Exercise type, musculoskeletal health and injury risk factors in adolescent middle-distance runners

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Statement of sources

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Abstract

Background: Adolescent growth provides a unique opportunity for the growing body to adapt to external stimuli. A positive association between site-specific mechanical loading and increases in regional bone mineral content (BMC) during adolescence is established. Mechanical loads associated with middle-distance running expose the skeleton to a combination of compressive ground reaction forces and muscular contraction. Previous studies concerning musculoskeletal health in active adolescents are largely limited to planar, two-dimensional measures of bone mineral status, using Dual X-ray Absorptiometry (DXA). Intrinsic bone material properties are accurately measured using DXA. However, the interaction between bone material and structural properties that reflects the mechanical integrity of bone require a combination of imaging modalities. Magnetic Resonance Imaging (MRI) provides a three-dimensional geometric and biomechanical assessment of bone. When MRI is integrated with DXA technology, an effective non-invasive method of assessing in vivo bone strength is achieved. The impact of high training volumes on musculoskeletal development of male and female adolescent athletes engaged in repetitive, high magnitude mechanical loading has not been investigated. Specifically, differences in total body and regional bone mineral, bone and muscle geometry, bone biomechanical indices and bone strength at differentially-loaded skeletal sites have not been compared between adolescent middle-distance runners and age- and gender-matched non-athletic controls.

Objectives: (i) to investigate the effects of intense sports participation involving mechanical loading patterns on bone mineral, bone and muscle geometry, biomechanical indices and estimated regional bone strength between elite adolescent male and female middle-distance runners and age- and gender-matched controls (ii) to examine factors predictive of total body BMC, distal tibial bone geometry, distal tibial bone strength, and Hip Strength Analysis (HSA)- derived indicators of bone strength at the femoral neck.

Methods: Four groups of 20 adolescents were comprised of male (mean (SD) age 16.8 ± 0.6 yr, physical activity 14.1 ± 5.7 $hr.wk^{-1}$) and female (age 16 ± 1.7 yr, physical activity 8.9 ± 2.1 $hr.wk^{-1}$) middle-distance runners, and male (16.4 ± 0.7 yr, physical activity 2.2 ± 0.7 $hr.wk^{-1}$) and female (age 16 ± 1.8 yr, physical activity 2.0 ± 0.07 $hr.wk^{-1}$) controls. Total body and regional BMC were calculated using DXA. Distal tibial bone and muscle cross-sectional areas (CSA) were assessed using MRI. To calculate distal tibial bone strength index (BSI), a region of interest representing 10% of the mid distal tibia was

analysed for DXA-derived bone mineral and was combined with bone geometry and biomechanical properties from MRI assessments. Calculations for femoral neck strength were acquired from DXA-derived HSA software.

Results: No differences were found between male athletes and controls for unadjusted BMC at total body or regional sites. After covarying for fat mass (kg), male athletes displayed greater BMC at the lumbar spine ($p = 0.001$), dominant proximal femur ($p = 0.001$) and dominant leg ($p = 0.03$) than male controls. No differences were found in distal tibial bone geometry, bone strength at the distal tibia or HSA-derived indicators of bone strength at the femoral neck between male athletes and controls. Lean tissue mass and fat mass were the strongest predictors of total body BMC ($R^2 = 0.71$), total muscle CSA explained 43.5% of variance in BSI at the distal tibia, and femur length and neck of femur CSA explained 33.4% of variance at the femoral neck. In females, athletes displayed greater unadjusted BMC at the proximal femur (+3.9 ±1.4 g, $p = 0.01$), dominant femoral neck (+0.5 ± 0.12 g, $p = 0.01$) and dominant tibia (+4.1 ± 2.1 g, $p = 0.05$) than female controls. After covarying for fat mass (kg), female athletes displayed greater ($p = 0.001$) total body, dominant proximal femur and dominant leg BMC than female controls. Female athletes also showed greater distal tibial cortical CSA (+30.9 ± 9.5 mm^2, $p = 0.003$), total muscle (+240.2 ± 86.4 mm^2, $p = 0.03$) and extensor muscle (+46.9 ±19.5 mm^2, $p = 0.02$) CSA, smaller medullary cavity (-32.3 ± 14.7 mm^2, $p = 0.03$) CSA and greater BSI at the distal tibia (+28037 ± 8214.7 $g/cm^3.mm^4$, $p = 0.002$) than female controls. Lean tissue mass and fat mass were the strongest predictors of total body BMC ($R^2 = 65$), hours of physical weekly activity and total muscle CSA explained 58.3% of the variance of distal tibial BSI, and neck of femur CSA accounted for 64.6% of the variance in a marker of femoral neck HSA.

Conclusion: High training loads are associated with positive musculoskeletal outcomes in adolescent middle-distance runners compared to non-athletic controls. Exposure to similar high training loads may advantage female adolescent athletes, more than male adolescent athletes compared with less active peers in bone strength at the distal tibia.

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They say you can't choose your parents……..but I'd choose mine every time.

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CHAPTER ONE

INTRODUCTION

1.1 Rationale

Adolescence is a period of growth resulting in unprecedented physiological change. Rapid increases in height and weight, development of secondary sex characteristics and changes to the musculoskeletal and cardiorespiratory systems occur (Naughton, Farpour-Lambert, Carlson, Bradney & Van Praagh, 2000). Despite these changes, adolescence provides a unique opportunity for the growing body to adapt to external stimuli. Potentially, exercise produces beneficial osteogenic effects during growth (Seeman, 2002).

Weight bearing physical activity generates forces of greater magnitude on the musculoskeletal system than those associated with normal life (Maffulli, 1990). Increasing secretions of sex steroids and growth hormone combine with greater bone turnover to achieve large gains in bone mass (Bass, 2000). Positive musculoskeletal adaptations to increasing weight bearing exercise are described in both adolescent (Duncan, Blimkie, Cowell, Burke, Briody & Howman-Giles, 2002) and pre-adolescent populations (Dyson, Blimkie, Davison, Webber & Adachi, 1997).

In contrast, an increase in cortical porosity, is associated with an increased risk of fracture from repetitive mechanical loads in athletic adolescents (Parfitt, 1994). Ligaments are two to three times stronger than bone, therefore cartilage at the growth plate may be at its weakest during intense growth (Micheli, 1996). Injuries may permanently affect both growing bone and soft tissues (Maffulli, 1990).

The impact of high training volumes on musculoskeletal development of adolescents has a limited understanding. The relatively small number of adolescent studies includes a

comparison of bone mineral density (BMD) and bone mineral content (BMC) in female adolescents involved in soccer and competitive rope-skipping (Petterson, Nordstrom, Alfredson, Henriksson-Larsen & Lorentzen, 2000). Both high-activity groups had significantly higher BMD at most weight-bearing sites compared than controls. Mechanical loading patterns on BMD were investigated involving elite adolescent female cyclists, runners, swimmers, triathletes and controls. Runners demonstrated greater femoral neck and leg BMD compared with athletes from predominately nonweight-bearing sports (Duncan et al, 2002).

The relative contribution of other factors affecting bone status and injury during adolescence such as maturational stage and nutrition requires investigation. Children of identical chronological age can differ by as much as six years developmentally and endocrine events in maturation influence the rate of advancement through developmental stages (Bailey, 1997). Sex steroids and growth hormone significantly influence bone status during and beyond puberty. Bone accrual, bone turnover, linear growth, apposition of bone on the endosteal surface and epiphyseal closure are greatly influenced by testosterone in boys and oestrogens girls (MacKelvie, Khan & McKay, 2002). Oestrogen and testosterone are critical hormones affecting bone mineral mass during puberty and the optimisation of peak BMD during late adolescence or early adulthood (Blimkie et al, 1996).

Nutrition is considered a modifiable lifestyle factor influencing bone adaptations from childhood to older adulthood. Increasing calcium demands of the growing skeleton must be provided from dietary sources for essential increases in calcium between birth and adulthood (Heaney, 1991). Results of studies examining the role of calcium in contributing to higher BMD values during growth are generally equivocal (Lloyd, Andon, Rollings, Martel, Landis, Demers et al, 1993; Matkovic, Fontana, Tominac, Goel and Chestnut, 1990). Furthermore, the influence of calcium intake on bone mineral in highly active adolescent male and female athletes remains unclear.

1.2 Aims

The primary aims of this study are:

(i) To investigate the effects of intense sports participation involving relatively high mechanical loading patterns on bone mineral, bone material properties, bone geometry, biomechanical indices and estimated bone strength.

(ii) To compare skeletal adaptations (above) between male and female athletic and non-athletic adolescents.

The secondary aim is:

To determine relationships between exercise-related skeletal adaptations and: injury history, training load, sex hormones, habitual physical activity, body composition, nutrition, muscle strength and morphology, biomechanical measures, pubertal status and menstrual status for post-menarcheal girls.

1.3 Hypotheses

The following hypotheses will be addressed:

(i) male adolescent middle-distance runners will display greater total body and regional BMC and distal tibial bone geometry than age-matched non-athletic controls

(ii) female adolescent middle –distance runners will display greater total body and regional BMC and distal tibial bone geometry than age-matched non-athletic controls

(iii) male and female adolescent middle-distance runners will display greater distal tibial bone strength and HSA-derived indicators of bone strength at the femoral neck than age- and gender-matched non-athletic controls

1.4 Limitations

The following limitations are recognized within the research conducted for this thesis:

(i) The volume, type and intensity of training undertaken by athletes varied according to coaching. Controlling training loads were beyond the scope of the research project. Training load was however, monitored as an independent variable .

(ii) A record of injury history was obtained from athletes. Injured athletes who met the major criteria of completing ≥6 hrs/week of training during the previous 2 years were therefore included in the study. A limitation of the study was an inability to control for injury history of the adolescent athletes. A classification of sporting injury history was an independent variable.

(iii) Nutritional practices were therefore outside the control of the researchers. Nutritional information, ascertained through the use of a 3 day food diary, provided an estimate of total daily energy and calcium intake. Estimates of calcium and total energy intake were used as independent variables.

(iv) Pubertal maturation and associated sex hormones varied between participants. Criteria for inclusion accepted all stages of pubertal development within participants. Pubertal data generated an important independent variable.

(v) The cross-sectional study design limits the generalisability of the findings beyond adolescent middle-distance runners and well matched controls.

1.5 Delimitations

(i) The number of participants was restricted to 20 male and 20 female middle distance runners, with 20 male and 20 female age-matched controls.

(ii) Participants were aged 14 to 18 years.

(iii) Athletic participants competed at the state level and were training ≥6 hrs/week for the past 2 years and met the following criteria:

- Caucasian ethnicity
- of similar age and gender to control participants
- in good health and no recent (past two years) hospitalisation and no history of systemic illness lasting more than 2 weeks
- no known history of metabolic bone disease and no medication, hormones (including the Oral Contraceptive Pill), or calcium preparations that may influence bone metabolism taken in the past preceding 6 months
- athletic females who are post menarcheal will need to have a normal menstrual cycle (≥8 menstrual cycles in the past 12 months).
- musculoskeletal parameters were selected as the major dependant variables. Specifically, measurements and calculations were derived using Dual X-ray Absorptiometry (DXA) and Magnetic Resonance Imaging (MRI). Based on previous findings, secondary outcome variables were restricted to biological (pubertal, body composition, lower limb biomechanics, muscle morphology, sex hormones, and injury history) and modifiable factors (nutrition, training load, habitual physical activity and muscle strength).

(iv) Adolescent controls met the following criteria:

- <3 hrs/week of physical activity, including activities during school hours
- Caucasian ethnicity
- of similar age and gender to athletic subjects
- in good health and no recent (past two years) hospitalisation and no history of systemic illness lasting more than 2 weeks
- no known history of metabolic bone disease and no medication, hormones (including the Oral Contraceptive Pill), or calcium preparations that may influence bone metabolism taken in the past preceding 6 months
- control females who are post menarcheal will need to have a normal menstrual cycle (≥8 menstrual cycles in the past 12 months).
- musculoskeletal parameters were selected as the major dependant variables. Specifically, measurements and calculations were derived using Dual X-ray

Absorptiometry (DXA) and Magnetic Resonance Imaging (MRI). Based on previous findings, secondary outcome variables were restricted to biological (pubertal, body composition, lower limb biomechanics, muscle morphology, sex hormones, and injury history) and modifiable factors (nutrition, training load, habitual physical activity and muscle strength).

(v) Gene-environment interactions are increasingly recognized as contributing to variance in osteogenic responses when the exercise exposure is similar. Financial constraints precluded the exploration of potential genetic contributions to differences between athletic and control groups.

1.6 Definitions

The following definitions apply to the most cited terms in this thesis and presented within the following categories; Bone, Bone geometry; Bone measures:

BONE

Bone: Connective tissue consisting of calcified matrix (mainly calcium phosphate) and collagen fibres

Cortical bone: Bone consisting of a central Haversian canal surrounded by concentric rings of bone tissue intruded by minute canals called canaliculi

Trabecular bone: Bony tissue found predominately in the distal portions of long bones, flat bones and vertebral bodies. Bone is arranged in a lattice-like structure consisting of small needle-like or flat pieces of mineralized bars called trabeculae.

Bone mineral: The absolute amount of hydroxyapatite (calcium phosphate crystal) present in bone

Bone mineral content (BMC): The amount of bone mineral in the skeleton or within a skeletal region

Bone mineral density (BMD): Relative value of bone mineral per measured bone area

Areal bone mineral density (aBMD): Value of bone mineral per measured bone region based on a two-dimensional image

Volumetric bone mineral density (vBMD): Value of bone mineral per measured bone region based on a three-dimensional image

BONE GEOMETRY

Cross-sectional area (CSA): Measurement of the area of a section formed by a plane cutting through an object, usually at right angles to an axis

Cross-sectional moment of inertia (CSMI): Measure of the distribution of material around a given axis

Bone strength index (BSI): Combination of bone material and structural properties to represent bone resistance to external loads, based on the formula by Ferretti, Capozza and Zanchetta, 1996 (BSI = CSMI x Volumetric Cortical BMD)

BONE MEASURES

Dual X-ray Absorptiometry (DXA): Imaging procedure involving collimated radiation beams that permit quantification of bone mineral content, density, fat mass and lean tissue mass

Magnetic Resonance Imaging (MRI): Imaging procedure involving a strong magnetic field to excite hydrogen nuclei present in bodily fluid and lipid molecules.

Peripheral Quantitative Computed Tomography (pQCT): Imaging procedure involving collimated radiation beams to quantify bone compartments, bone cross-sectional area and mineralization at the periphery of long bones; namely distal radius and tibia.

CHAPTER TWO

REVIEW OF LITERATURE

This review of literature begins with a systemic, biological description of bone, bone metabolism and bone growth. The influence of lifestyle factors such as nutrition and physical activity on musculoskeletal development is examined relative to weight-loaded and weight-supported activities. Musculoskeletal health issues arising from serious sports participation during adolescence are also explored. Technologies currently used to assess bone mineral and bone architecture are discussed, with a particular emphasis on biomechanical properties of bone.

2.1 Musculoskeletal Growth

2.1.1 Bone Growth

The human skeleton is a framework that provides protection of internal organs, support against gravity, a lever system enabling movement and a reserve of ions for the maintenance of serum homeostasis (Van Wynsberghe, Noback & Carola, 1995). Bone is composed of a tough organic matrix that is strengthened by deposits of calcium salts. The matrix consists of approximately 70% mineral salts and 30% collagenous fibres (Tortora, 1991). Calcium and phosphates combine to form a major crystalline salt known as hydroxyapatite, which is deposited in the organic matrix. Collagen fibres and hydroxyapatite crystals are bound together and generate a high degree of bone strength. Collagen fibres have advanced tensile strength whereas calcium salts, which are similar in physical properties to marble, have great compressional strength (Guyton, 1991).

Osteoblasts are bone lining cells that initially form bone and are found on the outer surfaces of bone and in bone cavities. Once osteoblasts become isolated in the bony matrix they are called osteocytes or mature bone cells. Osteocytes assist in maintaining daily cellular activities of bone tissue (Tortora, 1991).

Osteoclasts are large multinucleated cells responsible for bone resorption (degradation). Through the release of acidic protons, osteoclasts dissolve hydroxyapatite crystals and collagen fibres. The development, growth, maintenance, and repair of bone are important functions of osteoclasts.

Bone formation occurs through a process called ossification. Formation commences in the sixth or seventh week of embryonic life and continues throughout adulthood. Two methods of bone formation occur. Intramembranous ossification results from a cluster of osteoblasts that secrete intercellular substances composed of collagenous fibres. The framework, or matrix formed becomes calcified through the deposition of calcium salts. A trabeculae is created when a cluster of osteoblasts is completely surrounded by calcified matrix. Increasing numbers of trabeculae fuse together to create a latticework appearance and the space between the trabeculae is filled with red marrow. The periosteum develops from the original connective tissue that surrounds the growing mass of the bone. Successive layers of bone will be destroyed and re-formed as the bone reaches a final size and shape (Tortora, 1991).

Endochondral ossification is the replacement of cartilage by bone and is the process through which most bones are formed. A nutrient artery, midway along the shaft of a cartilage model, penetrates the surface and stimulates inner layer cells to become osteoblasts. A periosteal collar of compact bone around the middle of the diaphysis begins to form. As cartilage cells degenerate and are replaced by osteoblasts, bone gradually expands toward the epiphyses and steadily increases in length. The periosteal collar thickens with the deposition of successive layers of bone on the outer surface. Osteoclastic activity inside the bone results in the formation of a medullary cavity. Bone completely replaces cartilage except at the articular surface of the epiphyses and the region between the epiphysis and diaphysis, known as the epiphyseal plate. During growth, cartilage cells produced on the epiphyseal side of the plate are destroyed and replaced by bone on the diaphyseal side. This replacement process allows bone to increase in length while maintaining epiphyseal plate thickness. Eventually cartilage

cells stop dividing and cartilage is replaced by bone, producing a new structure called the epiphyseal line (Tortora, 1991).

A dense layer of calcified tissue, known as the cortex or compact bone forms the external surface of bone. Trabecular bone is a network of thin, calcified trabeculae occupying the internal space toward the metaphysis and epiphysis. Cortical and trabecular bone are comprised of the same matrix elements, but differ both structurally and functionally. The volume of calcified compact bone is 80% to 90% whereas only 15% to 20% of trabecular bone is calcified. As a result, 70% - 85% of the contact between bone and soft tissue occurs at the internal or endosteal bone surface. Cortical bone fulfils a mechanical and protective role while trabecular bone provides a metabolic function (Guyton, 1991).

Growth, modelling and re-modelling, are processes allowing bone tissue to be in a constant state of change throughout life. While the three processes can function simultaneously, a single process may dominate at different times during the lifespan. The genetically programmed process of enlargement of the entire skeleton is known as growth and occurs without regard to concurrent regional changes in shape in response to local loading factors (Bailey, Faulkner and McKay, 1996).

Modelling refers to the sculpting of bone in response to extraneous factors such as mechanical strains. Modelling mainly occurs during growing years and is distinguished from growth by a regional response (Bailey, 1996). Modelling can increase the periosteal perimeter and cortical bone mass and trabeculae (Bass and Myburgh, 2000). Weight bearing physical activity in the young produces a modelling response that results in a reserve of bone beyond normal activity responses. Areal bone mineral density (aBMD) for the total, radial, femoral neck and lumbar spine sites increases more rapidly for between 10 to 16 years of age than at times prior to or following this period (Blimkie, Chilibeck and Davidson, 1996). Areal BMD is calculated by dividing bone mass by the projected area of the region (g/cm^2) and although it does not account for depth, it is the unit most commonly reported (Bass et al, 2000).

The two years following menarche correspond with the largest gains for radial and femoral neck BMD and gains in lumbar spine BMD occur between Tanner stages 3 and 5 of pubertal development. Rates of gain in radial and lumbar spine BMD abate substantially in females during the later stages of puberty and adolescence. In males, radial and lumbar spine BMD increases from late puberty through to early adulthood (Blimkie et al, 1996). Studies containing BMD values unadjusted for bone size may have overestimated bone density changes during the growth years. An equation was devised to control for effect of bone geometry (Katzman, Bachrach, Carter and Marcus, 1991). The equation was derived following results from comparisons of mineral status among bones of similar shape but different size in 45 healthy prepubertal and pubertal girls. Results showed that 99% of the change in whole body mineral was attributed to bone expansion rather than to an increase in bone mineral per unit volume.

A consensus on site specific gender variations in bone mineral content (BMC) during growth is difficult to obtain (Bailey et al 1996). BMD and BMC have been used synonymously by some investigators when defining bone mass while others have used poorly contolled for differences in bone size. Despite problematic interpretations of data, results consistently demonstrate greater BMC or BMD values in males than females at total body, femoral neck and radial sites at various stages of growth.

Differences in maturational rates between males and females has produced inconsistent data comparing gender differences at the lumbar spine. No gender differences in lumbar spine BMC during adolescence were reported in a study involving Canadian children however greater lumbar spine BMD values in females until 15 years of age were also published (Grimston, Morrison, Harder and Hanley, 1992; Rubin, Schirduan, Gendreau, Sarfarazi, Mendola and Dalsky, 1993). A marked attenuation in lumbar spine BMD following puberty in females contrasted with a steep increase for males between 15 to 17 years (Gilsanz, Boechat, Roe, Loro, Sayre, & Goodman, 1994). In general, at skeletal maturity, males have

greater BMC than females. Skeletal sites containing relatively greater amounts of cortical bone are a result of greater skeletal size and cortical shell in males (Forwood, Bailey, Beck, Mirwald, Baxter-Jones and Uusi-Rasi, 2004; Bailey et al, 1996).

Remodelling is the process responsible for modifying bone shape and mass throughout life. Fatigue – damaged bone is replaced through a biologically coupled activation – resorption – formation sequence. Cycles of bone resorption (osteoclastic activity) and formation (osteoblastic activity) occur throughout skeletal life (Nattiv and Armsey, 1997). The cyclical process assists in maintaining calcium homeostasis and maintains mechanical integrity of the skeleton through bone renewal (Bailey, 1996). Remodelling over the lifespan however, results in a net loss of bone as new bone never fully replaces the bone that has been resorbed. As a result, a decrease in bone mineral accompanies ageing (Bailey et al, 1996).

Peak bone mass reflects the maximal lifetime amount of bone mineral accrued in individual bones and the whole skeleton (Blimkie et al, 1996). Peak bone mass value is a consequence of net accrual of bone during childhood and the balance between accrual and resorption in adulthood (Bass et al, 2000). Theoretically, because bone loss occurs with ageing, people who acquire maximal bone mass in early years should be at a reduced risk of skeletal fragility and fracture in later life. Agreement on the age at which peak bone mass is achieved remains illusive and site specific. Early studies suggested that peak bone mass was attained following skeletal maturation in a 10 to 15 year period of bone consolidation (Bailey, 1996). Most bone acquisition occurs during the adolescent growth spurt with approximately 90% of peak skeletal mass present by 18 years of age (Nattiv et al, 1997). Australian research examining the tempo and change in bone growth during puberty found considerable changes in BMC and BMD in girls in the 12 months prior to and following menarche (Magarey, Boulton, Chatterton, Schultz, Nordin and Cockington, 1999). Approximately 80-85% of peak bone mass is accrued by the onset of menarche and half of this bone mass is achieved during prepubertal growth (10 to 12 years of age). The other half of peak bone mass accrual is achieved very rapidly in the 2 to 4 year period of pubertal growth (Bass, Delmas, Pearce, Hendrich, Tabensky and Seeman, 1999).

Conflicting reports on the attainment of peak bone mass may be due to the site specific nature of skeletal development. A rapid and marked cessation of bone mineral accrual at most bone sites was observed by approximately 18 years of age (Matkovic, Jelic, Wardlaw, Ilich, Goel, Wright et al, 1994). Bone mineral gains however, continued at the distal radius until 22 years of age and spine regions until 27 years of age. Other studies of females reported peak BMD at the lumbar spine occurred between 20 and 25 years of age and peak BMD at the proximal femur occurred at approximately 30 years of age (Gilsanz et al, 1988).

The attainment of peak bone mass also differs among individuals when lifestyle behaviours, such as physical activity and nutrition are considered in addition to biological and genetic influences. Peak bone mass is most likely to occur when lifestyle and hereditary factors are temporally synchronised and operating at an optimal level. The synchronisation of modifiable and biological factors may vary according to different skeletal sites, individuals and populations (Blimkie et al, 1996). The prevailing opinion is that peak bone mass is achieved at an earlier age than previously considered and probably shortly after the cessation of linear growth (Bailey et al, 1996).

2.1.2 Prepubertal growth

Few physiological differences are evident in males and females prior to puberty. Anatomical and physiological tests reveal no physical differences between the sexes in flexibility, strength, height or body mass (Blanksby, Bloomfield, Elliot, Ackland and Morton,1994). Bone modelling and growth occurs in both axial and appendicular skeleton during childhood until puberty, however differences between the sexes in BMC are negligible (Whiting and Zernicke, 1998). Areal BMD increases between infancy and at the onset of puberty in both sexes for total body, femoral neck and lumbar spine. Rates of gain in BMD between infancy and puberty vary from 1.2% - 3.9% per year in females to greater values of 1.5% - 7.7% per year for males (Blimkie et al, 1996).

Prior to the onset of puberty, as bones increase in length the formation of bone beneath the periosteal envelope widens the bone shaft and the removal and replacement of a small volume of bone on the enodocortical surface produces a medullary cavity. As periosteal apposition exceeds resportion of bone on the endocortical surface, the enlarging long bone develops a thicker cortex that is displaced further from the neutral axis (Seeman, 2003).

2.1.3 Pubertal growth

Puberty is the time of the greatest sex differentiation since the early intra-uterine months (Tanner, 1978). Development of secondary sex characteristics, changes in body composition and increased lineal growth denote physical changes during puberty. Reproductive hormone secretion rates also increase dramatically in both genders (Grumbach and Styne, 1992).

Substantial increases in total body, radial, femoral neck and lumbar spine BMD have been reported with advancing sexual maturity in male and females during puberty (Blimkie et al, 1996). For females, the largest gains occur during the first two years following the onset of menarche for radial BMD, durig the year prior to menarche for femoral neck BMD and between Tanner stages 3 and 5 for lumbar spine BMD. Large gains in radial, femoral neck and lumbar spine BMD during two years following the onset of menarche abate substantially in females during the later stages of puberty (Theintz, Buchs, Rizzoli, Slosman, Clavien, Sizonenko and Bonjour, 1992). In males, radial and lumbar spine BMD continue to increase from late puberty into early adulthood (Blimkie et al, 1996). Males appear to have slightly higher radial, femoral neck and lumbar spine areal BMD than females by full sexual maturity during the later years of adolescence.

Estrogen in females inhibits periosteal bone formation by limiting the diameter of the bone and simultaneously promoting net bone formation on the endocortical surface. The inner diameter of longs bones is thus reduced in size which contributes approximately 15% of final cortical thickness. In males, pubertal androgen production increases periosteal apposition, bone diameter and cortical thickness. Continued widening of the inner diameter displaces the

cortex further from the neutral axis than it is displaced in females. Periosteal apposition partially maintains the cross-sectional area of the bone despite erosion on the endocortical surface. Males have greater periosteal apposition during aging than females and therefore have less net bone loss (Seeman, 2003).

Muscle development substantially increases during the pubertal growth period in concert with increases in lineal growth. Increases in muscle strength however, lags behind increases in muscle development by several months. Strength increases linearly in males until approximately 13 to 14 years of age, when there is a marked acceleration in strength development. Usually, maximum strength development during male adolescence occurs after maximum growth in weight and height. In females, strength increases steadily and linearly until 15 to 16 years, with a tendency to taper off by 18 years of age (Wheeler, 1991).

A synergistic relationship between bone and muscle development is believed to occur during growth. As muscle mass responds to increased loading, a corresponding adaptation in bone geometry may result from increased muscular contraction (Frost and Schonau, 2000). Several studies (Schoenau et al, 2002; Burr, 1997; Nordstrom, Thorsen, Bergstrom and Lorentzon, 1996) have demonstrated a direct cause-and-effect relationship between muscle strength and bone variables such as bone mass and strength. Furthermore, a site-specific relationship between bone and muscle geometry has been shown in pre- and post-pubertal females (Heinonen et al, 2001) and male and female young adults (Rittweger et al, 2000).

2.1.4 Section summary

Endochondral ossification involves the replacement of cartilage with bone during the formative years, establishing both cortical and trabeculae bone within an enlarging (width and length) bone structure. Axial and appendicular bone growth and modeling occurs steadily between infancy and the onset of puberty, after which lineal growth increases rapidly. Weight-bearing physical activity during the pubertal growth period augments regional gains in bone

mass however, the attainment of peak bone mass appears influenced by the synchronization of modifiable lifestyle and hereditary factors.

2.2 Biological factors influencing musculoskeletal growth in adolescence

2.2.1 Genetics

Research from twin and family studies demonstrate that heredity is the major determinant of bone mineral status, accounting for up to 60-90% of BMD variance in the population (Bailey et al, 1996; Kelly, Morrison, Sambrook, Nguyen, Eisman, 1995; Krall and Dawson-Hughes, 1993). Twin studies compare the correlation of bone density variance in monozygotic twins with BMD variance in dizygotic twins. Any observed differences are, therefore, assumed to be due to greater geometric sharing in monozygotic twins. In a study of premenopausal twin pairs, BMD was significantly more highly correlated in monozygotic then in dizygotic twins for the spine, proximal femur and forearm (Pocock, Eisman, Hopper, Yeates, Sambrook and Eberl, 1987). In family studies, daughters of mothers with hip and spine fractures have demonstrated low bone density at the hip and spine, respectively (Seeman, Hopper, Bach, Cooper, Parkinson, McKay et al, 1989). Similar mother-daughter relationships of low bone density are reported for the femoral neck (Seeman, Tsalamandris, Formica, Hopper and McKay, 1994).

Specific genes responsible for regulating bone density remain uncertain (Freenfield and Goldberg, 1997). Peak bone mass is influenced by multiple genes with small effects rather than a few genes with large effects (Gueguen, Jouanny, Guillemin, Kuntz, Pourel and Siest, 1995). Early studies indicate however, that allelic variations in the vitamin D receptor (VDR) gene play an important role on the intensity of the genetic effect on bone density (Sainz, van Tornout, Loro, Sayre, Roe and Gilsanz, 1997; Morrison, Qi, Tokiikita, Kelly, Crofts and Nguyen, 1994).

The association between VDR alleles and BMD has been extensively reviewed (Cooper and Umbach, 1996) and support for the initial hypothesis that allelic polymorphism ("*B*" for the absence of the polymorphic site and "*b*" for its presence) in the VDR gene contributes to BMD appears divided. Researchers studied twins as well as unrelated postmenopausal women and found "*b*" homozygotes (*bb* genotype) had significantly higher BMD compared with "*B*" homozygotes (*BB* genotype). Women with the "*Bb*" genotype displayed intermediate BMD (Morrison et al, 1994). In contrast, a large-scale study of peak BMD determinants in Caucasian women aged between 18-35 years confirmed a significant association between VDR (*BB*) genotype and BMD at the proximal femur (Rubin, Hawker, Peltekova, Fielding, Ridout and Cole, 1999).

The possibility of an interaction between genetics and environmental factors, such as physical activity, and its synergistic influence on bone mass has also been explored. During gene-environment interaction research with young people however, difficulties arise in comparing studies because of pre- and postmenopausal cohorts. Researchers investigated the relation between VDR genotype and physical activity on bone mass in a retrospective study of young adult women using calcaneal broadband ultrasound attenuation (BUA) and speed of sound (SOS) (Omasu, Kitagawa,Koyama, Asakawa, Yokouchi, Ando et al, 2004). A partially significant association between bone mass and VDR genotype was found however, physical activity during puberty more strongly influenced bone mass than the VDR genotype. Findings from a cross-sectional study (Nakamura, Ishii, Ando, Amagai, Oto, Imafuji et al, 2002) support a gene-environment interaction in active males who carry a specific VDR genotype. Highly trained athletes with an absence of the endonuclease *Fok* I restriction site (FF) displayed greater bone volume at the lumbar spine compared with nonathletic controls (Ff). In a large study examining the influence of VDR genotype and physical activity on bone, unrelated postmenopausal women with *BB* genotype had greater lumbar spine BMD, prompting researchers to claim that women carrying the *BB* genotype could benefit more from physical activity than women carrying the *bb* genotype (Blanchet, Giguere, Prud'homme, Dumont, Rousseau and Dodin, 2002). It should be noted however, that the relationship between VDR genotype and bone mass in postmenopausal women is more difficult to accurately assess

because genetic factors are confounded with environmental factors throughout life. The period of exposure to environmental factors therefore, is shorter in the young to middle-aged than in the elderly.

More detailed analyses of genetic contributions to bone mass are available using animal models because researchers can perform experiments in a standardized environment and control breeding to overcome the problem of genetic heterogeneity. In a study examining the genetic components of peak BMD, selective breeding was used to create high and low BMD strains of mice (Klein, Shea, Gunness, Pelz, Belknap, and Orwoll, 2001). Femoral shaft cortical area and thickness, vertebral trabecular bone volume, and failure load and stiffness in the femoral neck, shaft and L6 vertebrae were all greater in high-BMD mice. Although measurements were obtained from adult mice, researchers hypothesized the increased peak bone mass in the high-BMD mice arose from increased bone formation during skeletal growth. Bone remodeling processes, which predominate in adulthood, were reversed in the high-BMD mice because they were less susceptible to mechanical stress due to their greater bone mass. Researchers suggest a genetically determined lower mechanostat set point may produce greater osteogenic effects of weight bearing and muscle tension during growth resulting in increased bone mass and skeletal integrity.

2.2.2 Hormones

Contributions of growth hormone, insulin like growth factor (IGF-1), thyroid hormones and sex steroids alter during growth. Prepubertal increases in BMC appear largely growth hormone and IGF-1 dependent whereas pubertal increases in BMC involve the interaction of growth and sex hormones (Parfitt, 1994). Oestrogen and testosterone are critical hormones affecting bone mineral mass during puberty and the optimisation of peak BMD during late adolescence or early adulthood (Blimkie et al, 1996). Oestrogen may also increase the efficiency of intestinal absorption of calcium, decrease urinary calcium loss and suppress the rate of bone remodelling (Heaney, Recker, Stegman and Moy, 1989).

The presence of growth hormone is required for testosterone to exert an influence on musculoskeletal growth (Tanner, 1986). Greater acceleration in the axial than appendicular skeleton during puberty is demonstrated by increasing gains in the trunk than in the legs. Appendicular growth accelerates slightly then slows while accelerated axial growth dominates (Seeman, 2002). The axial skeleton is relatively more dependent on sex hormones but the appendicular skeleton is dependent on growth hormone (Parfitt, 1994).

2.2.3.1 *Section summary*

Twin and family studies provide evidence that bone mineral status in adolescence is strongly influenced by genetics. The precise influence of specific genes on bone mineral status, remains elusive. Additionally, evidence of gene-environment interactions contributing to variance in osteogenic responses to physical activity, is further challenged by the incompatibility of study cohorts for comparisons and generalisability. Controlled breeding of high- and low-BMD adult mice however, appears suggestive of a genetically determined mechanostat set point. The interaction of growth and sex hormones during adolescence is also recognised as an influencing factor on musculoskeletal growth.

2.3 Behavioural factors influencing musculoskeletal growth in adolescence

2.3.1 Nutrition

2.3.1.1 Energy

Bone turnover and bone mass are directly influenced by nutritional habits (Heaney, 1993). Osteogenic responses to mechanical loading are typically site specific whereas the influence of diet is more diffuse, acting on the whole skeleton. The interaction however, of loading and nutrition may enhance skeletal integrity of active individuals. Physically active individuals experience greater energy expenditure and subsequently have a greater demand for selected fuels, namely muscle and liver glycogen (Brooks and Mercier, 1994; Hagerman, 1992). If

energy expenditure from physical activity is greater than energy intake, an energy deficit will result. Prolonged periods of energy deficit culminates in reduced body weight, altered body composition, a reduction in bone mass and disturbances in endocrine function (Warren, Brooks-Gunn, Fox, Holderness, Hyle and Hamilton, 2002; Hotta, Fukuda, Sato, Hizuka, Shibasaki and Takano, 2000; Hotta, Shibasaki, Sato and Demura, 1998).

Bone loss due to increased bone turnover can result from endocrine changes that mobilize stored fuels. An attenuation of insulin release during prolonged physical activity coupled with inadequate energy intake, produces increasing quantities of tissue protein as a substrate for gluconeogenesis (Wagenmakers, Beckers and Brouns, 1991). A subsequent decrease in plasma levels of insulin and insulin-like growth factor 1 (IGF-1) with a concomitant increase in plasma concentrations of cortisol and growth hormone, exerts a direct effect on the function of bone cells. Osteoblast function is retarded while osteoclast activity is accelerated (Bressot, Meunier, Chapuy, Lejeune, Edovard and Darby, 1979).

Young athletes can fail to consume sufficient total kilocalories (kcals) to balance energy expenditure and growth demands. Energy intake, particularly for female gymnasts and ballet dancers is frequently lower than expected. The average daily energy intake of 1838 kcals has been reported in adolescent gymnasts and ballet dancers (Loosli, Bensen, Gillien & Bourdet, 1986). Reports from Swiss gymnasts showed an average intake of 1544 kcals daily (Bensen, Allermann, Theintz & Howald, 1990). Normative data from sedentary adolescents displays energy expenditure of between 1.4 to 1.6 times basal metabolic rate (BMR). Reported energy intake in elite gymnasts is below this level despite 3-4 hours of training each day (Westerterp & Saris, 1992).

Inadequate caloric intake relative to energy expenditure predisposes young women to menstrual dysfunction and potential detrimental effects on bone (Nattiv et al, 1997). Exercise and / or diet-associated amenorrhea are attributable to ovarian suppression, with energy deficit recognised as the primary stimulus (Laughlin and Yen, 1996). Inadequate energy intake appears to reduce bone formation and retard bone turnover. The limited efficacy of sex

hormone treatment for women with exercise and / or diet-associated amenorrhea appears to exclude sex hormone deficiency as the primary cause of bone remodeling imbalance (Golden, Lanzkowsky, Schebendach, Palestro, Jacobson and Shenker, 2002).

Insufficient energy intake in association with intense athletic training in sports emphasising small body size may also compromise linear growth in adolescent athletes. Anthropometric measurements of 14 year old female gymnasts revealed delayed growth rates consistent with not having attained menarche (Caldarone, Leglise, Giampietro & Berlutti, 1986). Junior elite gymnasts, aged between 11 and 14 years, were at 20th percentile for weight-for-age and height-for-age (Benardot and Czerwinski, 1991). Energy deficit states restrict normal maturational growth and place young athletes at risk of stress injury to bone (Theintz, Howald, Weiss and Sizonenko, 1993; Nattiv et al, 1997).

If energy deficit is a primary stimulus for disturbed bone turnover, including decreased bone formation, sufficient energy intake commensurate with the physical demands of training and competition appears an obvious recommendation. Carbohydrate consumption in particular, should be sufficient to replace depleted glycogen stores and prevent the attenuation of insulin release. Positive energy balance that produces weight gain in active individuals with exercise and / or diet-associated amenorrhea appears an effective treatment however, implementation of effective treatment strategies may be considered undesirable or impractical to individuals who deliberately manipulate their energy balance for performance reasons.

2.3.1.2 Calcium

Adequate calcium intake is considered one of the most important preventative interventions contributing to optimal bone health in children and young adults (Nattiv et al, 1997). Recommended dietary intakes for calcium ensure gains in bone mass during adolescence and minimise bone loss that occurs with ageing (Hawley et al, 1998). Childhood and adolescent years are important in terms of the influence of calcium on bone status. The skeleton of a new-born contains approximately 25 grams of calcium. A healthy adult contains

over 1000 grams of calcium. Increasing calcium demands of the growing skeleton must be provided from dietary sources for essential increases in calcium between birth and adulthood (Heaney, 1991).

Results of studies examining the role of calcium in contributing to higher BMD values during growth are generally equivocal. Nutritional status is suggested to have an independent effect on bone mineral development during the formative growth years (Henderson and Hayes, 1994). Research examining calcium intake and BMC of the radius in children aged 2 to 16 years found higher BMC Z-scores in children ingesting over 1000 mg calcium per day than Z scores in the children consuming less than 1000 mg per day (Chan, 1991). A significant association between calcium intake and bone density at the hip was also reported for girls aged 15 to 17 years (Turner, Gilchrist, Ayling, Hassall, Hooke and Sadler, 1992). In addition, calcium intake was significantly associated with lumbar spine BMD in girls aged 8 to 18 years (Sentipal, Wardlaw, Mahan and Matkovic, 1991). Beyond general populations, calcium intake in sporting populations also appears to exert an influence. A positive linear correlation exists between dietary calcium and lumbar spine BMD in amenorrheic and eumenorrheic elite female athletes (Wolman, Clark, McNally, Harries and Reeve, 1990).

Other studies however, fail to demonstrate significant correlations between calcium intake and BMD at the spine, femur, radius or whole body sites in young females (Bachrach, Guido, Katzman, Litt and Marcus, 1990; Katzman et al, 1991). Similarly, a retrospective study found no correlation between calcium intake during adolescence and bone mass or density in adult females (Welten, Kemper, Post, van Mechelen, Twisk, Lips et al 1994).

Prospective studies involving calcium supplementation provide strongest evidence in support of the importance of calcium for bone mineral development during growth. The BMD in 7 pairs of identical twins was assessed during a 3 year supplementation trial (Johnston, Miller, Slemenda, Reister, Hui, Christian et al, 1992). One of the twins received a calcium supplement of 1000 mg and the other a placebo. Supplementation resulted in significant increases in mid-shaft radial (5.1%) and lumbar spine BMD (2.8%). No effects of

supplementation were detected in twin pairs who were pubertal at the start of the study or who became pubertal during the study. Another 18-month supplementation trial of 500 mg calcium involving initially premenarcheal girls, resulted in increases in lumbar spine BMC (4.7%), lumbar spine BMD (2.9%) and total body BMD (1.3%) in the supplemented group compared to the placebo group (Lloyd et al, 1993).

Premenarcheal skeletal growth may be more responsive to higher calcium intakes than peri- or post-menarcheal bone tissue. Forearm BMD gains and nutrient intake were examined in boys and girls aged 8 to 16 years (Gunnes and Lehmann, 1996). Results indicate calcium intake should be encouraged at prepubertal age in order to increase bone density. Calcium supplementation over a 2 year period however, failed to result in significant increases in radial or lumbar spine BMD in postmenarcheal adolescent females compared to controls with low calcium intake (Matkovic et al, 1990). The extent to which calcium supplementation influences adolescent bone is uncertain. The contention that high levels of calcium supplements are much less effective postmenarche than premenarche is not strongly supported. Despite advocacy for higher levels of supplementation to increase bone mineral accrual during postmenarcheal growth, research does not consistently support the underlying assumption. An increased calcium intake of adolescent females from 250 to 1600 mg per day did not produce significant increases in urinary calcium (Matkovic et al, 1990). Other evidence however, suggests peak bone mass is achieved with average daily intakes of calcium below 1000 mg and 850 mg for males and females respectively (Peacock, 1991). Despite the lack of strong evidence a recommended daily allowance (RDA) of 1250mg of calcium during childhood and 1450 mg during adolescence is frequently cited (Andon, Lloyd and Matkovic, 1994).

Beneficial effects of calcium supplementation on bone density may not however, be maintained when supplementation ceases. An 18-month follow-up study conducted after an 18-month calcium supplementation trial revealed benefits of calcium supplementation disappeared after treatment was withdrawn (Lee, Leung, Leung and Cheng, 1996). Bone mineral density in one year post-supplementation demonstrated that significant differences

between the supplemented and unsupplemented twins disappeared (Slemenda, Resiter, Peacock and Johnston, 1993). High calcium intakes would need to be maintained indefinitely for the difference in bone density to persist.

2.3.2 Exercise / Physical activity

2.3.2.1 Adaptive response of bone to mechanical loading

Genetics determine the basic morphology of the skeleton but its final bone mass and architecture are modulated by adaptive mechanisms sensitive to mechanical loading (Forwood, 2001). Wolff, in 1892, first documented observations of changes in bone mass that accompany different mechanical loadings. Alterations to the internal architecture and external structure occur as a consequence of primary changes in mechanical stressors (Marquet and Furlong, 1986).

Induced mechanical strain has been recognised as the key intermediate variable between mechanical loading and bone adaptation. Increased loading produces minute changes in the surface curvature of bone inducing a strain gradient signal that activates bone cell response (Bailey et al, 1996). Strains however, must be above or below threshold levels for bone to have an adaptive response (Frost, 1987). Figure 2.1 illustrates this concept, known as the “mechanostat theory”.

The Mechanostat Model

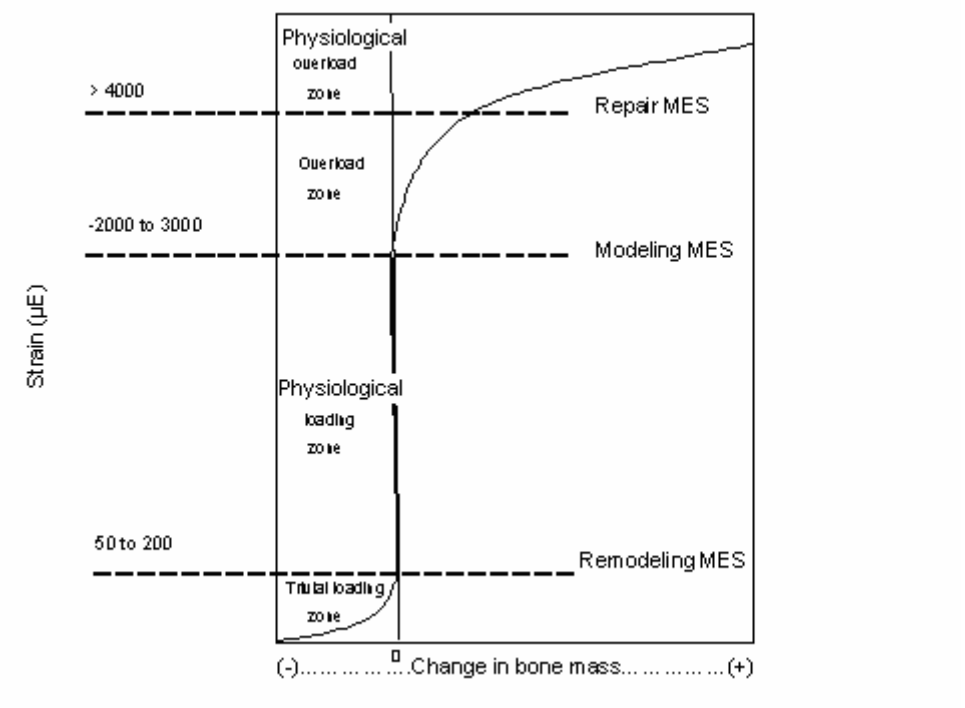


Figure 2.1: Mechanostat model

Figure 2.1 presents the mechanostat model and shows that when strains are low in the trivial loading zone resulting an increased remodeling and subsequent loss of bone occurs. Sufficient strain is maintained in the physiological loading zone and bone is neither gained nor lost. In the overload zone, modeling is stimulated to add more bone and to respond to further high strains. Bone enters a repair mode at very high strains resulting in disorganised bone being added to meet immediate needs (Forwood and Turner, 1995).

Recent evidence suggests that induced mechanical strain signal may be amplified by interstitial fluid flow through canalicular channels. The contents of the bone fluid compartment relocate surfaces of greater concavity to surfaces of greater convexity when bone is deformed in bending (Burr, Robling and Turner, 2002). Dynamic loading and relaxation of bone create shear stresses on cells that allow an adaptive response. Rates of fluid flow through the bone fluid compartment are proportional to the sheer stresses generated on bone cells. Higher strain rates produce greater fluid velocity and an increase in sheer stress (Robling, Burr and Turner, 2000). The application of a compressive end-load to the ulnae of growing male rats

10 min/day for 2 weeks was examined in three treatment groups who received static loading at 8.5 Newtons (N), static loading at 17N, or dynamic loading at 17N (Robling, Duijvelaar, Geevers, Ohashi and Turner, 2001). Dynamic loading proportionally increased osteogenesis significantly on both periosteal and endocortical surfaces, which supported the concept that fluid flow mediates the mechanical signal.

Bone reacts differently when it is stretched (tensile strain) or compressed (compressive strain). Curved or bent bone exposes the concave side to compressive stresses and the convex side to tensile stresses (Burr et al, 2002). A neutral axis exists at some point between the two surfaces where the strains are zero. Theoretically the load placed on the periosteal surface will be greater than loads placed on the endocortical surface because of the distance from the neutral axis (Hayes and Gerhart, 1985).

Mechanical loading increases bone apposition at the surfaces in a state of net formation. During childhood net formation occurs on the periosteal surface and net resorption on the endocortical surface (Ruff, Walker and Trinkaus, 1994). Late in puberty however, the predominant effect of mechanical loading is endocortical apposition. Increased diameter of bone distributes material further from the neutral axis and improves resistance to bending or torsional loads (Einhorn, 1992).

2.3.2.2 Influence of high-intensity physical activity

Numerous studies indicate that increased mechanical loading through the application of resistance or increased weight-bearing activity augments bone mass. Mechanical loads that generate high load magnitudes are more likely to produce an osteotrophic effect than low intensity loads (Taaffe, Robinson, Snow and Marcus, 1997; Heinrich, Going, Pamenter, Perry, Boydon, Lohman, 1990; Heinonen, Oja, Kannus, Sievanen, Manttari and Vuori, 1993).

A study of athletes from high-impact sports (running, gymnastics, tumbling and dance) with elite swimmers found greater femoral neck BMD in athletes from the high-impact sports than

the swimmers. The findings of higher densities at the proximal femur suggests the femur may experience greater mechanical loading during high-impact activities than other skeletal sites (Grimston, Willows and Hanley, 1993). Adolescent male weight lifters had significantly greater BMD at femoral neck and lumbar spine compared with age-matched controls (Conroy, Kraemer, Maresh, Fleck, Stone, Fry et al,1993). A comparison of 35 athletes (Judo N=21, Karate N=14 and Water Polo N=24) involved in training 3 $h.day^{-1}$, 6 $day.wk^{-1}$ and age-matched nonathletic individuals resulted in higher total BMD in athletes compared with controls (Andreoli, Monteleone, Van Loan, Promenzio, Tarantino and De Lorenzo, 2001).

Eumenorrheic gymnasts, who trained on average 4 $h.day^{-1}$, 5 $day.wk^{-1}$ displayed significantly greater lumbar spine BMD (+1.3%) than age-matched controls after 27 weeks of gymnastics training (Nichols, Sanborn, Bonnick, Ben-Ezra, Gench and Di Marco, 1994). A comparison of pre-adolescent female gymnasts with a history of high volume impact loading and healthy nonathletic controls showed greater whole body, femoral neck and lumbar spine areal BMD (aBMD) in gymnasts than controls. The conclusion was that high-volume impact loading increased whole body and regional BMD in pre-adolescent female gymnasts (Dyson et al, 1997). Results from a similar study reported greater BMD in prepubertal female gymnasts compared with female swimmers and controls. All athletes were involved in high intensity exercise during the previous 3 years although only the gymnastic group experienced impact loading on the skeleton (Courteix, Obert and Germain, 1997). In order to quantify impact loading in gymnastics, ground reaction forces of common gymnastic manoeuvres were measured. Assessment of ground reaction forces from male gymnastics training sessions revealed 102 to 217 impacts on upper and lower extremities with peak magnitudes of 3.6 to 10.4 times body weight (Daly, Rich, Klein and Bass, 1999). An investigation of the influence of two different types of high-impact weight-bearing activity (rope skipping and soccer) in BMD in late adolescent females resulted in significantly higher BMD at most loaded sites in both high activity groups compared with controls (Petterson et al, 2000).

2.3.2.3 Cross-sectional studies

Cross-sectional studies allow comparisons of different physical activity patterns such as sporting and non-sporting people. Activities in which body weight is carried create forces on skeletal sites sufficient to stimulate gains in bone mass (Kemper and Niemeyer, 1995). The current review of cross-sectional studies will focus on the high impact loading sport of gymnastics, comparative studies of gymnasts and runners, weight-loaded sports, weight-loaded sports versus weight-supported sports and unilateral loading sports such as tennis and squash.

i) Gymnastics

A number of studies have examined the effect of gymnastics training on bone density in preadolescent, adolescent and young adult populations. Gymnastics is characterised by very high impact through repeated jumps and body contact with hard surfaces. As previously discussed, preadolescent gymnasts with a history of high volume impact loading had significantly greater femoral neck and trochanter aBMD and higher total body, femoral neck and lumbar spine BMAD than healthy nonathletic controls (Dyson et al, 1997). A similar study revealed premenarcheal gymnasts with significantly higher proximal femur, femoral neck, trochanter, Ward's triangle and lumbar spine BMD than healthy age, height and weight-matched controls (Nickols-Richardson, Modlesky, O'Connor and Lewis, 2000). Greater lumbar spine BMD in young adult female gymnasts than controls was observed after 27 weeks intensive training, compared to controls. Prior to the training program gymnasts also displayed significantly higher mean lumbar and femoral neck BMD than controls (Nichols et al, 1994).

The effect of high impact loading forces on BMD in upper limbs of young adult female gymnasts was recently described (Proctor, Adams, Shaffrath and Van Loan, 2002). Vaulting studies show forces on the upper extremity of between 1.13 to 1.57 times body weight and 3.1 times body weight on uneven bars. Compared with controls, gymnasts had significantly

higher lumbar spine, total right proximal, left proximal femur and total body BMD. Higher BMC was also found in gymnasts compared with controls at all sites.

ii) Gymnasts and Runners

Peripubertal Caucasian female gymnasts, runners and controls were studied over 12 months to determine the effect of high impact loading on the growing skeleton. The 12-month follow-up data were adjusted for age, height, Tanner stage, BMD at baseline and increases in height and weight. Gymnasts showed greater increases in femoral neck and trochanter BMD than runners or controls. Lumbar spine BMD did not differ between groups (Lehtonen-Veromaa, Mottonen, Irjala, Nuotio, Leino and Viikari (2000). Another study of young adult female gymnasts, runners and controls found similar results with gymnasts showing higher femoral neck BMD than runners and controls (Robinson, Snow-Harter, Taaffe, Gillis, Shaw, Marcus, 1995). In addition, runners showed lower total body BMD values compared with gymnasts. Collectively, the studies highlight the superior site-specific BMD profile at the femoral neck of gymnasts compared with other sport participants and less active controls.

iii) Weight-loaded sports

The BMD of children involved in sports producing significant impact loading on the skeleton was compared with children in non-weight bearing sports (Grimston et al, 1993). Eight males and 9 females were recruited from impact loading sports such as running, gymnastics, tumbling and dance and were matched for race, gender, stage of puberty and body weight with competitive swimmers. Impact-load children had greater femoral neck BMD and a tendency for greater lumbar spine BMD than swimmers. Non sporting weight bearing activity appears to have a similar impact on the bone mineral of children. Seventy-eight female pre- and early pubertal ballet dancers were compared with 52 age-matched controls. Mean weekly hours of formal dance training were 4.6 $h.wk^{-1}$ and all dancers had attended dance classes on average for the past 4.3 years. Participation in dance training was associated with 4.5% greater BMD and 7% greater aBMD at the femoral neck. Differences could not be explained by maturity, size or body composition differences between groups (Bennell, Khan, Matthews, Cook, Holzer, McKay et al, 2000).

Another study compared late adolescent females participating in competitive rope skipping training, female soccer players and nonathletic healthy controls. The skipping group had significantly higher BMD at total body, lumbar spine, trochanter and the diaphyses of femur and tibia than controls. Soccer players also had greater BMD at proximal femur, total femur and the diaphyses of femur and tibia than controls. No significant differences were found between the two high-activity groups except for a higher radial BMD in the skipping group than the soccer group (Pettersson et al, 2000). Activities which involve significant impact loading on the skeleton appear to result in greater gains in site-specific BMD compared with participants exposed to reduced impact loading.

iv) Weight-loaded v's weight-supported sports

Higher regional and total body BMD in competitive young adult female gymnasts was reported in a comparative study with runners, swimmers and nonathletic females (Taaffe et al, 1997). Two cohorts were studied. Cohort 1 included 26 gymnasts, 36 runners and 14 controls. Percent change in lumbar spine BMD after 8-months was greater in gymnasts than runners or controls. An increase in femoral neck BMD in gymnasts was also greater than runners. Cohort 2 included 8 gymnasts, 11 swimmers and 11 nonathletic controls. Gymnasts gained greater lumbar spine BMD and experienced greater change in femoral neck than swimmers and controls. Similar research compared young adult male runners, cyclists, a group comprising both runners and cyclists, and age-matched controls. All athletic participants had competed for a minimum of 3 years and trained for a minimum of 4 $h.wk^{-1}$. Compared with controls, runners had greater total body and leg BMD, cyclists had reduced lumbar spine BMD and athletes of the combined group had greater total body and arm BMD (Stewart and Hannan, 2000).

Similar research evaluated the effects of 3 years of intensive sport training on BMD in elite prepubertal females. Athletes were engaged in a sport with significant impact loading on the skeleton (gymnastics) or a sport without impact loading (swimming). Compared with controls, gymnasts had significantly greater mid-radius, distal radius, lumbar spine, Ward's triangle and femoral neck BMD. No difference between swimmers and controls for all BMD measurements

were recorded (Courteix, Lespessailles, Peres, Obert, Germain and Benhamou, 1998). In a similar study, young adult female runners were compared with a non-weight bearing activity (swimmers) and controls. Greater total body and femoral neck BMD were found in runners compared with swimmers and controls. Furthermore, swimming exercise had no effect on BMD (Emslander, Sinaki, Muhs, Chao, Wahner, Bryant et al, 1998). In young adult male athletes, participants from high impact sports such judo and karate were compared with weight-supported athletes (water polo) and age-matched controls. Total body BMD was higher in judo and karate athletes in comparison to water polo athletes and controls. Furthermore, no difference was detected in total body BMD between water polo athletes and controls (Andreoli et al, 2001).

More recently the influence of different mechanical loading patterns on BMD in elite adolescent female cyclists, runners, swimmers, triathletes and controls was investigated. Differences in BMD between weight-bearing (running), nonweight-bearing (swimming and cycling) sports, and a sport that incorporates both types of loading conditions (triathlon) were compared. Runners had greater total body, lumbar spine, femoral neck and leg BMD than controls. Runners also had higher total body, femoral neck and leg BMD than swimmers and greater leg BMD than cyclists (Duncan et al, 2002). Collectively, the studies show that athletes engaged in weight-loaded activities appear advantaged in total body and regional BMD compared with athletes involved in weight-supported activities.

v) Unilateral loading

Comparisons of sporting people with non-sporting people can be problematic because intervening factors such as differences in genetic background, nutrition, self-selection, compliance, activity level and body composition can explain differences found in bone mass. Confounding however, can be partly overcome by within-subjects comparisons (Kemper et al, 1995). To avoid genetic confounding, bilateral intra-subject variations in BMC and BMD for dominant and nondominant limbs in children aged 8 to 16 years were examined (Faulkner, Houston, Bailey, Drinkwater, McKay and Wilkinson, 1993). Greater BMC and BMD in the dominant arm compared to the nondominant arm were attributed to greater habitual loading.

In a similar study, site specific effects of loading were examined in pre, peri and post pubertal female tennis players. Humeral BMC was greater in the loaded arms of prepubertal players due to greater periosteal apposition. Results suggest loading further enhances structural changes produced during growth (Bass, Saxon, Daly, Turner, Robling, Seeman et al, 2002).

Post-pubertal differences in BMC of the playing and non-playing arms of adult female national level tennis and squash players have also been examined (Kannus, Haapasalo, Sankelo, Sievanen, Pasanen, Heinonen et al, 1995). Selection criteria included 5 or more years of participation and mean starting age of playing careers was 16 years. Compared to controls, the dominant – to – nondominant side difference in BMC was significantly greater in players at proximal humerus, humeral shaft, radial shaft and distal radius. More recently, a similar examination of impact loading on bone mass and size in young adult female tennis and squash players was undertaken (Kontulainen, Sievanen, Kannus, Pasanen and Vuori, 2003). Players were divided into two groups according to the age at which they commenced tennis or squash training to examine possible differences in bone structure and density. At the humeral shaft, the loaded arm contained greater BMC with the greatest side-to-side difference detected in young starters compared to old starters and controls.

A similar study involving male adult national level tennis players examined bilateral bone characteristics of the humerus with age, height and weight-matched controls (Haapasalo, Kontulainen, Sievanen, Kannus, Jarvinen and Vuori, 2000). Players demonstrated greater BMC in proximal humerus, humeral shaft, distal humerus, radial shaft and distal radius in dominant arm compared to nondominant arm. Controls also showed significant dominant – to – nondominant side differences however differences were small and significantly lower than those of players. Despite large differences in side-to-side differences in BMC, volumetric bone density was identical in dominant and nondominant arms of players and controls. High playing-arm BMC was due to increases in trabecular bone, cross-sectional area of marrow cavity, cortical bone and cortical wall thickness. Sports involving unilateral loading provide further support of greater changes in BMC and BMD in loaded than unloaded regions.

Results from cross-sectional studies provide compelling evidence to suggest physical activity produces gains in bone mass. Weight-bearing activities in particular, appear more osteogenic than weight-supported activities. Similarly, athletes exposed to unilateral loading reveal greater gains in site-specific bone mineral compared with unloaded regions. Cross-sectional studies however, only highlight possible differences between heterogeneous groups. Intervention studies enable researchers to control participant exposure to stimuli, thereby producing more robust research outcomes.

2.3.2.4 Intervention Studies

Exercise intervention studies challenge the hypothesis that exercise during childhood and adolescence increases bone density. Studies recruiting children from distinct maturity stages seek to determine the phase of growth during which bone responds optimally to physical loading (MacKelvie et al, 2002). Research has progressed from weight bearing generic activities, higher intensity circuit-based loading, resistance and plyometric training, to highly prescriptive jumping activities.

The effect of weight-bearing physical activity on bone mineral was examined in 71 premenarcheal girls, aged 9 to10 years. A school-based intervention program assessed lean mass, strength and bone mineral responses to activities which included aerobics, soccer, football, step aerobics, dance, skipping, ball games and weight training (Morris, Naughton, Gibbs, Carlson and Wark, 1997). Each session was 30 minutes in duration and was performed 3 times per week for 10 months. A 20 station weight-bearing, strength building circuit operated for 10 weeks during the study. Significantly more total body (+5.5%) and femoral neck (+4.5%) BMC and total body (+2.3%), lumbar spine (+3.6%) and proximal femur (+3.2%) aBMD was reported in the intervention group compared with controls.

A similar school-based intervention involving weight-bearing physical activity was examined in prepubertal boys. Twenty boys participated in three sessions of 30 minutes of moderate exercise per week over an 8 month period. Controls received no additional exercise beyond 2

hours of physical education per week and were matched for age, sitting height, standing height and baseline aBMD. Decreased endocortical (medullary) diameter produced an increase in femoral midshaft cortical thickness in the exercise group. Lumbar spine, leg and total body aBMD also increased in the intervention group (Bradney, Pearce, Naughton, Bass, Beck et al, 1998).

Bone mineral accrual in prepubertal and early pubertal girls was studied using a school-based physical education exercise intervention program. Forty-four prepubertal (Tanner stage 1) and 43 early pubertal (Tanner stage 2 & 3) girls completed a high-impact, weight-bearing exercise session for 10 to 12 minutes, 3 times per week for 7-months. Five circuit training stations involved a range of jumping exercises and produced ground reaction forces of 3.5 to 5 times body weight. Twenty-six prepubertal and 64 early pubertal healthy controls completed 10 minute stretching sessions 3 times per week. Early pubertal girls in the intervention group gained 1.5% to 3.1% more bone at the femoral neck and lumbar spine than early pubertal controls. No changes were reported in bone parameters between prepubertal intervention and control groups following the 7-month intervention (MacKelvie, McKay, Khan and Crocker, 2001).

A less vigorous, circuit-based intervention program involved prepubertal and early pubertal Asian and white girls and boys. Children completed games and circuits involving jumping or skipping and 10 tuck jumps during each physical education class, 3 times per week for 8-months. Children in the intervention group had significantly greater (1.4%) aBMD at the trochanter than the control group. After controlling for initial status and difference in growth, the less vigorous nature of the school based 8-month intervention may explain the lack of difference in aBMD at other skeletal sites (McKay, Petit, Shutz, Prior, Barr and Khan, 2000).

The effect of weight-bearing, high-impact exercise on BMC in two distinct groups of pubertal girls was investigated. Twenty-five premenarcheal and 39 postmenarcheal girls completed a step-aerobic program twice a week for 9-months. Thirty-three premenarcheal and 29 postmenarcheal girls served as controls. In premenarcheal girls, BMC increased more in the

intervention group than controls at lumbar spine and femoral neck. Postmenarcheal girls showed no significant post-training differences in BMC. The authors concluded the 9-month exercise intervention program produced large additional bone gain in exercising premenarcheal girls but not exercising postmenarcheal girls (Heinonen, Sievanen, Kannus, Oja, Pasanen and Vuori, 2000).

The consequence of site-specific loading through progressive resistance training on BMC and BMD was examined in postmenarcheal adolescent girls. Four sets of 13 exercises were performed 3 times per week for 26 weeks using hydraulic resistance machines. Despite a trend towards a transient increase in lumbar spine bone mineral during the first 13 weeks, there were no significant changes in total body or lumbar spine bone mineral between intervention and control groups (Blimkie, Rice, Webber, Martin, Levy and Gordon, 1996).

Using a similar maturational cohort, the effects of plyometric jump training on bone mass was investigated in postmenarcheal adolescent girls aged 13 to 15 years. Twenty-five girls completed plyometric training for approximately 30 to 45 minutes per session, 3 times per week for 9-months (Witzke and Snow, 2000). Progressive resistance training and simple plyometrics were performed for the first 3 months of the program. In the final 6-months of the program advanced plyometrics including jumps, depth jumps, bounding and hopping were introduced. Twenty-eight controls were matched by age and months past menarche. Intervention girls failed to show greater changes in BMC than controls despite trends in BMC improvements across skeletal sites. The authors suggested the postpubertal skeleton is not as responsive to exercise training as the prepubertal skeleton.

A highly intensive, repetitive, box jumping program involving 44 prepubertal girls and boys was implemented to examine changes in BMC. Sessions included 100 two footed drop landings from a height 61 cm, 3 times per week for 7-months. Participants progressed from 50 jumps per session (no box) to 80 jumps per session (from box) in the first 4 weeks. From week 5 to the conclusion of the study, 100 jumps from 61 cm box were completed. Significant

increases in lumbar spine (+3.1%) and femoral neck (+4.5%) BMC and lumbar spine (+2.0%) aBMD occurred in the jumping group compared with controls (Fuchs, Bauer and Snow, 2001).

Difficulties in comparing the responsiveness of the skeleton to structured physical loading during growth are compounded by timing and onset of maturational events. Intervention studies involving children and adolescents have predominantly focused on females and have examined osteogenic responses across the spectrum of maturational stages. Results suggest changes in bone mass and structure appear particularly responsive to physical loading in the pre- and peripubertal years. The prescription of exercise to maximise osteogenic responses in children and adolescents however, remains equivocal. The majority of school-based intervention studies involve 10 to 20 minutes per day of weight-bearing impact activities with 3 or more sessions per week. The prescription of 3 days of exercise per week may potentially advance oestogenic responses in children and adolescents however, longitudinal studies are required to ascertain the sustainability of gains in bone mineral.

2.3.2.5 Longitudinal Studies

The long term effects of mechanical loading on bone mineral accretion in child and adolescent populations are not fully understood. Cross-sectional studies generally report a positive association between mechanical loading and bone mineral accrual throughout childhood and adolescence however, longitudinal consequences of the sustainability of exercise-induced bone gains are relatively unknown.

Longitudinal studies which span the entire pubertal period must control for considerable maturational differences in children and adolescents of the same chronological age. A six year study which annually evaluated the relationship between physical activity and bone mineral accrual in children passing through adolescence, used peak bone mineral content velocity (PBMCV) as a common maturational landmark to compare participants (Bailey, McKay, Mirwald, Crocker and Faulkner, 1999). Children in the highest activity quartile for physical activity accrued more bone across the six year period than inactive children. Highly

active children also demonstrated great total body BMC one year after PBMCV compared with least active children. At regional sites, active children had 18% greater BMC at the lumbar spine compared with the least active group. Results provide more credible evidence to suggest physical activity positively influences bone mineral accrual in the growing skeleton.

A similar study examined the effect of physical activity on bone mineral accretion in males and females aged 9 to 16 years (Sundberg, Gardsell, Johnell, Karlsson, Ornstein, Sandstedt et al, 2001). Highly active boys and girls between ages 9 to 13 demonstrated greater femoral neck BMC and BMD than less active controls. Three years of further high and low activity did not yield any increased differences in bone size and mass in either girls or boys. Baseline data of BMC / BMD and physical activity however, were gained at age 13 using a cross-sectional study design, raising concerns about sampling bias. Furthermore, physical activity levels from 9 to 13 years were evaluated retrospectively by questionnaire in order to determine annual activity level. Despite methodological concerns, results suggest physical activity may advance gains in bone mineral in prepubertal years but provide no additional gains in the peripubertal period.

Favourable bone geometric changes and gains in bone mineral were found in prepubertal males participating in a school-based, high-impact, 20-month circuit intervention program (MacKelvie, Petit, Khan, Beck and McKay, 2004). Intervention and control participants had similar baseline height, weight, physical activity scores and calcium intakes. Greater periosteal and endosteal bone expansion at the narrow neck of the femur in addition to greater femoral neck BMC, was found in intervention participants compared with controls. As the study was limited to a single maturational cohort, comparisons between pre- and peripubertal participants was not possible.

Conflicting results were gained from a three year longitudinal study which compared changes in total body and regional BMC / BMD in pre- and peripubertal female gymnasts with non-exercising controls (Courteix et al, 1999). Annual measurements over a three year period showed gymnasts displayed greater areal BMD than controls at all sites except for the total

body. Gains in BMD at the lumbar spine, femoral neck, trochanter, Ward's triangle, and distal and mid radius were achieved by gymnasts despite maturational progression. Results highlight the benefits of weight-bearing exercise on bone mass acquisition throughout the pre- and peripubertal period.

In contrast to the prepubertal years, a three year longitudinal study examined the effect of physical activity on the accumulation of bone mass in postpubertal male athletes and controls (Gustavsson, Olsson, Nordstrom, 2003). At baseline, athletes from badminton and ice hockey displayed greater total body, dominant and non-dominant humeral and femoral neck BMD than age-matched controls. At the three year follow-up, athletes had greater BMD at all sites. Bone mass differences between athletes and controls were greatest at sites exposed to high mechanical loading, such as the femoral neck and dominant humerus. Although results suggest physical activity positively influences bone mineral accrual in healthy adolescent males after puberty, selection bias from comparing cross-sectional data at 16 and 19 years of age cannot be discounted.

A similar study compared changes in bone mass in male and female postpubertal track and field athletes with non-athletic controls over a 12-month period (Bennell, Malcolm, Khan, Thomas, Reid, Brukner et al, 1997). At baseline, male and female athletes displayed greater bone mass at loaded sites compared with control groups however, a genetic predisposition to higher bone mass in athletes warrants acknowledgement. Modest but significant increases were found in total body BMC and femur BMD in athletes and controls during the 12-month study. Athletes also displayed greater increases in bone density at the lumbar spine when compared to controls. Baseline results indicate an association between greater bone mass and participation in track and field training. Longitudinal data after 12-months of athletic training suggest additional gains in bone mass can be achieved in the postpubertal period.

Longitudinal analysis of data gained from intervention studies minimizes the influence of selection bias inherent in cross-sectional study design. A 20-month follow-up study assessed the effect of a 9-month jumping intervention on bone gain in growing girls (Kontulainen,

Kannus, Pasanen, Sievanen, Heinonen, Oja et al, 2002). Immediately after the jumping intervention, trainees displayed 3.6% greater BMC at the lumbar spine than controls. At the 20-month follow-up, BMC gains at the lumbar spine had increased to 4.9% in trainees when compared with controls. No other between group differences were found in BMC at the 20-month follow-up. Despite continued participation in step-aerobic training by a third of trainees between the 9-month intervention and 20-month follow-up, previous participation in high impact training appears to produce residual bone gain in growing girls.

Residual gains in bone mass however, were not evident in adult females after a 6-month withdrawal of exercise following a 12-month jumping and lower body resistance training intervention study (Winters and Snow, 2000). Trainees displayed greater BMD at the trochanter compared with controls at the conclusion of a 12-month intervention period. Lumbar spine and total body BMD values were not different between groups and remained near baseline levels throughout the training and detraining periods. A foam gymnastics mat used as a landing surface may have prevented the delivery of sufficient forces to affect lumbar spine. After a 6-month period of detraining, BMD for trainees returned to baseline values, despite a third of trainees refusing to stop exercising. Although continued participation in community and home-based exercise classes were of lower intensity than the intervention study, failure to comply with detraining guidelines may have attenuated observed changes in bone mass. Results suggest 12-month gains in bone mass from jumping and lower body resistance training are not maintained after 6-months of detraining in adult females.

A similar pattern of gains in bone mass from training and bone loss from detraining were found in a two year longitudinal study involving postpubertal female gymnasts (Snow, Williams, LaRiviere, Fuchs and Robinson, 2001). In the initial 12-month period, total body, hip, and lumbar spine BMD increased during the 8-month training season but BMD values decreased in the 4-month off season. The same pattern of bone gain and loss occurred throughout the second 12-month period. Several limitations related to study design however, detract from the longitudinal findings. A lack of control group prevents the determination of whether gains in BMD were related to gymnastics training or normal growth-related bone

accrual. Reproductive endocrine status was not established despite athletes with menstrual irregularities, calcium intake was not recorded, and a small sample size of eight participants limits the applicability of results.

Selection bias and other confounding factors inherent in cross-sectional studies potentially obscure our understanding of skeletal adaptations to exercise. Longitudinal studies therefore, provide a greater opportunity to examine the influence of mechanical loading on bone mineral accretion over time. Few longitudinal studies however, encompass the entire pubertal period due to time, long-term compliance demands and financial restrictions. Several studies have used cross-sectional data to evaluate the effect of physical activity on bone mass at specific maturational stages. Although longitudinal studies based on intervention data provide a more accurate representation of skeletal responses to loading, few studies spanning the pubertal years have been completed. Differences in study design, athletic groups, maturational stages and densitometry preclude the comparison of longitudinal studies of bone mass accrual in child and adolescent populations.

2.3.3 Section summary

Behavioural factors influencing musculoskeletal growth in adolescence include nutritional practices, namely energy and calcium intake, and mechanical loading from physical activity. Inadequate energy and calcium intake retards bone mineral accrual and produces sub-optimal peak bone mass. Reversal of energy and calcium intake to adequate levels has produced equivocal results, particularly concerning the sustainability of gains in bone mineral. Mechanical loading from physical activity appears a vital osteogenic stimulator of bone mineral. Active adolescents have achieved gains in bone mineral from large mechanical loads that exceed pre-set strain thresholds. Despite equivocal findings, the adaptive responsiveness of bone to mechanical loading appears heightened during the early pubertal / pre-menarcheal period. The sustainability of residual bone gains in the post-pubertal period however, remains unclear.

2.4 Musculoskeletal health issues in serious sport participation during adolescence

2.4.1 Growth factors

Intense physical training can have profound effects on growth and sexual development particularly in sports requiring weight control. Participation in sports where weight control is not required does not appear to delay pubertal timing or alter growth (Malina, 1994). Delay in growth and sexual development is well documented among particular female athletics groups, most notably gymnasts. The impact of intense physical training during puberty on growth potential in adolescent female athletes is demonstrated in a frequently cited study of young gymnasts compared with swimmers (Theintz et al, 1993). A marked stunting of leg-length growth was observed in gymnasts from 12 years of bone age compared with less active peers. Heavy training in gymnastics (>18 $h.wk^{-1}$) altered growth rates to such an extent that full adult height was not reached. Compromised growth was also evident in the sitting height of 83 active female gymnasts. Active gymnasts had delayed skeletal maturation of 1.3 years and reduced height, sitting height and leg length (Bass, Bradney, Pearce, Hendrich, Inge, Stuckey et al, 2000). Similarly, slower growth velocities were observed in female adolescent gymnasts when compared with an inactive control group (Lindholm, Hagenfeldt and Ringertz, 1994). Gymnasts did not experience the expected growth spurt common during adolescence and 27% of gymnasts had less than expected adult heights based on parental height.

Several studies have compared age at menarche among female athletes with normative values from less active peers. Within a group of gymnasts and swimmers aged 12.7 ± 1.1 years, approximately 7% of gymnasts had reached menarche compared with 50% of age-matched swimmers (Theintz et al, 1993). A similar study of elite female rhythmic gymnasts, aged 11 to 23 years, reported a 1.3 year delay in skeletal maturation (Georgopalous, Markou, Theodoropoulou, Paraskeropoulou, Varaki, Kazantzi et al, 1999). Pubertal development followed bone age rather than chronological age and mean age of menarche was significantly delayed in the gymnasts when compared with their mothers and sisters. Mean age of menarche was positively correlated to intensity of training and to the difference between

chronological age and bone age. A delay in the onset of menarche was also seen when three groups of intensively trained gymnasts, swimmers and tennis players were compared (Baxter-Jones, Helms, Baines-Preece and Preece, 1994). Gymnasts reported a mean age at menarche of 14.3 years compared with 13.3 years for swimmers and 13.2 years for tennis players. Only gymnasts displayed a difference in mean age at menarche when compared to a population reference value of 13.0 years.

Research concerning highly active male athletes indicate normal growth rates and normal and advanced states of skeletal and sexual maturation (Rogol, Clark and Roemmich, 2000). A study which evaluated height velocity in elite distance runners over a two year period found runners and controls did not differ in standing or sitting height however, the maturity level of runners was not recorded (Seefeldt et al, 1988). A similar cross-sectional study compared growth patterns of male adolescent wrestlers with non-athletic controls (Housh, Johnson, Stout and Housh, 1993). Controls were taller than wrestlers however, gains in height were not different between groups.

A plethora of additional variables require consideration when examining the effect of intense physical training on growth and sexual development during adolescence. Training intensity varies between and within sports. Female adolescents who train less than 15 hours per week are less likely to experience alterations in growth and pubertal maturation (Bonen, 1992), however individual variations preclude acceptable recommendations for fixed hours of participation. More importantly, psychological and emotional stressors associated with parental and/or coaching expectations may influence growth and pubertal timing (Malina, 1994). Pubertal timing is also strongly influenced by maternal menarcheal age (Baxter-Jones and Helms, 1996). In a study of female gymnasts, swimmers and tennis players, mean ages of menarche were higher than the national average (United Kingdom) but athlete menarcheal age strongly correlated with maternal menarcheal age. Reduced energy intake, particularly in sports that emphasize strict weight control, is also considered a major factor for disordered growth (Rogol et al, 2000).

Selection bias and genotype however, remain important variables that must be acknowledged when considering the impact of athletic training on growth and sexual development. Athletes with genetically determined delayed puberty and / or short stature seek out sports requiring smaller body types. Delayed menarche favours continued participation in sports such as gymnastics, diving and dance, which in turn may lead to more intense training (Roemmich, Richmond and Rogol, 2001). It appears more likely that activities such as gymnastics, diving and dance select participants who demonstrate desirable genetic anthropometric characteristics.

Results from studies of maturation and training imply a causal relationship exists between intense physical training and delayed growth and sexual development however, no convincing evidence supporting a relationship has been established. Athletic training has no apparent deleterious effect on growth and sexual maturation in children and adolescents, with perhaps the exception of elite female gymnasts (Claessens, 1999). Longitudinal studies involving athletes in weight-controlled sports and appropriately matched control groups require a more wholistic and inclusive approach when examining the effect of intense physical training on growth and sexual development during the adolescent years.

2.4.2 Overuse Injuries

Overuse injuries of the lower extremity occur most frequently in running-based activities (Hreljac, 2004). Studies of recreational (Casperson, Powell, Koplan, Shirley, Cambell and Sikes, 1984) and competitive runners (Rochcongar, Pernes, Carre and Chaperon, 1995; Lysholm and Wiklander, 1987) estimate that up to 70% of runners experience an overuse injury during any 12-month period.

Overuse injuries occur when an activity fatigues a specific structure due to repetitive submaximal loading. Microtrauma develops from inadequate recovery and stimulates an inflammatory response that damages local tissue. Cumulative microtrauma from further

repetitive activity produces degenerative changes leading to weakness, loss of flexibility and chronic pain (DiFiori, 1999).

Overuse injuries which preclude athletic competition are increasingly common in young athletes (Rowland, 1998). The rising occurrence of overuse injuries in young athletes are due to increased participation in organised sport, a tendency towards increased specialisation in one or two sports and a growing emphasis on increased duration and complexity of training at younger ages (Michelli, 1996). An investigation of sports injuries in children and adolescents treated at a sports injury clinic reported half of the 394 sports injuries presented to the clinic were classified as overuse. Athletes with overuse injuries lost 54% more time from training and competition than athletes with acute injuries (Watkins and Peabody, 1996). Examples of overuse injuries experienced by adolescent runners include stress fractures, epiphyseal plate injuries and apophysitis (Osgood Schlatter's disease and Sever's disease).

2.4.2.1 Stress Fractures

Stress fractures are caused by repetitive trauma usually associated with vigorous weight-bearing activities such as running or jogging (Martin and Martine, 2002). A variety of theories are proposed to explain the cause of stress fractures. The most common theory suggests stress fractures result from a phenomenon known as fatigue failure. When each repetitive loading cycle produces a minute amount of microdamage, fatigue failure occurs. Under normal conditions of healing, microdamage will occur but not accumulate because sufficient rest allows the loaded site to repair. However, bones repetitively loaded over short periods without sufficient time for a reparative response, will exhibit fatigue failure after several cycles of loading (Einhorn, 1992). Another theory suggests physical exercise leads to muscle fatigue with resultant excessive concentration of force being transmitted to sites on the underlying bone. A third suggested mechanism of stress fracture involves repetitive mechanical loading resulting in increased muscle activity and greater concentration of excess force of muscle acting on bone (Coady and Micheli, 1997).

Stress fractures are reported in a range of athletic populations but the incidence of stress fractures involving runners suggests the activity predisposes athletes to suffer stress injuries of bone. The combination of continuous, repetitive muscular activity and weight-bearing skeletal loading exposes runners to excessive skeletal stress which may exceed the body's ability to adapt (Jones, Harris, Vinh and Rubin, 1989). Sports-related injuries in collegiate male and female athletes were studied over a two year period. In total, 34 stress fractures were diagnosed and track and field accounted for 64% of stress fractures in women and 50% in men (Johnson, Weiss and Wheeler, 1994).

Track athletes are at highest risk for stress fracture because 5 to 6 times body weight is experienced during running (Knapp and Garrett, 1997). Limited studies however, have reported the incidence of stress fractures in terms of exposure, which prevents a valid comparison of stress fracture risk in a range of diverse sports (Bennell and Brukner, 1997). A 12 month prospective study examined the incidence and distribution of stress fractures in 95 track and field athletes and expressed stress fracture incidence rates in terms of exposure (Bennell, Malcolm, Thomas, Wark and Brukner, 1996). The incidence of stress fracture per 1000 hours of training was 0.70. Female athletes sustained 0.86 stress fractures per 1000 training hours and males sustained a rate of 0.05 injuries per 1000 training hours. Results revealed a high annual incidence of stress fractures in competitive track and field athletes.

Stress fractures are most common in bones of the lower extremity such as the tibia, fibula and metatarsals. Bone scans of stress fractures in 145 male and 175 female athletes revealed the tibia as the most frequent site of injury (49%), which was followed by the tarsals (25%). Tibial and fibular stress fractures were more common in younger athletes (Matheson, Clement, McKenzie, Taunton, Lloyd-Smith and Macintyre, 1987). In a study involving 351 male army recruits, 24% presented with stress fractures (Milgrom, Finestone, Shlamkovitch, Rand, Lev, Simkin et al, 1994). Results revealed the tibia was the most common site of injury and the risk of stress fracture was inversely proportional to age. Each year of increase in age above 17 years reduced the risk of fracture by 28%. The tibia was the most common site of stress fracture in track and field athletes with 12 tibial injuries from a total of 26 stress fractures

(Bennell et al, 1997). A review of 180 stress fractures also revealed the tibia as the most common stress fracture site among track and distance runners (Brukner, Bradshaw, Khan, White and Crossley, 1996). A similar study of runners found 34% of stress fractures occurred in the tibia followed by 24% in the fibula and 18% in the metatarsals (Monteleone, 1995).

Limited knowledge exists on the incidence of stress fractures involving adolescent athletes. A retrospective analysis of 368 stress fractures during a 14 year period revealed 9% of stress fractures occurred in children less than 15 years of age. Adolescents aged between 16 to 19 years incurred 32% of stress fractures (Hulkko and Orava, 1987). A study of stress fractures in children less than 14 years of age revealed a similar distribution of stress fractures between adult and paediatric athletes. The tibia was the predominant site of injury in both children and adults followed by metatarsal stress fractures (Yngve, 1988).

Stress fractures are an important consideration in the diagnosis of extremity pain in athletically active adolescents. Track athletes and distance runners are at the highest risk for stress fracture however, a lack of extensive data involving adolescent track athletes and distance runners prevent accurate investigation of risk factors and incidence rates.

2.4.2.2 Epiphyseal plate injuries

During growth, bone matrix formation may exceed the rate of bone mineralization resulting in a temporary state of weakness in bone (Parfit, 1994). Consequently, epiphyseal growth centres of long bones may be two to five times weaker than structures supporting bone (Maffulli and Helms, 1988). Acute trauma normally resulting in ligamentous injury in adults may produce a serious fracture within the epiphysis of a growing child. Most epiphyseal injuries associated with sports activity occur at the onset of the adolescent growth spurt (Maffulli, 1990).

Epiphyseal plates do not tolerate extreme compressive loads. Damage to cells in the proliferating zone of the growth plate may result in premature growth arrest, limb length

discrepancy and abnormal limb angulation (Maffulli, 1990). High-impact compressive loads are primarily the most common type of forces experienced in gymnastics, particularly in springing and landing activities. A critical appraisal of the literature concerning the relationship between repetitive physical loading and radial growth in gymnastics included the vulnerability of competitive female gymnasts to stress-related epiphyseal injuries of the distal radius (Caine, Howe, Ross and Bergman, 1997). Distal radius stress reactions, characterized by a widened and irregular physis, were reported in all 38 case reports however, premature closure of the distal-radial growth plate was only documented in four cases of skeletally immature gymnasts. Repetitive physical loading in excess of tolerance limits was considered the principal etiologic factor in epiphyseal plate injuries at the distal radius. Despite the plausibility of stress-related inhibition of bone growth at the distal radius in female gymnasts, poor research design produced insufficient conclusive evidence.

Stress-related damage to epiphyseal growth plates has also been documented in young athletes involved in baseball pitching. Repetitive strain of overhand throwing causes degenerative and inflammatory changes at the elbow and in extreme cases, premature arrest of the proximal radial epiphysis (Cain, Dugas, Wolf and Andrews, 2003).

Epiphyseal plate injuries have not been reported however, in other sports involving child and adolescent athletes (Adirim and Cheng, 2003). Overuse running injuries in children and adolescents occur as frequently as injuries sustained by adult runners yet damage to epiphyseal growth plates are rarely documented (Apple, 1985). An evidence-based trend for epiphyseal injuries as a consequence of compressive loading experienced in long distance running is not available. Similarly, no evidence supports the notion that children or adolescents, who participate in organized sport, disproportionally incur more epiphyseal fractures or are at greater risk of growth arrest caused by epiphyseal injuries than non-athletic children and adolescents (Anderson, Grieseman, Johnson, Martin, McLain, Rowland et al, 2000).

2.4.2.3 Apophysitis

During periods of accelerated growth, an elongation of the musculotendinous unit occurs in response to an increase in bone length that occurs at the growth plate. Rapid bone growth creates an increase in tightness of the muscle-tendon unit as muscle lengthening lags behind bone lengthening (Dalton, 1992). Apophysitis or strain at the apophyses is caused by micro-avulsions at the bone-tendon junction and are common in early adolescence. Most common sites of apophysitis include the knee (Osgood Schlatter disease) and heel (Sever's disease) (Adirim et al, 2003).

Osgood Schlatter's disease involves damage to the insertion of the patella tendon at the tibial tubercle and is the most frequent adolescent overuse injury of the knee. Lesions occur generally between 8 and 13 years in girls and 10 and 15 years in boys (Peck, 1995). Limited information is available on site-specific injury rates in adolescent runners. A prospective study of 48 adolescent runners reported an injury rate of 40%, with knee pain and apophysitis accounting for 17% of all injuries incurred (Orava and Saarela, 1978). A cross-sectional study of 257 high school track athletes, observed over one season (77days), revealed 20% of the 41 reported injuries occurred at the knee however, the specific nature of knee injuries was not disclosed (Watson and DiMartino, 1987). Difficulties arise in accurately determining the prevalence of Osgood Schlatter's disease amongst active children and adolescents because symptoms of pain or discomfort respond rapidly to rest and activity modification. As a result, many children or adolescents with symptomatic conditions do not present to physicians or sports injury clinics (Dalton, 1992).

Sever's disease is a comparable condition to Osgood Schlatter's disease and involves damage to the insertion of the Achilles tendon into the calcaneous (Micheli and Ireland, 1987). Sever's disease typically occurs between ages 7 to 10 years in physically active children who are contracted in the gastrocnemius-soleus muscle complex (Adirim et al, 2003). The condition often occurs in running and jumping sports, particularly soccer for boys and gymnastics for girls (Madden and Medden, 1996). A retrospective review of diagnosis and

treatment of calcaneal apophysitis in 85 male and female athletic children and adolescents revealed repetitive microtrauma, or overuse, as the common cause of Sever's disease (Micheli et al, 1987). In males, soccer most exacerbated symptoms followed by basketball, gymnastics and running. Only a quarter of athletes with Sever's disease were female.

To summarise, positive health outcomes are associated with regular physical activity but intensive exercise participation can often result in adverse health consequences. The growing body is vulnerable to overuse injury arising from repeated microtrauma. Repetitive loading can produce stress fractures at locally-loaded sites, potentially arrest growth through trauma to epiphyseal plates and cause apophyseal damage as evidenced in diseases such as Osgood Schlatter's and Sever's. Epidemiological data on the prevalence of overuse injury in adolescent sporting populations remains sparse.

2.4.3 Menstrual Dysfunction

A variety of hormones are released throughout the menstrual cycle that co-ordinate the readiness of the female reproductive system for conception (Marsh and Jenkins, 2002). Fluctuating hormone levels throughout a typical 28-day menstrual cycle are shown in Figure 2.2.

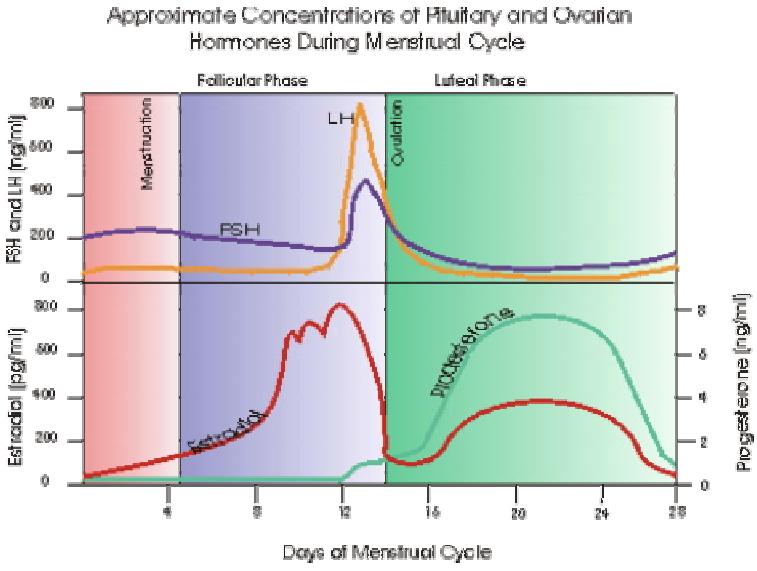


Figure 2.2: Pituitary and Ovarian hormone fluctuations throughout a typical 28-day menstrual cycle. (http://sprojects.mmi.mcgill.ca/menstrualcycle/physiology.html)

A normal menstrual cycle is divided into three phases: the follicular phase in which the follicle matures, the ovulatory phase in which the egg is released and the luteal phase in which the endometrium prepares for the fertilised ovum (Harmon, 2002). The hypothalamus secretes gonadotrophin-releasing hormone (GnRH) every 60 to 90 minutes which stimulates the pituitary to release follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Rose, Lee, Maffulli and Patrizio, 2001).

During the follicular phase, FSH acts on the ovarian follicle and results in oestrogen production. During the follicular phase, the increasing level of oestrogen causes the pituitary to release a large amount of LH and results in ovulation. Oestrogen also stimulates the development of the endometrial lining of the uterus. Decreasing levels of oestrogen and progesterone toward the end of the luteal phase initiates menses (Fieseler, 2001).

Females who undertake chronic strenuous exercise are at risk of a range of menstrual disturbances. Menstrual changes can be classified as luteal phase deficiency, anovulatory oligomenorrhea, and exercise-associated amenorrhea. A disruption to the delicate balance of carefully timed hormonal events needed for regular ovulation and menstruation usually produces luteal phase deficiency (Shangold, 1994).

Luteal phase deficiency is the least severe form of menstrual dysfunction and occurs when the luteal phase of the menstrual cycle is decreased in length. Shortened time spent in the luteal phase means that follicular development is suboptimal and progesterone secretion is inadequate after ovulation (Rose et al, 2001). Suppressed GnRH production results in decreased fertility during the cycles in which it occurs. Subtle hormonal imbalances causing luteal phase defects may affect bone mass even when menses is regular. Trained runners with regular normal length menstrual cycles had similar lumbar BMD to sedentary controls with regular normal length cycles. Despite the mechanical stress placed on the bones of

runners, it is possible that mild hormonal imbalances in runners limit the potential to increase BMD above sedentary controls (Heinrich, Going, Pamenter, Perry, Boyden and Lohman, 1990).

Athletic females may also have reduced BMD due to low circulating progesterone. Inadequate production of progesterone in cycles with short luteal phases was associated with accelerated bone loss despite normal production of oestradiol and the preservation of normal cycle intervals (Prior, Vigna, Schechter and Burgess, 1990). Since oestrogen inhibits bone resorption and progesterone stimulates bone formation, a reduction in circulating levels of either hormone may result in decreased BMD. The role of progesterone however, in the maintenance of bone density remains controversial. Young women who exercised with luteal phase deficiency demonstrated decreased progesterone but not decreased bone density (DeSouza, Miller, Sequenzia, Luciano, Ulreich, Stier et al, 1997).

Anovulation is the cessation of follicular development prior to ovulation. Insufficient levels of progesterone create an environment of unopposed oestrogen production, which leads to continuous endometrial stimulation (Shangold, 1994). Irregular bleeding occurs, ranging from short menstrual cycles of less than 21 days to oligomenorrhea of 35 to 150 days. Oligomenorrhea is defined as 9 or fewer menstrual periods per year (Tanner, 1998).

The most severe manifestation of menstrual dysfunction occurs when there is no follicular development which results in insufficient production of oestrogen and progesterone. In the absence of oestrogen the endometrial lining does not proliferate resulting in amenorrhea (Rose et al, 2001). Primary amenorrhea includes an absence of menstrual period by 14 years, an absence of growth or development of secondary sex characteristics, or no period by 16 years regardless of the presence of secondary sex characteristics. Secondary amenorrhea can be defined as the absence of a period for a length of time equivalent to at least 3 of the previous cycle intervals or 6-months of amenorrhea (Tanner, 1998). Reduced bone density in amenorrheic athletes results from failure to gain bone mass, bone loss, or an interactive force of both processes. Primary amenorrhea in athletes is associated with insufficient gains in

bone mass which contrasts to bone losses associated in athletes with secondary amenorrhea (Rutherford, 1993). In the first year of secondary amenorrhea, up to 4% of trabecular bone can be lost and bone loss may continue for at least 2 years (Otis, 1992). The BMD values at four skeletal sites were reduced from 56% to 82% below normal, in a study conducted 2 years after the resumption of normal menses in recovered anorexics (Bachrach, Katzman and Litt, 1991).

Loss of bone mass in amenorrheic and oligomenorrheic athletes is linked to a reduction of circulating oestrogen. Females become hypoestrogenic in response to the removal of the inhibitory oestrogenic effect of parathyroid hormone (PTH) on osteoblasts in bone. Loss of oestrogen production by the ovaries can cause losses in bone mass and the loss can be rapid in athletes with low calcium and vitamin D intakes (Anderson, Stender, Rondano, Bishop & Duckett, 1998). Two groups of amenorrheic and eumenorrheic female marathon runners were compared (Drinkwater, Nilson, Chestnut, Brenner, Shainholtz and Southworth, 1984). Amenorrheic runners completed 60 $km.wk^{-1}$ and eumenorrheic runners 40 $km.wk^{-1}$. Amenorrheic runners had lower lumbar spine BMD and lower oestrogen levels than eumenorrheic athletes. In a follow-up study, return of menses in 7 former amenorrheic athletes resulted in a 6% increase in lumbar spine BMD. Runners who remained amenorrheic continued to lose bone in lumbar vertebrae averaged at a loss of 3% (Drinkwater, Nilson, Ott and Chestnut, 1986). Low BMD in athletes with amenorrhea is not limited to the axial skeleton but is also present in appendicular weight-bearing bones. Amenorrheic athletes had lower BMD than eumenorrheic athletes at the proximal femur, and femoral mid-shaft (Myburgh, Bachrach, Lewis, Kent and Marcus, 1993). Similarly, amenorrheic runners displayed lower BMD values at the femoral neck, lumbar spine, lower leg and arms when compared with performance-matched eumenorrheic runners (Tomten, Falch, Birkeland, Hemmersbach and Hostmark, 1998)

Exercise-induced hypoestrogenism has also been linked to increased rates of musculoskeletal injuries among recreational distance runners (Lloyd, Triantafyllou, Barker, Houts, Whiteside, Kalenak et al, 1986). A retrospective study revealed injured athletes were

more likely to have had irregular or absent menses than a group of non-injured runners. Furthermore, a review of medical records showed athletes with menstrual irregularity were at increased risk of bone fracture. Only 9% of athletes with regular menses experienced bone fractures in comparison to 24% of athletes with irregular or absent menses. A similar investigation addressed stress fracture prevalence in competitive distance runners with menstrual history (Barrow and Saha, 1988). Runners were classified according to their menstrual history: very irregular (0 to 5 menses / yr), irregular (6 to 9 menses / yr) and regular (10 to 13 menses / yr). Stress fractures occurred in 49% of very irregular runners, 39% of irregular runners and 29% of regular runners. The majority of stress fractures occurred at the tibia.

Intense physical training however, may provide protection against bone mineral loss in adolescent females who experience reproductive hormone deficiency during menstrual dysfunction (Blimkie et al, 1996). Amenorrheic runners and figure skaters maintained whole body BMC and BMD when compared with eumenorrheic sedentary individuals (Slemenda & Johnston, 1993). Female gymnasts with a high prevalence of menstrual dysfunction had higher whole body, lumbar spine and femoral neck BMD than controls and age-matched runners (Robinson et al, 1995). Amenorrheic athletes displayed lower bone density at the spine and higher or normal bone density at weight-bearing sites when compared with less active peers. Adolescent female ballet dancers with a high prevalence of menstrual dysfunction demonstrated normal or slightly higher than normal BMD in weight-bearing regions. However, reduced BMD was found at non weight-bearing sites of ballet dancers (Young, Formica, Szmukler and Seeman, 1994). Lower bone density at weight-bearing sites may occur with longer periods of oligomenorrhea (Pearce, Bass, Young, Formica and Seeman, 1996). A cross-sectional study of dancers compared females with menstrual irregularity of less than 40 months and females with menstrual irregularities longer than 40 months. Dancers with menstrual irregularity of less than 40 months had a higher bone density than controls at weight-bearing sites and normal bone density at non weight-bearing sites. Bone density of dancers with menstrual irregularities greater than 40 months was normal at weight-bearing sites and low at non weight-bearing sites.

In summary, female adolescents who engage in strenuous physical training are at risk of experiencing a range of menstrual disturbances including, luteal phase deficiency, anovulatory oligomenorrhea and exercise-associated amenorrhea. Athletes who subsequently become hypoestrogenic are also at increased risk of injury, such as the development of stress fractures. High intensity sports participation may increase BMD in site specific skeletal regions despite the presence of amenorrhea however, a decrease in BMD at non-weight bearing sites has also been found.

2.4.4 Training errors

Training errors have been identified as the most common cause of running injuries (Knapp and Garrett, 1997). Previous studies (Maitra and Johnson, 1997; Lysholm et al, 1987) estimate that over 60% of running injuries, including 25% of stress fractures, are attributed to training errors. Rapid increases in weekly running distance or intensity, continued exposure to hard training surfaces, worn or poorly fitted footwear, and anthropometric and biomechanical variables are factors recognized as causes of overuse injuries (Hreljac, 2004).

Abrupt increases in the total volume and intensity of training often lead to the development of overuse injuries in young athletes (Micheli, 1996). Sport specialization training camps can expose young athletes to rapid increases in training and intensity and may be sufficient to encourage the onset of a tibial or fibular stress fracture (DiFiori, 1999). A direct correlation between injury risk and training distance in runners has also been established, with risk of injury significantly increasing at distances greater than 40 km per week. Risk of injury also appears greater if the same weekly training distance is completed over fewer days (Knutzen and Hart, 1996). Athletes with a history of stress fractures report more weekly hours of training and greater weekly distances compared with athletes who have never sustained a stress fracture (Cameron, Telford, Wark, 1992). Conversely, a study examining injury risk and training distance found 48% of injured runners were completing less than 20 miles per week and only 2% of injured runners were running more than 80 miles per week (Macera, Pate, Powell, Jackson, Kendrick and Craven, 1989). Despite equivocal findings concerning injury

risk and weekly training distances completed by runners, it is generally accepted that in young athletes, an increase in training distances, volume or intensity of more than 10% per week is considered potentially injurious (Micheli, 1996).

The association between playing surface and athletic injury is well accepted, particularly when athletes who normally train outdoors switch to indoor artificial training surfaces (Dalton, 1992). The stiffness of a surface affects impact forces and can result in overload to bone, muscle and connective tissue (Murphy, Connolly and Beynnon, 2003). Stress fracture risk assessment among elite collegiate female runners found athletes who sustained stress fractures trained on harder surfaces than athletes who did not develop stress fractures (Zernicke, McNitt-Gray and Otis, 1993). Similar research has shown females have a five times greater risk of injury running on concrete compared with non-concrete surfaces (Macera et al, 1989). The rapid introduction of cambered, tilted or uneven surfaces can also contribute toward the occurrence of lower extremity stress fractures (DiFiori, 1999).

Worn or poorly fitted footwear is an additional potential contributing factor in overuse running injuries. Footwear with an appropriate sole, sufficient shock-absorptive material, flexibility at the forefoot and the addition of orthotic support to correct malalignment, assist in the prevention of lower limb injuries (Dalton, 1999). A semi-rigid insole device significantly reduced the incidence of femoral and metatarsal stress fractures in military recruits (Simkin, Leichter and Giladi, 1989). Conversely, a study of the effect of insoles and age of running shoes on the incidence of stress fractures in over 3000 marine recruits, revealed no difference in the incidence of stress fractures between recruits wearing polymer or standard insoles (Gardner, Dziados, Jones, Brundage, Harris, Sullivan et al, 1988). An increasing trend of stress fractures was also found with increasing age of running shoes. Running shoes can lose more than 40% of their shock-absorbing capacity after 250 to 500 miles and require replacement at regular intervals (DiFiori, 1999). In track and field athletes, anecdotal evidence suggests the use of running spikes may influence the likelihood of stress fractures however a direct link is yet to be established (Brukner, 2000).

Anthropometric and biomechanical features may predispose young athletes to stress fractures by increasing area of stress concentration in bone or promoting muscle fatigue (Brukner et al, 2001). High longitudinal arches (pes cavus), leg-length discrepancies and magnitude of impact forces are implicated as causes of overuse running injuries. The majority of research however, linking these variables with overuse running injuries has been conducted using military recruits, with minimal data pertaining to athletes (Murphy, Connolly and Beynnon, 2003).

High longitudinal arches have been associated with an increased risk of stress fracture at femoral and tibial sites (Milgrom, Giladi, Stein, Kashtan, Marguiles, Chisin et al, 1985). In a prospective study of 295 male military recruits, the incidence of stress fracture in a low-arched group was 10% compared with 40% in the high-arched group. A similar study used digitized photographs to measure arch height in military trainees and found arch height was a significant predictor of foot and lower limb overuse injuries (Cowan, Jones and Robinson, 1993). Conversely, results from a retrospective study of 304 runners completing a marathon training program over 12-months, reported no association between arch height and overuse running injuries (Wen, Puffer and Schmalzried, 1997). Methods of quantifying an abnormally high or low arch differ greatly among researchers and as a consequence, disparate results exist.

Leg-length discrepancy has also been postulated as a potential risk factor for overuse running injuries due to asymmetries in loading, bone torsion and muscle contraction. However, conflicting results exist. A radiological analysis of 130 military recruits found that greater leg length was associated with 73% of tibial, metatarsal and femoral fractures (Friberg, 1982). A positive correlation between the degree of leg-length inequality and stress fracture incidence was established. In female athletes, leg length discrepancy has also been found to be associated with increased stress fracture incidence. A leg-length difference of more than 0.5 cm was found in 70% of female athletes with stress fractures compared with 36% of non-injured athletes (Bennell et al, 1996). In contrast, a prospective study found no difference in

leg- length inequality in injured and non-injured physical education students (Twellaar, Verstappen, Huson and van Mechelen, 1997).

Impact forces experienced during running have also been identified as a risk factor for overuse injuries. Impact forces are defined as forces resulting from the collision of two bodies over a relatively short period of time (Hreljac, 2004). During running, forces vary in magnitude from approximately 1.5 to 5 times body weight. A study of previously injured female runners with a history of stress fractures, exhibited greater impact ground reaction forces than non-injured female runners (Feber, McClay-Davis, Hamill, Pollard and McKeown, 2002). Similar studies, comparing previously injured runners with runners who had never sustained an overuse injury found previously injured runners displayed greater vertical impact forces than uninjured runners (Hreljac, Marshall and Hume, 2000; Grimston, Nigg, Fisher and Ajemian, 1993). Unlike other factors, general agreement appears to exist among researchers that greater impact forces during running expose athletes to an increased risk of overuse injury.

A summary of the additional potential risks to injuries shows that an athlete, who exceeds normal training distance or intensity, alters the surface on which they train, wears poorly fitted footwear, has high longitudinal arches, a limb length discrepancy and experiences high-impact ground reaction forces, appears at greatest risk of developing an overuse running injury. Modifications to individual training programs based on the particular training error producing deleterious effects may assist in overuse injury prevention.

2.4.5 Gender differences in running injuries

Stress fractures are a common overuse injury among male and female runners. A 12-month prospective study of 95 track and field athletes revealed stress fractures at the tibia were the most commonly diagnosed injury, accounting for 21% of injuries sustained by runners (Bennell et al, 1996). Conflicting data exists however, concerning which gender is at greatest risk of suffering a stress fracture.

Military studies suggest females experience a disproportionately higher number of stress fractures than men. Studies involving new recruits over an 8-week training period report stress fracture incidence rates of 13.9% in females and 3.2% in males (Jones, Bovee, Harris and Cowan, 1993; Jones et al, 1989). Variability of stress fracture incidence rates among male and female recruits could not be explained by differences in exposure to loading because the amount and intensity of basic training was rigidly controlled. Female recruits displaying lower initial physical fitness compared to male recruits was recognized as a potential explanation for gender differences in stress fracture incidence rates (Bennell et al, 1997).

Females may be more at risk of stress fracture due to their smaller body size. In a retrospective case-controlled analysis of over 2000 running injuries, females with a BMI of less than 21 kg/m^2 were at a higher relative risk of experiencing tibial stress fractures (Taunton, Ryan, Clement, McKenzi, Lloyd-Smith and Zumbo, 2002). The validity of BMI among athletic populations however, is of considerable concern because a larger proportion of total body mass can often be attributed to lean tissue. Nonetheless, female runners with a low BMI may have had insufficient musculature to adequately compensate for the stresses experienced in running. A 12-month prospective study, which examined risk factors for stress fractures in 111 male and female track and field athletes, found low lean mass in the lower limb was an independent predictor of stress fracture in females (Bennell et al, 1996). During running, ground reaction forces subject the tibial region, where most stress fractures occur, to large forward-bending moments (Scott and Winter, 1990). The gastrocnemius and soleus muscles contract to control the rotation of the tibia and oppose the large forward-bending moment. Insufficient lower leg musculature may be unable to produce adequate force to counteract ground reaction forces and attenuate excessive strain experienced at the tibia. For every 1 cm decrease in calf girth, the risk of sustaining a stress fracture increased fourfold (Bennell et al, 1996).

Bone width is also cited as a potential factor for differences in stress fracture incidence rates between the genders. Anthropometric data indicate that females have relatively narrow bone

in comparison with bone width of males (Ohta-Fukushima, Mutoh, Takasugi, Iwata and Ishii, 2002). Previous research suggests female athletes are considered more susceptible to stress fractures of the lower limbs because a narrow tibia was identified as a risk factor for stress fracture (Giladi, Milgrom, Simkin and Danon, 1991).

In contrast to military studies, a gender difference in stress fracture rates is not as evident in athletic populations. A direct comparison of stress fracture incidence rates between male and female track and field athletes revealed no difference between genders even when incidence rates were expressed in terms of exposure (Bennell et al, 1996). Females sustained 0.86 stress fractures per 1000 training hours compared with 0.54 in males. Similar research examining the incidence, distribution and type of musculoskeletal injuries sustained by track and field athletes during a 12-month period, found no difference in injury incidence rates comparing males and females (Bennell et al, 1996). A possible explanation for a lack of gender difference in stress fracture incidence rates among athletic populations, concerns fitness levels. Stress fracture risk may be lessened in female athletes as they may be more conditioned to exercise than female military recruits. Furthermore, the difference in fitness levels between male and female athletes may be closer than potential fitness differences between male and female recruits (Brukner et al, 2001). A similar prospective study of stress fracture risk factors, incidence and distribution in male and female collegiate runners found a trend for a higher incidence of stress fractures in females however, differences between genders were not significant (Nattiv, Puffer, Casper, Dorey, Kabo, Hame, 2000).

Difficulties arise in comparing studies of stress fracture incidence rates among male and female runners. Some studies include the number of stress fractures that have occurred over a specific period of time (Ohta-Fukushima et al, 2002), some assess the number of athletes that have sustained stress fractures (Korpelainen, Orava, Karpakka, Siira and Hulkko, 2001), while other studies express stress fracture rates relative to exposure (Bennell et al, 1996). Diagnostic criteria for stress fractures also varies between studies. Bone scans, computed tomography, radiographs, MRI, and compartment pressure readings have been used (Ohta-Fukushima et al, 2002; Taunton et al, 2002; Nattiv et al, 2000; Bennell et al, 1996), making

incidence rates difficult to compare. Furthermore, exposure to varying conditions and individual differences in training programs present challenges when comparing stress fracture incidence rates among male and female athletes.

2.4.6 *Section summary*

Regular physical activity is associated with positive health outcomes but adverse health consequences can result from serious sports participation during adolescence. Despite evidence of delayed growth and menarche in active female athletes, only a causal relationship exists between intense physical activity and disrupted growth patterns. Immature bone in the growing body however, is vulnerable to overuse injuries for repeated microtrauma. Female athletes in particular, are at further risk of musculoskeletal injury if training intensity disturbs normal menstrual patterns. A hypoestrogenic status is unable to negate bone resorption and the capacity to stimulate further bone formation is dampened. Despite the presence of menstrual disturbances, regional gains in bone mineral in high intensity athletic populations appear to marginally offset the deleterious effects of reduced circulating levels of oestrogen. Training errors, recognised as potential contributors toward musculoskeletal injury during adolescence, appear largely avoidable if training strategies, support and advice are developmentally appropriate. Although military studies highlight an increased incidence of injury to female participants, less conclusive evidence exists in athletic populations to support a gender-based difference in injury occurrence.

2.5 Issues in Methods used on Bone, Exercise and Growth

A variety of bone densitometry techniques are used to non-invasively assess bone mineral content (BMC), density (BMD), geometry and strength in children and adolescents. Dual-energy X-ray Absorptiometry (DXA), magnetic resonance imaging (MRI) and peripheral quantitative computed tomography (pQCT) are currently used by clinicians and researchers to assess bone parameters however, techniques vary considerably in precision, radiation

exposure and cost. Recent advancements in measurement of bone strength using biomechanical principles such as cross-sectional moments of inertia (CSMI) and hip structural analyses (HSA) have also been made.

2.5.1 Dual-energy X-ray Absorptiometry (DXA)

For more than a decade, assessment of bone mineral status predominantly involved non-invasive, planar DXA technology (Bolotin, Sievanen and Grashuis, 2003). DXA uses narrow, tightly collimated X-ray beams that are generated below a supine participant. The X-rays travel upward through the patient and are detected above by banks of electronic detectors, as shown in Figure 2.3.

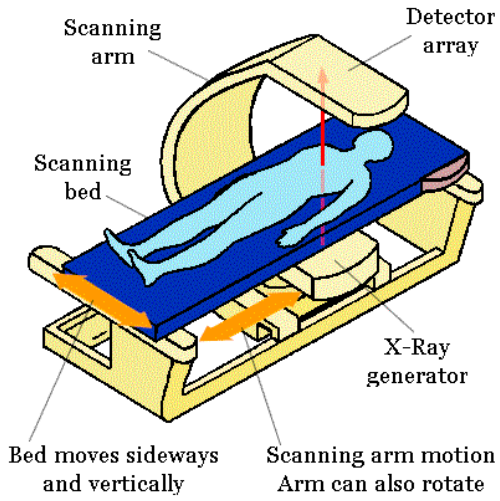


Figure 2.3: Illustration of a typical DXA instrument.

(http://www.bcm.edu/bodycomplab/dxaschemapage.htm)

DXA relies on two distinct energy peaks. One peak is absorbed mainly by soft tissue and the other peak is absorbed by bone. Soft tissue can be eliminated by subtracting the high energy image from the lower energy image. A residual image results, whereby the magnitude of the image pixels is proportional to the chosen tissue mass ie: bone (Tothill, Avenell and Reid, 1994). The amount of radiation used in a typical DXA scan is very low. The effective dose for a total body scan is less than one tenth of a standard chest X-ray (Njeh, Fuerst, Hans, Blake and Genant, 1999).

Initially, clinically important sites such as lumbar and proximal femur regions as well as total body scans were undertaken to assist in the diagnosis of osteoporosis and monitor changes in bone density. Recent advancements in image analysis algorithms, X-ray generation technology and modification of data acquisition protocols have resulted in an increase in DXA use in quantifying soft-tissue composition. Three major components of the body, namely fat mass, lean tissue mass and BMC can be precisely quantified using a single total body DXA scan (Albanese, Diessel and Genant, 2003).

The most basic densitometric parameter is BMC and is defined as either the mass of mineral contained in an entire bone (g) or as the mass of mineral per unit length (g/cm). The most widely used densitometric parameter at present however, is areal BMD. Areal BMD, also referred to as apparent BMD, is defined as the mineral mass of a bone divided by its projection area in a given direction (g/cm^2) (Schoenau and Frost, 2002). The most common scanning direction using DXA is the antero-posterior (AP) plane. Recent advances in software capabilities permit site-specific analyses of clinically relevant regions such as the femoral neck. Hip strength analysis (HSA) is a measure of bone mass and geometric bone distribution at the femoral neck and consists of eight measurements including neck CSA, neck length, neck and shaft angles, and neck CSMI.

However, concerns for technical limitations of DXA-based measurements, particularly the assessment of BMD, have been highlighted in recent years (Schoenau et al, 2002; Seeman, 2002; Faulkner, 2000). The planar two dimensional assessment capabilities of DXA present difficulties in accurately scanning a three dimensional bone structure. Although bone length and width can be measured, depth cannot be detected by DXA technology. An increase in “density” may be due to greater bone size and not necessarily an increase in mass per unit volume of bone (Seeman, 2002). Concerns about bone size are most relevant when research involves participants of varying size or where bone size may change rapidly during a study, such as studies of growing children and adolescents (Haapasalo et al, 2000). Areal BMD is therefore size dependent, particularly in children.

The influential effects of bone size on BMC and areal BMD values have lead to the measurement of total volumetric BMD (vBMD). Total vBMD is defined as the mass of mineral divided by the volume enclosed by the periosteal bone surface (Schoenau et al, 2002). In studies involving children and adolescents, concerns about DXA-derived vBMD however, also exist. An increase in vBMD can only occur if periosteal bone apposition is proportionally greater than any increase in bone size (Seeman, 2002). Increased bone mineral accrual and changes in bone size however, are dissociated in time during growth. Reduced vBMD is most apparent at the age of 12 to 13 years when bone mineral accrual and bone size are most dissociated (Fournier, Rizzoli, Slosman, Theintz and Bonjour, 1997). Assessment of vBMD is therefore dependent upon relative changes in bone mineral accrual and bone size. If bone size remains constant, an increase in vBMD may be due to a number of factors such as, increased cortical thickness, trabecular number or thickness, or increased density of these structures (Seeman, 1998). During growth however, bone size does not remain constant. Furthermore, DXA is unable to accurately measure the underlying contribution that differences in bone mineral make to vBMD.

DXA-derived in vivo BMD values are also subject to inherent systematic inaccuracies that potentially and adversely influence measurement outcomes. DXA studies involving in situ / in vitro cadaveric analysis (Lochmuller, Eckstein, Kaiser, Zeller, Landgraf, Putz et al, 1998), absorptiometrically realistic phantom studies (Bolotin, Sievanen, Grashius, Kuiper and Jarvinen, 2001), and in vivo bone-site studies (Bolotin, 1998), collectively highlight the need for re-evaluation of the reliability and accuracy of DXA-measured in vivo BMD. DXA technology is subject to the “two-component DXA limitation” (Tothill et al, 1994). Inaccuracies in BMD result from the inability of planar DXA methodology to distinguish more than two absorptiometrically distinguishable components in a scan region of interest (ROI). After acknowledging bone material as one component, DXA technology presumes the composition and distribution of soft tissues constitute a second component. All in vivo bone sites however, are comprised of bone material, intraosseous soft tissue (red / yellow marrow) and a combination of lean tissue and fat mass that together constitute at least four components in a DXA scan ROI (Bolotin et al, 2001). DXA attributes any difference in specific bone marrow

and extraosseous soft tissue composition within a bone-scan ROI to bone material. As a result, violation of the two-component DXA limitation produces an under- or overestimation of BMD.

In summary, in vivo measurement of BMC, areal BMD and vBMD by DXA is widely accepted as the methodology of choice in bone densitometry due to relative safety, reliability and convenience. Known technical limitations however, highlight the need for caution in interpreting DXA-derived findings. Bone size is recognised as a confounder in the calculation of BMC and areal BMD, vBMD is less size dependent but remains an estimated value based upon analysis algorithms and violation of the two-component DXA limitation produces an inaccurate BMD measurement. At present, the integration of DXA technology with other densitometric methods may circumvent, or at least significantly attenuate recognised DXA limitations.

2.5.2 Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) is a phenomenon involving magnetic fields and radio frequency (RF) electromagnetic waves. The combination of a strong magnetic field with RF pulses produce differences in tissue signal intensity which are processed and reconstructed by computer (Johnson and Steinbach, 2004).

Fundamentally, MRI images protons in hydrogen atoms which are in abundance throughout human tissue (bone, fluid, muscle, fat). Spinning, unpaired protons placed in an external magnetic field line up in the direction of the magnetic source. If RF waves are emitted into the patient, some protons alter their alignment as a result of the new magnetic field. When the RF pulse is removed, protons realign or “relax” along the dominant magnetic field and subsequently generate a faint signal as they return to their original alignment. The resultant signal intensity is reconstructed by computer to visually represent the scanned region or tissue (Hashemi and Bradley, 1997). The presence of disease and / or injury alters proton relaxation characteristics within normal tissue and these changes in proton relaxation

characteristics become visible as areas of abnormal signal intensity (McRobbie, Moore, Graves and Prince, 2003).

MRI images are comprised of thousands of small squares known as “pixels” (picture elements). A pixel is the front surface of a three dimensional volume of tissue known as a “voxel”. Each pixel in a reconstructed image is a visual representation of signal intensity relative to a specific location. Pixels are organised into rows and columns known as a “matrix”, with 256 or 512 the most common matrices used. Once a matrix is full, data, as a function of time, is transformed to represent a function of frequency. The transformation from “signal vs time” to “signal vs frequency” is achieved by a mathematical procedure known as “Fourier transformation” (McRobbie et al, 2003).

The high precision of contrast resolution in MRI is vastly superior to other imaging modalities. MRI’s ability to distinguish difference among soft tissues such as fat and muscle make it superior to computed tomography (CT), particularly in the early diagnosis of pathological processes within bone marrow (Johnson et al, 2004). Patients with a clinical suspicion of stress fracture can be effectively diagnosed using MRI mainly because depictions of osseous abnormalities are often available weeks before the development of radiographic abnormalities. Stress injury to bone results in localised oedema which MRI is highly sensitive in detecting. An oedema-sensitive imaging sequence such as short tau inversion recovery (STIR) or a fat-suppressed T2 sequence are used for the detection of early changes of stress reaction in periosteal, muscle or bone marrow oedema. The increased water content associated with oedema results in high signal intensity against a dark background of suppressed fat (Spitz and Newbery, 2003).

A comparison of imaging modalities (MRI, bone scintigraphy and radiographs) was undertaken using a classification of osseous stress injury from Grade 1 (mild) to Grade 4 (severe) involving 14 symptomatic runners (Fredericson, Bergman, Hoffman, Dilligham, 1995). MRI more precisely defined the anatomic location and extent of injury and allowed for more accurate recommendations for rehabilitation and return to activity. The prognostic value

of MR imaging to bone stress injury has also been studied in a more diverse group of thirty-five patients (Yao and Johnson, 1998). Using the same MR imaging classification system as Fredericson et al (1995), MRI correlated with total duration of symptoms and time to return to sports activity. The appearance of a fracture, fatigue line or a cortical signal abnormality using MRI was also predictive of a longer symptomatic period and indicated more severe stress injury to bone.

The clinical significance of bone marrow oedema however, depends on the severity of findings. A STIR sequence was used to image ankles and feet of 20 runners and 12 non-runners (Lazzarini, Troinano and Smith, 1997). Although 16 of the runners displayed bone marrow oedema, compared with 3 in the non-runners, all participants with positive MR images were asymptomatic. Bone marrow oedema observed on STIR imaging of the runners may have been caused by exercise alone.

Similarly, a finding of bone marrow oedema may not necessarily represent osseous stress injury. Numerous pathologic conditions such as acute bone bruise, osteomyelitis, avascular necrosis and transient osteoporosis may cause bone marrow oedema. The potentially misleading appearance of excessive oedema should always be considered with reference to individual clinical history (Spitz et al, 2003).

Despite MRI's non-invasive, non-ionizing radiation exposure, superior contrast resolution and recognised diagnostic capabilities, a few limitations exist. For example, during operational use, MRI is prone to a large number of artefacts, in particular metal artefacts. A small piece of metal (eg: earring) can produce a large artefact and obscure anatomic information. Electrical appliances such as pacemakers can also malfunction inside the strong magnetic field of a MRI scanner. Metal foreign bodies within the eye have been known to migrate and cause ocular injury and blindness (Johnson et al, 2004). The claustrophobic environment of the MRI scanner can also increase patient anxiety although recent advances in image quality from open MRI units have been made. An additional limitation of MRI relates to cost. MRI is

considered the most expensive imaging modality in routine use, mainly due to the large imaging suite required to accommodate the unit and initial capital expenditure.

In summary, MRI uses magnetic fields and RF waves to detect and represent differences in tissue signal intensity. Signals are processed and reconstructed by computer to provide high contrast resolution of soft tissue. Superior contrast resolution allows for the early diagnosis of bone stress injury through the detection of increased localised oedema. A classification of osseous stress injury has accurately defined and diagnosed injury and has assisted in rehabilitation recommendations. Despite its numerous benefits, the strong magnetic field in MRI scanners can cause problems with metal objects, placing greater importance of patient pre-screening. Some patients may also find the small magnet bore claustrophobic. The high cost of MRI, relative to other imaging modalities, remains a challenge for clinicians and researchers.

2.5.3 Peripheral Quantitative Computed Tomography (pQCT)

Computed tomography (CT) uses x-rays to produce tomographic images by transmitting a thinly collimated beam through a patient. The amount of x-ray radiation received by a detector is a function of the amount of radiation absorbed by tissues and objects along the course of the beam. A reconstructed image results from the mathematical manipulation of multiple contiguous slices of data (Johnson et al, 2004). Tomographic images allow specific anatomical locations to be viewed in slices without the intervening tissues that can obscure the region of interest.

CT produces higher contrast resolution of images compared to plain radiography and is less expensive than MRI however, the large radiation exposure to patients restricts its use, particularly if screening involves repeated measurements of apparently healthy individuals (Sievanen, Koskue, Rauhio, Kannus, Heinonen and Vuori, 1998). Using CT, typical radiation doses administered during musculoskeletal imaging range from 5 to 15 mSv. In comparison, radiation exposure involving plain radiography ranges from 0.1 to 2.0 mSv (Johnson et al,

2004). As a result, peripheral quantitative computed tomography (pQCT) scanners have been developed to provide clinical users and researchers with the benefits of large CT systems but at lower cost and considerably lower radiation exposure.

A pQCT densitometer measures volumetric BMD at peripheral skeletal sites independent of skeletal size. In contrast to DXA technology, pQCT allows for selective measurement of trabecular and cortical components of bone and provides precise information on cross-sectional bone geometry (Haapasalo et al, 2000). The tomographic nature of pQCT images allows trabecular bone to be analysed without interference from cortical structures. The terms "vBMD-trab" (Neu, Manz, Rauch, Merkel and Schoenau, 2001), "Tr.Dn" (Haapasalo et al, 2000), and "TrD" (Sievanen et al, 1998) represent an integrated measure of trabecular number, thickness, and mean density and provide clinicians and researchers the opportunity to examine changes in bone microarchitecture. Trabecular bone is metabolically active tissue and can therefore demonstrate considerable responses to various treatments (Boonen, Cheng, Nijs, Nicholason, Verbeke, Lesaffre et al, 1997) and mechanical loading (Heinonen, Sievanen, Kannus, Oja and Vuori, 2002; Uusi-Rasi, Sievavnen, Pasanen, Oja and Vuori, 2002).

The three-dimensional nature of pQCT reduces potential misinterpretations arising from the two-dimensional planar nature of DXA-derived data. Size-dependent BMD data from DXA technology can provide a false indication of actual bone density. Using DXA, BMD at the distal femur can be four times higher compared to the distal radius however, using pQCT, trabecular density is actually similar at both sites (Sievanen, Kannus, Nieminen, Heinonen, Oja and Vuori, 1996; Heionen, Oja, Kannus, Sievanen, Haapasalo, Manttari et al, 1995).

Despite pQCT's superior ability to differentiate between trabecular and cortical bone compartments and its ability to precisely examine bone geometry, limitations derived from *in vitro* and *in vivo* precision studies exist (Sievanen et al, 1998; Augut, Gordon, Lang, Iida and Genant, 1998; Grampp, Lang, Jergas, Gluer, Mathur, Engelke et al, 1995). A lack of spatial resolution prevents the precise identification of areas where a thin cortical rim of bone exists,

such as at the ultra distal radius. Standard geometric analysis assigns the outer 55% of the radial cross-section as “cortical and subcortical” bone and the remaining area is considered trabecular bone. As actual cortical thickness in adults is considerably smaller, the percentage of CSA consisting of cortical bone is much lower than 55%. Determining cortical volumetric BMD at the ultra distal region appears subject to analysis imprecision (Neu et al, 2001).

Gross movements during scanning and inconsistencies in the alignment of target bones with respect to direction of the tomographic slices, are considered additional sources of imprecision. Distorted pQCT scans, similar to other disrupted imaging scans, do not convey accurate information within acceptable limits (Sievanen et al, 1998). Reliance upon visual checking of correct limb alignment introduces considerable variability, particularly at the physeal sites of long bones. Reduced precision of CSA measurements of bone ends occurs because cross-sectional geometry changes rapidly along the longitudinal axis of bone (Takada, Engelke, Hagiwara, Grampp and Genant, 1996). Standard fixation tubes used for correct limb alignment require refinement to allow more consistent positioning between participants.

In summary, the emergence of pQCT as an alternate imaging modality provides clinicians and researchers the opportunity to selectively examine bone compartments and geometry at lower cost and radiation exposure than CT technology. The calculation of volumetric BMD, independent of bone size, allows pQCT to avoid the size-related concerns inherent in DXA-derived volumetric data. Limitations however, include poor spatial resolution, particularly in areas of thin cortical bone, gross movements during scanning and inconsistent limb alignment between participants.

2.5.4 Section summary

A variety of imaging techniques are used by clinicians and researchers to non-invasively assess BMC, BMD, bone geometry and bone strength in adolescents. DXA technology is widely accepted as the methodology of choice in bone densitometry despite increasing

recognition of limitations concerning single plane scanning technology. The influential effects of bone size on BMC and areal BMD are partly circumvented by the calculation of vBMD however, the required proportionality of periosteal bone growth to whole bone growth to asses vBMD appears dissociated during adolescence. MRI technology offers a non-ionizing imaging method with superior contrast resolution capable of detecting early changes in bone oedema. MRI offers clinicians an accurate prognostic tool, albeit at considerable financial expense. The three-dimensional nature of pQCT enables the selective assessment of bone compartments, such as trabecular bone, as well as the calculation of vBMD independent of bone size. Despite the benefits of assessing bone material and structural properties, adolescent exposure to high radiation remains a concern.

2.6 Biomechanical properties of bone

From an engineering perspective, strength in structure relies on the materials available for construction (material properties) and the size, shape and geometry of the structure (structural properties). The human skeleton is a unique biological system whose composition (material properties) and organization (structural properties) reflect the functional demands of intense physical activity with a lightweight design to allow energy-saving locomotion (Einhorn, 1992). The response of bone material to applied loads requires the understanding of the terms “stress” and “strain”, while the proportional relationship of stress to strain is known as “Young’s modulus”. When whole bones are exposed to applied loads structural properties such as bone geometry are also challenged. The geometric parameter used to quantify the effect of altered bone geometry on bone strength is known as cross-sectional moment of inertia (CSMI). Material properties of bone tissue and the structural properties of whole bone combine to influence bone strength (Khan, McKay, Kannus, Bailey, Wark and Bennell, 2001).

2.6.1 Material properties

2.6.1.1 Stress / strain (Young's modulus)

When a force is applied to bone, deformation occurs coupled with the generation of internal resistance to counter the applied force. The internal reaction, known as stress, is equal in magnitude but opposite in direction to the applied force (Einhorn, 1992). Stress is defined as force per unit area and is reported in units of Newtons per square metre (N/m^2) or Pascals (Pa). An applied force can be directed at bone from any angle however, most stresses can be classified as compressive, tensile or shear (Turner and Burr, 1993). Compression results from two forces that are directed towards each other along the same line, tension is produced when two forces are directed away from each other along the same line, and when two forces are directed parallel to each other but not along the same line, shear stresses result (Einhorn, 1992).

Strain is defined as the deformation of material relative to its own dimensions and is therefore calculated by dividing change in bone dimension by original bone dimension. Strain is a non-dimensional measurement and is often reported as a fraction or a percentage. For example, if a material is stretched to 101% of its original length, the reported strain is 0.01 or 1% (Khan et al, 2001).

The relationship between forces applied to a structure and the amount of deformation in response to the applied load is known as the "stress-strain curve" (Figure 2.4).

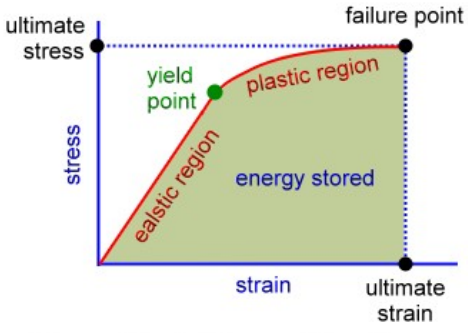


Figure 2.4: stress / strain curve

(http://www.pt.ntu.edu.tw/hmchai/BM03/BMmaterial/Bone.htm)

The stress-strain curve is divided into an elastic deformation region and a plastic deformation region. If a load on bone is released with the elastic deformation region, bone returns to its original shape. Typically, a linear relationship exists between stress and strain until a yield point is reached. An increasing load on bone beyond the yield point may lead to permanent bone deformation and is represented on the stress-strain curve as the non-linear portion of the plastic deformation region (Turner et al, 1993). The slope of the stress-strain curve within the elastic region for compressive and tensile loading represents material stiffness and is known as elastic modulus or Young's modulus. For shear loading, material stiffness is known as shear modulus (Martin, Werner, Andresen, Schober and Schmitz, 1998).

Young's modulus varies according to the direction in which a load is applied. Bone materials that exhibit different mechanical properties in different directions are termed anisotropic. For example, the femur is better suited to resisting compressive loads than tensile loads. The ultimate tensile strength of femoral bone in the longitudinal direction is 135 megapascals (MPa) whereas compressive strength is 205 MPa (Heinonen, 2001).

The degree of anisotropy also varies within anatomical regions (cortical and trabecular bone). Calculation of Young's modulus within trabecular bone becomes more difficult because trabecular bone has a material stiffness, which is the stiffness of each trabeculae, and a structural stiffness, which is the stiffness of the trabecular structure. Due to the difficulties associated with the measurement of trabecular material properties, trabecular structural properties are commonly reported in biomechanical studies (Turner et al, 1993). Trabecular orientation, a structural property of trabecular bone, can alter Young's modulus from 0.1 to 4.5 gigapscals (GPa) (Demetropoulos, Willis and Goldstein, 1993).

2.6.2 Structural properties

Geometric characteristics of bone combine with material properties to produce a functional skeleton capable of withstanding externally applied loads. For example, bending and torsional loads are ideally resisted by the tubular shape of long bones whereas the widened ends of

long bones complete with trabecular architecture, are designed to dissipate compressive forces often experienced from contact with external surfaces (Currey, 2001). Skeletal adaptations to loading typically involve remodeling responses that generate favourable increases in bone geometry. Despite decreasing mass and architectural decay at the proximal femur during aging, net bone loss is offset by the re-distribution of bone mass further from the neutral bone axis, particularly in males (Beck, Oreskovic, Stone, Ruff, Ensrud, Nevitt et al, 2001). By altering its own structural properties, bone is capable of maintaining its strength.

Youngs' modulus, a measure of the intrinsic stiffness of bone material in bending (compression and tension) is related to the cross-sectional geometry of bone (Forwood, 2001). Stiffness determines the amount of deformation engendered in a bone for a given load and is the product of Young's modulus and the areal moment of inertia of the cross-section about the axis of bending, commonly known as cross-sectional moment of inertia (CSMI).

2.6.2.1 Cross-sectional moment of inertia (CSMI)

CSMI is a measure of the distribution of material around a given axis. During a bending test, the axis of bending that contains the centre of mass of the cross-section is known as the neutral axis because no compressive or tensile stresses are experienced along the axis. Compressive or tensile stresses increases linearly with distance from the neutral axis, therefore the highest stresses are experienced on the outside surface of bone (Turner et al, 1993). Maximal CSMI is identified when cross-sectional bone area is measured at the greatest distance possible from the neutral axis. As a result, a stronger bone is achieved. Importantly, a small change in bone CSA created by periosteal apposition results in a large change in CSMI because CSMI is proportional to the fourth power of the radius. CSMI for a circular cross-section is represented by the following mathematical equation: CSMI = (∏/4) x $(r_1^4 - r_2^4)$ where CSMI = cross-sectional moment of inertia, r_1 = outer radius and r_2 = inner radius.

CSMI can be calculated using rectangular elements (pixels) of a bone cross-section from a digital image. CSMI is comprised of the sum of products of pixel areas of cortical bone and the squared distance from each pixel to the areas passing through the cross-sectional mass centre (Ferretti, Capozza and Zanchetta, 1996).

The contribution of CSMI to bone strength, particularly at the proximal femur, has lead to the development of an alternate method of geometric assessment known as “Hip Structural Analysis” (HSA). CSMI principles are incorporated with bone mineral data acquired with conventional DXA technology to assess whether bone is appropriately placed to resist mechanical stresses leading to fracture (Beck, Ruff, Warden, Scott and Rao, 1990). Cross-sectional studies involving postmenopausal woman (Beck et al, 2001), prepubertal female gymnasts (Faulkner, Forwood, Beck, Mafukidze, Russell and Wallace, 2003) and intervention studies involving pre- and early pubertal females (Petit, McKay, MacKelvie, Heinonen, Khan and Beck, 2002) highlight the adaptive geometric response of the proximal femur to different mechanical loads.

2.6.3 Bone strength index (BSI)

From a biomechanical perspective, bone strength depends on the intrinsic stiffness (material properties) and the architectural distribution (structural properties) of bone mineral (Ferretti, Cointry, Capozza and Frost, 2003). In contrast, densitometrically assessed bone mass is recognised as a poor independent predictor of bone strength due to the inability of DXA technology to account for changes in bone geometry. Numerous studies (Kontulainen et al, 2003; Haapasalo et al, 2000; Jarvinen, Kannus, Sievanen, Jolma, Heinonen and Jarvinen, 1998) have reported improvements in bone strength due to altered spatial distribution of bone mineral without simultaneous gains in bone mass.

The intrinsic stiffness of bone is represented by Young’s modulus of cortical BMC (Turner et al, 1993). The relationship between Young’s modulus and BMC is closely linear within the physiological range for cortical bone during bending and tension (Currey, 1998). The

architectural distribution of bone mineral is indicated by CSMI with reference to the neutral axis during bending conditions. Both Young's modulus and CSMI have been associated to represent bone strength due to the incorporation of bone mineralization and geometric bone properties (Ferretti et al, 1996; Barker and Haugh, 1979). Bone material properties (volumetric cortical BMD) and bone architectural parameters (CSMI) are represented by a single bone strength index (BSI) which is supported by strong correlations between Young's modulus and CSMI (Ferretti, Capozza, Mondelo and Zanchetta, 1993). Assessment of bone material in the femoral diaphyses of rodents revealed a high correlation value when ultimate resistance and stiffness of femoral bone was compared to femoral CSMI.

The CSMI and volumetric cortical BMD of rodent femoral mid-shafts were non-invasively assessed to predict actual bending breaking force using pQCT technology (Ferretti et al, 1996). Results showed BSI correlated more strongly with actual fracture load than either BMD or CSMI alone. The BSI formula has been applied using different imaging modalities. MRI and DXA technology have been combined to assess mid-femoral BSI in female adolescent athletes (triathletes, swimmers, cyclists and runners) with non-active female adolescent controls (Duncan, Blimkie, Kemp, Higgs, Cowell, Woodhead et al, 2002). Volumetric cortical BMD was derived as the quotient of DXA-derived mid-femoral BMC divided by MRI-derived mid-femoral cortical bone volume.

The contiguous acquisition of multiple cross-sectional slices throughout the mid-femoral region by MRI analysis allowed the accurate assessment of cortical bone volume (cross-sectional area x slice thickness x number of slices). Validation of MRI as a technique to assess bone geometry at the mid-femur has been shown, with acceptable accuracy and repeatability in healthy and osteoporotic participants (Woodhead, Kemp, Blimkie, Briody, Duncan, Thompson et al, 2001).

BSI is based on the integration of volumetric cortical BMD and CSMI. This integration is superior to other bone strength measurement techniques because the formula is inclusive of material and structural bone properties. Previous studies (Faulkner et al, 2003; Petit et al,

2002; Beck et al, 1990) have reported section modulus as an indicator of bone bending strength at the proximal femur using DXA-derived HSA software. Section modulus is calculated as CSMI divided by half the subperiosteal width for the region of interest. Assumptions concerning cross-sectional bone shape however, are employed because of limitations associated with the single plane nature of DXA. Furthermore, the reporting of a strength index (SI) based on the normalizing of section modulus by dividing section modulus by limb length, is also based on the assumption that bone is cylindrical in shape (Faulkner et al, 2003). The standard 5 mm cross-section of bone used by HSA software at the femoral neck for example, does not accurately represent bone strength throughout the femoral neck region. Likewise, a 5 mm cross-section of bone at 2 cm distal to the midpoint of the lesser femoral trochanter, divided by femur length, does not represent bone strength throughout the entire femoral shaft. Assumptions of bone shape are avoided by MRI technology. The three dimensional geometric capability of MRI complements densitometric measures of BMC and more accurately assess the material and structural properties of bone strength than DXA technology alone.

2.6.4 Section summary

In summary, bone strength relies on material and structural properties to achieve a robust yet lightweight structure. Deformation of bone material due to an applied force produces a linear relationship between stress and strain until a yield point is reached. The proportional relationship of stress to strain is known as Young's modulus. Changes to structural (geometric) properties assist in the maintenance of bone strength by re-distributing bone mineral further from the neutral axis. CSMI is a measure of the distribution of material around an axis of bending. The integration of Young's modulus and CSMI using three dimensional imaging technology such as pQCT or MRI with single plane technology such as DXA, allows for the accurate calculation of in vivo bone strength. Volumetric cortical BMD and CSMI combine to quantify BSI. DXA-derived bone strength calculations based on HSA software appear inferior to BSI measurements due to imaging limitations and assumptions of bone shape.

2.7 Chapter summary

Current advances in bone analyses have insufficiently explored inherent advantages to physical activity among adolescents. Middle-distance running is a repetitive, weight-bearing activity characterised by high training volumes. The opportunity for increased training intensity during adolescence, is tempered by risks associated with, and exacerbated by maturational development. Osteogenic responses in highly-active male and female adolescents exposed to similar habitual loading patterns, particularly at the tibia have not been investigated. Furthermore, potential differences in bone geometric and bone strength parameters at load bearing sites between gender and activity groups, remain unknown.

CHAPTER 3

METHODS

3.1 Ethical approval

The study was jointly approved by the ethics committees of the Australian Catholic University and The Children's Hospital, Westmead.

3.2 Research design

The research design was a matched, case controlled cross-sectional study designed to compare markers of musculoskeletal health between elite, adolescent middle-distance runners and age- and gender-matched controls.

3.3 Recruitment of participants

Based on power calculations (see Section 3.9), forty male and forty female adolescents were recruited for the study. The recruitment process involved strong support from the New South Wales Institute of Sport (NSWIS) (Appendix 3.6) and the Parramatta Diocese of the New South Wales Catholic Education Office (CEO) (Appendix 3.4). Information outlining the purpose of the study (Appendix 3.7) as well as pamphlets for parents and potential participants explaining the type of tests conducted in the study (Appendix 3.8) were provided. Interested participants contacted the principal investigator (DG) by phone and if inclusion criteria were met, an appointment for testing was made.

3.3.1 Athlete inclusion criteria

To be included in the study, athletic participants were required to be:

(i) aged between 14 – 18 years

(ii) competing at the state and / or national level for middle-distance athletic events (800 metre, 1500 metre) for the previous 2 years

(iii) completing more than 6 hours of training / competition per week for the previous 2 years

(iv) in good health with no recent illness (previous 2 weeks)

(v) no hospitalisation during the previous 2 years

(vi) no history of medical conditions or medication usage (including the Oral Contraceptive Pill) or calcium preparations known to affect bone metabolism in the past 6 months

(vii) of Caucasian ethnicity

(viii) athletic females who are post menarcheal will need to have a normal menstrual cycle (≥8 menstrual cycles in the past 12 months).

3.3.2 Control inclusion criteria

To be included in the study, control participants were required to be:

(i) aged between 14 – 18 years

(ii) completing no more than 3 hours of physical activity per week, including structured physical activities at school during the past 12 months

(iii) in good health with no recent illness (previous 2 weeks)

(iv) no recent hospitalisation (previous 2 years)

(v) no history of medical conditions or medication usage (including the Oral Contraceptive Pill) or calcium preparations known to affect bone metabolism in the past 6 months

(vi) of Caucasian ethnicity

(vi) control females who are post menarcheal will need to have a normal menstrual cycle (≥8 `menstrual cycles in the past 12 months).

3.4 Study methods

Investigation of each participant involved:

(i) completion of a medical history / injury record questionnaire; self-reported pubertal / menstrual status questionnaire; training intensity questionnaire (athletes only)

(ii) a 3-hour visit to The Children's Hospital, Westmead for assessment and measurement

(iii) completion of a 3 day food diary and 3 day physical activity record

3.4.1 Questionnaires

3.4.1.1 Medical history / injury record

Participants were asked to complete a 2-year retrospective medical history / injury record questionnaire during their hospital visit (Appendix 3.9)

3.4.1.2 Self-reported pubertal status / menstrual status

Participants were provided with a private area to complete a self-reported pubertal status questionnaire for pubic hair and genital / breast development (Appendix 3.10). Pubertal status was determined by each participant using illustrations depicting the five stages of genital and pubic hair development, as described by Tanner (1962). Participants were asked to select an illustration comparable with breast (girls) or genital (boys) size and an illustration comparable with pubic hair development. Determination of pubertal status using Tanner staging is considered a reliable and valid measure (Duke, Litt and Gross, 1980). Female participants were also asked to complete a menstrual status questionnaire (Appendix 3.11).

3.4.1.3 Training intensity

Athletic participants were asked to complete a training intensity questionnaire (Appendix 3.12). The questionnaire was used to determine number of training sessions completed per week, distances covered, evidence of periodised training and the number of competitive events entered during the previous 12 months.

3.4.1.4 Three-day food diary

Dietary calcium (mg) and energy intake (kJ) were determined using a 3-day (two week days and one weekend day) food diary (Appendix 3.13). Instructions regarding completion of a 3-

day food diary were provided to all participants by the same investigator. Self-addressed stamped envelopes to assist in the return of completed diaries were provided. Participants were requested to complete the diary in as much detail as possible. Completed diaries were analysed using Foodworks™ Food Analysis program (Xyris Software 1999, Version 2.04.104) by the same investigator. Calcium (mg) and energy intake (kJ) were calculated as absolute daily intake and expressed as mean values.

3.4.1.5 Three-day physical activity assessment

Physical activity levels were assessed using a prospective Bouchard Three-day Physical Activity Record (two week days and one weekend day)(Appendix 3.14). Activities were ranked on a scale from 1 to 9 according to energy expenditure with the least vigorous activity scoring 1 and the most vigorous activity scoring 9. Activity level was recorded every 15 minutes for three, 24 hour periods. The simultaneous completion of the 3-day physical activity record and the 3-day food diary was encouraged with all participants. Results were analysed by the same investigator and expressed as mean kilojoules of energy expenditure per three days. The Bouchard Three-day Physical Activity Record has moderate reliability (Aaron, Kriska, Dearwater, Cauley, Metz and LaPorte, 1995).

3.4.2 Descriptive measures

3.4.2.1 Height / Weight / Body Mass Index

Standing height was measured to the nearest 0.1 cm using a stadiometer (Wedderburn UW150, Sydney, Australia). Body weight was measured using an electronic scale accurate to 500 g (Wedderburn UW150, Sydney, Australia) with participants dressed in light clothing and without shoes. Body mass index (BMI) was calculated by dividing body mass (kg) by height (m) squared ($kg.m^2$).

3.4.2.2 Determination of pubertal status

See section 3.4.1.2

3.4.3 Muscle strength

Muscle strength was measured using a Cybex Norm isokinetic dynamometer (Lumex, Inc., Ronkonkoma, NY). Plantar flexion and dorsi flexion torque (Nm) of the preferred foot were measured at $60^{o}.s^{-1}$ with standard positioning and stabilizing procedures for the legs and torso. Testing protocol involved a standardised warm-up procedure (5 repetitions at $60^{o}.s^{-1}$), one minute rest then 5 maximal continuous contractions at $60^{o}.s^{-1}$. Peak torque (Nm) values were accepted as the criterion strength measure. Flexion and extension torque can be measured in children with moderate to high reproducibility and reliability (Gaul, 1996).

3.4.4 Endocrine status

Participants were encouraged to provide a blood sample however, refusal to provide a sample did not exclude participants from the study. Samples were obtained from 39 male and 39 female participants. Venous blood (10 ml), drawn from the median cubital vein was centrifuged, stored at $-80^{o}C$ and analysed in a batch to minimise inter-assay error. Participants were not instructed to fast prior to blood draw. Blood sampling did not coincide with a specific phase of the menstrual cycle. Serum oestradiol was determined by ultrasensitive E_2 assay (pmol/L) using a modification of a commercial radioimmunoassay (Clinical assay ™ Oestradiol – 2, Diasorin S.R.L. 13040 Caluggia, V.C, Italy), with an intra-assay CV <3.0%. Sex hormones, testosterone (nmol/L), insulin-like growth factor (IGF-1) (pmol/L) and IGFBP3 (pmol/L), were also determined by ultrasensitive assay. Testosterone intra- and inter-assay CV's were <3.0% and <4.4%, respectively. IGF-1 intra- and inter-assay CV's were <2.8% and 4.1%, respectively. IGFBP3 intra- and inter-assay CV's were <3.2% and <4.6%, respectively.

3.4.5 Leg dominance

Leg dominance was determined by asking participants which leg was their preferred leg. The preferred leg was used in DXA, MRI and muscle strength analyses.

3.4.6 Dual X-ray Absorptiometry

3.4.6.1 Body composition

Lean tissue mass and fat mass were determined following analysis by total body dual x-ray absorptiometry (DXA) (Lunar Model Prodigy – high performance fan beam; Lunar Radiation Corp., Madison, WI). The CV's for lean tissue mass and fat mass were <1.1% and 1.8%, respectively, using an in house aluminium phantom.

3.4.6.2 Total body bone mineral

Total body bone mineral content (g) was measured using a high performance fan beam (Lunar Model Prodigy – Lunar Radiation Corp., Madison, WI) total body scanner (Figure 3.1).

Figure 3.1: Lunar Prodigy high performance fan beam scanner

The same investigator completed and analysed scans using standard analysis protocols and same scan mode. The technique and measurement procedure, including quality control, have been previously described (Lu, Briody, Ogle, Morley, Humphries, Allen et al, 1994). The Lunar Prodigy has excellent precision with a CV of 1.6% for two repeat total body (BMC) scans on 5 participants.

3.4.6.3 Regional bone mineral

Lumbar spine BMC (g) was assessed from lumbar vertebrae 2-4 using the “medium” scan mode. Left and right proximal femur BMC (g) and left and right femoral neck BMC (g) were assessed using Dual Femur software. Dominant tibial BMC (g) was assessed from total body scans by placing a region of interest (ROI) box over the entire tibia (see section 3.5.1). Distal tibial BMC (g) was measured using Anterior-Posterior (AP) spine software in “thin” scan mode. Rice bags were used to secure the dominant leg (see section 3.5.2). Measurement precision was established from two repeat lumbar spine, proximal femur, femoral neck and distal tibia scans on 5 participants. CV’s were <1.0% for lumbar spine, <1.1% proximal femur, <1.1% femoral neck and <1.6% distal tibial BMC scans.

3.4.6.4 Radiation exposure

According to the National Safety Council of Australia (1996), a limit of 1mSv per year for non-radiation workers is acceptable with increases up to 5 mSv per year provided that lifetime average of radiation exposure does not exceed 1 mSv per year. The effective radiation dose from DXA total body and regional scans is less than 1mSv, which is less than the average exposure (7 mSv) to background sources of radiation per day. Procedures and levels of exposure for this study were similar to other studies approved by the Children’s Hospital Westmead Ethics committee (Duncan et al, 2000; Woodhead et al, 2000).

3.4.7 Magnetic Resonance Imaging (MRI)

Bone geometry of the distal third tibia and femoral neck were measured using an MRI (1.5 Tesla Phillips INTERA, the Netherlands) unit with a manufacturer supplied knee coil (Figure 3.2).

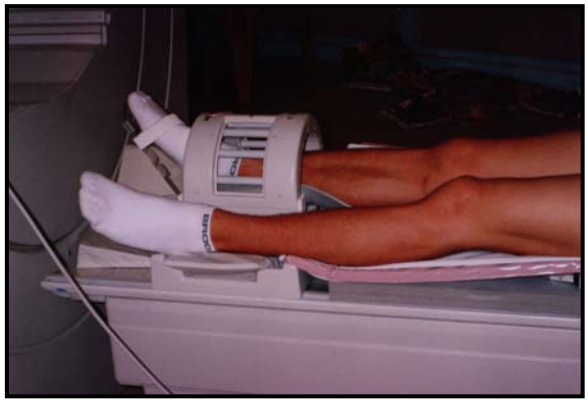


Figure 3.2: Phillips MRI manufacturer supplied knee coil

For the distal tibial scan, participants were required to remain in a supine position in the magnet aperture with the dominant leg firmly supported in a custom built holding device for approximately 45 minutes. Participants were encouraged to listen to their own music through headphones during the scanning procedure to minimise anxiety. A series of transverse images (T1 weighted TSE, 24 x 3 x 0.3 mm) were acquired starting at the most proximal point of the distal tibial epiphysis. A region of interest was demarcated from coronal scout views with contiguous transverse scans (3 mm thick) of the entire distal third of the tibia. Images were acquired with an axial turbo spin proton density weighted sequence and were saved in DICOM format.

For femoral neck scans, a coronal scout view (T1 weighted TSE, 28 x 3 x 0.3 mm) was used to demarcate a ROI of the entire femoral neck. Femoral neck scans were acquired from a series of transverse images (T2 weighted TSE, 20 x 3 x 0.3 mm) starting at the distal end of the femoral neck coinciding with the intersection of the neck / shaft axes. Transverse images were acquired using a dual echo proton density weighted sequence and were saved in DICOM format. Participants were required to stand and walk slowly for five minutes prior to repositioning for repeat image acquisitions. Scans were completed on 10 male participants, irrespective of athletic status.

MRI scans did not involve ionising radiation. All measurements were performed by the same MRI radiographer and all subsequent analyses were performed off-line by a single investigator blinded to participant group allocation. The CV for femoral neck CSA (mm^2) was <1.5% for 15 repeat analyses.

3.5 Calculations available from DXA

3.5.1 Dominant tibial BMC

Dominant tibial BMC (g) was assessed from a total body scan using the "custom" analysis function. Magnification of the total body scan was used to assist in visually locating tibial anatomical landmarks. A ROI box was placed over the entire tibia from the proximal, medial tibial plateau to the base of the medial malleolus. All measurements and analyses were completed by a single investigator. The CV for tibial BMC (g) was <0.8% for 10 repeat analyses.

3.5.2 Distal tibial BMC

An AP spine scan (thin mode) was used to measure distal tibial BMC (g). After calculating tibial length (see section 3.5.3), a ROI box was positioned distally between 20% and 30% of tibial length, using custom analysis. The size of each ROI box was proportional to individual tibial length. The positioning of the ROI box was directly related to MRI-acquired cross-sectional slices. Distal tibial BMC CV was < 1.6% for the same investigator based on 10 repeat analyses.

3.5.3 Axial and appendicular length

From a total body scan, the ruler function was used to measure axial length from skull base to symphysis pubis. Femoral length was measured from femoral head mid-point to the base of

the femoral condyle and tibia length was the distance between the proximal, medial tibial plateau and the base of the medial malleolus. The CV's for measured sites were 0.3%, 0.6% and 1.3% for axial, femoral and tibial length respectively, based on 10 repeat analyses per region. All measurements were completed by the same investigator.

3.5.4 Hip strength analysis

Values for hip strength analysis (HSA) of the dominant femoral neck were determined using DXA (Proximal Femoral Neck scan, standard speed, Prodigy; Lunar Radiation Corp., Madison, WI) (Figure 3.3). Head, neck axis and shaft axis ROI's were manually positioned in accordance with manufacturer protocols. A 4 mm (width) ROI was placed over the mid-point of the femoral neck, ensuring soft tissue in all four corners of the ROI. ROI size was selected to correspond with MRI slice thickness. CSMI, CSA, neck diameter, neck length and subperiosteal width of the femoral neck are automatically calculated by the HSA software. Intra-investigator HSA CV was 0.9% to 1.8% (N = 10). All measurements were completed by the same investigator.

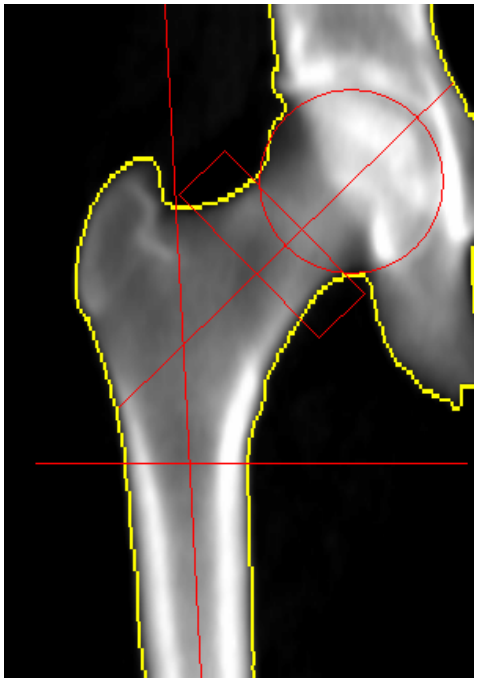


Figure 3.3: DXA-derived Hip strength analysis regions of interest

3.6 Calculations available from MRI

3.6.1 Cortical cross-sectional area

Distal tibial cortical cross-sectional area (mm^2) was calculated using a dedicated software program (Analyse, Mayo Foundation, Rochester: MN, version 5.0) at an off-line workstation. A series of transverse images (T1 weighted TSE, 24 x 3 x 0.3mm) were acquired starting at the most proximal point of the distal tibial epiphysis. The image corresponding to the distal 25% of the tibia was used for analysis. The image was analysed using the region of interest function in 3D mode. Grey scaling was manipulated for optimal viewing and differentiation of bone tissue compartments. Cortical bone cross-sectional area (mm^2) was obtained using the auto trace function. All measurements were performed by a single investigator. The CV for distal tibial cortical cross-sectional area (mm^2) was 0.8% based on 5 repeat analyses of 3 randomly selected scans (N = 15).

3.6.2 Medullary cavity cross-sectional area

Distal tibial medullary cavity cross-sectional area (mm^2) was calculated using the procedure outlined in section 3.6.1. All images were analysed by the same investigator. The CV for distal tibial medullary cavity cross-sectional area (mm^2) was 1.4% based on 5 repeat analyses of 3 randomly selected scans (N = 15).

3.6.3 Muscle cross-sectional area

3.6.3.1 Extensor muscle cross-sectional area

Extensor muscle cross-sectional area (mm^2) was calculated using the same procedure outlined in section 3.6.1. The auto trace function was used to measure cross-sectional area of the extensor muscle following manual separation of the extensor muscle group and use of the

limit function (Figure 3.4). The limit function ensured clear boundaries were achieved around individual muscle compartments.

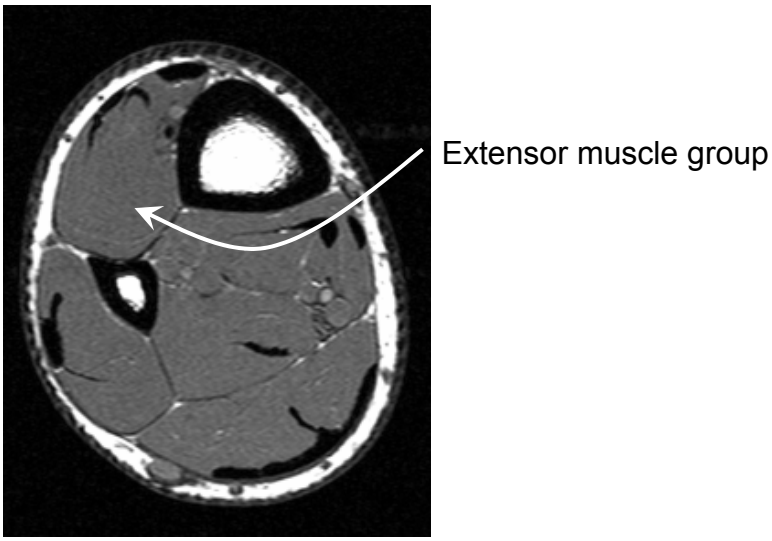


Figure 3.4: MRI distal tibial cross-sectional slice showing extensor muscle group

All images were analysed by the same investigator. The CV for extensor muscle cross-sectional area was 1.3% based on 5 repeat analyses of 3 randomly selected scans (N = 15).

3.6.3.2 Flexor muscle cross-sectional area

Flexor muscle cross-sectional area (mm^2) was calculated using the same procedure outlined in section 3.6.1. The auto trace function was used to measure cross-sectional area of the flexor muscle following manual separation of the flexor muscle group, and the use of the limit function (Figure 3.5).

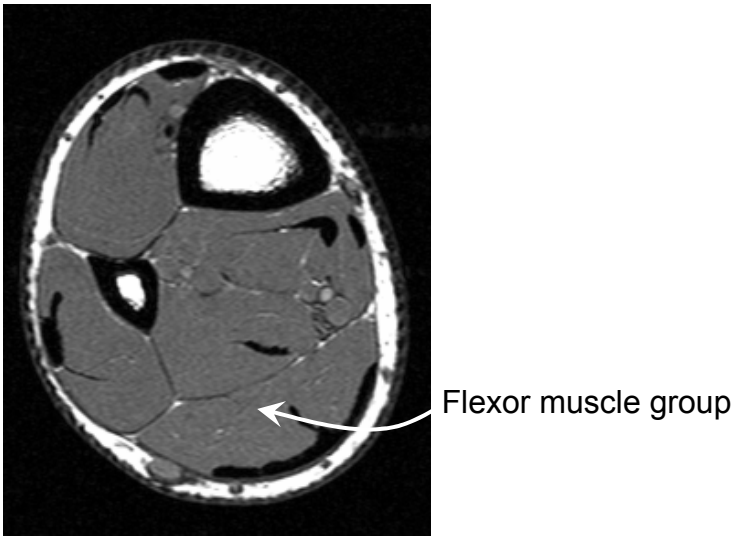


Figure 3.5: MRI distal tibial cross-sectional slice showing flexor muscle group

All images were analysed by the same investigator. The CV for flexor muscle cross-sectional area was 1.2% based on 5 repeat analyses of 3 different scans (N = 15).

3.6.4 Cortical bone volume

The calculation of distal tibial cortical bone volume (mm^3) was derived from the sum of cortical bone cross-sectional areas (mm^2) multiplied by individual slice thickness (3 mm). The number of cross-sectional slices included in the calculation of cortical bone volume corresponded with a 10% ROI (5% either side of the distal 25% mark). The 10% ROI used to analyse MRI-derived cortical bone volume, coincided with the measurement of DXA-derived distal tibial BMC (g) for the same 10% ROI. All measurements were completed by the same investigator.

3.6.5 Cross-sectional moment of inertia

Cross-sectional moment of inertia (CSMI) was calculated from MRI images imported from CD-ROM in DICOM format using Scion Image® (Frederick, Maryland: Version-Beta 3B) software and a custom macro program. Analyses were performed at the most proximal, mid and most distal slices of the 10% ROI of the tibia and the narrowest slice of the femoral neck

using a constant grey scale with a threshold range of 230-255. The Scion Image macro calculates the squared distance of pixel areas from the horizontal and vertical axis which pass through the centre of the cross-sectional mass (neutral axis), with the largest value represented as *Imax*. The macro integrates this procedure over the entire cross-section and about all possible neutral planes (Figure 3.6). *Imax* was used as the criterion CSMI and was measured in mm^4. Due to the automated analysis procedure, no variation in intra- or inter-observer values existed. All measurements were performed by the same investigator.

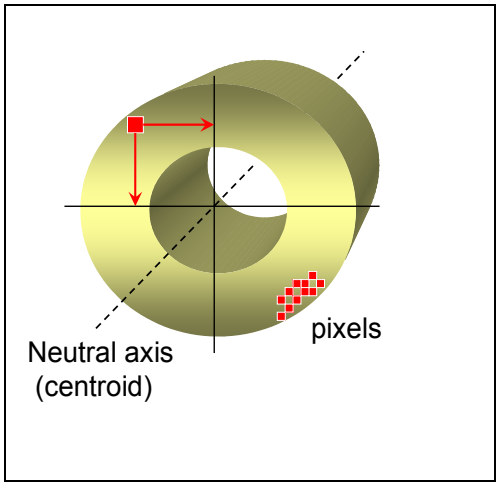


Figure 3.6: Calculation of CSMI from bone cross-section using the sum of pixel areas and their squared distance from the vertical and horizontal axes.

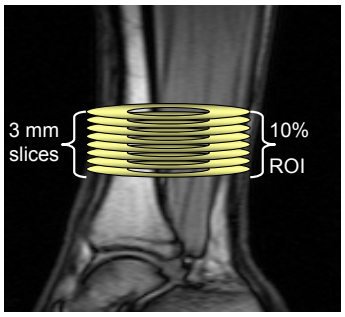
3.7 Calculations combining DXA and MRI

3.7.1 Volumetric cortical bone mineral density

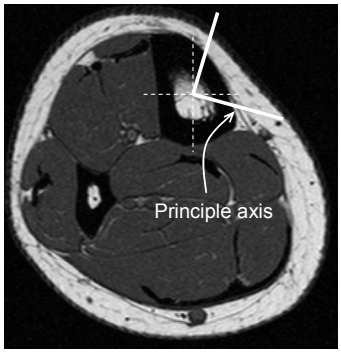
The integration of three-dimensional technology (MRI) with traditional densitometric measures of bone mineral (DXA) provides for the calculation of volumetric cortical bone mineral density. Volumetric cortical bone mineral density was calculated by dividing MRI–derived distal tibial cortical bone volume (cm^3) by DXA–derived distal tibial BMC (g), based on the same 10% ROI. All measurements and calculations were proportional to individual tibial length and were performed by the same investigator.

3.7.2 Bone strength index

Bone strength index (BSI) (g.cm^3 x mm^4) was determined using a previously published equation; BSI = CSMI x Volumetric Cortical BMD (Ferretti et al, 1996). BSI quantifies both the accumulation of bone mass and its distribution within bone. To calculate BSI, the average CSMI for the most proximal, mid and most distal slices of the 10% ROI was multiplied by volumetric cortical BMD for the corresponding 10% ROI (Figure 3.7). All measurements and calculations were proportional to individual tibial length and were performed by the same investigator.



Coronal view of distal tibia



Cross-sectional view of distal tibia

$$\frac{\text{MRI-derived cortical bone volume (cm}^3\text{)}}{\text{DXA-derived BMC (g)}} \quad \text{Eg: } \frac{81.6\ \text{cm}^3}{8.9\ \text{g}}$$

Volumetric cortical BMD (g.cm^3) = 9.2 g.cm^3

X

Cross-sectional moment of inertia (mm^4) Eg: 10039.8 mm^4

Therefore: 9.2 g.cm^3 x 10039.8 mm^4

Bone strength index = 92366.2 g.cm^3 x mm^4

Figure 3.7: Bone strength index (BSI) formula: volumetric cortical BMD (g.cm^3) x cross-sectional moment of inertia (mm^4) = BSI (g.cm^{3}xmm^4)

3.8 Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 10 – 11.5.1. Data were initially checked for normal distribution using differences between mean and median values, multiples of standard deviations compared with means, skewness and kurtosis. An additional criteria of normality occurred when skewness was also divided by standard error with a result less than 1.96 considered normally distributed. When two or more breaches of normality were observed, data was treated with non-parametric statistics.

Descriptive presentation of data included means and standard deviations for dependent and independent variables. Bivariate relationships between dependent and key independent variables, based on previously published demonstrated relationships, were completed using Pearson's correlation analysis. Independent variables with reasonable correlations ($p < 0.01$) were included in a multiple linear regression model, commencing with the strongest correlated variable, to assess the contribution of independent variables to predicted variability in selected dependent variables. If outliers were present they were eliminated to reduce bias to the regression estimate. Variables were entered into the model using the "enter" method to ensure any change in standard error with each subsequent entered variable did not exceed 10%. Regression coefficients were accepted as significant if $p < 0.05$. The independent effects of lean tissue mass and fat mass on group differences in dependent variables (namely BMC and BSI) were also analysed using analysis of covariance (ANCOVA). For interpretive purposes, data were also presented as population specific Z scores, after BMC was modelled by regression analysis as a function of fat mass and lean tissue mass.

3.9 Statistical power

Group sample size was based on observed effect sizes for total body and site-specific measures of areal BMD (gm/cm^2) ranging from 0.5 - 2.0 SD in cross-sectional studies involving healthy adolescents (Duncan et al, 2002; Nichols et al, 1994). A sample size of 20

participants in each of the athlete and control groups was expected to detect significant differences at $p < 0.05$ with a statistical power of 80%.

CHAPTER 4

4.1 Musculoskeletal health in elite male adolescent middle-distance runners

Adolescence is a period of growth resulting in unprecedented physiological change that provides an extraordinary opportunity for the growing body to adapt to external stimuli. The positive influence of physical activity on bone mineral accrual during the growing years has been extensively studied (Petit et al, 2002; Duncan et al, 2002; Bradney et al, 1998). Osteotrophic responses to increased mechanical loading are greatest during the adolescent growth spurt (Seeman, 2002) but more understanding of specific loading is required.

Mechanical loads cause bone tissue within a loaded region to deform. The deformation stimulates adaptation. Mechanical loads that impose high load magnitudes are more likely to produce an osteotrophic effect than low intensity loads (Robinson et al, 1995). Results from cross sectional studies of athletes in different sports compared with non-athletic controls suggest weight bearing activities more positively influence bone mineral status than non-weight bearing activities (Grimston et al, 1993). During adolescence athletes can be exposed to high magnitudes of skeletal loading such as weightlifting or activities that elicit relatively high ground reaction forces such as running. Greater whole body and regional bone density is observed in young female athletes exposed to activities that habitually impose moderate to high ground reaction forces (Duncan et al, 2002; Pettersson et al, 2001; Dyson et al, 1997;). Relatively less is known about musculoskeletal adaptations in male than female adolescent athletes.

Musculoskeletal adaptations to exercise are also influenced by muscle contraction (Frost, 1991). Large bone strains result from the contraction of muscle groups using levers to counteract the effects of gravity and body weight (Stewart et al, 2000). Comparisons of densitometric surrogates of bone strength with muscle cross-sectional area (CSA) report increased risk of fracture when the ratio of bone mineral content (BMC) to muscle CSA was

low (Schoenau et al, 2002). The muscle-bone relationship in physically active adolescents remains relatively under reported.

The impact of high training volumes on musculoskeletal adaptations of male adolescent athletes engaged in repetitive, high magnitude mechanical loading has not been investigated. A matched, case-controlled cross-sectional study design was used. The primary purpose of the study in this chapter was to compare total body and regional BMC and mid distal bone geometry in elite male adolescent middle distance runners and age-matched controls. The secondary purpose was to examine factors predictive of total body BMC from independent variables including muscle CSA, body composition profiles, lower limb muscular strength and macronutrient intakes. The hypothesis was that male adolescent middle-distance runners would display greater total body and regional BMC and distal tibial bone geometry than age-matched non-athletic controls.

Details of the methods used in this chapter are outlined in Chapter 3. However, to reiterate, the primary outcome measures were total body and regional BMC (proximal femur, femoral neck, lumbar spine) and distal tibial cortical and medullary cavity CSA. The secondary outcome measures and potential factors predictive of total body BMC included total muscle, extensor, and flexor CSA, lean tissue mass, fat mass, plantar flexion and dorsi flexion muscular strength and calcium intake.

Following tests for normality, independent t-tests were used to determine differences between groups. Bivariate relationships between total body BMC, selected descriptive statistics and independent variables were determined by correlation analysis (Pearson correlation coefficient) for combined groups. Multiple linear regression analyses were used to assess the contribution of strongly correlated variables to predicted variability in total body BMC. To generate population specific Z scores, BMC was modelled by regression analysis as a function of fat mass and lean tissue mass.

4.2 Results

4.2.1 Descriptive characteristics

Descriptive characteristics of participants are summarized in Table 4.1.

Table 4.1: Descriptive characteristics of male adolescent middle-distance runners and age-matched controls (N = 20 per group)

	Athlete Mean (SD)	Control Mean (SD)	p value
Age (y)	16.8 (0.6)	16.4 (0.7)	0.81
Height (m)	1.76 (0.6)	1.77 (0.8)	0.60
Weight (kg)	64.4 (6.2)	75.4 (14.7)	0.005
BMI ($kg.m^2$)	20.68 (1.6)	23.80 (3.5)	0.001
Fat mass (kg)	5.6 (2.4)	16.5 (10.7)	0.001
Body Fat (%)	8.6 (3.4)	20.7 (9.7)	0.001
Lean tissue (kg)	56.2 (5.2)	55.5 (6.2)	0.711
Plantar flexion (Nm)	56.9 (12.9)	59.4 (16.1)	0.593
Dorsi flexion (Nm)	21.5 (5.3)	25.2 (6.5)	0.058
Hrs Phys Act (wk^{-1})	14.1 (5.7)	2.2 (0.7)	0.001
Energy intake ($kj.d^{-1}$)	9589.3 (2713.4)	12663.5 (2177.8)	0.62
Calcium intake (mg)	1822.5 (620.2)	1287.5 (486.3)	0.09
No train - injury past 12 mo (wk^{-1})	5.25(1.4)	N/A	N/A

Compared with athletes, non-athletic controls had greater weight (+10.97 ± 3.56 kg, p = 0.005), fat mass (+10.93 ± 2.46 kg, p = 0.001) and percentage of body fat (-12.07 ± 2.31 percent, p = 0.001). Athletes displayed greater hours of physical activity per week (+11.92 ± 1.49 hours, p = 0.001) and lower body mass index (BMI) (-3.11 ± 0.86 $kg.m^2$, p = 0.001) than non-athletic controls. Macronutrient and calcium content did not differ between groups. Mann-Whitney *U* tests were conducted on the categorical data of self-reported Tanner stage for maturational development. Reported median values were not different between groups for genital (p = 0.262) or pubic hair (p = 0.406) development.

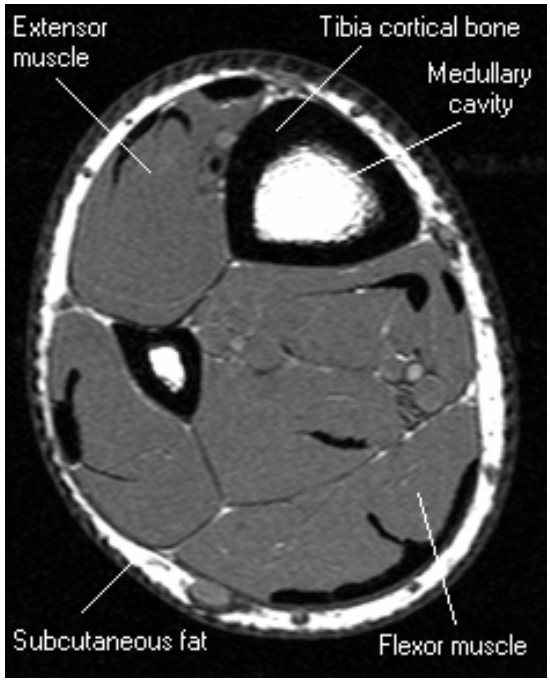
4.2.2 Bone geometry

Unadjusted values for mid-distal tibia, muscle and subcutaneous fat cross-sectional areas (CSA) are shown in Figure 4.1 and Table 4.2. Athletes and non-athletic controls were not different for tibial bone CSA (cortical and medullary cavity), muscle CSA (extensor, flexor and total muscle) and muscle to bone CSA ratio. Non-athletic controls had greater subcutaneous fat CSA (+580.20 ± 150.62 g, p = 0.001) than athletes.

Table 4.2: Mid distal tibia, muscle and subcutaneous fat cross-sectional areas (mm^2) for adolescent males and age-matched controls.

Site	Athlete Mean (SD)	Control Mean (SD)	p value
Tibia cortical	423.1 (47.3)	447.5 (65.1)	0.183
Medullary cavity	187.9 (47.3)	213.3 (69.4)	0.185
Extensors	486.9 (124.8)	470.8 (88.9)	0.261
Flexors	542.2 (454.4)	418.3 (169.5)	0.642
Subcutaneous fat	665.9 (169.4)	1246.1 (651.9)	0.001
Total muscle	2206.3 (786.7)	2040.7 (314.9)	0.387

Figure 4.1: MRI scan (Transverse slice – mid distal tibia) of a male adolescent middle-distance runner



4.2.3 Bone Mineral Content

Unadjusted total body and regional BMC results are summarized in Table 4.3.

Table 4.3: Unadjusted total body and regional BMC (grams) in male adolescent athletes and age-matched controls.

	Athlete Mean (SD)	Control Mean (SD)	p value
Total body	2801.3 (322.1)	2964.3 (415.2)	0.174
Lumbar spine	53.8 (7.1)	51.5 (8.3)	0.359
Right Proximal femur	41.2 (4.3)	38.1 (3.6)	0.019
Left Proximal femur	40.5 (4.2)	38.6 (3.8)	0.148
Right Femoral neck	5.9 (0.7)	5.7 (0.5)	0.319
Left Femoral neck	5.9 (0.6)	5.8 (0.5)	0.65

Compared with non-athletic controls, athletes had greater right proximal femur BMC (+3.08 ± 1.25 g, p = 0.019). No other differences in total body or regional BMC were observed between groups (p > 0.05). A comparison of population specific Z scores for regional BMC as a function of fat mass at the lumbar spine, dominant proximal femur and dominant leg is presented in Figure 4.2.

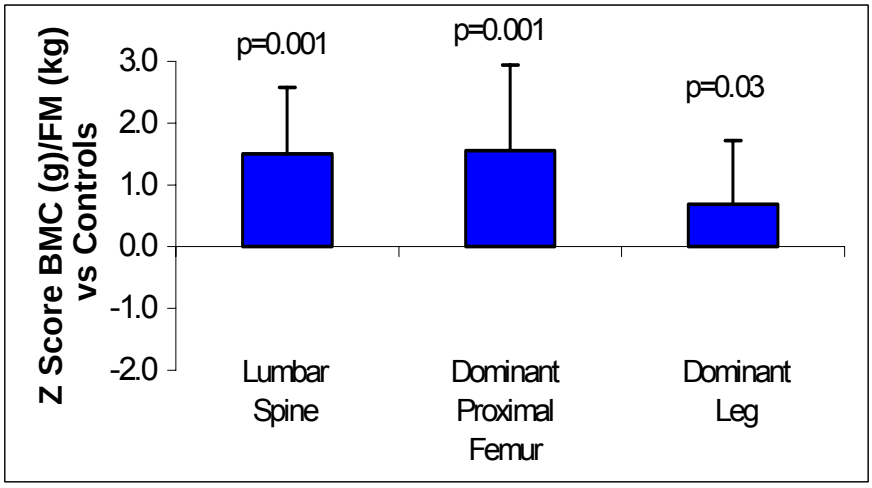


Figure 4.2: Z score regional BMC per kg fat mass in adolescent middle-distance runners. Zero on the y axis denotes the control group. Differences between athletes and controls from independent t-tests are denoted by p values, solid bars represent means and error bars represent standard deviations.

Athletes had greater Z scores for BMC / fat mass at the lumbar spine, dominant proximal femur and dominant leg. Similar modelling of BMC for lean tissue mass did not produce differences in Z scores between athletic and control groups. Bivariate correlations between measures of BMC and selected descriptive variables are presented in Table 4.4.

Table 4.4: Bivariate relationship (Pearson Correlation Coefficient) between BMC and selected descriptive variables for adolescent males (N = 40)

	Total Body	Lumbar Spine	Proximal Right Femur	Proximal Left Femur	Right Femoral Neck	Left Femoral Neck
Height	0.549**	0.516**	0.410**	0.432**	0.277	0.357
Weight	0.74**	0.457**	0.194	0.255	0.212	0.339
Fat mass	0.554**	0.243	-0.074	0.014	0.008	0.133
Lean tissue	0.701**	0.393	0.428	0.527**	0.300	0.336
Body Fat (%)	0.455**	0.179	-0.177	-0.100	-0.049	0.077
Plantar flexion	0.479**	0.354	0.137	0.102	0.214	0.226
Dorsi flexion	0.343	0.120	0.114	0.119	0.268	0.227
Hrs Phys Act. wk^{-1}	-0.351	0.023	0.207	0.104	0.075	0.000

** $p < 0.01$

Height correlated with all BMC measures ($p < 0.05$) except right and left femoral neck BMC ($p < 0.05$). Weight, fat mass, lean tissue mass, percent body fat and plantar flexion correlated with total body BMC. Weight also correlated with lumbar spine BMC and lean tissue mass correlated with left proximal femur BMC. No other significant correlations between BMC sites and selected descriptive variables were reported. Outcomes for the secondary purpose of the study were obtained using multiple regression to evaluate the effect of two or more predictor variables on total body BMC. Total body lean mass and total body fat mass explained 72% of the variance in total body BMC for combined groups (N= 40). The regression equation was Total body BMC = 286.5 + 0.0426*lean mass + 0.0189*fat mass ($R^2 = 0.716$).

4.3 Discussion

This investigation is the first to examine BMC or bone and muscle CSA in elite level, male adolescent middle distance runners and age-matched controls. Results showed no differences between athletes and non-athletic controls in total body and minimal differences in regional BMC. Consistent reports of greater levels of BMC or areal bone mineral density

(BMD) in female children, adolescents and adults in a variety of weight bearing activities (Dyson et al, 1997; Pettersson et al, 2001) are not supported by the findings in the present chapter. Limited research involving male runners has focussed on adult athletes with extensive years of training (Stewart and Hannan, 2000). It is possible that male adolescent athletes have not been exposed to a similar volume of training and that adaptations may occur later with continued participation.

However, in young populations, most studies report higher BMD in participants of sports characterised by weight bearing loads (Dyson et al, 1997; Grimston et al, 1993). Differences in BMD between weight bearing (running), non-weight bearing (swimming and cycling) sports, and a sport that incorporates both types of loading conditions (triathlon) were compared in adolescent females. Runners had greater total body and regional BMD than swimmers and controls (Duncan et al, 2002). Substantial sex-related differences in hormonal activity, particularly in adolescence may at least partially explain the difficulties in detecting differences between young male athletes and their age-matched controls.

The association between lean tissue mass in weight bearing exercise and bone density is well-established in adults. Mechanical forces generated by increased body weight are positively associated with skeletal density (Schoenau et al, 2000). However, the impact of additive mechanical loading of bone from body fat remains unclear. Equivocal research findings show sedentary individuals with greater fat and body mass have higher (Reid, Legge, Stapleton, Evans and Grey, 1995) and lower (Goulding, Jones, Taylor, Williams and Manning, 2001) bone densities than lean individuals with lower body mass. Within the study described in this chapter, comparison of body composition values showed athletes had less total body mass (10.97 kg) and fat mass (10.93kg) than non-athletic peers. Differences were also evident at the subcutaneous fat level through MRI technology. Distal tibia subcutaneous fat CSA was less in athletes than non-athletes. If bones respond to loads imposed by total body mass, then the heavier group could be expected to display superior bone properties. However, when the additional load is comprised of fat mass, the beneficial mechanical loading during exercise may be minimal and or, unsustainable. The impact of fat mass on

bone mineral content in males in the present chapter is demonstrated in the regression equation. After adjusting for lean tissue mass per kg of body weight, no difference in total body BMC was found between groups.

Body weight is frequently reported to be a significant predictor of total body BMC (Duncan et al, 2002) however, concerns with collinearity arise. Body weight is comprised of BMC, fat mass, lean tissue mass (muscle), fluids and connective tissues. Because BMC is a principle component of body weight, the regression analysis in the present study excluded body weight as a predictive variable. A negative impact of body fat and a positive impact of lean mass were identified as the strongest independent predictors of total body BMC (R^2 = 0.71).

Mechanical loading induces a strain gradient signal that activates a bone cell response. Frost's "mechanostat" theory states that a minimum effective strain needs to be exceeded to initiate bone modeling and potentiate an increase in bone mass (Frost, 1991). Mechanical loading stimulates modeling at bone sites until adaptation occurs and a new minimum effective strain set point is established. Higher mechanical loads are then required to maintain or increase bone mass. Athletes, in the present study, averaged 14 hours of physical activity per week and were exposed to repetitive impact forces and strain rates of 3 to 5 times body weight (Robinson et al, 1995). When greater regional BMC at the dominant proximal femur of the athletic compared with non-athletic controls is considered, adaptations to training loads appear to support the mechanostat theory. Longer-term exposure to specific loading through middle distance running training might increase regional differences between groups (Stewart et al, 2000).

The moderate ground reaction forces of running training may not generate the substantial bone adaptations observed in other weight bearing sports involving high impact forces. A study of gymnasts and middle distance athletes reported greater BMD at all sites for gymnasts compared with runners (Robinson et al, 1995). Higher impact forces resulting from gymnastics training were more stimulating to bone than the lower, repetitive impact forces of running. A similar study found ground reaction forces in children during running averaged 3

times body weight whereas ground reaction forces imposed by landing from gymnastics, tumbling and dance reached values as high as 10 times body weight (Grimston et al, 1993). At least in adolescent males involved in the present study, running training may be producing sub-optimal bone adaptations.

Magnetic resonance imaging (MRI) provides an accurate assessment of cross-sectional bone geometric structure. Exercise-induced changes in geometric structure may positively affect bone strength without subsequent changes in bone density (Haapasalo et al, 2000). Increases in periosteal diameter produce greater bone strength, as measured by cross-sectional moments of inertia, section modulus and bone strength index (Petit et al, 2002). In the present study, no differences in cortical bone or medullary cavity CSA were found between the male athletes and non-athletic participants. Conflicting data and methodology issues within the literature at present preclude an agreed understanding of an opportune time during growth for exercise to be linked to the greatest osteotrophic effect (Petit et al, 2002; Bradney et al, 1998).

Differences in CSA of the humerus between dominant and non-dominant arms in young adult tennis players have been reported (Haapasalo et al, 2000). Greater CSA of the humerus and cortical bone, and greater bone strength index were found in dominant playing arms. Non-significant differences in tibial cortical bone and medullary cavity CSA between groups of males in the study suggest long bone adaptation to mechanical loading is site specific. Furthermore, the assessment of one transverse section of the distal tibia does not necessarily represent the entire bone response to the mechanical loading associated with middle distance running. More substantial cortical bone and medullary cavity CSA differences may be evident elsewhere in the body. Favourable geometric and biomechanical adaptations using MRI images of the femur have been reported previously in adolescent female runners (Duncan et al 2002). However, the distal tibia was scanned in the present chapter because of the research group's interest in trabecular micro-architecture.

Current methods for assessing bone densitometry using DXA lack sensitivity in examining bone micro-architecture (Haapasalo et al, 2000). Adaptations of trabecular bone to loading may include greater trabecular density and subsequent mechanical competence as well as decreased risk of fracture. Bone mass can account for 40-60% of bone mechanical strength and indices of bone architecture may be linked to an additional 25-40% (Oden, Selvitelli and Hayes, 1998). High-resolution MRI may permit the acquisition of images sufficient to discern changes in trabecular architecture to mechanical loading. MRI scans taken for the present study remain suitable for further analysis of the acquired scans at the cortical micro-architectural level given imminent advancements in MRI analysis software.

A paucity of data prevents an accurate comparison of the influence of intensive training on musculoskeletal health in adolescent athletes. Research also varies in the populations studied, methodology used, and types and severity of injuries reported. A longitudinal study of intensive training in weight bearing and non-weight bearing sports reported a low injury rate of less than one injury per 1000 hours of training in children aged between 8 to 16 years (Baxter-Jones, Helms, Maffulli, Baines-Preece and Preece, 1996). In the present chapter, within a limitation of low numbers in the regression equation using only data from athletes, the number of weeks of training and competition missed through injury could not explain the variance in total body BMC (R^2 = 0.016).

Testosterone is the most active stimulator of the anabolic processes in muscle and bone development in males during adolescence. In absolute terms and when normalized for body mass, differences in muscle strength, mass, and CSA between genders are most evident during adolescent growth. Current discussion of the muscle bone unit postulates a protective role of muscle during excessive compressive and torsional forces (Schoenau et al, 2002). It is possible that in the present chapter, increased muscle development experienced during adolescence may have provided a gender-enhanced protective effect to offset the influence of mechanical loading. BMC differences between athletes and controls may subsequently be more difficult to detect in males than females during adolescence. Furthermore, an osteogenic response to loading in males may occur across pubertal stages as opposed to a

smaller window of opportunity for osteogenic change in females (Forwood et al, 2004). However, more articulate comparisons of bone material properties and muscle CSA between genders in highly trained adolescent athletes may further explain the influence of hormones on musculoskeletal development.

In conclusion, this chapter of the thesis profiled musculoskeletal health in elite level, male adolescent middle distance runners. Total body and most regional BMC did not differ between athletes and age-matched non-athletic participants. Differences in total body mass and fat mass were found between groups, with athletes displaying leaner body composition. The predictors of total body BMC were related to lean body mass and fat mass. High training volumes were not considered detrimental to the musculoskeletal health assessed by DXA and MRI images of muscle and bone CSA.

CHAPTER 5

5.1 Musculoskeletal health in elite female adolescent middle-distance runners

Peak bone mass and architecture are modulated by adaptive mechanisms sensitive to mechanical loading (Mosley and Lanyon, 2002). A positive association between site-specific mechanical loading and increases in regional bone mineral content (BMC) during childhood and adolescence is well established (MacKelvie et al, 2001; Petit et al, 2002). Weight bearing physical activities, such as middle distance running generate forces of greater magnitude on the musculoskeletal system, than loads imposed with normal living. Results from intervention studies suggest the two years surrounding peak bone velocity during adolescence may be the critical period for augmenting bone acquisition by additional physical activity (MacKelvie et al, 2001; Petit et al, 2002). Approximately 26% of adult bone is accumulated during the two years surrounding peak bone velocity (Bailey, 1997). However, the understanding of consequences of repetitive mechanical loading on total body and regional musculoskeletal development during adolescence remains imperfect.

Existing studies on bone in active adolescents are largely limited to methods using Dual-energy X-ray Absorptiometry (DXA). The mechanical integrity of bone is determined by an interaction between bone material and structural properties (Kontulainen et al, 2003). Intrinsic material properties, such as the mass of mineral contained within the entire bone (g) or the mass of mineral per unit of bone length (g/cm), can be evaluated using DXA. However, concerns for potential misconceptions resulting from DXA-based estimates of bone density based on areal mineral density (BMD g/cm^2) (Seeman, 2002) support the reporting of data in units of BMC (g) in the present study. Despite DXA precision and accuracy in the measurement of bone mineral content, short examination time and low radiation dose, the anterior – posterior planar nature of DXA also compromises the assessment of true bone volume and bone geometry (Kontulainen et al, 2003). The planar two-dimensional assessment capabilities of DXA present difficulties in accurately scanning a three-dimensional

bone structure. Although bone length and width can be measured, depth cannot be detected by DXA technology. Magnetic Resonance Imaging (MRI) provides a more accurate determination of bone geometry using circumferential, cortical and medullary cavity cross-sectional areas (CSA) (Woodhead et al, 2001). The third-dimensional capability of MRI is derived from slice depth. Compared with the two-dimensional nature of DXA, MRI serves as an accurate musculoskeletal assessment tool, allowing further clarification and understanding of geometric adaptations to mechanical loading from middle distance running in adolescent populations.

Middle distance running exposes the skeleton to repeated mechanical loads through a combination of compressive ground reaction forces and muscular contraction (Lanyon, Hampson and Goodship, 1975; Milgrom, Finestone, Levi, Simkin, Ekenman, Mendelson, 2000). While this may result in benefits via skeletal adaptation, injuries may also ensue. Stress fractures, especially at the tibia (Bonjour, Chavelley, Ammann, Slosman and Rizzoli, 2001), are the most common type of injury sustained by middle distance runners. Low tibial bone mineral density (BMD) and narrow tibial bone width are associated with the development of stress fractures (Bennell et al, 1996; Mosley et al, 2002). The study in this chapter compares bone and muscle geometry at the distal tibia, and total body and regional BMC in adolescent female middle distance runners with healthy, non-athletic controls. The distal tibia was examined because of a potential to demonstrate in detail the osteogenic adaptations to compressive and tensile forces experienced in middle distance running (Milgrom, Giladi, Simkin, Rand, Kedem, Kashtan et al, 1989). Our investigation also explores the relationship between muscle forces and skeletal adaptation in highly trained, female adolescent middle distance runners using MRI determined distal tibial muscle CSA and DXA-derived tibial BMC. Muscular contraction is theorized as a major modulator of bone strain (Burr, 1997) and muscle CSA is a limited but acceptable surrogate of muscle contraction (Schoenau et al, 2002).

The primary purpose of the study in this chapter was to compare mid distal tibial bone and muscle geometry, and total body and regional BMC in elite female adolescent middle distance

runners and age and sex-matched controls. The secondary purpose was to examine factors predictive of bone geometry and total body BMC from independent variables including muscle cross-sectional area, body composition profiles, lower limb muscular strength, macronutrient intakes and menstrual histories. The hypothesis was that female adolescent middle –distance runners would display greater total body and regional BMC and distal tibial bone geometry than age-matched non-athletic controls.

Details of the methods used in this chapter are outlined in Chapter 3. However, to reiterate, the primary outcome measures were mid distal tibial bone and muscle geometry (CSA) and total body and regional BMC. Secondary and potential explanatory factors predictive of bone geometry and total body BMC included total muscle, extensor, and flexor CSA, lean tissue mass, fat mass, plantar flexion and dorsi flexion muscular strength, calcium intake and menstrual histories.

To address the primary purpose similar statistical analyses were applied in this chapter to the statistics in Chapter 4. Specifically, following tests for normality, independent t-tests were used to determine differences in bone and muscle geometry, and total body and regional BMC between groups of female athletes and controls. Bivariate relationships between total body and regional BMC, and independent variables selected on the basis of previously reported strong associations were determined by correlation analysis (Pearson correlation coefficient) for combined groups. The strong influence of fat mass and lean tissue mass on total body and regional BMC was further explored with a modelled regression analysis and expressed as population-specific Z scores. To address the secondary purpose of the study in this chapter, a number of multiple linear regression analyses were performed to ascertain the contribution of highly correlating independent measures to variation in bone geometry (cortical bone CSA) at the distal tibia and total body BMC. Analysis of covariance (ANCOVA) was used to determine the independent effect of height on group differences in total body BMC. Non-parametric comparisons were applied to categorical data.

5.2 Results

5.2.1 Descriptive characteristics

Descriptive characteristics of the female participants are summarized in Table 5.1. Compared with non-athletic controls, athletes were lower in weight (-6.47 ± 2.7 kg, p = 0.027), body mass index (BMI) (-3.45 ± 0.9 kg.m^2, p = 0.002) and fat mass (-11.3 ± 1.9 kg, p = 0.001). Athletes were taller (+3.9 ± 1.7 cm, p = 0.030), consumed more calcium (+572.7 ± 201.2 mg, p = 0.007), had greater lean tissue mass (+5.48 ± 1.2 kg, p = 0.001) and engaged in more hours of physical activity per week (+6.9 ± 1.07 hours, p = 0.001) than non-athletic controls. Greater height in athletes compared to controls was explained by greater femoral (+1.31 ± 0.05 cm, p = 0.03), but not tibial (+1.05 ± 0.06 cm, p = 0.06) or axial length (+0.9 ± 0.08 cm, p = 0.91). No between group differences were observed for age, plantar or dorsi flexion torque at the ankle (p > 0.05).

Menstrual status in athletes showed categorical distributions of 10% premenarcheal, 30% with age-related oligomenorrhea and 60% with eumenorrhea. Similarly, the relative distribution for the 3 categories of menstrual status in the control group was 8% premenarcheal, 30% with age-related oligomenorrhea and 62% eumenorrhea. Pearson chi-square analysis demonstrated no differences in menstrual status between athlete and control groups (p > 0.05). Mann-Whitney *U* tests were conducted on the categorical data of self-reported Tanner stage for maturational development. Reported median values were not different between groups for breast (p = 0.16) or pubic hair (p = 0.12) development.

Table 5.1: Descriptive and modifiable lifestyle characteristics of adolescent female athletes and age matched controls (N = 20 per group). Values are reported in means and standard deviation

	Athlete Mean (SD)	Control Mean (SD)	p value
Age (y)	15.9 (1.6)	16.0 (1.8)	0.894
Height (m)	1.66 (0.5)	1.62 (0.6)	0.030
Axial length (cm)	59.48 (2.9)	59.38 (2.6)	0.913
Femur length (cm)	43.30 (1.8)	42.02 (1.8)	0.031
Tibia length (cm)	35.3 (1.9)	34.1 (1.8)	0.063
Weight (kg)	52.2 (6.1)	58.7 (10.8)	0.027
BMI ($kg.m^2$)	18.7 (1.5)	22.2 (4.1)	0.002
Fat mass (kg)	8.2 (2.8)	19.5 (8.4)	0.001
Lean tissue (kg)	41.8 (4.2)	36.3 (3.3)	0.001
Plantar Flexion (Nm)	46.7 (11.1)	46.3 (16.3)	0.928
Dorsi Flexion (Nm)	18.2 (5.2)	18.5 (8.9)	0.881
Physical Activity ($h.wk^{-1}$)	8.9 (4.1)	2.0 (0.7)	0.001
Calcium (mg)	1271.7 (122)	698.9 (79.4)	0.007
Age at menarche (y)	12.5 (4.3)	12.4 (3.1)	0.953
Oestrogen (pmol/L)	146.95 (36.2)	152.20 (43.6)	0.862

5.2.2 Bone and muscle geometry

Unadjusted cross-sectional areas (CSA) for mid-distal tibia cortical and medullary compartments, muscle compartments and total subcutaneous fat are shown in Table 5.2. Athletes had greater tibial cortical bone (+30.9 $\pm$ 9.5 mm^2, p = 0.003) and smaller medullary cavity CSA (-32.3 $\pm$ 14.7 mm^2, p = 0.035) than non-athletes, but no differences in total bone CSA. Extensor muscle (+46.9 $\pm$ 19.5 mm^2, p = 0.021) and total muscle CSA (+240.2 $\pm$ 86.4 mm^2, p = 0.035) was greater in athletes than controls. Athletes and non-athletes were not different in flexor muscle CSA. Athletes also had less subcutaneous fat CSA (-563.8 $\pm$ 140.8 mm^2, p = 0.001) than non-athletes. Corresponding mean tibial total bone, cortical bone and medullary cavity CSA between athletes and controls are shown in Figure 5.1.

Table 5.2: Mid distal tibia, muscle and subcutaneous fat cross-sectional areas (mm^2) of female adolescent athletes and age matched controls. Values are reported in means and standard deviation

Site	Athlete Mean (SD)	Control Mean (SD)	p value
Total tibia	349.26 (33.3)	348.63 (60)	0.968
Tibia cortical	207.08 (35)	176.17 (24.5)	0.003
Medullary cavity	140.18 (41.8)	172.46 (51.1)	0.035
Extensors	378.8 (70.7)	326.89 (51.4)	0.021
Flexors	419.45 (66.6)	304 (49.9)	0.135
Total muscle	1695.36 (128.3)	1455.16 (227.8)	0.035
Subcutaneous fat	920.74 (255.1)	1484.57 (375.9)	0.001

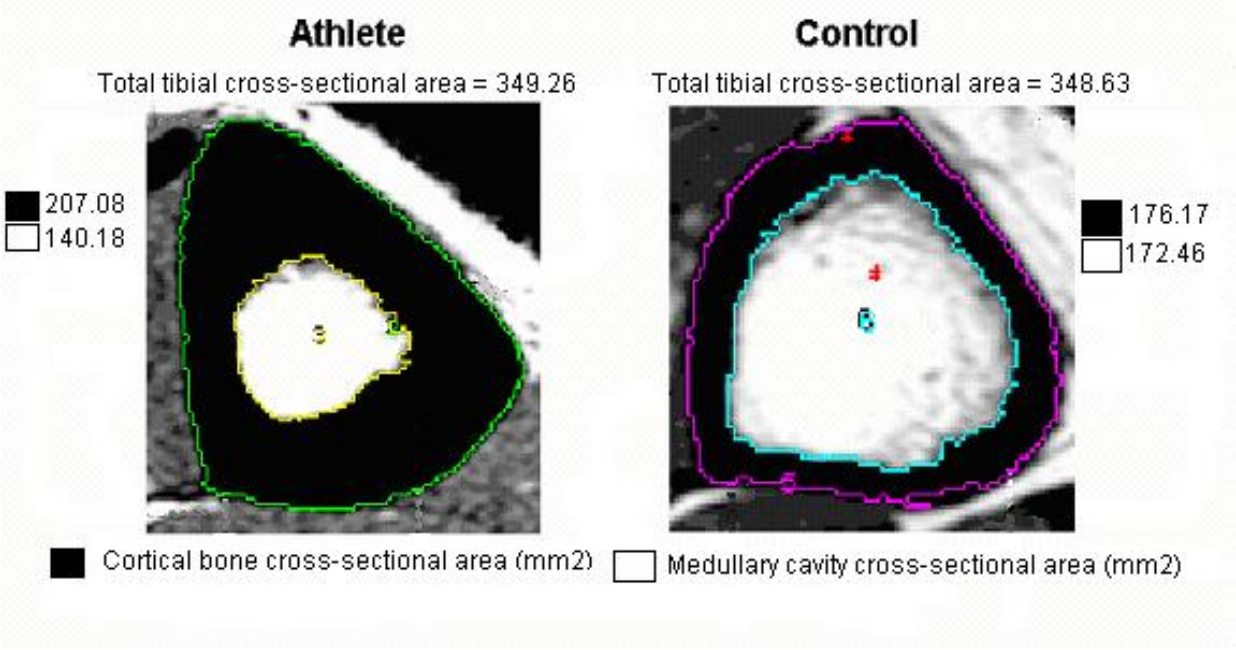


Figure 5.1: Corresponding mid distal tibial cross-sectional slices – mean values (mm^2) of an adolescent female athlete and an age-matched control

5.2.3 Bone mineral content

Unadjusted total body and regional BMC results are summarized in Table 5.3. Compared with non-athletic controls, athletes had greater BMC at the dominant proximal femur (+4.7 ± 1.3 g, p = 0.001), non-dominant proximal femur (+3.9 ± 1.4 g, p = 0.011), dominant femoral neck (+0.5 ± 0.12 g, p = 0.010) and dominant tibia (+4.1 ± 2.1 g, p = 0.050). No other differences in total body or regional BMC were observed between groups. A comparison of population-

specific Z scores generated for BMC as a function of fat mass at the total body, dominant proximal femur and dominant leg is presented in Figure 5.2. Athletes had greater Z scores for BMC per kg of fat mass at the total body, dominant proximal femur and dominant leg (p = 0.001). Similar modeling of BMC per kg of lean tissue mass did not produce differences in Z scores between athletic and control groups.

Table 5.3: Unadjusted total body and regional BMC (grams) in adolescent athletes and age and sex-matched controls

	Athlete Mean (SD)	Control Mean (SD)	p value
Total body	2287.15 (291.6)	2310.50 (328.8)	0.813
Lumbar spine	41.8 (8.1)	38.8 (6.8)	0.227
Dominant Proximal Femur	33.3 (4.2)	28.5 (4.4)	0.001
Non Dominant Proximal Femur	32.9 (4.4)	28.9 (4.7)	0.011
Dominant Femoral Neck	5.0 (0.5)	4.5 (0.6)	0.010
Non Dominant Femoral Neck	4.8 (0.6)	4.5 (0.6)	0.272
Dominant Tibia	49.4 (7.7)	45.3 (4.7)	0.050

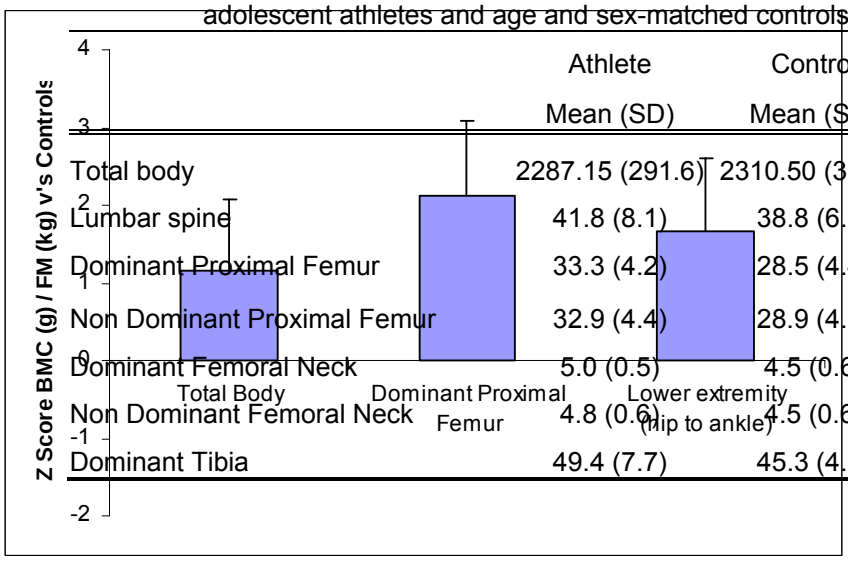


Figure 5.2: Difference in Z score between female adolescent athletes and controls in total body and regional BMC, as predicted by total body fat (kg). Zero on the y-axis represents controls (p = 0.001).

Bivariate correlations between measures of BMC and selected descriptive and independent variables are presented in Table 5.4. Correlations values are reported. Level of significance is $p < 0.01$. Lean tissue mass correlated with all BMC measures except non-dominant femoral neck. Height correlated with all BMC measures except non-dominant femoral neck and distal tibial CSA. Weight (r = 0.74), fat mass (r = 0.45) and lean tissue mass (r = 0.56) correlated with total body BMC. Weight also correlated with non-dominant proximal femur (r = 0.46) and distal tibial CSA (r = 0.42). Height (r = 0.51), lean tissue mass (r = 0.70), and hours of physical activity per week (r = 0.44) correlated with dominant tibial BMC. Calcium correlated with dominant (r = 0.44) and non-dominant femoral neck (r = 0.42) BMC. No other significant correlations between BMC sites and selected descriptive variables were observed. ANCOVA

was used to adjust for differences in height between athletes and controls. Between group differences in total body BMC, were reported after covarying for height.

Table 5.4: Bivariate relationship (Persons Correlation Coefficient) between BMC at multiple sites and tibial bone geometry with selected descriptive variables for combined participants (N = 40)

	TB	LS	DPF	NDPF	DFN	NDFN	DT	DTCSA
Height	0.60*	0.69*	0.63*	0.62*	0.61*	0.32	0.51*	0.32
Weight	0.74*	0.05	0.39	0.46*	0.34	0.29	0.32	0.42*
Fat mass	0.45*	-0.22	-0.31	0.05	-0.04	0.12	-0.47	0.20
Lean tissue	0.56*	0.50*	0.81*	0.78*	0.73*	0.32	0.70*	0.42*
Planar flexion	0.36	0.20	0.27	0.28	0.17	0.25	0.35	0.39
Dorsi Flexion	0.20	0.15	0.17	0.19	0.10	0.17	0.26	0.20
Hrs Phys. Act.wk^{-1}	0.15	0.15	0.41	0.32	0.27	-0.10	0.44*	0.01
Calcium	0.19	0.37	0.34	0.35	0.44*	0.42*	0.17	0.03

* correlation is significant at the 0.01 level (2 tailed).

TB = Total body, LS = Lumbar spine, DPF = Dominant proximal femur
NDPF = Non-dominant proximal femur, DFN = Dominant femoral neck
NDFN = Non-dominant femoral neck, DT = Dominant tibial BMC
DTCSA = Distal tibial cross-sectional area

5.2.4 Predictors of bone geometry and total body BMC

Linear regression analysis was used to examine the relationship between cortical bone CSA and total muscle CSA. The linear relationship is shown in Figure 5.3. Total muscle CSA of the mid distal tibia explained 73% of the variance in cortical CSA. The regression equation was Cortical CSA = 65.28 + 0.08*total muscle CSA (R^2 = 0.73).

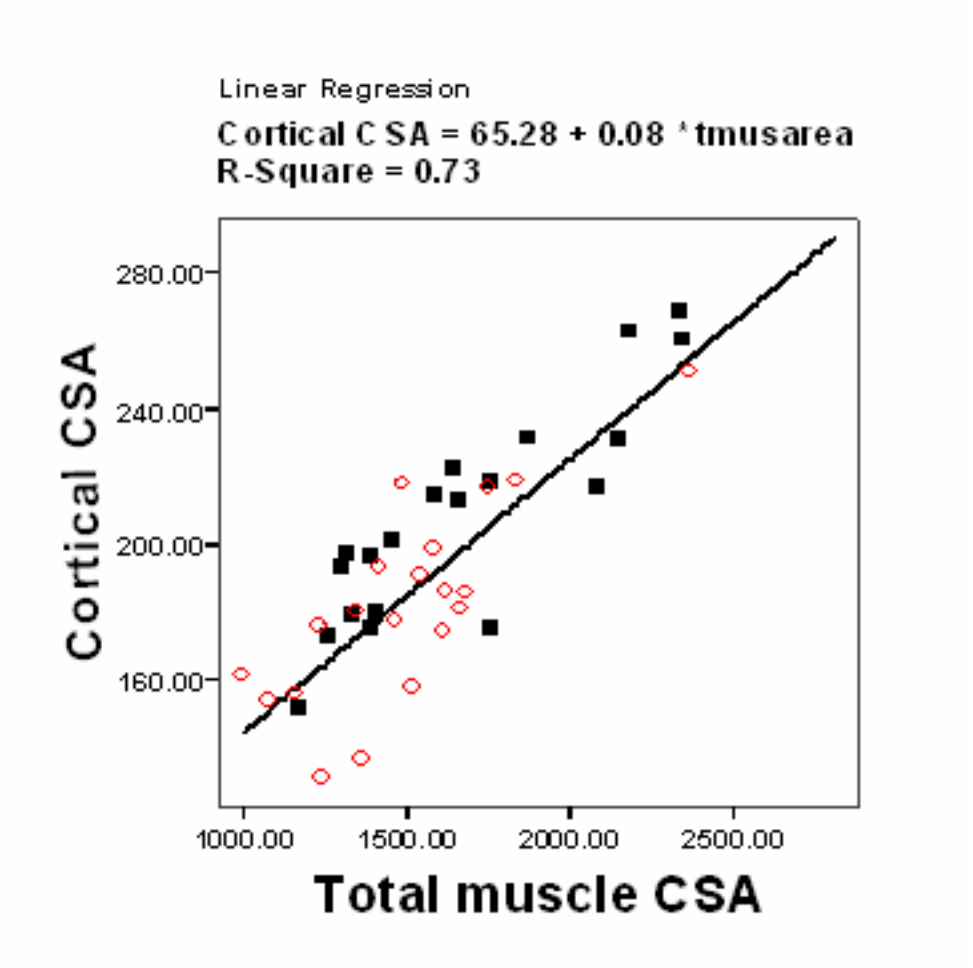


Figure 5.3: Relationship between cortical bone CSA (mm^2) and total muscle CSA (mm^2) at mid distal tibia in adolescent females (N = 40). Cortical CSA = 65.28 + 0.08 * total muscle area, R^2 = 0.73 ■ = Athletes ○ = Control

Multiple regression analysis was used to determine the strongest predictive factors of variability in total body BMC. Total body lean mass and fat mass explained 65% of the variance in total body BMC for the combined groups (N = 40). The regression equation was Total Body BMC = 276.58 + 0.441*lean mass + 0.212*fat mass (R^2 = 0.65).

5.3 Discussion

5.3.1 Primary outcomes: Bone and muscle geometry

The primary purpose of the study in this chapter was to compare bone and muscle geometry at the mid distal tibia as well as BMC at multiple sites, in elite, female adolescent middle distance runners and age and sex-matched controls. Results demonstrated differences between groups in bone and muscle geometric properties at the mid distal tibia. The female athletes displayed greater tibial cortical and smaller medullary cavity CSA than non-athletes, despite an absence of between group differences in total tibial CSA. In addition, the female athletes had greater dominant tibial BMC, even though tibial lengths were similar between athletes and controls. Athletes also displayed greater total and extensor muscle compartment CSA than non-athletes. Differences were detected between athletes and non-athletic controls in total body and regional BMC. When BMC results were adjusted for fat mass, the female athletes showed greater BMC than controls at the total body, dominant proximal femur and dominant lower extremity (hip to ankle). In addition, differences in total body BMC between athletes and controls remained after covarying for height.

5.3.2 Secondary outcomes: Predictive factors of bone geometry and total body BMC

The secondary purpose of the study in this chapter was to examine factors predictive of bone geometry and total body BMC from independent variables. Muscle mass (total muscle CSA) at the distal tibia explained 73% of the variance in tibial geometry (cortical CSA). Lean mass and fat mass combined to explain 65% of the variance in total body BMC. Weight was excluded from the total body regression analysis due to colinearity.

5.3.3 Bone and muscle geometry

In normal populations, after the cessation of growth, limited periosteal apposition coupled with continued endocortical resorption produces a thinning cortical shell, compromising the mechanical competence of bone in later life. The generation of a larger cortical CSA, at either the medullary or periosteal surface is considered an optimal adaptation to resisting compressive and tensile forces in long bones (Heinonen et al, 2002). In contrast, for resisting

bending or torsional forces, increased bone adaptation at the periosteal surface is more favourable. The results from the study in this chapter, indicate that athletes displayed greater cortical and smaller medullary cavity cross-sectional area (CSA) than non-athletes at the mid distal tibia, despite similar total tibial CSA. Other cross-sectional studies of post-menarcheal adolescent runners have similarly displayed either increased apposition or reduced resorption at the medullary surface of the mid-femur, compared with cyclists and swimmers, with no differences between groups in total femur CSA (Duncan et al, 2002). The asymmetrical shape of the tibia at the distal site among participants precluded the standardized measurement of tibial width. Therefore tibial CSA was preferred as a more accurate indication of bone size. Loading during adolescence may further enhance site specific increases in bone mineral and cortical CSA, as well as increase peak BMD, that may potentially advantage current bone strength parameters and protect against, delay or attenuate age-related bone loss (Hernandez, Beaupre, and Carter, 2003). The prospective longitudinal studies required to definitely support the popularly acclaimed link between adolescence and older age musculoskeletal health are yet to be conducted (Seeman, 2002).

Muscle groups must overcome the effects of gravity, body weight and a relatively inefficient lever system to produce movement (Lu, Taylor, O'Connor and Walker, 1997). Given the shared functional role in movement, bone must adapt appropriately to strains induced by muscular contraction. A muscle and bone interaction can be measured using densitometric surrogates of bone strength, such as bone mineral content (BMC), with surrogates of muscle force, namely muscle CSA, to examine musculoskeletal adaptation. Muscle CSA has been reported as a surrogate for muscle force because measurement precision of actual force can be affected by factors that include motivation and mood, particularly in children and adolescents (Schoenau et al, 2002). In the study presented in this chapter, the interrelationship between muscle force surrogate (CSA) and bone strength surrogate (tibial cortical CSA) is evident in the regression analysis, in which 73% of the variance in tibial cortical bone CSA was explained by total muscle CSA. Similarly strong correlations between muscle and cortical bone CSA were shown in healthy children, adolescents, and young adults aged 6 to 22 years (Schoenau and Frost, 2000) and healthy adults aged 18 – 30 years

(Rittweger, Beller, Ehrig, Jung, Ramolla, Schmidt et al, 2000). Subsequently the larger cortical bone CSA displayed by athletes in the present chapter may be influenced by greater muscle CSA at the mid distal tibia compared with controls. However, a limitation is imposed when muscle CSA is used as a surrogate measure of strength given the potential of genetic pre-selection of young females with greater lean tissue mass being attracted to events such as middle-distance running.

5.3.4 Mechanical loading and BMC

Cross-sectional findings of greater BMC and/or areal bone mineral density (BMD) in female children (Bass et al, 2002), adolescents (Duncan et al, 2002) and adults (Kontulainen et al, 2003) participating in a variety of weight bearing activities support the results of the present chapter. Axial load-induced bending and compressive strains generated by ground reaction forces in most of these weight-bearing sports (Duncan et al, 2002) may have strongly influenced the observed adaptations in whole body and site specific BMC. A comparison of BMD in adolescent females from sports associated with axial load-induced bending and tensile forces (running) with weight-supported sports (swimming and cycling), report greater total body and regional BMD in runners compared with swimmers and controls (Duncan et al, 2002). Results from the study in this chapter, highlight that athletes displayed greater tibial BMC compared with controls despite similar bone size between groups. Greater site specific BMC, coupled with enhanced localized bone geometric changes suggest positive adaptations to middle distance running may have occurred. A similar degree of adaptation in BMC in the dominant arms of young female tennis players suggests that other types of mechanical loading may be equally osteogenic (Bass et al, 2002; Kontulainen et al, 2003). To reiterate however, it is possible that the observed differences in BMC and bone geometry between athletes and controls in the present chapter, may have been substantially influenced by genetic factors and selection bias. Athletic females may have benefited from a genetic predisposition to advanced bone structure and a selection bias of young, lean females into athletic pursuits. Self-selection is a recognized potential confounder in all cross-sectional studies therefore findings concerning loading effects between athletes and controls should be

tempered in light of this limitation. Additional bias is also acknowledged in the delimitation of injury free selection criteria for young female athletes in the present chapter.

5.3.5 Moderate loading

Not all studies associate optimal musculoskeletal advantages with running in adolescent females. Sports of different impact loads frequently demonstrate dose-responsive bone adaptations. For example, the high mechanical forces in gymnastics are more likely associated with significant adaptation than lower impact sports such as running or weight-supported sports such as swimming and cycling (Duncan et al, 2002). Greater total body, lumbar spine, proximal femur and femoral neck BMD were found in gymnasts compared with runners (Robinson et al, 1995) and non-athletic participants (Nichols et al, 1994). Our findings of increased BMC in athletes, particularly at the tibia, appear to somewhat oppose previous findings of dose-responsive bone adaptations to high impact loads. We speculate that the low to moderate impact forces experienced in our female adolescent middle distance runners may have been sufficient to produce an osteogenic response in the form of changes to bone geometry and increased bone mineral accrual.

5.3.6 Oestrogen

Circulating oestrogen influences the optimization of bone mineral accrual, increases intestinal absorption of calcium, decreases urinary calcium loss and slows bone resorption. Despite these benefits, females who undertake chronic strenuous exercise are at risk of compromising circulating levels of oestrogen (Shangold and Mirkin, 1994). In the study in the present chapter, although the number of menstrual cycles in the previous 12 months was compared in athletes and controls, it is acknowledged that blood sampling did not coincide with a specific phase of the menstrual cycle for all participants. No exercise–associated primary or secondary amenorrhea was reported by athletes and reports of irregular menstruation (age-related oligomenorrhea) were similar in athletes and controls.

Concomitantly, circulating levels of oestrogen were not different between groups. Subsequently, the findings in this study are unable to support the hypothesis that an increase in circulating oestrogen lowers the mechanostat set point on endosteal bone surfaces (Seeman, 2003). Frost's "mechanostat" theory states that a minimum effective strain needs to be exceeded to initiate bone modeling and potentiate an increase in bone mass (Frost, 1991). Given that oestrogen did not differ between athletes and controls then more beneficial bone and muscle geometry observed in the athletes cannot be associated with differences in female hormone activity. A surrogate of muscle strength (muscle CSA) which may be genetically and / or environmentally determined, best explained variation in distal tibial bone geometry.

5.3.7 Differences in calcium

The female athletes in this study consumed, on average, nearly double the daily calcium intake of controls. The mean daily calcium intake of non-athletic participants was approximately 300 mg less than the recommended dietary intake (RDI), while athletes reported intakes well above this RDI. Calcium however, did not correlate with either total body BMC ($r = 0.19$) or tibial BMC ($r = 0.17$). Results of studies examining the role of calcium in contributing to higher BMD values during growth are equivocal. Some cross-sectional studies of adolescents indicate that higher calcium intakes are associated with higher bone mass at measured skeletal sites (Illich et al, 1998), while others (Bachrach et al, 1990) fail to demonstrate significant correlations between calcium and whole body and regional BMD. Prospective studies involving calcium supplementation provide strongest evidence to support the positive association between calcium intake and bone mineral development during growth (Bonjour et al, 2001). The synergistic or compensatory effects of both weight-bearing exercise and adequate calcium consumption may have produced an increase in total body and regional BMC in the present chapter. However, the limitations previously outlined concerning cross-sectional study design sensibly restrict further speculation of calcium results.

In conclusion, the study presented in this chapter compared bone and muscle geometry, and total body and regional BMC of elite, female adolescent middle-distance runners with age and sex-matched controls. The study also examined factors predictive of bone geometry at the distal tibia as well as total body BMC. Female athletes displayed greater tibial cortical and smaller medullary cavity CSA, greater total and extensor muscle compartment CSA and greater total body and regional BMC than non-athletes. Muscle strength, which was represented by total muscle CSA, predicted distal tibial bone geometry, while lean tissue and fat mass combined to predict total body BMC. The findings support the notion that bone and muscle geometry and BMC at multiple sites in injury-free adolescent females may be advantaged by participation in middle-distance running.

CHAPTER 6

6.1 Assessment of bone strength at differentially-loaded skeletal regions in adolescent middle-distance runners

Understanding of growth-related responses to muscle forces and gravitational loads associated with weight-bearing activity, produce gains in bone mineral content (BMC) that show bone apposition at endosteal or periosteal surfaces (Petit et al, 2002) but geometric advantages associated with bone apposition remain underexplored. Increased bone material and favourable geometric adaptations positively influence bone strength, particularly if bone is accumulated at the greatest distance from the neutral axis (Beck et al, 2001) however geometric data are harder to acquire and less frequently reported.

Structural adaptation of long bones to meet the demands of mechanical loading is highly site-specific (Bass et al, 2002; Heinonen et al, 2002; Heinonen, Sievanen, Kyrolainen, Perttunen and Kannus, 2001). Results from animal (Mosley and Lanyon, 1998) and human studies (Heinonen, McKay, MacKelvie, Whithall, Forster and Khan, 2001; Haapasalo et al, 2000) reveal a greater response to loading in distal than proximal regions of long bones. Subsequently, the type of loading forces experienced at regional skeletal sites can influence adaptive processes. Structural responses to extreme loads imposed in weightlifting for example, reflect differential results for the bending forces imposed in the upper body (distal radius) and the axial, compressive-loading of the lower body (distal femur) (Heinonen et al, 2002). To date, limited data is available on geometric adaptations to mechanical loading in growing girls (Duncan et al, 2002; Heinonen et al, 2000) and boys (Bradney et al, 1998).

Traditional densitometric measures of bone mineral status, such as BMC or areal bone mineral density (aBMD), using Dual X-ray Absorptiometry (DXA), are poor independent indicators of bone strength (Bolotin and Sievanen, 2001). Consequently, densitometry-based techniques for assessing bone structural parameters have been developed. Hip strength analysis (HSA) provides a useful means of estimating bone strength from a two-dimensional image of the proximal femur and is widely reported in paediatric and adult populations. Limitations inherent with single-plane DXA technology and assumptions of bone shape at the femoral neck, particularly in growing children, are well recognised (Nelson and Koo, 1999). Imaging techniques, such as peripheral quantitative computed tomography (pQCT), provide a detailed analysis of cortical and trabecular bone however, bone measures are restricted to

peripheral sites and therefore exclude the proximal femur. An alternate, non-invasive method of assessing *in vivo* bone strength involving a combination of imaging techniques is available. Magnetic Resonance Imaging (MRI) - based cortical bone volume and cross-sectional moment of inertia (CSMI) combined with DXA-derived BMC was applied to adolescent populations (Duncan et al, 2002). Results show female adolescent athletes involved in weight-loaded sport (running) had greater bone strength index (BSI) at the mid femur than athletes engaged in weight-supported sports (swimming and cycling) and non-active controls. Examination of markers of bone strength at regional skeletal sites in groups exposed to different mechanical forces using HSA and BSI will advance existing knowledge of site-specific loading in adolescent sporting populations.

A limited number of studies (McKay, Sievanen, Petit, MacKelvie, Forkheim, Whittall et al, 2004; Link, Vieth, Langenberg, Meier, Lotter, Newitt et al, 2003; Woodhead, Kemp, Blimkie, Briody, Duncan, Thompson et al, 2001) however, have investigated the level of agreement between MRI and DXA to assess bone geometry and biomechanical bone properties. Despite assumptions of cylindrical bone shape at the femoral neck using DXA-derived HSA software, comparability between MRI and DXA at a common regional skeletal site (femoral neck) may allow imaging methods to be used interchangeably. Comparability however, is confounded if one method has poor repeatability, therefore repeat femoral neck scans using MRI or DXA is required (Bland and Altman, 1986).

The primary purpose of the study in this chapter was to explore differences in CSMI derived from HSA at the femoral neck and BSI at the distal tibia between elite, adolescent male and female middle-distance runners and non-active, age- and gender-matched controls. The secondary purpose was to examine factors predictive of HSA at the femoral neck and BSI at the distal tibia for each gender from dependent and independent variables including limb length, lower limb muscular strength, lean tissue mass, fat mass, bone and muscle cross-sectional area (CSA), hours of physical activity per week, and calcium intake. A third purpose was to examine comparability between MRI-derived CSMI values at the femoral neck and

DXA-derived CSMI values at the same region. Additionally, repeatability of CSMI from MRI to biomechanically assess the femoral neck region was undertaken.

The primary hypothesis was that male and female adolescent middle-distance runners will display greater distal tibial bone strength and HSA-derived indicators of bone strength at the femoral neck than age- and gender-matched non-athletic controls. The secondary hypothesis was that a level of agreement between MRI- and DXA-derived CSMI values at the femoral neck will be established following high repeatability of MRI femoral neck scans of adolescents.

Details of the methods used in this study are outlined in Chapter 3. However, to reiterate, the primary outcome measures were BSI at the distal tibia and a marker of HSA at the femoral neck, namely CSMI. Potential factors predictive of distal tibial BSI and femoral neck CSMI included total muscle, extensor, and flexor CSA, lean tissue mass, fat mass, plantar flexion and dorsi flexion muscular strength, calcium intake and hours of physical activity per week.

Following tests for normality, independent t-tests were used to determine differences between groups. Bivariate relationships between distal tibia BSI and femoral neck CSMI with selected descriptive statistics and independent variables were determined by correlation analysis (Pearson correlation coefficient) for combined groups. Multiple linear regression analyses were performed to assess the contribution of strongly correlated variables to predicted variability in distal tibial BSI and femoral neck CSMI. A means-vs-differences (Bland et al, 1986) plot was used to examine comparability of MRI and DXA technologies and repeatability of MRI scans. Kendall's tau correlation coefficient was used to test for the presence of consistent bias (Medcalc®, Version 7.4.4.1., Mariakerke, Belgium)

6.2 Results

6.2.1 Descriptive characteristics

Descriptive characteristics of participants are summarised in Table 6.1.

Females: Compared with non-athletic controls, female athletes were lower in weight (mean -6.47 ± 2.7 kg, p = 0.027), body mass index (BMI) (-3.45 ± 0.9 kg.m^2, p = 0.002) and fat mass (-11.3 ± 1.9 kg, p = 0.001). Female athletes were taller (+3.9 ± 1.7 cm, p = 0.031), consumed more calcium (+572.7 ± 201.2 mg, p = 0.007), had greater LTM (+5.48 ± 1.2 kg, p = 0.001) and engaged in more hours of physical activity per week (+6.9 ± 1.07 hours, p = 0.001) than non-athletic controls. Greater height in female athletes compared to controls was explained by greater femoral length (p = 0.03), not tibial length (p = 0.06). No between group differences were observed for age, plantar or dorsi flexion torque at the ankle, age at menarche, menstrual status or oestrogen levels (p > 0.05). Menstrual status in athletes showed categorical distributions of 10% premenarcheal, 30% with age-related oligomenorrhea and 60% with eumenorrhea. Similarly, the relative distribution for the 3 categories of menstrual status in the control group was 8% premenarcheal, 30% with age-related oligomenorrhea and 62% eumenorrhea. Pearson chi-square analysis demonstrated no differences in menstrual status between groups (p > 0.05). Mann-Whitney *U* tests were conducted on the categorical data of self-reported Tanner stage for maturational development. Reported median values were not different between groups for breast (p = 0.168) or pubic hair (p = 0.129) development.

Males: Compared with non-athletic controls, male athletes were lower in weight (-10.97± 3.5 kg, p = 0.005), BMI (-3.11 ± 0.86 kg.m^2, p = 0.001) and fat mass (-10.93 ± 2.4 kg, p = 0.001). Male athletes displayed greater hours of physical activity per week (+11.9 ± 1.5 hours, p = 0.001) than non-athletic controls. Calcium intake (mg) did not differ between groups (p > 0.05). Mann-Whitney *U* tests were conducted on the categorical data of self-reported Tanner stage for maturational development. Reported median values were not different between groups for genital (p = 0.262) nor pubic hair (p = 0.406) development.

Table 6.1: Descriptive characteristics of adolescent middle-distance runners and age- and gender-matched controls (N = 20 per group)

	Female Athlete Mean (SD)	Female Control Mean (SD)	p value	Male Athlete Mean (SD)	Male Control Mean (SD)	p value
Age (y)	15.9 (1.6)	16.0 (1.8)	0.894	16.8 (0.6)	16.4 (0.7)	0.811
Height (m)	1.66 (0.5)	1.62 (0.6)	0.031	1.76 (0.6)	1.77 (0.8)	0.601
Femur length (cm)	43.3 (1.8)	42.1 (1.8)	0.031	45.7 (1.6)	46.1 (2.3)	0.601
Tibia length (cm)	35.3 (1.9)	34.1 (1.8)	0.063	38.6 (1.7)	39.3 (2.6)	0.282
Weight (kg)	52.2 (6.1)	58.7 (10.8)	0.027	64.4 (6.2)	75.4 (14.7)	0.005
BMI ($kg.m^2$)	18.7 (1.5)	22.2 (4.1)	0.002	20.7 (1.6)	23.8 (3.5)	0.001
Fat mass (kg)	8.2 (2.8)	19.5 (8.4)	0.001	5.6 (2.4)	16.5 (10.7)	0.001
Lean tissue (kg)	41.8 (4.2)	36.3 (3.3)	0.001	56.2 (5.2)	55.5 (6.2)	0.711
Plantar flexion (Nm)	46.7 (11.1)	46.3 (16.3)	0.928	56.9 (12.9)	59.4 (16.1)	0.593
Dorsi flexion (Nm)	18.2 (5.2)	18.5 (8.9)	0.881	21.5 (5.3)	25.2 (6.5)	0.058
Physical activity ($h.wk^{-1}$)	8.9 (4.1)	2.0 (0.7)	0.001	14.1 (5.7)	2.2 (0.7)	0.001
Calcium (mg)	1271 (122)	689 (79)	0.007	1822 (620)	1287 (486)	0.090
Oestrogen (pmol/L)	146.9 (36.2)	152.2 (43.6)	0.862	n/a	n/a	n/a
Age at menarche (y)	12.5 (4.3)	12.4 (3.1)	0.953	n/a	n/a	n/a
Testosterone (nmol/L)	n/a	n/a	n/a	17.4 (7.9)	16.6 (5.2)	0.701

6.2.2 Distal tibia

Absolute values for distal tibial BMC, and bone and muscle geometric properties for all participants are presented in Table 6.2.

Females: Compared with non-athletic controls, female athletes displayed greater BMC (+0.95 $\pm$ 0.40 g, p = 0.028), cortical bone volume (+17.88 $\pm$ 5.30 cm^3, p = 0.002), volumetric cortical BMD (+1.18 $\pm$ 0.54 g/cm^3, p = 0.034), CSMI (+1998.05 $\pm$ 661.80 mm^4, p = 0.005) and BSI (+28037.48 $\pm$ 8214.70 $g/cm^3.mm^4$, p = 0.002) at the 10% ROI. Female athletes had greater tibial cortical bone (+27.23 $\pm$ 9.09 mm^2, p = 0.005) and smaller medullary cavity CSA (-40.41 $\pm$ 17.11 mm^2, p = 0.023) than controls however, no between-group differences were evident in total tibial bone CSA (p > 0.05). Total muscle (+240.2 $\pm$ 86.4 mm^2, p = 0.033) and extensor muscle CSA (+46.9 $\pm$ 19.5 mm^2, p = 0.021), but not flexor muscle CSA (p > 0.05), were greater in female athletes than controls. ANCOVA was used to adjust for differences in LTM and fat mass between female athletes and controls. Group mean differences in BSI were reported after covarying for fat mass (p = 0.011) but not LTM (p > 0.05). Covarying for tibial length was not required because between-group differences in tibial length were not evident.

Males: No differences in BMC or bone and muscle geometric properties at the distal tibial 10% ROI were found between male athletes and controls ($p > 0.05$).

Table 6.2: Distal tibial bone mineral, and bone and muscle geometric properties for all participants (N = 20 per group)

	Female Athletes Mean (SD)	Female Controls Mean (SD)	p value	Male Athletes Mean (SD)	Male Controls Mean (SD)	p value
BMC (g)	8.9 (1.5)	8.0 (0.9)	0.028	12.7 (1.6)	12.7 (1.6)	0.971
Cortical bone volume (cm^3)	81.6 (20.8)	63.7 (11.5)	0.002	80.9 (13.4)	80.4 (8)	0.870
Vol. cortical BMD ($g.cm^3$)	9.2 (1.9)	8.0 (1.5)	0.034	6.4 (1.3)	6.4 (1.0)	0.942
Total CSA (mm^2)	363.1 (31.4)	364.4 (43.7)	0.761	610.9 (83.6)	660.8 (131.8)	0.161
Cortical CSA (mm^2)	195.3 (32.9)	158.1 (23.8)	0.005	423.1 (47.3)	447.5 (65.1)	0.182
Medullary cavity CSA (mm^2)	165.7 (56.5)	206.2 (51.5)	0.023	187.9 (47.2)	213.2 (69.4)	0.183
CSMI (mm^4)	10039.8 (2105.6)	8041.8 (2090.1)	0.005	14438.3 (3885.4)	14279.3 (2740.3)	0.881
BSI ($g.cm^3 \times mm^4$)	92950.3 (30545.6)	64912.8 (20410.5)	0.002	95766.5 (41346.3)	90668.7 (16579.3)	0.610
Total muscle CSA (mm^2)	1695.3 (428.03)	1455.2 (227.8)	0.033	2206.4 (786.8)	2040.7 (319.9)	0.392
Extensor muscle CSA (mm^2)	373.8 (70.7)	326.9 (51.4)	0.021	486.9 (124.8)	418.6 (169.5)	0.263
Flexor muscle CSA (mm^2)	419.5 (36.5)	304.1 (41.6)	0.135	542.2 (134.)	418.3 (169.5)	0.644

6.2.3 Neck of femur

Absolute values for dominant neck of femur bone mineral and geometric properties for all participants are presented in Table 6.3.

Females: Compared with non-athletic controls, female athletes displayed greater BMC (+0.11 ± 0.05 g, $p = 0.04$) and neck length (+3.56 ± 1.30 mm, $p = 0.02$). No other differences between female athletes and controls were found ($p > 0.05$). An ANCOVA was used to adjust for differences in LTM, fat mass and femur length between female athletes and controls. Group mean differences in femoral neck BMC and neck length were reported after covarying for fat mass ($p = 0.007$ and $p = 0.034$, respectively) but not LTM or femur length ($p > 0.05$).

Males: No differences in BMC or bone geometric properties at the femoral neck were found between male athletes and controls ($p > 0.05$).

Table 6.3: Dominant neck of femur bone mineral and bone geometric properties for all participants (N = 20 per group)

	Female Athletes (Mean, SD)	Female Controls (Mean, SD)	p value	Male Athletes (Mean, SD)	Male Controls (Mean, SD	p value
BMC (g)	1.30 (0.15)	1.20 (0.17)	0.04	1.60 (0.20)	1.50 (0.14)	0.41
CSA (mm^2)	153.4 (18.9)	138.7 (19.4)	0.21	184.4 (21.4)	180.2 (17.8)	0.52
CSMI (mm^4)	9233.5 (1750.5)	8583.9 (1949.5)	0.27	13724.1 (3724.3)	15416.5 (3387.9)	0.14
Section modulus (Z)	618.7 (97.6)	581.9 (101.0)	0.25	792.7 (203.1)	873.7 (122.5)	0.14
Neck length (mm)	48.2 (4.4)	44.6 (4.3)	0.02	51.8 (4.9)	53.7 (6.4)	0.30
Neck diameter (mm)	29.1 (1.6)	28.7 (1.9)	0.51	33.0 (1.6)	33.1 (4)	0.84

6.2.4 Correlations of distal tibial BSI and neck of femur CSMI

Females: Bivariate correlations of distal tibial BSI and HSA-derived CSMI at the femoral neck for selected descriptive and independent variables are presented in Figure 6.1. Hours of physical activity per week, total muscle CSA, LTM, and extensor and flexor muscle CSA correlated with distal tibial BSI (r = 0.48 to 0.68). Femoral neck CSA and LTM correlated with HSA-derived CSMI (r = 0.62 to 0.82). No other significant correlations between BSI and selected descriptive and independent variables or femoral neck CSMI and selected descriptive and independent variables emerged.

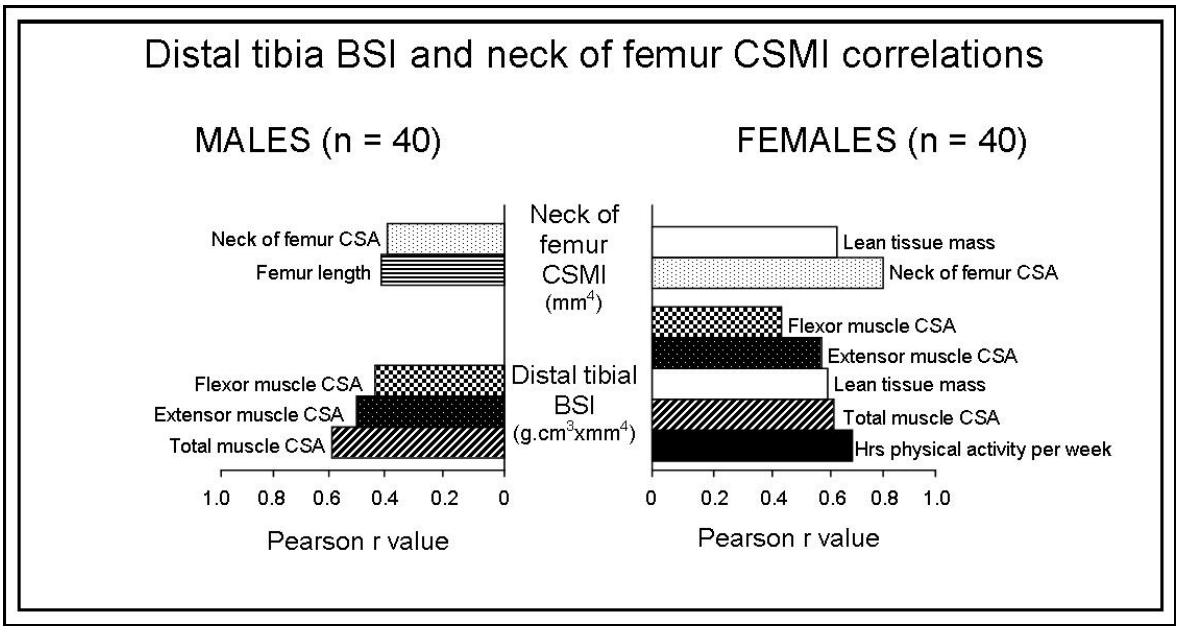


Figure 6.1: Distal tibial BSI and HSA-derived neck of femur CSMI correlations for male and female participants (p < 0.01) (N = 80).

Males: Bivariate correlations of distal tibial BSI and HSA-derived CSMI at the femoral neck for selected descriptive and independent variables are presented in Figure 6.1. Total, extensor and flexor muscle CSA correlated with distal tibial BSI (r = 0.43 to 0.59). Femur length and femoral neck CSA correlated with HSA-derived CSMI (r = 0.41 to 0.42). No other significant correlations between BSI and selected descriptive and independent variables or femoral neck CSMI and selected descriptive and independent variables emerged.

6.2.5 Predictors of distal tibial BSI and neck of femur CSMI

Multiple regression analyses were used to determine the strongest predictive factors of variability in distal tibial BSI and femoral neck CSMI for male and female participants (Figure 6.2).

Females: Hours of physical activity per week and total muscle CSA explained 58.3% of the variance in distal tibial BSI for combined female groups (N = 40). The regression equation was BSI = 24098.7 + 3171*hours of physical activity per week^{-1} + 24.6*total muscle CSA (R^2 = 0.58). Neck of femur CSA explained 64.6% of the variance in neck of femur CSMI for combined female groups. The regression equation was CSMI = -2333.9 + 66.1*neck of femur CSA (R^2 = 0.64).

Males: Total muscle CSA explained 43.5% of the variance in distal tibial BSI for combined male groups (N = 40). The regression equation was BSI = 28065 + 30.7*total muscle CSA (R^2 = 0.43). Femur length and neck of femur CSA explained 33.4% of the variance in neck of femur CSMI for combined male groups. The equation was CSMI = -29349 + 692*femur length + 66.4*neck of femur CSA (R^2 = 0.33).

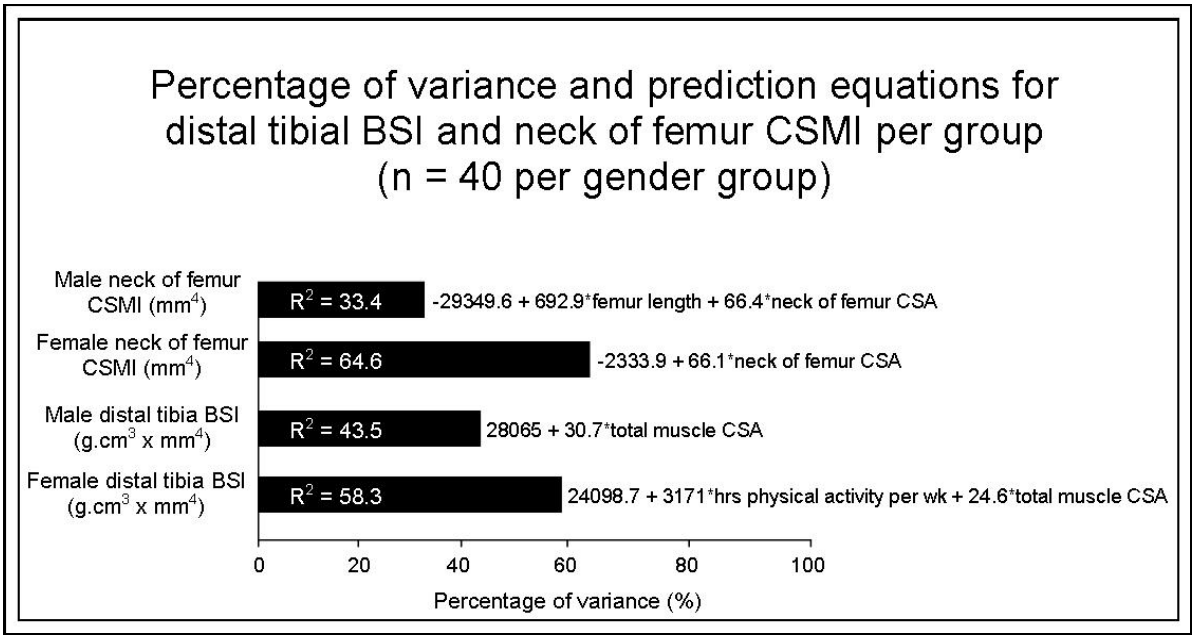


Figure 6.2: Percentage of variance and prediction equations for distal tibial BSI and HSA-derived femoral neck CSMI per group (N = 40 per gender group).

6.2.6 Agreement between methods and repeatability

A mean-vs-differences plot examined the comparability of MRI and DXA technologies, using neck of femur scans (Figure 6.3).

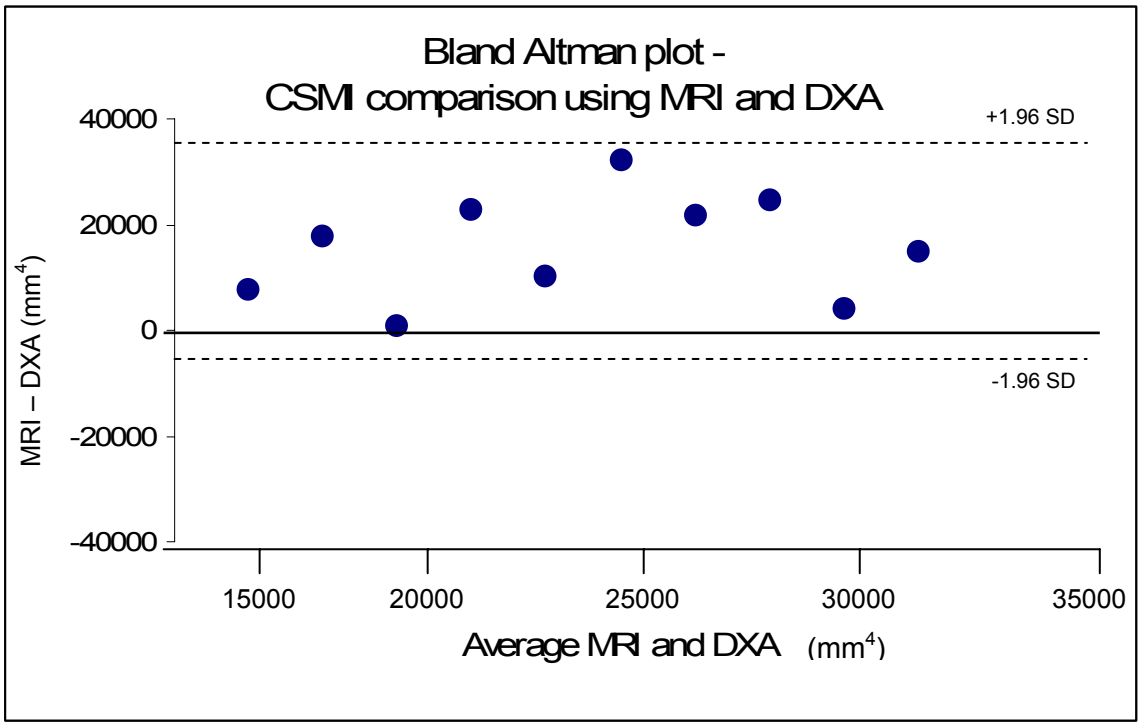


Figure 6.3: Bland Altman plot – CSMI comparison using MRI and DXA at the femoral neck (N = 10)

Results of differences between MRI and DXA were scattered above the line of zero difference indicating a level of agreement between the two imaging techniques. Kendall's tau correlation coefficient was also used to test for the presence of consistent bias. Kendall's tau (b = 0.378, p = 0.152) confirmed a consistent bias and a subsequent lack of systematic bias between the two instruments (Peat, Williams, Xuan and Mellis, 2002). A repeatability analysis of MRI neck of femur scans (N = 20) after repositioning was undertaken (Figure 6.4).

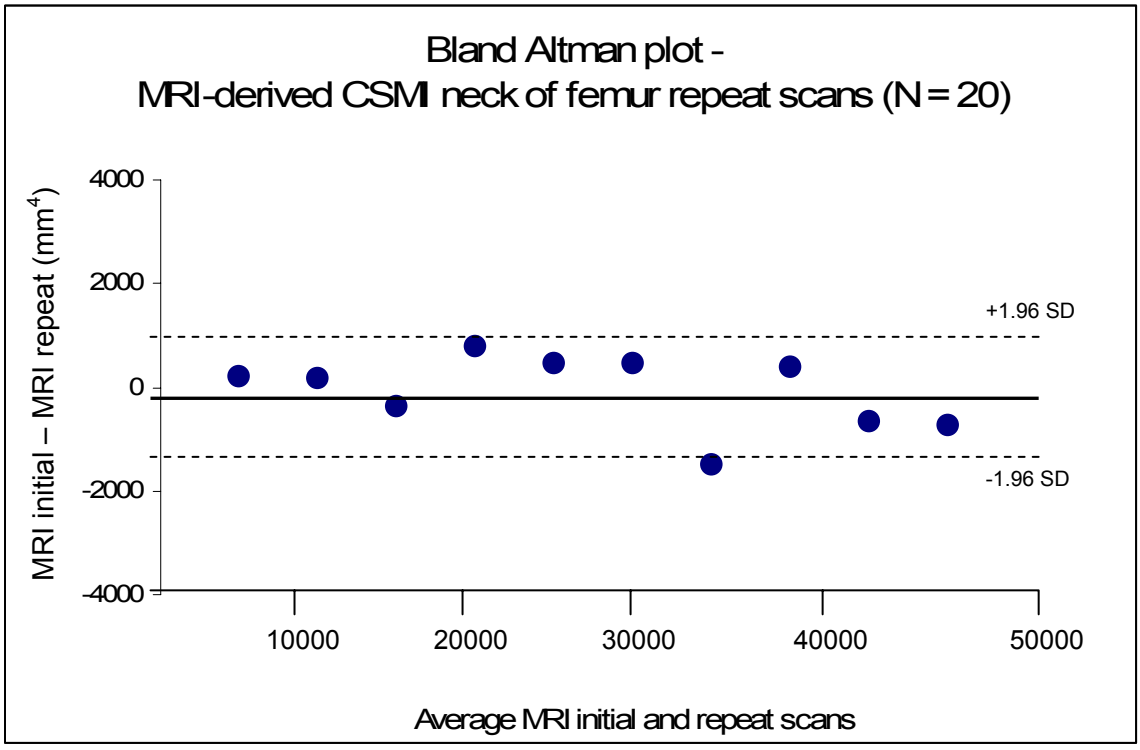


Figure 6.4: Bland Altman plot – MRI-derived CSMI neck of femur scans (N = 20)

Kendall's tau (b = 0.198, p = 0.324) showed a high level of repeatability between the initial neck of femur scans (N = 10) and repeat scans after repositioning.

6.3 Discussion

The relatively novel use of MRI software assisted in the assessment of structural differences at the distal tibia. MRI is a non-invasive imaging technique with superior resolution capable of accurately measuring bone and muscle cross-sectional geometry (Hong, Hipp, Mulkern, Jaramillo and Synder, 1998; Mitsiopopoulos, Baumgartner, Heymsfield, Lyons, Gallagher and Ross, 1998). This is the first investigation to compare regional differences in bone strength markers through HSA at the femoral neck and BSI at the distal tibia between elite, adolescent male and female middle-distance runners and non-active, age- and gender-matched controls. Differences in bone mineral, and bone and muscle geometry were apparent at the distal tibia in female participants however, differences were not evident at the femoral neck. Specifically, female athletes displayed greater BSI, CSMI, cortical bone CSA, total and extensor muscle CSA, volumetric cortical BMD and BMC at the distal tibia than non-active female controls. Differences in femoral neck BMC and neck length between female athletes and controls were removed after covarying for femur length. No differences were found in HSA at the femoral neck or BSI at the distal tibia between male athletes and controls.

6.3.1 Females

Compared with controls, female athletes showed superior distal tibial BSI due to a greater accumulation of bone material (volumetric cortical BMD) and a more optimal distribution of bone (CSMI) about the neutral axis. The findings of greater BSI at the distal tibia in female athletes support the positive bone geometric outcomes previously reported at the mid-femur in weight-loaded athletes. Recently, MRI-derived cortical bone volume (cm^3) and CSMI (mm^4) were combined with DXA-derived BMC (g) to reveal greater mid-femoral BSI in female adolescent athletes involved in a weight-loaded activity (running) compared with athletes engaged in weight-supported activities (swimming and cycling) and non-active controls (Duncan et al, 2002).

Ultimate bone strength varies according to the type of stress applied to bone. In cortical bone, compressive strength is superior to both tensile and shear strength whereas trabecular bone strength varies according to apparent density and trabecular orientation (Reilly and Burstein, 1975; Carter and Hayes, 1977). The two regions examined in the present chapter vary in cortical and trabecular bone composition. The neck of femur is a highly trabecular site enclosed by a thin cortical shell. In contrast, the distal tibial ROI, measured distally between 20% - 30% of tibial length, is largely comprised of cortical bone with a well-defined medullary cavity. The results from the study in the present chapter indicate that female athletes with greater cortical CSA at the distal tibia appear ideally suited to resisting axial compressive strains which predominate during running (Milgrom, Finestone, Segev, Olin, Arndt and Ekenman, 2003). The lesser influence of bending loads during running appears evident because bone size as indicated by total tibial CSA between athletic and control female groups did not differ. It appears the adaptive response at the distal tibia to predominately axial compressive strains during running may be associated with a thicker cortical wall rather than increasing bone diameter to resist lesser-strains from bending loads. The results support similar findings of greater cortical CSA at the distal femur in female weight-lifters exposed to axial compressive loads (Heinonen et al, 2002). Furthermore, examination of the site-specific response of tibial geometry to ground reaction forces in growing females suggests bones tend to be loaded preferentially in compression as opposed to bending (Heinonen et al, 2001).

In contrast to the tibia, the femoral neck can be viewed as a short cantilever beam, fixed at its lateral end and loaded through the centre of the femoral head. As a result, the femoral neck predominately experiences bending (compressive and tensile) stresses although axial compressive loads along the femoral neck are also recognised (Beck et al, 1990). Geometric adaptations at the femoral neck, as evidenced by increased CSMI and section modulus (a measure of bone stiffness), may confer a considerable strength advantage due to the displacement of bone further from the neutral axis. Densitometry-based HSA software integrates geometric information and absorptiometry data by applying engineering beam theory to provide a measure of bone bending strength.

Results in the present chapter concerning HSA-derived indicators of bone strength at the femoral neck are not supported by previous findings. An 8-month exercise intervention program involving pre- and early-pubertal females revealed increased CSA at the femoral neck and a subsequent increase in bone strength in exercising females compared to controls (Petit et al, 2002). A similar exercise intervention study, originally involving pre-pubertal males engaged in a 12-minute high-impact circuit program, three times per week for 20 months, showed greater femoral neck BMC, CSMI and section modulus in exercising males compared to controls (MacKelvie et al, 2004). In the present chapter, differences between female groups in femoral neck BMC and neck length were no longer evident after adjusting for femur length. Previous intervention studies covaried for changes in height between intervention and control groups however, no adjustments for changes in limb length were made. Differences in axial and appendicular growth rates inherent in adolescence do not appear to have been considered. Furthermore, we hypothesize that a lack of difference in bone mineral and bone geometric properties between female athletes and controls at the femoral neck in the present study may be due to load attenuation and a subsequent inability to achieve a minimum effective strain (Frost, 1987). Numerous studies (Bemben, Buchanan, Bemben, Knehans, 2004; Taaffe, Robinson et al, 1997; Robinson et al, 1995) have established a link between high-impact loading activities, such as gymnastics, and increased bone mineral, geometry and bone strength at the femoral neck. Repetitive, low-intensity loads from moderate ground reaction forces experienced in middle-distance running may be insufficient to produce an osteogenic effect at the femoral neck, particularly if loads were reduced by an efficient, well trained execution of musculoskeletal activity.

A synergistic relationship between bone and muscle development is believed to occur during growth. As muscle mass responds to increased loading, a corresponding adaptation in bone geometry may result from increased muscular contraction (Frost and Schonau, 2000). Proportionality allows muscle CSA to be used as a surrogate of muscle strength (Powell, Roy, Kanim, Bello and Edgerton, 1984). Several studies (Schoenau et al, 2002; Burr, 1997; Nordstrom, Thorsen, Bergstrom and Lorentzon, 1996) have demonstrated a direct cause-and-effect relationship between muscle strength and bone variables such as bone mass and

strength. Furthermore, a site-specific relationship between bone and muscle geometry has been shown in pre- and post-pubertal females (Heinonen et al, 2001) and male and female young adults (Rittweger et al, 2000). Results from the study presented in this chapter show that female athletes displayed greater total muscle and extensor muscle CSA at the distal tibia than female controls. The strong influence of total muscle CSA on distal tibial BSI is highlighted in the BSI correlation and regression analyses. Aside from hours of physical activity per week, local musculature at the distal tibia and whole-body LTM correlated strongly with distal tibial BSI. The findings are consistent with the assumption that greater muscle size places a greater strain on bone and produces a corresponding increase in bone mass and strength (Schoenau et al, 2002). Greater muscle CSA in female athletes however, did not produce an increase in bone size compared with controls. Similar tibial CSA between groups coupled with a smaller medullary cavity CSA in female athletes suggests bone acquisition in female athletes may have occurred on the endosteal bone surface. We postulate that axial compressive forces experienced during running are more dominant forces at the distal tibia than bone strain from muscle contraction and as such, the protective effect of greater bone mineral accrual on endosteal bone surfaces to withstand axial compressive forces has emerged.

6.3.2 Males

In contrast to marked differences in bone mineral, and bone and muscle geometry at the distal tibia between female athletes and controls, no differences were found between male participants in bone mineral, bone and muscle geometry or bone strength at the distal tibia or HSA-derived indicators of bone strength at the femoral neck. A possible explanation for the observed lack of difference between male groups concerns the greater propensity for muscle development inherent in adolescent males. In order the minimise damage to bone, muscles assist in maintaining strain levels and strain rates within normal physiological ranges (Wosk and Voloshin, 1981). Strain gauges mounted in the mid diaphysis of the medial tibia revealed a dissipation of higher strains associated with high impact exercises (Milgrom et al, 2000). Tibial strains during 52 cm drop jumps were identical to strains measured during running.

Attenuation of strain levels were postulated to have occurred due to the shock absorbing, protective nature of supporting muscles. Similar to female groups, the influence of muscle CSA on distal tibial BSI in male participants is evident in the correlation and regression analyses. Total muscle CSA alone explained 43.5% of the variance in bone strength at the distal tibia, highlighting the synergistic bone / muscle relationship. Furthermore, differences in relative lean tissue mass composition between athletes and controls were 18% in females and 13% in males. The greater difference between female athletes and controls in relative lean tissue mass may partially explain greater differences than males in bone mineral and geometric properties

The results presented in this chapter however, are not fully supported by previous research examining muscle size and bone strength in adolescent tennis players. Side-to-side differences in muscle CSA accounted for only 12-16% of the variance in bone properties, namely bone mass, geometry and strength. Common genes regulating both muscle and bone size, as well as nutrition and hormonal factors, were suggested as potential mediators of the bone / muscle relationship. Muscle development, and to a lesser extent bone development, is influenced by the anabolic effects of testosterone (Crawford, Liu, Kean, Bleasel and Handelsman, 2003). In adolescent males, peak plasma testosterone levels occur during Tanner stages 3 to 4 (Rogol, 1994). Male athletes in the present study did not differ from controls in testosterone or muscle CSA. Furthermore, differences in body mass between male athletes and controls were explained by fat mass not LTM. It is possible that stronger homogeneity of muscle development in boys than girls, commensurate with elevated sex hormones during adolescence, may have offset the osteogenic influence of mechanical loading.

In the study presented in this chapter, reported hours of physical activity per week for male and female controls were similar. Although it was beyond the scope of the present chapter to quantify intensity of physical activity, we speculate that male controls may have engaged in more vigorous physical activity than female controls. Examination of physical activity patterns and exercise intensity throughout childhood and adolescence reveals a consistent gender

difference, with males more active and more likely to engage in vigorous physical activity than females (Rowlands, Ingledew, Powell and Eston, 2004;Trost, Pate, Sallis, Freedson, Taylor, Dowda et al, 2002). We postulate that within the minutes of reported physical activity a greater potential for vigorous activity may have occurred in male controls than female controls. Gender differences in intensity of physical activity may partially explain the lack of difference in markers of musculoskeletal health between male runners and controls. The gap between sedentary and vigorous physical activity in adolescent males is postulated to be less than activity intensity with adolescent females.

6.3.3 Strengths and limitations of the chapter

A limited number of studies (Daly et al, 2004; Duncan et al, 2002; Bass et al, 2002) have combined MRI with DXA-derived data to examine bone strength in children and adolescents. To our knowledge, no study has explored two differentially-loaded skeletal sites using MRI and DXA technology to examine bone strength at the distal tibia and indicators of bone strength at the femoral neck in male and female adolescent athletes exposed to similar training loads. The planar nature of DXA, which provides an acceptable indication of bone mineral status, was supplemented with MRI-derived measurements of bone geometry to achieve a more accurate assessment of bone strength at the distal tibia. Current methods of examining bone strength at the femoral neck however, are largely limited to DXA-based HSA software. Methodological limitations inherent with DXA technology, such as the calculation of CSMI and section modulus in the scan plane only, could therefore not be avoided (Nelson et al, 1999). Results from animal (Mosley, March, Lynch and Lanyon, 1997) and human studies (Heinonen et al, 2001) show that skeletal adaptation to loading varies in the medial-lateral and anterior-posterior planes.

Results presented in this chapter also highlight comparability between MRI and DXA technologies. A comparison of imaging methods designed to biomechanically assess osteogenic responses in active and non-active populations is infrequently reported (McKay et

al, 2004; Link et al, 2003; Woodhead et al, 2001). A high level of repeatability for MRI scans at the femoral neck supported the level of agreement between MRI and DXA. Despite the small sample size, results suggest MRI may be used interchangeably with DXA, particularly in paediatric populations repeatedly exposed to radiation sources.

The cross-sectional nature of the present study restricts the impact of the findings presented in this chapter. It cannot be discounted that observed differences in distal tibial BSI between female athletes and controls were due to genetic bias and / or self selection. Results from animal research however, suggests the propensity of bone to respond to mechanical loading is only partially influenced by genes (Robling and Turner, 2002). Inbred strains of mice with large bone cross-sections did not appear genetically predisposed to greater mechanosensitivity than mice with smaller bone cross-sections. Results stimulate debate on that the extent of the role of genetics in influencing skeletal adaptation.

In conclusion, the study presented in this chapter explored two differentially-loaded skeletal regions using innovative imaging assessment techniques. The techniques, selected for their appropriateness at each skeletal site, revealed greater bone strength at the distal tibia in female athletes compared with non-athletic, female controls. Similar differences in bone strength between female groups however, were not found at the femoral neck. Despite similar exposure to female training loads, male athletes did not appear advantaged in bone strength at the distal tibia or femoral neck when compared with non-athletic, male controls. A level of agreement between imaging methods was established in addition to a high level of repeatability for MRI scans at the femoral neck. The findings in the present study suggest that a positive musculoskeletal response to repetitive mechanical loading, particularly at the distal tibia, appear enhanced in female middle-distance runners but not male middle-distance runners.

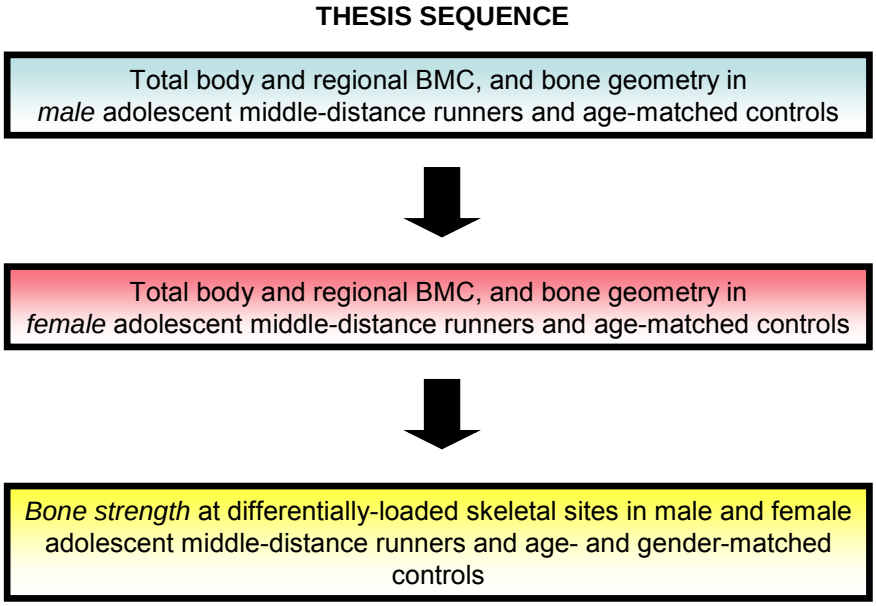
CHAPTER 7

Conclusion

7.1 General overview

This is the first investigation to examine markers of musculoskeletal health in elite, male and female adolescent middle-distance runners and age- and gender-matched controls. The application of DXA and MRI provided new information about bone and muscle material and geometric properties in male and female adolescents exposed to two different levels of habitual mechanical loading. The results advance the current understanding of the potential musculoskeletal responses to loading imposed on the distal tibia by middle-distance running using a combination of imaging modalities.

7.1.1 Thesis sequence



The intention of the studies in this thesis was to define musculoskeletal adaptation in male and female adolescent athletes with highly repeatable measures of bone mineral and bone material properties. The selected sequence of studies provided benchmarks for comparisons

with current literature and theories. Current criticisms of the use of DXA technology for BMD encourages a higher order of precision from alternative measures such as MRI. Despite the acknowledged advances in the precision of MRI, DXA analysis provides an exciting and relevant medium for reporting bone mineral at differentially-loaded skeletal sites throughout the body. The opportunity to combine DXA and MRI advances the possibilities to report bone material, geometric and biomechanical properties. The focus on distal tibial adaptations in adolescent middle-distance runners supports a trend in the literature to target measures at optimal sites for bone loading and high risks of fracture. Continued use and combinations of DXA and MRI technologies among athletic populations are supported by the findings in Chapters 4 to 6.

Differences in bone and muscle geometry advantaged female athletes compared with age- and gender-matched controls however, similar differences between male groups were not evident. Bone biomechanical analyses at the distal tibial revealed a similar pattern. Female athletes exhibited greater CSMI than female controls yet no differences in distal tibial CSMI were evident between male groups. Combining DXA and MRI technologies to estimate *in vivo* bone strength revealed further evidence of a gender-based trend. Greater bone strength at the distal tibia in female runners compared to female controls was again, unsupported by similar findings between male participants.

7.1.2 Hypotheses

Three hypotheses were tested in this thesis:	
(i)	male adolescent middle-distance runners will display greater total body and regional BMC and distal tibial bone geometry than age-matched non-athletic controls
(ii)	female adolescent middle –distance runners will display greater total body and regional BMC and distal tibial bone geometry than age-matched non-athletic controls
(iii)	male and female adolescent middle-distance runners will display greater distal tibial bone strength and HSA-derived indicators of bone strength at the femoral neck than age- and gender-matched non-athletic controls

(i)

The hypothesis that total body and regional BMC and distal tibial bone geometry would be greater in male adolescent middle-distance runners compared to age-matched controls, was *partially* supported.

Specifically, no differences in unadjusted values of total body, lumbar spine, proximal femur and femoral neck BMC between athletes and controls were found. However, after adjusting for the influence of fat mass, athletes displayed greater BMC at the lumbar spine, dominant proximal femur and dominant leg than non-athletic controls. No differences in bone or muscle geometry at the distal tibia were found between groups.

(ii) The hypothesis that total body and regional BMC and distal tibial bone geometry would be greater in female adolescent middle-distance runners compared to age-matched controls, was *fully* supported.

Specifically, athletes displayed greater unadjusted BMC at the proximal femur, dominant femoral neck and dominant tibia than controls. Furthermore, after adjusting for the influence of fat mass, greater total body, dominant proximal femur and leg (hip to ankle) BMC was shown by athletes compared to controls. Female athletes also displayed greater distal tibial cortical CSA, extensor and total muscle CSA, subcutaneous fat CSA, and a smaller medullary cavity CSA than non-athletic controls.

(iii) The hypothesis that male and female adolescent middle-distance runners would display greater bone strength at the distal tibia and greater HSA-derived indicators of bone strength at the femoral neck than age- and gender-matched controls, was *partially* supported.

Specifically, female athletes displayed greater BSI at the distal tibia compared to female controls. No differences in HSA-derived indicators of bone strength at the femoral neck were found between female athletes and controls. Male athletes and controls did not differ in distal tibial bone strength or HSA-derived indicators of bone strength at the femoral neck.

7.2 Strengths and limitations

7.2.1 Strengths

A strength of this thesis was the integration of current imaging technologies to assess bone geometric and bone strength parameters. In particular, the application of MRI technology to examine a regional skeletal site in middle-distance athletes at high fracture risk was novel.

Traditional densitometric measures of bone mineral status were exceeded in this thesis. Access to DXA and MRI technology ensured the capacity to report geometric and

biomechanical analyses of the distal tibia. The knowledge acquired extends the understanding of potential responses to tibial loading during adolescence.

Advancing the understanding of osteogenic responses in active adolescents remains an important strength of this study. Adolescent middle-distance runners are habitually exposed to considerable impact loads during high volumes of training and competition. The potential to identify risk of fracture or compromised musculoskeletal health with high loading remains critical in young populations. Furthermore, the selection of distal tibia is supported by current concerns regarding the potentially injurious nature of the skeletal site during running. Results from the thesis were unable to identify any compromise to bone properties at the distal tibia in male and female athletes. An understanding of the osteogenic response to athletic training during growth may assist in the establishment of developmentally appropriate training programs.

7.2.2 Limitations

The cross-sectional study design limits the generalisability of the findings beyond adolescent middle-distance runners and well matched controls. Differences in markers of musculoskeletal health between athletes and controls may be influenced by genetic bias and self-selection factors. An absence of analysis of genetic bone markers is an acknowledged limitation of the study. Furthermore, cross-sectional studies cannot directly predict the degree of variance in bone parameters attributed to training responses. A future investigation into gene-environment interactions in bone responses to training is supported by the results of this thesis.

An additional limitation may have occurred in the use of networks of people to recruit athletes. A potential bias is acknowledged by recruiting support from coaches and athletes associated with the New South Wales Institute of Sport and the homogeneity of training loads and competition schedules inherent in Institute programs.

The nature and type of loading at the distal tibia during the action of running was assumed to be a combination of axial, compressive and bending forces. It was beyond the scope of the thesis to quantify load at the distal tibia. Field-based assessment of load quantities would strengthen future research with adolescent middle-distance runners.

7.3 Concluding remarks

The thesis advances the knowledge of musculoskeletal health in active and non-active adolescents. Female adolescent athletes appear more responsive to loading than male adolescent athletes. Although speculative, repetitive bone loading associated with habitual activity may less frequently achieve minimum effective strain thresholds in non-athletic females than non-athletic males. Furthermore, male controls may be inherently more "vigorous" in their physical activity patterns than female controls. It is also possible that female controls may be more sedentary during inactive periods than male controls.

Despite the uncertainty of causal mechanisms, disparity between active and non-active adolescents in lean tissue and fat mass is reduced in male participants compared to female participants. The positive musculoskeletal outcomes reported in this thesis appear to support current coaching / training principles of adolescent athletes and highlight the benefits of engaging in regular physical activity during growth.

The thesis provides further support of site-specific skeletal responses to different types of loading forces. Results highlight the need to move from bone mass to bone geometry and bone strength as sensitive and clinically desirable indicators of bone health. Additionally, results support the muscle / bone unit paradigm and highlight the protective role of muscle during excessive compressive and bending forces. Attenuation of potential osteogenic responses to axial, compressive loads appears evident between the distal tibia and femoral neck region. The usefulness of MRI three-dimensional technology to examine bone geometry and bone biomechanical indices has been highlighted throughout the thesis. Furthermore, support for the integration of imaging modalities to estimate *in vivo* bone strength has been shown.

7.4 Recommendations

The opportunity to track musculoskeletal changes in pre-, peri, and post-pubertal middle-distance runners and non-active peers would provide additional understanding of musculoskeletal adaptations throughout maturation. Gains in bone mineral, geometry and strength throughout the pubertal growth period would strengthen the awareness of gender-specific trends.

The application of MRI technology to examine trabecular adaptations to loading would greatly advance the knowledge of osteogenic responses at the micro-architectural level. Improvements in MRI resolution and associated software would provide a unique and non-invasive perspective of how individual trabeculae respond to different forces, how trabecular bone collectively protects against impact loads and how trabecular bone responds to loading throughout growth.

The effectiveness of MRI technology as an alternate method of assessing bone strength has been shown in the present study. If radiation dosage from repeated DXA scans during growth prohibits continued exposure, MRI may serve as a viable assessment tool. Despite concerns of radiation exposure and peripheral limitations, peripheral quantitative computed tomography has emerged as an alternative for trabecular analyses. Access to all three imaging modalities may be required to address musculoskeletal concerns in future research with young active populations.

Portable load devices could be worn by athletes to examine ground reaction forces during running to accurately quantify load, assess cumulative loading and load attenuation throughout a training session or periodised program. Bone mineral and geometric adaptations could be analysed according to load intensity and cumulative load exposure.

A follow-up study after the cessation of athletic training would provide an indication of the sustainability of musculoskeletal gains. Retrospective assessment of retired athletes

commonly relies on athlete recall of previous training volumes. In the present study, exposure time to weight-loading activities has acceptable reliability and the outcomes from the thesis provide the foundation for extending to a prospective study involving the current participants.

Appendix 3.1 Ethics application

<u>SHORT TITLE OF PROJECT</u>: Exercise Type, Musculoskeletal Development and Injury Risk Factors In Elite Adolescent Athletes

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SCIENTIFIC PROTOCOL

1. AIM OF PROJECT/HYPOTHESIS TO BE TESTED

The primary aims of this study are: **(i) To investigate whether intense sports participation involving different mechanical loading patterns on bone, influences bone mineral, material properties, geometry, micro-architecture, biomechanical indices, estimated bone strength, and bone anabolic factors and metabolism, and (ii) To compare skeletal adaptations (above) between athletic and non-athletic adolescents, and female and male elite adolescents athletes participating in the same sport.**

The secondary aim is: To determine whether exercise-related skeletal adaptations correlate with: injury history, training load, sexual hormones, daily activity, aerobic fitness, body composition, nutrition, muscle strength and morphology, biomechanical measures, pubertal status, & menstrual status for post-menarcheal girls.

2. **SIMPLE DESCRIPTION -** Bone-related injuries in young athletes place them at risk of decreased performance and career prospects. An investigation of bone status and associated variables is proposed to predict factors contributing to bone-related injury in elite adolescent male and female athletes involved in sports of different mechanical loads. Dual X-ray Absorptiometry (DXA), ultrasound, and magnetic resonance imaging (MRI) will be used to examine bone mineral, material properties geometry, and biomechanical and strength indices. Data will be combined with potential causative factors (eg biomechanical analysis of lower extremities, nutrition, activity, and hormonal profiles) to determine factors that may modulate risks of bone injury. The study will advance knowledge in bone injury detection, clinical management, and ultimately injury prevention in young sports participants.

3. BACKGROUND/LITERATURE REVIEW

Sports specific changes in bone status among adolescents - Sports differentiation in bone mineral content, geometry, and micro-architecture can result when participation imposes repetitive mechanical loads on specific skeletal segments (Branca, 1999). Research from the Children's Hospital has good reliability and validity of techniques and protocols to study bone mineral, material properties, geometry and micro-architecture. Results indicate that increasing weight-bearing exercise in adolescent (Duncan *et al.* 2001, Farpour-Lambert *et al.* 2000) and pre-adolescent (Wiebe *et al.* 2000, Woodhead *et al.* 2000) populations induces positive musculoskeletal adaptations. Because lower areal BMD has been observed in amenorrheic adolescent and adult female distance runners when compared with eumenorrheic athletes from the same (Pettersson *et al.* 1999) or different training backgrounds (Louis *et al.* 1999) in some but not all (Moen *et al.* 1998) studies, more research is required.

Bone related injuries among adolescent athletes – Although current research is limited, bone stress in lower extremities of younger athletes is more likely to occur in the tibia than fibula (as is the case in adults) (Micheli, 1996) and are also more prevalent in the proximal than distal tibia (Davies *et al.* 1988, Kozlowski *et al.* 1992, Tyrell *et al.* 1994). Conditions such as tarsal navicular stress (Torg *et al.* 1987), varus alignment and pes planus can frequently appear with lower limb bone stress reactions (Bruns & Maffulli, 2000). Podiatory assessment is therefore warrented.

Summary - The primary measures and relative contribution of factors affecting bone status and injury require further research (Nattiv & Armsey, 1997). Large biological variations among individuals make identifying a single marker of risk of bone injury elusive. Nevertheless, injuries may prematurely and perhaps permanently damage young athletes' prospects. Therefore, knowledge gained from the proposed study will be used to prescribe more effective screening, management and prevention of bone injury in active adolescents.

4. METHODS:

a) PARTICIPANT SELECTION AND RECRUITMENT

Adolescent Athletes. Athletes recruited for this study will be 20 male & 20 female middle distance runners, and 20 male & 20 female soccer players (aged 14-18 years) who are associated with the NSW Institute of Sport or who are representing their sport at a state level. As well as the inclusion criteria listed for controls, athletes will need to have been training in their sport for ≥6 hr/week for the past 2 years.

Adolescent Controls. The control group will comprise of 40 females and 40 males will be recruited for the control group from peers of athletic participants. Should insufficient non-athletic controls be available via this recruitment method, local schools will be approached for additional volunteers. Adolescent controls should participate in <3 hr/week of organised activity including activities during school hours. Adolescents will be eligible to participate if they meet each of the following criteria: (i) caucasian ethnicity (height and weight ± 2 SD – NHANES II) (ii) of similar gender and age to sporting groups [Sporting groups will be matched for gender and pubertal status] (iii) in good health and no recent (past two years) hospitalisation and no history of systemic illness lasting more than 2 weeks (iv) no known history of metabolic bone disease and no medication, hormones (including the Oral Contraceptive Pill), or calcium preparations that may influence bone metabolism taken in the preceding

6 months. (v) control girls who are post menarcheal will need to have a normal menstrual cycle (≥8 menstrual cycles in the past 12 months).

b) **POWER ANALYSIS -** The proposed sample size is based on observed sports-associated effect sizes for total body and site-specific measures of areal (gm/cm^2) bone mineral density ranging from 0.5 - 2.0 SD in studies involving adolescents (Soderman *et al.* 2000, Pettersson *et al.* 2000). We will accept a moderate effect size of 0.75 SD difference for clinical significance between non-athletic control and athletic groups for the areal BMD measures in this study. Using ANOVA, a sample size of 20 in each of the athlete and control groups would detect statistically differences at $P<0.05$ with a statistical power of 80%

c) **INTERVENTION**- No direct experimental intervention is involved in this study. The case control study will involve two phases. Phase 1 is cross-sectional and involves 80 athletes and 80 non-athletic controls. Phase 2 is longitudinal and involves follow-up analyses of participants (providing data on 2 years of adolescent growth).

d) MEASURING INSTRUMENTS – (i) PRIMARY OUTCOMES

Bone Mineral - A Lunar Scanner dual-x-ray absorptiometry (DXA)) will measure areal bone mineral density (BMD g/cm^2) for the total body, lumbar spine (L_{2-4}), both hips (Dual Femur software) & tibia (AP Spine software). DXA also gives reliable estimates of lean and fat mass. (Coefficients of variation {CV} range between 1.0% for lumbar spine & <1.0% for total body (Lunar))

Bone Material Properties - Quantitative ultrasound of the calcaneum will estimate the properties of elastic modulus and breaking force of the bone as well as the trabecular orientation and pattern (In vivo CV ranges 0.43% - 2.5%, CHW).

Bone Geometry and Trabecular Micro-architecture - Magnetic resonance imaging-MRI (Philips 1.5 Tesla) will be used to measure bone geometry of the mid-third of the dominant proximal tibia and to enable calculations of bone organ, medullary cavity and cortical bone cross-sectional areas and volumes (ANALYZE software). (CV of 3.2% for volume measures (Woodhead *et al.* 1998)).

Trabecular Bone Architecture. Trabecular bone architecture of the proximal third of the tibia will be determined using data from MRI and a protocol modified from the work of Ouyang *et al.* (1997). (Short term CV of <2.25%, & long term CV of <5.1%)

Bone Stress Reaction - An MRI "STIR" sequence (Short Tau Inversion Recovery) will be used to detect bone stress (oedema & periosteal reaction) in the proximal tibia, navicular bone and metatarsals (Arendt & Griffith 1997).

Bone Biomechanical Characteristics - MRI dedicated software will enable investigators to determine the Cross-sectional Moment of Inertia (CSMI)(mm4) (CV <3%, Yoshikawa *et al.* 1995) and Bone Strength Index (Beck *et al.* 1996). Additional software will enable assessment of Hip Strength Index using data from the DXA scan.

Foot Biomechanics - Ankle motion, tibial alignment, subtalar motion, and static and dynamic characteristics of the foot arch will be assessed by a podiatrist. The podiatrist will be blinded to the results of other primary and secondary outcomes.

(ii) SECONDARY OUTCOMES

Bone Markers and Anabolic Bone Factors - Fasting blood samples of 30 ml will be collected in all participants. Markers of bone formation and resorption (Szulc *et al.* 2000) will be assessed. The insulin-like growth factor I (IGF-I) and its associated binding proteins IGF-$BP_{1,3,5}$. will provide indicators of growth processes.

Personal / medical history - **Participants will complete the Medical Questionnaire that is currently used for all athletes at the NSW Institute of Sport.**

Anthropometry and Body Composition - Height and weight will be measured and lean and fat mass will be determined using software from DXA scans (Lunar).

Pubertal Development - Pubertal status will be self-determined (Duke *et al.* 1980, Tanner, 1962). Respective samples of testosterone and estrogen will assist in describing pubertal stage in male and female adolescents participating in the study.

Nutrition - Nutrient intake will be determined from 3 day food records (including one weekend day) (Crawford *et al.* 1994) (Foodworks™ Version 2.1).

Training load and daily activity (ActiWatch, Questionnaires) - Activity for the previous 12 months and for the 3 days corresponding to diet assessment will be determined by questionnaires (Aaron, 1995). Training diaries of athletes will also be reviewed. Objective measures of activity will be obtained over a 7-day period using of a motion sensor worn on the wrist (ActiWatch) (Finn & Specker, 2000)

Maximal aerobic capacity - Aerobic fitness is best measured in children with a peak oxygen consumption (VO_2max) test using gas analysis (Rowland, 1996).

Muscular Leg Strength - Maximal voluntary isokinetic (60 & 300 degrees/second) knee extension and flexion strength will be assessed using a Cybex II dynamometer.

d)**ANALYSIS OF DATA/STATISTICS –** Group differences in primary key outcome variables (eg areal bone density, volumetric bone density, tibial volumes and cross-sectional areas, calcaneal ultrasound) will be determined by ANOVA, or ANCOVA for variables such as lean mass, and pubertal status. Differences among groups will be tested by an appropriate post hoc analysis (significant at $P< 0.05$). Bivariate correlation and multiple regression analyses will describe relationships among dependent (primary key outcomes) and independent (eg bone injury history, pubertal status, muscle size, blood markers of bone metabolism and growth) variables.

(d) **INTERPRETATION OF RESULTS –** The results may show differences in male and female athletes from sports involving similar or different types of mechanical loads. The results may also help identify the contribution of secondary factors that best predict bone related injuries in adolescents.

5. QUESTIONNAIRES TO BE USED

The food record questionnaire will be the same as that used in the study by Cowell and colleagues entitled “The effect of intense exercise training on bone density and architecture in children”, which received CHW Ethical approval. Activity questionnaires have been previously used in studies of Prof. Blimkie and colleagues. The Sports Injury History survey has been provided from the NSW Institute of Sport.

6. REFERENCES

Arendt E & Griffiths H The use of MR imaging in the assessment and clinical management of stress reactions of bones in high-performance athletes. **Clin Sports Med** 16(2): pp 291-306, 1997
Beck T *et al* Dual-energy X-ray absorptiometry derived structural geometry for stress fracture prediction in male U.S. Marine Corps recruits. **J Bone & Min Res** 11:645-653, 1996
Branca F. Physical activity, diet and skeletal health. **Pub Health Nutr**. 2(31): 391-6, 1999.
Bruns W, Maffulli N, Lower limb injuries in children in sports, **Clin Sports Med** 19(4):637-62, 2000

Davies AM *et al*. MRI of fatigue fractures of the long bones of the lower limb. **Br J Radiol** 67: 332-8, 1994

Duke PM *et al.* Adolescents' self-assessment of sexual maturation. **Pediatrics** 66: 918-920, 1980.
Duncan C *et al.* Bone mineral density in adolescent (15-18 y) female athletes: Relationship to exercise type, anatomical distribution of loads and muscle strength. (submitted) **Med Sci Sports & Exerc** 2001
Farpour-Lambert *et al* Bone material properties in children with IDDM (Abstract) **Bone** 27(4 Supp) 15S, 2000
Finn K & Specker B. Comparison of Actiwatch activity monitor and children's activity rating scale in children. **Med. Sci Sports & Exer**. 32, 1794-1797, 2000
Kozlowski K *et al.* Midshaft tibial stress fractures in children (report of four cases). **Australas Radiol** 21: 173-6, 1992
Louis O *et al.* Low vertebral bone density values in young non-elite female runners. **Int J Sports Med** 12(2): 214-7, 1991
Nattiv A & Armsey T Stress injury to bone in the female athlete. **Clin Sports Med** 16: 197-224, 1997
Micheli LJ Overuse injuries in the young athlete. In **The Child & Adolescent Athlete** 1996 pp189-201
Moen SM *et al.* Lumbar bone mineral density in adolescent female runners. **J Sports Med Phys Fitness** 38(3): 234-9, 1998
Ouyang *et al.* High resolution magnetic resonance imaging of the calcaneus: Age-related changes in trabecular structure and comparison with dual X-ray absorptiometry measurements. **Calcif Tissue Int** 60(2):139-47, 1997
Pettersson U *et al* Effect of high impact activity on bone mass and size in adolescent females. **Calcif Tissue Int** 64:117-25, 1999.

Rowland TW **Developmental Exercise Physiology**, Human Kinetics, USA, 1996
Soderman K *et al.* Bone mass and muscle strength in young female soccer players. **Calcif Tissue Int** 67: 297-303, 2000

Szulc P *et al* Biochemical measurements of bone turnover in children and adolescents. **Osteoporos Int** 11(4):281-94 2000
Tanner JM **Foetus into Man**, Oxford, London 1962
Torg JS *et al*. Overuse injuries in sport: the foot. **Clinics in Sports Med** 6(2):291-320, 1987.
Tyrell PNM & Davies AM. MRI of fatigue fractures of the long bones of the lower limb. **Br J Radiol**. 67: 332-8, 1994
Wiebe P *et al.* Relationship between BMD, muscle strength and physical activity in prepubertal girls. **Med Sci Sports & Ex** 32(5): (Suppl), S299, 2000
Woodhead H *et al*. Mid-femoral bone geometry: validity and reproducibility of magnetic resonance imaging and dual energy x-ray absorptiometry measurements. **J Bone Min Res** 23:5 (Supplement), S408, #W487, 1998.
Woodhead H *et al.* Skeletal adaptations to intense exercise in pre-pubertal boys (Abstract) **Bone** 27(4Supp) 16S, 2000
Yoshikawa T *et al.* Geometric structure of the femoral neck measured using DXA **J Bone Min Res** 9(7): 1053-64, 1994

ETHICAL ANALYSIS

1. POTENTIAL RISKS - DXA involves exposure to low levels of ionising radiation. The cumulative skin surface entrance radiation dosage during the course of the study will be 37 μSv, for the AP spine, and < 1 μSv for the total body. The whole body effective dose is < 3 μSv which is considerably less than the average daily exposure from natural background sources (7 μSv). The procedures and levels of exposure are similar to other studies previously approved by Hospital Ethics (Farpour-Lambert *et al.* Duncan *et al.* Wiebe *et al.* Woodhead *et al.*).

Magnetic resonance imaging (MRI) involves exposure to high magnetic fields and radio waves. This procedure involves exposure to non-ionizing radiation and there are no known clinical risks associated with this technique. The CHW Ethics committee has also previously approved MRI for use in healthy young populations (Farpour-Lambert *et al.* Duncan *et al.* Wiebe *et al.* Woodhead *et al.*). Participants may feel claustrophobic during the MRI procedure, and will be permitted to listen to music through headphones to minimise anxiety and improve perceived comfort.

Blood sampling (30 ml) will provide important information on the markers of growth, pubertal status, anabolic activity, menstrual status, and bone formation and resorption. Blood sampling might cause pain and discomfort and bruising can occur. An anaesthetic cream will be applied before taking blood.

There is a slight risk of injury associated with **strength testing**, but this will be minimised by proper technique training and appropriate warm-up and cool down procedures. Testing sessions will be administered by trained personnel.

2. POTENTIAL BENEFITS - Sports participation can be associated with desirable and undesirable effects on bone. Identifying sports-related and associated factors that can adversely alter skeletal

growth can advance current practices in injury detection, clinical services, and prevention and. The study will also provide information for athletes, coaches and sports science professionals about factors that contribute to positive bone health in well-trained adolescents. From a public health perspective, an long-term goal of such strategies is to minimise the risk of fracture in later life. On an individual basis, participants will be informed about their individual bone health status. Any participants with a BMD ≤ 1SD will be informed with a letter of referral to their nominated physician, within 2 days of obtaining the finding.

RESEARCH PLAN

1. **PROPOSED DATE OF COMMENCEMENT –** Pending Ethical approval, the study will commence in May 2001.
2. **ESTIMATED DURATION -** It is anticipated that the collection of this data on 160 adolescents will be completed with in 3.5 years.
3. **BUDGET** Costs per participant are: DXA analysis ($30), MRI ($150), Podiatry/biomechanical assessment ($50) and blood analysis (≈$150). Therefore funding per participant is $380 and the total funding sought is $45,600.
4. **SOURCE OF FUNDS -**Partial funding will be requested from the following sources; The NSW Institute of Sport, the NSW Sports Injuries Committee's Research and Injury Prevention Scheme, & the CHW Small Grants Schemes. Other opportunities will be pursued via the CHW and associated Universities.
5. **STAFFING –** The DXA Technician, Ms Julie Briody and MRI Radiographer, Mr. Allan Kemp will train a CHISM researcher on the analysis. The dietician will be consulted through the NSW Institute of Sport.
6. **CARE OF PARTICIPANTS -** Upon recruitment, a letter about the study will be provided to participant's preferred practitioner to inform him/her about the study. Upon completion of the study, two reports (using group data only) will be made. One will be sent to participants. The other will be sent to the NSW Institute of Sport and the respective physicians and coaches who have supported the study.
7. **REVIEW OF PROGRESS -** A review of the data will occur every 3 months. Because the sports-related factors in bone health can impact either negatively or positively, it is unlikely that the study terminate prematurely.
8. **MANAGEMENT OF ADVERSE EVENTS -** Should injury occur testing phases, participants will be referred to the physician of their choice.
9. **WINDING UP PROCEDURES -** Each participant will receive a personal feedback report at the completion of the study. The report will include a brief explanation and interpretation of individual key outcome variables in relation to available standards, as well as a comparison of individual results to the group average results. Thank you letters will be sent to all participants and cooperating authorities at the completion of the project.
10. **ACCESS TO DATA -** Only the chief investigators and research personnel will access individual data.

11. **WILL DATA BE COLLECTED FROM A FEDERAL GOVERNMENT AGENCY -** Data will not be collected for or provided to the Federal Government.

12. **STORAGE OR DISPOSAL OF DATA -** After the 5 year period, primary data will be destroyed (paper questionnaires shredded and computed files deleted).

DECLARATIONS

We undertake to carry out the research project **Exercise Type, Musculoskeletal Development and Injury Risk Factors in Elite Adolescent Athletes** as described in this application and to comply with the general and specific conditions laid down by the Ethics Committee. We do not have a commercial interest in the outcome of the study. We also undertake to notify the Ethics Committee should any changes to the protocol be necessary should any unexpected complications or adverse events take place or should the study be abandoned for any reason. Results will be reported to the Ethics Committee annually during the study and at its conclusion. Copies of abstracts or publications will be submitted to the Research & Development Office.

	_____	___/___/01
Geraldine Naughton		
Nathalie Farpour Lambert	_____	___/___/01
Ian F. Anderson	_____	___/___/01
Caroline Broderick	_____	___/___/01
Chris Cowell	_____	___/___/01
	_____	___/___/01
Ken Crichton		
Kenneth Graham	_____	___/___/01
Robert Howman-Giles*	_____	___/___/01
Allan Kemp	_____	___/___/01
	_____	___/___/01
Michael Kinchington		
Dr. Adam Steinberg* – Medical Imaging	_____	___/___/01

I support the aims of this project and am prepared to have it carried out in CHISM

Prof. Peter Gunning* – HEAD OF RESEARCH	_____	___/___/01

Associate Investigators

In addition to the expertise support for this project from within the Children's Hospital, there are a number of listed Associate Investigators who work outside of the Children's Hospital.

Dr. Ian Anderson is the most experienced clinical specialist in the imaging of sports injuries in Australia. He was in charge of radiology at the Sydney Olympic Games and contributes a wealth of expertise to the project.

Dr. Caroline Broderick has gained recognition for her sports medicine consultancy positions with several of Australia's leading sporting teams. Her expertise and research interests in Women and Sport will be particularly valuable in the proposed study. Dr. Broderick also has been a sports medicine consultant for the Institute of Sports Medicine in the Children's Hospital at Westmead.

Dr Ken Crichton is among Australia's most eminent Sports Physicians. He was Deputy Chief Medical Officer and Head of Athlete Care for the Sydney 2000 Olympic and Paralympic Games. He is also internationally recognised particularly for his work with young gymnasts and dancers. Ken is a Sports Physician consultant at the Children's Hospital's Institute of Sports Medicine and has a strong interest in injury prevention for adolescent athletes.

Mr. Kenneth Graham is Coordinator for Sports Science at the New South Wales Institute of Sport. He also leads the Medical Services Management team at the Institute. His strong scientific background and quest for best practice of athlete management and longevity provide a unique professional expertise in this project.

Mr. Michael Kinchington has emerged as one of the foremost Sports Podiatrists in Australia. He was Director of Podiatry Services at the Olympic Games and Paralympic Games, Sydney 2000. He is team podiatrist to the Sydney Swans AFL team and Sydney Roosters NRL team. Among his other consultancies are NSW Cricket team and Northern Spirits NSL team. Michael will conduct the biomechanical assessment of participants in this study.

Appendix 3.2 Approval letter from Ethics Committee,

The Children's Hospital, Westmead

the children's hospital at Westmead

Research and Development
Tel: 02 9845 1316
Fax: 02 9845 1317

Corner Hawkesbury Road
and Hainsworth Street
Locked Bag 4001
Westmead NSW 2145
Sydney Australia
DX 8213 Parramatta
Tel +61 2 9845 0000
Fax +61 2 9845 3489
www.chw.edu.au
ABN 53 188 579 090

G:\DATA\Research\ETHICS\EC\2002\2002-02\2002-02finalapproval.doc

15 April 2002

A/Prof Geraldine Naughton
CHISM

Dear A/Prof Naughton,

Project Title: Exercise type, musculoskeletal development and injury risk factors in elite adolescent athletes
Project Number: 2001/028
Patient Information Sheet: Version 3, 17/04/02

This is to confirm that final approval has been granted for this study. The amendments suggested by the Ethics Committee have been satisfied and your approval is valid.

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

Yours sincerely,

A O'Neill
Secretary, Ethics Committee

Appendix 3.3 Approval letter from New South Wales Sporting Injuries Committee

SPORTING INJURIES COMMITTEE

28 Pages
FAXED
Robyn
8.11.02

Level 4
92-100 Donnison St
Gosford, NSW 2250
Tel: (02) 4321 5392
Fax: (02) 9287 5392

31 October 2002

RECEIVED
07 NOV 2002

Dr Stewart Sharlow
Research Services
Australian Catholic University
Locked Bag 4115
Fitzroy Vic 3065

Our Reference:

Your Reference

Dear Dr Sharlow

RESEARCH & INJURY PREVENTION SCHEME
APPLICATION OF ASSOC PROFESSOR GERALDINE NAUGHTON
"Exercise type, musculoskeletal development and injury risk factors in elite female adolescent athletes"

I wish to advise that the Committee considered Associate Professor Naughton's application at its October meeting and has approved a grant of $23,617.

I attach for your information a copy of my letter to Associate Professor Naughton containing the terms and conditions of the grant.

Please do not hesitate to contact me if you have any queries.

Yours faithfully

John Anderson
Executive Officer

Robyn,
Please liaise with Geraldine and Prof. Coll RE acceptance of the grant. If Geraldine is happy with the amount and the associated conditions please draft a letter which accepts the conditions for Prof. Coll to sign asap.
Stewart
8/11/02.

Appendix 3.4 Approval letter from

Catholic Education Office

(Parramatta Diocese)

CATHOLIC EDUCATION OFFICE, DIOCESE OF PARRAMATTA

12 Victoria Road, Parramatta
All correspondence:
Locked Bag 4, North Parramatta NSW 1750
Phone: (02) 9840 5600 Fax: (02) 9840 5678

KS:kc
Reference: SU/02/0078

3rd June, 2002.

Mr. David Greene and
Associate Professor Geraldine Naughton,
Institute of Sports Medicine,
The Children's Hospital,
Locked Bag 4001,
WESTMEAD. NSW 2145

Dear Mr. Greene and Ms. Naughton,

Our Executive Director of Schools, Dr. Anne Benjamin, is happy for you to approach the following secondary schools in the diocese in order to carry out research on ***Exercise Type, Musculoskeletal Development, and Injury Risk Factors in Elite Adolescent Athletes:***

- Patrician Brothers' College, Blacktown
- Catherine McAuley, Westmead
- Parramatta Marist High School, Westmead

We always stress the following points in relation to research requests:

- It is the School Principal who gives final permission for research to be carried out in his/her school.
- Confidentiality needs to be observed in reporting.
- There should usually be some feedback to schools and a copy of the final report forwarded to the Catholic Education Office.
- This letter of approval should accompany any approach to schools.

Best wishes,

Yours sincerely,

Mr. Kerry Stirling,
Acting Director,
Department of Religious Education and Educational Services.

Appendix 3.5 Australian Catholic University

letter of support

AUSTRALIAN CATHOLIC UNIVERSITY

15 November 2002

Mr John Anderson
Executive Officer
Sporting Injuries Committee
Level 4
92-100 Donnison St
GOSFORD NSW 2250

Dear Mr Anderson,

The University welcomes the opportunity to complete this research with the support of the NSW Sporting Injuries Committee. We hereby accept the terms and conditions of the grant approved under the Research and Injury Prevention Scheme as set out in your letter of 31 October 2002 to Assoc. Prof. Naughton.

Assoc. Prof. Naughton has provided the following response to the independent assessor's concern regarding "the use of 3-day food intake data as a proxy for general nutrition":

The grant reviewer was correct in indicating that a three day record is not sufficient to be representative of general food intake. A 3-day food record was reported as an appropriate method for dietary assessment and estimate of nutrient intake in girls aged 9 and 10 years (Crawford PB, et al., Comparative advantage of 3-day food records over 24-hour recall and 5-day food frequency validated by observation of 9- and 10-year-old girls. American Dietetics Association, Jun; 94(6):626-30 1994) and older populations (Schroder H, et al. Use of a three-day estimated food record, a 72 hour recall and a food-frequency questionnaire for dietary assessment in a Mediterranean Spanish population. Clinical Nutrition 2001, Oct: 20 (5): 429-37). Data are intended to be presented in the same way that other studies present data using the 3-day food record i.e. in macronutrient or dietary assessment tables without reference to general or representative food sampling.

Please contact Assoc. Prof. Naughton directly (02) 9845 0765 if you require further discussion on this methodological issue. Geraldine wishes to convey sincere thanks on behalf of her research partners for the recognition and opportunities the NSW Sporting Injuries Committee provides applied researchers in this State.

Pro-Vice-Chancellor (Research and International): Professor John C. Coll
40 Edward Street, North Sydney NSW 2060 • PO Box 968, North Sydney NSW 2059, Australia
Telephone: (61 2) 9739 2911 Facsimile: (61 2) 9739 2964 Email: J.Coll@acu.edu.au
BALLARAT • BRISBANE • CANBERRA • MELBOURNE • SYDNEY
www.acu.edu.au
Australian Catholic University Limited ABN 15 050 192 660

For any queries in relation to the administrative aspects of this funding agreement, please do not hesitate to contact Dr Robyn Munro on (02) 9701 4159. The grant cheque of $23,617 plus GST should be addressed to Australian Catholic University Limited, and sent to:

Dr Robyn Munro
Research Services
Australian Catholic University
Locked Bag 2002
STRATHFIELD NSW 2135

Yours sincerely,

John C Coll
Pro-Vice-Chancellor (Research and International)

Appendix 3.6 New South Wales Institute of Sport letter of support

"PROUDLY SUPPORTED BY THE NEW SOUTH WALES GOVERNMENT"
www.nswis.com.au
NSWIS
NEW INSTITUTE OF SPORT

The Sporting Injuries Committee
Research and Injury Prevention Scheme
Level 4, 447 Kent St.
SYDNEY, NSW 2000

Dear Committee Members

I am writing this letter in support for the Application "Exercise Type, Musculoskeletal Development and Injury Risk Factors in Elite Adolescent Female Athletes" being submitted by Dr Geraldine Naughton, Dr Ken Crichton and Mr Michael Kinchington of Children's Hospital Institute of Sports Medicine, The Children's Hospital Westmead.

The NSW Institute of Sport is the organisation charged with the specific statutory responsibility for developing and assisting high performance NSW athletes and as part of its operations provides comprehensive support for a number of elite adolescent athletes.

The NSW Institute of Sport is also committed to undertaking co-operative research with key partners to maintain an understanding of best practice and enhance the services we provide to our athletes.

Based on a concern for best practice in injury prevention and management in young athletes, the New South Wales Institute of Sport is supportive of the proposed project.

Yours sincerely

RALPH DOUBELL

Director

20 February 2002

NSWIS

an Olympic Training Centre
Endorsed by the Australian Olympic Committee

PO BOX 476 SYDNEY MARKETS NSW 2129 AUSTRALIA • TELEPHONE +61 2 9763 0222 • FACSIMILE +61 2 9763 0250
SYDNEY ATHLETIC CENTRE EDWIN FLACK AVENUE OLYMPIC PARK HOMEBUSH BAY NSW 2140 AUSTRALIA

CLUBSNSW
PRINCIPAL PARTNER

Appendix 3.7 Participant information sheet and consent forms

the children's hospital at Westmead

Children's Hospital Institute of Sports Medicine

Corner Hawkesbury Road
and Hainsworth Street
Locked Bag 4001
Westmead NSW 2145
Sydney Australia
DX 8213 Parramatta
Tel +61 2 9845 0000
Fax +61 2 9845 3489
www.chw.edu.au
ABN 53 188 579 090

Participant Information Sheet

Research Project: Exercise Type, Musculoskeletal Development and Injury Risk Factors In Elite Adolescent Athletes

Investigators: Assoc. Prof Geraldine Naughton, (phone 9 845 0765) Dr.Nathalie. Farpour-Lambert, Mr. Allan Kemp, Dr. Chris Cowell, Dr. Robert Howman-Giles, Assoc Prof Ian Anderson, Dr. Ken Crichton, Mr. Kenneth Graham, Mr. Michael Kinchington, Dr. Caroline Broderick, Assoc. Prof Kathy North.

We would like you and your child to consider taking part in a research study investigating the potential association of adolescents' sports participation and several measures of bone health. Reports of bone injuries in athletic adolescents are a particular concern, because bone injuries may decrease performance, interrupt, or prematurely end good sporting prospects.

What is the purpose of the research?

The primary purposes of this study are **(i) To investigate whether serious training for different sports, influences bone properties and the way bone and muscles develop and function in the body, and (ii) To compare bone properties and markers of development (above) between athletic and not so athletic adolescents, as well as comparing female and male elite adolescents athletes who participate in the same sport.**

The secondary aim is: To determine whether exercise-related bone changes are related to: injury history, training loads, hormones in the blood, daily activity, aerobic fitness, body composition, nutrition, muscle strength and size, biomechanical measures, and maturity. The study will involve one, or possibly two**,** visits to the Children's Hospital at Westmead for most of the testing and one visit for the biomechanical assessment to at either the South Sydney Sports Medicine Centre (ANZAC Parade, Kensington) or the MLC Medical Centre (Martin Place, Sydney). Parent will be able to choose their preferred venue for the biomechanical assessment.

Should we be fortunate enough to get funding for two years of testing, we will be asking your child to complete the testing once per year over a two year period (this means 3 testing times – at the start, end of year one, and end of year two).

Who can participate?

To be involved in the study, adolescents must be aged between 14 and 18 years. In the athletic groups for the study, we are looking for middle distances runners and soccer players who are associated with the NSW Institute of Sport or who are representing their sport at a state level.

As well as the inclusion criteria listed for non-sporting adolescents, the adolescent athletes that we want to do the study will need to have been training in their sport for ≥6 hr/week for the past 2 years.

Non-athletic adolescents who are interested in being part of the research should participate in **no more than 3 hours per week of organised activity including activities during school hours.**

All adolescents will be eligible to participate if they meet each of the following requirements

(i) caucasian ethnicity
(ii) within the normal range for height and weight
(iii) in good health and no recent (past two years) hospitalisation
(iv) no known history of metabolic bone disease and no medication, (including the Oral Contraceptive Pill), or calcium preparations that may influence bone taken in the past 6 months.

If you and your child are interested in participating in this study, it would involve assessment on one, or possibly two, separate occasions. The study will be undertaken at the Children's Hospital at Westmead. Each visit would require approximately 2 hours, and would involve the following :

GENERAL INFORMATION

1. QUESTIONS THAT ARE ASKED AT THE START OF THE STUDY

(a) At the start of the first visit to the Children's Hospital at Westmead, a researcher will interview your child about his/her medical history, with particular reference to disease, medication and injury. Where appropriate we will ask female adolescents for details about menstruation (periods). Questions regarding osteoporosis and fractures in the family will also be asked.

(b) During this interview, the researcher will explain the **food and physical activity records** that we would like your child to complete Your child will be asked to fill these out over a period of **3** days, **2** week and **1** weekend days. For the food record, your child will need to write down all the food and drink that is consumed over the period, including cooking methods, brand names and recipes where applicable. An activity record will be kept over the same period, and your child will be asked to put the activity into categories for particular times of the day. Another questionnaire will be completed regarding your child's physical activity (including sport and less organised physical activities) in the 12-month period prior to the study. Athletes in the study, will

be asked to include a brief summary of schedule of their training and competitions for the past 12 months

(c) An important part of this research is to determine the stage of puberty of each adolescent. To avoid the embarrassment of a formal examination, we will be showing your child diagrams of the 5 stages of pubertal development and asking him/her to report the stage of development that most closely resembles their own. This process will be completed as discretely as possible.

2. LEG MUSCLE STRENGTH TESTING

Strength of the muscles in the "dominant" leg (kicking leg) will be measured using a specially designed machine (Cybex dynamometer) at the Children's Hospital. This will involve sitting in a special chair and moving in a kicking action through the leg's maximal range of motion 3 times at **2** predetermined speeds. This test will take about 20 minutes.

3. BONE SCAN

This scan measures the bone density and the amount of body fat compared to muscle tissue in the body. It is performed using a "DXA" (Dual Energy Xray Absorptiometry) machine, where two Xray beams are passed through the body to give us a computerised image of the bones and soft tissues.

This will involve your child lying still on a table for approximately 30 minutes while we obtain 3 separate scans. There is a chance to stretch in between each scan. We will look at the whole body, as well as the spine, and hip bone. Because this technique uses X-ray there is exposure to ionizing radiation; however, the cumulative skin surface entrance radiation dosage during the course of the study will be 38 micro Sv, or an effective (beneath the surface) dose of less than 3 micro Sv. This amount of radiation is considerably less than the average annual exposure from natural background sources (2000 – 2500 micro Sv).

4. ULTRASOUND

This scan, using a machine similar to that used for pregnant women to examine their unborn babies, measures bone architecture and elastic properties by analysing the speed of sound waves travelling through bone. The scan will require approximately 15 minutes of sitting still, and is preceded by a foot length measure. **The foot is then placed on a platform designed to take the shape of the foot**. An ultrasound wave is then passed through the heel. This process is repeated once again, after repositioning of the foot. Ultrasound measures of the foot involve exposure to non-ionizing, rather than ionizing radiation, and hasn't any known risks.

5. MAGNETIC RESONANCE IMAGING (MRI)

In this study, Magnetic Resonance Imaging (MRI)/Spectroscopy (MRS) will produce microscopic images of the tibia (a bone in the lower leg), so that its structure/ content can be analysed by computer. It involves your child lying quietly on a table that moves in and out of a tunnel shaped scanning device for **60** minutes while the body is scanned. The machine at our hospital is

larger and roomier than the usual models but is still quite noisy. Your child may bring a CD to listen to while the test is performed (the machine is equipped with a CD player). If your child feels claustrophobic or becomes distressed at any time, we will stop the test.

6. BLOOD TEST

We will need to collect 30 mls of blood (approximately 6 teaspoons) from a vein in the arm. Experienced personnel will take this blood in order to assess growth factors, pubertal status, markers of bone development and genetic markers of muscle structure and function. The genetic marker testing is part of a larger study being conducted Associate Professor Kathy North who investigates "genetic" factors or characteristics we inherit from our parents. Hopefully the research will help explain specific genetic characteristics associated with success in particular sports that have not been established. We aim to address this problem by conducting genetic analysis on blood collected from outstanding and aspiring athletes and comparing the results with those obtained for people who have not achieved sporting success. From the initial blood draw, 5-10 mls will be used for genetic testing with your permission. Genetic testing has some important ramifications. Please read the Genetic Testing form attached to the consent form carefully. Your child's name will not appear on the blood sample, and his/her identity will therefore be completely unknown by researchers conducting the analysis. For this reason, it will not be possible for you to receive feedback concerning your individual results. A report of the study may be submitted for publication(s) but individual participants will not be identifiable in such a report.

Withdrawing 30 mls of blood is not harmful to your child. Blood sampling however can be painful and bruising can occur. In this study we will try to reduce the pain by a local anaesthetic cream. We would like all participants to provide us with a blood sample but an additional signature on the consent form will be necessary for genetic analysis of the blood.

7. PODIATRIST ASSESSMENT OF LOWER LEGS AND FEET

We are lucky that the Head of Sports Podiatry at the Sydney 2000 Olympic and Paralympic Games, Mr. Michael Kinchington is able to assess the lower legs and feet of the participants in this study. The testing involves an examination of each participant's athletic shoes, standing and simple movements such as stretching and walking or jogging short distances. Where appropriate a biomechanical video taping of the movement of the lower extremities will also take place. The assessment will take place at either the South Sydney Sports Medicine Centre (ANZAC Parade, Kensington) or the MLC Medical Centre (Martin Place, Sydney) and will take about one hour. Parents will be able to choice the venue that suits them.

Should you decide to participate, we will be able to give you detailed information on your child's growth, body composition, nutrition, physical activity, muscle strength, bone density and architecture. If you wish, your family doctor will be notified of your child's participation in this study.

Participation in this study is voluntary and if you decide not to take part or to withdraw at any stage this will not otherwise affect your child's care at the Hospital. Individual data (using codes rather than names) will only be accessible to the chief investigators and researchers in this project. Group averages only will be presented at public and scientific presentations. In agreement with the requirements of the Ethics Committee at the Children's Hospital, data will be maintained for 5 years and then destroyed (paper questionnaires shredded and computed files deleted)..

If you have any concerns about the conduct of this project, please do not hesitate to discuss them with Associate Professor Geraldine Naughton (☎: 02 9845 0765; Fax: 02 9845 3076) or with Anne O'Neill (☎: 02 9845 1316), Secretary of the Hospital Ethics Committee, which has approved this project.

If you have any concerns about the genetics testing this study, please do not hesitate to discuss them with Dr Kathryn North (☎ 9845 3011), or with Anne O'Neill (☎ 9845 1316), the secretary of the Ethics Committee which has approved this project.

A copy of the Information Sheet and signed consent form will be provided to parents of participants in this study.

Appendix 3.8 Information pamphlet for potential participants

What will we need to measure?

Your muscle strength
You sit on a special chair and kick your leg as hard and as fast as you can.

Your bone mineral content
You lie on a bed and a special machine scans your body.

Your bone material properties
We do this on your heel with an ultrasound machine similar to the one the doctor might have used to see you before you were born.

Your bone shape
You just lie there and the MRI machine scans your legs.

Your bone metabolism
When you come to the Hospital, we take some blood to measure your hormones and minerals. We can minimize the pain by a local anaesthetic patch.

All measurements that need to be done have been checked for safety by the Children's Hospital

Should you decide to participate, we will be able to give you detailed information on your growth, body composition, nutrition, physical fitness, muscle strength, bone density and architecture.

If you want to join our research project just call the Children's Institute of Sports Medicine and ask for David Greene or leave your name and a contact number.

Address:
Children's Hospital
Institute of Sports Medicine
Suite 12-13
Children's Hospital Medical Centre
Hainsworth Street
Westmead 2145. NSW.

Phone: CHISM
(02) 9845 0761 or
(02) 9845 0000

Children's Hospital Institute of Sports Medicine

How Strong are Your Bones?

The Children's Hospital

Are you:

- Between 14 -18 years old, and
- involved in less than 3 hours per week of organised physical activity
- in good health (no history of illness lasting more than 2 weeks or hospitalisation in the past 2 years).

Why participate in this study?

The testing, using the latest equipment at the Children's Hospital, involves **no expense!**

You will be provided with a detailed analysis of your bone health together with muscle strength and current nutritional habits.

What do we want you to do?

If possible, we would like to compare your bones with those of elite adolescent athletes around the same age.

All you have to do is allow us to conduct some tests (explained later in the pamphlet) in order to gain some information.

Testing will take approximately an hour and a half.

What will we need to know about you?

Your personal/medical history

We will ask you and your parents a few questions about your health status and injuries you may have had in the past.

Diet and activity

We will need to know what you eat and will ask you or your parents to fill in a 3 day food diary. You will also be asked to keep an physical activity record over the same days.

Appendix 3.9 Medical history / injury record

Children's Hospital Institute of Sports Medicine

Athlete Medical History / Injury Record Questionnaire

Name: _____ Age: _____ School: _____

General Health

YES	NO	(Please Tick)
'	'	Have you ever had a heart abnormality or murmur diagnosed by a doctor?
'	'	Do you have asthma, (wheezing), or coughing spells after exercise?
'	'	Do you have a chronic illness or see a physician regularly for any particular problem? **Please List** _____ _____
'	'	Do you take any medications, or have you taken any medication in the last six months? **Please List** _____ _____
'	'	Do you have any allergies to medications or any other agents? **Please List** _____ _____
'	'	Do you have only one of any paired organ? (eyes, ears, kidneys, testicles) **Please Circle**
'	'	Have you had any surgery or hospitalizations? **Please List** (inc. date) _____ _____ _____ _____

Children's Hospital Institute of Sports Medicine

Injury Record

YES	NO	(Please Tick)
'	'	Have you had any injuries that interfered with your sporting career?
'	'	Have you ever broken a bone, had to wear a cast or had an injury to any joint?
'	'	Do you wear any protective equipment? **Please List: _____** _____

$ NB. IF YOU HAVE ANSWERED YES TO ANY OF THE INJURY RECORD QUESTIONS, PLEASE FILL OUT THE INJURY RECORD FORM ON THE FOLLOWING TWO PAGES.

For each injury:

1) Type of injury _____

Approx date ___/___/___

Treatment _____

_____

Any residual problems? _____

How many weeks did you stop training due to the injury? _____

2) Type of injury _____

Approx date ___/___/___

Treatment _____

_____

Any residual problems? _____

How many weeks did

_____

you stop training due to the injury?

3) Type of injury _____

Approx date ___/___/___

Treatment _____

_____

Any residual problems? _____

How many weeks did you stop training due to the injury? _____

4) Type of injury _____

Approx date ___/___/___

Treatment _____

_____

Any residual problems? _____

How many weeks did you stop training due to the injury? _____

Appendix 3.10 Self-assessment pubertal status (male and female)

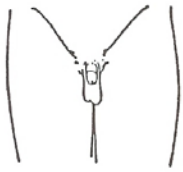
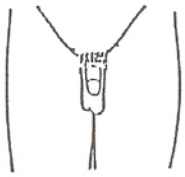
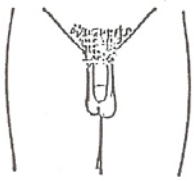
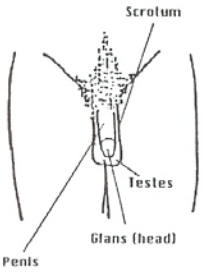
the children's hospital at Westmead

Children's Hospital Institute of Sports Medicine

Exercise Type, Musculoskeletal Development, and Injury Risk Factors in Elite Adolescent Athletes

Self Assessment for boys (genitalia)

The following pictures show different stages of growth of the testes, scrotum, and penis. A boy goes through each of the 5 stages as shown. Please look at each of the pictures. Read the sentences. Put an X on the line above the picture which is closest to your stage of growth.

Picture 1 _____	Picture 2 _____	Picture 3 _____	Picture 4 _____	Picture 5 _____
				
The testes, scrotum, and penis are about the same size and shape as they were when you were a child.	The testes and scrotum are bigger. The skin of the scrotum has changed. The scrotum (the sack holding the testes) has gotten lower. The penis has gotten only a little bigger.	The penis has grown in length. The testes and scrotum have grown and dropped lower than in picture 2.	The penis has gotten even bigger. It is wider. The glans (the head of the penis) is bigger. The scrotum is darker than before. It is bigger because the testes are bigger.	The penis, scrotum and testes are the size and shape of that of an adult man.

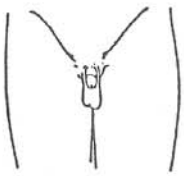
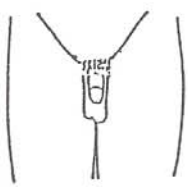
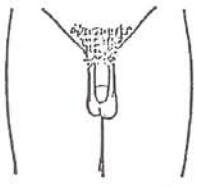
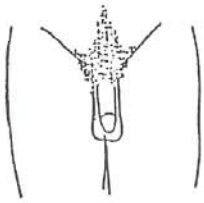
the children's hospital at Westmead

Children's Hospital Institute of Sports Medicine

Exercise Type, Musculoskeletal Development, and Injury Risk Factors in Elite Adolescent Athletes

Self Assessment for boys (pubic hair)

The drawings on this page show different amounts of male pubic hair. Please look at each of the drawings and read the sentences under the drawings. Then check the drawing that is closest to your stage of hair development.

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

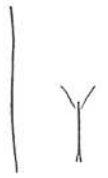
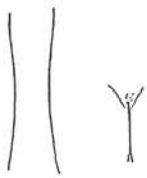
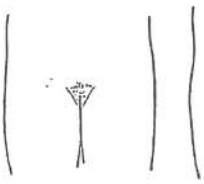
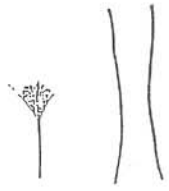
the children's hospital at Westmead

Children's Hospital Institute of Sports Medicine

Exercise Type, Musculoskeletal Development, and Injury Risk Factors in Elite Adolescent Athletes

Self Assessment for girls (pubic hair)

The drawings on this page show different amounts of female pubic hair. Please look at each of the drawings and read the sentences under the drawings. Then check the drawing that is ***closest*** to your stage of hair development.

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

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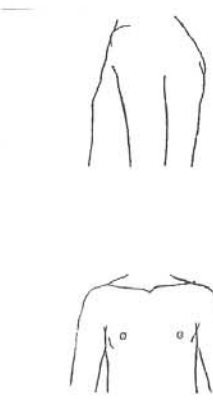
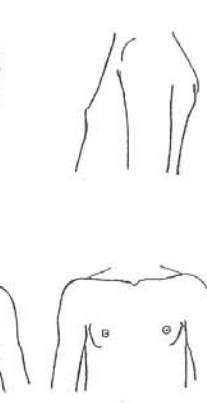
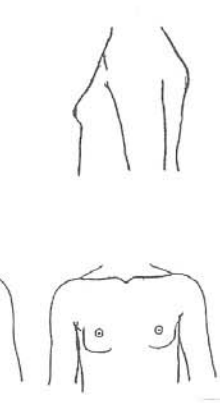
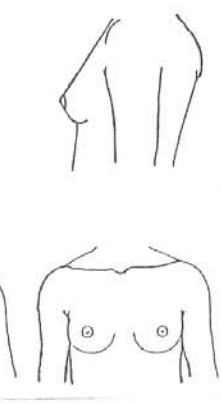
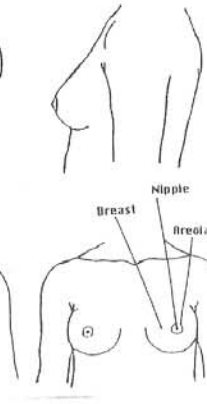
Children's Hospital Institute of Sports Medicine

Exercise Type, Musculoskeletal Development, and Injury Risk Factors in Elite Adolescent Athletes

Self Assessment for girls (breast development)

The following pictures show different stages of how the breasts grow. A girl can go through each of the 5 stages as shown. Please look at each of the pictures. Read the sentences. Put an X on the line above the picture which is ***closest*** to your stage of growth.

Picture 1 _____	Picture 2 _____	Picture 3 _____	Picture 4 _____	Picture 5 _____
				
The nipple is raised a little in this stage. The rest of the breast is still flat.	This is the breast at bud stage. In this stage the nipple is raised more than in stage 1. The breast is a small mound. The areola is larger than in stage 1.	The areola and the breast are both larger than in stage 2. The areola does not stick away from the breast.	The areola and the nipple make up a mound that sticks up above the shape of the breast.(This stage may not happen at all for some girls. Some girls go from stage 3 to stage 5)	This is the mature adult stage. The breasts are fully grown. Only the nipple sticks out in this stage. The areola has moved back to the general shape of the breast.

Appendix 3.11 Menstrual status questionnaire

Name: _____ Date _____

The Children's Hospital Institute of Sports Medicine

Menstrual Status Questionnaire

We would like you to answer the following questions related to your menstrual cycle as accurately as possible.

1. At what age did you first start menstruating?
   (Can you please tell us to the nearest half year eg: 12½ years) _____

2. How regularly are you currently menstruating? (Please tick a box)

Menstrual cycles in the last year	Yes	No
9	☐	☐
6	☐	☐
3	☐	☐
Less than three	☐	☐
Unable to recall	☐	☐

3. Is the time between cycles predictable?
   (ie: is the time between your periods the same with each cycle?) YES / NO

4. If you menstruate regularly, how many days between the end of your period and the start of the next period? _____

5. How heavy is the flow of your period: (Please tick a box)

Flow of the menstrual cycle	Yes	No
Very light	☐	☐
Light	☐	☐
Moderate	☐	☐
Heavy	☐	☐

Very Heavy	☐	☐

6. How long is the flow of your period? (Please tick a box)

Flow of the menstrual cycle	Yes	No
1 - 2 days	☐	☐
2 - 3 days	☐	☐
3 - 5 days	☐	☐
5 - 7 days	☐	☐
>7 days	☐	☐

7. Do you experience any pain or discomfort during your periods? YES / NO

8. Do you know when your Mother started menstruating? YES / NO
If yes, what was her age? _____

Appendix 3.12 Training intensity questionnaire

The Children's Hospital Institute of Sports Medicine

TRAINING INTENSITY QUESTIONNAIRE

Dear _____,

Would be kind enough to complete the following questionnaire with the help of your coach and parents.

1. On average, how many training sessions per week do you complete? _____
2. How long does each training session last? (eg: 50 minutes) _____
3. Please complete the table:

PERIODIZED YEAR	WHICH MONTHS OF THE YEAR?	SESSIONS PER WEEK?
Pre – season		
Competition season		
Out of season		

4. How many club races have you competed in during the past 12 months? _____
5. Please list major championships (eg: State and National level) that you have completed in the past 12 months:

Championship Event	Date

6. Do you taper your training prior to competition (YES / NO)

(i) If yes, how many weeks prior to competition do you taper?

_____

7. Do you have any rest periods throughout the year when you are not training and / or competing? (YES / NO)

(i) If yes, what has been the total accumulated time of rest during the past 12 months (eg: 35 days) _____

7. Please record an example of a typical **PRE-SEASON** training session.

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

8. Please record an example of a typical training session during

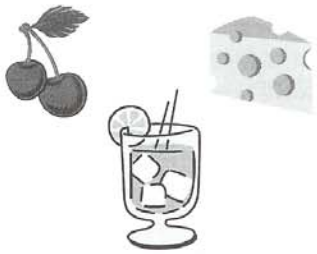
COMPETITION.

_____
_____
_____
_____
_____
_____
_____

Appendix 3.13 3-day food diary

This record book belongs to :_____

Date of birth : _____



MY FOOD AND ACTIVITY RECORD BOOK

Section 1 : Activity record
Section 2 : Food record

Three day food and activity record :
Two week and one weekend days

DAY 1 - Date : _____
DAY 2 - Date : _____
DAY 3 - Date : _____

For further information, please contact David Greene (phone 9845 0741, or 9845 0000 and ask for the Institute of Sports Medicine).

SECTION 2 : FOOD RECORD

Please write down everything you eat and drink for the same three days that you keep your activity record.

This is not a test. There are no right or wrong answers. Please do not report the foods you think you should be eating or the foods eaten by someone else in your household.

HOW TO FILL IN YOUR RECORD

- Fill in the date and day of the week at the top of the record sheet.
- Use as many pages as you need for each day's record (number each page).
- Start a new page for a new day.

Column 1 – Time

- Every time you have something to eat or drink, write down the time you started.
- Write down « am » for morning and « pm » for afternoon or evening.

Column 2 – What you are measuring

Name and full description of all food and drink.

- Write down everything you eat and drink. This include snacks, water, vitamins and mineral supplements. Eat as you normally would !
- For each food and drink use a new line.
- Measure each food individually, for example, bread and margarine are each separate foods and are recorded on separate lines.
- Always record cooking methods such as boiling, frying, etc.
- Give a detailed description of the food or drink and brand names, for example :

  Arnott's Milk arrowroot Biscuit
  Tip Top White Bread

- Record directly into this book while you still remember, such as while you are making a school lunch.
- Write down a cut of meat, that is lamb loin chop, chicken leg, rump steak etc.
- Write down if the fat on meat or skin on chicken was eaten or not eaten.

Column 3 – Amount eaten

In order to get the best estimate of your nutrient intake we need an accurate estimate of quantities of food and drink consumed.

- Estimate everything as accurately as possible in either **metric cups or spoonfuls** eg teaspoons, tablespoons (level or rounded) such as for breakfast cereal, rice, vegetables or spaghetti, or use a **metric measuring tape or ruler** to give length and width such as for sausage rolls, bananas, etc.

DATE :_____ DAY OF WEEK : _____

Column 1 Time	Column 2 Name, type, brand cooking method	Column 3 Amount	Leave blank

Appendix 3.14 3-day physical activity record

SECTION 1 - ACTIVITY RECORD

A) THREE DAY PHYSICAL ACTIVITY RECORD

In each box, write the number which corresponds to the ativity which you have carried out during this 15 minute period. Please consult the activity card that follow to establish the proper coding.
If an activity is carried out over a long period (e. g. 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, we suggest that you take a look at the example that follows.

DAY 1 - Date :

Minute / Hour	1-15	16-30	31-45	46-60
Midnight - 1 am				
1 am - 2 am				
2 am - 3 am				
3 am - 4 am				
4 am - 5 am				
5 am - 6 am				
6 am - 7 am				
7 am - 8 am				
8 am - 9 am				
9 am - 10 am				
10 am - 11 am				
11 am - midday				
Midday - 1 pm				
1 pm - 2 pm				
2 pm - 3 pm				
3 pm - 4 pm				
4 pm - 5 pm				
5 pm - 6 pm				
6 pm - 7 pm				
7 pm - 8 pm				
8 pm - 9 pm				
9 pm - 10 pm				
10 pm - 11 pm				
11 pm - midnight				

ACTIVITY CODES FOR THE BOUCHARD THREE DAY PHYSICAL ACTIVITY RECORD

Category of activity	Example of activity for each category	Approximative energy expenditure (kcal/kg/15 min)
1	Lying down : • Sleeping • Resting in bed	0.26
2	Seated : • Listening in class, writing by hand or typing, reading • Eating • Listening to the radio or T.V. • Taking a bath	0.38
3	Standing ; light activity : • Washing oneself, shaving, combing hair • Dusting, cooking	0.57
4	• Getting dressed • Taking a shower • Driving a car • Taking a walk (strolling)	0.70
5	• Moderately quick walking (going to school, shopping) • Housework (washing windows, sweeping, etc). • Doing the bed • Riding a moped • Light manual work : Tailor, baker, printer, brewer, cobbler, mechanic, electrician, painter, lab-work, carpentry, masonry, driving a farm tractor, cleaning trees, working in the chemicals or electric industries, feeding animals on a farm.	0.83
6	Light sport or leisure activities : • Light canoeing • Volleyball • Table tennis • Baseball (except the pitcher) • Golf • Rowing • Archery • Ninepins • Croquet • Sailing • Cycling (leisure)	1.20
7	Moderate manual work : Machine operating, repairing a fence, loading bags or boxes, plantation work, forest work (machine sawing and log handling), mine work, shovelling snow.	1.40
8	Moderate sport or leisure activities : • Baseball (pitcher) • Badminton • Canoeing • Cycling (race bike) • Dancing • Tennis • Jogging (slow running) • Horseback riding • Alpine-skiing • Cross-country skiing (leisure) • Swimming • Gymnastics • Brisk walking	1.00
9	Intense sport or leisure activities : • Running in a race • Boxing • Mointain-climbing • Squash • Cross-country skiing • Ice-hockey • Basketball • Football or soccer • Racquetball Intense manual work : • Felling a tree with an axe • Sawing with hand-saw • Working with a pitchfork (on a farm) • Cutting tree branches	1.95

Appendix 4.1 Correlation matrix

Correlations

Correlations

		Height	Weight	Lumbar spine bmc	Total body bmc
Height	Pearson Correlation	1	.591**	.516**	.549**
	Sig. (2-tailed)	.	.000	.001	.000
	N	40	40	40	40
Weight	Pearson Correlation	.591**	1	.457**	.740**
	Sig. (2-tailed)	.000	.	.003	.000
	N	40	40	40	40
Lumbar spine bmc	Pearson Correlation	.516**	.457**	1	.562**
	Sig. (2-tailed)	.001	.003	.	.000
	N	40	40	40	40
Total body bmc	Pearson Correlation	.549**	.740**	.562**	1
	Sig. (2-tailed)	.000	.000	.000	.
	N	40	40	40	40
Total body fat mass	Pearson Correlation	.223	.805**	.243	.554**
	Sig. (2-tailed)	.166	.000	.131	.000
	N	40	40	40	40
Total body lean mass	Pearson Correlation	.632**	.526**	.393*	.701**
	Sig. (2-tailed)	.000	.000	.012	.000
	N	40	40	40	40
Plantar flex	Pearson Correlation	.247	.384*	.354*	.479**
	Sig. (2-tailed)	.125	.014	.025	.002
	N	40	40	40	40
Dorsi flex	Pearson Correlation	.188	.377*	.120	.343*
	Sig. (2-tailed)	.244	.016	.460	.030
	N	40	40	40	40
Left tibia length	Pearson Correlation	.774**	.521**	.187	.542**
	Sig. (2-tailed)	.000	.001	.248	.000
	N	40	40	40	40
Right tibia length	Pearson Correlation	.755**	.516**	.175	.546**
	Sig. (2-tailed)	.000	.001	.280	.000
	N	40	40	40	40
Dominant leg BMC	Pearson Correlation	.261	.481**	.418**	.607**
	Sig. (2-tailed)	.104	.002	.007	.000
	N	40	40	40	40
Calcium	Pearson Correlation	-.196	-.147	-.059	-.145
	Sig. (2-tailed)	.266	.406	.741	.412
	N	34	34	34	34
Hrs phys act per wk	Pearson Correlation	-.185	-.464**	.023	-.351*
	Sig. (2-tailed)	.295	.006	.899	.042
	N	34	34	34	34
Total tibial CSA	Pearson Correlation	.492**	.378*	-.003	.224
	Sig. (2-tailed)	.001	.016	.986	.164
	N	40	40	40	40
Dorsiflexion CSA	Pearson Correlation	.117	.251	.451**	.368*
	Sig. (2-tailed)	.472	.118	.003	.020
	N	40	40	40	40
Plantar CSA	Pearson Correlation	-.110	-.060	.229	.100
	Sig. (2-tailed)	.501	.713	.155	.540
	N	40	40	40	40
Total muscle CSA	Pearson Correlation	-.018	.059	.315*	.159
	Sig. (2-tailed)	.912	.718	.048	.326
	N	40	40	40	40

Correlations

		Total body fat mass	Total body lean mass	Plantar flex	Dorsi flex
Height	Pearson Correlation	.223	.632**	.247	.188
	Sig. (2-tailed)	.166	.000	.125	.244
	N	40	40	40	40
Weight	Pearson Correlation	.805**	.526**	.384*	.377*
	Sig. (2-tailed)	.000	.000	.014	.016
	N	40	40	40	40
Lumbar spine bmc	Pearson Correlation	.243	.393*	.354*	.120
	Sig. (2-tailed)	.131	.012	.025	.460
	N	40	40	40	40
Total body bmc	Pearson Correlation	.554**	.701**	.479**	.343*
	Sig. (2-tailed)	.000	.000	.002	.030
	N	40	40	40	40
Total body fat mass	Pearson Correlation	1	.118	.135	.297
	Sig. (2-tailed)	.	.468	.407	.063
	N	40	40	40	40
Total body lean mass	Pearson Correlation	.118	1	.454**	.277
	Sig. (2-tailed)	.468	.	.003	.084
	N	40	40	40	40
Plantar flex	Pearson Correlation	.135	.454**	1	.425**
	Sig. (2-tailed)	.407	.003	.	.006
	N	40	40	40	40
Dorsi flex	Pearson Correlation	.297	.277	.425**	1
	Sig. (2-tailed)	.063	.084	.006	.
	N	40	40	40	40
Left tibia length	Pearson Correlation	.266	.602**	.188	.237
	Sig. (2-tailed)	.097	.000	.245	.141
	N	40	40	40	40
Right tibia length	Pearson Correlation	.271	.606**	.191	.243
	Sig. (2-tailed)	.091	.000	.238	.130
	N	40	40	40	40
Dominant leg BMC	Pearson Correlation	.472**	.446**	.215	.263
	Sig. (2-tailed)	.002	.004	.183	.101
	N	40	40	40	40
Calcium	Pearson Correlation	-.168	.047	.415*	.016
	Sig. (2-tailed)	.343	.793	.015	.930
	N	34	34	34	34
Hrs phys act per wk	Pearson Correlation	-.530**	-.031	-.076	-.173
	Sig. (2-tailed)	.001	.860	.669	.327
	N	34	34	34	34
Total tibial CSA	Pearson Correlation	.171	.323*	.244	.203
	Sig. (2-tailed)	.291	.042	.129	.209
	N	40	40	40	40
Dorsiflexion CSA	Pearson Correlation	.063	.245	.198	-.035
	Sig. (2-tailed)	.700	.128	.222	.830
	N	40	40	40	40
Plantar CSA	Pearson Correlation	-.120	.084	-.034	-.275
	Sig. (2-tailed)	.460	.605	.836	.085
	N	40	40	40	40
Total muscle CSA	Pearson Correlation	-.065	.149	.014	-.239
	Sig. (2-tailed)	.692	.360	.931	.138
	N	40	40	40	40

Correlations

		Left tibia length	Right tibia length	Dominant leg BMC	Calcium
Height	Pearson Correlation	.774**	.755**	.261	-.196
	Sig. (2-tailed)	.000	.000	.104	.266
	N	40	40	40	34
Weight	Pearson Correlation	.521**	.516**	.481**	-.147
	Sig. (2-tailed)	.001	.001	.002	.406
	N	40	40	40	34
Lumbar spine bmc	Pearson Correlation	.187	.175	.418**	-.059
	Sig. (2-tailed)	.248	.280	.007	.741
	N	40	40	40	34
Total body bmc	Pearson Correlation	.542**	.546**	.607**	-.145
	Sig. (2-tailed)	.000	.000	.000	.412
	N	40	40	40	34
Total body fat mass	Pearson Correlation	.266	.271	.472**	-.168
	Sig. (2-tailed)	.097	.091	.002	.343
	N	40	40	40	34
Total body lean mass	Pearson Correlation	.602**	.606**	.446**	.047
	Sig. (2-tailed)	.000	.000	.004	.793
	N	40	40	40	34
Plantar flex	Pearson Correlation	.188	.191	.215	.415*
	Sig. (2-tailed)	.245	.238	.183	.015
	N	40	40	40	34
Dorsi flex	Pearson Correlation	.237	.243	.263	.016
	Sig. (2-tailed)	.141	.130	.101	.930
	N	40	40	40	34
Left tibia length	Pearson Correlation	1	.996**	.229	-.252
	Sig. (2-tailed)	.	.000	.156	.150
	N	40	40	40	34
Right tibia length	Pearson Correlation	.996**	1	.235	-.252
	Sig. (2-tailed)	.000	.	.144	.150
	N	40	40	40	34
Dominant leg BMC	Pearson Correlation	.229	.235	1	-.146
	Sig. (2-tailed)	.156	.144	.	.412
	N	40	40	40	34
Calcium	Pearson Correlation	-.252	-.252	-.146	1
	Sig. (2-tailed)	.150	.150	.412	.
	N	34	34	34	34
Hrs phys act per wk	Pearson Correlation	-.249	-.244	-.249	.170
	Sig. (2-tailed)	.156	.165	.156	.343
	N	34	34	34	33
Total tibial CSA	Pearson Correlation	.558**	.536**	.131	-.269
	Sig. (2-tailed)	.000	.000	.421	.124
	N	40	40	40	34
Dorsiflexion CSA	Pearson Correlation	.042	.050	.156	-.106
	Sig. (2-tailed)	.797	.759	.337	.552
	N	40	40	40	34
Plantar CSA	Pearson Correlation	-.146	-.142	.063	-.068
	Sig. (2-tailed)	.369	.383	.700	.704
	N	40	40	40	34
Total muscle CSA	Pearson Correlation	-.111	-.107	.063	-.081
	Sig. (2-tailed)	.494	.510	.700	.650
	N	40	40	40	34

Correlations

		Hrs phys act per wk	Total tibial CSA	Dorsiflexion CSA
Height	Pearson Correlation	-.185	.492**	.117
	Sig. (2-tailed)	.295	.001	.472
	N	34	40	40
Weight	Pearson Correlation	-.464**	.378*	.251
	Sig. (2-tailed)	.006	.016	.118
	N	34	40	40
Lumbar spine bmc	Pearson Correlation	.023	-.003	.451**
	Sig. (2-tailed)	.899	.986	.003
	N	34	40	40
Total body bmc	Pearson Correlation	-.351*	.224	.368*
	Sig. (2-tailed)	.042	.164	.020
	N	34	40	40
Total body fat mass	Pearson Correlation	-.530**	.171	.063
	Sig. (2-tailed)	.001	.291	.700
	N	34	40	40
Total body lean mass	Pearson Correlation	-.031	.323*	.245
	Sig. (2-tailed)	.860	.042	.128
	N	34	40	40
Plantar flex	Pearson Correlation	-.076	.244	.198
	Sig. (2-tailed)	.669	.129	.222
	N	34	40	40
Dorsi flex	Pearson Correlation	-.173	.203	-.035
	Sig. (2-tailed)	.327	.209	.830
	N	34	40	40
Left tibia length	Pearson Correlation	-.249	.558**	.042
	Sig. (2-tailed)	.156	.000	.797
	N	34	40	40
Right tibia length	Pearson Correlation	-.244	.536**	.050
	Sig. (2-tailed)	.165	.000	.759
	N	34	40	40
Dominant leg BMC	Pearson Correlation	-.249	.131	.156
	Sig. (2-tailed)	.156	.421	.337
	N	34	40	40
Calcium	Pearson Correlation	.170	-.269	-.106
	Sig. (2-tailed)	.343	.124	.552
	N	33	34	34
Hrs phys act per wk	Pearson Correlation	1	-.290	-.067
	Sig. (2-tailed)	.	.097	.709
	N	34	34	34
Total tibial CSA	Pearson Correlation	-.290	1	-.010
	Sig. (2-tailed)	.097	.	.951
	N	34	40	40
Dorsiflexion CSA	Pearson Correlation	-.067	-.010	1
	Sig. (2-tailed)	.709	.951	.
	N	34	40	40
Plantar CSA	Pearson Correlation	.125	-.216	.713**
	Sig. (2-tailed)	.482	.181	.000
	N	34	40	40
Total muscle CSA	Pearson Correlation	.019	-.114	.886**
	Sig. (2-tailed)	.913	.483	.000
	N	34	40	40

Correlations

		Plantar CSA	Total muscle CSA
Height	Pearson Correlation	-.110	-.018
	Sig. (2-tailed)	.501	.912
	N	40	40
Weight	Pearson Correlation	-.060	.059
	Sig. (2-tailed)	.713	.718
	N	40	40
Lumbar spine bmc	Pearson Correlation	.229	.315*
	Sig. (2-tailed)	.155	.048
	N	40	40
Total body bmc	Pearson Correlation	.100	.159
	Sig. (2-tailed)	.540	.326
	N	40	40
Total body fat mass	Pearson Correlation	-.120	-.065
	Sig. (2-tailed)	.460	.692
	N	40	40
Total body lean mass	Pearson Correlation	.084	.149
	Sig. (2-tailed)	.605	.360
	N	40	40
Plantar flex	Pearson Correlation	-.034	.014
	Sig. (2-tailed)	.836	.931
	N	40	40
Dorsi flex	Pearson Correlation	-.275	-.239
	Sig. (2-tailed)	.085	.138
	N	40	40
Left tibia length	Pearson Correlation	-.146	-.111
	Sig. (2-tailed)	.369	.494
	N	40	40
Right tibia length	Pearson Correlation	-.142	-.107
	Sig. (2-tailed)	.383	.510
	N	40	40
Dominant leg BMC	Pearson Correlation	.063	.063
	Sig. (2-tailed)	.700	.700
	N	40	40
Calcium	Pearson Correlation	-.068	-.081
	Sig. (2-tailed)	.704	.650
	N	34	34
Hrs phys act per wk	Pearson Correlation	.125	.019
	Sig. (2-tailed)	.482	.913
	N	34	34
Total tibial CSA	Pearson Correlation	-.216	-.114
	Sig. (2-tailed)	.181	.483
	N	40	40
Dorsiflexion CSA	Pearson Correlation	.713**	.886**
	Sig. (2-tailed)	.000	.000
	N	40	40
Plantar CSA	Pearson Correlation	1	.896**
	Sig. (2-tailed)	.	.000
	N	40	40
Total muscle CSA	Pearson Correlation	.896**	1
	Sig. (2-tailed)	.000	.
	N	40	40

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Appendix 4.2 Population-specific Z-scores

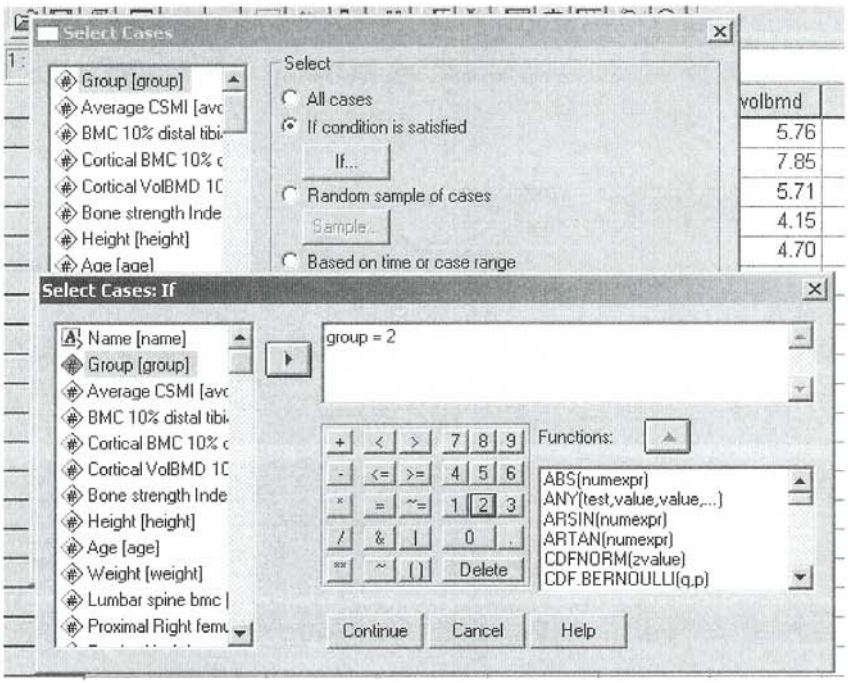
APPENDIX 4.2

Population-specific Z-scores

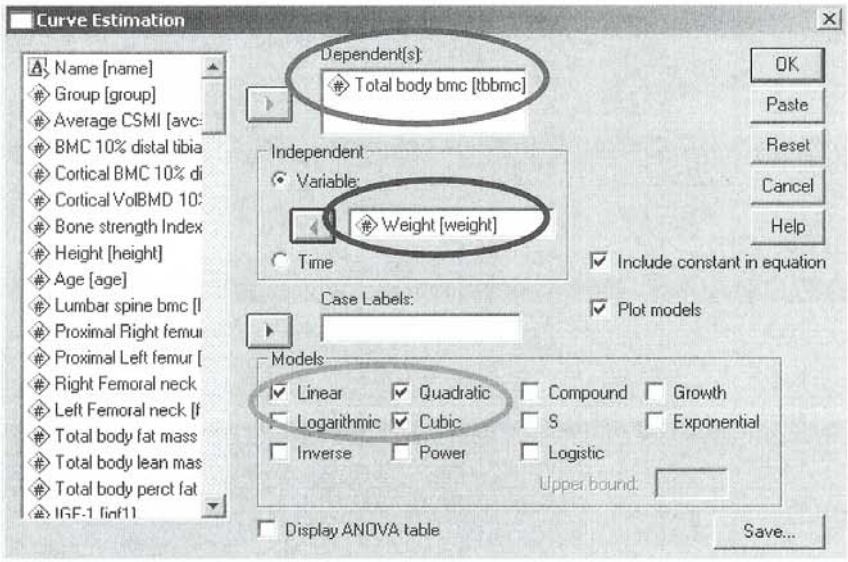
The following steps were undertaken to establish population-specific Z-scores using SPSS Version 11.5.1:

1/ Data – select cases

2/ If – Group = 2



3/ Analyse – regression – curve estimation



4/ Dependent variable = Total body BMC (g)

Independent variable = Weight (kg)

5/ Select a few models (linear, quadratic, cubic)

6/ Check for the greatest R square

```
MODEL:  MOD_1.
Independent:  WEIGHT


  Dependent Mth   Rsq  d.f.      F  Sigf      b0      b1   b2     b3

   TBBMC    LIN  .703    18   42.60  .000 1175.97 23.7289
   TBBMC    QUA  .739    17   24.04  .000 -581.52 68.1841  -.2707
 9 TBBMC    CUB  .742    17   24.43  .000 -43.696 46.9581      -.0011
```

7/ Accept a model and include ANOVA

8/ Record S.E value eg: 222.98846

```
MODEL:  MOD_2.
Dependent variable.. TBBMC              Method.. CUBIC

Listwise Deletion of Missing Data

Multiple R           .86134
R Square             .74191
Adjusted R Square    .71154
Standard Error     222.98846
```

9/ Create regression equation eg:

y = -43.695862 + 46.958121*weight -.001113*weight3**

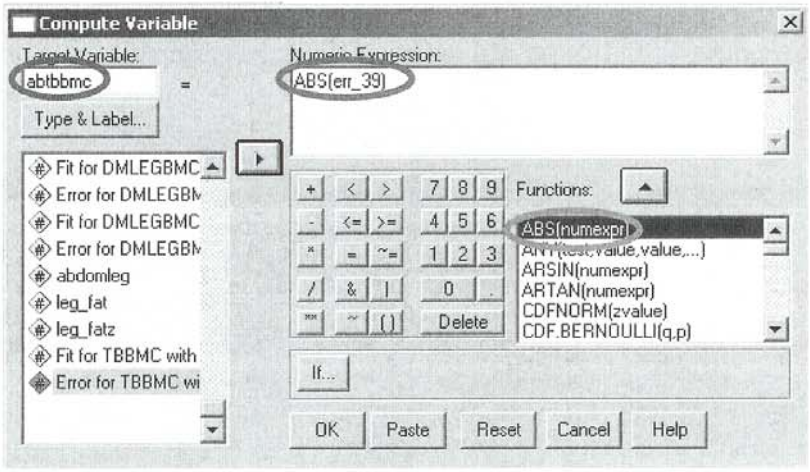
---------- Variables in the Equation ----------

Variable	B	SE B	Beta	T	Sig T
WEIGHT	46.958121	14.916027	1.659193	3.148	.0059
WEIGHT**3	-.001113	.000695	-.844162	-1.602	.1276
(Constant)	-43.695862	807.089582		-.054	.9575

10/ Transform – compute

11/ Create a new title eg: abtbbmc

12/ Select ABS and include error variable



13/ Analyse – regression – curve estimation

14/ Dependent variable = abtbbmc

Independent variable = weight (kg)

15/ Include ANOVA

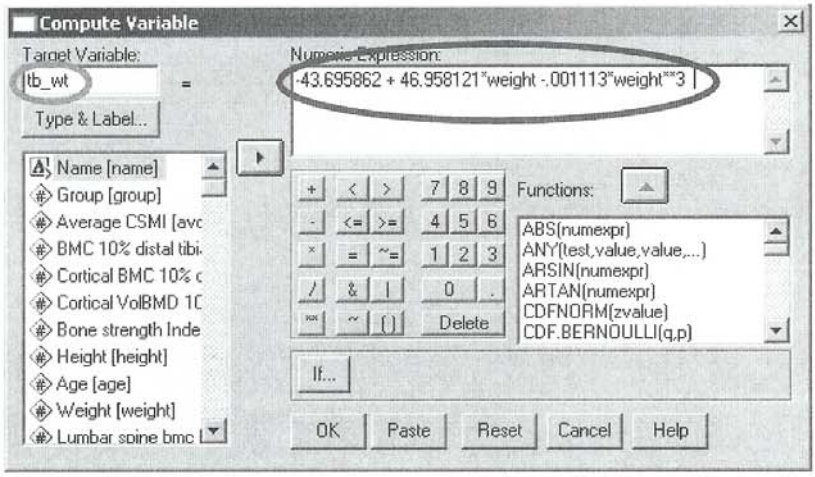
16/ Check significance of residuals

17/ Unselect cases

18/ Transform – compute

19/ Create title eg: tb_wt

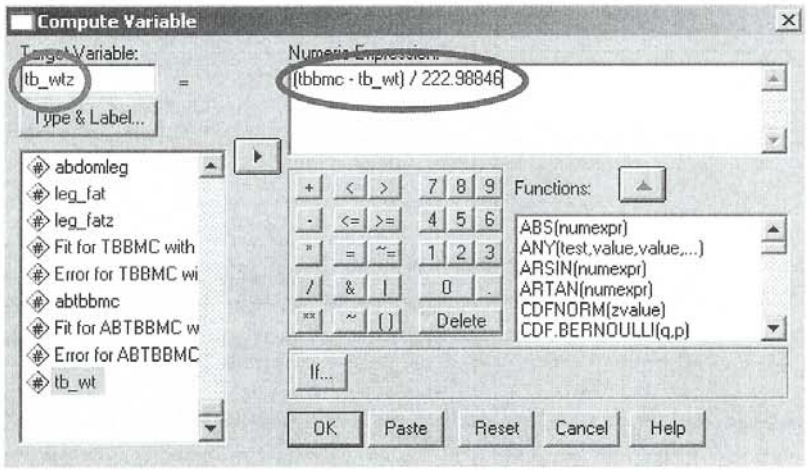
20/ Enter regression equation



21/ Transform – compute

22/ Create title eg: tb_wtz

23/ Select (tbbmc – tb_wt) / S.E (222.98846)



24/ Run independent t- test for difference between groups

T-Test

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
TB_WTZ	athletic male	20	.5671	1.22355	.27359
	control male	20	-.0002	.94591	.21151

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
TB_WTZ	Equal variances assumed	1.056	.311	1.641	38	.109	.5674	.34582	-.13272	1.26743
	Equal variances not assumed			1.641	35.734	.110	.5674	.34582	-.13418	1.26889

Appendix 4.3 Regression model (Total body BMC)

Regression

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	Total body lean mass[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: Total body bmc

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.701[a]	.491	.477	271.77919

a. Predictors: (Constant), Total body lean mass

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2705162.2	1	2705162.167	36.624	.000[a]
	Residual	2806829.3	38	73863.928		
	Total	5511991.4	39			

a. Predictors: (Constant), Total body lean mass

b. Dependent Variable: Total body bmc

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	287.333	431.028		.667	.509
	Total body lean mass	.046	.008	.701	6.052	.000

a. Dependent Variable: Total body bmc

Regression

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	Total body fat mass, Total body lean mass[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: Total body bmc

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.846[a]	.716	.701	205.63564

a. Predictors: (Constant), Total body fat mass, Total body lean mass

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	3947408.8	2	1973704.397	46.675	.000[a]
	Residual	1564582.6	37	42286.018		
	Total	5511991.4	39			

a. Predictors: (Constant), Total body fat mass, Total body lean mass

b. Dependent Variable: Total body bmc

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	286.508	326.127		.879	.385
	Total body lean mass	.043	.006	.644	7.302	.000
	Total body fat mass	.019	.003	.478	5.420	.000

a. Dependent Variable: Total body bmc

Appendix 5.1 Correlation matrix

Correlations

		Average CSMI 10% distal tibia	BMC 10% distal tibia	Cortical BMC 10% distal tibia (cm3)	Bone strength Index (mm4.gcm3)	Height	Weight	Femur length
Average CSMI 10% distal tibia	Pearson Correlation	1	.796**	.597**	.733**	.691**	.601**	.442**
	Sig. (2-tailed)	.	.000	.000	.000	.000	.000	.004
	N	80	80	80	80	80	80	40
BMC 10% distal tibia	Pearson Correlation	.796**	1	.407**	.289**	.810**	.676**	.515**
	Sig. (2-tailed)	.000	.	.000	.009	.000	.000	.001
	N	80	80	80	80	80	80	40
Cortical BMC 10% distal tibia (cm3)	Pearson Correlation	.597**	.407**	1	.862**	.319**	.262*	.465**
	Sig. (2-tailed)	.000	.000	.	.000	.004	.019	.003
	N	80	80	80	80	80	80	40
Bone strength Index (mm4.gcm3)	Pearson Correlation	.733**	.289**	.862**	1	.270*	.229*	.386*
	Sig. (2-tailed)	.000	.009	.000	.	.016	.041	.014
	N	80	80	80	80	80	80	40
Height	Pearson Correlation	.691**	.810**	.319**	.270*	1	.639**	.880**
	Sig. (2-tailed)	.000	.000	.004	.016	.	.000	.000
	N	80	80	80	80	80	80	40
Weight	Pearson Correlation	.601**	.676**	.262*	.229*	.639**	1	.052
	Sig. (2-tailed)	.000	.000	.019	.041	.000	.	.750
	N	80	80	80	80	80	80	40
Femur length	Pearson Correlation	.442**	.515**	.465**	.386*	.880**	.052	1
	Sig. (2-tailed)	.004	.001	.003	.014	.000	.750	.
	N	40	40	40	40	40	40	40
Plantar Flexion strength (Nm)	Pearson Correlation	.481**	.572**	.208	.158	.455**	.486**	.269
	Sig. (2-tailed)	.000	.000	.064	.161	.000	.000	.093
	N	80	80	80	80	80	80	40
Dorsi Flexion strength (Nm)	Pearson Correlation	.276*	.431**	.122	.021	.407**	.453**	.242
	Sig. (2-tailed)	.013	.000	.280	.856	.000	.000	.133
	N	80	80	80	80	80	80	40
Calcium	Pearson Correlation	.255*	.387**	.285*	.129	.307**	.109	.392*
	Sig. (2-tailed)	.028	.001	.014	.272	.008	.353	.012
	N	74	74	74	74	74	74	40
Estrogen (pmol/L)	Pearson Correlation	.113	.043	.066	.120	.423**	.110	.374*
	Sig. (2-tailed)	.488	.790	.688	.459	.007	.499	.017
	N	40	40	40	40	40	40	40

Correlations

		Height	Weight	Femur length	Plantar Flexion strength (Nm)	Dorsi Flexion strength (Nm)	Calcium	Estrogen (pmol/L)	Tibia total CSA
Fat Mass (kg)	Pearson Correlation	-.214	.865**	-.262	.131	.132	-.281	.039	.202
	Sig. (2-tailed)	.186	.000	.102	.422	.418	.079	.811	.211
	N	40	40	40	40	40	40	40	40
Lean Mass (kg)	Pearson Correlation	.668**	.328*	.575**	.380*	.336*	.372*	.115	.442**
	Sig. (2-tailed)	.000	.039	.000	.016	.034	.018	.480	.004
	N	40	40	40	40	40	40	40	40

Correlations

		Medullary CSA	Cortical CSA	Tibia BMC	Dorsiflex CSA	Plantarflex CSA	Total muscle CSA	Total Body BMC	Lumbar Spine BMC
Height	Pearson Correlation	.011	.422**	.519**	.249	.132	.225	.600**	.698**
	Sig. (2-tailed)	.946	.007	.001	.122	.416	.164	.000	.000
	N	40	40	40	40	40	40	40	40
Weight	Pearson Correlation	.294	.202	.320*	.157	-.060	.152	.741**	.059
	Sig. (2-tailed)	.066	.211	.044	.335	.713	.348	.000	.717
	N	40	40	40	40	40	40	40	40
Femur length	Pearson Correlation	-.109	.416**	.428**	.214	.171	.212	.431**	.516**
	Sig. (2-tailed)	.505	.008	.006	.185	.291	.188	.006	.001
	N	40	40	40	40	40	40	40	40
Plantar Flexion strength (Nm)	Pearson Correlation	.296	.139	.350*	.062	-.221	-.071	.366*	.206
	Sig. (2-tailed)	.064	.393	.027	.703	.171	.663	.020	.201
	N	40	40	40	40	40	40	40	40
Dorsi Flexion strength (Nm)	Pearson Correlation	.189	.030	.262	.041	-.189	-.061	.202	.155
	Sig. (2-tailed)	.243	.855	.103	.800	.243	.708	.212	.339
	N	40	40	40	40	40	40	40	40
Calcium	Pearson Correlation	-.154	.273	.175	.319*	.135	.240	.192	.373*
	Sig. (2-tailed)	.343	.088	.280	.045	.405	.135	.234	.018
	N	40	40	40	40	40	40	40	40
Estrogen (pmol/L)	Pearson Correlation	.075	.015	.066	.009	-.178	-.132	.295	.305
	Sig. (2-tailed)	.645	.927	.685	.957	.272	.419	.064	.056
	N	40	40	40	40	40	40	40	40
Tibia total CSA	Pearson Correlation	.760**	.291	.400*	.193	-.053	.126	.553**	.264
	Sig. (2-tailed)	.000	.069	.011	.232	.745	.438	.000	.100
	N	40	40	40	40	40	40	40	40
Medullary CSA	Pearson Correlation	1	-.389*	-.054	-.339*	-.510**	-.446**	.270	.048
	Sig. (2-tailed)	.	.013	.740	.032	.001	.004	.092	.769
	N	40	40	40	40	40	40	40	40
Cortical CSA	Pearson Correlation	-.389*	1	.704**	.779**	.682**	.856**	.413**	.301
	Sig. (2-tailed)	.013	.	.000	.000	.000	.000	.008	.059
	N	40	40	40	40	40	40	40	40
Tibia BMC	Pearson Correlation	-.054	.704**	1	.656**	.399*	.601**	.598**	.392*
	Sig. (2-tailed)	.740	.000	.	.000	.011	.000	.000	.012
	N	40	40	40	40	40	40	40	40

Correlations

		Average CSMI 10% distal tibia	BMC 10% distal tibia	Cortical BMC 10% distal tibia (cm3)	Bone strength Index (mm4.gcm3)	Height	Weight	Femur length
Dominant Femoral Neck BMC	Pearson Correlation	.722**	.799**	.487**	.415**	.692**	.537**	.442**
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	.004
	N	80	80	80	80	80	80	40
Non dominant Femoral Neck BMC	Pearson Correlation	.678**	.735**	.306**	.304**	.651**	.570**	.185
	Sig. (2-tailed)	.000	.000	.006	.006	.000	.000	.252
	N	80	80	80	80	80	80	40
Dominant Proximal Femur BMC	Pearson Correlation	.763**	.812**	.516**	.467**	.743**	.553**	.514**
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	.001
	N	80	80	80	80	80	80	40
Non dominantProximal Femur BMC	Pearson Correlation	.743**	.793**	.497**	.447**	.746**	.591**	.506**
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	.001
	N	80	80	80	80	80	80	40
Dominant Leg BMC	Pearson Correlation	.789**	.806**	.500**	.464**	.709**	.716**	.590**
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	.000
	N	80	80	80	80	80	80	40
Fat Mass (kg)	Pearson Correlation	-.047	-.054	-.058	-.067	-.077	.592**	-.262
	Sig. (2-tailed)	.682	.632	.606	.554	.495	.000	.102
	N	80	80	80	80	80	80	40
Lean Mass (kg)	Pearson Correlation	.800**	.896**	.389**	.355**	.831**	.668**	.575**
	Sig. (2-tailed)	.000	.000	.000	.001	.000	.000	.000
	N	80	80	80	80	80	80	40

Appendix 5.2 Regression model

(Cortical CSA and total muscle CSA)

Regression

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	Hours Physical Activity per week[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: Bone strength Index (mm4.gcm3)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.678[a]	.460	.443	20978.96157

a. Predictors: (Constant), Hours Physical Activity per week

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.24E+10	1	12359196069	28.082	.000[a]
	Residual	1.45E+10	33	440116828.75		
	Total	2.69E+10	34			

a. Predictors: (Constant), Hours Physical Activity per week

b. Dependent Variable: Bone strength Index (mm4.gcm3)

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	58113.601	5813.781		9.996	.000
	Hours Physical Activity per week	4091.395	772.076	.678	5.299	.000

a. Dependent Variable: Bone strength Index (mm4.gcm3)

Regression

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	Total muscle CSA, Hours Physical Activity per week[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: Bone strength Index (mm4.gcm3)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.733[a]	.538	.509	19704.80695

a. Predictors: (Constant), Total muscle CSA, Hours Physical Activity per week

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.45E+10	2	7229055038.7	18.618	.000[a]
	Residual	1.24E+10	32	388279416.89		
	Total	2.69E+10	34			

a. Predictors: (Constant), Total muscle CSA, Hours Physical Activity per week

b. Dependent Variable: Bone strength Index (mm4.gcm3)

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	24098.765	15615.853		1.543	.133
	Hours Physical Activity per week	3171.424	826.111	.526	3.839	.001
	Total muscle CSA	24.620	10.589	.318	2.325	.027

a. Dependent Variable: Bone strength Index (mm4.gcm3)

Appendix 5.3 Regression model (Total body BMC)

Regression

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	Total muscle CSA[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: Bone strength Index (mm4.gcm3)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.587[a]	.345	.328	25577.72236

a. Predictors: (Constant), Total muscle CSA

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.31E+10	1	13102957141	20.028	.000[a]
	Residual	2.49E+10	38	654219881.29		
	Total	3.80E+10	39			

a. Predictors: (Constant), Total muscle CSA

b. Dependent Variable: Bone strength Index (mm4.gcm3)

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	28065.226	15109.494		1.857	.071
	Total muscle CSA	30.681	6.856	.587	4.475	.000

a. Dependent Variable: Bone strength Index (mm4.gcm3)

Appendix 6.1 Correlation matrix

Correlations

		Average CSMI 10% distal tibia	BMC 10% distal tibia	Cortical BMC 10% distal tibia (cm3)	Bone strength Index (mm4.gcm3)	Height	Weight	Femur length
Dominant Femoral Neck BMC	Pearson Correlation	.722**	.799**	.487**	.415**	.692**	.537**	.442**
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	.004
	N	80	80	80	80	80	80	40
Non dominant Femoral Neck BMC	Pearson Correlation	.678**	.735**	.306**	.304**	.651**	.570**	.185
	Sig. (2-tailed)	.000	.000	.006	.006	.000	.000	.252
	N	80	80	80	80	80	80	40
Dominant Proximal Femur BMC	Pearson Correlation	.763**	.812**	.516**	.467**	.743**	.553**	.514**
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	.001
	N	80	80	80	80	80	80	40
Non dominantProximal Femur BMC	Pearson Correlation	.743**	.793**	.497**	.447**	.746**	.591**	.506**
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	.001
	N	80	80	80	80	80	80	40
Dominant Leg BMC	Pearson Correlation	.789**	.806**	.500**	.464**	.709**	.716**	.590**
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	.000
	N	80	80	80	80	80	80	40
Fat Mass (kg)	Pearson Correlation	-.047	-.054	-.058	-.067	-.077	.592**	-.262
	Sig. (2-tailed)	.682	.632	.606	.554	.495	.000	.102
	N	80	80	80	80	80	80	40
Lean Mass (kg)	Pearson Correlation	.800**	.896**	.389**	.355**	.831**	.668**	.575**
	Sig. (2-tailed)	.000	.000	.000	.001	.000	.000	.000
	N	80	80	80	80	80	80	40

Appendix 6.2 Regression model (Bone strength index – females)

Regression

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	Total muscle CSA[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: Bone strength Index (mm4.gcm3)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.587[a]	.345	.328	25577.72236

a. Predictors: (Constant), Total muscle CSA

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.31E+10	1	13102957141	20.028	.000[a]
	Residual	2.49E+10	38	654219881.29		
	Total	3.80E+10	39			

a. Predictors: (Constant), Total muscle CSA

b. Dependent Variable: Bone strength Index (mm4.gcm3)

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	28065.226	15109.494		1.857	.071
	Total muscle CSA	30.681	6.856	.587	4.475	.000

a. Dependent Variable: Bone strength Index (mm4.gcm3)

Appendix 6.3 Regression model (Neck of femur CSMI – females)

Regression

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	Dominant NOF CSA (mm2), Dominant femur length (mm)[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: Dominant NOF CSMI (mm4)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.578[a]	.334	.298	3030.58754

a. Predictors: (Constant), Dominant NOF CSA (mm2), Dominant femur length (mm)

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.70E+08	2	85220338.011	9.279	.001[a]
	Residual	3.40E+08	37	9184460.864		
	Total	5.10E+08	39			

a. Predictors: (Constant), Dominant NOF CSA (mm2), Dominant femur length (mm)

b. Dependent Variable: Dominant NOF CSMI (mm4)

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-29349.594	11739.822		-2.500	.017
	Dominant femur length (mm)	692.982	263.093	.367	2.634	.012
	Dominant NOF CSA (mm2)	66.391	25.745	.359	2.579	.014

a. Dependent Variable: Dominant NOF CSMI (mm4)

Appendix 6.4 Regression model (Bone strength index – males)

Regression

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	Total muscle CSA[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: Bone strength Index (mm4.gcm3)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.587[a]	.345	.328	25577.72236

a. Predictors: (Constant), Total muscle CSA

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.31E+10	1	13102957141	20.028	.000[a]
	Residual	2.49E+10	38	654219881.29		
	Total	3.80E+10	39			

a. Predictors: (Constant), Total muscle CSA

b. Dependent Variable: Bone strength Index (mm4.gcm3)

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	28065.226	15109.494		1.857	.071
	Total muscle CSA	30.681	6.856	.587	4.475	.000

a. Dependent Variable: Bone strength Index (mm4.gcm3)

Appendix 6.5 Regression model
(Neck of femur – males)

Regression

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	Dominant NOF CSA (mm2), Dominant femur length (mm)[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: Dominant NOF CSMI (mm4)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.578[a]	.334	.298	3030.58754

a. Predictors: (Constant), Dominant NOF CSA (mm2), Dominant femur length (mm)

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.70E+08	2	85220338.011	9.279	.001[a]
	Residual	3.40E+08	37	9184460.864		
	Total	5.10E+08	39			

a. Predictors: (Constant), Dominant NOF CSA (mm2), Dominant femur length (mm)

b. Dependent Variable: Dominant NOF CSMI (mm4)

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-29349.594	11739.822		-2.500	.017
	Dominant femur length (mm)	692.982	263.093	.367	2.634	.012
	Dominant NOF CSA (mm2)	66.391	25.745	.359	2.579	.014

a. Dependent Variable: Dominant NOF CSMI (mm4)

Appendix 7.1 Publications

Publications

Greene, D.A., Naughton, G.A., Briody, J.N., Kemp, A., Woodhead, H., and Farpour-Lambert, N. (2004). Musculoskeletal health in elite male adolescent middle-distance runners. *Journal of Science and Medicine in Sport*, 7 (3), 373-383.

Greene, D.A., Naughton, G.A., Briody, J.N., Kemp, A., Woodhead, H., and Corrigan, L. (2004). Bone strength index in adolescent females: Does physical activity make a difference? *British Journal of Sports Medicine*. (Accepted - in press).

Greene, D.A., Naughton, G.A., Briody, J.N., Kemp, A., Woodhead, H., and Farpour-Lambert, N. (2004). Bone and muscle geometry in female adolescent middle-distance runners. *Pediatric Exercise Science*. (Accepted – in press)

Greene, D.A., Naughton, G.A., Briody, J.N., Kemp, A., Woodhead, H. (2004) Assessment of bone strength at differentially-loaded skeletal regions in adolescent middle-distance runners. *Bone*. (Submitted November, 2004)

Appendix 7.2 Conference presentations

Conference presentations

2003 **Greene, D.A**., Naughton, G.A., Briody, J. Towards an understanding of musculoskeletal health in elite male adolescent middle-distance runners. Australian Conference of Science and Medicine in Sport.Tackling Barriers to Participation and Performance. 25-28 October, Canberra, Australian Capital Territory.

2004 **Greene, D.A**. Factors predictive of musculoskeletal health in male and female adolescent middle distant runners (invited talk) New South Wales Conference of Science and Medicine in Sport, 6 March, Sydney, New South Wales.

2004 **Greene D.A**., Naughton G.A., Briody J., Kemp A., Woodhead H., Fapour-Lambert, N. Musculoskeletal health in elite male adolescent middle distance runners. Amercian College of Sports Medicine Conference, June 2004, Indianapolis, IN, USA.

2004 **Greene D.A**., Naughton G.A., Briody J., Kemp A., Woodhead H., Fapour-Lambert, N. Predicting musculoskeletal health in adolescent middle distance runners, Amercian College of Sports Medicine Conference, June 2004, Indianapolis, IN, USA.

2004 **Greene, D.A**., Naughton, G.A., Kemp, A., Briody, J., Corrigan, L. Bone strength index in adolescent females: Does physical activity make a difference? Australian Conference of Science and Medicine in Sport. Hot topics from the Red Centre. 7 – 9 October, Alice Springs, Northern Territory.

Appendix 7.3 Awards / Grants

Awards

2002-2004	Australian Catholic University Post Graduate Scholarship (PhD) for the project Exercise type, musculoskeletal development and injury risk factors in elite adolescent athletes.
2002 -	CHISM Research Team: Member of the Outstanding research team gold medal. NSW Sports Safety Awards - $1,500.
2003 -	Ken Maguire Award for Best Young Investigator – Clinical Science Australian Conference of Science and Medicine in Sport award - $2,500.
2003 -	New South Wales Most Outstanding New Research Talent in Applied Sports Medicine. NSW Sports Safety Awards - $15,000.

Grants

2002 -	Greene D A. The Children's Hospital, Westmead Hospital Small Grants Scheme Markers of bone adaptation in young populations using hip structural analysis - $5,360.
2003 -	The LCM Sports Medicine Trust - $16,485.

References

Aaron, D. J., Krista, A. M., Dearwater, S. R., Cauley, J. A., Metz, K. F., & LaPorte, R. E. (1995). Reproducibility and validity of an epidemiological questionnaire to assess past year physical activity in adolescents. *American Journal of Epidemiology, 142* (2), 191-201.

Abrams, S., & Stuff, J. (1994). Calcium metabolism in girls: current dietary intakes lead to low rates of calcium absorption and retention during puberty. *American Journal of Clinical Nutrition, 60* (5), 739-743.

Adirim, T. A., & Cheng, T. L. (2003). Overview of injuries in the young athlete. *Sports Medicine, 33* (1), 75-81.

Anai, T., Miyazaki, F., Tomiyasu, T., & Matsuo, T. (2001). Risk of irregular menstrual cycles and low peak bone mass during early adulthood associated with age at menarche. *Pediatrics International, 43* (5), 438-438.

Anderson, S. J., Griesemer, B. A., Johnson, M. D., Martin, T. J., McLain, L. G., Rowland, T. W., et al. (2000). Intensive training and sports specialization in young athletes. *Pediatrics,106 (*1), 154-157.

Andon, M., Lloyd, T., & Matkovic, V. (1994). Supplementation trials with calcium citrate malate: evidence in favor of increasing the calcium RDA during childhood and adolescence. *Journal of Nutrition, 124* (8 Suppl), 1412S-1417S.

Andreoli, A., Monteleone, M., Van Loan, M., Promenzio, L., Tarantino, U., & De Lorenzo, A. (2001). Effects of different sports on bone density and muscle mass in highly trained athletes. *Medicine & Science in Sports & Exercise, 33* (4), 507-511.

Apple, D. F. (1985). Adolescent runners. *Clinics in Sports Medicine, 4*, 641-655.

Arena, B., Maffulli, N., Maffulli, F., & Morleo, M. (1995). Reproductive hormones and menstrual changes with exercise in female athletes. *Sports Medicine, 19* (4), 278-287.

Augut, P., Gordon, C. L., Lang, T. F., Iidia, H., & Genant, H. K. (1998). Accuracy of cortical and trabecular bone measurements with peripheral quantitative computed tomography (pQCT). *Physics in Medicine and Biology, 43*, 2873-2883.

Bachrach, L. K., Guido, D., Katzman, D., Litt, I. F., & Marcus, R. (1990). Decreased bone density in adolescent girls with anorexia nervosa. *Pediatrics, 86* (3), 440-447.

Baer, J., Taper, L., Gwazdauskas, F., Walberg, J., Novascone, M., Ritchey, S., et al. (1992). Diet, hormonal, and metabolic factors affecting bone mineral density in adolescent amenorrheic and eumenorrheic female runners. *Journal of Sports Medicine & Physical Fitness, 32* (1), 51-58.

Bailey, D. (1997). The Saskatchewan pediatric bone mineral accrual study: bone mineral acquisition during the growing years. *International Journal of Sports Medicine, 18* (3), S191-194.

Bailey, D., Faulkner, R., & McKay, H. (1996). Growth, physical activity, and bone mineral acquisition. *Exercise & Sport Sciences Reviews, 24*, 233-266.

Bailey, D., McKay, H., Mirwald, R., Crocker, P., & Faulkner, R. (1999). A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the university of Saskatchewan bone mineral accrual study. *Journal of Bone & Mineral Research, 14* (10), 1672-1679.

Bailey, D. A. (2000). Physical activity and bone mineral acquisition during adolescence. *Osteoporosis International, 11* (Suppl. 4), S2-S3.

Baker, S., Cochran, W., Flores, C., Georgieff, M., Jacobson, M., Jaksic, T., et al. (1999). American Academy of Pediatrics. Committee on Nutrition. Calcium requirements of infants, children, and adolescents. *Pediatrics, 104* (5 Pt 1), 1152-1157.

Ballabriga, A. (2000). Morphological and physiological changes during growth: an update. *European Journal of Clinical Nutrition, 54* (1), S1-6.

Bamman, M., Newcomer, B., Larson-Meyer, D., Weinsier, R., & Hunter, G. (2000). Evaluation of the strength-size relationship *in vivo* using various muscle size indices. *Medicine & Science in Sports & Exercise, 32* (7), 1307-1313.

Barker, J. L., & Haugh, C. G. (1979). Mechanical properties of bone: a review. *Trans Am Soc Agr Eng, 22*, 678-687.

Barrow, G., & Saha, S. (1988). Menstrual irregularity and stress fractures in collegiate female distance runners. *American Journal of Sports Medicine, 16* (3), 209-216.

Bass, S. (2000). The prepubertal years: a uniquely opportune stage of growth when the skeleton is most responsive to exercise? *Sports Medicine, 30* (2), 73-78.

Bass, S., Bradney, M., Pearce, G., Hendrich, E., Inge, K., Stuckey, S., et al. (2000). Short stature and delayed puberty in gymnasts: influence of selection bias on leg length and the duration of training on trunk length. *Journal of Pediatrics, 136* (2), 149-155.

Bass, S., Delmas, P., Pearce, G., Hendrich, E., Tabensky, A., & Seeman, E. (1999). The differing tempo of growth in bone size, mass, and density in girls is region-specific. *Journal of Clinical Investigation, 104* (6), 795-804.

Bass, S., Saxon, L., Daly, R., Turner, C., Robling, A., Seeman, E., et al. (2002). The effect of mechanical loading on the size and shape of bone in pre-, peri-, and postpubertal girls: a study in tennis players. *Journal of Bone & Mineral Research, 17* (12), 2274-2280.

Baxter-Jones, A.D.G., Helms, P.J. (1996). Effects of training at a young age: a review of the training of young athletes study. *Pediatric Exercise Science*, 8 (4), 310-327.

Baxter-Jones, A., Helms, P., Baines-Preece, J., & Preece, M. (1994). Menarche in intensively trained gymnasts, swimmers and tennis players. *Annals of Human Biology, 21*, 407-415.

Baxter-Jones, A., & Maffulli, N. (2002). Intensive training in elite young female athletes. Effects of intensive training on growth and maturation are not established. *British Journal of Sports Medicine, 36* (1), 13-15.

Baxter-Jones, A., Mirwald, R., McKay, H., & Bailey, D. (2003). A longitudinal analysis of sex differences in bone mineral accrual in healthy 8-19-year-old boys and girls. *Annals of Human Biology, 30* (2), 160-175.

Baxter-Jones, A. D., Helms, P., Maffulli, N., Baines-Preece, J. C., & Preece, M. (1995). Growth and development of male gymnasts, swimmers, soccer and tennis players: a longitudinal study. *Annals of Human Biology, 22* (5), 381-394.

Beck, T., Ruff, C., Warden, K., Scott, W. J., & Rao, G. (1990). Predicting femoral neck strength from bone mineral data. A structural approach. *Investigative Radiology, 25* (1), 6-18.

Beck, T. J., Oreskovic, T., Stone, K. L., Ruff, C. B., Ensrud, K., Nevitt, M. C., et al. (2001). Structural adaptation to changing skeletal load in the progression toward hip fragility: the study of osteoporotic fractures. *Journal of Bone and Mineral Research, 16* (6), 1108-1119.

Bemben, D. A., Buchanan, T. D., Bemben, M. G., & Knehans, A. W. (2004). Influence of type of mechanical loading, menstrual status, and training season on bone density in young woman athletes. *Journal of Strength and Conditioning Research, 18* (2), 220-226.

Benito, M., Gomberg, B., Wehrli, F., Weening, R., Zemel, B., Wright, A., et al. (2003). Deterioration of trabecular architecture in hypogonadal men. *Journal of Clinical Endocrinology & Metabolism, 488* (4), 1497-1502.

Bennell, K., Malcolm, S., Khan, K., Thomas, S., Reid, S., Brukner, P., et al. (1997). Bone mass and bone turnover in power athletes, endurance athletes, and controls: a 12-month longitudinal study. *Bone, 20* (5), 477-484.

Bennell, K. L., & Crossley, K. (1996). Musculoskeletal injuries in track and field: incidence, distribution and risk factors. *Australian Journal of Science and Medicine in Sport, 28* (3), 69-75.

Bennell, K. L., Malcolm, S. A., Thomas, S. A., Reid, S. J., Brukner, P. D., & Ebling, P. R. (1996). Risk factors for stress fractures in track and field athletes. *American Journal of Sports Medicine, 24* (6), 810-820.

Black, R., Williams, S., Jones, I., & Goulding, A. (2002). Children who avoid drinking cow milk have low dietary calcium intakes and poor bone health. *American Journal of Clinical Nutrition, 76* (3), 675-680.

Blanchet, C., Giguere, Y., Prudhomme, D., Dumont, M., Rosseau, F., & Dodin, S. (2002). Association of physical activity and bone: influence of vitamin D receptor genotype. *Medicine and Science in Sports and Exercise, 34*, 24-31.

Blimkie, C., Rice, S., Webber, C., Martin, J., Levy, D., & Gordon, C. (1996). Effects of resistance training on bone mineral content and density in adolescent females. *Canadian Journal of Physiology & Pharmacology, 74* (9), 1025-1033.

Boehm, H., Raeth, C., Monetti, R., Mueller, D., Newitt, D., Majumdar, S., et al. (2003). Local 3D scaling properties for the analysis of trabecular bone extracted from high-resolution magnetic resonance imaging of human trabecular bone: comparison with bone mineral density in the prediction of biomechanical strength in vitro. *Investigative Radiology, 38* (5), 269-280.

Bollen, A. (2000). A prospective longitudinal study of urinary excretion of a bone resorption marker in adolescents. *Annals of Human Biology, 27* (2), 199-211.

Bolotin, H. H. (1998). Analytic and quantitative exposition of patient-specific systematic inaccuracies inherent in planar DXA-derived in vivo BMD measurements. *Med Phys, 25*, 139-151.

Bolotin, H. H., & Sievanen, H. (2001). Inaccuracies inherent in dual x-ray absorptiometry in vivo bone mineral density can seriously mislead diagnostic / prognostic interpretations of patient-specific bone fagility. *Journal of Bone and Mineral Research, 16* (5), 799-805.

Bolotin, H. H., Sievanen, H., & Grashuis, J. L. (2003). Patient-specific DXA bone mineral density inaccuracies: quantitative effects og nonuniform extraosseous fat distributions. *Journal of Bone and Mineral Research, 18*, 1020-1027.

Bolotin, H. H., Sievanen, H., Grashuis, J. L., Kuiper, J. W., & Jarvinen, T. L. N. (2001). Inaccuracies inherent in patient-specific DXA bone mineral density measurements: comprehensive phantom-based evaluation. *Journal of Bone and Mineral Research, 16*, 417-426.

Bonen, A. (1992). Recreational exercise does not impair menstrual cycles: a prospective study. *International Journal of Sports Medicine, 13*, 110-120.

Bonjour, J., Carrie, A., Ferrari, S., Clavien, H., Slosman, D., Theintz, G., et al. (1997). Calcium-enriched foods and bone mass growth in prepubertal girls: a randomized, double-blind, placebo-controlled trial. *Journal of Clinical Investigation, 99* (6), 1287-1294.

Bonjour, J.P., T. Chavelley, Ammann, P., Slosman, D., and Rizzoli, R. (2001). Gain in bone mineral mass in prepubertal girls 3.5 years after discontinuation of calcium supplementation: a follow-up study. *Lancet,* 358 (9289):1208-1212.

Boonen, S., Cheng, X. G., Nijs, J., Nicholson, P. H., Verbeke, G., Lesaffre, E., et al. (1997). Factors associated with cortical and trabecular bone loss as quantified by peripheral computed tomography (pQCT) at the ultradistal radius in aging women. *Calcified Tissue International, 60*, 164-170.

Bouchard, C., Tremblay, A., Leblanc, C., Lortie, G., Savard, R., & Theriault, G. (1983). A method to assess energy expenditure in children and adults. *American Journal of Clinical Nutrition, 37* (3), 461-467.

Bradney, M., Pearce, G., Naughton, G., Sullivan, C., Bass, S., Beck, T., et al. (1998). Moderate exercise during growth in prepubertal boys: changes in bone mass, size, volumetric density, and bone strength: a controlled prospective study. *Journal of Bone & Mineral Research, 13* (12), 1814-1821.

Brandenburger, G. (1993). Clinical determination of bone quality: is ultrasound an answer? *Calcified Tissue International, 53* (Suppl 1), S151-156.

Bressot, C., Meunier, P. J., Chapuy, M. C., Lejeune, E., Edovard, C., & Darby, A. J. (1979). Histomorphometric profle, pathology and reversibility of corticosteroid-induced osteoporosis. *Metabolic Bone Disease and Related Research, 1*, 303-311.

Brooks, G. A., & Mercier, J. (1994). Balance of carbohydrate and lipid utilization during exercise: the "crossover" concept. *Journal of Applied Physiology, 76*, 2253-2261.

Brukner, P. (2000). Exercise-related lower leg pain: bone. *Medicine and Science in Sports and Exercise, 32* (3 Suppl), S15-26.

Brukner, P., & Bennell, K. (1997). Stress fractures in female athletes. Diagnosis, management and rehabilitation. *Sports Medicine, 24* (6), 419-429.

Burr, D., Robling, A., & Turner, C. R. (2002). Effects of biomechanical stress on bones in animals. *Bone, 30* (5), 781-786.

Burrows, M., Nevill, A., Bird, S., & Simpson, D. (2003). Physiological factors associated with low bone mineral density in female endurance runners. *British Journal of Sports Medicine, 37* (1), 67-71.

Cain, E. L., Dugas, J. R., Wolf, R. S., & Andrews, J. R. (2003). Elbow injuries in throwing athletes: a current concepts review. *American Journal of Sports Medicine, 31* (4), 621-645.

Caine, D., Howe, W., Ross, W., & Bergman, G. (1997). Does repetitive physical loading inhibit radial growth in female gymnasts? *Clinical Journal of Sport Medicine, 7* (4), 302-308.

Calbet, J., Dorado, C., Diaz-Herrera, P., & Rodriguez-Rodriguez, L. (2001). High femoral bone mineral content and density in male football (soccer) players. *Medicine & Science in Sports & Exercise, 33* (10), 1582-1587.

Callahan, L. (2000). The evolution of the female athlete: progress and problems. *Pediatric Annals, 29* (3), 149-153.

Cameron, K.R., Telford, R.D., and Wark, J.D. (1992) Stress fractures in Australian competitive runners. In *Australian Sports Medicine Federation Annual Scientific Conference in Sports Medicine*, Perth, Australia.

Carrie Fassler, A., & Bonjour, J. (1995). Osteoporosis as a pediatric problem. *Pediatric Clinics of North America, 42* (4), 811-824.

Carter, D. R., & Hayes, W. C. (1977). The compressive behaviour of bone as a two-phase porous structure. *Journal of Bone and Joint Surgery, 59A*, 954-962.

Carter, L., Whiting, S., Drinkwater, D., Zello, G., Faulkner, R., & Bailey, D. (2001). Self-reported calcium intake and bone mineral content in children and adolescents. *Journal of the American College of Nutrition, 20* (5), 502-509.

Casperson, C. J., Powell, K. E., Koplen, J. P., Shirley, R. W., Cambell, C. C., & Spikes, R. K. (1984). The incidence of injuries and hazards in receational and fitness runners. *Medicine & Science in Sports & Exercise, 16*, 113-114.

Chan, G., Hoffman, K., & McMurry, M. (1995). Effects of dairy products on bone and body composition in pubertal girls. *Journal of Pediatrics, 126* (4), 551-556.

Chatterjee, S., & Bandyopadhyay, A. (1993). Effect of continuous slow-speed running for 12 weeks on 10-14-year-old Indian boys. *British Journal of Sports Medicine, 27* (3), 179-185.

Cheng, J., Leung, S., Lee, W., Lau, J., Maffulli, N., Cheung, A., et al. (1998). Determinants of axial and peripheral bone mass in Chinese adolescents. *Archives of Disease in Childhood, 78* (6), 524-530.

Clarkson, P., & Haymes, E. (1995). Exercise and mineral status of athletes: calcium, magnesium, phosphorus, and iron. *Medicine & Science in Sports & Exercise, 27* (6), 831-843.

Claessens, A.L. (1999). Elite female gymnasts: a kinanthropometric overview. In F.E. Johnston, P. Eveleth, and B. Zemel (Eds.), *Human growth in context*. London: Smith-Gordon and Company.

Cobb, K., Bachrach, L., Greendale, G., Marcus, R., Neer, R., Nieves, J., et al. (2003). Disordered eating, menstrual irregularity, and bone mineral density in female runners. *Medicine & Science in Sports & Exercise, 35* (5), 711-719.

Conroy, B., Kraemer, W., Maresh, C., Fleck, S., Stone, M., Fry, A., et al. (1993). Bone mineral density in elite junior Olympic weightlifters. *Medicine & Science in Sports & Exercise, 25* (10), 1103-1109.

Cooper, C., Cawley, M., Bhalla, A., Egger, P., Ring, F., Morton, L., et al. (1995). Childhood growth, physical activity, and peak bone mass in women. *Journal of Bone & Mineral Research, 10* (6), 108-114.

Cooper, G. S., & Umbach, D. M. (1996). Are vitamin D receptor polymorphisms associated with bone mineral density? A meta-analysis. *Journal of Bone and Mineral Research, 12*, 1841-1849.

Courteix, D., Lespessailles, E., Peres, S., Obert, P., Germain, P., & Benhamou, C. (1998). Effect of physical training on bone mineral density in prepubertal girls: a comparative study between impact-loading and non-impact-loading sports. *Osteoporosis International, 8* (2), 152-158.

Courteix, D., Lespessailles, E., Jaffre, C., Obert, P., and Benhamou, C.L. (1999). Bone material acquisition and somatic development in highly trained girl gymnasts. *Acta Paediatrica*, 88 (8), 803-808.

Cowan, D. N., Jones, B. H., & Robinson, J. R. (1993). Foot morphologic characteristics and risk of exercise-related injury. *Archives of Family Medicine, 2*, 773-777.

Cowin, S. (1983). The mechanical and stress adaptive properties of bone. *Annals of Biomedical Engineering, 11* (3-4), 253-295.

Crawford, B. A. L., Liu, P., Kean, M. T., Bleasel, J. F., & Handelsman, D. J. (2003). Randomized placebo-controlled trial of andrigen effects on muscle and bone in men requiring long-term systemic glucocorticoid treatment. *Journal of Clinical Endocrinology and Metabolism, 88*, 3167-3176.

Cromer, B., & Harel, Z. (2000). Adolescents: at increased risk for osteoporosis? *Clinical Pediatrics, 39* (10), 565-574.

Cupisti, A., D'Alessandro, C., Castrogiovanni, S., Barale, A., & Morelli, E. (2002). Nutrition knowledge and dietary composition in Italian adolescent female athletes and non-athletes. *International Journal of Sport Nutrition & Exercise Metabolism, 12* (2), 207-219.

Currey, J. D. (1969). The mechanical consequences of variation in the mineral content of bone. *Journal of Biomechanics, 2*, 1-11.

Currey, J. D. (1988). The effect of porosity and mineral content on the Young's modulus of elasticity of compact bone. *Journal of Biomechanics, 21*, 131-140.

Currey, J. D. (2001). Bone strength: what are we trying to measure? *Calcified Tissue International, 68*, 205-210.

Cutler, G. J. (1997). The role of estrogen in bone growth and maturation during childhood and adolescence. *Journal of Steroid Biochemistry & Molecular Biology, 61* (3-6), 141-144.

Dalton, S. E. (1992). Overuse injuries in adolescent athletes. *Sports Medicine, 13* (1), 58-70.

Daly, R., Rich, P., Klein, R., & Bass, S. (1999). Effects of high-impact exercise on ultrasonic and biochemical indices of skeletal status: A prospective study in young male gymnasts. *Journal of Bone & Mineral Research, 14* (7), 1222-1230.

Daly, R. M., Saxon, L., Turner, C. H., Robling, A. G., & Bass, S. (2004). The relationship between muscle size and bone geometry during growth and in response to exercise. *Bone, 34*, 281-287.

Damsgaard, R., Bencke, J., Matthiesen, G., Petersen, J., & Muller, J. (2000). Is prepubertal growth adversely affected by sport? *Medicine & Science in Sports & Exercise, 32* (10), 1210-1215.

de Ridder, C., & Delemarre-van de Waal, H. (1998). Clinical utility of markers of bone turnover in children and adolescents. *Current Opinion in Pediatrics, 10* (4), 441-448.

Delvaux, K., Lefevre, J., Philippaerts, R., Dequeker, J., Thomis, M., Vanreusel, B., et al. (2001). Bone mass and lifetime physical activity in Flemish males: a 27-year follow-up study. *Medicine & Science in Sports & Exercise, 33* (11), 1868-1875.

Demetropoulos, C. K., Willis, C. B., & Goldstein, S. A. (1993). Biomechanical characteristics of rat trabecular bone. *Transactions of the Orthopedic Research Society, 23*, 1103-1113.

DiFiori, J. P. (1999). Overuse injuries in children and adolescents. *The Physician and Sportsmedicine, 27* (1), 1-10.

Dixon, M., & Fricker, P. (1993). Injuries to elite gymnasts over 10 yr. *Medicine & Science in Sports & Exercise, 25* (12), 1322-1329.

Drinkwater, B., Bruemner, B., & Chesnut, C. R. (1990). Menstrual history as a determinant of current bone density in young athletes. *Journal of American Medical Association, 263* (4), 545-548.

Drinkwater, B., Nilson, K., Ott, S., & Chesnut, C. R. (1986). Bone mineral density after resumption of menses in amenorrheic athletes. *Journal of American Medical Association, 256* (3), 380-382.

Drubach, L., Connolly, L., D'Hemecourt, P., & Treves, S. (2001). Assessment of the clinical significance of asymptomatic lower extremity uptake abnormality in young athletes. *Journal of Nuclear Medicine, 42* (2), 209-212.

Duke, P. M., Litt, I. F., & Gross, R. T. (1980). Adolescent's self assessment of sexual maturation. *Pediatrics, 66* (6), 918-920.

Duncan, C., Blimkie, C., Cowell, C., Burke, S., Briody, J., & Howman-Giles, R. (2002). Bone mineral density in adolescent female athletes: relationship to exercise type and muscle strength. *Medicine & Science in Sports & Exercise, 34* (2), 286-294.

Duncan, C., Blimkie, C. J., Kemp, A., Higgs, W., Cowell, C. T., Woodhead H., et al. (2002). Mid-femur geometry and biomechanical properties in 15- to 18-yr-old female athletes. *Medicine & Science in Sports & Exercise, 34* (4), 673-681.

Dyson, K., Blimkie, C., Davison, K., Webber, C., & Adachi, J. (1997). Gymnastic training and bone density in pre-adolescent females. *Medicine & Science in Sports & Exercise, 29* (4), 443-450.

Einhorn, T. (1992). Bone strength: the bottom line. *Calcified Tissue International, 51* (5), 333-339.

Eisenmann, J., & Malina, R. (2002). Growth status and estimated growth rate of young distance runners. *International Journal of Sports Medicine, 23* (3), 168-173.

Elgan, C., Dykes, A., & Samsioe, G. (2002). Bone mineral density and lifestyle among female students aged 16-24 years. *Gynecological Endocrinology, 16* (2), 91-98.

Eliakim, A., Brasel, J., Mohan, S., Barstow, T., Berman, N., & Cooper, D. (1996). Physical fitness, endurance training, and the growth hormone-insulin-like growth factor I system in adolescent females. *Journal of Clinical Endocrinology & Metabolism, 81* (11), 3986-3992.

Emans, S. (1997). Menarche and beyond--do eating and exercise make a difference? *Pediatric Annals, 26* (2 Suppl), S137-141.

Emslander, H., Sinaki, M., Muhs, J., Chao, E., Wahner, H., Bryant, S., et al. (1998). Bone mass and muscle strength in female college athletes (runners and swimmers). *Mayo Clinic Proceedings, 73* (12), 1151-1160.

Faulkner, K. (2000). Bone matters: are density increases necessary to reduce fracture risk? *Journal of Bone & Mineral Research, 15* (2), 183-187.

Faulkner, R., Forwood, M., Beck, T., Mafukidze, J., Russell, K., & Wallace, W. (2003). Strength indices of the proximal femur and shaft in prepubertal female gymnasts. *Medicine & Science in Sports & Exercise, 35* (3), 513-518.

Ferber, R., McClay-Davis, I., Hamill, J., Pollard, C. D., & McKeown, K. A. (2002). Kinetic variables in subjects with previous lower extremity stress fractures. *Medicine & Science in Sports & Exercise, 34*, S5.

Ferretti, J., Cointry, G., Capozza, R., & Frost, H. (2003). Bone mass, bone strength, muscle-bone interactions, osteopenias and osteoporoses. *Mechanisms of Ageing & Development, 124* (3), 269-279.

Ferretti, J. L., Capozza, R. F., Mondelo, N., & Zanchetta, J. R. (1993). Interrelationships between densitometric, geometric, and mechanical properties of rat femora: inferences concerning mechanical regulation of bone modeling. *Journal of Bone and Mineral Research, 8* (11), 1389-1396.

Ferretti, J. L., Capozza, R. F., & Zanchetta, J. R. (1996). Mechanical validation of a tomographic (pQCT) index for noninvasive estimation of rat femur bending strength. *Bone, 18* (2), 97-102.

Fielsler, C. M. (2001). The Female Runner. In F. G. O'Connor & R. P. Wilder (Eds.), *Textbook of Running Medicine* (pp. p. 435-446). New York: McGraw Hill.

Foldes, A., Danziger, A., Constantini, N., & Popovtzer, M. (1997). Reduced ultrasound velocity in tibial bone of young ballet dancers. *International Journal of Sports Medicine, 18* (4), 296-299.

Foldes, A., Rimon, A., Keinan, D., & Popovtzer, M. (1995). Quantitative ultrasound of the tibia: a novel approach for assessment of bone status. *Bone, 17* (4), 363-367.

Forwood, M.R., Bailey, D.A., Beck, T.J., Mirwald, R.L., Baxter-Jones, A.D., and Uusi-Rasi, K. (2004). Sexual dimorphism of the femoral neck during the adolescent growth spurt: a structural analysis. *Bone*, 35 (4), 973-981.

Forwood, M. (2001). Mechanical effects on the skeleton: are there clinical implications? *Osteoporosis International, 12* (1), 77-83.

Fournier, P. E., Rizzoli, R., Slosman, D. O., Theintz, G., & Bonjour, J. (1997). Asynchrony between the rates of standing height gain and bone mass accumulation during puberty. *Osteoporosis International, 7*, 525-532.

Fredericson, M., Bergman, A., Hoffman, K., & Dillingham, M. (1995). Tibial stress reaction in runners. Correlation of clinical symptoms and scintigraphy with a new magnetic resonance imaging grading system. *American Journal of Sports Medicine, 23* (4), 472-481.

Friberg, O. (1982). Leg length asymmetry in stress fractures. A clinical and radiological study. *Journal of Sport Medicine, 22*, 485.

Frost, H. (1983). A determinant of bone architecture: the minimum effective strain. *Clinical Orthopaedics, 175*, 286-292.

Frost, H., & Schonau, E. (2000). The "muscle-bone unit" in children and adolescents: a 2000 overview. *Journal of Pediatric Endocrinology & Metabolism, 13* (6), 571-590.

Frost, H. M. (1987). Bone "mass" and the "mechanostat": a proposal. *Anatomical Record, 219*, 1-9.

Frost, M., Blake, G., & Fogelman, I. (2001). Quantitative ultrasound and bone mineral density are equally strongly associated with risk factors for osteoporosis. *Journal of Bone & Mineral Research, 16* (2), 406-416.

Fuchs, R., & Snow, C. (2002). Gains in hip bone mass from high-impact training are maintained: a randomized controlled trial in children. *Journal of Pediatrics, 141* (3), 357-362.

Gardner, L. I., Dziados, J. E., Jones, B. H., Brundage, J. F., Harris, J. M., Sullivan, R., et al. (1988). Prevention of lower extremity stress fractures: a controlled trial of a shock absorbent insole. *American Journal of Public Health, 78* (12), 1563-1567.

Gaul, C.A. (1996). Muscular strength and endurance. In D. Docherty (Ed.), *Measurement in Pediatric Exercise Science.* Champaign, Illinois: Human Kinetics.

Genant, H., Gluer, C., & Lotz, J. (1994). Gender differences in bone density, skeletal geometry, and fracture biomechanics. *Radiology, 190* (3), 636-640.

Georgopoulos, N., Markou, K., Theodoropoulou, A., Benardot, D., Leglise, M., & Vagenakis, A. (2002). Growth retardation in artistic compared with rhythmic elite female gymnasts. *Journal of Clinical Endocrinology & Metabolism, 87* (7), 3169-3173.

Georgopoulos, N., Markou, K., Theodoropoulou, A., Paraskevopoulou, P., Varaki, L., Kazantzi, Z., et al. (1999). Growth and pubertal development in elite female rhythmic gymnasts. *Journal of Clinical Endocrinology & Metabolism, 84* (12), 4525-4530.

Giangregorio, L., & Blimkie, C. (2002). Skeletal adaptations to alterations in weight-bearing activity: a comparison of models of disuse osteoporosis. *Sports Medicine, 7* (32), 459 - 476.

Gilsanz, V., Boechat, M., Roe, T., Loro, M., Sayre, J., & Goodman, W. (1994). Gender differences in vertebral body sizes in children and adolescents. *Radiology, 190* (3), 673-677.

Golden, N. H., Lanzkowsky, L., Schebendach, J., Palestro, C. J., Jacobson, M., & Shenker, I. R. (2002). The effect of estrogen-progestin treatment on bone mineral density in anorexia nervosa. *Journal of Pediatric and Adolescent Gynecology, 15*, 135-143.

Gomberg, B., Saha, P., Song, H., Hwang, S., & Wehrli, F. (2000). Topological analysis of trabecular bone MR images. *IEEE Transactions on Medical Imaging, 19* (3), 166-174.

Goulding, A., Jones, I., Taylor, R., Williams, S., & Manning, P. (2001). Bone mineral density and body composition in boys with distal forearm fractures: a dual-energy x-ray absorptiometry study. *Journal of Pediatrics, 139* (4), 509-515.

Grampp, S., Lang, P., Jergas, M., Gluer, C. C., Mather, A., Engelke, K., et al. (1995). Assessment of the skeletal status by peripheral quantitative computed tomography of the forearm: short-term precision in vivo and comparison to dual x-ray absorptiometry. *Journal of Bone and Mineral Research, 10*, 1566-1576.

Gremion, G., Rizzol, i. R., Slosman, D., Theintz, G., & Bonjour, J. (2001). Oligo-amenorrheic long-distance runners may lose more bone in spine than in femur. *Medicine & Science in Sports & Exercise, 33* (1), 15-21.

Grimston, S., Willows, N., & Hanley, D. (1993). Mechanical loading regime and its relationship to bone mineral density in children. *Medicine & Science in Sports & Exercise, 25* (11), 1203-1210.

Grumbach, M.M., and Styne, D.M. (1992). Puberty: ontogeny, neuroendocrinology, physiology and disorders. In J.D. Wilson, and D.W. Foster (Eds.), *Textbook of Endocrinology*. Philadelphia: W.B.Saunders

Gunnes, M., & Lehmann, E. (1996). Physical activity and dietary constituents as predictors of forearm cortical and trabecular bone gain in healthy children and adolescents: a prospective study. *Acta Paediatrica, 85* (1), 19-25.

Gustavsson, A., Olsson, T., & Nordstrom, P. (2003). Rapid loss of bone mineral density of the femoral neck after cessation of ice hockey training: a 6-year longitudinal study in males. *Journal of Bone and Mineral Research, 18* (11), 1964-1969.

Haapasalo, H., Kannus, P., Sievanen, H., Heinonen, A., Oja, P., & Vuori, I. (1994). Long-term unilateral loading and bone mineral density and content in female squash players. *Calcified Tissue International, 54* (4), 249-255.

Haapasalo, H., Kontulainen, S., Sievanen, H., Kannus, P., Jarvinen, M., & Vuori, I. (2000). Exercise-induced bone gain is due to enlargement in bone size without a change in volumetric bone density: a peripheral quantitative computed tomography study of the upper arms of male tennis players. *Bone, 27* (3), 351-357.

Hagerman, F. C. (1992). Energy metabolism and fuel utilization. *Medicine and Science in Sports and Exercise, 24*, S309-S314.

Hashemi, R. H., & Bradley, W. G. (1997). *MRI the basics*. Baltimore: Williams and Wilkins.

Hawley, J., & Peterson, P. (1988). Calcium requirements for optimal skeletal development in young females. *Calcif. Tissue Int.*, 49:176-183.

Heaney, R. P. (1993). Nutritional factors in osteoporosis. *Annual Review of Nutrition, 13*, 287-316.

Heinonen, A. (2001). Biomechanics. In K. Khan, H.A. McKay, P. Kannus, D.A. Bailey, J. Wark, & K. Bennell (2001). *Physical Activity and Bone Health*. Champaign, IL: Human Kinetics.

Heinonen, A., Kannus, P., Sievanen, H., Oja, P., Pasanen, M., Rinne, M., et al. (1996). Randomised controlled trial of effect of high-impact exercise on selected risk factors for osteoporotic fractures. *Lancet, 348* (9038), 1343-1347.

Heinonen, A., McKay, H. A., MacKelvie, K. J., Whithall, K. P., Forster, B. B., & Khan, K. M. (2001). High-impact exercise and tibial polar moment of inertia in pre- and early pubertal girls: a quantitative MRI study. *Journal of Bone and Mineral Research, 16*, S482.

Heinonen, A., Oja, P., Kannus, P., Sievanen, H., Haapasalo, H., Manttari, A., et al. (1995). Bone mineral density in female athletes representing sports with different loading characteristics of the skeleton. *Bone, 17*, 197-203.

Heinonen, A., Sievanen, H., Kannus, P., Oja, P., Pasanen, M., & Vuori, I. (2000). High-impact exercise and bones of growing girls: a 9-month controlled trial. *Osteoporosis International, 11* (12), 1010-1017.

Heinonen, A., Sievanen, H., Kannus, P., Oja, P., & Vuori, I. (2002). Site-specific skeletal response to long-term weight training seems to be attributable to principal loading modality: a pQCT study of female weightlifters. *Calcified Tissue International, 70* (6), 469-474.

Heinonen, A., Sievanen, H., Kryolainen, H., Perttunen, J., & Kannus, P. (2001). Mineral mass, size, and estimated mechanical strength of triple jumpers' lower limb. *Bone, 29* (3), 279-285.

Heinrich, C. H., Going, S. B., Pamenter, R. W., Perry, C. D., Boydon, T. W., & Lohman, T. G. (1990). Bone mineral content of cyclically menstruating female resistance and endurance trained athletes. *Medicine and Science in Sports and Exercise, 22* (5), 558-563.

Hergenroeder, A. (1994). Effects of calcium intake and exercise on bone mineral density. *Journal of Pediatrics, 125* (1), 169.

Hernandez, C.J., Beaupre, G.S., and Carter, D.R. (2003). A theoretical analysis of the relative influence of peak BMD, age-related bone loss and menopause on the development of osteoporosis. *Osteoporosis International*, 14, 843-847.

Hogler, W., Blimkie, C. J. R., C.T., C., Kemp, A. F., Briody, J., Wiebe, P., et al. (2003). A comparison of bone geometry and cortical density at the mid-femur between prepuberty and young adulthood using magnetic resonance imaging. *Bone, 33*, 771-778.

Hong, J., Hipp, J. A., Mulkern, R. V., Jaramillo, D., & Synder, B. D. (2000). Magnetic resonance imaging measurements of bone denisty and cross-sectional geometry. *Calcified Tissue International, 66,* 74-78.

Hotta, M., Fukuda, I., Sato, K., Hizuka, N., Shibasaki, T., & Takano, K. (2000). The relationship between bone turnover and body weight, serum insulin-like growth factord (IGF-1), and serum IGF-binding protein levels in patients with anorexia nervosa. *Journal of Clinical Endocrinology & Metabolism, 85*, 200-206.

Hotta, M., Shibasaki, T., Sato, K., & Demura, H. (1998). The importance of body weight history in the occurrence and recovery of osteoporosis in patients with anorexia nervosa: evaluation by dual x-ray absorptiometry and bone metabolic markers. *European Journal of Endocrinology, 139*, 276-283.

Housh, T. J., Johnson, G. O., Stout, J., & Housh, D. J. (1993). Anthropometric growth patterns of high school wrestlers. *Medicine & Science in Sports & Exercise, 25*, 1141-1151.

Hreljac, A. (2004). Impact and oversuse injuries in runners. *Medicine & Science in Sports and Exercise, 36* (5), 845-849.

Hwang, S., & Wehrli, F. (2002). Subvoxel processing: a method for reducing partial volume blurring with application to in vivo MR images of trabecular bone. *Magnetic Resonance in Medicine, 47* (5), 948-957.

Ilich, J., & Kerstetter, J. (2000). Nutrition in bone health revisited: a story beyond calcium. *Journal of the American College of Nutrition, 19* (6), 715-737.

Iuliano-Burns, S., Saxon, L., Naughton, G., Gibbons, K., & Bass, S. (2003). Regional specificity of exercise and calcium during skeletal growth in girls: a randomized controlled trial. *Journal of Bone & Mineral Research, 18* (1), 156-162.

Janse de Jonge, X. (2003). Effects of the menstrual cycle on exercise performance. *Sports Medicine, 33* (11), 833-851.

Jarvinen, T. L., Kannus, P., Sievanen, H., Jolma, P., Heinonen, A., & Jarvinen, M. (1998). Randomized controlled study of effects of sudden impact loading on rat femur. *Journal of Bone and Mineral Research, 13* (9), 1475-1482.

Johnson, T. R., & Steinbach, L. S. (2004). *Essentials of musculoskeletal imaging*. Illinois: American Academy of Orthopaedic Surgeons.

Jones, B. H., Bovee, M. W., Harris, J. M., & Cowan, D. H. (1993). Intrinsic factors for exercise-related injuries among male and female army trainees. *American Journal of Sports Medicine, 21*, 705-710.

Jones, B. H., Harris, J. M., Vinh, T. N., & Rubin, C. (1989). Exercise-induced stress fractures and stress reactions of bone: epidemiology, etiology, and classification. *Exercise & Sport Sciences Reviews, 17*, 379-422.

Jones, G., & Dwyer, T. (1998). Bone mass in prepubertal children: gender differences and the role of physical activity and sunlight exposure. *Journal of Clinical Endocrinology and Metabolism, 83* (12), 4274-4279.

Juul, A., Dalgaard, P., Blum, W., Bang, P., Hall, K., Michaelsen, K., et al. (1995). Serum levels of insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3) in healthy infants, children, and adolescents: the relation to IGF-I, IGF-II, IGFBP-1, IGFBP-2, age, sex, body mass index, and pubertal maturation. *Journal of Clinical Endocrinology & Metabolism, 80* (8), 2534-2542.

Kannus, P., Haapasalo, H., Sankelo, M., Sievanen, H., Pasanen, M., Heinonen, A., et al. (1995). Effect of starting age of physical activity on bone mass in the dominant arm of tennis and squash players. *Annals of Internal Medicine, 123* (1), 27-31.

Kannus, P., Haapasalo, H., Sievanen, H., Oja, P., & Vuori, I. (1994). The site-specific effects of long-term unilateral activity on bone mineral density and content. *Bone, 15* (3), 279-284.

Kaptoge, S., Dalzell, N., Jakes, N., Wareham, N. E., Day, K. T., Beck, T. J., et al. (2003). Hip section modulus, a measure of bending resistance, is more strongly related to reported physical activity than BMD. *Osteoporosis International, 14*, 941-949.

Karlsson, M., Linden, C., Karlsson, C., Johnell, O., Obrant, K., & Seeman, E. (2000). Exercise during growth and bone mineral density and fractures in old age. *Lancet, 355* (9202), 469-470.

Karlsson, M., Magnusson, H., C., K., & Seeman, E. (2001). The duration of exercise as a regulator of bone mass. *Bone, 28* (1), 128-132.

Katzman, D., Bachrach, L., Carter, D., & Marcus, R. (1991). Clinical and anthropometric correlates of bone mineral acquisition in healthy adolescent girls. *Journal of Clinical Endocrinology & Metabolism, 73* (6), 1332-1339.

Kaufman, J., & Einhorn, T. (1993). Ultrasound assessment of bone. *Journal of Bone & Mineral Research, 8* (5), 517-525.

Kelly, P. J., Morrison, N. A., Sambrook, P. N., Nguyen, T. V., & Eisman, J. A. (1995). Genetic influences on bone turnover, bone density and fracture. *European Journal of Endocrinology, 133*, 265-271.

Kemper, H., Bakker, I., Twisk, J., & van Mechelen, W. (2002). Validation of a physical activity questionnaire to measure the effect of mechanical strain on bone mass. *Bone, 30* (5), 799-804.

Kerstetter, J. (1995). Do dairy products improve bone density in adolescent girls? *Nutrition Reviews, 53* (11), 328-332.

Khan, K., Liu-Ambrose, T., Sran, M., Ashe, M., Donaldson, M., & Wark, J. (2002). New criteria for female athlete triad syndrome? As osteoporosis is rare, should osteopenia be among the criteria for defining the female athlete triad syndrome? *British Journal of Sports Medicine, 36* (1), 10-13.

Khan, K., McKay, H., H., H., Bennell, K., Forwood, M., Kannus, P., et al. (2000). Does childhood and adolescence provide a unique opportunity for exercise to strengthen the skeleton? *Journal of Science & Medicine in Sport, 3* (2), 150-164.

Khan, K., McKay, H. A., Kannus, P., Bailey, D. A., Wark, J., & Bennell, K. (2001). *Physical Activity and Bone Health*. Champaign, IL: Human Kinetics.

Kibler, W. B., & Sarfran, M. R. (2000). Musculoskeletal injuries in the young tennis player. *Clinics in Sports Medicine, 19* (4), 781-792.

Klein, R. F., Shea, M., Gunnes, M. E., Pelz, G. B., Belknap, J. K., & Orwoll, E. S. (2001). Phenotypic characterisation of mice bread for high and low peak bone mass. *Journal of Bone and Mineral Research, 16* (1), 63-73.

Knapp, T. P., & Garrett, W. E. (1997). Stress fractures: general concepts. *Clinics in Sports Medicine, 16* (2), 339-356.

Knutzen, K., & Hart, L. (1996). Running. In D. J. Caine, C. G. Caine & K. J. Lidner (Eds.), *Epidemiology of Sports Injuries*. Champaign, Illinois: Human Kinetics.

Kontulainen, S., Kannus, P., Pasanen, M., Sievanen, H., Heinonen, A., Oja, P., et al. (2002). Does previous participation in high-impact training result in residual bone gain in growing girls? One year follow-up of a 9-month jumping intervention. *International Journal of Sports Medicine, 23* (8), 575-581.

Kontulainen, S., Sievanen, H., Kannus, P., Pasanen, M., & Vuori, I. (2003). Effect of long-term impact-loading on mass, size, and estimated strength of humerus and radius of female racquet-sports players: a peripheral quantitative computed tomography study between young and old starters and controls. *Journal of Bone & Mineral Research, 18* (2), 352-359.

Korpelainen, R., Orava, S., Karpakka, J., Siira, P., & Hulkko, A. (2001). Factors for recurrent stress fractures in athletes. *American Journal of Sports Medicine, 29* (3), 304-310.

Krall, E. A., & Dawson-Hughes, B. (1993). Heritable and lifestyle determinants of bone mineral density. *Journal of Bone and Mineral Research, 8*, 1-9.

Laughlin, A., & Yen, S. S. C. (1996). Nutritional and endocrine-metabolic aberrations in amenorrheic athletes. *Journal of Clinical Endocrinology & Metabolism, 81*, 4301-4309.

Lazzarini, K. M., Troinano, R., & Smith, R. (1997). Can running cause the appearance of marrow edema on MR images of the foot and ankle? *Radiology, 202*, 540-542.

Lee, W., Leung, S., Leung, D., & Cheng, J. (1996). A follow-up study on the effects of calcium-supplement withdrawal and puberty on bone acquisition of children. *American Journal of Clinical Nutrition, 64* (1), 71-77.

Lehtonen-Veromaa, M., Mottonen, T., Irjala, K., Nuotio, I., Leino, A., & Viikari, J. (2000). A 1-year prospective study on the relationship between physical activity, markers of bone metabolism, and bone acquisition in peripubertal girls. *Journal of Clinical Endocrinology & Metabolism, 95* (10), 3726-3732.

Libanati, C., Baylink, D., Lois-Wenzel, E., Srinvasan, N., & Mohan, S. (1999). Studies on the potential mediators of skeletal changes occurring during puberty in girls. *Journal of Clinical Endocrinology & Metabolism, 84* (8), 2807-2814.

Lima, F., De Falco, V., Baima, J., Carazzato, J., & Pereira, R. (2001). Effect of impact load and active load on bone metabolism and body composition of adolescent athletes. *Medicine & Science in Sports & Exercise, 33* (8), 1318-1323.

Lin, Y., Lyle, R., McCabe, L., McCabe, G., Weaver, C., & Teegarden, D. (2000). Dairy calcium is related to changes in body composition during a two-year exercise intervention in young women. *Journal of the American College of Nutrition, 19* (6), 754-760.

Lindholm, C., Hagenfeldt, K., & Ringertz, B. M. (1994). Pubertal development in elite juvenile gymnasts: effects of physical training. *Acta Obstetrics and Gynecology Scandinavica, 73*, 269-273.

Lindholm, C., Hagenfeldt, K., & Ringertz, H. (1995). Bone mineral content of young female former gymnasts. *Acta Paediatrica, 84* (10), 1109-1112.

Link, T.M., Veith, V., Langenberg, R., Meier, N., Lotter, A., Newitt, D., et al. (2003). Structure analysis of high resolution magnetic resonance imaging of the proximal femur: in vitro correlation with biomechanical strength and BMD. *Calcified Tissue International*, 72, 156-165.

Lloyd, T., Beck, T., Lin, H., Tulchinsky, M., Eggl, i. D., Oreskovic, T., et al. (2002). Modifiable determinants of bone status in young women. *Bone, 30* (2), 416-421.

Lloyd, T., Chinchilli, V., Johnson-Rollings, N., Kieselhorst, K., Eggli, D., & Marcus, R. (2000). Adult female hip bone density reflects teenage sports-exercise patterns but not teenage calcium intake. *Pediatrics,* 106 (pt 1), 40-44.

Lloyd, T., Triantafyllou, S., Baker, E. R., Houts, P., Whiteside, J., Kalenak, A., et al. (1986). Women athletes with menstrual irregularity have increased musculoskeletal injuries. *Medicine & Science in Sports & Exercise, 18* (4), 374-379.

Lochmuller, E. M., Eckstein, F., Kaiser, D., Zeller, J. B., Landgraf, J., Putz, R., et al. (1998). Prediction of vertebral failure loads from spinal and femoral dual energy x-ray absorptiometry and calcaneal ultrasound: an insitu analysis with intact soft tissue. *Bone, 23*, 417-424.

Long, G., Cooper, J., & Gibbon, W. (1999). Magnetic resonance imaging of injuries in the child athlete. *Clinical Radiology, 54* (12), 781-791.

Loosli, A., Bensen, H., Gillien, J., & Bourdet, B. (1986). Development of bone mass and bone density of the spine and femoral neck – a prospective study of 65 children and adolescents. *Bone Miner*, 23:171-182.

Lu, P.W., Briody, J.N., Ogle, G.D., Morley, K., Humphries, I.R.J., Allen, J., et al (1994). Bone mineral density of total body, spine, and femoral neck in children and young adults: a cross-sectional and longitudinal study. *Journal of Bone and Mineral Research*, 9, 1451-1458.

Lu, T., Taylor, S., O'Connor, J., & Walker, P. (1997). Influence of muscle activity on the forces in the femur: an in vivo study. *Journal of Biomechanics, 30* (11-12), 1101-1106.

Lysholm, J., & Wiklander, J. (1987). Injuries in Runners. *American Journal of Sports Medicine, 15*, 168-171.

Macera, C. A., Pate, R. R., Powell, K. E., Jackson, K. L., Kendrick, J. S., & Craven, T. E. (1989). Predicting lower extremity injuries among habitual runners. *Archives of Internal Medicine, 149* (11), 2565-2568.

MacKelvie, K., Khan, K., & McKay, H. (2002). Is there a critical period for bone response to weight-bearing exercise in children and adolescents? a systematic review. *British Journal of Sports Medicine, 36* (4), 250-257.

MacKelvie, K., Khan, K., Petit, M., Janssen, P., & McKay, H. (2003). A school-based exercise intervention elicits substantial bone health benefits: a 2-year randomized controlled trial in girls. *Pediatrics, 112* (6 Pt 1), e447.

MacKelvie, K., McKay, H., Khan, K., & Crocker, P. (2001). Lifestyle risk factors for osteoporosis in Asian and Caucasian girls. *Medicine & Science in Sports & Exercise, 33* (11), 1818-1825.

MacKelvie, K., McKay, H., Khan, K., & Crocker, P. (2001). A school-based exercise intervention augments bone mineral accrual in early pubertal girls. *Journal of Pediatrics, 139* (4), 501-508.

MacKelvie, K., McKay, H., Petit, M., Moran, O., & Khan, K. (2002). Bone mineral response to a 7-month randomized controlled, school-based jumping intervention in 121 prepubertal boys: associations with ethnicity and body mass index. *Journal of Bone & Mineral Research, 17* (5), 834-844.

MacKelvie, K. J., Petit, M. A., Khan, K. M., Beck, T. J., & McKay, H. A. (2004). Bone mass and structure are enhanced following a 2-year randomized controlled trial of exercise in prepubertal boys. *Bone, 34*, 755-764.

Madden, C. C., & Mellion, M. B. (1996). Sever's disease and other causes of heel pain in adolescents. *American Family Physician, 54* (6), 1995-2000.

Maffulli, F., & Helms, P. (1988). Controversies about intensive training in young athletes: a current concepts review. *Archives of Disease in Childhood, 63* (11), 1405-1407.

Maffulli, N. (1990). Intensive training in young athletes. The orthopaedic surgeon's viewpoint. *Sports Medicine, 9* (4), 229-243.

Magarey, A., Boulton, T., Chatterton, B., Schultz, C., Nordin, B., & Cockington, R. (1999). Bone growth from 11 to 17 years: relationship to growth, gender and changes with pubertal status including timing of menarche. *Acta Paediatrica, 88* (2), 139-146.

Magnusson, H., Linden, C., Karlsson, C., Obrant, K., & Karlsson, M. (2001). Exercise may induce reversible low bone mass in unloaded and high bone mass in weight-loaded skeletal regions. *Osteoporosis International, 12* (11), 950-955.

Maitra, R. S., & Johnson, D. L. (1997). Stress fractures - Clinical history and physical examination. *Clinics in Sports Medicine, 16* (2), 259-274.

Malina, R. (1994). Physical activity and training: effects on stature and the adolescent growth spurt. *Medicine & Science in Sports & Exercise, 26*, 759-766.

Malina, R. (1994). Physical growth and biological maturation of young athletes. *Exercise & Sport Sciences Reviews, 22*, 389-343.

Marsh, S., & Jenkins, D. (2002). Physiological responses to the menstrual cycle - Implications for the development of heat illness in female athletes. *Sports Medicine, 32* (10), 209-215.

Martin, H., Werner, J., Andresen, R., Schober, H. C., & Schmitz, K. P. (1998). Noninvasive assessment of stiffness and failure load of human vertebrae from CT-data. *Biomedical Technology, 43* (3), 82-88.

Martin, R. B., Burr, D. B., & Sharkey, N. A. (1998). *Skeletal Tissue Mechanics*. New York: Springer.

McCreadie, B., & Goldstein, S. (2000). Biomechanics of fracture: is bone mineral density sufficient to assess risk? *Journal of Bone & Mineral Research, 15* (12), 2305-2308.

McGuigan, F., Murray, L., Gallagher, A., Davey-Smith, G., Neville, C., Van't Hof, R., et al. (2002). Genetic and environmental determinants of peak bone mass in young men and women. *Journal of Bone & Mineral Research, 17* (7), 1273-1279.

McKay, H., Bailey, D., Mirwald, R., Davison, K., & Faulkner, R. (1998). Peak bone mineral accrual and age at menarche in adolescent girls: a 6-year longitudinal study. *Journal of Pediatrics, 133* (5), 682-687.

McKay, H., Petit, M., Schutz, R., Prior, J., Barr, S., & Khan, K. (2000). Augmented trochanteric bone mineral density after modified physical education classes: a randomized

school-based exercise intervention study in prepubescent and early pubescent children. *Journal of Pediatrics, 136* (2), 156-162.

McKay, H., Sievanen, H., Petit, M., MacKelvie, K.J., Forkheim, K.M., Whittall, K.P., et al (2004). Application of magnetic resonance imaging to evaluation of femoral neck structure in growing girls. *Journal of Clinical Densitometry*, 7 (2), 161-168.

McRobbie, D. W., Moore, E. A., Graves, M. J., & Prince, M. R. (2003). *MRI From proton to proton*. Cambridge: Cambridge University Press.

Micheli, L. J., & Ireland, M. L. (1987). Prevention and management of calcaneal apophysitis in children: an overuse syndrome. *Journal of Pediatric Orthopedics, 7*, 34-38.

Milgrom, C., Finestone, A., Levi, Y., Simkin, A., Ekenman, I., & Mendelson, S. (2000). Do high impact exercises produce hiher tibial strains than running? *British Journal of Sports Medicine, 34*, 195-199.

Milgrom, C., Finestone, A., Segev, S., Olin, C., Arndt, T., & Ekenman, I. (2003). Are overground or treadmill runners more likely to sustain tibial stress fracture? *British Journal of Sports Medicine, 37*, 160-163.

Milgrom, C., Giladi, M., Simkin, A., Rand, A., Kedem, R., Kashtan, H., et al. (1989). The area moment of inertia of the tibia: a risk factor for stress fractures. *Journal of Biomechanics, 22* (11-12), 1243-1248.

Mitsiopoulos, N., Baumgartner, R. N., Heymsfield, S. B., Lyons, W., Gallagher, D., & Ross, R. (1998). Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerised tomography. *Journal of Applied Physiology, 85*, 115-122.

Mittlmeier, T., Mattheck, C., & Dietrich, F. (1994). Effects of mechanical loading on the profile of human femoral diaphyseal geometry. *Medical Engineering & Physics, 16* (1), 75-81.

Molgaard, C., Thomsen, B., & Michaelsen, K. (1999). Whole body bone mineral accretion in healthy children and adolescents. *Archives of Disease in Childhood, 81* (1), 10-15.

Molgaard, C., Thomsen, B., & Michaelsen, K. (2001). The influence of calcium intake and physical activity on bone mineral content and bone size in healthy children and adolescents. *Osteoporosis International, 12* (10), 887-894.

Mora, S., Pitukcheewanont, P., Nelson, J., & Gilsanz, V. (1999). Serum levels of insulin-like growth factor I and the density, volume, and cross-sectional area of cortical bone in children. *Journal of Clinical Endocrinology & Metabolism, 84* (8), 2780-2783.

Morrison, N. A., Qi, J. C., Tokita, A., Kelly, P. J., Crofts, L., Nguyen, T. V., et al. (1994). Prediction of bone density from vitam D receptor alleles. *Nature, 367*, 284-287.

Mosley, J., & Lanyon, L. (2002). Growth rate rather than gender determines the size of the adaptive response of the growing skeleton to mechanical strain. *Bone, 30* (2), 314-319.

Mosley, J. R., & Lanyon, L. E. (1998). Strain rate as a controlling influence on adaptive modelling in response to dynamic loading of the ulna in growing male rats. *Bone, 23*, 313-318.

Mosley, J. R., March, B. M., Lynch, J., & Lanyon, L. E. (1997). Strain magnitude related changes in whole bone architecture in growing rats. *Bone, 20*, 191-198.

Murphy, D. F., Connolly, D. A., & Beynnon, B. D. (2003). Risk factors for lower extremity injuries: a review of the literature. *British Journal of Sports Medicine, 37*, 13-29.

Myburgh, K., Bachrach, L., Lewis, B., Kent, K., & Marcus, R. (1993). Low bone mineral density at axial and appendicular sites in amenorrheic athletes. *Medicine & Science in Sports & Exercise, 25* (11), 1197-1202.

Nattiv, A., & Armsey, T. J. (1997). Stress injury to bone in the female athlete. *Clinics in Sports Medicine, 16* (2), 197-225.

Nattiv, A., Puffer, J. C., Casper, J., Dorey, F., Kabo, J. M., & Hame, S. (2000). Stress fracture risk factors, incidence and distribution: a 3-year prospective study in collegiate runners. *Medicine and Science in Sports and Exercise, (suppl 5)*, S347.

Naughton, G., Farpour-Lambert, N., Carlson, J., Bradney, M., & Van Praagh, E. (2000). Physiological issues surrounding the performance of adolescent athletes. *Sports Medicine, 30* (5), 309-325.

Nelson, D. A., & Koo, W. W. (1999). Interpretation of absorptiometric bone mass measurements in the growing skeleton: issues and limitations. *Calcified Tissue International, 65*, 1-3.

Neu, C., Manz, F., Rauch, F., Merkel, A., & Schoenau, E. (2001). Bone densities and bone size at the distal radius in healthy children and adolescents: a study using peripheral quantitative computered tomography. *Bone, 28* (2), 227-232.

Neville, C., Murray, L., Boreham, C., Gallagher, A., Twisk, J., Robson, P., et al. (2002). Relationship between physical activity and bone mineral status in young adults: the Northern Ireland Young Hearts Project. *Bone, 30* (5), 792-798.

Nichols, D., Sanborn, C., Bonnick, S., Ben-Ezra, V., Gench, B., & DiMarco, N. (1994). The effects of gymnastics training on bone mineral density. *Medicine & Science in Sports & Exercise, 26* (10), 1220-1225.

Nichols, D., Sanborn, C., & Love, A. (2001). Resistance training and bone mineral density in adolescent females. *Journal of Pediatrics, 139* (4), 494-500.

Nickols-Richardson, S., Modlesky, C., O'Connor, P., & Lewis, R. (2000). Premenarcheal gymnasts possess higher bone mineral density than controls. *Medicine & Science in Sports & Exercise, 32* (1), 63-69.

Njeh, C. F., Fuerst, T., Hans, D., Blake, G. M., & Genant, H. K. (1999). Radiation exposure in bone mineral density assessment. *Applied Radialogical Isotopes, 50* (1), 215-236.

Omasu, F., Kitagawa, J., Koyama, K., Asakawa, K., Yokouchi, J., Ando, D., et al. (2004). The influence of VDR genotype and exercise on ultrasound parameters in young adult Japanese woman. *Journal of Physiology, Anthropology and Applied Human Science, 23* (2), 49-55.

Orava, S., & Saarela, J. (1978). Exertion injuries to young athletes: a follow up research of orthopaedic problems of young track and field athletes. *American Journal of Sports Medicine, 6*, 68-74.

Otis, C., Wilson, G.L., & Pearce, G. (1992). Amenorrhea and the female athlete. *Calcif Tissue Int,* 22:54-60.

Parfitt, A. (1994). The two faces of growth: benefits and risks to bone integrity. *Osteoporosis International, 4* (6), 382-398.

Parfitt, A. (2002). High bone turnover is intrinsically harmful: two paths to a similar conclusion. The Parfitt view. *Journal of Bone & Mineral Research, 17* (8), 1558-1559.

Pearce, G., Bass, S., Young, N., Formica, C., & Seeman, E. (1996). Does weight-bearing exercise protect against the effects of exercise-induced oligomenorrhea on bone density? *Osteoporosis International, 6* (6), 448-452.

Peat, J.K., Williams, K., Xuan, W., and Mellis, C. (2002). *Health Science Research: a handbook of quantitative methods.* Sage Publications: London, United Kingdom.

Peck, D. M. (1995). Apophyseal injuries in the young athlete. *American Family Physicians, 51* (8), 1891-1895.

Petit, M., McKay, H., MacKelvie, K., Heinonen, A., Khan, K., & Beck, T. (2002). A randomized school-based jumping intervention confers site and maturity-specific benefits on bone structural properties in girls: a hip structural analysis study. *Journal of Bone & Mineral Research, 17* (3), 363-372.

Pettersson, U., Nordstrom, P., Alfredson, H., Henriksson-Larsen, K., & R, L. (2000). Effect of high impact activity on bone mass and size in adolescent females: A comparative study between two different types of sports. *Calcified Tissue International, 67* (3), 207-214.

Powell, P. L., Roy, R., Kanim, P., Bello, M. A., & Edgerton, V. R. (1984). Predictability of skeletal muscle tension from architectural determinations in guinea pig hind limbs. *Journal of Applied Physiology, 57*, 1715-1721.

Prior, J., Vigna, Y., Schechter, M., & Burgess, A. (1990). Spinal bone loss and ovulatory disturbances New England. *Journal of Medicine, 323* (18), 1221-1227.

Proctor, K., Adams, W., Shaffrath, J., & Van Loan, M. (2002). Upper-limb bone mineral density of female collegiate gymnasts versus controls. *Medicine & Science in Sports & Exercise, 34* (11), 1830-1835.

Reeve, J. (2002). A role for mechanical strain in the preservation of trabecular number (density). *Journal of Bone & Mineral Research, 17* (8), 1556-1557.

Reid, I. (2002). Relationships among body mass, its components, and bone. *Bone, 31* (5), 547-555.

Reid, I. R., Legge, M., Stapleton, J. P., Evans, M. C., & Grey, A. B. (1995). Regular exercise dissociates fat mass and bone density in premenopausal women. *Journal of Clinical Endocrinology and Metabolism, 80* (6), 1764-1768.

Reilly, D. T., & Burstein, A. H. (1975). The elastic and ultimate properties of compact bone tissue. *Journal of Biomechanics, 8*, 393-405.

Rice, S., Blimkie, C., Webber, C., Levy, D., Martin, J., Parker, D., et al. (1993). Correlates and determinants of bone mineral content and density in healthy adolescent girls. *Canadian Journal of Physiology & Pharmacology, 71* (12), 923-930.

Rittweger, J., Beller, G., Ehrig, J., Jung, C., Koch, U., Ramolla, J., et al. (2000). Bone-muscle strength indices for the human lower leg. *Bone, 27* (2), 319-326.

Robinson, T., Snow-Harter, C., Taaffe, D., Gillis, D., Shaw, J., & Marcus, R. (1995). Gymnasts exhibit higher bone mass than runners despite similar prevalence of amenorrhea and oligomenorrhea. *Journal of Bone & Mineral Research, 10* (1), 26-35.

Robling, A., Hinant, F., Burr, D., & Turner, C. (2002). Improved bone structure and strength after long-term mechanical loading is greatest if loading is separated into short bouts. *Journal of Bone & Mineral Research, 17* (8), 1545-1554.

Robling, A., Hinant, F., Burr, D., & Turner, C. (2002). Shorter, more frequent mechanical loading sessions enhance bone mass. *Medicine & Science in Sports & Exercise, 34* (2), 196-202.

Robling, A. G., & Turner, C. H. (2002). Mechanotransduction in bone: genetic effects on mechanosensitivity in mice. *Bone, 31* (5), 562-569.

Rochcongar, P., Pernes, J., Carre, F., & Chaperon, J. (1995). Occurrance of running injuries: a survey among 1153 runners. *Science in Sports, 10*, 15-19.

Rockwell, J., Sorensen, A., Baker, S., Leahey, D., Stock, J., Michaels, J., et al. (1990). Weight training decreases vertebral bone density in premenopausal women: a prospective study. *Journal of Clinical Endocrinology & Metabolism, 71* (4), 988-993.

Roemmich, J., Clark, P., Mai, V., Berr, S., Weltman, A., Veldhuis, J., et al. (1998). Alterations in growth and body composition during puberty: III. Influence of maturation, gender, body composition, fat distribution, aerobic fitness, and energy expenditure on nocturnal growth hormone release. *Journal of Clinical Endocrinology & Metabolism, 83* (5), 1440-1447.

Roemmich, J., Richmond, R., & Rogol, A. (2001). Consequences of sport training during puberty. *Journal of Endocrinological Investigation, 25* (9), 708-715.

Rogers, A., Hannon, R., & Eastell, R. (2000). Biochemical markers as predictors of rates of bone loss after menopause. *Journal of Bone & Mineral Research, 15* (7), 1398-1404.

Rogol, A. (2000). Sex steroid and growth hormone supplementation to enhance performance in adolescent athletes. *Current Opinion in Pediatrics, 12* (4), 382-387.

Rogol, A., Clark, P., & Roemmich, J. (2000). Growth and pubertal development in children and adolescents: effects of diet and physical activity. *American Journal of Clinical Nutrition, 72* (2 Suppl), 521S-528S.

Rogol, A. D. (1994). Growth at puberty interaction of androgens and growth hormone. *Medicine and Science in Sports and Exercise, 26* (2), 767-770.

Rourke, K., Brehm, B., Cassell, C., & Sethuraman, G. (2003). Effect of weight change on bone mass in female adolescents. *Journal of the American Dietetic Association, 103* (3), 369-372.

Rowlands, A. V., Ingledew, D. K., Powell, S. M., & Eston, R. G. (2004). Interactive effects of habitual physical activity and calcium intake on bone density in boys and girls. *Journal of Applied Physiology, 97*, 1203-1208.

Rubin, K., Schirduan, V., Gendreau, P., Sarfaraz, i. M., Mendola, R., & Dalsky, G. (1993). Predictors of axial and peripheral bone mineral density in healthy children and adolescents, with special attention to the role of puberty. *Journal of Pediatrics, 123* (6), 863-870.

Rubin, L. A., Hawker, G. A., Peltekova, V. D., Fielding, L. J., Ridout, R., & Cole, D. E. (1999). Determinants of peak bone mass: clinical and genetic analyses in a young female Canadian cohort. *Journal of Bone & Mineral Research, 14* (4), 633-643.

Ruiz, J., Mandel, C., & Garabedian, M. (1995). Influence of spontaneous calcium intake and physical exercise on the vertebral and femoral bone mineral density of children and adolescents. *Journal of Bone & Mineral Research, 10* (5), 675-683.

Russo, C., Lauretani, F., Bandinelli, S., Bartali, B., Di Iorio, A., Volpato, S., et al. (2003). Aging bone in men and women: beyond changes in bone mineral density. *Osteoporosis International, 14* (7), 531-538.

Sandstrom, P., Jonsson, P., Lorentzon, R., & Thorsen, K. (2000). Bone mineral density and muscle strength in female ice hockey players. *International Journal of Sports Medicine, 21* (7), 524-528.

Schiessl, H., Frost, H., & Jee, W. (1998). Estrogen and bone-muscle strength and mass relationships . *Bone, 22* (1), 1-6.

Schoenau, E., & Frost, H. (2002). The "muscle-bone unit" in children and adolescents. *Calcified Tissue International, 70* (5), 405-407.

Schoenau, E., Neu, C., Beck, B., Manz, F., & Rauch, F. J. (2002). Bone mineral content per muscle cross-sectional area as an index of the functional muscle-bone unit. *Journal of Bone & Mineral Research, 17* (6), 1095-1101.

Schonau, E. (1998). The development of the skeletal system in children and the influence of muscular strength. *Hormone Research, 49* (1), 27-31.

Scott, S. H., & Winter, D. A. (1990). Internal forces at chronic running injury sites. *Medicine and Science in Sports and Exercise, 22*, 357-369.

Seeman, E. (1998). Growth in bone mass and size-are racial and gender differences in bone mineral density more apparent than real? *Journal of Clinical Endocrinology & Metabolism, 83* (5), 1414-1419.

Seeman, E. (2002). An exercise in geometry. *Journal of Bone & Mineral Research, 17* (3), 373-380.

Seeman, E. (2003). Periosteal bone formation-a neglected determinant of bone strength. *New England Journal of Medicine, 349* (4), 320-323.

Seeman, E. (1997). From density to structure: growing up and growing old on the surfaces of bone. *Journal of Bone & Mineral Research, 12* (4), 509-521.

Seeman, E., Hopper, J., Young, N., Formica, C., Goss, P., & Tsalamandris, C. (1996). Do genetic factors explain associations between muscle strength, lean mass, and bone density? A twin study. *American Journal of Physiology, 270* (2 Pt 1), E320-327.

Seeman, E., Hopper, J. L., Bach, L. A., Cooper, M. E., Parkinson, E., McKay, J., et al. (1989). Reduced bone mass in daughters of women with osteoporosis. *New England Journal of Medicine, 320*, 554-558.

Seeman, E., Tsalamandris, C., Formica, C., Hopper, J. L., & McKay, J. (1994). Reduced femoral neck bone density in the daughters of women with hip fractures: the role of low peak bone denisty in the pathogensis of osteoporosis. *Journal of Bone and Mineral Research, 9*, 739-743.

Seefeldt, V., Haubenstricker, J., Branta, C.F., Evans, S. (1988) Physical characteristics of adult distance runners. In E.W. Brown and C.F. Branta (Eds.), *Competitive sports for children and youth.* Champaign, Illinois: Human Kinestics.

Shangold, M.M., and Mirkin, G. (1994). *Women and Exercise: Physiology and Sports Medicine* (2nd ed). Philadelphia: F. A Davis Company

Sievanen, H., Kannus, P., Nieminen, V., Heinonen, A., Oja, P., & Vuori, I. (1996). Estimation of various mechanical characteristics of human bones using dual energy x-ray absorptiometry: methodology and precision. *Bone, 18*, 175-275.

Sievanen, H., Koskue, V., Rauhio, A., Kannus, P., Heinonen, A., & Vuori, I. (1998). Peripheral quantitative computered tomography in human long bones: evaluation of in vitro and in vivo precision. *Journal of Bone & Mineral Research, 13*, 871-882.

Simkin, A., Leichter, I., & Giladi, M. (1989). Combined effect of foot arch structure and an orthotic device on stress fractures. *Foot Ankle, 10*, 25.

Slemenda, C., Reister, T., Hui, S., Miller, J., Christian, J., & Johnston, C. J. (1994). Influences on skeletal mineralization in children and adolescents: evidence for varying effects of sexual maturation and physical activity. *Journal of Pediatrics, 125* (2), 201-207.

Smolak, L., Murnen, S., & Ruble, A. (2000). Female athletes and eating problems: a meta-analysis. *International Journal of Eating Disorders, 27* (4), 371-380.

Snow, C., Rosen, C., & Robinson, T. (2000). Serum IGF-I is higher in gymnasts than runners and predicts bone and lean mass. *Medicine & Science in Sports & Exercise, 32* (11), 1902-1907.

Snow, C., Williams, D., LaRiviere, J., Fuchs, R., & Robinson, T. (2001). Bone gains and losses follow seasonal training and detraining in gymnasts. *Calcified Tissue International, 69* (1), 7-12.

Soyka, L. A., Fairfield, W. P., & Klibanski, A. (2000). Hormonal determinants and disorders of peak bone mass in children. *J Clin Endocrinol Metab, 85* (11), 3951-3963.

Specker, B. (2001). The significance of high bone density in children. *Journal of Pediatrics, 139* (4), 473-475.

Spitz, D. J., & Newberg, A. H. (2003). Imaging of stress fractures in the athlete. *Magnetic Resonance Imaging Clinics in North America, 11*, 323-339.

Srinivasan, S., Weimer, D., Agans, S., Bain, S., & Gross, T. (2002). Low-magnitude mechanical loading becomes osteogenic when rest is inserted between each load cycle. *Journal of Bone & Mineral Research, 17* (9), 1613-1620.

Stewart, A., & Hannan, J. (2000). Total and regional bone density in male runners, cyclists, and controls. *Medicine & Science in Sports & Exercise, 32* (8), 1373-1377.

Sundberg, M., Duppe, H., Gardsell, P., Johnell, O., Ornstein, E., & Sernbo, I. (1997). Bone mineral density in adolescents. Higher values in a rural area - a population-based study of 246 subjects in southern Sweden. *Acta Orthopaedica Scandinavica, 68* (5), 456-460.

Sundberg, M., Gardsell, P., Johnell, O., Karlsson, M., Ornstein, E., Sandstedt, B., et al. (2001). Peripubertal moderate exercise increases bone mass in boys but not in girls: a population-based intervention study. *Osteoporosis International, 12* (3), 230-238.

Sundberg, M., Gardsell, P., Johnell, O., Ornstein, E., Karlsson, M., & Sernbo, I. (2003). Pubertal bone growth in the femoral neck is predominantly characterized by increased bone size and not by increased bone density--a 4-year longitudinal study. *Osteoporosis International, 14* (7), 548-558.

Szulc, P., Seeman, E., & Delmas, P. (2000). Biochemical measurements of bone turnover in children and adolescents. *Osteoporosis International, 11* (4), 281-294.

Taaffe, D., Duret, C., Cooper, C., & Marcus, R. (1999). Comparison of calcaneal ultrasound and DXA in young women. *Medicine & Science in Sports & Exercise, 31* (10), 1484-1489.

Taaffe, D., Robinson, T., Snow, C., & Marcus, R. (1997). High-impact exercise promotes bone gain in well-trained female athletes. *Journal of Bone & Mineral Research, 12* (2), 255-260.

Takada, M., Engelke, K., Hagiwara, S., Grampp, S., & Genant, H. K. (1996). Accuracy and precision study in vitro for peripheral quantitative computed tomography. *Osteoporosis International, 6*, 207-212.

Takeda, Y., Ikata, T., Yoshida, S., Takai, H., & Kashiwaguchi, S. (1998). MRI high-signal intensity in the menisci of asymptomatic children. *Journal of Bone & Joint Surgery - British Volume, 80* (3), 463-467.

Tami, A., Nasser, P., Verborgt, O., Schaffler, M., & Knothe, T. M. (2002). The role of interstitial fluid flow in the remodeling response to fatigue loading. *Journal of Bone & Mineral Research, 17* (11), 2030-2037.

Tanner, T. M. (1968). *Growth in adolescence* (2nd ed.). Oxford: Blackwell Scientific Publications.

Teegarden, D., Lyle, R., Proulx, W., Johnston, C., & Weaver, C. (1999). Previous milk consumption is associated with greater bone density in young women. *American Journal of Clinical Nutrition, 69* (5), 1014-1017.

Theintz, G., Howald, H., Weiss, U., & Sizonenko, P. (1993). Evidence for a reduction of growth potential in adolescent female gymnasts. *Journal of Pediatrics, 122* (2), 306-311.

Tomten, S., Falch, J., Birkeland, K., Hemmersbach, P., & Hostmark, A. (1998). Bone mineral density and menstrual irregularities. A comparative study on cortical and trabecular bone structures in runners with alleged normal eating behavior. *International Journal of Sports Medicine, 19* (2), 92-97.

Tothill, P., Avenhill, A., & Reid, R. M. (1994). Precision and accuracy of measurements of whole-body bone mineral: comparison between Hologic, Lunar and Norland dual energy absorptiometers. *British Journal of Radiology, 67*, 1210-1217.

Trost, S. G., Pate, R. R., Sallis, J. F., Freedson, P. S., Taylor, W. C., Dowda, M., et al. (2002). Age and gender differences in objectively measured physical activity in youth. *Medicine and Science in Sports and Exercise, 34* (2), 350-355.

Turner, C., Owan, I., & Takano, Y. (1995). Mechanotransduction in bone: role of strain rate. *American Journal of Physiology, 269* (3 Pt 1), E438-442.

Turner, C., Wang, T., & Burr, D. (2001). Shear strength and fatigue properties of human cortical bone determined from pure shear tests. *Calcified Tissue International, 69* (6), 373-378.

Turner, C. H., & Burr, D. B. (1993). Basic biomechanical measurement of bone: a tutorial. *Bone, 14*, 595-606.

Twellaar, M., Verstappen, F. T., Huson, A., & Van Mechelen, W. (1997). Physical characteristics as risk factors for sports injuries: a four year prospective study. *International Journal of Sports Medicine, 18*, 66-71.

Uusi-Rasi, K., Sievanen, H., Pasanen, M., Oja, P., & Vuori, I. (2002). Associations of calcium intake and physical activity with bone density and size in premenopausal and postmenopausal women: a peripheral quantitative computed tomography study. *Journal of Bone & Mineral Research, 17* (3), 544-552.

Valentino, R., Savastano, S., Tommaselli, A., D'Amore, G., Dorato, M., & Lombardi, G. (2001). The influence of intense ballet training on trabecular bone mass, hormone status, and

gonadotropin structure in young women. *Journal of Clinical Endocrinology & Metabolism, 86* (10), 4674-4678.

Valimaki, M., Karkkainen, M., Lamberg-Allardt, C., Laitinen, K., Alhava, E., Heikkinen, J., et al. (1994). Exercise, smoking, and calcium intake during adolescence and early adulthood as determinants of peak bone mass. Cardiovascular Risk in Young Finns Study Group. *British Medical Journal, 309* (6949), 230-235.

van der Meulen, M., Ashford, M. J., Kiratli, B., Bachrach, L., & Carter, D. (1996). Determinants of femoral geometry and structure during adolescent growth. *Journal of Orthopaedic Research, 14* (1), 22-29.

Virvidakis, K., Georgiou, E., Korkotsidis, A., Ntalles, K., & Proukakis, C. (1990). Bone mineral content of junior competitive weightlifters. *International Journal of Sports Medicine, 11* (3), 244-246.

Wagenmakers, A. J. M., Beckers, E. J., & Brouns, F. (1991). Carbohydrate supplementation, glycogen depletion and amino acid metabolism during exercise. *American Journal of Physiology, 260*, E883-E890.

Wallace, L., & Ballard, J. (2002). Lifetime physical activity and calcium intake related to bone density in young women. *Journal of Womens Health & Gender-Based Medicine, 11* (4), 389-398.

Warren, M., Brooks-Gunn, J., Fox, R., Holderness, C., Hyle, E., & Hamilton, W. (2002). Osteopenia in exercise-associated amenorrhea using ballet dancers as a model: a longitudinal study. *Journal of Clinical Endocrinology & Metabolism, 87* (7), 3162-3168.

Warren, M., & Perlroth, N. (2001). The effects of intense exercise on the female reproductive system. *Journal of Endocrinology, 170* (1), 3-11.

Watson, M. D., & DiMartino, P. P. (1987). Incidence of injuries in high school track and field athletes and its relation to performance ability. *American Journal of Sports Medicine, 15* (3), 251-254.

Weaver, C. (2000). Calcium requirements of physically active people. *American Journal of Clinical Nutrition, 72* (2 Suppl), 579S-584S.

Weaver, C., Peacock, M., & Johnston, C. J. (1999). Adolescent nutrition in the prevention of postmenopausal osteoporosis. *Journal of Clinical Endocrinology & Metabolism, 84* (6), 1839-1843.

Welsh, L., Rutherford, O., James, I., Crowley, C., Comer, M., & Wolman, R. (1997). The acute effects of exercise on bone turnover. *International Journal of Sports Medicine, 18* (4), 247-251.

Welten, D., Kemper, H., Post, G., Van Mechelen, W., Twisk, J., Lips, P., et al. (1994). Weight-bearing activity during youth is a more important factor for peak bone mass than calcium intake. *Journal of Bone & Mineral Research, 9* (7), 1089-1096.

Wen, D. Y., Puffer, J. C., & Schmalzried, T. P. (1997). Lower extremity alignment and risk of overuse injuries in runners. *Medicine & Science in Sports & Exercise, 29* (10), 1291-1298.

Whiting, S., Vantanparast, H., Baxter-Jones, A., Faulkner, R. A., Mirwald, R., & Bailey, D. A. (2004). Factors that affect bone mineral accrual in the adolescent growth spurt. *Journal of Nutrition, 134* (3), 696S-700S.

Whiting, S., and Zernicke, R. (1998). Sequental changes in weight, density and percentage ash weight of human skeletons from an early fetal period through old age. *Anat. Rec,* 179:1-18.

Winters, K., Adams, W., Meredith, C., Loan, M., & Lasley, B. (1996). Bone density and cyclic ovarian function in trained runners and active controls. *Medicine & Science in Sports and Exercise, 28* (7), 776-785.

Winters, K. M., & Snow, C. M. (2000). Detraining reverses positive effects of exercise on the musculoskeletal system in premenopausal women. *Journal of Bone & Mineral Research, 15* (12), 2495-2503.

Witzke, K., & Snow, C. (2000). Effects of plyometric jump training on bone mass in adolescent girls. *Medicine & Science in Sports and Exercise, 32* (6), 1051-1057.

Woodhead, H.J., Kemp, A.F., Blimkie, C.J.R., Briody, J.N., Duncan, C.S., Thompson, M., et al (2001). Measurement of midfemoral shaft geometry: repeatability and accuracy using magnetic resonance imaging and dual-energy x-ray absorptiometry. *Journal of Bone and Mineral Research*, 16 (12), 2251-2259.

Wosk, J., & Voloshin, A. S. (1981). Wave attenuation in skeleton of young healthy persons. *Journal of Biomechanics, 14*, 261-267.

Yao, L., & Johnson, C. (1998). Stress injuries of bone: analysis of MR imaging staging criteria. *Acad Radiology, 23*, 472-481.

Zernicke, R., McNitt-Gray, J., & Otis, C. (1993). *Stress fracture risk assessment among elite collegiate women.* Paper presented at the International Society of Biomechanics XIVth Congress.