age related cross-sectional profiles of bone growth demonstrate that in puberty males add bone mostly on the periosteal surface whereas females tend to add bone more on the endocortical surface (Schoenau et al., 2001). Garn, Sandusky, Miller, & Nagy (1972) showed in their landmark study using metacarpal dimensions that females add bone at both the periosteal and endocortical surfaces during the pubertal growth spurt. However growth related changes could be influenced by the nature of physical activity to which children are exposed. Children habitually performing gross motor tasks were associated with greater tibial periosteal and

endosteal circumferences indicating a greater CSMI than children who routinely engaged in fine motor tasks (Specker & Binkley, 2003). In addition, high impact loads from serious and longitudinal involvement in gymnastics have been associated with significantly greater size-adjusted CSMI (Faulkner et al., 2003). An intervention study assessing the effects of high-intensity jumping on hip and lumbar spine bone mass, revealed that jumpers had greater increases at the femoral neck area than controls (Fuchs et al., 2001). Similarly, after a 10-month, high-impact, strength building exercise program the FN bone area increased at a significantly greater rate in the exercise group compared with the controls (Morris et al., 1997).

Of the recent studies that have investigated bone geometry and biomechanics in children (Rauch, & Schoenau, 2001; Robling et al., 2002; Schoenau et al., 2002b; Dyson et al., 1997; Haapasalo et al. 2000; Heinonen et al., 2001) none have examined these properties in prospective, randomised, controlled exercise studies in the prepubertal population.

The purpose of this study therefore was to assess the effects of single-leg drop-landing exercises from different heights on mid-femoral bone geometry and biomechanical properties in prepubertal girls using a newly developed and validated technique that incorporates both DXA and MRI imaging techniques (Hoegler et al., 2003; Woodhead et al., 2001). The mid-femoral region was selected because it represents the largest functional muscle-bone unit in the human body, providing a model that allows investigation of the influences of both voluntary muscle forces and gravitational loading during weight-bearing activities including locomotion, and most children's sports and exercise programs.

6.2 Methods

Details of the methods used in this chapter have been presented in Chapter 3. Primary outcome geometrical measures included cross sectional areas. The cross-sectional areas involved cortical, medullary cavity and total bone areas (as well as within-group pre- and post-intervention comparisons). Primary outcome biomechanical measures involved proximal middle and distal slices of the mid femoral shaft. The biomechanical measures included torsional, maximum and minimum cross-sectional moments of inertia (as well as within-group pre- and post-intervention

comparisons). Additionally, differences in bone strength index (Ferretti, Capozza, & Zanchetta, 1996) for the non-dominant and dominant mid femoral shaft were analysed.

Differences among groups at baseline as well as pre-post changes that occurred over time were assessed using analysis of variance (ANOVA). Baseline primary bone cross-sectional areas and cross-sectional moment of inertia measures were analysed using ANCOVA, with adjustments for the covariates baseline body and fat mass. Between group differences for changes in bone geometrical and biomechanical outcomes were analysed using ANCOVA, with adjustments for baseline body and fat mass and the change in lean tissue mass. Dependent samples t-tests were completed to determine within group differences between dominant and non-dominant legs. All statistical comparisons were subjected to Bonferroni adjustments.

6.3 Results

6.3.1 Physical and Descriptive Characteristics

6.3.1.1 Anthropometry

Baseline and change data for the physical and descriptive characteristics are summarised in Table 6.1. Data that were not normally distributed were log transformed before analysis. Data for the control group participant who refused to submit to the MRI analysis were withdrawn in an attempt to compare only measured participants across the 3 groups.

6.3.1.2 Physical Activity, Calcium and Estradiol

No ($p>0.05$) differences among the 3 groups at baseline and no differences ($p>0.05$) among groups in magnitude of change in leisure time physical activity level or dietary calcium intake over the duration of the intervention were detected (Table 6.1). Serum E_2 levels were similar among groups at the end of the study (Table 4.1).

6.3.1.3 Bone Geometry Measures

No differences ($p>0.05$) in cortical bone area at either proximal, mid or distal slices of the mid femoral shaft for non-dominant or dominant legs among the 3 groups for baseline measures were identified. The dominant leg femoral shaft distal cortical bone area of the HD group had a greater change (approaching significance) than the change occurring in the LD group (Table 6.2). When the dominant leg data were covaried for body mass, fat mass and change in LTM (Figure 6.6; Appendix O4), the difference became significant ($p=.023$). No other adjusted data differences ($p>0.05$) in cortical bone area at either proximal, mid or distal slices of the mid femoral shaft for non-dominant or dominant leg were discernable (Figures 6.1 – 6.6; Appendix O4). Furthermore, no differences ($p>0.05$) in medullary cavity measures were discernable for unadjusted (Table 6.3) or after adjusting for body mass, fat mass and the change in LTM (Figures 6.7 – 6.12; Appendix O5). A reduction in medullary cavity area (indicating endocortical apposition) was observed in the HD mean but the reduction was no different ($p=.447$) from the other mean changes. The cortical and medullary cavity cross sectional area (CSA)s were added together to obtain a measure of total bone CSA for each of the slices. In the unadjusted data, LD mean for the non-dominant leg distal slice total bone area was greater than that of the controls (Table 6.4). However, after adjusting for body mass, fat mass and the change in LTM the significance disappeared (Figure 6.18; Appendix O6). Furthermore, no other differences were detected (Figures 6.13 – 6.18; Appendix O6).

Table 6.1 Physical and lifestyle characteristics and leg torque. Baseline Means±SD: Change Means±SD

	Baseline				***Change following intervention***			
	Controls (C)	***Low Drop (LD)***	***High Drop (HD)***		***Controls (C)***	***Low Drop (LD)***	***High Drop (HD)***	
	N=13	N=13	N=13	***p***	N=13	N=13	N=13	***p***
Age (yr)	7.87 ± 0.77	7.79± 0.94	7.89± 1.12	.957	0.77± 0.05	0.79± 0.03	0.77± 0.03	.241
Height (cm)	125.24 ± 6.00	128.82± 5.95	127.13± 2.89	.213	3.41± 2.49	2.95± 2.24	4.45± 2.69	.294
Body Mass (kg)	24.35 ± 3.15	27.51± 5.17	27.99± 4.30	.065	1.37± 2.45	2.41± 0.68	2.14± 1.28	.257
Lean Tissue (kg)	17.93 ± 2.33	19.09± 2.53	18.99± 2.27	.379	1.59± 0.87	2.42± 0.83	2.42± 1.36	.069
Fat Mass (kg)	4.80 ± 1.39	6.89± 3.22	7.38± 3.20	**.042***	0.30± 1.00	-0.24± 1.41	-0.16± 0.91	.410
% Body Fat	20.21 ± 4.14	24.72± 6.14	26.25± 7.93	**.042***	-0.26± 3.66	-2.49± 3.94	-2.71± 3.37	.169
Leisure Time Physical Activity ($h \cdot w^{-1}$)	3.03± 1.97	2.39± 1.96	2.18 ± 1.62	.474	0.99± 1.77	-0.17± 1.77	0.38± 3.04	.422
Calcium Intake (mg. d^{-1})	537± 245	604± 291	582 ± 251	.798	-132± 254	-131± 243	-51± 335	.698
ND Extensor Muscle Torque (Nm)	38.69± 8.75	43.69± 10.40	44.69± 9.94	.254	2.92± 6.10	6.69± 9.22	0.77± 4.36	.098
NDFlexor Muscle Torque (Nm)	18.62± 5.08	20.15± 6.82	24.15± 5.40	.055	3.69± 4.55	4.85± 5.94	1.15± 7.03	.280
D Extensor Muscle Torque (Nm)	37.54± 9.80	40.46± 10.09	46.62± 11.04	.087	3.39± 4.96	4.85± 13.52	-0.39± 8.64	.375
DFlexor Muscle Torque (Nm)	23.31± 6.16	21.62± 3.99	26.54± 6.51	.093	0.31± 6.28	5.92± 7.90	1.69± 6.24	.108

* High Drop mean significantly greater than Controls ($p<0.05$)

Table 6.2 Unadjusted Data - Cortical bone cross sectional area Baseline Means±SD: Change Means±SD

	Baseline				***Change following intervention e***			
	Controls	***Low Drop (LD)***	***High Drop (HD)***		***Controls***	***Low Drop (LD)***	***High Drop (HD)***	
N	N=13	N=13	N=13	***p***	[φ]N=12	13	N=13	***p***
ND Leg Proximal (mm^2)	159.36 ± 24.93	176.48 ± 30.45	176.27 ± 18.91	.153	20.62 ± 19.71	13.99 ± 8.90	20.21 ± 11.54	.414
ND Leg Mid (mm^2)	163.28 ± 19.90	179.71 ± 29.79	172.60 ± 16.59	.197	15.84 ± 8.57	17.83 ± 6.67	21.40 ± 12.30	.341
ND Leg Distal (mm^2)	144.39 ± 22.75	160.69 ± 26.04	155.74 ± 13.78	.155	13.17 ± 12.16	16.91 ± 9.24	13.99 ± 8.94	.626
	N=13	N=13	N=13		N=13	N=13	N=13	
D Leg Proximal (mm^2)	162.69 ± 24.83	179.77 ± 30.14	175.48 ± 12.60	.175	14.71 ± 8.82	11.09 ± 9.36	12.36 ± 13.13	.681
D Leg Mid (mm^2)v	161.61 ± 22.36	180.02 ± 29.46	175.13 ± 13.77	.117	16.84 ± 10.01	14.55 ± 7.15	15.86 ± 10.23	.825
D Leg Distal (mm^2)	149.22 ± 23.95	167.59 ± 26.60	158.79 ± 12.68	.117	11.53 ± 5.25	7.58 ± 9.09	15.13 ± 8.33	.057

*Significant difference between (a) LD and Controls (p=.028) (b) LD and HD groups (p=.005)

[φ]N=12 - one non-dominant post-intervention control MRI scan unable to be analysed due to movement artefact

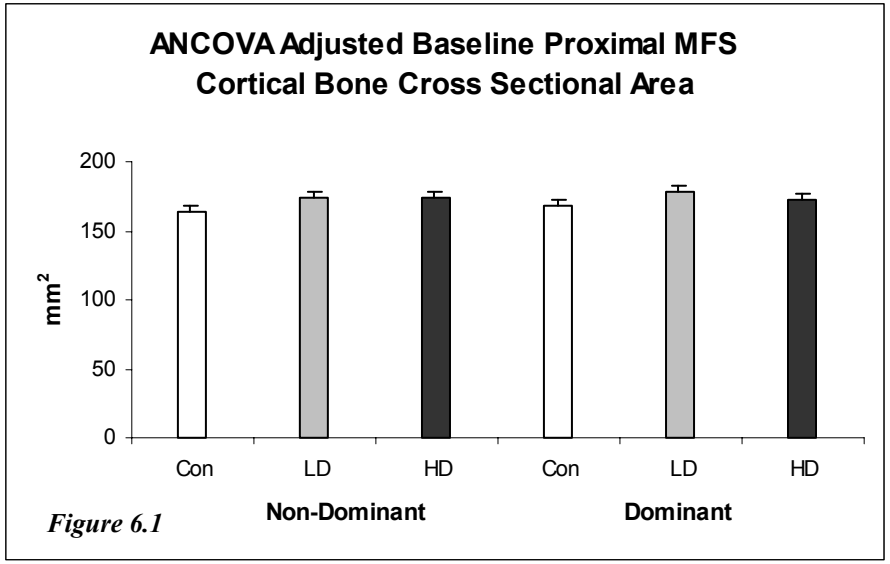


Figure 6.1

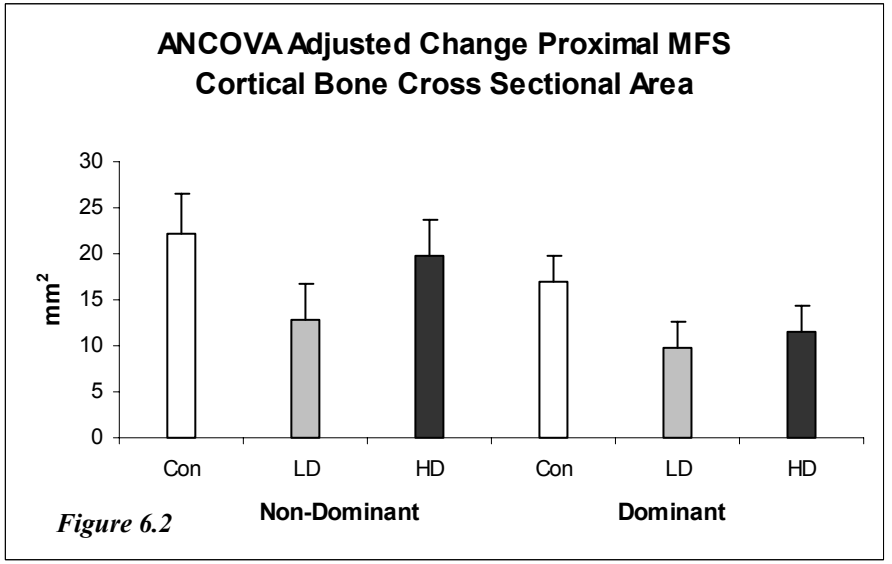


Figure 6.2

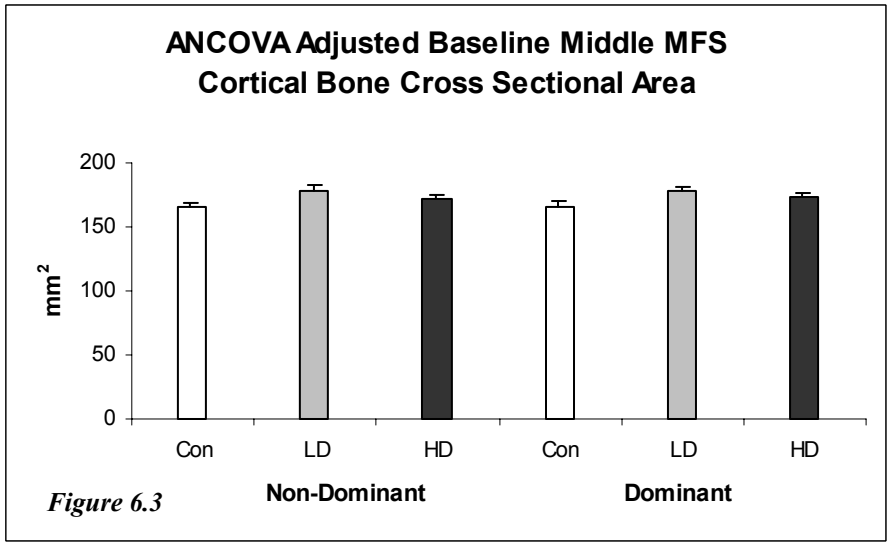


Figure 6.3

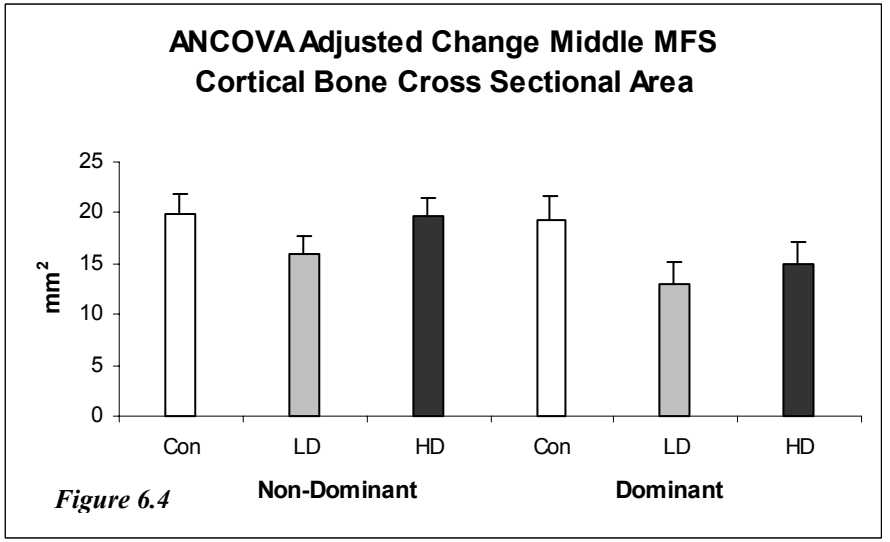


Figure 6.4

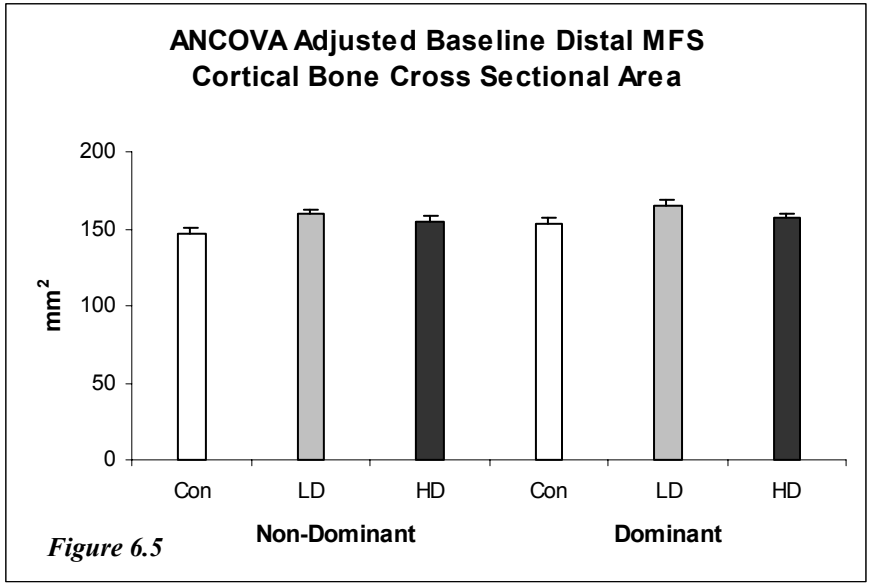


Figure 6.5

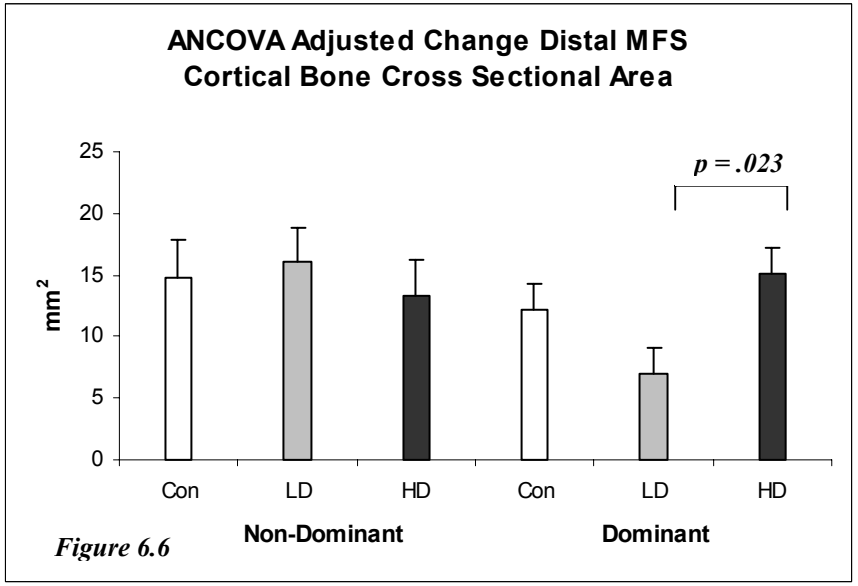


Figure 6.6

Table 6.3 Unadjusted Data – Medullary cavity area Baseline Means±SD: Change Means±SD

	Baseline				***Change following intervention***			
	Controls	***Low Drop (LD)***	***High Drop (HD)***		***Controls***	***Low Drop (LD)***	***High Drop (HD)***	
N	N=13	N=13	N=13	***p***	[φ]N=12	N=13	N=13	***p***
ND Leg Proximal (mm^2)	90.91 ± 22.50	88.67 ± 15.17	94.95 ± 36.60	.828	4.31 ± 9.08	4.33 ± 5.16	-2.24 ± 22.83	.432
ND Leg Mid (mm^2)	77.68 ± 23.31	74.88 ± 15.51	85.37 ± 24.46	.443	2.74 ± 4.88	5.54 ± 4.35	3.54 ± 4.61	.303
ND Leg Distal (mm^2)	119.56 ± 29.95	122.84 ± 29.11	139.05 ± 32.50	.233	3.88 ± 11.61	11.17 ± 7.04	10.97 ± 10.09	.120
	N=13	N=13	N=13		N=13	N=13	N=13	
D Leg Proximal (mm^2)	90.87 ± 21.13	88.36 ± 17.11	91.70 ± 28.27	.926	5.70 ± 4.51	7.81 ± 5.65	5.03 ± 6.09	.407
D Leg Mid (mm^2)v	78.53 ± 22.68	76.29 ± 15.40	85.74 ± 24.63	.503	3.32 ± 3.35	4.31 ± 4.84	5.26 ± 3.87	.420
D Leg Distal (mm^2)	110.52 ± 25.30	123.04 ± 27.81	137.07 ± 34.66	.086	8.49 ± 6.95	10.62 ± 7.56	6.68 ± 6.63	.372

[φ]N=12 - one non-dominant post-intervention control MRI scan unable to be analysed due to movement artefact

Table 6.4 Unadjusted Data - Total bone area Baseline Means±SD: Change Means±SD

	Baseline				***Change following intervention***			
	Controls	***Low Drop (LD)***	***High Drop (HD)***		***Controls***	***Low Drop (LD)***	***High Drop (HD)***	
N	N=13	N=13	N=13	***p***	[φ]N=12	N=13	N=13	***p***
ND Leg Proximal (mm^2)	250.27 ± 37.09	265.16 ± 41.11	271.21 ± 49.14	.446	24.94 ± 14.74	18.32 ± 6.81	17.97 ± 22.84	.494
ND Leg Mid (mm^2)	240.97 ± 36.33	254.59 ± 40.09	257.97 ± 32.27	.459	18.58 ± 8.43	23.36 ± 7.02	24.94 ± 9.60	.162
ND Leg Distal (mm^2)	263.95 ± 47.05	283.54 ± 49.37	294.79 ± 40.81	.236	17.05 ± 6.55	28.08 ± 7.40	24.96 ± 12.99	***.019****
	N=13	N=13	N=13		N=13	N=13	N=13	
D Leg Proximal (mm^2)	253.57 ± 37.70	268.13 ± 42.93	267.18 ± 37.07	.577	20.41 ± 10.95	18.91 ± 7.96	17.39 ± 10.42	.739
D Leg Mid (mm^2)v	240.14 ± 37.17	256.32 ± 40.37	260.87 ± 31.84	.328	19.92 ± 10.14	18.86 ± 5.89	21.12 ± 7.74	.777
D Leg Distal (mm^2)	259.73 ± 43.52	290.63 ± 49.76	295.86 ± 42.82	.105	20.02 ± 7.46	9.68 ± 32.41	21.81 ± 8.50	.255

*LD significantly greater than controls ($p<.05$)

[φ]N=12 - one non-dominant post-intervention control MRI scan unable to be analysed due to movement artefact

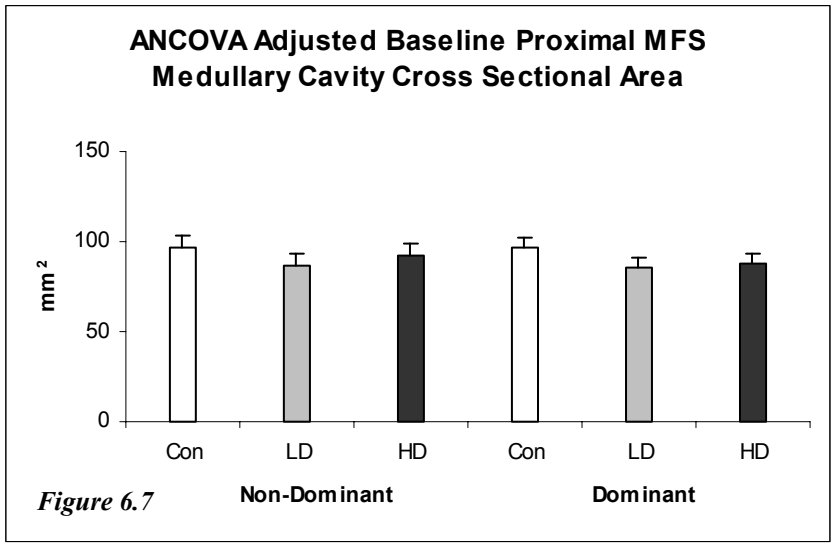


Figure 6.7

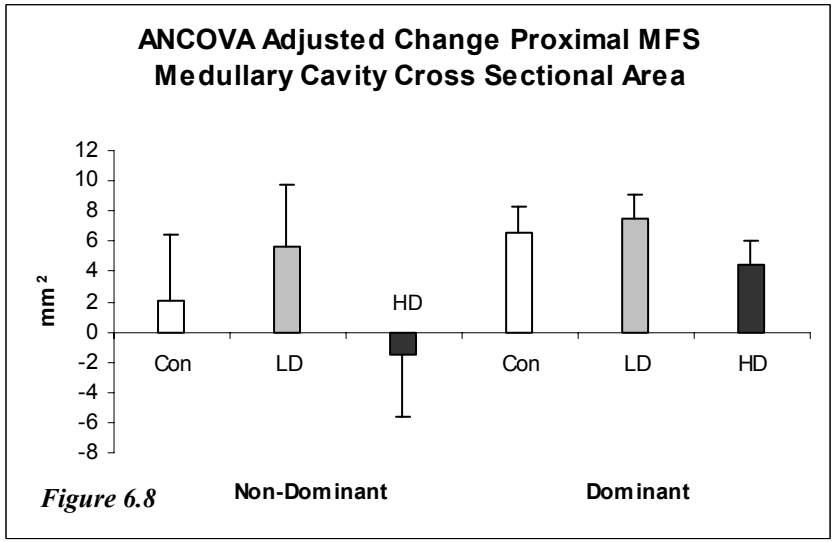


Figure 6.8

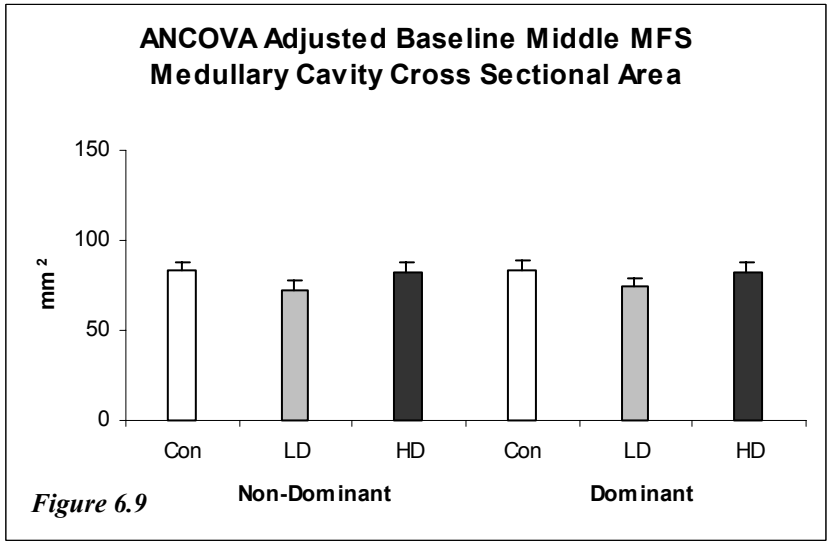


Figure 6.9

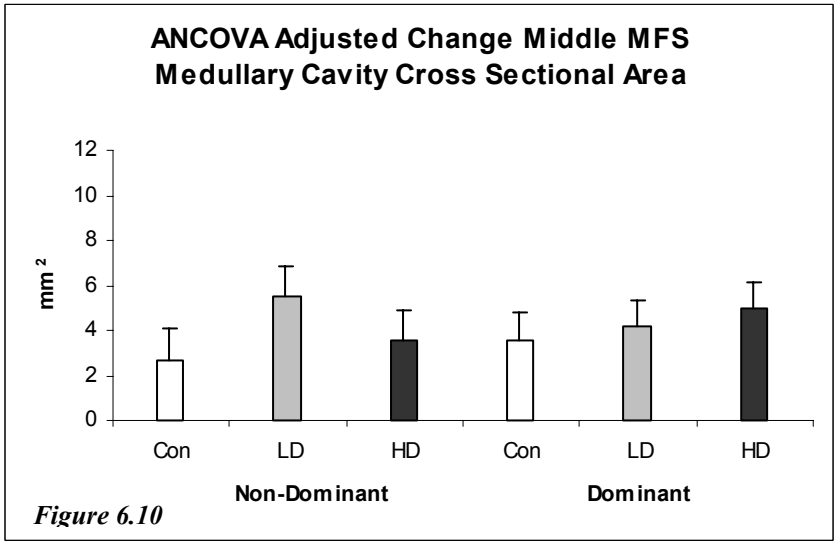


Figure 6.10

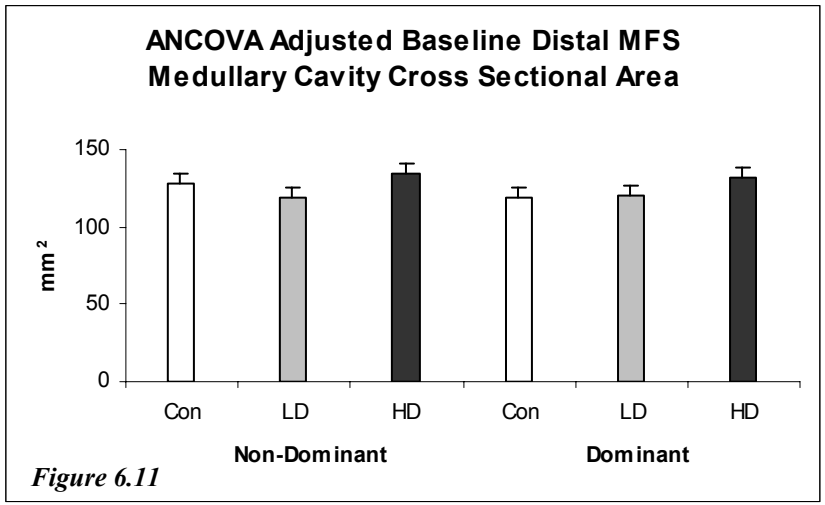


Figure 6.11

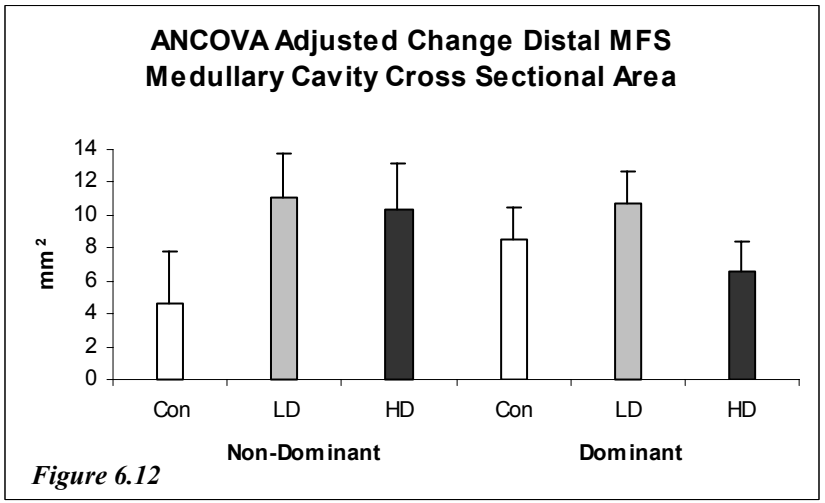


Figure 6.12

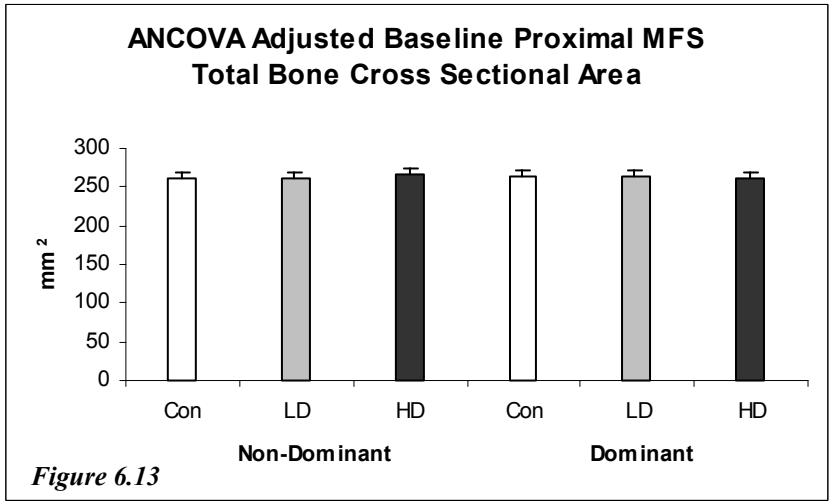


Figure 6.13

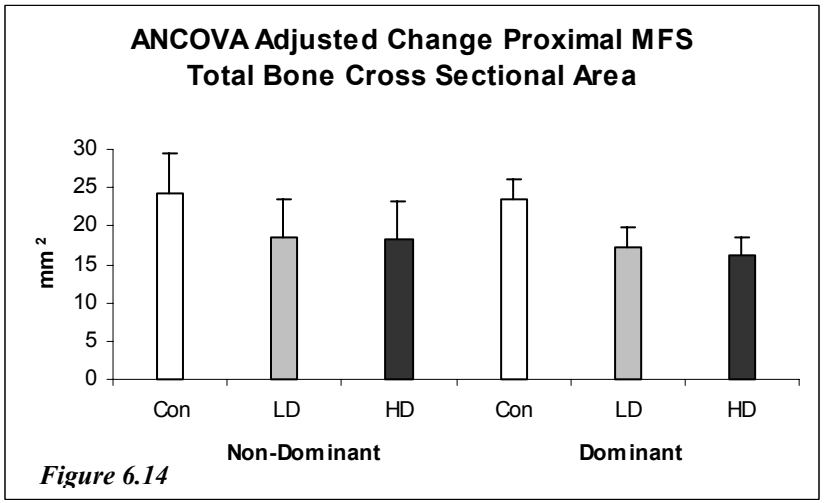


Figure 6.14

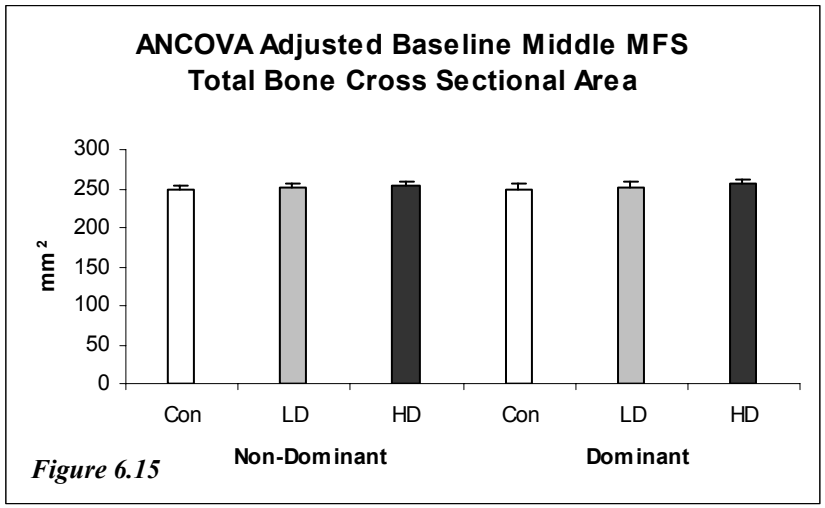


Figure 6.15

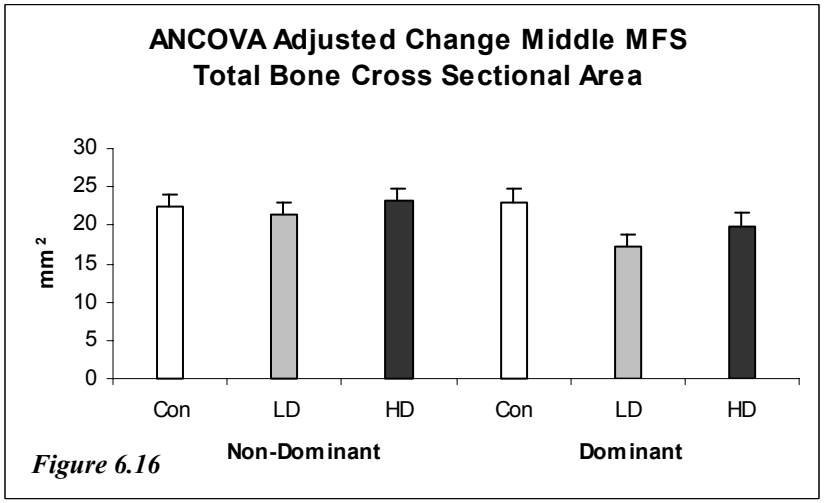


Figure 6.16

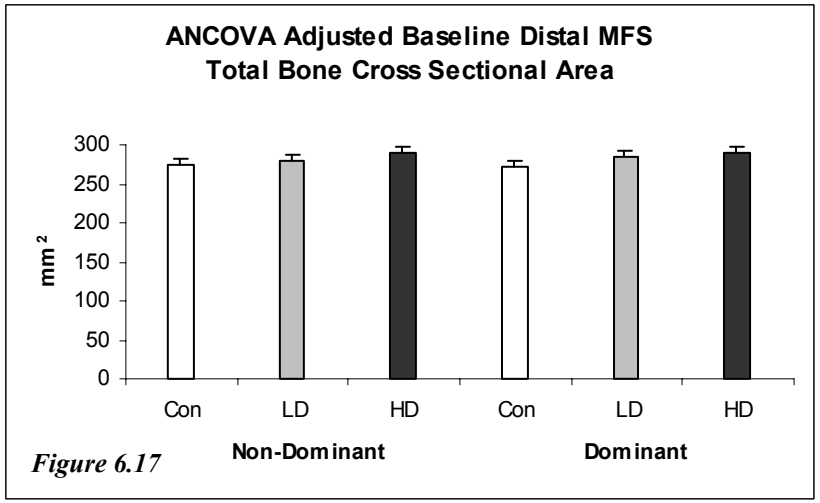


Figure 6.17

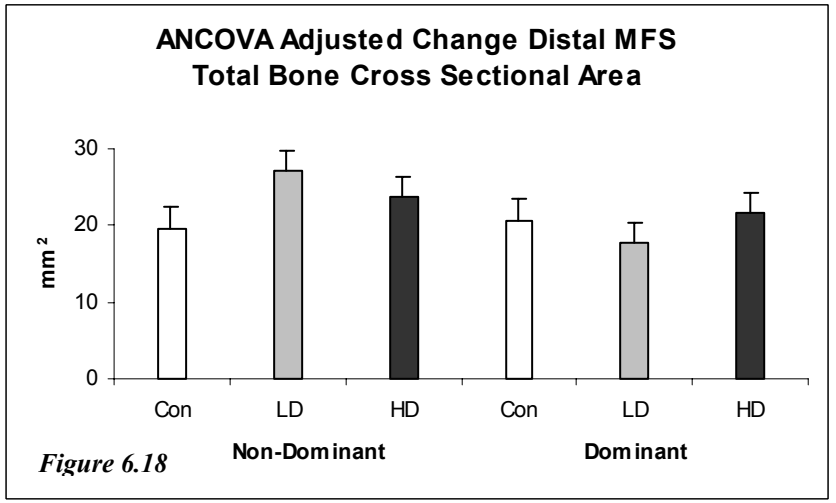


Figure 6.18

6.3.1.4 Biomechanical Measures

No differences (p>0.05) were evident among groups for unadjusted baseline or post-intervention change measures at any site for any of the biomechanical (J0, Imax, Imin) variables (Tables 6.5 - 6.7). When ANCOVA adjustments for baseline body and fat mass were accounted for no differences (p>0.05) among groups or significant group x leg interaction effects were observed at any site for biomechanical measures at the outset of the study for proximal (Figures 6.19, 6.21, 6.23; Appendix O7) middle (Figures 6.25, 6.27, 6.29; Appendix O8) or distal (Figures 6.31, 6.33, 6.35; Appendix O9) mid femoral shaft slices. Similarly, no group differences (p>0.05) were detected for biomechanical change data after adjustments were made for baseline body mass, fat mass and change in lean tissue mass for proximal (Figures 6.20, 6.22, 6.24; Appendix O7) middle (Figures 6.26, 6.28, 6.30: Appendix O8) and distal (Figures 6.32, 6.34, 6.36; Appendix O9) mid femoral shaft slices.

6.3.1.5 Bone Strength Index

Differences (p>0.05) in bone strength index could not be detected at either the proximal, mid or distal slices for the non-dominant or dominant legs among the 3 groups at baseline (Table 6.8) or when the data were adjusted for body mass and fat mass (Figures 6.37,6.38; Appendix O10).

6.3.1.6 Between Leg Comparisons

Unadjusted pre- and post-intervention comparisons of bone CSA measures for non-dominant and dominant legs showed no significant differences (Table 6.9) (p>0.05). After adjusting for body mass and fat mass no differences (p>0.05) for any of the bone CSA variables between the exercised (non-dominant) and non-exercised (dominant) leg at baseline or after the completed intervention remained (Appendix O11). Similarly, the unadjusted pre- and post-intervention comparisons of biomechanical (CSMI) measures for non-dominant and dominant legs showed no (p>0.05) differences (Table 6.10). After adjusting for body mass and fat mass no differences (p>0.05) were observed for any of the CSMI variables between the exercised (non-dominant) and non-exercised (dominant) legs at baseline or in post- intervention changes (Appendix O12).

Table 6.5 Unadjusted Data – Proximal mid-femoral shaft slice cross-sectional moment of inertia (CSMI). Baseline Means±SD: Change Means±SD

	Baseline				***Change following intervention***			
	Controls (C)	***Low Drop (LD)***	***High Drop (HD)***		***Controls (C)***	***Low Drop (LD)***	***High Drop (HD)***	
N	[γ]N=12	[φ]N=12	13	***p***	N=12	N=12	N=13	***p***
Non-Dominant Leg J0 (mm^4)	7195 ± 1954	8638 ± 2620	7986 ± 1839	.273	1366 ± 671	1394 ± 771	1319 ± 952	.323
Non-Dominant Leg Imax (mm^4)	4231 ± 1193	4955 ± 1453	4697 ± 1196	.386	754 ± 428	900 ± 445	702 ± 585	.592
Non-Dominant Leg Imin (mm^4)	2964 ± 826	3683 ± 1204	3289 ± 680	.176	612 ± 304	494 ± 348	617 ± 483	.680
N	N=13	N=13	N=13		N=13	N=13	N=13	
Dominant LegJ0 (mm^4)	7191 ± 2007	8551 ± 2512	8151 ± 1939	.297	1180 ± 710	1348 ± 861	1444 ± 1022	.877
Dominant Leg Imax (mm^4)	4174 ± 1138	5038 ± 1474	4711 ± 1105	.244	647 ± 452	854 ± 518	850 ± 609	.556
Dominant Leg Iymin(mm^4)	3017 ± 918	3512 ± 1072	3440 ± 867	.396	532 ± 279	494 ± 353	594 ± 436	.788

[γ]N=12 - one non-dominant baseline control group MRI scan unable to be analysed due to movement artefact

[φ]N=12 - one non-dominant baseline LD MRI scan unable to be analysed due to movement artefact

Table 6.6 Unadjusted Data – Middle mid-femoral shaft slice CSMI. Baseline Means±SD: Change Means±SD

	Baseline				***Change following intervention***			
	Controls (C)	***Low Drop (LD)***	***High Drop (HD)***		***Controls (C)***	***Low Drop (LD)***	***High Drop (HD)***	
N	[γ]N=12	[φ]N=12	N=13	***p***	N=12	N=12	N=13	***p***
Non-Dominant Leg J0 (mm^4)	6580 ± 1893	8554 ± 2676	7644 ± 1708	.089	1326 ± 1033	1564 ± 772	1969 ± 1792	.395
Non-Dominant Leg Imax (mm^4)	3816 ± 1203	5031 ± 1577	4460 ± 1202	.098	776 ± 592	964 ± 572	1198 ± 1027	.398
Non-Dominant Leg Imin (mm^4)	2764 ± 733	3523 ± 1135	3184 ± 531	.096	550 ± 458	600 ± 225	771 ± 804	.589
N	N=13	N=13	N=13		N=13	N=13	N=13	
Dominant Leg J0 (mm^4)	6583 ± 1819	8225 ± 2638	7802 ± 1562	.139	1517 ± 875	1552 ± 864	1717 ± 880	.201
Dominant Leg Imax (mm^4)	3791 ± 1095	4767 ± 1528	4550 ± 1062	.144	923 ± 604	894 ± 562	1024 ± 522	.831
Dominant Leg Imin (mm^4)	2792 ± 769	3458 ± 1145	3252 ± 565	.162	594 ± 301	658 ± 328	692 ± 469	.807

[γ]N=12 - one non-dominant baseline control group MRI scan unable to be analysed due to movement artefact

[φ]N=12 - one non-dominant baseline LD MRI scan unable to be analysed due to movement artefact

Table 6.7 Unadjusted Data – Distal mid-femoral shaft slice CSMI. Baseline Means±SD: Change Means±SD

	Baseline				***Change following intervention***			
	Controls (C)	***Low Drop (LD)***	***High Drop (HD)***		***Controls (C)***	***Low Drop (LD)***	***High Drop (HD)***	
N	[γ]N=12	[φ]N=12	N=13	***p***	N=12	N=12	N=13	***p***
Non-Dominant Leg J0 (mm^4)	7046 ± 2328	9203 ± 2714	8598 ± 1777	.072	1704 ± 1277	2131 ± 735	1920 ± 1286	.923
Non-Dominant Leg Imax (mm^4)	3940 ± 1329	5192 ± 1605	4881 ± 1052	.072	1031 ± 837	1194 ± 467	1013 ± 671	.768
Non-Dominant Leg Imin (mm^4)	3106 ± 1019	4012 ± 1142	3718 ± 750	.083	673 ± 467	937 ± 294	907 ± 645	.369
N	N=13	N=13	N=13		N=13	N=13	N=13	
Dominant Leg J0 (mm^4)	7851 ± 2711	9711 ± 3139	9066 ± 1669	.210	968 ± 936	1563 ± 731	1715 ± 1059	.408
Dominant Leg Imax (mm^4)	4352 ± 1558	5353 ± 1896	5040 ± 917	.259	570 ± 515	918 ± 401	968 ± 609	.134
Dominant Leg Imin (mm^4)	3499 ± 1200	4358 ± 1271	4027 ± 800	.172	398 ± 433	645 ± 372	747 ± 478	.133

[γ]N=12 - one non-dominant baseline control group MRI scan unable to be analysed due to movement artefact

[φ]N=12 - one non-dominant baseline LD MRI scan unable to be analysed due to movement artefact

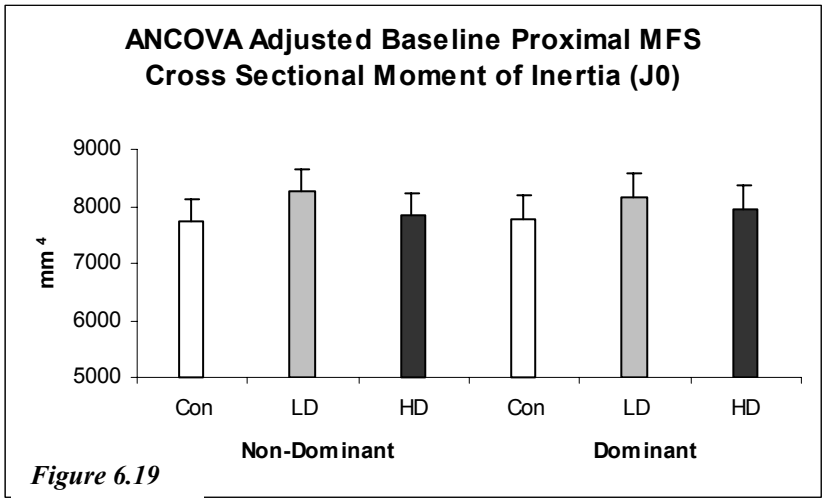
Table 6.8 Unadjusted Data - Bone strength index (BSI) Baseline Means±SD: Change Means±SD

	Baseline				***Change following intervention e***			
	Controls	***Low Drop (LD)***	***High Drop (HD)***		***Controls***	***Low Drop (LD)***	***High Drop (HD)***	
N	[γ]N=12	[φ]N=12	N=13	***p***	N=12	N=12	N=13	***p***
[φ]Non-Dominant Leg BSI ($mm^4.g/cm^3$)	4467 ± 1466	5945 ± 2053	5497 ± 1211	.086	932 ± 457	973 ± 502	1006 ± 1015	.970
	N=13	N=13	N=13		N=13	N=13	N=13	
Dominant Leg BSI ($mm^4.g/cm^3$)	4527 ± 1303	5771 ± 1963	5527 ± 1297	.127	808 ± 498	981 ± 772	1177 ± 754	.416

Calculation of BSI using average of proximal, mid and distal slice CSMI (Imax) multiplied by mid femoral shaft DXA and MRI derived volBMD

[γ]N=12 - one non-dominant baseline control group MRI scan unable to be analysed due to movement artefact

[φ]N=12 - one non-dominant post-intervention control MRI scan in LD group unable to be analysed due to movement artefact



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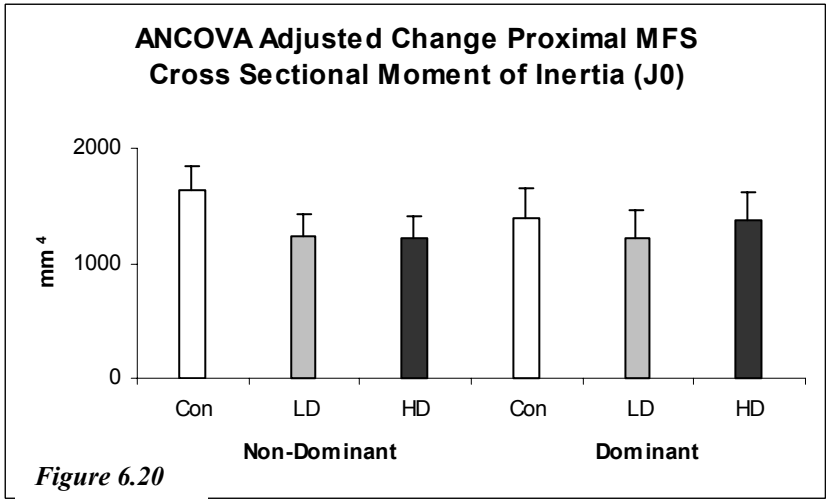

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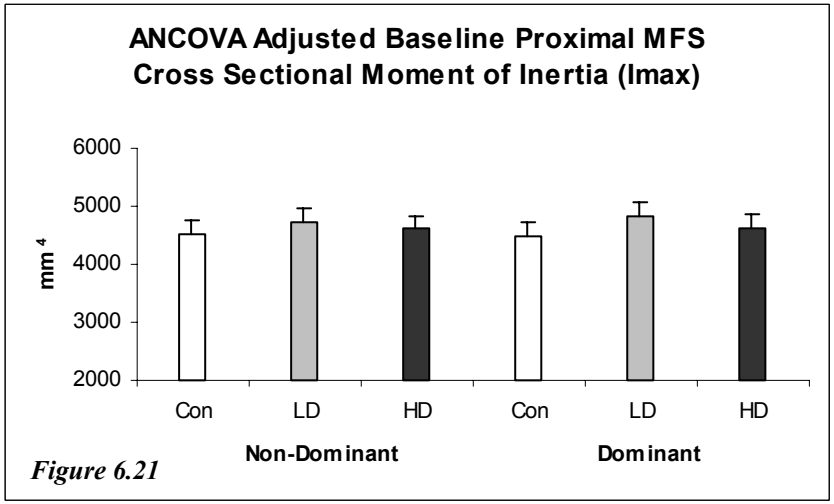

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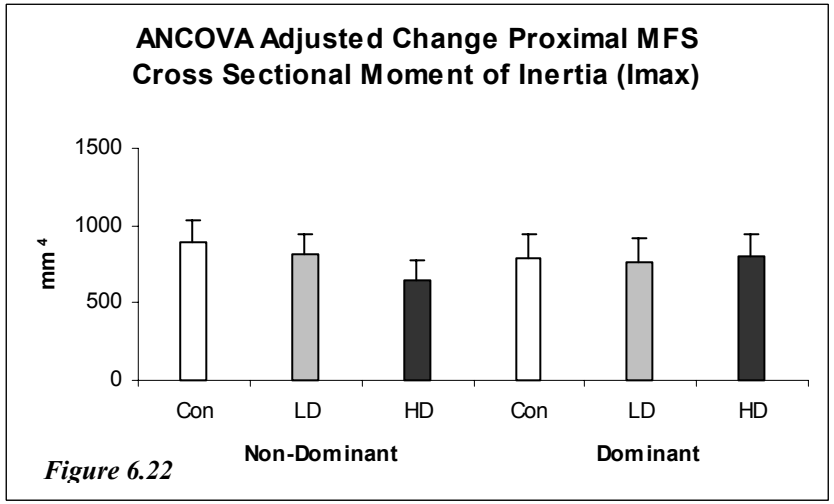

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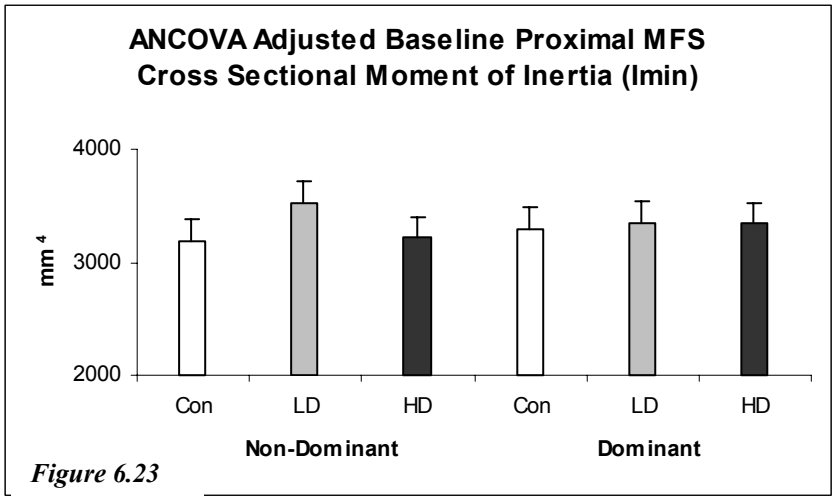

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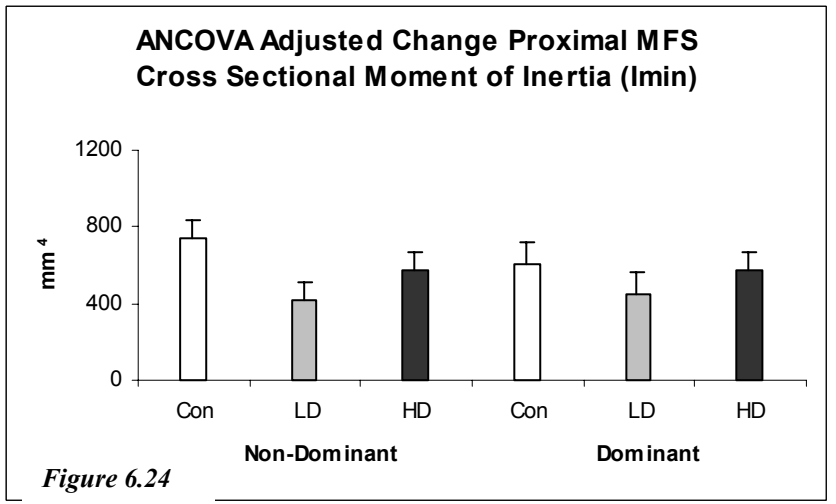

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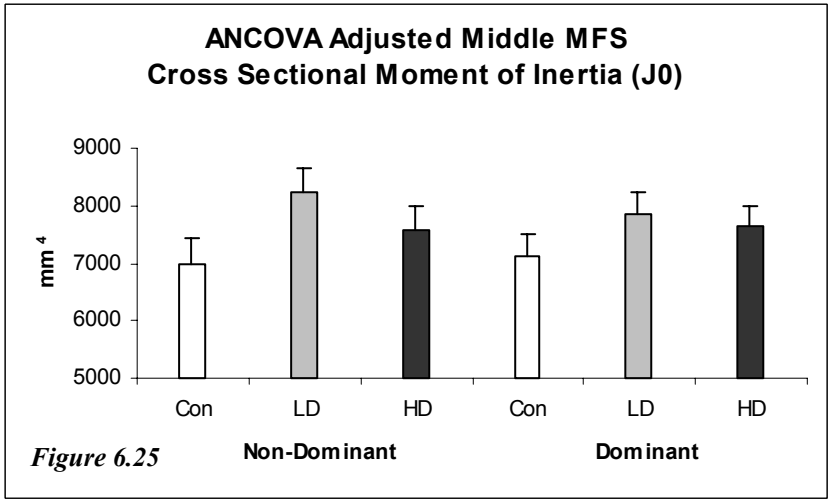

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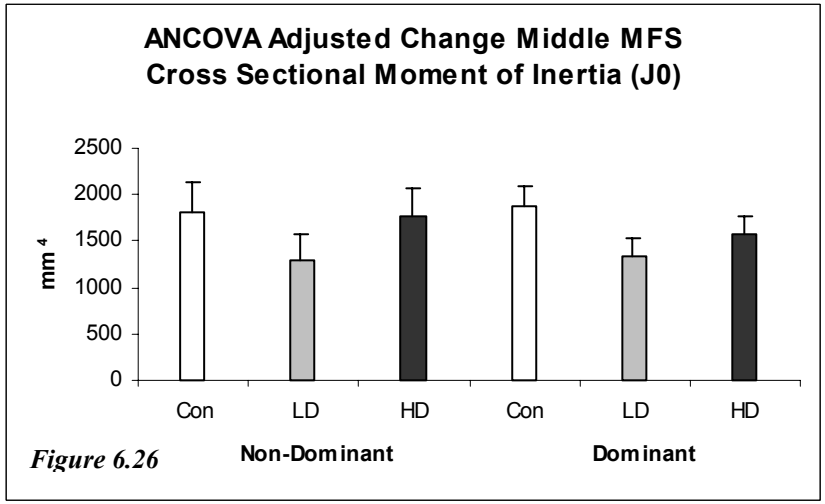

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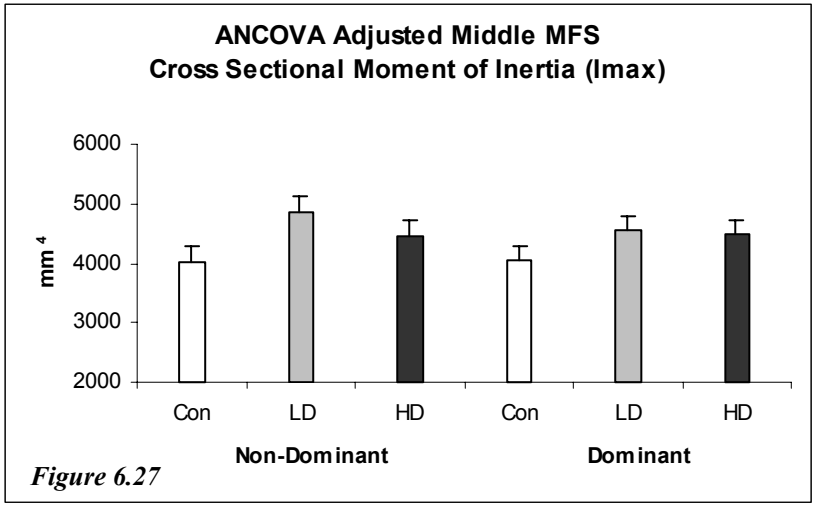

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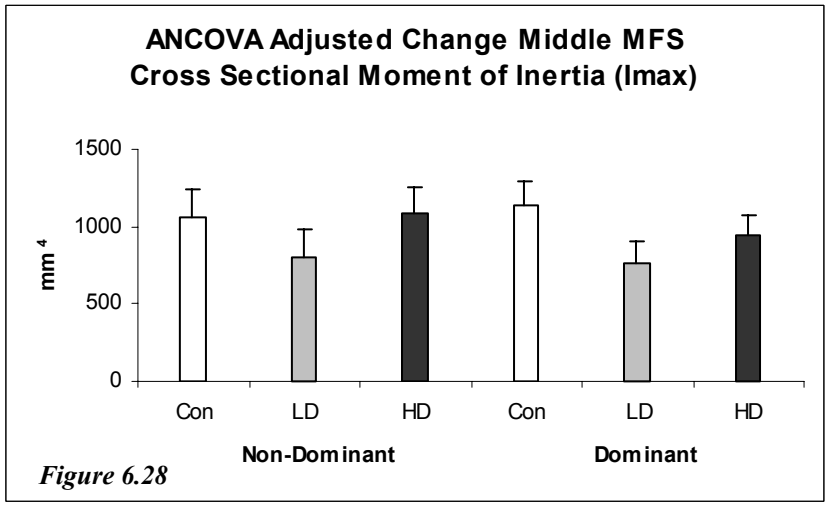

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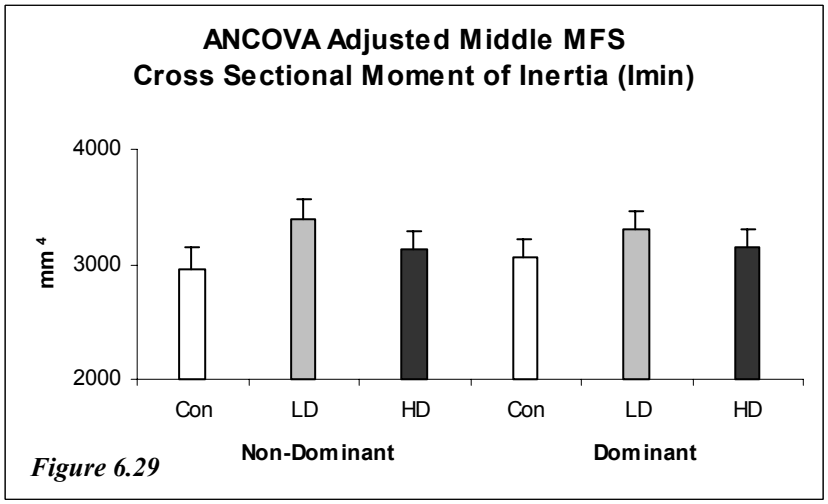

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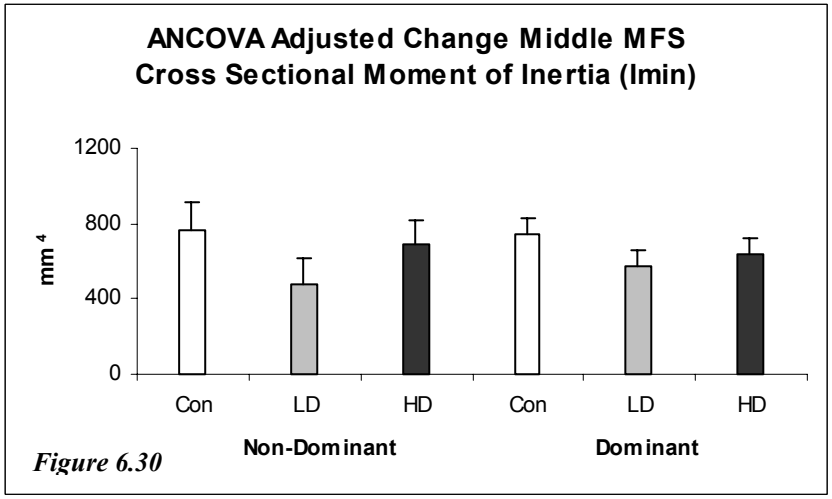

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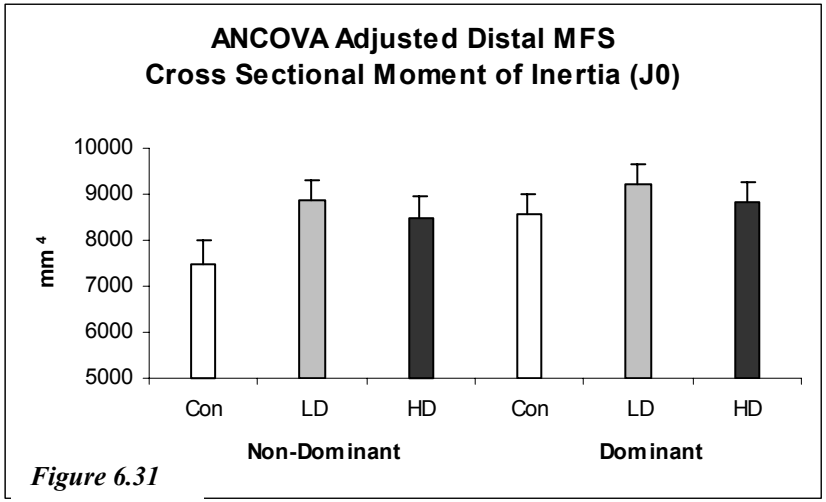

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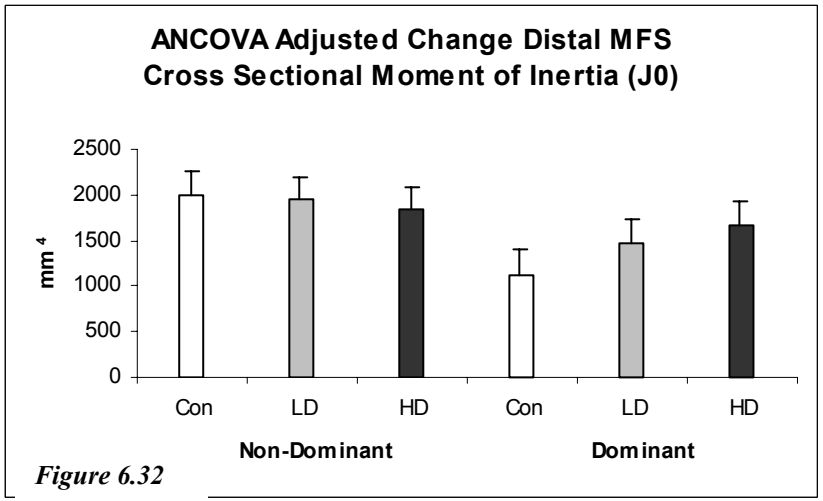

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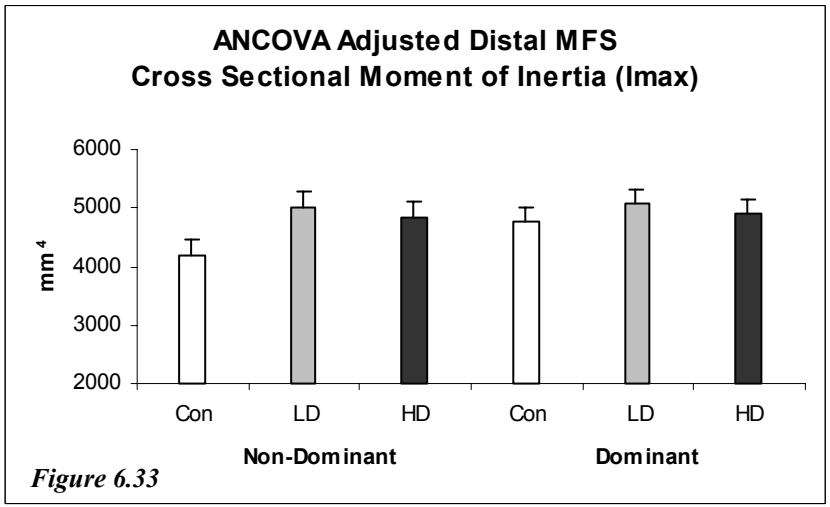

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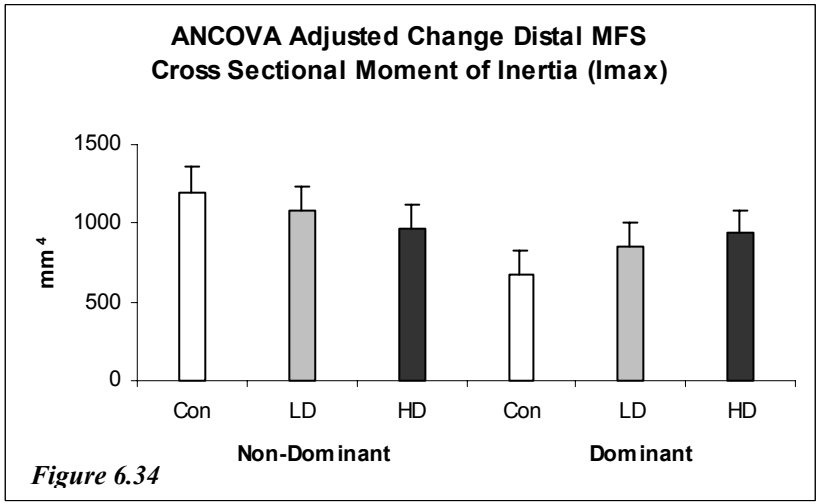

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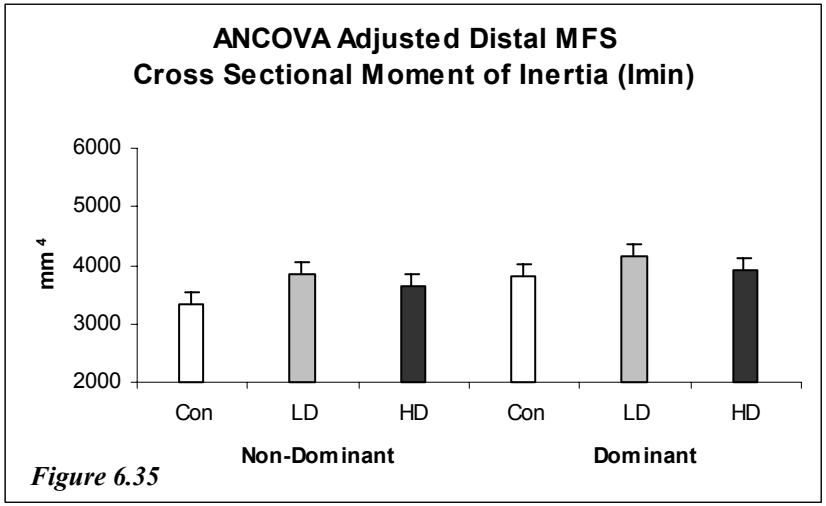

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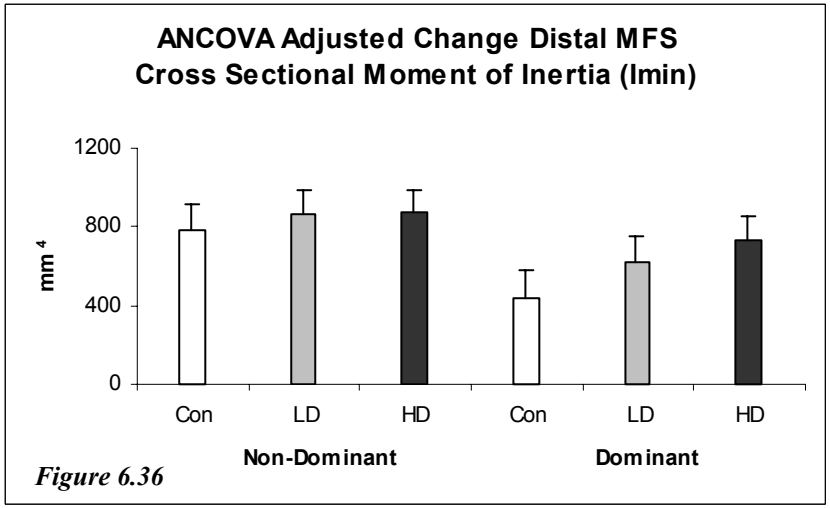

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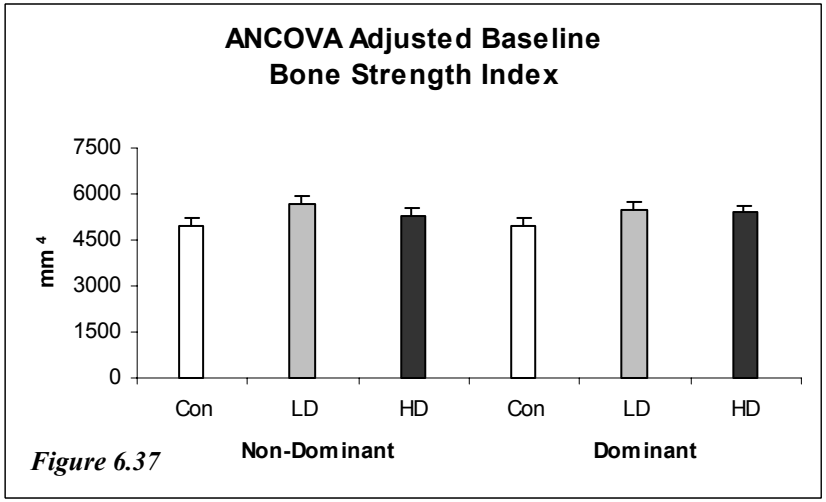

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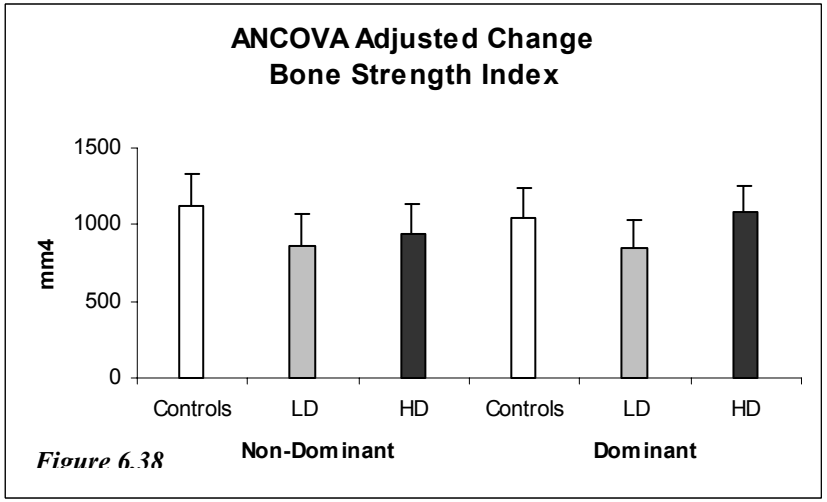

Figure 6.38

Table 6.9 Within-group baseline and post-intervention comparisons of differences between cortical, medullary cavity and total bone cross-sectional areas for proximal, mid and distal mid-femoral shaft slices of non-dominant and dominant legs: Means±SD

	Baseline			***Post Intervention***		
	Non-Dominant Leg	***Dominant Leg***		***Non-Dominant Leg***	***Dominant Leg***	
Controls	(N =13)	(N =13)	***p***	(N =12)	(N =12)	***p***
Proximal cortical CSA (mm^4)	159.36 ±24.93	162.69 ±24.83	.735	182.09 ±25.43	180.17 ±27.33	.860
Proximal medullary CSA (mm^4)	90.91 ±22.42	90.87 ±21.13	.997	96.90 ±24.61	98.21 ±22.87	.894
Proximal total bone CSA (mm^4)	250.27 ±37.09	253.57 ±37.70	.824	278.99 ±40.78	278.37 ±44.21	.972
Mid cortical CSA (mm^4)	163.28 ±19.90	161.61 ±22.36	.842	181.64 ±24.39	181.83 ±24.73	.989
Mid medullary CSA (mm^4)	77.69 ±23.31	78.53 ±22.68	.926	82.27 ±23.36	83.21 ±22.89	.922
Mid total bone CSA (mm^4)	240.97 ±36.33	240.14 ±37.17	.955	263.91 ±40.29	264.98 ±41.26	.949
Distal cortical CSA (mm^4)	144.39 ±22.75	149.22 ±23.95	.603	159.74 ±22.07	162.92 ±22.83	.732
Distal medullary CSA (mm^4)	119.56 ±29.95	110.52 ±25.30	.414	126.82 ±27.27	121.63 ±26.33	.640
Distal total bone CSA (mm^4)	263.95 ±47.05	259.73 ±43.52	.815	286.56 ±45.56	284.55 ±46.01	.915
Low Drop [φ] (N =12)						
Proximal cortical CSA (mm^4)	176.48 ±30.45	179.77 ±30.14	.785	190.48 ±29.86	190.86 ±27.33	.973
Proximal medullary CSA (mm^4)	88.67 ±15.17	88.36 ±17.11	.961	93.00 ±17.83	96.17 ±19.58	.670
Proximal total bone CSA (mm^4)	265.16 ±41.11	268.13 ±42.93	.858	283.48 ±42.01	287.04 ±43.09	.833
Mid cortical CSA (mm^4)	179.71 ±29.79	180.02 ±29.46	.979	197.54 ±33.35	194.57 ±27.09	.805
Mid medullary CSA (mm^4)	74.88 ±15.50	76.29 ±15.40	.818	80.42 ±17.55	80.60 ±16.77	.978
Mid total bone CSA (mm^4)	254.59 ±40.09	256.32 ±40.37	.914	277.96 ±45.05	275.17 ±40.59	.870
Distal cortical CSA (mm^4)	160.69 ±26.04	167.59 ±26.60	.511	177.60 ±25.65	175.16 ±23.35	.802
Distal medullary CSA (mm^4)	122.84 ±29.11	123.04 ±27.81	.986	134.01 ±29.76	133.66 ±30.71	.977
Distal total bone CSA (mm^4)	283.54 ±49.37	290.63 ±49.76	.718	311.61 ±50.62	308.82 ±48.91	.888
High Drop (N =13)						
Proximal cortical CSA (mm^4)	176.27 ±18.91	175.48 ±12.60	.901	196.47 ±21.93	187.84 ±13.97	.243
Proximal medullary CSA (mm^4)	94.95 ±36.60	91.70 ±28.27	.802	92.71 ±25.88	96.73 ±32.13	.729
Proximal total bone CSA (mm^4)	271.21 ±49.14	267.17 ±37.07	.815	289.18 ±43.21	284.56 ±40.79	.782
Mid cortical CSA (mm^4)	172.60 ±16.59	175.13 ±13.77	.676	194.00 ±21.58	190.99 ±20.01	.716
Mid medullary CSA (mm^4)	85.37 ±24.46	85.74 ±24.63	.970	88.91 ±22.73	91.00 ±25.25	.826
Mid total bone CSA (mm^4)	257.97 ±32.27	260.87 ±31.84	.820	282.91 ±37.66	281.99 ±35.85	.950
Distal cortical CSA (mm^4)	155.74 ±13.78	158.79 ±12.68	.563	169.74 ±19.74	173.92 ±17.24	.570
Distal medullary CSA (mm^4)	139.05 ±32.50	137.07 ±34.66	.882	150.02 ±36.07	143.75 ±33.90	.652
Distal total bone CSA (mm^4)	294.80 ±40.81	295.86 ±42.82	.949	319.76 ±48.68	317.66 ±43.67	.909

[γ]N=12 - one non-dominant post-intervention control group MRI scan unable to be analysed due to movement artefact

[φ]N=12 - one non-dominant post-intervention LD group MRI scan in unable to be analysed due to movement artefact

Table 6.10 Within-group baseline and post-intervention comparisons of differences between cross-sectional moments of inertia for proximal, mid and distal mid-femoral shaft slices of non-dominant and dominant legs: Means±SD

	Baseline			***Post Intervention***		
	Non-Dominant Leg	***Dominant Leg***		***Non-Dominant Leg***	***Dominant Leg***	
Controls [ϒ] ***(N =12)***			**p**			**p**
Proximal J0 (mm^4)	7195 ±1954	71911 ±2007	.996	8561 ±2518	8371 ±2532	.855
Proximal Imax (mm^4)	4231 ±1193	4174 ±1138	.997	4985 ±1549	4821 ±1451	.859
Proximal Imin (mm^4)	2964 ±826	3017 ±918	.443	3576 ±1054	3549 ±1112	.953
Mid J0 (mm^4)	6580 ±1893	6583 ±1819	.905	7906 ±2687	8100 ±2582	.883
Mid Imax (mm^4)	3816 ±1203	3791 ±1095	.958	4592 ±1677	4714 ±1584	.928
Mid Imin (mm^4)	2764 ±733	2792 ±769	.493	3314 ±1064	3386 ±1050	.396
Distal J0 (mm^4)	7046 ±2328	7851 ±2711	.791	8750 ±2890	8820 ±2785	.953
Distal Imax (mm^4)	3940 ±1329	4352 ±1558	.857	4971 ±1725	4922 ±1671	.869
Distal Imin (mm^4)	3106 ±1019	3499 ±1200	.944	3779 ±1197	3897 ±1165	.809
Low Drop [φ] *(N =12)*						
Proximal J0 (mm^4)	8638 ±2620	8551 ±2512	.934	10032 ±3079	9899 ±2672	.911
Proximal Imax (mm^4)	4955 ±1453	5038 ±1474	.765	5855 ±1726	5892 ±1549	.787
Proximal Imin (mm^4)	3683 ±1204	3512 ±1072	.676	4177 ±1405	4007 ±1166	.963
Mid J0 (mm^4)	8554 ±2676	8225 ±2638	.890	10117 ±3158	9778 ±2928	.717
Mid Imax (mm^4)	5031 ±1577	4767 ±1528	.681	5994 ±1954	5661 ±1742	.891
Mid Imin (mm^4)	3523 ±1135	3458 ±1145	.824	4123 ±1239	4116 ±1235	.490
Distal J0 (mm^4)	9203 ±2714	9711 ±3139	.956	11334 ±3177	11274 ±3097	.749
Distal Imax (mm^4)	5192 ±1605	5353 ±1896	.664	6386 ±1960	6271 ±1966	.989
Distal Imin (mm^4)	4012 ±1142	4358 ±1271	.888	4948 ±1255	5003 ±1170	.913
High Drop (N =13)						
Proximal J0 (mm^4)	7986 ±1839	8151 ±1939	.826	9305 ±2315	9595 ±2395	.756
Proximal Imax (mm^4)	4697 ±1196	4711 ±1105	.807	5399 ±1342	5561 ±1419	.922
Proximal Imin (mm^4)	3289 ±680	3440 ±867	.495	3906 ±1010	4034 ±1011	.789
Mid J0 (mm^4)	7644 ±1708	7802 ±1562	.976	9612 ±2541	9518 ±2269	.626
Mid Imax (mm^4)	4460 ±1202	4550 ±1062	.841	5658 ±1649	5574 ±1479	.755
Mid Imin (mm^4)	3184 ±531	3252 ±565	.685	3954 ±997	3944 ±951	.320
Distal J0 (mm^4)	8598 ±1777	9066 ±1669	.767	10519 ±2590	10781 ±2355	.750
Distal Imax (mm^4)	4881 ±1052	5040 ±917	.893	5894 ±1475	6007 ±1337	.979
Distal Imin (mm^4)	3718 ±750	4027 ±800	.839	4625 ±1146	4774 ±1060	.733

[ϒ] N=12 - one non-dominant post-intervention control group MRI scan unable to be analysed due to movement artefact

[φ] N=12 - one non-dominant post-intervention LD group MRI scan in unable to be analysed due to movement artefact

6.4 Discussion

The results of this study suggest that drop landing exercises, without follow-up countermovement jumping, from heights of up to 28 cm was not effective in eliciting osteogenic adaptations in bone geometry, biomechanical properties or the bone strength index which is a surrogate measure of the bone's resistive force to breaking in prepubertal girls. These findings are consistent with the findings in Chapter 5 in which no influence of drop-landing exercise on measures of whole body and regional BMC, as well as volumetric cortical BMD in the same population was detected.

Previous studies involving children in association with, or in response to, exercise have reported aspects of bone geometry including bone cross sectional area (Bass et al., 2002; Duncan et al., 2002; Dyson et al., 1997; Fuchs et al., 2001; Petit et al., 2002; Morris et al., 1997), cortical diameter and thickness (Bass et al., 2002; Duncan et al., 2002; Bradney et al., 1998; Petit et al., 2002; Heinonen et al., 2000). Fewer studies have examined biomechanics in association with exercise response (Bradney et al., 1998; Bass et al., 2002; Duncan et al., 2002; Heinonen et al., 2000; Faulkner et al., 2003). High-impact exercise appears to be the type of exercise to which bone cross sectional area responds (Duncan et al., 2002). In exercise regimens including high-impact as opposed to low impact (Heinonen et al., 2000), exercise for 7 (Fuchs et al., 2001; Petit et al., 2002) or more (Morris et al., 1997) months, bone cross sectional area at the femoral neck was significantly greater in the early maturing as well as (although equivocally Petit et al., 2002) prepubertal exercise groups. Additionally, the femoral neck increase was maintained for at least 7 months post-intervention (Fuchs et al., 2002). Moreover a femoral midshaft cortical thickness increase accompanied by an endocortical diameter decrease was observed after 3 sessions of 30-minute weight-bearing physical education weekly for 8 months (Bradney et al., 1998). Correspondingly, as a result of high-impact exercise, both prepubertal (Faulkner et al., 2003) and preadolescent (Dyson et al., 1997) female gymnasts had significantly greater femoral neck cross sectional area and cross-sectional moment of inertia measures than healthy non-athletic controls.

The most widely used technique for bone measurements is DXA. A recently emerging and recognised drawback for DXA-determined geometry and biomechanical parameters is the inability of this technology to account for the large changes in body and skeletal size that occur during

growth (Prentice et al., 1994). Therefore the validity of reporting measures derived from DXA is further limited in longitudinal studies in children (Gilsanz, 1998). Results of studies using more acceptable and validated MRI (Woodhead et al., 2000; Hong et al., 2000) and QCT (Ferretti et al., 1996) techniques confirm the adaptability of bone geometry and biomechanical measures to exercise (Dyson et al., 1997; Duncan et al., 2000; Heinonen et al., 2000). It is important to note that, to date, there have been no prospective, controlled training studies that have incorporated valid methods for measuring bone geometry and biomechanical outcomes in children, and none that have done so concurrently with adaptations in bone mineralization to permit comparisons of the relative responsiveness of these biological fronts to exercise.

Despite 7 months of moderate and intensive drop-landing exercise no training effects were evident in any of the bone geometry or biomechanical variables assessed in this study. These findings are consistent with the findings of Petit et al., (2002) who reported no differences in cortical thickness, endosteal diameter, or section modulus at FN, intertrochanter or femoral shaft regions following a similar period of weight-bearing exercise in children. Equivocally, after 8 months of 30-minute sessions of weight-bearing physical education lessons three times weekly, the cortical thickness and section modulus of the femoral midshaft increased in a cohort of prepubertal boys indicating a skeletal sensitivity to moderate weight-bearing exercise undertaken before puberty (Bradney et al., 1998).

In studies of athletic populations the skeleton has shown geometrical and biomechanical adaptation to exercise. Elite runners had significantly greater size-adjusted CSMI and BSI than elite swimmers, cyclists or triathletes (Duncan et al., 2002). Prepubertal female gymnasts had significantly greater size-adjusted strength indices at the narrow neck and shaft of the femur (Faulkner et al., 2003) and prepubertal competitive tennis players exhibited greater cortical area due to periosteal expansion at the mid and distal humerus (Bass et al., 2002). Furthermore, periosteal apposition before puberty appeared to account for the increase in torsional resistance with accompanying modification of bone shape (Bass et al., 2002).

These findings differ, however, from those reported recently by Faulkner et al., (2003) for axial (2.40 cm^2) and bending (1.13 cm^3) strength biomechanical parameters in elite pre-menarcheal gymnasts who were training for at least 15 hours per week and had been competing for a minimum of two years. Differences between studies may be explained in part by (a) the potential for selection bias in their prospective but not randomised study (Faulkner et al., 2003) (b) differences in magnitude of loading between these elite gymnasts and the healthy but non-athletic children in this study, and perhaps also by (c) developmental differences between the study populations. On the latter point, the girls in this study were confirmed to be pre-pubertal by an ultra-sensitive estrogen test at the completion of the study. However, the gymnasts in the study by Faulkner et al (2003) had advanced into the early stages of puberty, which may have conferred increased skeletal trainability, although this hypothesis remains to be proven.

In this chapter, the moderate nature of the impact loading resulted in large SDs in analysis of the changes in cortical, medullary cavity and total bone area (Tables 6.2, 6.3 and 6.4 respectively). This indicates large individual variation of participant values within each or the groups suggesting a random and unexplained result from the understanding of how bone adapts to exercise. Another possible explanation for large variability of results might be that with training, the non-dominant leg may have become the preferred leg for load bearing, in effect unloading the dominant leg and causing endocortical resorption. This suggestion can be supported in the LD group since an increase in the cortical CSA for the proximal, mid and distal slices of the exercised (non-dominant) leg was accompanied by and increase in the medullary cavity CSA (indicative of endocortical resorption). However, in the HD group the increase in the proximal slice cortical CSA was accompanied by a decrease in proximal slice medullary cavity CSA indicating endocortical apposition (Tables 6.2 and 6.3). This result was not observed in the dominant leg and the difference in medullary cavity CSA change between the two legs was not significant. Further investigation of the HD group proximal slice mean reduction in medullary cavity CSA compared to the proximal slice mean increase in medullary cavity CSA for LD and controls revealed no differences ($p=.432$).

Initially, the change in the distal slice total bone CSA for the LD group appeared greater (p=.019) than that of the controls however, after adjusting for baseline body and fat mass and change in LTM the difference disappeared (Table 6.4 and Figure 6.19).

The tendency for the change in control group dominant leg distal slice cortical CSA to be greater than that of the LD group, was highlighted after adjusting for baseline body and fat mass and change in LTM remains difficult to explain (Table 6.2 and Figure 6.7).

In a two year longitudinal study, a differing tempo and direction of growth of the periosteal and endocortical surfaces was observed in girls (Bass et al., 1999) which may in part explain this occurrence. It is difficult to attribute a direct cause–effect relationship here. On the one hand the dominant leg was not the exercised leg and owing to its dominant nature, any differences would most likely be due to normal habitual activity and growth. If indeed, a change such as this can be achieved with no training, this would seem to be a further justification for the null results observed in the exercised leg.

The study that is closest to the present study, and the only one in which participants remained prepubertal post intervention, was conducted by Fuchs, Bauer and Snow (2001). In contrast to the findings of the present study, the Fuchs et al., (2001) study reported significant increases in (i)BMC and aBMD at the lumbar spine (ii) BMC at the femoral neck and (iii) significantly greater increases in bone area at the femoral neck - after 7 months of high impact drop-jumping eliciting GRIF's of eight times body weight. Fuchs et al., (2001) described a protocol that began with stepping up onto a box, and then involved uni-directional, bilateral leg drops from a 61-cm-hight landing flatfooted, with straight posture and knees slightly bent followed by a walk/skip/run to the next box before stepping up again and jumping. Eventually, after 5 weeks 100 jumps were performed for the remaining 58 sessions. The protocol in this chapter involved isolated uni-directional single-leg loading landing on the ball of the non-dominant foot bending slightly at the knee and hip on contact to decelerate the body's centre of gravity. Furthermore, this study controlled for the positive work and related disparity involved in climbing to the top of each set of steps, whereas, the muscle-generated loads involved in climbing to the top of the boxes in the Fuchs et al., (2001) study were

unaccounted for. Differences in the findings between these two studies may be explained by several factors including differences in the strains distribution patterns and magnitudes, and possibly also by differences in total loading, as the number of jumps by themselves, not including the secondary exercise patterns in the Fuchs et al., (2001) study were substantially higher than in this study.

6.5 Conclusion

The null findings cannot be explained by poor compliance as all participants completed all required exercise sessions and bouts, performing 4,200 single-leg drop-landings. Concern for joint safety of the participants in this study and purposely limiting the maximum step riser height to 28cm consequently, limited the applied load magnitude. In retrospect, the training stimulus may have been below the minimum effective strain for modeling, limiting the likelihood of geometric or biomechanical adaptation. Alternatively, the engendered GRF during drop landing may have been sufficiently attenuated by eccentric muscular action (Bauer et al., 2001; Dufek, & Bates, 1990) to minimize transferal to bone.

CHAPTER 7

CONCLUSIONS

7.1 General Overview

This is the first intervention study to combine DXA and MRI measures in prepubertal children to understand skeletal response to controlled uni-modal, uni-directional mechanical loading. It is also the first to confirm prepubertal status of the participants by using an ultra sensitive estrogen assay technique.

In general, studies presented in chapters 4 to 6 aimed to determine whether (a) adaptive responses in the mineral, material and geometric properties of bone are dependent upon magnitude of impact loading exercise and (b) geometric (rather than mineral and material) properties of bone are dependent upon magnitude of impact loading exercise.

7.1.1 Training Prescription

The hypothesis that bone adaptive responses would be threshold-dependent reflecting two loading conitions in the female prepubertal population was not supported.

Specifically, no differences in bone mineral, bone geometry or bone biomechanical properties were found between the low drop (LD) group at 14cm step riser height and the high drop (HD) group at 28cm step riser height for drop-landing. Thus, at these threshold magnitudes, no adaptive response was discernable after the 28 week intervention.

The exercise prescription dose consisted of a pre-training week and training weeks. In the pre-training week there were 15 drop-landings in the 1st session, 30 drop-landings in the 2nd session and 45 drop-landings in the 3rd session (90 drop-landings in week 1). The dose for the training weeks consisted of 3 sessions per week of 50 drop-landings per session (150 per week for 28

weeks). The total workload consisted of 90 + 4200 (or 4290 drop-landings in total). At this training dose, no adaptive response was observed.

7.1.2 Impact Exercise

The hypothesis that impact exercise would have a smaller effect on BMD than on the other properties of bone in the female prepubertal population was not supported. Specifically, the impact of the exercise had no effect on BMD or BMC in the female prepubertal population. This result occurred following repeated, uni modal, uni-directional, weight-bearing, muscular contractions at ground reaction forces toward the end of the eight-month training period of between 4.54 –4.82 times body weight compared to a control group matched for age and pubertal status.

7.1.3 Exercised Leg Bone Density of Different Training Loads

The hypothesis that the trained (non-dominant) leg aBMD ($gm.cm^{-2}$) and volBMD ($gm.cm^{-3}$) in prepubertal girls would be significantly greater than that of the trained leg of the age- and pubertal stage- matched control group was not upheld. No differences were evident between groups at baseline, or in the measured change from pre to post-intervention in the trained (or untrained) leg bone mineral density variables. Additionally, there were no differences between groups at baseline, or in the measured change from pre to post-intervention in the trained (or untrained) mid-femoral shaft bone mineral density variables.

7.1.4 Regional and Site Specific Bone Density

The hypothesis of significant differences in regional and site-specific bone geometry between trained and non-trained legs of prepubertal girls involved in eight months of weight-bearing training was not confirmed. No differences in regional or site-specific bone geometry were detected between trained and non-trained legs of prepubertal girls involved in the intervention compared to a control group matched for age and pubertal status.

7.1.5 Muscle Hypertrophy and Muscle Size of Exercise Group

The hypothesis that both exercise groups would demonstrate muscle hypertrophy and greater muscle size than controls in the trained legs was not observed. No differences in muscle hypertrophy were detected between trained and non-trained legs after the conclusion of the intervention.

Nevertheless, multiple variables representing body size and composition, modifiable lifestyle behaviour (activity and nutrition), fitness (strength) and biological (estrogen status) influences, each of which are considered plausible determinants of BMD in humans were included in the multiple linear regression analysis. Results indicated that in this study, neither past year physical activity, muscle strength, dietary calcium intake nor estradiol status emerged as significant independent predictors of BMD in a narrow age-band of prepubertal girls. Body mass, however, and especially the lean tissue component, seems an important determinant of BMD at most sites in this population. These findings point to the importance of attaining and maintaining as high a lean tissue mass as possible for the promotion of bone health, even in prepubertal period.

By minimizing the effect of variable strain distribution patterns and ensuring the uni-modal drop-landing exercise pattern in this study directional variability was kept to a minimum allowing analysis of one only mode of force application. However, it is possible that the magnitude of loading in this study was below the minimum effective strain level for modeling, as the PGRIFs fell within the range of PGRIFs typical of many free play and sport activities in which children regularly participate (Janz, Rao, Baumann, & Schultz, 2003). Additionally, despite the within participant, between-leg design, the study could have been underpowered to detect statistical differences among group. This result may in part be from an over zealous estimate of the effect size that was largely based on published cross-sectional comparative studies of young athletes, prior to initiating this study. The prospective studies demonstrating more modest effects sizes were not published until after this study was initiated. Doubling the sample size of the exercise group increased power ($0.13 \leq \beta \leq 0.72$) for the primary bone mineral outcomes, but still failed to demonstrate an exercise effect, suggesting that the exercise protocol was not osteogenic in this prepubertal population. The null findings are not likely explained by the brevity of the exercise regimen, lasting only 28 weeks, as

this is comparable to other recently published studies demonstrating significant positive effects of training in prepubertal children. Although extremely time consuming, the findings would have been strengthened by the measurement of PGRIFs during training in all participants, not just the sub-samples selected from the control, LD and HD training groups. Complexities of existing devices to assess ground reaction forces prohibit on-going measurement during field-based physical activity interventions in relatively large groups of children.

While good technology has been used in these studies if more advanced technologies are applied a higher order of adaptation could be investigated. High radiation exposure however, would be a legitimate drawback in the use of alternative technologies such as Quantitative Computerised Tomography for whole body analysis.

Nevertheless, the study substantially advances what is currently known about musculoskeletal health in children. The study investigates the most comprehensive and developmentally selective group of potential determinants of musculoskeletal factors including age, height, body mass, lean tissue mass, fat mass, percent body fat, physical activity level, calcium intake, isokinetic knee flexion and extension strength and endocrine status.

The present study is the most definitive study to date in creating an application-controlled, quantifiable load to which the bone could be exposed. There was an advantage of being able to report MFS cortical volumetric BMD by a new validated technique combining BMC from DXA measurements with MRI measured bone volume. However, it is still possible that, without the ability to quantify trabecular density, the micro-architectural adaptations to *compressive* forces resulting in increased trabecular density might have been unable to be detected.

Lack of osteogenic mineral, geometrical or biomechanical response could not be attributed to poor compliance or length of administration of training. The results obtained have only minimal agreement in the prepubertal area however, only a few studies can be legitimately compared to this study. The studies (MacKelvie et al., 2001; Petit et al., 2002) strengthen the contention that pre-pubertal loading may not be effective for osteogenesis and maturation-related hormones are

required. The contentions of Khan et al., (2000), that Tanner stage II and III appears to be the maturational stage when the association between exercise and BMD becomes manifest can now, more confidently, be confirmed.

Prepubertal girls with a measured amount of definitively prepubertal estradiol would appear to lack hormonal osteogenic stimulus. Conceivably, a relatively lean group of participants, normally active may achieve sufficient loading in every day play activity to sustain developmentally appropriate skeletal development without the need for additive loading. This would seem to be a further justification for the null results observed in the exercised leg.

The limited applied load magnitude, it would appear, delivered with a uni-directional, uni-modal restriction was not a sufficient training stimulus for modeling obviating geometrical or biomechanical adaptation. The ground reaction forces may have been sufficiently attenuated by eccentric muscular action to reduce any impact generated load below the level required for modeling.

7.2 Recommendations

(1) No bone impact can be isolated from the muscle bone unit in which exits. More research needs to be directed at quantifying load in relation to muscle-bone interaction. Habitual loading thresholds, which are a developmental characteristic of childhood exposure to repeated activities, need to be accurately identified and then exceeded to generate an osteogenetic stimulus. Additionally, load attenuation by improved muscle/skill efficiency needs to be monitored.

(2) The same exercise prescription in pubertal and post-pubertal groups of females and males needs to be undertaken to determine the influence of pubertal stage on skeletal adaptation.

(3) A change in intervention regimes incorporating 2-foot drop jumps, higher step riser heights and increased doses (eg 4 times per week) of training may be appropriate in future research.

(4) The use of a relatively sedentary group for comparison rather than an active control group would allow a more definitive analysis.

(5) Portable load devices, with a similar temporal feedback capacity as an accelerometer or heart rate monitor, would be able record single and cumulative loading for analysis.

(6) Investigation of trabecular micro-architectural adaptations to compressive loading may be possible in studies of a similar nature by involving pQCT or validated MRI technology

(7) The impact of multidimensional, but quantifiable, loads needs to be explored using safe and developmentally appropriate activities.

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APPENDICIES

Appendix A – Information Sheet

An opportunity to be involved in a
University research project
linked to the New Children's Hospital

What's This All About?

The New Children's Hospital
at Westmead together with
Australian Catholic University

wants to see if exercise can make bones stronger.

Some studies indicate that increasing physical activity in childhood has a good effect on bone later in life. Little is known though, about the best way to make bone stronger in childhood. It seems that exercise in children leads to more bone, bigger bone and heavier bone.

By hopping from different heights for a short training time over a number of months the muscles will contract with different forces when you land. If one height is shown to be better than others for making bones stronger then we can use this information to make a training program to increase bone strength in childhood.

Benefits

This study will help us understand how we might be able to lower the risk of breaking bones in later life.

It will also give **YOU** information about:

- your own bone health
- the relationship between physical activity, diet and bone health
- your own body composition
- your leg muscle strength measurements

By volunteering you will become a member of a **special research team**

INTERESTED?

Please read the pamphlet for **parents** and the pamphlet for **research team members** that came with this letter.

Appendix B – Initial Medical Status Questionnaire

Telephone Questionnaire NAME:_____

PHONE:_____

MEDICAL/HEALTH

1. Has your daughter seen a doctor in the last 6 months for a medical concern or injury? Yes ❒ No ❒
If yes, what was the reason for her visit?_____

2. Has there been any change in your daughter's general health during the last 6 months? Yes ❒ No ❒
If yes, please describe the nature of the change:

3. Has your daughter been hospitalised in the last year? Yes ❒ No ❒
If yes, please indicate the medical condition(s) that was being treated.

4. Is your daughter currently taking any prescribed medications? Yes ❒ No ❒
If yes, which medications is she taking?

5. What are these medications for?

6. Has your daughter ever taken any of the following medications? Please indicate at what age she began to use them and for how long she used them.

Medication	**Currently Using**	**Age at Start**	**Duration of Use**
eg Insulin		6 years old	2 years
Calcium Preparations			
Antacids			
Inhaled steroids			
Anabolic steroids			
Fluoride			
Vitamin D compounds			
Calcitonin			
Diuretics			
Heparin			
Cortisone (oral)			
Corticosteroids			
Anti-inflammatories			
Thyroid preparations			

Has your daughter ever been treated for any of the following conditions?
(Hyper = excess Hypo = deficiency)

food allergies	yes ☐	no ☐	asthma	yes ☐	no ☐
other allergies	yes ☐	no ☐	kidney disease	yes ☐	no ☐
back pain	yes ☐	no ☐	liver problems	yes ☐	no ☐
scoliosis	yes ☐	no ☐	gastrointestinal disease	yes ☐	no ☐
epilepsy	yes ☐	no ☐	muscular dystrophy	yes ☐	no ☐
osteoporosis	yes ☐	no ☐	osteoarthritis	yes ☐	no ☐
rheumatoid arthritis	yes ☐	no ☐	anemia	yes ☐	no ☐
diabetes	yes ☐	no ☐	malabsorption	yes ☐	no ☐
excess urinary calcium	yes ☐	no ☐	excess blood	yes ☐	no ☐
hyperthyroidism	yes ☐	no ☐	calcium	yes ☐	no ☐
hypothyroidism	yes ☐	no ☐	hyperparathyroid	yes ☐	no ☐
food allergies	yes ☐	no ☐	hypoparathyroid	yes ☐	no ☐
			other?		

SPORT INVOLVEMENT
Does your daughter currently take part in any organised activity
(eg gymnastics, dance , sport)? Yes ☐ No ☐

If yes, when did she start participating? Year_____ Month_____

How many hours does she train per week? Hours_____

How many hours of competition per week? Hours_____

How many months a year does she train? _____

1. Your daughter is cleared to enter the study Yes ☐ No ☐ OR
2. There are some things that need to be checked out I will phone you back with the results

Are you familiar with the carpark at the Children's Hospital?

- Please park in the carpark - refer to the map on the last page of the pamphlet sent home with your daughter. We will cover the cost of the parking.
- Please have her at the hospital this Saturday/Sunday at _____(time)
- Please have her wear a T-shirt and shorts
- Please ensure that there is no metal in what she wears (eg zippers, buttons)
- Please inform us if she is wearing braces on any of her teeth.

Availability for Testing (3 at each session initially)

Preferred Time	Sat 13th Mar	Sun 14th Mar	Sat 20th Mar	Sun 21st Mar	Sun 28th Mar	Sun 29th Mar		
8.30 - 11 30								
12.30 - 3.30								
4.30 - 7.30								

Appendix C – Medical History Questionnaire

DATA RECORDS - PARTICIPANT INFORMATION

ID	
NAME	
CONTACT PHONE NUMBER	
AGE **BIRTHDATE**	Years _____Months_ Day Month Year
HEIGHT	
WEIGHT	
PUBERTAL STATUS	**TANNER STAGE** **B =** **P =**
DOMINANT LEG	

Has your daughter had a bone scan or diagnostic x-ray in the last year? Yes ☐ No ☐

If yes, what part of the body was x-rayed? _____

Has your daughter ever had a fractured bone? Yes ☐ No ☐

If yes, please indicate which bone(s) were fractured and when the fractures occurred:

1st fracture: body part_____ _____Month_____Year
2nd fracture: body part_____ _____Month_____Year
3rd fracture: body part_____ _____Month_____Year

Has your daughter ever been hospitalised or confined to bed for any reason - or had a limb immobilised (eg arm in a cast) for 21 days or longer? Yes ❒ No ❒

If yes, please list the condition, approximate date it occurred and the length of time she was hospitalised or immobilised.

Injury Type	Date of Injury	Time Immobilised
eg Wrist fracture	July 1998	6 weeks

Daughter's Medical Declaration

Does your daughter have any medical or health condition which might prevent her from participating in this study? Yes ☐ No ☐

I (parent/guardian) certify that the information provided in this form is correct.

Signature of parent/guardian _____

Signature of daughter _____

Date_____

Appendix D – Past Year Leisure Time Physical Activity Questionnaire

PAST YEAR LEISURE-TIME PHYSICAL ACTIVITY

Tick all the activities that you did at least 10 times in the PAST YEAR. Do not include time spent in school physical education classes. Make sure you include all sports teams that you participated in during the last year.

Aerobics
Band/Drill Team
Baseball
Basketball
Bicycling
Bowling
Cheerleading
Dance Classes
Gardening/Yard work
Gymnastics
Hiking
Ice Skating
Netball
Roller Skating
Rugby
Running for exercise
Skateboarding
Snow Skiing
Soccer
Softball
Swimming (Laps)
Tennis
Volleyball
Water Skiing
Weight Training (Competitive)
Wrestling
Others:
_____
_____
_____

List each activity you ticked above in the “Activity” box below
Tick the months you did each activity and then estimate the amount of time spent in each activity.

Activity	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Months Per Year	Days Per Week	Minutes Per Day

Modifiable Activity Questionnaire for Adolescents

DATE_____ NAME_____ ID_____ SCHOOL CLASS_____

1. How many times in the past 14 days have you done at least 20 minutes of exercise hard enough to make you breathe heavily and make your heart beat fast? (Hard exercise includes, for example, playing basketball, jogging, or fast bicycling; include time in physical education class)

( ) None
( ) 1 to 2 days
( ) 3 to 5 days
( ) 6 to 8 days
( ) 9 or more days

2. How many times in the past 14 days have you done at least 20 minutes of light exercise that was not hard enough to make you breathe heavily and make your heart beat fast? (Light exercise includes, for example, playing basketball, walking or slow jogging, include time in physical education class)

( ) None
( ) 1 to 2 days
( ) 3 to 5 days
( ) 6 to 8 days
( ) 9 or more days

3. During a normal week how many hours a day do you watch television and videos or play computer or video games before or after school

( ) None
( ) 1 hour or less
( ) 2 to 3 hours
( ) 4 to 5 hours
( ) 6 or more hours

1. During the past 12 months, how many team or individual sports or activities did you participate in on a competitive level, such as in sports period at school or out -of -school (weekend) sports?

( ) None
( ) 1 activity
( ) 2 activities
( ) 3 activities
( ) 4 or more activities

Which activities did you compete in?

_____ _____

_____ _____

BOUCHARD THREE-DAY PHYSICAL ACTIVITY RECORD

Day 1 **Date: _____/_____/_____**
day month year

Last Name:_____

First Name:_____

In each box write the number which corresponds to the activity which you have carried out during this 15 minute period. Please consult the activities on the back of this sheet to record the proper coding. If an activity is carried out over a long period (eg sleeping) you can draw a continuous line in the rectangular boxes which follow until such a time when there is a change in activity. To understand this better please see the example that is attaches.

In this example the subject gets up at 8am and showers combs hair and gets dressed (30 mins) then eats breakfast (30 mins). At 9am subject walks to school (15 mins) and plays a game for 15 mins before class. Subject does not play at recess and goes back into school till lunchtime at 12.30pm. Subject eats lunch for 30 mins and then plays for 30 mins before going back to class at 1.30pm for the afternoon. At 3.30pm Subject walks home from school (15 mins) and walks (15 mins) to netball practice where she trains hard for 45 of the 60 mins training time. Subject walks home (15 mins) and has tea for 30 mins at 5.15pm. Now its homework for 30 mins and then TV till bedtime at 8.30pm.

Minute/	0-15	16-30	31-45	46-60
0 (Midnight)				
1				
2				
3				
4				
5				
6				
7				
8	**4**		**2**	
9	**5**	**6**	**2**	
10				
11				
12 (Noon)			**2**	
13	6		2	
14				
15			5	
16	8	9		
17	5	2		
18				
19				
20			1	
21				
22				
23				

Activity Codes for the Bouchard Three Day Physical Activity Record

Category of Activity	Example of activity of each category
1	Lying down: - sleeping - resting in bed
2	Seated - listening in class - eating - writing by hand or typing - reading - listening to the radio or TV - taking a bath
3	Standing light activity - washing oneself - shaving - combing hair - cooking
4	Getting dressed Taking a shower Driving a car Taking a walk (strolling)
5	Light physical work - housework - making the bed - feeding animals on a farm - riding a bicycle Moderately quick walking (going to school, shopping)
6	Light sport or leisure activities - light canoeing - archery - volleyball - croquet - table tennis - sailing - softball (except pitcher)- cycling (leisure) - golf - rowing
7	Moderate physical work - loading bags - lifting boxes - cutting the grass
8	Moderate sport or leisure activities - canoeing - horseback riding - tennis - skiing - dancing - swimming - softball (pitcher) - gymnastics - brisk walking - jogging (slow running)
9	Intense physical work - felling a tree with an axe (appropriate?) - cutting tree branches (appropriate?) Intense sport or leisure activities - running in a race - field hockey - basketball - racquetball - mountain climbing - cross country skiing

Activity Codes for the Bouchard Three Day Physical Activity Record

Category of Activity	Example of activity of each category	Approximate energy expenditure (kcal/kg/15 min)
1	Lying down: - sleeping - resting in bed	0.26
2	Seated - listening in class - eating - writing by hand or typing - reading - listening to the radio or TV - taking a bath	0.38
3	Standing light activity - washing oneself - shaving - combing hair - cooking	0.57
4	Getting dressed Taking a shower Driving a car Taking a walk (strolling)	0.70
5	Light physical work -housework -making the bed -feeding animals on a farm -riding a bicycle Moderately quick walking (going to school, shopping)	0.83
6	Light sport or leisure activities -light canoeing -archery -volleyball -croquet -table tennis -sailing -softball (except pitcher) -cycling (leisure) -golf -rowing	1.20
7	Moderate physical work -loading bags -lifting boxes -cutting the grass	1.40
8	Moderate sport or leisure activities - canoeing -horseback riding -tennis -skiing -dancing -swimming -softball (pitcher) -gymnastics brisk walking -jogging (slow running)	1.50
9	Intense physical work -felling a tree with an axe (appropriate?) -cutting tree branches(appropriate?) Intense sport or leisure activities - running in a race -field hockey -basketball - racquetball -mountain climbing - cross country skiing	1.95

Appendix F – Dietary Calcium Intake Questionnaire

CALCIUM

HOW TO ANSWER

How often did you eat these foods last week?

Not last week N
Times a week` 1W, 2W, 3W (and so on)
Times a day 1D, 2D, 3D (and so on)
Please give an answer for every food!

DAIRY FOODS AND EGGS

		How Often?	**Comments**
Glass of plain milk (excludes milk on cereal and in hot drinks)	medium glass		
Glass of flavoured milk	medium glass		
Milk shake	regular size		
Thick shake	regular size		
Cheese (includes cheddar, colby, edam, brie, camembert)	20g (1 slice)		
Reduced fat cheese	20g (1 slice)		
Cottage cheese	100g (½ carton)		
Cheese spread	25g (1 tblspoon)		
Cheese sauce/cream sauce (eg on meat/pasta)	3 tblspoons		
Cream	(1 tblspoon)		
Yoghurt	200g (1 carton)		
Ice cream	2 scoops		
Custard	½ cup		
Custard (no added sugar)	½ cup		
Fried egg	1 egg		
Boiled egg/poached	1 egg		
Omelette/scrambled eggs	2 eggs		

DAIRY FOODS AND EGGS I CONSUMED THAT HAVE NOT BEEN MENTIONED:

If you had any other dairy foods or eggs in the last 7 days (last week) that have not been mentioned, please write them down below and tell us how often them - using the same code as before (ie: 1D, 3W)

Name of food	**Your usual serve size**	**How often?**

Please circle one number:

Q1 When you drank milk or added it to cereal etc. Did you use:

1. Whole milk
2. Shape
3. Reduced fat milk (eg: litre white)
4. Skim milk
5. Farmer's best
6. Something else - Please describe:_____

Q2 When you ate yoghurt what type was it?

1. Plain
2. Plain, low fat
3. Fruit flavoured
4. Fruit flavoured, low fat
5. Diet fruit flavoured (sweetened with Nutrasweet)
6. I did not eat yoghurt

Thank you

Appendix G – Dual-Energy X-ray Absorptiometry Consent Form

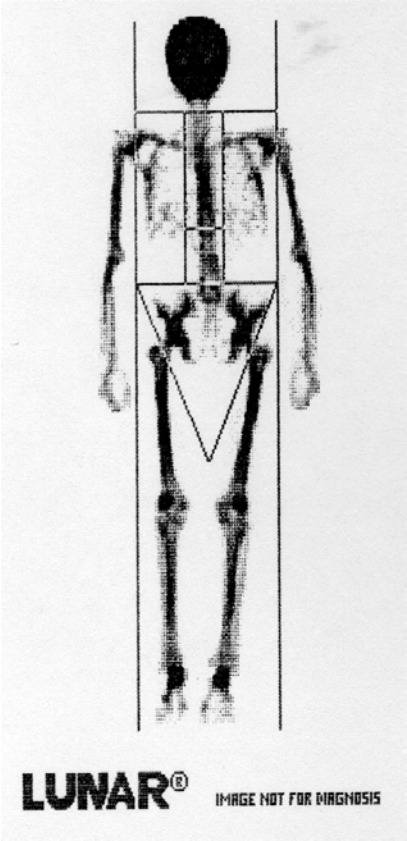
DEXA (Dual Energy X-Ray Absorptiometry)

Information Sheet

DEXA or DXA is short for "Dual-Energy X-ray Absorptiometry". DEXA is used to measure the amount of bone mineral in the bone and thus give an estimate of bone density. DEXA is also becoming more widely used to measure body composition as the technique also measures the amount of lean tissue and fat tissue.

At the NCH, DEXA measurements are made of the total body, lumbar spine and the top of the femur. The effective radiation dose for the three scans is less than 1 μSv, which is less than that normally received daily from natural sources of radiation (6 μSv/day).

DEXA is an easy, painless test. There are no needles or injections. All you need to do is lie on a table for approximately 30 minutes. Whilst you lie on the table, an x-ray beam is passed through your body. As the x-ray travels through your body, air, bone and tissue stop some of the x-ray. By knowing how much x-ray your body stops, the machine can work out how much bone and tissue your body contains. (It also uses the information to make a picture like that shown on this page.)



Consent Form

DEXA (Dual Energy X-Ray Absorptiometry)

I/We have read the Information Sheet regarding this procedure. I understand that this procedure involves no discomfort to me/my child, and there are no known risks, except for a small radiation exposure of 1 μSv The cost of the procedure will be met by the Department of Nuclear Medicine. I have been assured that the records will be kept confidential and that the information released or published will not disclose my identity without my permission.

I am aware that I may withdraw myself/my child from the procedure at any time and by doing so, that this will not jeopardise me/my child's management at this hospital.

I hereby consent to participate.

NAME OF SUBJECT:

SIGNATURE OF SUBJECT:
(12 years and older)

.....

NAME OF PARENT:

.....

SIGNATURE OF PARENT:

NAME OF WITNESS : .....

SIGNATURE OF WITNESS : .....

DATE:

.....

The persons to contact about this procedure:

.....

Dr Robert Howman-Giles, Dr Roger Uren or Julie Briody
Dept of Nuclear Medicine .....
New Children's Hospital,
WESTMEAD.
Phone: 9845 2890

Appendix H – Magnetic Resonance Imaging Consent Form

MRI PRE-SCAN QUESTIONNAIRE

Your child has been booked to have a MRI procedure. MRI stands for Magnetic Resonance Imaging. This test utilises a powerful magnet (which is always "ON") to create the images. Magnets affect certain substances, so it is important that you read this sheet carefully, answer the questions and follow the staff's instructions. This will ensure the best investigation of your child.

WEIGHT (kg).....

SURNAME..... **GIVEN NAMES.....**

DATE OF SCAN..... **MEDICAL RECORD NUMBER .....**
(if applicable)

Unless your child is having a general anaesthetic, you may accompany your child into the MRI room for the procedure. Therefore you will need to consider the questions for yourself as well as your child. **Please circle YES or NO to the following questions.**

DO YOU OR YOUR CHILD HAVE ANY OF THE FOLLOWING?
(Please complete both boxes):

	CHILD	**PARENT**	**PARENT**
A cardiac pacemaker?	**Yes/No**	**Yes/No**	**Yes/No**
Neurostimulators?	**Yes/No**	**Yes/No**	**Yes/No**
A cochlea implant?	**Yes/No**	**Yes/No**	**Yes/No**
Brain aneurysm clips?	**Yes/No**	**Yes/No**	**Yes/No**
An artificial heart valve?	**Yes/No**	**Yes/No**	**Yes/No**
Metal in eyes?	**Yes/No**	**Yes/No**	**Yes/No**
Any other implants (after operation)?	**Yes/No**	**Yes/No**	**Yes/No**

If you have answered "YES" to any of the above questions, please notify the MRI staff immediately.

Artificial joints	Yes/No	Yes/No	Yes/No
Metal rods/plates/screws/nails?	Yes/No	Yes/No	Yes/No
Wire sutures/surgical clips?	Yes/No	Yes/No	Yes/No
Shrapnel injuries?	Yes/No	Yes/No	Yes/No

SIGNATURE..... DATE.....
(of parent/guardian if under 18yrs)

Prior to the procedure you and/or your child will be required to remove all metal jewellery and any loose objects. You may also need to remove other items (which could either be damaged by the magnet, or cause the images to be distorted). A secure locker will be provided, and you may keep the key with you. Depending on the clothing your child is wearing, they may be required to change into a hospital gown for the examination.

Please turn off your mobile phones.

If you have any further questions, please don't hesitate to ask. Please do not ask the radiographers to review the images with you after the examination as this can only be done with your referring doctor.

Revised March, 2003

PRELIMINARY TRAINING PROTOCOL

PRELIMINARY SESSION

- **Floor Sequence**
    - ✶ Walk around gym

- **Stretch sequence (Before and after each training session)**
    - ✶ Gastroc stretch Lean to wall - press body to floor
    - ✶ Quad stretch - bend leg pull toe back
    - ✶ Hamstring stretch bend over - hang and let gravity pull - do not touch toes

- **Activity sequence**
    - ✶ Bound on two legs
    - ✶ Hop on kicking leg
    - ✶ Hop on non-kicking leg

- **Stair bench sequence**
    - ✶ Low bench - both training groups
    - ✶ Both legs : Bound down - holding rail for balance - head up (absorb force and pause)
    - ✶ Technique: One set then split higher training group
    - ✶ Single leg (non-kicking leg) balance don't twist - absorb force - pause hold rail for balance - head up

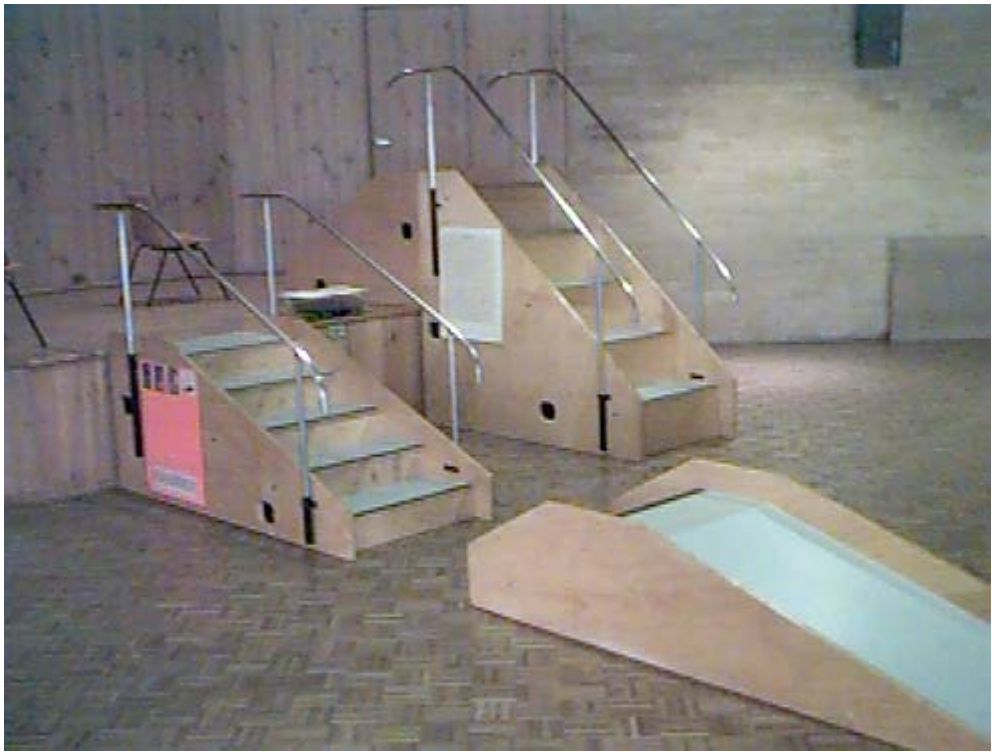
- **Low Drop group**
    - ✶ On low bench - three sets

- **High Drop group**
    - ✶ On high bench
    - ✶ Bound down - two feet - holding rail - pause to absorb force - head up
    - ✶ Hop down - non-kicking foot - holding rail - pause to absorb force - head up 3 sets

Stretch sequence to conclude

Appendix J – Exercise Intervention Equipment Arrangement

Training Set Up (Front View)



Training Set Up (Side View)

Appendix K – Progress Chart Records

Compliance Record for Low Drop Training Group

Low Drop Training Group	*Week 1*			*Week 2*		
	1	**2**	**3**	**1**	**2**	**3**
Participant#1	*	*	*	*	*	*
Participant#2	*	*	*	*	*	*
Participant#3	*	*	*	*	*	*
Participant#4	*	*	*	*	*	*
Participant#5	*	*	*	*	*	*
Participant#6	*	*	*	*	*	*
Participant#7	*	*	*	*	*	*
Participant#8	*	*	*	*	*	*
Participant#9	*	*	*	*	*	*
Participant#10	*	*	*	*	*	*
Participant#11	*	*	*	*	*	*
Participant#12	*	*	*	*	*	*
Participant#13	*	*	*	*	*	*
Participant#14	*	*	*	*	*	*

* = completed session

Appendix L – Calculated Distances

Reserach

Low Drop					**High Drop**				
Group #2		cms	metres		Group #3		cms	metres	
1 hop		14	0.14		1 hop		28	0.28	
5 hops /set		70	0.7		5 hops /set		140	1.4	
10 sets/bout		700	7		10 sets/bout		1400	14	
3 bouts/week		2100	21		3 bouts/week		4200	42	
7 m=28w		58800	588		7 m=28w		117600	1176	
Distance					**Distance**				
Week	1	2100	21		Week	1	4200	42	
Week	2	4200	42		Week	2	8400	84	
Week	3	6300	63		Week	3	12600	126	
Week	4	8400	84		Week	4	16800	168	
Week	5	10500	105		Week	5	21000	210	
Week	6	12600	126		Week	6	25200	252	
Week	7	14700	147	**Harbour Bridge - 132m**	Week	7	29400	294	
Week	8	16800	168		Week	8	33600	336	**Sydney Tower - 300m**
Week	9	18900	189		Week	9	37800	378	
Week	10	21000	210		Week	10	42000	420	
School Hols					School Hols				
Week	11	23100	231		Week	11	46200	462	
Week	12	25200	252		Week	12	50400	504	
Week	13	27300	273		Week	13	54600	546	
Week	14	29400	294		Week	14	58800	588	
Week	15	31500	315	**Sydney Tower - 300m**	Week	15	63000	630	
Week	16	33600	336		Week	16	67200	672	
Week	17	35700	357		Week	17	71400	714	
Week	18	37800	378		Week	18	75600	756	
Week	19	39900	399		Week	19	79800	798	
Week	20	42000	420		Week	20	84000	840	
School Hols					School Hols				
Week	21	44100	441		Week	21	88200	882	
Week	22	46200	462		Week	22	92400	924	**3 Sisters – 906m - 918m - 922m**
Week	23	48300	483		Week	23	96600	966	
Week	24	50400	504		Week	24	100800	1008	
Week	25	52500	525		Week	25	105000	1050	
Week	26	54600	546		Week	26	109200	1092	
Week	27	56700	567		Week	27	113400	1134	
Week	28	58800	588		Week	28	117600	1176	

Appendix M – Pain or Discomfort Weekly Record Form

Weekly Training REPORT **DATE:** _____

ATTENDANCE AND CONDITION CHECK **Training Group #1**

Record any pain or discomfort after training that has not been felt before in hip/knee/ankle

	ATTENDANCE			CONDITION	
Training Group #1	***Training Session 1***	***Training Session 2***	***Training Session 3***	**Pain Location**	**Description**
1. Name					
2. Name					
3. Name					
4. Name					
5. Name					
6. Name					
7. Name					
8. Name					
9. Name					
10. Name					
11. Name					
12. Name					
13. Name					
14. Name					
TIME OF SESSION					

Name of Recorder _____

Appendix N – Individual Participant Results Report

Participant Information for:

NAME

Age at beginning of study: 8.1yrs

M	First Results	Final Results	% Change	
Height (cm)	127.9	131.3	2.7% more	☯
Weight (kg)	38.0	39.9	4.2% more	⊙
Activity per week (hrs)	5.4	3.3	39% less	●
% Body Fat	40.6			●
Total Body BMD	.899			●
% Fat per day	47			●
Calcium per day (mg)	791	347	56% less	⊙
Ultrasound (Left Heel)	1637	1654	1%more	☯
Strength Left Leg (kg)	54	78	44%more	●
Strength Right Leg (kg)	38	77	103%more	●
Total Calories per day (Kcal)	10615			●

Change in Measurements		
Small change compared to control or total group	Average change compared to control or total group	Large change compared to control or total group
⊙	☯	●

EXPLANATION OF MEASURES

Age:	was measured in years from the day you were born to the beginning month of the study.
Height:	was measured in centimetres standing tall with no shoes looking straight ahead. Unless you are in a growth spurt this will not change much over the length of the study.
Weight:	measures by how much the force of the earth is pulling you to its centre. You will likely get heavier over the length of the study.
Activity:	This is the average number of hours you do activity each week. This will change if you do more activities or spend more time doing the same activities.
% Body Fat	If your body was split into 100 pieces then this measure tells how many of the pieces would be fat.
BMD = Bone Mineral Density	tells how tightly packed all the minerals are in your bones.
% Fat	tells how much fat is in the things you eat. This will change if you eat different foods now than at the beginning of the study.
Calcium intake	This is a very important mineral for the bones, muscles, nerves, and your heart. This will change if you eat different foods now than you did at the beginning of the study.
Ultrasound	tells the difference in time it takes to send sound through your bones. It is a measure of how strong your bones are.
Strength Left Leg	is the strength in the thigh muscles in you left leg. This will change if your thigh muscles are getting stronger.
Strength Right Leg	is the strength muscles in you right leg. This will change if your thigh muscles are getting stronger.
Total Calories	tells how much energy is in the foods you eat. This will change if you eat different foods now than at the beginning of the study.

Appendix O – ANCOVA Tables – data from which figures were constructed

O1 Data Table for Figures 5.1-5.10 (inclusive)

ANCOVA Adjusted – BMC. Baseline$^{\nabla}$ Means±SEM and Change $^{\Psi}$ Means±SEM

	Baseline				***Change***			
	Controls	***Low Drop (LD)***	***High Drop (HD)***		***Controls***	***Low Drop (LD)***	***High Drop (HD)***	
N	14	13	13	***p***	14	13	13	***p***
Total Body BMC (g)	852.02 ± 16.43	872.93 ± 16.25	882.48 ± 16.54	.447	86.86 ± 7.67	95.07 ± 7.47	89.20 ± 7.58	.737
Non-Dominant Leg LEGBMC (g)	124.20 ± 6.08	131.23 ± 6.01	127.73 ± 6.11	.724	21.42 ± 2.63	24.21 ± 2.56	24.21 ± 2.60	.715
Non-Dominant Leg FNBMC (g)	1.69 ± 0.06	1.75 ± 0.06	1.78 ± 0.06	.642	0.18 ± 0.10	0.09 ± 0.10	0.12 ± 0.10	.848
Non-Dominant Leg GTBMC (g)	2.61 ± 0.24	3.19 ± 0.23	3.01 ± 0.24	.229	0.57 ± 0.28	-0.03 ± 0.27	0.13 ± 0.27	.332
$^{\delta}$Non-Dominant Leg MFSBMC (g)	20.65 ± 0.57	21.39 ± 0.55	21.27 ± 0.55	.630	4.16 ± 0.39	3.70 ± 0.35	3.31 ± 0.36	.317
Dominant Leg LEGBMC (g)	124.81 ± 5.39	127.03 ± 5.33	122.18 ± 5.42	.812	19.31 ± 2.47	23.17 ± 2.41	24.05 ± 2.44	.402
Dominant Leg FNBMC (g)	±1.76 ±0.06	±1.63 ±0.06	±1.67 ±0.06	.260	0.13 ± 0.10	0.18 ± 0.10	0.27 ± 0.10	.624
Dominant Leg GTBMC (g)	±2.97 ±0.20	±2.95 ±0.20	±2.72 ±0.20	.628	0.25 ± 0.26	0.34 ± 0.26	0.74 ± 0.26	.393
$^{\delta}$Dominant Leg MFSBMC (g)	20.73 ± 0.59	21.45 ± 0.57	±21.30 ±0.58	.677	4.45 ± 0.53	3.51 ± 0.48	3.34 ± 0.49	.306

$^{\nabla}$ Covariate analysis adjusted for baseline body mass and fat mass

$^{\Psi}$ Covariate analysis adjusted for baseline body mass, fat mass and change in lean tissue mass

$^{\delta}$ N= 13 for Control group. 1 subject DXA data unreadable for MFS unusable due to movement artifact

O2 Data Table for Figures 5.11-5.16 (inclusive)

ANCOVA Adjusted - Volumetric BMD for femoral neck, total mid femoral shaft and mid femur shaft cortex. Baseline$^{\nabla}$ Means±SEM and Change$^{\Psi}$ Means±SEM

	Baseline				***Change***			
	Controls	***Low Drop (LD)***	***High Drop (HD)***		***Controls***	***Low Drop (LD)***	***High Drop (HD)***	
N	14	13	13	***p***	14	13	13	***p***
ND femoral neck vol BMD (g/cm^3)	.648 ± 0.018	.610 ± 0.018	.624 ± 0.018	.351	-0.017 ± 0.020	0.036 ± 0.019	-0.003 ± 0.019	.150
ND mid fem shaft vol BMD (g/cm^3)	.725 ± 0.023	.735 ± 0.022	.724 ± 0.023	.928	-0.024 ± 0.017	0.010 ± 0.017	0.017 ± 0.017	.258
$^{\delta}$ND mid femoral shaft corticalvolBMD(g/cm^3)	1.169 ± 0.025	1.115 ± 0.022	1.150 ± 0.022	.263	-0.014 ± 0.031	-0.014 ± 0.025	-0.025 ± 0.025	.947
Dom femoral neck vol BMD (g/cm^3)	.630 ± 0.017	.547 ± 0.017	.586 ± 0.017	.068	0.005 ± 0.017	0.046 ± 0.016	0.029 ± 0.017	.242
Dom mid fem shaft vol BMD (g/cm^3)	.761 ± 0.023	.759 ± 0.023	.753 ± 0.024	.973	-0.012 ± 0.019	0.009 ± 0.018	-0.006 ± 0.018	.703
$^{\delta}$Dom mid fem shaft corticalvolBMD(g/cm^3)	1.125 ± 0.026	1.103 ± 0.023	1.143 ± 0.023	.464	0.013 ± 0.031	0.003 ± 0.026	0.011 ± 0.027	.967

$^{\nabla}$ Covariate analysis adjusted for baseline body mass and fat mass

$^{\Psi}$ Covariate analysis adjusted for baseline body mass, fat mass and change in lean tissue mass

$^{\delta}$ N= 11 for Control group. 1 subject refused to participate in the MRI procedure, 1subject DXA and 1 subject MRI images were unusable due to movement artifact.

O3 Data Table for Figures 5.17-5.20 (inclusive)

ANCOVA Adjusted - Dominant and non-dominant ultrasound. Baseline$^{\nabla}$ Means±SEM: Change$^{\Psi}$ Means±SEM

	Baseline				***Change***			
	Controls	***Low Drop (LD)***	***High Drop (HD)***		***Controls***	***Low Drop (LD)***	***High Drop (HD)***	
N	14	13	13	***p***	14	13	13	***p***
ND BUA (dB.MHz^{-1})	50.75 ± 2.72	49.02 ± 2.69	46.65 ± 2.73	.589	-0.92 ± 1.52	1.84 ± 1.48	-0.37 ± 1.51	.398
Dom BUA (dB.MHz^{-1})	50.79 ± 2.68	47.14 ± 2.65	45.55 ± 2.70	.407	-3.19 ± 1.77	1.34 ± 1.73	0.58 ± 1.76	.196
ND VOS (m.sec^{-1})	1658 ± 7	1659 ± 7	1655 ± 7	.913	-2.28 ± 4.30	2.22 ± 4.19	2.31 ± 4.25	.717
Dom VOS (m.sec^{-1}) $^{\Phi}$	1655 ± 8	1653 ± 8	1658 ± 8	.893	-0.15 ± 3.06	6.59 ± 3.11	1.00 ± 3.05	.273

$^{\nabla}$ Covariate analysis adjusted for baseline body mass and fat mass

$^{\Psi}$ Covariate analysis adjusted for baseline body mass, fat mass and change in lean tissue mass

$^{\Phi}$N=12 in Change data for LD group where adjustment made for 1 outlier greater than 2 SD

O4 Data Table for Figures 6.1-6.6 (inclusive)

ANCOVA Adjusted – Cortical bone cross sectional area Baseline[ƒ] Means±SEM: Change[t] Means±SEM

	Baseline				***Change***			
	Controls	***Low Drop (LD)***	***High Drop (HD)***		***Controls***	***Low Drop (LD)***	***High Drop (HD)***	
N	N=13	N=13	N=13	***p***	[φ]N=12	N=13	N=13	***p***
ND Leg Proximal (mm^2)	163.94 ± 4.02	174.26 ± 3.82	173.90 ± 3.89	.147	22.18 ± 4.25	12.91 ± 3.84	19.86 ± 3.86	.248
ND Leg Mid (mm^2)	165.43 ± 3.92	178.42 ± 3.73	171.74 ± 3.79	.075	19.83 ± 1.94	15.91 ± 1.76	19.64 ± 1.77	.233
ND Leg Distal (mm^2)	146.94 ± 3.87	159.30 ± 3.68	154.58 ± 3.74	.090	14.78 ± 3.14	16.07 ± 2.84	13.35 ± 2.86	.792
					N=13			
D Leg Proximal (mm^2)	167.66 ± 4.50	177.58 ± 4.46	172.86 ± 4.51	.322	16.85 ± 3.04	9.75 ± 2.84	11.57 ± 2.86	.263
D Leg Mid (mm^2)v	166.21 ± 3.95	177.88 ± 3.76	172.67 ± 3.82	.126	19.38 ± 2.31	12.91 ± 2.16	14.90 ± 2.17	.155
D Leg Distal (mm^2)	146.94 ± 3.87	159.30 ± 3.68	154.58 ± 3.74	.090	12.13 ± 2.18	6.97 ± 2.04	15.14 ± 2.06	***.023****

*Significant difference (p<.05) HD mean greater than LD mean

[ƒ] Covariate analysis adjusted for baseline body mass and fat mass

[t] Covariate analysis adjusted for baseline body mass, fat mass and change in lean tissue mass

[φ]N=12 - one non-dominant post-intervention control MRI scan unable to be analysed due to movement artefact

O5 Data Table for Figures 6.7-6.12 (inclusive)

ANCOVA Adjusted – Medullary cavity area Baseline[ƒ] Means±SEM: Change[t] Means±SEM

	Baseline				***Change***			
	Controls	***Low Drop (LD)***	***High Drop (HD)***		***Controls***	***Low Drop (LD)***	***High Drop (HD)***	
N	N=13	13	13	***p***	[φ]N=12	13	N=13	***p***
ND Leg Proximal (mm^2)	96.24 ± 6.81	86.46 ± 6.49	91.83 ± 6.60	.594	2.05 ± 4.45	5.69 ± 4.02	-1.52 ± 4.05	.447
ND Leg Mid (mm^2)	83.05 ± 5.22	72.70 ± 4.98	82.22 ± 5.05	.282	2.68 ± 1.45	5.54 ± 1.32	3.59 ± 1.32	.342
ND Leg Distal (mm^2)	127.39 ± 6.45	119.52 ± 6.14	134.51 ± 6.24	.234	4.68 ± 3.07	11.03 ± 2.78	10.37 ± 2.79	.300
					N=13			
D Leg Proximal (mm^2)	96.65 ± 5.49	86.01 ± 5.23	88.28 ± 5.31	.376	6.58 ± 1.66	7.47 ± 1.55	4.49 ± 1.56	.380
D Leg Mid (mm^2)v	83.72 ± 5.30	74.19 ± 5.05	82.66 ± 5.13	.360	3.55 ± 1.24	4.20 ± 1.16	4.97 ± 1.17	.725
D Leg Distal (mm^2)	118.68 ± 6.75	119.71 ± 6.43	132.24 ± 6.54	.286	8.49 ± 2.00	10.75 ± 1.87	6.54 ± 1.88	.282

[ƒ] Covariate analysis adjusted for baseline body mass and fat mass

[t] Covariate analysis adjusted for baseline body mass, fat mass and change in lean tissue mass

[φ]N=12 - one non-dominant post-intervention control MRI scan unable to be analysed due to movement artefact

O6 Data Table for Figures 6.13-6.18 (inclusive)

ANCOVA Adjusted – Total bone area Baseline[f] Means±SEM: Change[t] Means±SEM

	Baseline				***Change***			
	Controls	***Low Drop (LD)***	***High Drop (HD)***		***Controls***	***Low Drop (LD)***	***High Drop (HD)***	
N	N=13	13	13	***p***	[φ]N=12	13	N=13	***p***
ND Leg Proximal (mm^2)	260.18 ± 7.70	260.73 ± 7.33	265.73 ± 7.45	.850	24.23 ± 5.29	18.60 ± 4.78	18.35 ± 4.81	.686
ND Leg Mid (mm^2)	248.44 ± 6.18	251.12 ± 5.89	253.97 ± 5.99	.825	22.51 ± 1.59	21.45 ± 1.44	23.23 ± 1.45	.675
ND Leg Distal (mm^2)	274.33 ± 7.63	278.82 ± 7.27	289.13 ± 7.38	.383	19.46 ± 2.86	27.10 ± 2.59	23.72 ± 2.61	.175
					N=13			
D Leg Proximal (mm^2)	264.15 ± 7.32	263.59 ± 6.98	261.14 ±7.09	.952	23.43 ± 2.69	17.22 ± 2.51	16.06 ± 2.53	.149
D Leg Mid (mm^2)v	249.92 ± 6.30	252.07 ± 6.00	255.34 ± 6.09	.832	22.93 ± 1.85	17.11 ± 1.73	19.87 ± 1.74	.101
D Leg Distal (mm^2)	272.19 ± 7.92	285.26 ± 7.55	288.77 ± 7.67	.328	20.62 ± 2.80	17.72 ± 2.61	21.68 ± 2.63	.539

[f] Covariate analysis adjusted for baseline body mass and fat mass

[t] Covariate analysis adjusted for baseline body mass, fat mass and change in lean tissue mass

[φ]N=12 - one non-dominant post-intervention control MRI scan unable to be analysed due to movement artefact

O7 Data Table for Figures 6.19-6.24 (inclusive)

ANCOVA Adjusted – Proximal mid femoral shaft slice cross-sectional moment of inertia (CSMI). Baseline[f] Means±SEM: Change[t] Means±SEM

	Baseline				***Change***			
	Controls	***Low Drop (LD)***	***High Drop (HD)***		***Controls***	***Low Drop (LD)***	***High Drop (HD)***	
N	12	12	13	***p***	12	12	13	***p***
Non-Dominant Leg J0 (mm^4)	7723 ± 403	8268 ± 384	7840 ± 373	.587	1634 ± 210	1231 ± 195	1222 ± 188	.323
Non-Dominant Leg Imax (mm^4)	4531 ± 238	4738 ± 227	4620 ± 220	.824	896 ± 138	815 ± 128	649 ± 124	.415
Non-Dominant Leg Imin (mm^4)	3192 ± 190	3530 ± 182	3220 ± 176	.358	738 ± 101	416 ± 94	573 ± 91	.097
Dominant Leg J0 (mm^4)	7774 ± 427	8171 ± 407	7963 ± 395	.804	1385 ± 266	1222 ± 246	1372 ± 238	.877
Dominant Leg Imax (mm^4)	4481 ± 247	4828 ± 236	4621 ± 229	.605	785 ± 157	769 ± 145	802 ± 141	.986
Dominant Leg Imin (mm^4)	3293 ± 198	3344 ± 189	3341 ± 183	.980	600 ± 115	453 ± 106	570 ± 103	.609

[f] Covariate analysis adjusted for baseline body mass and fat mass

[t] Covariate analysis adjusted for baseline body mass, fat mass and change in lean tissue mass

O8 Data Table for Figures 6.25-6.30 (inclusive)

ANCOVA Adjusted – Middle mid femoral shaft slice cross-sectional moment of inertia (CSMI). Baseline[f] Means±SEM: Change[t] Means±SEM

	Baseline				***Change***			
	Controls	***Low Drop (LD)***	***High Drop (HD)***		***Controls***	***Low Drop (LD)***	***High Drop (HD)***	
N	12	12	13	***p***	12	12	13	***p***
Non-Dominant Leg J0 (mm^4)	6972 ± 446	8244 ± 425	7568 ± 412	.143	1818 ± 322	1285 ± 299	1772 ± 289	.395
Non-Dominant Leg Imax (mm^4)	4008 ± 283	4855 ± 270	4445 ± 262	.123	1054 ± 191	806 ± 178	1087 ± 172	.471
Non-Dominant Leg Imin (mm^4)	2964 ± 179	3389 ± 170	3123 ± 165	.239	764 ± 145	480 ± 135	685 ± 130	.345
Dominant Leg J0 (mm^4)	7112 ± 380	7866 ± 362	7646 ± 352	.375	1882 ± 209	1337 ± 193	1579 ± 187	.201
Dominant Leg Imax (mm^4)	4053 ± 237	4568 ± 226	4492 ± 220	.281	1142 ± 146	767 ± 136	939 ± 131	.212
Dominant Leg Imin (mm^4)	3058 ± 164	3297 ± 156	3154 ± 151	.580	740 ± 92	570 ± 85	640 ± 82	.436

[f] Covariate analysis adjusted for baseline body mass and fat mass

[t] Covariate analysis adjusted for baseline body mass, fat mass and change in lean tissue mass

O9 Data Table for Figures 6.31-6.36 (inclusive)

ANCOVA Adjusted – Distal mid femoral shaft slice cross-sectional moment of inertia (CSMI). Baseline[f] Means±SEM: Change[t] Means±SEM

	Baseline				***Change***			
	Controls	***Low Drop (LD)***	***High Drop (HD)***		***Controls***	***Low Drop (LD)***	***High Drop (HD)***	
N	12	12	13	***p***	12	12	13	***p***
Non-Dominant Leg J0 (mm^4)	7497 ± 482	8860 ± 459	8498 ± 446	.141	1985 ± 280	1941 ± 260	1837 ± 251	.923
Non-Dominant Leg Imax (mm^4)	4175 ± 280	5000 ± 267	4841 ± 259	.111	1197 ± 164	1079 ± 152	966 ± 147	.606
Non-Dominant Leg Imin (mm^4)	3322 ± 213	3860 ± 203	3657 ± 197	.217	788 ± 132	862 ± 123	871 ± 119	.895
Dominant Leg J0 (mm^4)	8566 ± 451	9239 ± 430	8843 ± 418	.568	1112 ± 291	1470 ± 270	1669 ± 261	.408
Dominant Leg Imax (mm^4)	4762 ± 262	5078 ± 249	4915 ± 242	.694	672 ± 158	852 ± 147	935 ± 142	.506
Dominant Leg Imin (mm^4)	3805 ± 212	4160 ± 202	3927 ± 196	.477	440 ± 140	618 ± 130	734 ± 125	.340

[f] Covariate analysis adjusted for baseline body mass and fat mass

[t] Covariate analysis of change data adjusted for baseline body mass, fat mass and change in lean tissue mass

O10 Data Table for Figures 6.37-6.38 (inclusive)

ANCOVA Adjusted – Bone strength index (BSI) Baseline[f] Means±SEM: Change[t] Means±SEM

	Baseline				***Change***			
	Controls	***Low Drop (LD)***	***High Drop (HD)***		***Controls***	***Low Drop (LD)***	***High Drop (HD)***	
N	12	12	13	***p***	12	12	13	***p***
Non-Dominant Leg BSI ($mm^4.g/cm^3$)	4973 ± 258	5649 ± 257	5281 ± 240	.204	1116 ± 219	863 ± 205	944 ± 189	.718
Dominant Leg BSI ($mm^4.g/cm^3$)	4977 ± 253	5476 ± 241	5385 ± 234	.357	1044 ± 196	849 ± 182	1081 ± 176	.617

[f] Covariate analysis adjusted for baseline body mass and fat mass

[t] Covariate analysis adjusted for baseline body mass, fat mass and change in lean tissue mass

O11 Data Table for Pre and Post Dominant and Non-Dominant Cross-Sectional Areas

ANCOVA Adjusted within-group comparisons of differences between cortical, medullary cavity and total bone cross-sectional areas (CSA) for proximal, mid and distal mid-femoral shaft slices of non-dominant and dominant legs: Baseline[ƒ] Means±SEM

	Baseline			***Post Intervention***		
	Non-Dominant Leg	***Dominant Leg***		***Non-Dominant Leg***	***Dominant Leg***	
Controls	**(N =13)**	**(N =13)**	**p**	**(N =12)**	**(N =12)**	**p**
Proximal cortical CSA (mm^4)	159.36 ±3.93	162.69 ±3.93	.554	182.09 ±4.55	180.17 ±4.55	.768
Proximal medullary CSA (mm^4)	90.91 ±4.65	90.87 ±4.65	.996	96.90 ±5.16	98.21 ±5.16	.859
Proximal total bone CSA (mm^4)	250.27 ±4.18	253.57 ±4.18	.582	278.99 ±6.02	278.37 ±6.02	.943
Mid cortical CSA (mm^4)	163.28 ±3.14	161.61 ±3.14	.711	181.64 ±3.32	181.83 ±3.32	.977
Mid medullary CSA (mm^4)	77.69 ±414	78.53 ±414	.887	82.27 ±4.36	83.21 ±4.36	.881
Mid total bone CSA (mm^4)	240.97 ±4.36	240.14 ±4.36	.895	263.91 ±4.88	264.98 ±4.88	.878
Distal cortical CSA (mm^4)	144.39 ±2.84	149.22 ±2.84	.242	159.74 ±3.13	162.92 ±3.13	.481
Distal medullary CSA (mm^4)	119.56 ±4.69	110.52 ±4.69	.187	126.82 ±4.44	121.63 ±4.44	.419
Distal total bone CSA (mm^4)	263.95 ±4.77	259.73 ±4.77	.539	286.56 ±6.23	284.55 ±6.23	.822
Low Drop [φ] (N =12)						
Proximal cortical CSA (mm^4)	176.48 ±4.62	179.77 ±4.62	.620	190.48 ±4.51	190.86 ±4.51	.952
Proximal medullary CSA (mm^4)	88.67 ±3.06	88.36 ±3.06	.943	93.00 ±3.69	96.17 ±3.69	.550
Proximal total bone CSA (mm^4)	265.16 ±6.22	268.13 ±6.22	.738	283.48 ±6.46	287.04 ±6.46	.701
Mid cortical CSA (mm^4)	179.71 ±4.33	180.02 ±4.33	.960	197.54 ±4.55	194.57 ±4.55	.649
Mid medullary CSA (mm^4)	74.88 ±3.19	76.29 ±3.19	.757	80.42 ±3.59	80.60 ±3.59	.971
Mid total bone CSA (mm^4)	254.59 ±6.02	256.32 ±6.02	.841	277.96 ±6.59	275.17 ±6.59	.768
Distal cortical CSA (mm^4)	160.69 ±4.32	167.59 ±4.32	.271	177.60 ±3.89	175.16 ±3.89	.662
Distal medullary CSA (mm^4)	122.84 ±5.48	123.04 ±5.48	.980	134.01 ±5.57	133.66 ±5.57	.965
Distal total bone CSA (mm^4)	283.54 ±7.62	290.63 ±7.62	.517	311.61 ±7.27	308.82 ±7.27	.788
High Drop (N =13)						
Proximal cortical CSA (mm^4)	176.27 ±3.30	175.48 ±3.30	.867	196.47 ±3.66	187.84 ±3.66	.109
Proximal medullary CSA (mm^4)	94.95 ±8.15	91.70 ±8.15	.781	92.71 ±7.35	96.73 ±7.35	.703
Proximal total bone CSA (mm^4)	271.21 ±9.74	267.17 ±9.74	.772	289.18 ±9.44	284.56 ±9.44	.732
Mid cortical CSA (mm^4)	172.60 ±2.94	175.13 ±2.94	.548	194.00 ±3.40	190.99 ±3.40	.538
Mid medullary CSA (mm^4)	85.37 ±6.01	85.74 ±6.01	.966	88.91 ±6.16	91.00 ±6.16	.813
Mid total bone CSA (mm^4)	257.97 ±6.30	260.87 ±6.30	.748	282.91 ±7.01	281.99 ±7.01	.927
Distal cortical CSA (mm^4)	155.74 ±2.15	158.79 ±2.15	.327	169.74 ±2.92	173.92 ±2.92	.322
Distal medullary CSA (mm^4)	139.05 ±7.57	137.07 ±7.57	.855	150.02 ±8.02	143.75 ±8.02	.586
Distal total bone CSA (mm^4)	294.80 ±8.19	295.86 ±8.19	.928	319.76 ±8.71	317.66 ±8.71	.867

[ƒ] Covariate analysis adjusted for baseline body mass and fat mass

[Y] N=12 - one non-dominant post-intervention control group MRI scan unable to be analysed due to movement artefact

[φ] N=12 - one non-dominant post-intervention LD group MRI scan in unable to be analysed due to movement artefact

O12 Data Table for Pre and Post Dominant and Non-Dominant CSMI

ANCOVA Adjusted within-group baseline and post-intervention comparisons of differences between cross-sectional moments of inertia for proximal, mid and distal mid-femoral shaft slices of non-dominant and dominant legs: Baseline[f] Means±SEM

	Baseline			***Post Intervention***		
	Non-Dominant Leg	***Dominant Leg***		***Non-Dominant Leg***	***Dominant Leg***	
Controls [ϒ] ***(N =12)***			**p**			**p**
Proximal J0 (mm^4)	7195 ±282	71910 ±282	.991	8561 ±315	8371 ±315	.674
Proximal Imax (mm^4)	4231 ±183	4174 ±183	.826	4985 ±224	4821 ±224	.610
Proximal Imin (mm^4)	2964 ±136	3017 ±136	.785	3576 ±131	3549 ±131	.887
Mid J0 (mm^4)	6580 ±284	6582 ±284	.995	7906 ±312	8100 ±312	.665
Mid Imax (mm^4)	3816 ±191	3791 ±191	.926	4592 ±217	4714 ±217	.696
Mid Imin (mm^4)	2764 ±116	2792 ±116	.867	3314 ±131	3386 ±131	.701
Distal J0 (mm^4)	7046 ±400	7851 ±400	.170	8750 ±361	8820 ±361	.893
Distal Imax (mm^4)	3940 ±242	4352 ±242	.243	4971 ±253	4922 ±253	.893
Distal Imin (mm^4)	3106 ±117	3499 ±117	.132	3779 ±132	3897 ±132	.534
Low Drop [φ] *(N =12)*						
Proximal J0 (mm^4)	8638 ±445	8551 ±445	.891	10032 ±524	9899 ±524	.859
Proximal Imax (mm^4)	4955 ±243	5038 ±243	.810	5855 ±276	5892 ±276	.925
Proximal Imin (mm^4)	3683 ±220	3512 ±220	.589	4177 ±267	4007 ±267	.656
Mid J0 (mm^4)	8554 ±413	8225 ±413	.581	10117 ±549	9778 ±549	.666
Mid Imax (mm^4)	5031 ±248	4767 ±248	.460	5994 ±356	5661 ±356	.516
Mid Imin (mm^4)	3523 ±175	3458 ±175	.796	4123 ±203	4116 ±203	.981
Distal J0 (mm^4)	9203 ±488	9711 ±488	.470	11334 ±525	11274 ±525	.937
Distal Imax (mm^4)	5192 ±281	5353 ±281	.689	6386 ±312	6271 ±312	.798
Distal Imin (mm^4)	4012 ±216	4358 ±216	.269	4948 ±226	5003 ±226	.866
High Drop (N =13)						
Proximal J0 (mm^4)	7986 ±385	8151 ±385	.765	9305 ±465	9595 ±465	.663
Proximal Imax (mm^4)	4697 ±226	4711 ±226	.966	5399 ±254	5561 ±254	.656
Proximal Imin (mm^4)	3289 ±171	3440 ±171	.540	3906 ±221	4034 ±221	.686
Mid J0 (mm^4)	7644 ±359	7802 ±359	.758	9612 ±426	9518 ±426	.878
Mid Imax (mm^4)	4460 ±250	4550 ±250	.801	5658 ±295	5574 ±295	.843
Mid Imin (mm^4)	3184 ±125	3252 ±125	.705	3954 ±191	3944 ±191	.970
Distal J0 (mm^4)	8598 ±314	9066 ±314	.303	10519 ±420	10781 ±420	.663
Distal Imax (mm^4)	4881 ±178	5040 ±178	.534	5894 ±231	6007 ±231	.733
Distal Imin (mm^4)	3718 ±153	4027 ±153	.167	4625 ±205	4774 ±205	.610

[f] Covariate analysis adjusted for baseline body mass and fat mass

[ϒ] N=12 - one non-dominant post-intervention control group MRI scan unable to be analysed due to movement artefact

[φ] N=12 - one non-dominant post-intervention LD group MRI scan in unable to be analysed due to movement artefact

Appendix P – Ethics Clearances

New Children's Hospital
search & Development

AO:ro g:\\ec\9906appvar.doc

Prof C Blimkie
Children's Hospital Institute of Sports Medicine

29 June 1999

Dear Prof Blimkie,

The efffects of mechanical load magnitude on skeletal adaptations to exercise in pre-pubertal girls
98072
(Variation dated: 31 May 1999)

At its meeting on 25th June 1999, the Ethics Committee approved amendments to this project. The changes recommended by the Form Review Committee must also be made for your ethical approval to be effective. Please submit copies of the revised forms for our records.

We wish you well with your project. Please contact us should you have any queries.

Yours sincerely,

A O'Neill
Secretary, Ethics Committee

awkesbury Rd &
orth St, Westmead

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AUSTRALIAN CA.THOLIC UNIVERSITY

MEMORANDUM

TO: Prof. Cameron Blimkie
(MacKillop Campus)
c.c. Executive Officer, University Research Projects Ethics Committee

FROM: Administrative Officer (Research)
Mount Saint Mary Campus

SUBJECT: Ethics clearance for a research project involving human participants

DATE: 5 March 1999

The University Research Projects Ethics Committee (URPEC) has considered the application for ethics clearance for the following project:

URPEC Register No.: N99-03
Project title: *The effects of mechanical load magnitude on skeletal adaptation to exercise in pre-pubertal girls.*
Principal Investigator: Prof. Cameron Blimkie (MacKillop Campus)
Co-Investigatorls: Dr C. T. Cowell (SCH)
Dr M. Braun (UTS)
Project duration: 1 November 1998 to 31 March 2000

The following is an extract of the relevant draft minute approved by the Chair of the University Research Projects Ethics Committee:

4.4. 1 *URPEC Register No: . N99-03 (ref: URPEC99/5) .*
Project title: The effects of mechanical load magnitude on skeletal adaptation to exercise in pre-pubertal girls. Principal Investigator: Prof Cameron Blimkie, MacKillop Other Investigators: Or C *T Cowell (SCH)*
Or M *Braun (UTS)*

The Committee received document URPEC99/5. Some members of the Committee were. concerned about the onerous tests to be performed on children, and whether or not the Information Letter to Parents
fully informed the parents about whatthey were really getting in

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to. Committee members were concerned about the use of language in the Information Letter to Parents, particularly paragraph three. The language should be in layman's terms so that the parents can make a fully informed decision about allowing their child to participate.

Concerns were also raised in relation to the safe dosage level of radiation. If there is any potential of danger from exposure to radiation then this needs to be pointed out in the Information Letter to Parents in simple terms. Members of the Committee noted that the Hospital Ethics Committee had already granted Ethics Approval and are in a better position to determine whether the Researchers are-using a 'safe dosage'rate;'" It was agreed that the Chair would request guidance from Or Frank Morgan (Medical Expert) regarding safe dosage rates, and would then sign off on his response on behalf of the Committee.

The Committee noted the inconsistency regarding the use of the ultrasound being 'entirely without side-effect' as stated in the Information Letter to Parents, or posing 'little clinical risks to subjects' as stated in the Ethics Application Form to the Hospital Ethics Committee. If there is a risk, however minor, then this must be stated in the Information Letter to Parents.

The Committee noted that this was a joint project b.etween ACU and the Hospital as Professor Blimkie, the Principal Investigator, is a member of staff at ACU. Therefore the letter head should use joint logos. Information Letters to Parents should indicate that it is a joint project and approved by both the Hospital Ethics Committee and the URPEC.

The Committee questioned the role of Mr Peter Weibe in the project. Mr Peter Weibe is not mentioned in the Ethics Application, but indicates in correspondence with the Administrative Officer (Research and Ethics) in NSW that the study is part of his PhD Thesis. Professor Blimkie is Mr Peter Weibe's Supervisor.

The Committee agreed to approve the project subject to:

(1) *The Information Letter to Parents being rewritten in lay language.*
(2) The Information Letter to Parents should indicate that this is a joint project between ACU and the Hospital.
(3) Clarification to be provided regarding the number of participants. A memorandum from Mr Weibe indicated that the total number of participants would be 45, *whilst the application referred to a total sample of '100 subjects'.*
(4) Clarification to be provided regarding whom is involved in the research project.
(5) An interpreter should be made available to participants if required.
(6) If there is any possible risk to participants from the use of radiation or ultrasound then this should be stated clearly in the Information Letter to Parents.

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