BONE MINERAL DENSITY: AGE, PHYSICAL ACTIVITY AND MENSTRUAL STATUS OF PREMENOPAUSAL WOMEN

LYNN M. SMITH

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
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ABSTRACT

Objective To examine the relationships of age, physical activity, and menstrual status to bone mineral density (BMD) of premenopausal women.
Design (i) Cross-sectional study of age and BMD in 130 premenopausal women. (ii) Cross-sectional study of BMD and data from self-reported questionnaires, n=95. BMD was compared in athlete and non-athlete groups and eumenorrheic and oligo/amenorrheic groups. Variables eg. weight, body fat, muscularity, parity, oral contraceptive use (OC), dietary calcium and assay results were investigated. (iii) Cross-sectional study of anorexia nervosa patients (AN, n=12) compared to two age-matched control groups; normal controls (NC, n=12) and oligo/amenorrheic athlete (OAC, n=12) controls. (iv) Longitudinal study, repeated BMD measurements in 14 athletes, interval of 360±11d.
Subjects 130 healthy, not pregnant or lactating, premenopausal women aged 18-48y, 12 anorexia nervosa (AN) patients, aged 15.6-38.5y diagnosed at least one year prior and 4 age matched control subjects, 15-17y.
Main outcome measure BMD (gm/cm^2) at the lumbar spine (L2-L4) and proximal femur, femoral neck (FN), Ward's triangle (WT) and Trochanter (FT) as measured by dual photon absorptiometry (DP3).
Measurements Anthropometry: height, weight, body girths, bone breadths, and skinfold measures. Questionnaires: general medical history, menstrual history, physical activity, and nutrition/dietary calcium. Mid-follicular blood samples from 65 subjects, collected prior to 0830 and in fasted, non-exercised state. Assays: serum estrone (E1), estradiol (E2), follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), cortisol (C), and osteocalcin (BGP). A calcium panel assay which included serum calcium, total and ionized, and parathyroid hormone was also completed.
Results (i) Relationship between age and BMD was not significant at the lumbar spine but was significant: FN, $p<0.0001$; WT, $p<0.0001$; FT, $p<0.05$. Stratified age groups indicated peak bone mass occurred <21y FN and WT,

<24.9y FT. Age and weight, respectively, explained asignificant portion of variation in BMD at FN (35%, 9%), WT (42%,8%) and FT (n.s.,22%). An estimate of total muscle mass was not as highly correlated with BMD as was weight. However, regional muscularity provided a good indication of regional BMD: triceps to L2-L4 and thigh to the FN.

(ii) Compared to the non-athletes, the athletes' BMD was 7-9% higher at the proximal femur sites and 3.8% higher at the lumbar site. At the femoral sites, the non-athletes showed a significant or near significant decline of BMD with age, ($p \leq 0.06$ at FT). The athletes' estimated BMD loss with age was significant only at WT. Total muscle mass was significantly greater in athletes ($p<0.02$). Athletes had a significantly lower sum of 6 skinfolds and % body fat than nonathletes ($p<0.0001$). Menarche was significantly later in athletes (13.2±0.21 y) compared to non-athletes (12.5±0.17y). Analysis of OC use showed the following trend of BMD at all sites: athlete non-user > athlete user > non-athlete non-users > non-athlete user.

Two groups were formed on the basis of menstrual history data; eumenorrheics (R) and oligo/amenorrheics (OA). Weight (55.9±1.0 vs 59.1±0.9k) and % body fat were significantly lower in the OA group. BMD was higher (n.s.) at all sites in the R group compared to the OA group. Considering both athletic and menstrual status, the following trend was evident for BMD at all sites: R athletes > OA athletes > R non-athletes > OA non-athletes: R athletes had significantly higher BMD at all sites compared to other groups except at L2-L4 in OA athletes (n.s.). The OA athletes had a significantly lower percentage of body fat than the R athletes and non-athletes. The highest BMD's at all sites were observed in the high calcium athletes' group. However, there were no significant differences in BMD in high and low calcium groups between athletes and nonathletes, controlling for menstrual group. The interaction effect of calcium and physical activity was significant at all four BMD sites ($p<0.05$). All hormonal assay mean results were within normal reference ranges. Significantly lower values for serum E1, E2 and LH were observed in the athletes. E2 (-31%), LH (-18%) and E1 (-17%) were lower while C (+5%), FSH (+11%) and PRL (+39%) were

higher in OA compared to R (n.s.). This general trend was observed for estradiol, estrone and LH: OA athlete <OA non-athlete <R athlete < R non-athlete. There were no significant relationships between assay results (E1, E2, C, PRL) and BMD.

(iii) The AN weight was significantly different from NC (45.7±2.3 vs 59.7±2.2k). AN had significantly lower mean BMD values than NC and OAC at all sites. At the L2-L4 site, the AN BMD was less than the fracture threshold of 0.98 g/cm^2. At the FN, 5 of the AN individual BMD values were below the fracture threshold of 0.75 g/cm^2. Significant negative correlations existed between the duration of anorexia nervosa and bone mineral density at all sites.

(iv) Repeated measurements in athletes showed bone gain at the proximal femur which was significantly different from cross-sectional estimate of bone loss (i) at L2-L4 and FT.

Conclusions Premenopausal women exhibited a model of differential involutional bone loss: proximal femur bone loss begins early in the third decade and the lumbar spine shows no significant bone loss prior to menopause. Strenuous physical activity was associated with higher BMD and may attenuate the age-related bone loss at the proximal femur. OA has moderate negative BMD effects and anorexia nervosa results in severe osteopenia. Weight, menstrual state, physical activity and dietary calcium are important influences of skeletal mass in premenopausal women. Primary prevention of osteoporosis depends upon maximizing the positive potential of these factors.

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LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AN	Anorexia Nervosa
BN	Bulimia Nervosa
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Bone Mass Index
DEXA	Dual Energy X-ray Absorptiometry
FN	Femoral neck
FT	Trochanter region of femur
L2-L4	Lumbar Spine, average of 2nd, 3rd and 4th vertebrae
WHR	Waist girth to Hip girth Ratio
WT	Ward's triangle
OA	Oligo/amenorrheic
OC	Osteocalcin
PBM	Peak Bone Mass
PI	Power Index
QCT	Quantitative Computed Tomography

TABLE OF CONTENTS

1. INTRODUCTION

1.0 Bone

More than two hundred bones are assembled in the human skeletal infrastructure to provide support, protection and enable locomotion. Bones also fulfill important functions related to hemopoiesis, the immune system and mineral homeostasis, particularly calcium and phosphate. In both its structural and metabolic roles, bone is a dynamic tissue capable of responding to many factors. Some predominant influences upon bone tissue are: physical stimuli, endocrine factors, dietary availability of ions, local bone regulatory factors, drugs and toxins.

Bone is a highly specialized connective tissue composed of cellular elements and an extracellular matrix which is mineralized (Robey, 1989). The cellular component consists of progenitor cells, osteoclasts, osteoblasts and osteocytes. The extracellular matrix is composed of mineral, collagen, proteins, molecules and ions (Boskey, 1990). Normally, the proportions of the organic and inorganic components make bone rigid yet compressible. These two properties permit bone a unique mechanical role. In abnormal states, a bone may be unable to withstand normal mechanical stimuli due to defective structure.

The skeleton is made up of two distinct types of bone, cortical bone approximately 80% and trabecular bone, 20% (Marcus & Carter, 1988). Cortical or compact bone is found on the surface of all bones and in the shafts of long bones. Trabecular or cancellous bone is located in the vertebral bodies, the small bones and the metaphyses of long bones. Trabecular bone is composed of bony spicules or trabeculae which in some bones are obviously arranged in response to lines of force. Cortical bone is composed of osteons which are packed tightly together in layers such that the specialized Haversian canal system provides nutrient delivery and waste removal. Although trabecular and cortical bone exhibit unique structural and functional properties, their cellular and extracellular components are the same. Both bone types may be influenced by similar factors, although perhaps to different degrees since for a given mass, trabecular bone has a larger surface area than cortical bone (Gallagher, 1990).

During the growth and development of the skeleton, the process of modeling occurs whereby bone structure and mass are modified by systemic and local factors. After skeletal maturation, bone responds to similar influences by a process called remodelling (Frost, 1964). The mechanisms by which these influences signal and modify bone remodelling remain largely undefined (Frost, 1987). In normal remodelling, the osteoclast and osteoblast are coupled so that resorption is followed by formation. The amount of bone resorbed is usually similar to the amount formed. Where this is not the case, there is an imbalance in remodelling (Eriksen, Steiniche, Mosekilde & Melsen, 1989). Normally, resorption takes about ten days and formation three months (Mundy, 1987; Parfitt, 1988). The osteoblast population is encapsulated in the matrix which it synthesizes (Martin, Ng & Suda, 1989). These cells, now termed osteocytes may form an intracellular network in bone. The osteoid becomes fully mineralized in the subsequent three to four months (Parfitt, 1988).

Remodeling occurs in discrete packets of bone throughout the skeleton (Parfitt, 1988). Different bone types and compartments undergo different remodelling processes (Eriksen et al, 1989). The remodelling process permits the skeleton to maintain its mechanical integrity through the renewal of bone (Eriksen et al, 1989). However, normal bone turnover and remodelling may be dramatically influenced by other factors which will affect the quality or quantity of new bone. When the remodelling balance is perturbed it may take months or years to reach a new steady state (Johnston, 1985).

1.1 Osteoporosis

Osteoporosis was originally defined by Albright and colleagues (1941) as a condition of "porous bone" wherein the quality of bone was not affected. There was putatively a reduction in the amount of bone but the bone which remained was structurally normal. Currently, controversy exists regarding the reputed normal composition of osteoporotic bone (Boskey, 1990; Heaney, 1987).

Osteoporosis is a disease which traditionally has been diagnosed upon its fracture outcome. Thus, osteoporosis can be defined as an increased

susceptibility to fracture with minimal trauma. Both fatigue damage and trabecular disconnection have been considered to contribute to fracture propensity. However, a reduction in bone mass is the main factor in fracture predisposition (Heaney, 1989b). Although arbitrary, a reduction of bone mass measured by densitometry to the level of two standard deviations below that of a young adult of the same sex, has been suggested to be diagnostic of osteoporosis (Nordin, 1987).

Osteoporosis is a heterogeneous disorder with both primary and secondary causes. The secondary causes are diseases or medical interventions which include: thyroid disorders, Cushing's disease, corticosteroid use, liver disease, diabetes, Paget's disease and rheumatoid arthritis (Bonnick, 1990). Primarily, osteoporosis is caused by involutional bone loss and menopause. Involutional bone loss is a universal process that causes rarefaction of skeletal mass with age. In females, this normal bone loss of the aging process is exacerbated by the estrogen deficient state of menopause (Riggs & Melton, 1986a). Up to 20% of women will become osteoporotic at menopause (Riggs & Melton III, 1990). Efforts to identify potentially high risk females have resulted in recognition of various risk factors. These risk factors for primary osteoporosis have been identified: "slight or slender build, fair skin, family history of osteoporosis or osteoporotic fractures, small muscle mass, sedentary life style, small peak adult bone mass, low calcium intake, early menopause or oophorectomy, cigarette smoking, excessive consumption of protein, fibre and caffeine, and one or more prior osteoporotic fractures" p. 85, (Heaney, 1987). The development of risk profiles to predict osteoporosis has not been effective (Bonnick, 1990) and a direct measurement of bone mass is necessary (Slemenda et al, 1990b).

In western countries, osteoporotic fractures are at epidemic proportions in the elderly (Heaney, 1983). Projections show that one in four Canadian women will suffer an osteoporotic fracture in her lifetime, with one in seven incurring a hip fracture, the most serious manifestation of excessive bone loss (Martin & Houston, 1987; Martin et al, in press). Acute care costs of osteoporotic fractures in the United States have been estimated at $6-$10 billion annually (Mundy, 1987), a figure that will increase rapidly as the proportion of elderly in the population continues to increase. By the

year 2020, it has been estimated that the annual economic impact of osteoporosis will approach $60 billion (Gallagher, 1990).

1.2 Bone Mineral Measurements

Low bone mineral density is the most important risk factor for osteoporotic fractures (Johnston, Melton III, Lindsay & Eddy, 1989). The accurate and precise measurement of bone mass is central to the detection of those at risk and to the monitoring of those being treated for osteoporosis. Precision is the standard deviation of the difference between repeated measurements divided by the mean of those measurements and is commonly expressed as a percentage. Similarly, accuracy is the coefficient of variation of the measured value relative to the true value. Currently, the most common methods are photon absorptiometry, dual energy X-ray absorptiometry (DEXA) and quantitative computed tomography (QCT). These techniques have been comprehensively reviewed elsewhere (Chestnut III, 1987; Firoonzia, Golimbu, Rafii & Schwartz, 1989; Fogelman, Rodin & Blake, 1990; Mazess & Barden, 1989; Mazess & Wahner, 1988; Wahner, 1989) and only a brief summary is presented herein.

Radiography The need for non-invasive techniques of bone mass measurement which were more sensitive than standard radiographs, was recognized as early as 1960 (Cameron & Sorenson, 1963). Radiography, which had been the main clinical tool, lacked the precision to detect osteoporosis at any time before fracture. A reduction in bone density by as much as 40% was necessary to detect differences on a radiograph (Marcus et al, 1988). Two other radiologic methods, radiogrammetry and radiographic densitometry saw limited use. Radiogrammetry, used in early cross-sectional research of bone mass, consisted of the measurement of cortical bone, usually the third metacarpal to calculate various indices based on cortical thickness. Radiographic densitometry measured the optical density of a radiograph against a phantom included in the view. None of these methods was adequate for osteoporosis research or clinical use where the aim was prevention or early

intervention. However, radiology may remain a useful technique in musculoskeletal imaging of later stages of metabolic bone disease (Cooper, 1989).

Photon absorptiometry Photon absorptiometry in various forms has been used for three decades. This method relies on an isotope as the source of low energy photons which are collimated and directed at the site of measurement. A scintillation detection system collects data on the transmission and attenuation of the beam as the source and counter move together in a rectilinear path. In 1963, the report published by Cameron and Sorenson introduced single photon absorptiometry (SPA) as the preferred method over radiography. SPA generally used ^{125}I as its low energy source to provide maximum discrimination between bone and soft tissue. To standardize the soft tissue path length, the appendage is immersed in tissue equivalent gel or water. SPA is normally limited to forearm sites and the os calcis. Radiation exposure is minimal (Johnston et al, 1989). Although excellent accuracy (4-5%) and precision (1-2%) have been reported for SPA, a major disadvantage has been its restriction to appendicular measurement sites. (Johnston et al, 1989). The remodelling activity and the stimuli for changes in remodelling differ for the trabecular and cortical bone as well as the different surfaces of cortical bone (Frost, 1987). Therefore the forearm site was not a reliable predictor of bone mass at other sites, particularly the common fracture sites, the lumbar spine and femoral neck (Mazess & Barden, 1990a).

Consequently, dual photon absorptiometry (DPA) using a dual energy isotope, commonly ^{153} Gd, was developed to measure the proximal femur and lumbar spine. Instrument modifications and software have been introduced to measure other sites, regions of interest and whole body bone mineral. The amount of bone mineral is expressed as bone mineral content (BMC, grams/cm of hydroxyapatite) and bone mineral density (BMD grams of hydroxyapatite per unit area, gm/cm^2). The ^{153} Gd radionuclide emits two energy peaks at 44 keV and 100 keV (Wahner, 1989). The additional information from the second energy level helps to reduce the confounding effect of soft tissue and irregular path length in the region of interest. Typically, DPA precision is about 2% at the lumbar spine and 3% at the

proximal femur (Mazess, 1990). The estimated accuracy at the lumbar spine is 3-6% and proximal femur 3-4%(Fogelman et al, 1990; Johnston et al, 1989). The radiation exposure is low, 5-10 millirems for the DP3 instrument. Although DPA has been recognized as a reasonably safe, accurate and precise method of measuring bone mineral, there are several limitations such as poor image resolution, drift in results due to source decay (Ross, Wasnich & Vogel, 1988b), expensive source replacement costs and unacceptable precision in older and/or osteoporotic subjects (Gluer, Steiger & Genant, 1988; Pouilles et al, 1988; Sartoris & Resnick, 1988).

In response to shortcomings of the existing instrumentation, dual energy X-ray absorptiometry (DEXA) was developed. DEXA has replaced the isotope source with an X-ray tube which generates the dual photon flux by rare earth filters or switching kilovoltage. The precision is 0.5-1.2% (Johnston et al, 1989) and accuracy of a spinal measurement 4-8%(Wahner, 1989). Other features are shorter scan times, higher resolution and lower radiation exposure than conventional DPA (Kelly, Slovik, Schonfeld & Neer, 1988; Mazess, 1990; Wahner, Dunn, Brown, Morin & Riggs, 1988).

Quantitative Computed Tomography QCT is another common method of measuring bone mineral. Precision and accuracy are respectively 1-3% and 5-10% (Johnston et al, 1989). The major advantage of QCT is that a specific volume of trabecular bone can be measured separately from cortical bone. This method employs a conventional CT scanner with a commercially available software package and a standard phantom which is included in each image (Wahner, 1989). Strict patient positioning and a scout view improve reproducibility (Wahner, 1989). Due to the high variability of density and composition of fat tissue, dual energy QCT has been developed but its precision is reduced. The main disadvantage of QCT is that radiation dosage is very high, particularly with dual energy QCT (200-1000 mrem/scan)(Mazess et al, 1989; Wahner, 1989).

Accurate and precise baseline measurement at various skeletal sites is currently available. A single measurement of the common fracture sites, spine and proximal femur, along with consideration of other risk factors provides

valuable information regarding fracture probability. Repeated measurements are capable of monitoring changes in bone mass and evaluating therapy.

1.3 Inter-site Variability

Bone mineral measurements at different skeletal sites are not highly correlated. In a set of measurements, the correlation between any two sites is approximately 0.6 (Ross, Wasnich & Vogel, 1988a). Thus, the measurement of a single site is a poor predictor for other sites (Mazess et al, 1990a). Inter-site variability exists partly because of the different proportions of trabecular and cortical bone. Trabecular bone has been reported to be more metabolically active than cortical bone because of its larger surface area and its proximity to the blood supply and the bone marrow (Fogelman et al, 1990). Trabecular and cortical bone have different mechanisms of loss (Eriksen et al, 1989). Another factor contributing to inter-site variability is that axial and appendicular areas exhibit different patterns of loss (Wahner, 1989). This may be due to differences in mechanical stressors and other systemic influences (Fogelman et al, 1990). Trabecular bone may contribute more to bone strength than cortical bone although site is an important factor in determining the properties of different bone types (Wahner, 1989).

The heterogeneity of the skeleton dictates that bone mineral measurements should be made at the sites under investigation. For diagnosis and monitoring, axial measurements are preferable to peripheral measurements (Mazess et al, 1989). In osteoporosis, the clinically relevant sites are the lumbar spine and the proximal femur. Early studies reported that bone loss was more evident at the axial skeleton than the appendicular forearm sites and this disparity led to the general belief that cortical bone was less vulnerable to aging than trabecular bone. Due to instrumentation, the typically measured sites consist of integrated bone, variable amounts of trabecular and cortical which demonstrate variable site-specific remodelling responses. Only QCT is capable of measuring wholly trabecular bone. General comparisons of axial and appendicular sites should include specification of the regions of interest and their particular proportions of bone. Thus, it may be more relevant to discuss regional bone loss patterns as opposed to trabecular versus cortical bone loss.

1.4 Age-related Changes in Bone Mass

Bone is a dynamic tissue whose density and composition are the result of mechanical, hormonal, nutritional, and genetic factors (Heaney, 1987). Each of these factors influences bone throughout life although the relative importance of any one factor may change at various phases (Slemenda, Christian, Williams & Johnston Jr., 1990a). Many models have been proposed to describe skeletal modeling and remodelling and how various factors might interact in a lifetime. Adult bone mass at any age reflects the peak bone mass (PBM) of early adulthood and the subsequent rate of loss (Parfitt, 1987).

Cross-sectional data suggest that after linear growth is completed, the female skeleton undergoes a consolidation phase of about 10 years (Garn, Rohmann & Wagner, 1967; Krolner & Neilsen, 1982; Nordin, 1966; Parfitt, 1979; Riggs et al, 1981). Reportedly, during this decade, an additional 10-15% bone mass can be accrued in attaining peak bone mass (PBM) (Avioli, 1984; Heaney, 1989b; Kanders, Dempster & Lindsay, 1988). This consolidation phase may be followed by a transient period of maintenance of peak bone mass and thereafter, the variable process of involutional bone loss occurs. One current, generally accepted concept of bone loss presented by Riggs and Melton (1990) states that bone loss associated with aging begins at about age 25 in both sexes and proceeds at a similar rate in cortical and trabecular bone to old age. In women, this age-related bone loss is transiently increased postmenopausally for 4 to 8 years, with a disproportionate reduction of trabecular bone (Riggs, 1990).

However, recent reports regarding both peak skeletal mass and subsequent loss are contradictory. Although most investigators recognize that osteoporosis is a heterogeneous disease, few agree on all the details of primary bone loss. There have been numerous models proposed to explain the pattern, rates and fracture profiles of osteoporosis. Most of these models have not specifically focussed on PBM and premenopausal bone mass. For young adult women, the parameters of peak bone mass and age-related bone loss have not been clarified in the literature and remain controversial

topics. Consequently in this group, the extent and the manner in which PBM and bone loss are influenced by genetic, mechanical, hormonal and nutritional factors have not been resolved.

1.5 Factors Affecting Bone Mass

1.5.0 Genetics

Genetics which for the purpose of this discussion include ethnicity and sex, contribute greatly to skeletal size. It is likely that a genetic program exists which dictates the process of skeletal development and peak bone mass for each individual. The parameters of physiological loss of bone due to aging may also be genetically programmed. Support for the strong genetic influence comes from several types of investigations and reviews. Males reportedly have denser bones than females and bone mineral density is higher in blacks than whites and orientals. A positive family history is a risk factor for osteoporosis. Thus, the contribution of genetics to bone growth and development is acknowledged but not well-defined by research. An extensive review of this topic (Pollitzer & Anderson, 1989) supports a major role for genetic determination of bone mass. Stevenson (1990) suggested that genetics largely determine peak bone mass. It has been reported that by the age of 14 or 15, girls have achieved an estimated 90% of PBM (Matkovic & Chestnut, 1987). It is also clear that genetic influences may be less important after the accrual of peak bone mass after which environmental influences may predominate (Stevenson, 1990).

1.5.1 Mechanical Stimuli

Mechanical loading has been recognized as a major modulator of bone mass through evidence from immobilization and physical activity investigations. Body weight or body mass provides simple evidence of the effect of weight bearing on the skeleton although fatness or lack of fat is a major complicating factor. Research concerning disuse (Nishiyama, Kuwahara & Matsuda, 1986), immobilization (Donaldson et al, 1970; Green, 1985) and weightlessness (Mack, Lachance, Vose & Vogt, 1967) points out the rapid and sometimes irreversible bone loss associated with inactivity in humans and animals. Animal studies have conclusively attested to physical activity as a primary modifier of bone mass. The effects of physical activity in humans are not as conclusive and this topic has been extensively reviewed elsewhere (Block, Smith, Friedlander & Genant, 1989; Dalsky, 1987; Smith & Gilligan, 1989a). Although high levels of physical activity in humans have been associated with greater bone mass, the supporting data have relied mainly on cross-sectional and non-randomized longitudinal studies. However, more recent intervention studies have reported that physical activity can attenuate bone loss or promote bone gain in postmenopausal and elderly females (Dalsky et al, 1988; Smith, Gilligan, Shea, Ensign & Smith, 1989b). The effect of high levels of physical activity on bone mineral density and age-related bone loss in young adult women has not been determined even though female involvement in strenuous activity is greater than ever before, is steadily increasing and often begins prior to adolescence.

1.5.2 Hormonal Factors

There are many hormones which act upon the skeleton both directly and indirectly. Among these hormones are: estrogens, prolactin, progesterone, glucocorticoids, androgens, growth hormone, parathyroid hormone, calcitonin, calcitriol and thyroid hormone (Compston, 1990). The effect of various factors such as insulin-like growth factors, epidermal growth factor, fibroblast growth factor, bone morphogenic proteins, interleukins, prostaglandins and calcitonin-related peptide portions, on bone cells is an area of rigorous investigation (Compston, 1990; Cotton, 1990). Although the interaction of all these hormones and factors likely determines the effect at the bone cell unit, research in females has identified that estrogens are particularly important to skeletal health. The estrogenic mechanism on bone is not known although estrogen receptors have been located by two groups (Eriksen et al, 1988; Komm et al, 1988).

A reduction of serum estradiol is a major hormonal change in amenorrhea. Estrogen deficiency at any age causes accelerated bone loss and eventually may result in osteoporosis. In both natural and artificial menopause, bone loss may be as high as 5-10% annually for five to eight years (Riggs et al, 1986b). The role of estrogen in the enhancement of the skeletal mass of menopausal women has been established convincingly in double blind controlled studies in which estrogen replacement prevented the expected bone loss (Christiansen, Christensen & Transbol, 1981; Riis, Thomsen & Christiansen, 1987).

1.5.3 Nutritional Factors

Bone health depends upon many components of nutrition. Skeletal tissue relies upon the ingestion and absorption of an adequate number of calories which contain vital substances required for maintenance and remodelling. Though calcium intake has dominated the literature, phosphate, protein, and the trace elements such as manganese, copper and zinc are important to skeletal health. Calcium remains a very controversial topic with regards to its prophylactic and therapeutic effectiveness in osteoporosis. Despite the fact that early research (Matkovic et al, 1979; Nordin, 1966) appeared to confirm the importance of dietary calcium, the recommended levels of dietary (RDA) calcium continue to generate disagreement (Heaney, 1990a; Martin et al, 1987). The recommended dietary allowance (RDA) for any nutrient is set at a level which meets the requirements of 97% of the population. Thus, reports of under-consumption must be reviewed with this in mind. On the other hand, excess dietary calcium has been reported as not harmful to normal people (Arnaud, 1990).

It has been acknowledged generally, though based on little data, that calcium is important to bone health particularly during growth and development. The effect of calcium during consolidation and after skeletal maturation, particularly during the premenopausal stage, has not been adequately researched. However, a considerable number of investigations concerning calcium and its interactions with other bone agents such as estrogen and calcitriol have been reported. The role of calcium, is secondary to estrogen and may be permissive rather than causative in osteoporosis (Heaney, 1986). It has been suggested that calcium inadequacy in later years may be an important factor in cortical bone loss and consequent hip fracture (Bonnick, 1990) although others would disagree (Riggs et al, 1987; Martin et al, 1987).

1.6 Skeletal Health of the Premenopausal Female

Our knowledge of the female skeleton has been gained through studies which have mainly focussed on the mature or menopausal subject. This emphasis has likely arisen due to the former practice of reacting to the end stage of the disease. The skeletal health of the premenopausal woman requires further investigation since the attainment and maintenance of a high peak bone mass in early adulthood is a primary strategy against osteoporosis (Riggs et al, 1982a). Recent investigations employing improved measurement methods have been initiated to examine the early predisposing factors to osteoporosis. These factors can be clarified by investigating these three states of premenopausal skeletal health: the normal woman, the amenorrheic athlete and the anorectic female.

1.6.0 The Premenopausal Woman

There is a lack of research on bone mineral density in premenopausal women. There have been few studies which have investigated the stage(s) between attainment of peak bone mass and the perimenopausal years. Thus, little is known about the timing of peak bone mass, consolidation and involution in young adult females. The effects of nutritional, endocrine and activity factors also require further investigation

1.6.1 Athletic Amenorrhea

With recent advances in the measurement of bone mineral density concerns have been raised about the skeletal integrity of young women with menstrual dysfunction. Reports of osteoporotic fractures in young women with anorexia nervosa and exercise-induced amenorrhea reinforce these concerns (Barrow & Saha, 1988; Drinkwater et al, 1984; Rigotti, Nussbaum, Herzog & Neer, 1984). Low serum estradiol has been implicated in the bone loss associated with athletic amenorrhea and not surprisingly, low bone density (Drinkwater et al 1984) and a high incidence of stress fractures in young amenorrheic athletes have been documented (Warren, Brooks-Gunn, Hamilton, Fiske Warren & Hamilton, 1986). The extent of bone loss and the underlying endocrine abnormalities associated with exercise-induced menstrual dysfunction are poorly understood. Both delayed menarche and early menopause are risk factors for osteoporosis, suggesting that the hypogonadal female athlete may be at risk for increased incidence of stress fractures and premature osteoporotic fractures.

1.6.2 Anorexia Nervosa

Osteopenia has been investigated in this eating disorder, in which amenorrhea is a major diagnostic criterion. Several investigators, utilizing varied bone measurement techniques, have reported that bone loss in women with anorexia nervosa is approximately 10-20% at various skeletal sites (Mazess, Barden & Ohlrich, 1990b; Rigotti et al, 1984; Szmukler, Brown, Parsons & Darby, 1985; Treasure, Fogelman & Russell, 1986). Recent studies have indicated that decreases in bone mineral density are more extreme than previously reported, particularly in the adolescent anorectic (Bachrach, Guido, Katzman, Litt & Marcus, 1990; Fosson, Knibbs, Bryant-Waugh & Lask, 1987). Though hip fracture is one of the most common debilitating traumas suffered by elderly women, evidence of a similar effect at the proximal femur in anorectics is limited. Further investigation of bone mineral measurements at the two clinically significant sites, the lumbar spine and the proximal femur, is warranted.

Research concerning the skeletal effects of anorexia nervosa is imperative due to the following reasons: (i) the frequency of anorexia nervosa is increasing (Lewis et al, 1986; National Eating Disorder Information Centre, 1988; Yates, 1989), (ii) the onset is manifested at a younger age and consequently may lead to a prolonged duration (Fosson et al, 1987; Maloney, McGuire, Daniels & Specker, 1989) and (iii) the occurrence is more generalized in the population than reported in the past (Kreipe & Forbes, 1990; National Eating Disorder Information Centre, 1988; Yates, 1989). These trends may have major repercussions for bone health and could have a profound impact on the bleak personal and public health projections for osteoporosis. It is likely that low bone density in anorectic females will result in the earlier onset of osteoporotic fractures because the onset and course of anorexia nervosa occurs during a time when bone mass should be increasing. It is therefore important to determine the severity of bone loss in young women with anorexia nervosa.

1.7 Summary

Skeletal health has become an important issue for females of all ages. However, few investigations have researched the specific concerns of bone mass in premenopausal females. Recent investigations have reported reduced bone mineral density in amenorrheic athletes and females with anorexia nervosa. Traditionally, osteoporosis has been associated with the postmenopausal and the elderly woman. Consequently, there exists a large body of research on bone mineral density in postmenopausal women. Although bone mass in postmenopausal women has been widely researched, several main topics remain controversial. Consensus has not been reached concerning the parameters of peak bone mass and the model of bone loss. The clearest agreement in the literature on the postmenopausal female is that women are at a clear disadvantage in terms of bone loss and that estrogen deficiency exacerbates involutional bone loss. Because the loss of BMD is a gradual process with a transient acceleration at menopause, there may be considerable opportunity to influence this loss prior to menopause.

Both physical activity and dietary calcium have been identified as important considerations for women. Physical activity tends to decline with aging and calcium deficiency, due to decreased intake and malabsorption is common in the elderly. Body weight and/or obesity have been cited as having positive skeletal effects whereas slenderness is a negative risk factor for osteoporosis.

1.8 Statement of the Problem

The literature is equivocal about many issues concerning skeletal health in premenopausal women. Most information regarding premenopausal women and BMD is from large cross-sectional studies which have included a small cohort of premenopausal subjects. Measurements were made on outdated BMD instruments. Only recently have data from clinically important axial and appendicular sites been collected together with very precise and accurate instruments. There is no model of bone loss which has been derived from data collected in this population. Information regarding the femoral sites during the time from adolescence to menopause is also lacking. Consequently, peak bone mass in young premenopausal women and involutional bone loss have not been well defined. It is important to further investigate: (i) the consolidation or transition phase, (ii) the age at PBM, (iii) the onset, pattern and rate of bone loss.

A postulated model of bone gain and loss in premenopausal women would facilitate the clarification of the factors which influence these processes. Two of the main modulators of bone mass are physical activity and estrogen. The manner by which these two factors cause changes in bone mass are not well understood. Similarly, their relationship to body weight has not been explained satisfactorily. These mechanisms require further investigation.

Mechanical loading of bones may increase formation whereas hypoestrogenism has been associated with increased resorption which is not matched by formation. Body weight/composition has been noted to be highly associated with both physical activity and estrogen status. This

association requires further investigation as there are several possible mechanisms for body weight to influence BMD. The bone-protective role of obesity in postmenopausal women may be a function of augmented mechanical loading and/or increased serum estrone. Likewise, in premenopausal women the body weight factor may work through mechanical stimuli and/or the endocrine pathway. It is unclear how reduced body fatness and increased muscularity affect BMD. However, athletic amenorrhea and anorexia nervosa have been identifed as two states of hypothalamic hypogonadism wherein there are marked decreases in bone mineral density.

The positive effect of physical activity on bone remodelling has not been examined in athletic premenopausal women. Though physical activity has been related to high bone mass, high levels of physical activity for some females seriously affect reproductive function and skeletal health. In athletes, amenorrhea related to intense training has been associated with reduced spinal BMD and increased fracture incidence. In athletic amenorrhea, increased resorption due to estrogen deficiency may be dominant over the activity-enhanced drive for formation at the bone remodelling unit. The effects of less severe menstrual dysfunction on lumbar spine and femoral BMD are not clear.

Dietary calcium and its contribution to bone mass is an ongoing topic of debate. In postmenopausal women, calcium plays a secondary role to estrogen replacement therapy. Although dietary calcium is considered vital to the growth and development of bones, few studies have investigated the association of habitual calcium consumption with BMD in premenopausal women. It is also important to address the interactive effects of estrogen, physical activity and calcium.

Anorectics, amenorrheics, athletic and non-athletic premenopausal women provide a wide spectrum of the effects of body weight, physical activity and menstrual status on bone mineral density. The amenorrheic athlete may exhibit some eating disorders symptoms and excessive activity may be observed in anorectics. Both the anorectic and the amenorrheic athlete are hypogonadal and have low body weight and a low percentage of

body fat. There are no studies which have compared the BMD in anorectics to that of amenorrheic athletes and normal controls.

The purpose of this study was to investigate bone mineral density in young premenopausal women in order to clarify the roles of physical activity, body fatness, menstrual history, selected endocrine factors and dietary calcium. The bone mineral density (BMD) of the lumbar spine (L2-L4) and three regions of the hip: femoral neck (FN), Ward's Triangle (WT) and trochanter (FT) were measured by dual photon absorptiometry in premenopausal women aged 19-48 years. This cross-sectional study examined bone mineral density and the pattern and rate of bone loss over the age range. The BMD values of athletes and non-athletes were compared to clarify the role of physical activity on skeletal mass. Two states, athletic amenorrhea and anorexia nervosa, were also evaluated with respect to bone mineral density to determine the extent of bone loss and thereby comment on the risk of osteoporotic fracture.

1.9 Delimitations and Limitations

1.9.0 Sample Size Estimation

An estimation of sample size was completed to determine the statistical power of this investigation's analyses. Although regression analyses, t-tests and analyses of variance statistics were employed, the following method was used for the cross-sectional bone mineral density data. The sample size was estimated according to the formula for studies involving two sample means ($n=2$ {PI x within group S.D./ difference between means}^{2} (Hassard, 1987). The published norms for 248, 30-39 year-old, women in the United States were consulted; 1.26 (L2-L4) and 0.99 (FN) g/cm^2 (Mazess et al, 1987a). An estimate of the difference between the means was based on a recent cross-sectional study in which the annual rate of bone loss was 1% per year from the mid 30's at the lumbar spine bone and 0.4% from the late 20's at the femoral neck (Rodin et al, 1990). By menopause, women were reported to have lost comparable amounts of trabecular (10%) and cortical (9%) bone. The power index (PI) of 3.60 was selected which included an α of 0.05 and a β of 0.05. The within group standard deviation was estimated to be 13% (L2-L4), 12% (FN) (Mazess et al, 1990a). A conservative estimate of the sample size was calculated to be 28 in each of the 30-39 and 40-49 year old groups for the lumbar spine investigation and 59 in each of the 20-29 and 40-49 year age groups for the femoral neck study.

To estimate the sample size for detecting a true difference between means in BMD between athletes and non-athletes, the norms and within group standard deviations cited previously were used (Mazess et al, 1990a; Mazess et al, 1987a). To estimate the possible difference between these two populations, previously reported differences were reviewed. A value 11% higher was reported for the lumbar BMC of female tennis players (Jacobsen, Beaver, Grubb, Taft & Talmage, 1984). There have been no data reporting the differences at the femoral sites between young adult female athletes and non-athletes. It has been stated that the positive effects of exercise, more strongly supported by cross-sectional than prospective studies, might be more evident in premenopausal women (Bailey & McCulloch, 1990). As a conservative

estimate, an 11% (L2-L4) and 8% (FN) difference between the means of the two groups was selected. Calculations resulted in these estimated sample sizes for each group to detect true differences between the means: L2-L4; 23 and proximal femur; 60. An adjustment of the power index is possible by using a one-tailed test. A recalculation resulted in these sample size estimates: L2-L4; 19 and proximal femur; 50.

Similar calculations were completed for the anorectic study in which the difference between means was estimated to be 20% (L2-L4) and 23%(FN). These figures were based on literature reports of BMD reductions of 14-24 % at L2-L4 and 23% at the proximal femur (Mazess et al, 1990b; Savvas et al, 1989; Treasure et al, 1986). The estimated sample size for each group was 6 for both the lumbar and femoral sites.

1.9.1 Delimitations

Subjects were delimited to current non-smoking premenopausal women aged 19-48 years. Pregnant and nursing women were excluded from the study. The use of oral contraceptives was documented. All subjects resided in Winnipeg or its vicinity. The sample groups were delimited by the athlete and non-athlete categorization assessed from survey information. The delimitations for the athlete group included: (i) national high performance stream athletes (ii) university athletes (iii) elite provincial athletes and (iv) master's athletes and (v) non-competitive athletes and former elite athletes. The non-athlete group was delimited to those who were excluded from the athlete category and who recorded low to moderate past and current activity levels. Current activity levels of not more than four hours per week of regular planned activity met this low to moderate category.

The bone mineral density measurements were delimited to dual photon absorptiometry of the lumbar spine and the proximal femur. The anthropometric measurements were delimited to height, weight, girths and skinfolds. Skinfolds measured were: triceps, subscapular, iliac crest, supraspinale, abdomen, anterior mid-thigh and medial calf. Girths

measured with a metal tape were: head, neck, mid-brachium, forearm, wrist, chest, waist, hip, gluteal, upper thigh, mid-thigh and calf.

The blood sample was delimited to one mid-follicular early morning fasting sample. The endocrine assays were delimited to: estradiol, estrone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, cortisol and osteocalcin. The calcium panel assay was delimited to: total calcium, ionized calcium, phosphate, albumin, creatinine, and parathyroid hormone (PTH).

The subjects were regrouped according to reported menstrual status. The groups were delimited by these criteria: amenorrhea, not more than 3 menses per year and not more than 1 period during the 6 months preceding the study; oligomenorrhea, 3-10 menses per year and only 1 period within the last 3 months; and eumenorrhea, 10.5-13.5 menses per year and menstrual cycles of 27-35 day intervals of which 3-7 days were menses.

The anorectic subjects, were patients of the Eating Disorders Clinic at the Health Sciences Center, Winnipeg, Manitoba. All were Caucasian females who met the third edition Diagnostic and Statistical Manual criteria for Anorexia Nervosa (American Psychiatric Association, 1987). We excluded patients with less than one year duration of anorexia. Restrictive and bulimic anorectics were included in this study.

1.9.2 Limitations

This study has several limitations. The major limitation is that this is a cross-sectional investigation. The subjects were recruited on the basis of two distinct levels of physical activity: prolonged, intense and moderate activity. The main criticism of this type of cross-sectional research design is self-selection bias. The longitudinal component contains a small number of subjects with a moderate amount of time between measurements. The amenorrheic athlete and the anorectic patient were included in the study design to permit comparative analyses between these groups and normal athletic and nonathletic subjects. The large individual variability of bone mineral results and assay results has also been a limiting factor. The measurement of bone mineral density was central to this investigation and a pilot study was conducted to determine short term and long term precision of the absorptiometer.

The surveys and questionnaires were retrospective and vulnerable to the problems with such methods. Subjects were required to recall and report past and current particulars regarding menstrual history, physical activity levels and calcium intake. Where possible, discrepancies were checked. The categorization of subjects on the basis of this information is another limiting factor. A further limiting factor is that the scope of this study initially focussed on the relationships of menstrual status and physical activity on bone mineral density. Body weight and body composition were investigated as necessary intermediary components of these relationships. The contribution of dietary calcium was also assessed. Dietary habits such as restriction of calories, excess fibre, excess caffeine, high protein and vegetarianism were not analysed in this thesis.

Other lifestyle factors which have been identified as risk factors for osteoporosis have not been investigated. Those subjects who reported being former smokers were determined to be light smokers who had not smoked regularly in the recent past. These former smoking habits and alcohol consumption were not addressed. The negative effects of smoking, alcohol and dietary extremes on the skeleton may be partly due to direct effects at the bone unit but are more likely mediated through changes in body weight/ composition and/or estrogen status.

1.10 Definition of terms

1.10.0 Bone mineral content (BMC)

Bone mineral content (BMC) is defined as the total amount of bone mineral measured. BMC, expressed as g/cm is also known as linear density because 1 cm long cadaver radii provide the reference value.

1.10.1 Bone mineral density (BMD)

Bone mineral density (BMD) is defined as the density of bone mineral measured in a projected area or volume of bone. Areal BMD expressed as g/cm^2, is used when the bone is measured by a two-dimensional scanning path. Volume BMD expressed as g/cm^3, is used when the bone is measured by a three dimensional scan.

1.10.2 Lumbar spine (L2-L4)

The lumbar spine site includes the second, third and fourth lumbar vertebrae. BMD is the average of these regions. The proportions of bone in this area are considered to be approximately 35% cortical and 65% trabecular (Wasnich, Ross, Vogel & Davis, 1989).

1.10.3 Femoral neck (FN)

The femoral neck is the narrow region of the proximal femur which joins the proximal femoral shaft to the head of the femur. The proportions of bone in this area are considered to be approximately 75% cortical and 25% trabecular (Wasnich et al, 1989).

1.10.4 Ward's triangle (WT)

Ward's triangle is an area of bone on the inferior distal neck where the arrangement of trabeculae forms a triangle, evident upon radiography. The proportions of bone in this area are considered to be approximately 40% cortical and 60% trabecular (Wasnich et al, 1989).

1.10.5 **Trochanter (FT)**

The trochanteric region is the area between the greater and lesser trochanter of the proximal femur. The proportions of bone in this area are considered to be approximately 50% cortical and 50% trabecular (Wasnich et al, 1989).

1.10.6 **Amenorrhea**

Amenorrhea is defined as not more than 3 menses per year and not more than 1 period during the last 6 months preceding entry to the study.

1.10.7 **Oligomenorrhea**

Oligomenorrhea is defined as 3-10 menses per year and only 1 period within the last 3 months of entry to the study.

1.10.8 **Eumenorrhea**

Eumenorrhea is defined as 10.5-13.5 menses per year with menstrual cycle characteristics of 27-35 day intervals, of which 3-7 days is menses.

1.10.9 **Menarche**

Menarche is defined as the initiation of menses related to the pubertal process. Menarche occurs at approximately 12.5 years with regular, ovulatory menstrual cycles being established by 14 years.

1.10.10 **Menopause**

Menopause is defined as the complete cessation of menses related to aging and the accompanying ovarian failure. Menopause occurs at approximately 50 years and is clinically diagnosed by lack of menses for one year, high circulating levels of gonadotropins, particularly FSH, and low or undetectable estradiol levels.

1.10.11 **Primary amenorrhea**

Primary amenorrhea is defined as the absence of menarche by 14 years or later.

1.10.12 **Secondary amenorrhea**

Secondary amenorrhea is amenorrhea as defined above which is not primary nor associated with menopause.

2. REVIEW OF LITERATURE

2.0 Introduction

The maintenance of ideal skeletal mass depends upon the establishment of an optimum amount of bone during the growth and development phases, the maximization of the consolidation phase and the minimization of subsequent bone loss (Parfitt, 1987). This chapter presents a review of the concepts of peak bone mass and bone loss followed by a brief overview of genetics, physical activity, hormonal milieu and dietary calcium. Where possible, current publications are favored and extensively reviewed due to the considerable developments in this field in the last decade. Several specific factors; physical activity, estrogen deficiency and dietary calcium have been identified as major, inter-related modulators of bone mass, particularly in young adult women. The review concludes with a summary of the literature of activity-induced menstrual dysfunction and anorexia nervosa, two conditions which have profound effects on premenopausal bone mass.

2.1 Peak Bone Mass

Peak bone mass, a simple concept introduced over three decades ago continues to generate controversy. Early literature regarding PBM reported that after linear growth ceased in late adolescence, there followed a phase during which skeletal mass was consolidated (Aloia, 1989). During this poorly documented period of some ten to fifteen years, skeletal mass could increase 10-15% (Avioli, 1984). Available information regarding PBM is based mainly on cross-sectional data which relied on inadequate, by today's standards, instrumentation. Recent investigations have challenged several aspects concerning PBM. The age at PBM and PBM sexual dimorphism are controversial. Moreover the consolidation phase and the homeostatic phase prior to involutional bone loss remain largely uninvestigated.

Hypothetically, optimal bone mass is the maximum amount of bone mass that an individual is capable of attaining. Ideally, peak bone mass should equal optimal bone mass. After the cessation of linear growth, consolidation is said to occur. During this stage, the skeleton is fully

mineralized and PBM, the highest bone mass value that an individual attains in a lifetime is achieved. The attainment of PBM may be followed by a plateau after which age-related bone loss begins (Mazess et al, 1987a). Heaney (1987) explained that the adolescent skeleton experienced an accelerated growth phase and a decade of consolidation allowed mineralization and reorganization of this fragile skeletal infrastructure .

Using radiogrammetry, Nordin derived an index of cortical thickness to bone width expressed as a percentage (Nordin, 1966). Countries selected on the basis of extreme intakes of protein and calcium were visited and hand and spine radiographs were randomly chosen from centers with X-ray departments. The spine films were graded for evidence of osteoporosis and the Nordin index was calculated from hand X-rays. From these data, Nordin concluded that osteoporosis was relatively rare in Africa and common in Japan and India. In women, there was an apparent reduction in the metacarpal index by age 40 years in all countries. A similar universal trend existed in males but was less pronounced than in females.

In another international retrospective investigation of osteoporosis, Garn (1967) employed radiogrammetry of the second metacarpal. Data were presented as cortical width, % cortical area and Nordin's ratio. Data were collected from over 13,000 subjects in seven countries. The conclusions from this cross-sectional research and a small subset of serial radiographs were that age loss is universal and that bone loss begins in both sexes at about 40 years of age, with a greater loss in women. Garn reported that the average annual rates of bone loss from 40-90 years were 0.03% for males and 0.08% for females.

These two large investigations of bone mass were important because they provided initial evidence of age-related bone loss. The radiographic measurement techniques lacked precision and the peripheral cortical bone measurements had limited power to detect changes at other skeletal sites. However, these studies agreed that involutional bone loss was universal. This loss began at approximately the fifth decade in women and men with the rate of bone loss higher in females than males. From these reports, it appears that PBM was attained at approximately 40 years in both sexes.

Since these early studies, the pattern of aging bone loss has been investigated largely through cross-sectional studies. Currently there is no agreement on the age at PBM which has been reported to be from 25 to 40 years (Garn et al, 1967; Krolner et al, 1982; Nordin, 1966; Parfitt, 1979; Riggs et al, 1981; Rodin et al, 1990; Ross, Wasnich & Vogel, 1987). This is a large age range and may reflect the limitations of cross-sectional data, the accuracy and precision of various bone instruments and the large inter-individual variability of bone mass. Also, Garn and Nordin's studies presented the pattern of bone mass changes as simply an early gain and later loss (Garn, 1970). This paradigm may oversimplify the pattern of bone gain and loss, thus obscuring the age of PBM. Consolidation and maintenance have only recently been considered as separate phases and may account for some of the variability regarding age at PBM.

There was only one publication of longitudinal data on PBM with both appendicular and axial BMD. Davies' group (1990c) investigated third decade bone gain by semi-annual DPA and SPA measurements of the spine and forearm in 185 healthy women between the ages of 18.5-25 years. At the end of four years, their results showed bone gain at the lumbar spine and forearm. The authors concluded that bone mass in females could be increased at both axial and appendicular sites during the third decade.

However, other evidence suggests that PBM may be achieved earlier with bone loss beginning during the third decade. A cross-sectional study by Fujii et al (Fujii et al, 1989) measured trabecular BMD (QCT) in normal and osteoporotic Japanese subjects. The authors stated that healthy women lost trabecular bone beginning at age 20 at a rate of 1.1% per year until 80 years of age. Similarly, Gilsanz (1988a) confirmed an early age at PBM. This conclusion was based on data from two age groups of females, adults (25-35y) and adolescents (14-19y) who had computed tomography (QCT) measurements of spinal BMD. Because the adolescents had a higher mean trabecular BMD than the adults, Gilsanz reported that peak vertebral bone mass in females was reached at the end of the second decade, at the time longitudinal growth ceases and the epiphyses close. The QCT assessment however does not reliably predict similar results for the total skeleton or other sites.

The most recent cross-sectional investigation of peak bone mass measured spinal and femoral neck BMD (DPA) in 225 Caucasian women, aged 18-52 (Rodin et al, 1990). This study concluded that spinal BMD increased from age 20 to peak in the mid-30's and the femoral neck BMD simply maintained the peak bone mass accrued at late adolescence with bone loss commencing in the late 20's (Rodin et al, 1990).

Stevenson and collaborators (1989) also challenged reports of fourth decade PBM. They stated that shortly after linear growth ceases, PBM is achieved. Thus, the determinants of PBM may be more important risk factors for osteoporosis than previously considered. However, valid conclusions regarding PBM require precise and accurate data from studies of young females which also investigate and clarify the impact of genetic and environmental factors.

2.2 Involutional Bone Loss

It is generally accepted that the total mass of the skeleton slowly declines with age. However, controversies exist regarding the onset and rate of bone loss at selected sites, the relative involvement of trabecular and cortical bone and sex-specific skeletal differences. Conflicting data have been collected primarily in cross-sectional studies utilizing a variety of research instruments. Consequently, the parameters of bone loss and the models proposed to characterize bone loss in females are controversial.

Models of involutional bone loss in females support either a generalized or differential pattern of bone loss. Variations of each of these patterns have been proposed to clarify the characteristics of bone loss with age in females and the effect of menopause. Discrepancies in models are evident due to research design, instrumentation and choice of site measured. Different models have evolved as instrumentation has permitted the more precise measurement of more informative axial and appendicular sites.

These models of bone loss have been identified in the literature. The general universal loss of bone with age was reported by several investigators (Garn et al, 1967; Riggs et al, 1982a; Riggs et al, 1982b) (Riggs et al, 1986a). This generalized loss of trabecular and cortical bone was

accelerated at menopause (Cann, Genant, Kolb & Ettinger, 1985a; Mazess, 1982). A second model supported the hypothesis that there was a differential loss of bone with preferential diminution of trabecular bone (Nordin, 1983; Riggs et al, 1981). Others confirmed that a differential bone loss pattern was accelerated at menopause (Fujii et al, 1989; Krolner et al, 1982; Nilas & Christiansen, 1987; Riggs et al, 1986a; Riggs et al, 1981; Riggs et al, 1986b).

However, other data supported a model of reduced total body bone mineral density with greater lifetime losses at the femur (Riggs et al, 1981; Riggs et al, 1982b) or early diminution of cortical bone (Schaadt & Bohr, 1988). Cortical bone loss was reported to be transiently augmented by menopause (Mazess, 1982; Riggs et al, 1981). Another model stated that neither trabecular nor cortical bone loss occurred until menopause (Aloia, Vaswani, Ellis, Yuen & Cohn, 1985; Nilas et al, 1987; Sambrook, Eisman, Furler & Pocock, 1987). Madsen's (Madsen, 1977) cross-sectional data on lumbar vertebrae showed unchanged bone mineral content until 50-60 years. Although the failure to detect changes at this site was possibly due to instrument imprecision, this early study suggested that trabecular vertebral bone loss was not significantly related to aging in premenopausal women.

The heterogeneity of bone loss was recognized by one pair of investigators who introduced a classification system. Riggs and Melton (Riggs et al, 1986a) concluded that two types of osteoporosis appeared to best fit the observed patterns of bone loss: Type I, initiated by diminished endogenous estrogen and Type II, involutional bone loss associated with aging. For both women and men, trabecular bone loss preceded cortical bone loss by at least a decade and in women, trabecular bone loss at menopause exceeded cortical bone loss. Following menopause, losses were as high as 5-10% annually for five to eight years (Riggs et al, 1986b). Postmenopausal (Type I) osteoporosis, is characterized by an accelerated rate of bone loss, particularly trabecular bone, decreased serum PTH, and decreased calcium absorption (Mundy, 1987).

Several investigators do not support the Type I and II classification. Gallagher (Gallagher, 1990) has suggested that the addition of a Type Ia for a combined pathophysiology and Type III for secondary osteoporosis would

complete the disease categories. Heaney (Heaney, 1989a) addressed several shortcomings and proposed that the osteoporotic effect is site-specific rather than discriminating between cortical and trabecular bone. Compston (Compston, 1990) suggested that both types affect menopausal women and fracture type is related not to Type I or II pathogenesis but to PBM. Gotfredsen (Gotfredsen, Nilas, Podenphant, Hadberg & Christiansen, 1989) reported that two distinct osteoporosis syndromes did not exist as bone loss is due to a general mechanism which elicits variable site-specific responses due to biological differences. This conclusion was based on a cross-sectional study which evaluated total body and six regional BMC and BMD (DPA) sites in healthy and osteoporotic females. In the group of normal subjects, the data on 73 premenopausal and 55 postmenopausal women showed that bone loss was generalized and amounted to a 20% reduction from age 20-80y. At all sites, bone loss was minimal premenopausally and accelerated after the menopause. Bone loss was also generalized in the osteoporotic patients but fracture patients illustrated regional bone loss bias.

Preferential bone loss at different skeletal sites was supported by BMD data collected from women aged 50-79 grouped as controls, osteoporotics and fracture patients (Mautalen, Vega, Ghiringhelli & Fromm, 1990). Those with spinal osteoporosis had similar bone loss at both the spine and hip whereas those with femoral osteoporosis showed a preferential loss at the femoral neck compared to the spine and the trochanter. In a recent paper, objections to clinical heterogeneity of osteoporosis are rebutted by Riggs and Melton (Riggs et al, 1990). Controversy surrounding the two distinct osteoporosis syndromes has not been settled. However, most investigators have recognized that differences between trabecular and cortical compartments are crucial factors.

One of the earliest studies to report that spinal density was not significantly reduced premenopausally had cross-sectional and longitudinal components and included postmenopausal subjects (Krolner et al, 1982). In the cross-sectional component, no significant relationship between age and premenopausal bone loss was observed. Longitudinal data on 27 premenopausal normal women consisted of two lumbar spine measurements, with a mean interval of 10 months. These data confirmed

that although there appeared to be bone gain until 34 years then a minimal decline, the lumbar spine loss prior to menopause was not significant. It is important to point out that the sample size was relatively small considering the brief observation period.

A cross-sectional study of BMC at the lumbar spine (DPA) and radius (SPA) was conducted in 159 normal women premenopausal women (Aloia et al, 1985). They concluded that there was no significant premenopausal decline of BMC.

Mazess (1987a) reported on a seven center cross-sectional study which measured spinal and hip BMD by DPA in 20-70 year old women. The author concluded that there was not an age related decline in spinal BMD in 20-39 year old women but at the femoral neck site, there was a low but significant relationship with a loss rate of 0.0047 gm/cm^2 per year in the 20-39 year old women. Prior to 50 years there was a 10% decrease per decade in spinal density in women and a similar 10% BMD decrement at all three femoral neck sites prior to age 40. A 25% total decrease in FN BMD was reported in the 40-70 year olds whereas the total reduction in this age group was 20% at the lumbar spine.

A cross-sectional study of age and bone mass in 57 premenopausal healthy women, aged 18-44y, excluded subjects who had a history of menstrual dysfunction, anorexia nervosa, excessive physical activity or used oral contraceptives within the last six months (Rosenthal et al, 1989). Several variables were explored in relationship to trabecular BMD (single energy and dual energy QCT). BMD was not significantly related to age overall or age by decade or age above and below 33y. There were no significant relationships between bone density and height, weight, parity, caloric intake or dietary calcium. The main conclusion was that there is minimal or no trabecular loss prior to menopause.

A cross-sectional study by Elliott's group investigated the BMD of the spine and hip by DPA in 462 females and 264 males aged 20-84 (Elliott et al, 1990). This paper reported that there was a significant age-related reduction of spinal BMD with aging and this relationship was non-linear. A re-analysis of the data by age subgroups showed that spinal BMD was

preserved until age 40. There was a subsequent rapid reduction between 40-60 years and thereafter a levelling out of bone loss.

Schaadt and Bohr (1988) studied the differential pattern of bone loss at the lumbar spine, femoral neck and femoral shaft in 113 healthy 20-89 year old women. The authors stated that the loss of bone at the L2-L4 site occurred mainly at menopause and at the FN there was a significant linear decline from young adulthood to old age. Bone loss at the midshaft femoral site was not evident until the seventh decade, at which point it was significant. Another investigation determined no correlation between age and BMD, measured by Hologic QDR, in the 57 normal controls included in a larger study (Davies, Hall & Jacobs, 1990d). Lumbar spine BMD was maintained across the 16-40 year age range.

Rodin's group (1990) utilized running averages to determine premenopausal bone loss at the lumbar spine and femoral neck. This cross-sectional study concluded that spinal BMD increased from age 20 to attain a peak in the mid-30's after which the rate of bone loss was 1% per year. There was no peak in femoral neck BMD and an annual 0.4% rate of loss began in the late 20's. By menopause, women were reported to have lost comparable amounts of trabecular (10%) and cortical (9%) bone.

The relationship of age and bone loss was analysed as part of a larger study of the effects of family resemblance and dietary calcium on femoral and spinal BMD (Lutz & Tesar, 1990). At the lumbar spine and trochanter region, the correlation between age and BMD for 37 females, age 20-35y, was not significant. The correlations were significant at the femoral neck and Ward's triangle. The authors concluded that bone loss commences at the femoral neck and Ward's triangle in young adult women. Also, the BMD values for the 37 premenopausal and postmenopausal women in this study were compared. The postmenopausal group had a significantly lower BMD at the lumbar spine and Ward's triangle.

The effects of age and menopause on proximal femur BMD (DPA) were the focus of a cross-sectional study of 263 normal women aged 20-84y (Hedlund & Gallagher, 1989). The data on 119 premenopausal women were divided into five year age groups. Regression analyses of the data illustrated that there was a significant decline of BMD with age at the FN and WT but

not at the FT. This study concluded that femoral BMD commenced to decrease in the early twenties.

In summary, the literature review indicates that age-related bone loss remains a controversial topic. Current reports have variably concluded that vertebral (trabecular) BMD is reduced from age 30y (Krolner et al, 1982; Rodin et al, 1990), age 40y (Davies et al, 1990d; Elliott et al, 1990; Mazess et al, 1987a) or not until menopause (Rosenthal et al, 1989; Schaadt et al, 1988). There is more agreement regarding bone loss with aging at the proximal femur, probably because this site has only recently been investigated by DPA or DEXA. Involutional loss of BMD at the femoral neck is reported to begin at age 20y or in young adulthood (Hedlund et al, 1989; Lutz et al, 1990; Mazess et al, 1987a; Rodin et al, 1990).

In spite of considerable research, the pattern of bone loss, particularly in premenopausal females, remains unsettled. Part of the controversy has been due to data which combine premenopausal and postmenopausal cohorts without controlling for age differences at menopause (Rosenthal et al, 1989). Another reason for the confusion of models of bone loss is that subjects are not always well characterized as healthy normal subjects prior to group allocation.

A substantial part of the controversy may be due to instrumentation and choice of measurement site. Most models were based on cross-sectional investigations which employed imprecise measurements of BMD at peripheral sites which were not clinically informative. Due to the available methods, the cortical bone component at the forearm was frequently investigated and few studies employed concurrent assessments of BMD at the lumbar spine and proximal femur. The site of measurement is important in the determination of pattern of bone loss. Because mechanisms of involutional bone loss may vary from site to site and may be specific to the type of bone, bone mass at one site is not a reliable predictor of other sites (Bohr & Schaadt, 1985). Recent investigations have utilized biomedical instruments with improved safety and increased accuracy and precision. New measurement capabilities have permitted the measurement of clinically more important and more informative sites. It is now clear that

there are differences between the initiation and the rates of loss throughout the skeleton.

Research continues to present information which may elucidate the differences of trabecular and cortical bone and portray a more accurate model of female gain and loss of bone. A study of ovariectomized rats illustrated that the weight-bearing cortical bone was more responsive to changes, showing earlier and greater differences in bone loss whereas trabecular bone involvement was slower and less extreme (Wronski, Dann & Horner, 1989). This report contradicts the tenet that trabecular bone is more vulnerable to changes because of its large surface area and consequent increased metabolic activity. Trabecular bone has been characterized as more susceptible than cortical bone to diminution during estrogen deficiency. Trabecular bone loss with aging was reported to be eight times greater than cortical bone loss due to a higher turnover rate of trabecular bone (Boden, Labropoulos & Saunders, 1990). Changes in trabecular bone, related to aging, may be secondary to the estrogenic induction of local growth factors. It may also be that cortical bone is more responsive than trabecular bone to environmental factors such as physical activity (Kanders et al, 1988). Alternatively, the trabecular component at integrated sites which may be contributing to the net bone loss may be incorrectly interpreted as cortical bone loss. Although the specifics remain to be determined, bone loss is highly site specific (Mazess et al, 1990a; Schaadt et al, 1988).

2.3 Factors Influencing Bone Mass

The attainment of peak bone mass is influenced by mechanical factors, physical activity, hormonal milieu and nutrition. These same factors act upon the skeleton throughout life but growing and developing bones are highly responsive to excesses or deficiencies. Thus, peak bone mass is dependent upon the interplay of environmental factors on a skeletal template. After peak bone mass is attained, the aging process of the skeleton, which in itself may be partly determined by genetics, is influenced by environmental factors.

2.3.0 Genetics

The genetic component of bone mass is poorly understood and there is limited research on this topic. It has been established that caucasians and orientals have lower bone mineral content than blacks (Mundy, 1987). Further conclusions regarding bone mass can be drawn from comparative studies and reviews which have generally concluded that genetics, ethnicity and sex are determinants of PBM and subsequent bone loss (Lutz et al, 1990; Pocock et al, 1987). Investigations have identified various characteristics of the genetic component such that the aging, white, North American female with a positive family history of osteoporosis is generally at high risk for osteoporosis.

Recent research on female bone mass and the contribution of genetics is sparse. It has been reported that PBM occurs earlier in females and that females have a smaller skeleton than males (Peck, 1984). Investigators have recently published evidence which suggests that females and males have similar PBM (Elliott et al, 1990) (Eisman et al, 1989).

Glastre and colleagues (1990) measured lumbar BMD (DEXA) in 135 healthy Caucasian 1-15 year old children. The BMD for sexes was similar at all ages except at 12 years when the BMD was significantly higher in girls. This was reportedly due to puberty being achieved earlier in females. In both sexes, puberty was accompanied by an acceleration in skeletal accumulation. Data from this study confirmed that BMD was highly correlated to height, weight, body surface and bone age. Calcium intake corrected for age was not correlated to BMD. By the age of 15, approximately 86% of young adult peak bone mass (DEXA) had been attained.

In a study of 101 children, aged 2-19 years, Gilsanz (1988b) used spinal BMD (QCT) and a derived cortical index to determine that bone density increased markedly during puberty and that at skeletal maturity, there was no significant difference in BMD between sexes. An analysis, controlling for puberty found that trabecular BMD was positively but weakly correlated with these variables; height, weight, surface area and body mass index.

A recent study investigated the spinal and femoral bone mineral densities in mother-daughter pairs (Lutz et al, 1990). There existed significant correlations between the pairs for femoral site BMD's and all the lumbar vertebrae except L2 where the p-value was 0.054. The authors stated that these data confirmed familial resemblance not solely geneticism.

Matkovic's group (1990) investigated the inheritance of bone mass in young adolescent females as part of a larger two year calcium balance and supplementation prospective study. The subjects were 24 parent-daughter pairs which included mothers, fathers and 14 year-old adolescent daughters. Bone measurements were assessed by radiogrammetry, SPA of the distal radius and DPA of the lumbar spine. Significant high correlations existed between the bone size, bone mass and bone density measurements of the parent-daughter pairs. By the age of 16 years, the daughters had attained 90-97% of the premenopausal mothers' bone mass. The authors suggested that PBM occurs early and bone mass is largely inherited but enviromental factors such as calcium also are important.

Bone mineral measurements by SPA (mid-radius) and DPA (L2-L4) were taken in a group of 183 healthy Caucasians males and females aged 6-18y (Rubin, Schirduan, Gendreau & Dalsky, 1989). Each of the developmental factors of age, height, weight, stage of puberty and grip strength were highly correlated with BMD and when considered together by multiple regression explained up to 83% of the BMD variation. The female high calcium group had a significantly higher mean spinal BMD than the low calcium group. There was no significant effect of physical activity level on BMD.

An investigation of college aged females (18-22y) and their mothers (36-50y) measured mid- and distal radial BMD (SPA) and several anthropometric and nutrition variables (Tylavsky, Bortz, Hancock & Anderson, 1989). These authors stated that genetics are important on the basis of significant relationships between familial paired BMCs and BMDs after controlling for the effect of body mass index. In the mothers, body mass index was significantly correlated to BMC but the effects of pregnancy, lactation and oral contraceptive use were not significant. Both body mass index and dietary calcium were found to be significant contributors to the daughters' BMD. In the daughters, the lower BMC but comparable width as their mothers was

cited as proof that during the subsequent decades about 5-10% of peak bone mass would be accrued. Heredity was stated to be the dominant component in the accrual of peak bone mass but two facets of nutrition, BMI and dietary calcium were important.

There have been reports of high correlations between mother and daughter bone measurements (Lutz et al, 1990; Seeman et al, 1989) (Tylavsky et al, 1989) and mother and father-daughter pairs (Matkovic et al, 1990). Daughters of osteoporotic women had lower mean bone mineral density than those women with non-osteoporotic mothers (Seeman et al, 1989). Twin studies have also confirmed the important genetic contribution to bone mass (Dequeker, Nijs, Verstraeten, Geusens & Gevers, 1987; Pocock et al, 1987; Smith, Nance, Kang, Christian & Johnston, 1973). However, in all of these studies, family lifestyle factors may have contributed to the postulated genetic influence on the skeleton.

What remains to be determined is what proportion of the variation in BMD is genetic and the impact of environmental factors (Slemenda et al, 1990a). Moreover, the relative contributions of nature and nurture are likely dissimilar for attainment of PBM and protection against involutional bone loss. It has been suggested that genetic factors may be more important in developmental stages and thus have a major effect on PBM whereas environmental influences are more critical for the maintenance of bone mass and attenuation of bone loss (Pollitzer et al, 1989).

2.3.1 Mechanical Loading and Physical Activity

A century ago, Wolff (1892) stated that mechanical loading markedly influenced bone mass. Wolff's law has been proven in cases of extreme unloading. Disuse (Nishiyama et al, 1986), bed rest (Donaldson et al, 1970), immobilization (Green, 1985), and weightlessness (Mack et al, 1967) cause immediate and severe bone loss, typically about 1% per week for trabecular bone. Much less is known about the effects of less severe changes in loading. Investigations in human and animal models which examined if bone densities differed between dominant and non-dominant limbs, support the tenet that weight-bearing or mechanical loading fosters bone accretion (Aloia, 1981; Montoye, 1987).

A seemingly obvious illustration of Wolff's law is that a greater body mass will be associated with an increased skeletal mass (Ott, 1990). However, the effects of body habitus or body weight are difficult to separate from endocrine effects on bone which are discussed in a subsequent section. Higher body weight as an indirect index of fatness and peripheral aromatization of estrogen have been considered protective of postmenopausal bone loss. However, body weight may also be important to the maintenance of bone mass via mechanical loading of bone. Low body weight for height is related to less dense bones than normal weight, and obesity in women has been associated with higher bone density (Parfitt, 1987). One group suggested that the protective effect of weight on bone mass is a factor when normal weight ranges are exceeded as in obesity (Rosenthal et al, 1989).

Simple anthropometric measurements such as height and weight and other indicators of body size such as body surface area and body mass index have been reported as determinants of BMD. In one study 119 premenopausal women were divided into five year age groups (Hedlund et al, 1989). The proximal femur BMD (DPA) correlated with age, height and weight and a multiple regression of these variables explained approximately 30% of the variation in BMD. In the study of aerobic and muscle building activities by Davee's group, over all subjects, BMI was the best predictor of L2-4 BMD ($r=0.42$, $p<0.03$) (Davee, Rosen & Adler, 1990). Higher BMI was

related to greater BMD at both radial sites in mother and daughter groups (Tylavsky et al, 1989).

The skeleton responds to the habitual load of body mass but only a dynamic load which induces normal tension and strain parameters will maintain bone density. Although the manner in which mechanical loading is translated to elicit bone changes is not known, there are several hypotheses. Some of the putative mechanisms are via the endocrine and immune systems, local direct effects on vasculature and blood flow, peripheral innervation and contraction of muscles (Silbermann et al, 1990).

Research has not validated the role of physical activity in the prevention of osteoporosis (Block et al, 1989). Hypothetically, physical activity, through optimization and maintenance of bone mass and the attenuation of bone loss, has tremendous prophylactic potential. Most cross-sectional studies (Aloia et al, 1978; Block, Genant & Black, 1986; Dalen & Olsson, 1974; Huddleston, Rockwell, Kulund & Harrison, 1980; Jacobsen et al, 1984; Lane et al, 1986) have reported greater bone mass in athletes than in sedentary controls. Other studies have reported a positive relationship between activity or fitness levels and bone mass (Bailey et al, 1986; Oyster, Morton & Linell, 1984; Pocock, Eisman, Yeates, Sambrook & Eberl, 1986; Talmage, Stinnett & Landwehr, 1986). There are cross-sectional studies which have shown no relationship between physical activity levels and bone mass (Smith, Khairi, Norton & Johnston, 1976; Sowers, Wallace & Lemke, 1985) or no difference between athletic and less active controls (Aloia et al, 1978; Brewer, Meyer, Keele, Upton & Hagan, 1983). All of these cross-sectional studies, reviewed elsewhere (Bailey et al, 1990; Block et al, 1989) had few features in common as subject categories, measurement sites and instrument selection varied considerably.

A number of longitudinal studies have examined the effects of exercise intervention on bone mass (Aloia et al, 1978; Chow, Harrison, Brown & Hajek, 1986; Dalsky et al, 1988; Krolner, Toft & Neilsen, 1983; Margulies et al, 1986; Sandler, Cauley, Hom, Sashin & Kriska, 1987; Simkin, Ayalon & Leichter, 1986; Smith, Reddan & Smith, 1981; Smith, Smith, Ensign & Shea, 1984; White, Martin, Yeater, Butcher & Radin, 1984; Williams, Wagner, Wasnich & Heilbrun, 1984). These have been extensively

reviewed elsewhere (Block et al, 1989; Pollitzer et al, 1989; Schapira, 1988; Smith, Smith & Gilligan, 1990; Tipton & Vailas, 1990). Generally, these studies have produced conflicting results and do not confirm a strong role for physical activity in promoting bone mass. Most exercise intervention studies have been conducted with male subjects or postmenopausal and elderly female subjects. Similar research on premenopausal females has not been conducted. Only two longitudinal studies, published prior to 1989 included premenopausal subjects (King, Lukert & Robinson, 1987; Smith et al, 1984).

One report by Smith's group (1984) was inconclusive although BMD was considered to have been maintained through the aerobic exercise program. The measurement sites for this study, the radius, ulna and humerus were not likely to reflect the effects of this intervention as mechanical loading would have been directed to the torso and legs. In a later publication, Smith and co-workers (1989b) reported a significant decrease in the rate of bone loss in three arm sites in the training group of females aged 35-65 years (mean age 50 y) compared to controls. The training program consisted of arm loading exercises as well as an aerobic component. Alternative site selection such as the lumbar spine and proximal femur may have provided stronger and more discriminatory evidence on behalf of physical activity.

The second report of longitudinal data investigated wrist and lumbar BMC (DPA) in 16 female recreational runners aged 29-62y (King et al, 1987). After 30 months of running an average of 26 miles per week, the lumbar BMC increased an average of 6.5% in 14 subjects. The two subjects with decreased BMC did not significantly change running mileage. The wrist BMC did not change significantly.

There are numerous cross-sectional reports regarding physical activity and bone mass in young adult females. These investigations have used radiography (Emiola & O'Shea, 1978), SPA (Brewer et al, 1983) (Jacobsen et al, 1984) (Kanders et al, 1988; Talmage et al, 1986) (Halioua, 1986) (Stillman, Lohman, Slaughter & Massey, 1986) photodensitometry (Brewer et al, 1983), and computed tomography, (Bailey et al, 1986; Kirk et al, 1989). Most of these studies reported that active females had higher bone density

than less active controls (Bailey et al, 1986; Brewer et al, 1983; Emiola et al, 1978; Halioua, 1986; Jacobsen et al, 1984; Kanders et al, 1988; Talmage et al, 1986) (Stillman et al, 1986).

Several cross-sectional studies have investigated the relationship of physical activity to BMD measured by SPA and DPA, at several sites in young premenopausal women (Jacobsen et al, 1984; Kanders et al, 1988; Talmage et al, 1986). Only one study (Kanders et al, 1988) specifically examined the young adult age group (25-34y) while the age ranges of the others were from late adolescence to several decades past menopause. In all cases, except for the radial site in one study (Kanders et al, 1988), the bone mineral density of the more active females was higher than those less active. The BMD of the lumbar site (Jacobsen et al, 1984; Kanders et al, 1988; Talmage et al, 1986) and the femoral neck (Talmage et al, 1986) site were higher in those females who were physically active.

Kirk and colleagues (1989) measured vertebral BMD (QCT) and radial BMD (SPA) in a group of younger eumenorrheic premenopausal women, aged 25-35y, older postmenopausal women, aged 55-65y and age-matched sedentary controls. All subjects were currently runners, having run for at least two years with a minimum weekly distance of twenty miles. From their results, the authors concluded that long-distance running has a positive effect on the vertebral BMD of young women as a higher BMD was evident in young runners compared to their controls. Similar effects of running were not evident in the spinal BMD of the postmenopausal group or cortical BMD of either runner's group. These results were taken as proof that exercise did not compensate for the effects of estrogen deprivation at menopause. Also, the lack of response of cortical bone to running was suggested to mean that either there was no systemic mechanism or systemic influences are less powerful in cortical bone.

McCulloch and colleagues (1990) studied lifestyle factors and bone density of the os calcis (QCT) in 101 healthy women aged 20-35y. They found no relationship of bone density to height, weight or age. Physical activity during childhood was the main determinant of calcaneal bone density. Those subjects, who in their youth participated in organized sports or fitness programs, had a significantly higher adult calcaneal bone density. No

relationship existed between the current level of avocational physical activity and bone density. Adequate mechanical loading, particularly during childhood, was deemed important to healthy bones.

An earlier study investigated the same site, os calcis (QCT) at the beginning and end of a six-month interval during which time subjects recorded activity and menstrual histories (Bailey, Martin, Houston & Howie, 1987). The subjects were females aged 18-29y who composed the following activity groups; 24 athletes, 13 high level recreation (5h/wk), 9 low level recreation(1-3 h/wk) and 5 controls (<1h/wk). The athlete group had significantly higher calcaneal bone density than the control group. Within the athlete group, the hierarchy of bone densities from highest to lowest was basketball, mid-short distance, long distance, and volleyball.

Predicted maximal oxygen (VO_2max) uptake as an indicator of habitual activity was studied among other factors in relationship to bone density (Pocock et al, 1986). Bone measurements were taken at the lumbar spine and femoral neck (DPA) and the distal forearm(SPA) in 84 caucasian women, aged 20-75y. In the 38 premenopausal women, femoral neck BMD was correlated to VO_2max ($r=0.32$, $p<0.05$) and age ($r=-0.3$. $p<0.05$, 1 tailed). At the lumbar spine, there were significant high correlations between VO_2max ($r=0.54$, $p<0.001$) , weight ($r=0.39$, $p<0.001$), height ($r=0.30$, $p<0.01$) and age ($r=-0.57$, $p<0.001$). A multiple regression analysis determined that weight was the sole significant predictor of lumbar BMD ($r=0.45$, $p<0.01$). Forearm bone density was said to be unaffected because walking or running was the major activity of subjects.

According to Drinkwater's (1990) recent review of physical activity and BMD, the increases at the lumbar spine reported by various researchers were 5-12% (QCT) and 6-11% (DPA). Comparable information on the proximal femur is not available. The consensus of the literature reviewed herein is that active women have greater skeletal mass than sedentary age-matched women.

The contribution of mechanical loading to either the attainment of peak bone mass and/or the maintenance of a robust skeleton requires further investigation. Although research has progressed, it is not clear what sites and which type of bone respond best to various types of physical exertion (Frost, 1987) (Smith et al, 1989a; Whalen, Carter & Steele, 1988).

Although the mechanism by which changes in mechanical strain act on the bone remodelling unit has not been clarified, the type of mechanical loading which elicits an optimal response from bone is also being investigated by others. This study has investigated the relationship of physical activity to BMD at the lumbar spine and the proximal femur. The effect of physical activity on involutional bone loss was also examined.

Type of Exercise Studies conducted by Lanyon and Rubin (1983) and Marcus and Carter (1988) have added considerable information concerning the local effect of exercise and strain and cycle characteristics in animal and human models. It is recognized that the specificity of loading or local effects may be important but the systemic effects of exercise are also suspected as mediating factors. The success of an exercise program may be determined by the ability to measure the loading history (Whalen, Carter & Steele, 1988). Thus, the need to distinguish between athletic activities which are mainly compressive or aerobic has been recognized (Ott, 1990). Activities which feature high intensity and low repetitions may be prescriptive for healthy bones (Rikli & McManis, 1990).

Thirty college-aged females were grouped as sedentary controls, eumenorrheic athletes and oligomenorrheic athletes in a study of BMD and hormones (Buchanan, Myers, Lloyd, Leuenberger & Demers, 1988). In these young women, the general aerobic training had no significant effect on spinal BMD (QCT). The authors suggested that specific back-loading exercises would be required to significantly increase vertebral bone mass.

One prospective study examined the effects of a 9 month weight training program of axial loading exercises on lumbar spine and femoral neck BMD (DPX) (Rockwell et al, 1990). The non-randomized study included ten premenopausal subjects, mean age 36 years and a group of sedentary controls. All subjects received a supplement of 500 mg/d calcium. Strength increased by 57% in the trained group and femoral neck BMD did not change. The lumbar spine BMD decreased significantly by the end of 9 months training. This significant decrease was evident by 4.5 months.

A cross-sectional study of 40 cyclically menstruating athletes aged 17-38 y and 18 inactive controls examined the effects of different types and levels of

activity on bone mineral content (Heinrich et al, 1990). The athlete groups included weight lifters, elite runners, recreational runners, and swimmers. All had regular menses for at least two years prior to the study and none had used oral contraceptives during this time. All groups exceeded the RDA for calcium. All subjects had fat free body mass determined by densitometry and bone measurements of the proximal and distal radius (SPA) and lumbar spine and proximal femur (DPA). Body weight and fat free body mass were higher in the body builders and swimmers than the runners but there was no difference in per cent body fat between athlete groups. In the athlete group, the fat free body mass correlated significantly with BMC at each site. Body builders had higher BMC than other groups at all sites and these differences reached significance at all sites in the inactive controls. The higher BMC in body builders also reached significance in other comparisons notably at the FN in swimmers and runners. The runners and swimmers had generally higher BMC than inactive controls and the BMC in the swimmers at the three femoral sites was higher than the recreational runners and in active controls.

Another cross-sectional study compared bone densities of the lumbar spine (DPA) and heel (SPA) in eumenorrheic intercollegiate volleyball players (12), basketball players (9), swimmers (10) and non-athletes (13) (Risser et al, 1990). Mean bone mineral densities, after adjustment for height and weight, were compared. At the lumbar spine, the swimmers' BMD was significantly lower than all other groups. The volleyball players had the highest L2-L4 BMD which was also significantly greater than the non-athletes. The calcaneal BMD was significantly higher in both basketball and volleyball players compared to both swimmers and non-athletes. The very low spinal BMD in the swimmers was not expected and needs further investigation.

Data on exercise pattern, nutrition, general anthropometry, three skinfold measures, body density by underwater weighing, serum insulin-like growth factor (IGF-1) and vertebral BMD (DPA) were collected in college females with a mean age of 24.5y (Davee, Rosen & Adler, 1990). The subjects were allocated to one of three groups; sedentary (<1h/wk), aerobically active (>2.5h/wk), and muscle building activity combined with aerobics (included at least 1h/wk of muscle building activities). There were no significant

differences between groups in most variables including total body weight, BMI and calcium intake. The L2-L4 BMD of the muscle building group was significantly greater than both the sedentary and aerobic groups which were similar. The IGF-1 levels, greatest in the muscle building group, were significantly and positively related to hours of loading exercises. Thus, the effect of increased BMD may be due to local and systemic effects of exercise of specific resistance exercises. Davee and colleagues (1990) concluded that college aged females who engaged in weight training exercises had higher spinal BMD than those who undertook aerobic programs.

A cross-sectional study of lumbar spine and femoral neck BMD (DPA) in 58 eumenorrheic athletes, mean age 25y, allocated the subjects into 5 groups according to activity modality (Westfall et al, 1989). The investigators reported that inactive controls had the lowest BMD. The body builders' group had the highest femoral neck BMD which was significantly greater than inactive controls, swimmers and recreational runners but not collegiate runners. The body builders' lumbar spine BMD was the highest and significantly greater than the inactive controls.

The results of the preceding studies which have attempted to determine the effect of specific exercise programs on bone density are mixed. The cross-sectional investigations (Davee et al, 1990; Heinrich et al, 1990; Risser et al, 1990; Westfall et al, 1989) have found a positive effect on BMC of weight training programs or sport-specific loading activities. Aerobic exercises and swimming do not increase bone mass to the extent of body building or specific compressive exercises. Inactivity is associated with the reduced bone mass. The one exercise intervention study did not find an increase in BMC although muscle strength increased significantly (Rockwell et al, 1990). Another weight training intervention study in postmenopausal women stated that although improvements in muscle strength may be evident by 6-8 weeks, bone changes may not be discernible in this time (Rikli et al, 1990). Similarly, the data collection at 4.5 and 9 months in Rockwell's study may have shown decreases at the spine and no change at the femoral neck because significant effects in bone mass require time for completion of remodelling cycles in a multitude of bone structural units. Also, in Rockwell's study, there were only 10 subjects in the treatment group. As well, the subjects were not

randomized and no additional information was provided on menstrual history, weight records and nutrition.

Deconditioning The decrease in physical activity with aging has been suggested as a mechanism for involutional bone loss. Preliminary results from Whalen and workers (1988) suggest that up to the age of 50 years, the age-related decline of daily walking activity is a major contributory factor to the decrease in bone density in both men and women. Conversely, Kanders and co-workers (1988) calculated that increments in activity, walking one mile per day, yielded an increase of 1.2% in vertebral BMD.

Two longitudinal studies in humans have found similar deconditioning effects on bone (Dalsky et al, 1988) (Lane et al, 1986). Although both of these investigations featured older women in different research designs, the conclusions were similar: detraining led to decreases in BMD.

Two recent studies in rats offer some insight into this topic. One study of rats showed that physical activity which starts before middle age and is continued into old age, will have beneficial effects on trabecular bone (Silbermann et al, 1990). The ability of bone to respond to physical activity appeared to be diminished with age. From a study of training and deconditioning conducted in rats, Yeh and Aloia (1990) concluded that exercise had positive effects on selected parameters of bone metabolism. Generally, formation indices increased and resorption decreased to result in increased bone mineral content. These changes were rapidly reversed with deconditioning.

Interactive role of physical activity An interactive role for physical activity has been acknowledged by several investigators studying other factors related to bone gain and loss. The interrelationships of physical activity with menstrual status and dietary factors such as increased fibre intake, lower fat, vegetarianism and reduced calories have been explored with reference to bone (Lloyd et al, 1987; Snow, Schneider & Barbieri, 1990). One prospective study which provided evidence of bone gain at the lumbar spine and forearm in the third decade stated that physical activity contributed to the beneficial effect of at least 900 mg of daily calcium on this bone accretion (Davies et al, 1989). Pollitzer and Anderson (1989) postulated that genetics contribute largely to bone mass to age 20 then bone mass is likely significantly influenced by dietary calcium and physical activity. They suggested that the adolescent augmentation of cortical bone and the protection of trabecular bone in later years is largely due to nutrition and exercise.

2.3.2 Endocrine Factors

Bone remodelling units are cyclically activated by various local and systemic factors (Krane, 1988; Raisz & Kream, 1983). The remodelling process is governed by the resorptive activity of osteoclasts and the formative action of osteoblasts (Parfitt, 1979). A balance between the resorptive and the formative stages is necessary to maintain bone mass. Because these two phases are normally tightly coupled, it is often difficult to locate the primary defect in bone turnover (Mundy, 1987). Also, the identification of autocrine and paracrine agents, their responses to endocrine factors and their coordinated actions on bone is in the preliminary stage.

The roles of the gonadal steroids, particularly estrogen and the calciotropic hormones: parathyroid hormone (PTH), calcitonin and the active vitamin D3 metabolite, 1,25 dihydroxycalciferol, have been extensively studied with regard to their complex roles in bone metabolism. Calcium is an important agent in the mineralization of newly formed bone (Anonymous, 1984; Avioli, 1984; Recker, 1987). Because the level of serum calcium is stringently regulated, bone mineral acts as a calcium reservoir

(Eastell & Riggs, 1987; Garel, 1987). PTH, secreted in response to low serum calcium, is a bone resorptive agent because its primary action is to promote osteoclastic activity leading to the demineralization of bone and elevation of serum calcium (Endres, Morgan, Garry & Omdahl, 1987; Gallagher, Riggs, Jerpbak & Arnaud, 1980). Calcitonin, a putative inhibitor of resorption, functions in response to hypercalcemia to promote a flux of excess serum calcium to the bone (McDermott & Kidd, 1987), but it is not believed to play an important long-term role in bone homeostasis. Calcitriol (1,25 (OH)-D3), a bone resorptive agent, works synergistically with PTH in a variety of calcium related functions (Tsai, Heath, Kumar & Riggs, 1984). This physiologically active form of vitamin D has as its main role the intestinal absorption of calcium.

Estrogen, progesterone, testosterone, growth hormone, insulin and somatomedins have been regarded as promoters of bone accretion (Mundy, 1987). Conversely, excess glucocorticoids, prolactin, and thyroid hormone have been associated with decreased bone density (Lam, Baker, Seeman & Pepperell, 1988). However, bone loss in hyperprolactinemic women has been attributed to estrogen deficiency rather than excess prolactin (Klibanski, Biller, Rosenthal, Schoenfeld & Saxe, 1988).

The use of biochemical indicators of resorption (urinary hydroxyproline) and formation (alkaline phosphatase) has aided both *in vivo* and *in vitro* investigations of various hormones and agents. Osteocalcin or bone GLA protein (BGP) is now considered to be a reliable marker of bone formation and serum levels are specific to bone (Peck et al, 1988). In cases where resorption and formation are not uncoupled, plasma BGP reflects bone turnover (Delmas, Stenner & Wahner, 1983). Higher levels of BGP have been found in postmenopausal osteoporotic females and with estrogen therapy these levels return to normal (Stepan, Presl & Pacovsky, 1987).

Estrogen is critical in maintaining bone mass (Albright et al, 1941; Lindsay et al, 1976; Nilas et al, 1987) though the mechanisms by which this is accomplished are not well understood. Some general hypotheses have been formed. It had been proposed that estrogen indirectly reduced osteoclastic activity through calcitonin (Stevenson et al, 1981), but recently

several independent investigators have reported the existence of estrogen receptors in osteoblasts (Eriksen et al, 1988; Komm et al, 1988). It appears as if the loss of estrogen leads to an unchecked drive to resorption which cannot be matched by formation (Kelly, Pocock, Sambrook & Eisman, 1989). An increased rate of bone turnover with net resorptive action leads to the chronic phase of bone loss (Eastell et al, 1988). It is possible that estrogen may act directly upon receptors to mediate bone turnover by inhibition of resorption and stimulation of formation (Takano-Yamamoto & Rodan, 1990). Alternative mechanisms suggest a role for estrogen in the desensitization of the parathyroid glands to calcium levels in the blood or the increase of the main enzyme resulting in enhanced calcitriol production (Wardlaw & Barden, 1989).

Natural menopause leads to an accelerated phase of bone loss (Parfitt, 1979). Young oophorectomized females also experience a rapid phase of bone loss in conjunction with the reduction of endogenous estrogen (Eriksen et al, 1990). Hypogonadism related to eating disorders (Rigotti et al, 1984), exercise (Drinkwater et al, 1984) and gonadotropin-agonist treatment (Johansen et al, 1988) have been characterized by reduced bone density. Recent studies suggest that females in these groups may be at risk for osteoporotic fractures. The hypogonadal female athlete has only recently been investigated from the perspective of skeletal health (Cann, Martin & Genant, 1984; Drinkwater, 1987; Drinkwater et al, 1984; Jones, Ravnikar, Tulchinsky & Schiff, 1985; Lindberg et al, 1984).

Exercise, weight and estrogen are highly related factors. The physical training undertaken by elite athletes would be expected to increase bone mass directly or indirectly. A direct increased drive of the formation stage of bone remodelling through unknown mechanisms may occur or may be indirectly mediated through systemic factors or augmented lean tissue effects. However, the associated major hormonal and metabolic changes associated with chronic exercise may augment resorption (Cumming & Belcastro, 1982; Schwartz et al, 1981). The cumulative data confirm that the anabolic effects of exercise on bone are minimized by a catabolic endocrine milieu if the exercise stressor is excessive: the rarefaction of trabecular bone is particularly evident in the literature.

The development of osteopenia in both anorectics and athletes appears to be largely due to hypoestrogenism associated with the low body fatness/weight and exercise stress. The endocrine profile of the amenorrheic athlete is similar to that of an anorectic female but the etiology of secondary amenorrhea in athletes may differ from that of anorectics (Fears, Glass & Vigersky, 1983). These modifications are mediated at the hypothalamic level with involvement of the pituitary-ovarian axis as well as the pituitary-adrenal axis.

Other events in the reproductive history of females have been investigated as possible modifiers of bone mass. The relationships of age at menarche, parity, pregnancy, lactation and use of oral contraceptives have not been fully elucidated. Pregnancy and lactation have considerable potential to modify bone mass through adaptations of sex steroid and calciotropic hormones as well as nutritional and mechanical loading changes. More definitive research is required but several papers reviewed in this chapter have included one or more of these variables in their investigations.

In a study of age and premenopausal bone mass, only age at menarche demonstrated a significant relationship to bone density (Rosenthal et al, 1989). This relationship was negative leading the authors to conclude that delayed puberty might lead to osteopenia.

Bone density and parity has also been investigated. McCulloch and collaborators (1990) reported no relationship between parity and calcaneal bone density. Other studies have reported no correlation between parity and lumbar bone density (Hreshchyshyn, Hopkins, Zylstra & Anbar, 1988a) (Lindquist, Bengtsson, Hansson & Roos, 1981; Smith, 1967) and a negative correlation between parity and femoral bone density (Hreshchyshyn et al, 1988a). A positive relationship between lactation and lumbar bone mineral density was noted in parous women (Hreshchyshyn et al, 1988a). Sowers' (1985) study of radial bone mass in 20-35 year old females showed no relationships existed between BMD and these variables; oral contraceptive use, parity, lactation, smoking and physical activity. However, pregnancy prior to 20y was negatively related to BMD.

The effect of oral contraceptives on bone mass has also been examined. All studies reviewed subsequently reported no significant relationship

between oral contraceptive use and bone mineral density (Mazess & Barden, 1991; Elliott et al, 1990; Davee, Rosen & Adler, 1990; Rodin et al, 1990) (Hreshchyshyn et al, 1988a). In Mazess and Barden's study (1991) of premenopausal bone mass, 300 females aged 20-39y were grouped into three classes of oral contraceptive use: never users, use of less than 5y and use of more than 5y. No significant associations existed between oral contraceptive use and BMD at any site. There were no significant differences in BMD's between groups and there was no relationship of change in BMD over two years and oral contraceptive use.

Elliott's group (1990) included in their study sample 145 current users and 65 past users after determining that their bone density measurements were the same as population bone measurements. These authors concluded that there was no effect of oral contraceptives on bone. Davee and colleagues (1990) also included subjects who currently used oral contraceptives as there were no relationships with any of the hormones measured nor with L2-L4 BMD. Oral contraceptive use was refuted as a confounding factor and was not an exclusion criteria in another study (Rodin et al, 1990). In normal women aged 35-65 there was no effect of estrogen and/or progestin use on either lumbar spine or femoral neck bone densities (Hreshchyshyn, Hopkins, Zylstra & Anbar, 1988b). Thus, current research supports a lack of effect of oral contraceptive use on BMD. Although there are no publications investigating various preparations and dosages of birth control pills in the young adult female, it appears as if levels of endogenous estrogen are sufficient to maintain BMD.

Dhuper and colleagues investigated the effects of endogenous hormones on acquisition of PBM in 43 white females aged 13-20 years (Dhuper, Warren, Brooks-Gunn & Fox, 1990). There were 28 dancers and 15 non-dancers. In the initial part of the study, weight, estrogen exposure score and testosterone levels were highly correlated with BMD of the wrist, spine and first metatarsal. The second aspect of the study investigated factors which determined PBM in a sub-set of 18-20 year-old subjects. The subjects with the lowest estrogen exposure index had significantly lower bone mineral density at the spine and wrist, lowest weight, lowest weight/height, lowest weight during adolescence, highest age at menarche and highest intake of dietary

fibre. Estrogen exposure score during adolescence and weight were the major variables affecting wrist and spinal BMD. In addition to these effects, foot BMD was negatively affected by activity status. The dancers were more active and more likely to be estrogen deficient. However this is further proof that estrogen deficiency undermined the benefits of physical activity. The authors concluded that PBM is considerably affected by adolescent hormonal events and that weight was a highly interdependent factor.

A group of thirty 18-22 year old females were subjects of a study of selected hormones and trabecular BMD (QCT) (Buchanan et al, 1988). The subject groups were sedentary, eumenorrheic athletes and oligomennorheic athletes. The athletic mode was primarily aerobic with no specific back-loading exercises. Selected adrenal precursors, androgens, estrogens, progesterone and pituitary hormones were sampled once a week for a month. Analyses of these data determined that estrogen (estradiol) and androgens (androstenedione, free and albumin-bound testosterone) independently and cumulatively determined peak trabecular bone mass. Also, in these young women, the general physical training had no significant effect on spinal BMD. The oligomenorrheic group displayed a significantly lower BMD than eumenorrheics after adjusting for androgen levels. The authors concluded that sex steroids were more important than activity and activity-induced menstrual dysfunction.

Clearly, estrogen is the major sex-steroid hormone in the maintenance of female bone mass. In situations where endogenous estrogen levels are deficient, bone mineral density is compromised. In postmenopausal women, estrone levels may partially offset the loss of estrogen. The levels of estrone are largely determined by the amount of adipose tissue available for peripheral aromatization of precursors. In younger, premenopausal women, the estrogen deficient state of hypogonadal amenorrhea is usually concomitant with decreased body fat so that estrone levels are also low. Although oral contraceptive use is not related to BMD, in estrogen deficient states such as postmenopause and athletic amenorrhea, estrogen replacement plays a significant role in assuring bone health. The role of progesterone and androgens in promotion and maintenance of bone mass in young women requires further research.

2.3.3 Calcium

To date, dietary calcium has been a major focus of investigators of osteoporosis. Calcium requirements at all ages are the subject of much controversy (Martin et al, 1987; Parfitt, 1987). Most investigations have attempted to settle the issue regarding the benefits of increased calcium intake in postmenopausal women. Although the debate continues, reviews of evidence from controlled trials do not support the contention that calcium therapy on its own is adequate intervention (Martin et al, 1987; Peck et al, 1988). The calcium and estrogen interaction is central to defining a role for calcium and determining appropriate daily intakes (Heaney, 1990b; Reid, 1989).

Dietary calcium may be particularly important during those years leading to the attainment of PBM, but no studies have established requirements. Heaney (1989a; 1990a) suggested that calcium intake may have appropriate levels for three critical skeletal stages in female life: primary prevention, secondary prevention and maintenance. Moreover, he estimated the calcium requirements for several age groups within these stages (Heaney, 1990a). Heaney's estimates for selected stages are: for 12-18 y (1500mg/d), 18-30 (1200 mg/d) and 30 to menopause (800-1000mg/d). The present recommended dietary allowances (RDAs) in the United States are 1200mg/d for adolescents and for pregnant or lactating women and 800mg/d for other groups (Anonymous, 1989b). The RDA levels are set to ensure a positive calcium balance for 97% of the population (Reid, 1989). In Canada, the most recent guidelines recommend 800 mg/d for all females with the following special requirements, 1100 mg/d for girls aged 10-12, 1000 mg/d for girls 13-15 and 250 mg/d for infants (Scientific Review Committee, 1990). For pregnancy and lactation, an additional 500 mg/d is recommended. The report listed skeletal accretion and maintenance as well as the earlier growth acceleration associated with puberty as rationale for the increases in the two female adolescent age groups.

Part of the dispute regarding the role of calcium in bone health has arisen due to methodology. Calcium balance studies are costly. Thus indirect assessments of calcium balance are used. There are many

problems with dietary assessments whether retrospective or prospective (Block, 1989). Moreover, the usual long-term intake is important in determining calcium intake effects on bone (Block, 1989). There are also shortcomings in dietary assessments which focus on one nutrient without consideration of the influence of other foods (Block, 1989).

A recent meta-analysis of twelve calcium investigations reported that the inconsistencies regarding calcium and bone health are due mainly to differences in research design, sample chronological and menopausal age and methodology (Cumming, 1990). The author concluded that the literature supported the positive effects of calcium on cortical bone in elderly and osteoporotic women with low baseline levels of calcium. In early postmenopausal women, the benefits of calcium are less obvious because of the strong influence of estrogen deficiency. Overall, however, a weak positive correlation exists between level of calcium intake and bone mass. Only seven studies of solely premenopausal women were included in this meta-analysis (Angus, Sambrook, Pocock & Eisman, 1988; Freudenheim, Johnson & Smith, 1986; Kanders et al, 1988; Riggs et al, 1987; Smith, Gilligan, Smith & Sempos, 1989a; Sowers et al, 1985; Stevenson et al, 1989). All but one (Riggs et al, 1987) of these showed a positive effect of calcium intake on BMD. The correlation coefficients reported in three studies (Freudenheim et al, 1986; Kanders et al, 1988; Stevenson et al, 1989) were higher than those in the postmenopausal reports. Thus, Cumming concluded that the evidence supported an important effect of calcium in premenopausal women. However, the issue is not settled.

The classic study of two Yugoslavian regions of high and low calcium intake supports the hypothesis that habitual high intakes of calcium during childhood are associated with higher adult bone mass (Matkovic et al, 1979). Hip fractures were approximately a third of the incidence in the low calcium region. This is however, not a definitive study and was excluded from Cumming's review because a number of variables confound the data.

Matkovic's (1990) recent study investigated the contribution of calcium intake to peak bone mass in adolescent females. In this study, a total of 28 subjects completed a two year study of calcium supplementation

using milk and calcium carbonate. Eight subjects were in the low calcium (~750mg/d) and 20 in the high calcium (~1640 mg/d) category. The bone measurements included radiogrammetry, distal radius SPA and L1-L4 DPA at 10, 18 and 24 months. Both groups demonstrated a significant increase in bone mass and density measures. The high calcium group showed higher but not significant increases than the low calcium group.

An investigation of 37 young females and their mothers, respective mean ages 25y and 52y, examined the role of calcium and bone mass (Lutz et al, 1990). The subjects generally met the RDA's for calcium as determined by 3 day dietary intakes, with 21 mothers and 13 daughters reporting calcium supplementation. Only the daughters' total calcium intakes correlated significantly with BMD at the L2-L4, FN and FT sites.

An interactive effect on bone between calcium and physical activity has been hypothesized. Shangold (1990) has stated that the benefits of exercise on bone can not be realized if dietary calcium is inadequate. Kanders specifically examined this interaction in 60 women aged 25-34y (Kanders et al, 1988). The mid-radius (SPA) and lumbar (DPA) BMD were measured. Calcium intake was assessed by a 24- hour dietary recall and a prospective 6-day dietary log. Activity levels were determined by questionnaire and a prospective 7-day recording by pedometer. The lumbar BMD was significantly related to activity level and to habitual calcium intake when the effects of activity were eliminated. Calcium intake significantly affected radial BMC. This study confirmed that mechanical loading of specific sites as in walking elicited the site specific positive response to the lumbar spine. Also, the evidence pointed to a calcium threshold of approximately 880-1000 mg/d, beyond which the positive relationship with vertebral BMD ceased. Davies (1989) reported that physical activity contributed to the beneficial effect of at least 900 mg of daily calcium on bone accretion during the third decade.

An earlier investigation of calcium and other reported determinants of PBM in females aged 20-35y measured radial bone mass (Sowers et al, 1985). The data showed a trend that current calcium intake was positively related to bone mass. More importantly, BMD was significantly higher in

those with a daily calcium intake of more than 800 mg than below this threshold.

Other research not considered in Cumming's analysis has addressed the issue of dietary calcium. According to Davee and workers (1990), dietary calcium did not contribute to the higher BMD in the muscle builders and was not correlated with L2-L4 BMD. No correlation of calcium intake to cortical radial or vertebral bone mass was evident in young eumenorrheic or older postmenopausal runners (Kirk et al, 1989). In the examination of growth in normal children by Glastre and workers (1990), calcium intake corrected for age was not correlated to BMD. Childhood milk consumption and current dietary calcium were not significant determinants of bone density of the os calcis of young women mean age 28.5y (McCulloch et al, 1990).

In summary, the conclusions of Cumming's meta-analysis and more recent literature do not agree on a positive role for calcium after skeletal accumulation and maturation. The postmenopausal woman may benefit from calcium supplementation with estrogen replacement therapy. The benefits of a high calcium intake in premenopausal years has not been confirmed by research although suggestions of a weak positive relationship between high bone mineral density and high dietary calcium have been recorded. It is likely that habitual calcium intake at recommended levels are important for attainment of bone mass but this too is equivocal. A threshold level of calcium intake probably exists so that consumption of less than approximately 800 mg/d may be detrimental to bone mass. The effects of chronically low levels of dietary calcium (~400-600 mg/d) on BMD in adolescent and young adult women requires further investigation. The effect of dietary calcium on bone depends upon individual calcium balance which in turn is determined by various other life-style factors. Physical activity and estrogen status modulate the effects of calcium on bone with estrogen likely being the critical determinant of the size and direction of the interaction.

2.4 Menstrual Dysfunction in Athletes

2.4.0 Introduction

Intense physical activity is associated with menstrual irregularities in girls and young women. Athletic females have a later menarche than their less active counterparts (Frisch, Wyshak & Vincent, 1980). A peripubertal deficiency of estrogen has been linked to delayed menarche (Malina, 1973; Shangold et al, 1990; Warren et al, 1986). The prepubertal athlete may exhibit delayed bone age and increased longitudinal growth of bones as epiphyseal fusion depends upon estrogen. The young athlete is also more vulnerable to secondary amenorrhea than the older athlete (Warren, 1980). Secondary amenorrhea is found most frequently in dancers, gymnasts and endurance runners, with an incidence close to 50% (Feicht, Johnson, Martin, Sparkes & Wagner Jr., 1978).

Consensus has not been reached regarding the etiology of exercise-induced amenorrhea. The problem is most severe in those activities that combine intense training with low body weight (Sinning & Little, 1987). Both decreased body fat and weight loss have been investigated but the manner in which they affect the menstrual cycle has not been satisfactorily elucidated. Frisch and McArthur's (1974) "critical fat" hypothesis has been disproved but leanness and body fat remain major considerations in the etiology of exercise-induced amenorrhea. The amount and the rate of weight loss are factors which may initiate the reproductive response of low gonadotropins and low estrogen levels. It has been shown that the levels of gonadotropins are highly correlated to the percent of ideal body weight (Abraham, Beumont, Fraser & Llewellyn-Jones, 1982). The reduction of adipose tissue in athletes may be important in that fat cells provide a secondary source of endogenous estrogen through the aromatization of precursors.

Exercise-induced menstrual dysfunction ranges from a slight shortening of the luteal phase to loss of ovulation and amenorrhea (Pirke, Schweiger, Broocks, Tuschl & Laessle, 1990; Prior, 1982). Athletes may move along the spectrum of menstrual function and dysfunction as estradiol levels fluctuate (Shangold et al, 1990). Secondary amenorrhea in athletes is characterized by a loss of the pulsatile release of gonadotropin releasing

hormone (GnRH) which decreases the pituitary secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) and results in the hypoestrogenic state (Baker, 1981; Fisher, Nelson, Frontera, Turksoy & Evans, 1986). Since the administration of GnRH has normalized LH concentrations in amenorrheic runners, it appears that the defect is at the hypothalamic level. This endocrine profile is similar to observations of anorectic amenorrheic females (Rigotti et al, 1984).

Several hormones other than the gonadal steroids have been implicated in athletic amenorrhea. Recent investigations point to disruptions in several endocrine systems which may be slow to recover and may in fact display subtle differences despite restoration of weight loss (Ohzeki et al, 1989; Smith et al, 1989b). Increased cortisol secretion and decreased secretion of ovarian steroids with a secondary decrease in prolactin are characteristics of athletes with impaired reproductive cycles (Schweiger, Tuschl, Broocks & Pirke, 1989). The mechanism of the athletic-induced hypogonadotropic and hypogonadal state may be in part due to the suppression of gonadotropins by increased synthesis of catechol estrogens (Blumenthal, Rose & Chang, 1985; Tan & Jacobs, 1985).

Exercise elicits a stress response from the body and stress-related hormones such as cortisol and endorphins have been investigated in the pathophysiology of athletic amenorrhea (Carr et al, 1981; Laatikainen, Virtanen & Apter, 1986). Increased cortisol was implicated in the disruption of normal follicular development and consequent LH decrease (Schweiger et al, 1989). Excess cortisol was stated to preferentially decrease trabecular bone (Biller et al, 1989; Newman & Halmi, 1989). However, a recent study reported that females on corticosteroid therapy showed greater losses in BMD at the femoral neck than the lumbar spine site (Sambrook et al, 1987). This finding indicates that cortical bone may also be susceptible to excess cortisol. Endorphins have also been implicated as inhibitors of normal pulsatile release of gonadotropins (Laatikainen et al, 1986; Ruffin IV, Hunter & Arendt, 1990).

Hyperprolactinemia may be a contributing factor in athletic amenorrhea and prolactin is known to increase bone loss (Koppelman et al, 1984). However, decreased resting levels of prolactin have also been reported

in amenorrheic athletes (Laatikainen et al, 1986; Schweiger et al, 1989). It has been stated that hyperprolactinemia is not detrimental to bone density if normal estrogen levels are maintained (Ciccarelli et al, 1988; Klibanski et al, 1988).

Menstrual dysfunction due to athletic training reflects disruptions in the normal secretory and feedback systems of various hormones. The balance between anabolic and catabolic hormones may ultimately determine the effect of intense physical activity on BMD. However, decreased estrogen is the primary endocrine characteristic of the amenorrheic athlete which concerns bone mineral density. The prolonged state of estradiol and estrone deficiency are likely to have a major impact at the skeletal level.

2.4.1 Implications for Bone

Reduced bone mass Research on osteopenic athletes was first published by Cann's group (1984) and supported by Drinkwater (1984). The initial findings of these two investigators emphasized the association between the amenorrheic state and low vertebral bone mineral density. Cann and colleagues compared the lumbar BMD (QCT) of age-matched groups of active women and eumenorrheic sedentary controls. In the active women, amenorrhea was associated with a decrease in bone mass. In Drinkwater's study, 25 year-old amenorrheic runners ran a significantly higher number of miles than eumenorrheic runners and had significantly lower estradiol concentrations. The lumbar BMD (DPA) of these non-menstruating athletes was significantly lower than their eumenorrheic controls (Drinkwater et al, 1984).

Since then, a number of studies have amplified this research. In a later study, the bone mineral content of amenorrheics was significantly correlated with the duration of amenorrhea (Cann et al, 1985a). In a third study, Cann and workers explored menstrual history, dietary factors, exercise patterns and smoking as predictors of trabecular bone mass in runners (Cann, Cavanaugh, Schnurpfiel & Martin, 1989). Only menstrual history, characterized by regular, oligomenorrheic (2-6 cycles/y) and amenorrheic groups (0-1 cycle/y), was highly correlated with the trabecular BMD (QCT).

The authors suggested that amenorrhea of 3 years duration or more indicated that bone loss was irreversible.

In Lindberg and workers' (1984) investigation of premenopausal runners aged approximately 30y, runners with menstrual disturbances were found to have longer training histories and greater weekly mileage than normal runners. The 11 amenorrheic runners had significantly lower BMD at two forearm sites (SPA) than 15 eumenorrheic runners and 14 normal controls. The spinal BMD (DPA) of 8 amenorrheic runners was lower than non-study normals.

The lumbar spine BMD (QCT) and radial BMD (SPA) of amenorrheic runners, eumenorrheic runners and sedentary controls were compared (Marcus et al, 1985). The amenorrheic runners had a mean lumbar BMD 20% lower than similar age eumenorrheic runners and 10% lower than controls.

Three groups of females, aged 18-43y, with secondary amenorrhea, were investigated for cortical radial osteopenia (SPA) and compared to 25 non-athletic regular menstruating controls (Jones et al, 1985). There were 8 runners, 20 weight loss patients and 11 patients identified with primary ovarian failure. The data analysis showed that only the peripheral BMD of the runners group was not significantly different from controls. However, this may be because the duration of amenorrhea was less in the runners. The duration of amenorrhea was significantly correlated with reduced BMD ($r=0.506$, $p<0.001$).

The mean lumbar BMD (DPA) of 11 amenorrheic runners was found to be significantly reduced compared to 17 eumenorrheic runners (Nelson et al, 1986). This difference could not be accounted for by differences in height, weight or body composition. A significant correlation was found between estradiol and lumbar BMD.

Another study investigated hormonal status and skeletal health in active and sedentary women aged approximately 19y (Baker & Demers, 1988). The activities participated in were lacrosse, field hockey and track and field. The following data were collected; trabecular BMD(QCT), body composition by skinfolds, and hydrodensitometry, 7-day dietary profile, menstrual history and 4 weekly blood samples which were assayed for estrone, estradiol, androstenedione, progesterone, FSH, LH, and prolactin. The age and weight

were similar in these subject groups; 12 sedentary, eumenorrheic controls (11-13 cycles/y), 10 eumenorrheic athletes and 6 oligomenorrheic athletes (3-7 cycles/y). The athletes had a greater lean body mass and were less fat than the controls. Menarche was later in the athletes, being most delayed in the oligomenorrheics. Estradiol was significantly lower in the oligomenorrheic athletes than eumenorrheics during the second and third weeks but rose in the fourth week. A trend of lower estrone was evident in the athletes. Despite the above differences, BMD was not significantly different between groups although the eumenorrheic athletes tended to have the highest BMD values.

A cross-sectional study of 46 athletes included rowers, runners and ballet dancers (Wolman, Clark, McNally, Harries & Reeve, 1990). These athletes were grouped according to menstrual status and activity. Amenorrhea was defined as no menses for 6 months prior to the study. The mean ages of these groups were 27-29 years. Bone mineral density measured by QCT was about 20% lower in the amenorrheic athletes compared to eumenorrheic athletes. The rowers had about 11% higher bone mineral density than the non-rowers when height and weight differences were controlled. In both comparisons, differences between groups were significant but there was no significant interaction between the two variables. The authors concluded that the effects of amenorrhea were partially offset by the intense loading of the spine in rowing.

Lumbar spine bone mineral density was measured by Hologic QDR in 200 amenorrheic fertility clinic patients and 57 age-matched normally menstruating controls (Davies et al, 1990d). The subjects were 16-40 year old Caucasians and the mean length of amenorrhea was approximately 3 years. Amenorrhea was defined as no menses in the 6 months prior to the study. The amenorrheic group's BMD was 15% lower than the eumenorrheic controls. Patients with primary amenorrhea had significantly lower BMD than secondary amenorrheics. No differences in BMD were found when subjects were grouped by smoking and exercise habits. These authors (Davies et al, 1990d) stated that the demineralisation associated with amenorrhea was due to estrogen deficiency. Their indices of estrogen deficiency included an estradiol assay, uterine size by ultrasound and a group of patients with primary amenorrhea due to gonadal dysgenesis.

In a recent review, the BMD of athletes with amenorrhea was reported by different investigators to be reduced by 9-24% compared to sedentary age-matched (Drinkwater, 1990). Osteopenia of trabecular bone is common among amenorrheic athletes (Hohtari, Elovainio, Salminen & Laatikainen, 1988). The larger surface area and higher metabolic activity of trabecular bone make highly trabecular sites such as vertebrae more sensitive indicators of bone homeostasis.

The reduction in bone mineral density is less severe in oligomenorrheic athletes than amenorrheic athletes and bone mineral density is normal or increased in eumenorrheic athletes (Cook et al, 1987; Lindberg et al, 1984; Marcus et al, 1985). The bone-promoting effects of weight-bearing activity are not able to compensate for the decrease in bone mineral density associated with hypoestrogenism. A study showed that amenorrheic athletes who decrease training, increase body weight and establish normal menstrual cycles, show significant increases in bone mineral density but this restoration of bone may not be complete. In one study, the amenorrheic athletes who regained menses showed a 6.3% increase in L1-L2 BMD over 14.4 months compared to a loss of 3.4% in the 2 chronic amenorrheics (Drinkwater, Nilson, Ott & Chestnut, 1986). In 4 runners there was an increase of 13.5% at the lumbar spine over 15 months, whereas the 3 amenorrheic runners who remained amenorrheic showed a decrease of 1% (Lindberg, Powell, Hunt, Ducey & Wade, 1987).

The publications reviewed in this chapter strongly support the negative impact of amenorrhea induced by activity on lumbar bone density. There is limited information regarding the proximal femur BMD and athletic activity. We found no publications of the effects of athletic amenorrhea on proximal femur BMD.

Fractures The large-scale occurrence of exercise-induced amenorrhea is too recent for the chronic manifestations of osteoporosis to be observed, although anecdotal reports of hip fractures in several amenorrheic athletes have been reported (Schwartz et al, 1981). Stress fractures associated with amenorrhea have been reported (Warren et al, 1986). In that survey of young ballet dancers, 62% reported fractures that were confirmed by radiography or bone scan and 69% of these were stress fractures primarily of the metatarsals. Those dancers with stress fractures reported both a higher incidence and longer duration of amenorrhea.

In another study, the incidence of stress fractures was significantly higher in amenorrheic runners than in eumenorrheic runners, 54% compared to 16% (Marcus et al, 1985). Similarly, amenorrheic runners were reported to have a 49% incidence of fractures (Lindberg et al, 1984). A retrospective analysis of female participants in two 10 km races revealed that runners with irregular or absent menses had a significantly higher incidence of musculoskeletal injuries than eumenorrheic runners (LLoyd et al, 1986). Fracture history was related to lower BMD and 10 patients had reported atraumatic fractures (Davies et al, 1990d).

Nelson and colleagues recorded stress fractures in 72% of 18 amenorrheic elite women runners and 36% of 75 currently eumenorrhic elite runners (Nelson, Clark, Otradovec & Evans, 1987). They related the amenorrheics' higher incidence of stress fractures to past body mass indices. The amenorrheic athletes had a history of lower BMI (16.7±1.2) which was significantly different from the currently eumenorrheic group (17.6± 1.3).

One recent investigation examined nutrition and stress fractures in 50 ballet dancers and 59 non-dancers (Frusztajer, Dhuper, Warren, Brooks-Gunn & Fox, 1990). Thirty subjects were assigned to three age, height and weight-matched groups of 10. Bone measurements were taken at the mid-radius (SPA), L2-L4 (DPA) and first metatarsal (DPA). There were no significant differences in wrist and spine BMD between the dancers with stress fractures, dancers without stress fractures and non-dancers without stress fractures. Both groups of dancers had a similar foot BMD which was higher than the non-dancers. The dancers with stress fractures displayed the following characteristics: trend to lower BMD, lower body weight due to

caloric restriction and anorectic tendencies. Marcus et al (1988) also suggested that the diet of amenorrheic athletes was probably low in calcium due restriction of calories and narrow diet regimes.

The exact mechanism of stress fractures is unknown but one of the major functions of bone remodelling is to repair microfractures caused by a too high load level and frequency (Simkin, Swissa, Milgrom & Giladi, 1987). According to Simkin and colleagues, when bone remodelling can not match the formation of new microfractures, a complete fracture is the outcome. Stress fractures seem to be an inevitable consequence of the low bone mineral density resulting from the exercise induced menstrual abnormalities. Also, a greater incidence of stress fractures may be found in those types of physical activity which chronically expose skeletal sites to high loads and frequencies.

In summary, there is a consensus that the long-term skeletal effects of athletic amenorrhea are reduced bone mineral density with a higher incidence of stress fractures. The main site currently evaluated is the lumbar vertebral region although foot and forearm assessments have been popular. The literature review has identified a lack of data concerning the proximal femur and the responses of this important region to athletic amenorrhea. The reductions in BMD due to activity-induced menstrual dysfunction may result in an increased risk of postmenopausal osteoporosis.

2.5 Anorexia Nervosa

2.5.0 Introduction

Eating disorders are complex and not well understood from several perspectives. The etiology and treatment of anorexia nervosa and bulimia nervosa have been reviewed elsewhere (Halmi, 1983; Leichner, 1985; National Eating Disorder Information Centre (NEDI), 1988). The prevalence of eating disorders, seldom reported in males have been reported to be as low as 1% of adolescent and young women and as high as 50% in ballet dancers (Brooks-Gunn, Warren & Hamilton, 1987). In Canada approximately 1% of adolescent and young women are suffering from anorexia and as many as 2-3% of the entire female population are suffering from bulimia nervosa (NEDI, 1988). Moreover, an additional 5% manifest sub-clinical symptoms of either eating disorder (NEDI, 1988). In younger females aged 12-25 years, the incidence is 1% for anorexia nervosa and 5% for bulimia and is said to be increasing (Lewis et al, 1986). A high prevalence of preoccupation with dieting and weight control has been recorded in adolescent females (Casper, Chatterton & Davis, 1979) and younger children (Maloney et al, 1989).

Anorexia nervosa and bulimia nervosa are multifactorial eating disorders which have severe psychological and physiological effects. An interaction between societal, individual and family factors result in a predisposition to the development of anorexia and bulimia. Approximately one half of eating disorder patients will manifest the two eating disorders of restriction and binge/purge (NEDI, 1988). It may be more correct to view anorexia and bulimia as a continuum rather than two distinct disorders, with depression being a major symptom of both disorders. The course of these eating disorders is usually protracted and resistant to recovery with anorexia nervosa having a worse prognosis than bulimia. Recovery depends upon early identification of the eating disorder and treatment which addresses both the issues concerning eating and weight control as well as the precipitating and underlying psychological issues (NEDI, 1988). Morbidity and mortality rates are significant for anorexia nervosa: the mortality rate for eating disorders ranges from 5-20% (NEDI, 1988).

There are several high risk groups which appear to be susceptible to the pathogenesis of anorexia nervosa. The typical anorectic patient is an adolescent female with a seriously distorted body image. Those individuals most at risk are females between the ages of 12 to 18 years with the most common age of onset being 14-15 years of age (NEDI, 1988). Recently, a survey of infertility patients found that among those with amenorrhea or oligomenorrhea, 58% had an eating disorder or a partial syndrome of anorexia or bulimia (Stewart, Robinson, Goldbloom & Wright, 1990).

Athletics or activities, which impose competitive standards for decreased body fatness, such as ballet, gymnastics, endurance running and modelling yield an increased incidence of eating disorders. The term "anorexia athletica" has been applied to those athletes who incorporate eating disorder practices into their training to attain a desired leanness (CMA Review, 1989) The balance or imbalance between caloric intake and caloric expenditure is of critical importance to those highly concerned with body image. Yates (1989), commenting on the commonality between athleticism and eating disorders, stated that females are more likely to choose diet whereas males choose exercise for weight control. Blumenthal and colleagues (Blumenthal et al, 1985) reviewed this topic and concluded that there was little evidence to support the view of the male runner as the female anorectic's counterpart. However, strategies for the pursuit and maintenance of a cachectic body may range from chronic undernutrition to chronic extreme activity. The manner in which these factors are manipulated to achieve the acceptable habitus may differ only by degree in the athletic and anorectic populations (McSherry, 1985). One study contrasted a 62% incidence of eating disorders in amenorrheic runners to a group of regularly menstruating runners with no symptoms of eating disorders (Gadpaille, Feicht Sanborn & Wagner, 1987). In contrast to the "anorexia athletica" sub-group, there is a category of primary anorectics which uses chronic vigorous physical activity to lose weight (CMA Review, 1989). According to Goldman (1988), the psychological profiles of these two groups are different. Gadpaille (1987) stated that these similarities existed between the anorectic and the amenorrheic athlete: low food intake, strict dietary regime, heightened energy and activity, amenorrhea and

compulsive behaviour. McSherry (1984) reported several distinguishing features between the anorectic and the female athlete; the most notable differences were increased exercise tolerance and higher lean mass of the athlete. However, the evidence clearly suggests that both the anorectic and the amenorrheic athlete demonstrate similar skeletal consequences.

2.5.1 Implications for Bone Mass

It is difficult to compare the magnitude of bone loss reported by different authors due to the variability in the age of subjects, choice of controls, measurement techniques and assessment sites. For example, Crosby's group (1985), using metacarpal bone morphometry in 14 adult anorectics (25±4.4y), reported an 11.6 % reduction in appendicular per cent cortical area which was equated to that of 60 year old women. This report seemingly confirmed an earlier report (Rigotti et al, 1984) that radial cortical bone assessed by single photon absorptiometry was decreased by 11% in anorectic patients, aged 19 to 36 years, compared to controls. Other reported bone losses in anorectics have ranged from 3% at the forearm measured by SPA to 32% at the spine assessed by single energy Quantitative Computer Tomography (QCT). Thus, the validity of comparing reports of anorectic bone loss is questionable particularly because measurement techniques have included bone morphometry, QCT, SPA, DPA and DEXA.

Davies et al (1990b) found radial and L2-4 BMC and BMD to be significantly lower than normal in a group of anorectics and bulimic anorectics. Their anorectic and anorectic/bulimic L2-4 BMD values were approximately 14% and 10% less than normals. Joyce and colleagues reported significantly decreased BMD at the L2-L4 and femoral sites over three categories of eating disorder subjects compared to younger normal controls (Joyce, Warren, Humphries, Smith & Coon, 1990). Anorectics, bulimics and non-specified eating disorders were included in that group.

Savvas' group (1989) reported a significant 17% difference between median bone densities at the lumbar spine, determined by DPA, of female adult anorectics and comparably aged females. Mazess (1990b) utilizing a DP4 scanner, reported a 10% decline in total body BMD of female anorectics with a preferential diminution of thoraco-lumbar bone density by 27% and a

lumbar density 22% lower than historic controls. Biller et al (1989), employing single-energy and dual-energy QCT observed reductions of 32% and 21% at the spine and a 3% non-significant loss of forearm BMD. They concluded that trabecular bone appeared to be preferentially rarefied. These authors found no differences between BMD in the anorectic or anorectic/bulimic patients (Biller et al, 1989). This confirms Joyce's observation of BMD reductions in non-specified eating disorders. Newman and Halmi (1989) assessed the spinal BMD of recovering anorectics and reported a 15% dimunition. Another group reported reductions in forearm and spine bone mineral density in adult anorectics compared to age-matched controls and these respective differences were 7% and 18% (Treasure et al, 1986). Wahner (1989) in a brief review of bone loss in anorectics recommended that spinal density in anorectic patients be routinely evaluated. However, no statement was issued regarding the appendicular skeleton.

Available data on the proximal femur is limited with only one study reporting data on anorectics and age-matched controls. Treasure and colleagues' study (1986) found a 23% lower BMD value in the DPA proximal femur measurement of adult anorectics. Treasure noted that this represented a preferential loss at the femur compared to the concurrently measured lumbar spine and radius. In a later report, Treasure (1987) reported significant losses at the wrist, spine and femur in anorectics and that the mean FN BMD was two standard deviations below normal.

Joyce's (1990) investigation of three groups: adult anorectics, bulimics and non-specified eating disorder patients, showed the combined eating disorders groups displayed a significantly lower BMD at the femoral sites than younger normal controls and bone loss was more exaggerated at the femoral site than the spine. Also, the anorectic sub-group had significantly lower BMD at the FT and FN sites. Savvas' group (1989) using DPA of the femur, documented a 23% difference between adult anorectics and controls, thereby supporting Treasure's report (1986). Mazess (1990b) reported a less drastic 13% reduction, between anorectics and other normative data, for femoral BMD difference assessed by DPX.

With generalized bone losses of this magnitude, it is highly probable that fractures will occur in anorectics but deleterious skeletal effects of anorexia have been reported largely through case study and anecdotal presentations (Baum, Kramer, Sanger & Pena, 1987; Brotman & Stern, 1985; Joyce et al, 1990; Kaplan & al, 1986; McAnarney, Greydanus, Campanella & Hoekelman, 1983; Treasure et al, 1987).

Brotman and Stern (1985) suggested that back and bone pain should be thoroughly investigated in anorectics as vertebral compression fractures were diagnosed in three cases. Another group presented a case report of rib fractures and anorexia nervosa (McAnarney et al, 1983). Kaplan (1986) reported a case study of a 35 year old female with a 17-year history of anorexia. Profound osteoporosis was present and radiography confirmed an intracapsular femoral neck fracture of the right hip. Three years post-fracture, the patient was maintaining 90% of optimum body weight and menses had been regular for 2.5 years. The lumbar spine bone mineral density, although increased from the immediate post fracture assessment, remained more than two standard deviations below age- and sex-matched normal controls. Joyce (1990) reported a positive fracture history in 3 of 33 eating disorder patients. One anorectic patient had a fractured clavicle and a fracture of the wrist and ankle were reported by two bulimic patients. In a group of 45 anorectic patients, those who had anorexia for more than 10 years accounted for 8 of the 9 instances of pathological fractures (Treasure et al, 1987). Baum (1987) described a case of multiple stress fractures in a female patient who at the age of 19 began a two year course of anorexia. The fractures of the tibia and bilateral mid-ulnae were sequelae to separate non-traumatic physical activities and signalled a deficiency of cortical bone.

2.5.2 Duration of Anorexia Nervosa

The severity of bone loss in anorectics has also been associated with the duration of the disease or the duration of amenorrhea. Investigators have reported a negative correlation between spinal bone density and duration of amenorrhea (Treasure et al, 1986) (Biller et al, 1989). Treasure (1986) also stated that femoral bone density was negatively correlated to duration of amenorrhea. Deficits in cortical bone density were correlated to the duration of anorexia (Ayers, Gidwani, Schmidt & Gross, 1984; Rigotti et al, 1984; Treasure et al, 1987). Others have failed to confirm this relationship (Joyce et al, 1990).

2.5.3 Mechanism of Bone Loss

In anorectics, the mechanism of bone loss is not known however, hypoestrogenism may be the primary factor in bone loss associated with prolonged anorexia nervosa (Biller et al, 1989). Menstrual irregularities affect an estimated 50% of bulimic patients (NEDI, 1988). The bulimic patient may also be at risk for osteopenia if her endocrine status is similar to the anorectic (Joyce et al, 1990). Investigations of the estrogen deficiency of menopause have conclusively documented that estrogen is critical to maintenance of bone mass. The effect of estrogen at the cell level is now known to be direct (Eriksen et al, 1988; Komm et al, 1988) and estrogen may not only inhibit resorptive agents but also promote bone formation (Ernst, Heath & Rodan, 1989). The prevention of osteoporosis by estrogenic agents in anorectic patients was reported as unsuccessful in one applicable case history. However, the authors identified a general lack of research concerning estrogen replacement in the anorectic population (Brotman et al, 1985). Treasure and colleagues (1987) dismissed the utility of estrogen in most young anorectics. However, a study which confirmed reduced bone density and decreased skin thickness and collagen content in anorectics, similar to postmenopausal women, concluded that estrogen was of critical importance in anorexia nervosa (Savvas et al, 1989).

Cortisol excess in anorectics has been identified as a promoter of bone loss and hypercortisolism was stated to preferentially decrease trabecular bone (Biller et al, 1989; Newman et al, 1989).

Studies have documented the profound effects of anorexia nervosa on bone but little is known regarding the disruptions at the cell physiology level. There is a lack of information concerning the anorectic-induced disruptions of the normal bone remodelling process of coupled resorption and formation. Ayer's group (1984) stated that the mechanism of bone loss in young anorectics was not similar to menopausal bone loss. They suggested a reduction in pubertal appositional bone rather than increased bone resorption accounted for anorectic osteopenia. Treasure (1986) supported the position that the bone loss associated with anorexia was different from the excessive trabecular diminution characteristic of menopause. Kaplan (1986) examined bone remodelling parameters in a morphometric study of a trans-iliac bone biopsy from a 35-year-old female with a history of 17 years of anorexia nervosa. There was a reduced level of bone remodelling with a delay in the onset and extent of bone formation due to decreased osteoblastic activity. This type of low bone turnover, described as Type II involutional bone loss may be due to the protracted time of illness and does not exclude that the anorectic patient could initially experience a Type I, rapid phase, bone loss associated with estrogen deficiency (Riggs et al, 1986a). In support of this postulate, Joyce's (1990) trans-iliac biopsy research showed that high turnover osteoporosis characterized the osteoporosis evident in five eating disorder patients with severely decreased BMD. Fonseca's (1988) investigation of the mechanism of osteopenia studied calcium, PTH, vitamin D and osteocalcin concentrations in 15 female and 2 male anorectic patients aged 13-47 years. Serum osteocalcin was below normal in the anorectics compared to age- and sex-matched controls. The authors concluded that reduced osteocalcin, a bone specific protein and a biochemical marker for bone formation, was a reflection of decreased osteoblastic activity, the cause of osteopenia. Thus, the osteopenia associated with anorexia nervosa may result from growth and development deficiencies and remodelling anomalies which appear to be initially characterized by high turnover, decreased formation and net bone loss.

2.5.4 Reversibility of Bone Loss

It is not known whether the bone rarefaction of anorexia would be completely restored upon refeeding. The literature does not define what is meant by skeletal recovery and prospective studies to date have not been long enough to clearly address reversibility of bone loss in anorectics. Treasure (1987) reported that weight gain was associated with increased BMD and that recovered anorectics had normal BMD. Other evidence does not support this view. Davies and colleagues (1990a) reported that statistical significance was not achieved between fractional changes in weight and BMC in 17 anorectic and anorectic/bulimic patients monitored for an average of 1.4 years. However, the authors suggested that there was evidence to suggest some restoration of bone mass with weight recovery.

In a case study report, BMD had improved after two and half years but was still two standard deviations below age- and sex-matched norms in a recovered female anorectic (Kaplan et al, 1986). Baum's group (1987) found that two years after resumption of menses and optimal weight in a recovered anorectic, BMD was below normal: L2-4 =82.5%,FN= 76.5%, WT = 69%,FT =56%. They concluded that bone loss associated with adolescent anorexia may not be reversible. Joyce (1990) observed that weight gain may not restore lost bone. Several reports have also pointed out that there may be a refractory phase after weight restoration before the hormonal milieu is normalized (Hsu, Crisp & Harding, 1979; Ohzeki et al, 1989; Smith et al, 1989b). Bachrach and colleagues' (1990) prospective study of 12 adolescent anorectics concluded that weight gain was accompanied by improved osteopenia even before menses was regained. In a group of 9 long-term recovered anorexia nervosa patients only 6 demonstrated a recovery of BMD to normal values. However, 3 recovered anorectics did not restore BMD which remained lower than two standard deviations below normal. The restoration of bone may depend upon the recovery of weight, resumption of normal reproductive cycles and the onset, duration and severity of the bone loss phase.

2.5.5 Early Onset Anorexia Nervosa

The onset of anorexia nervosa is usually peripubertal although a later onset in both juveniles and adults is common. Most investigators have examined chronic adult anorectics regardless of early or late onset. The effects of anorexia, malnutrition and delayed menarche, on the growth and development of bone have not been thoroughly investigated. During childhood, bone remodelling is accelerated and puberty is accompanied by an increase in both cortical and trabecular bone mass (Gilsanz, Varterasian, Senec & Cann, 1986). What must be determined are the roles of increased bone resorption and decreased bone formation in anorectic bone loss. Young bones are highly vulnerable as bone remodelling turnover rate is approximately ten times higher than in adults (Mazess & Cameron, 1974). Gilsanz (1988a) has recently stated that in females, peak vertebral trabecular density is reached at the end of the second decade about the time of longitudinal growth cessation and epiphyseal closure. The early onset of anorexia nervosa could be severely detrimental to bone mass by resulting in reduced adult height (Kreipe, Churchill & Strauss, 1989) and failure to attain PBM (Davies et al, 1990a).

The early onset of anorexia nervosa was investigated in a group of young anorectics with a mean age 16.7 years, who had been amenorrheic for an average of 26 months (Ayers et al, 1984). These authors reported that significant osteopenia, determined by radiographic measures of the carpal bones, was evident in the anorectics and that the degree of cortical thinning was related to the age at onset and the duration of amenorrhea. They suggested that the juvenile anorectic undergoes prolonged skeletal pubertal delay due to a decrease in cortical bone apposition and consequently will be an osteoporotic adolescent. Bachrach's (1990) group documented decreased BMD at the L2-4 lumbar site (18%), mid-radius (4.4%) and whole body (26%) as measured by SPA and DPA in adolescent anorectics compared to age-matched controls. This report concluded that bone loss is generalized, affecting both the axial and appendicular skeleton. In these anorectics, those with primary amenorrhea tended to have lower BMD. Fosson (1987) evaluated early onset anorexia nervosa and increased occurrence of spontaneous and traumatic fractures in 48 children aged 14 years or less.

For this group, which included 35 females, the authors predicted long term consequences of osteoporosis with prolonged illness. Thus, there is no definitive research on the reversibility of decreased BMD with anorexia. The prognosis may depend upon the time of onset and the duration of the disease as well as the extent of bone rarefaction. When the normal attainment of peak bone mass is interrupted by early onset anorexia nervosa, the skeletal consequences are likely to be severe and irreversible.

2.5.6 Anorexia Nervosa and Physical Activity

The role of activity in the pathophysiology and treatment of anorexia nervosa has not been resolved. Rigotti (1984) concluded that anorectics who chronically exercised had a higher bone mineral density than their non-exercising counterparts. Although exercise-associated amenorrhea has resulted in diminished bone mineral density, a high level of exercise appeared to attenuate bone loss in the anorectic population. However Bachrach's (1990) group suggested that regular physical exercise was reported to be inadequate prevention against the development of osteoporosis in anorexia nervosa (Bachrach et al, 1990). In anorexia, the lean body mass is also reduced, which could thereby reduce the mechanical strain on bones. Boden and colleagues (1990) stated that the younger population may be predisposed to fractures due to the high level of physical activity which increases the potential for higher energy trauma. Thus, anorectics, who exercise vigorously, may sustain a fracture although the decreased BMD is above the 'fixed' fracture threshold. Consequently, an individual fracture threshold which considers BMD and the biomechanics of activities may be more meaningful for prediction and prevention of anorectic fractures (Kreipe et al, 1990).

The maintenance or recovery of muscle mass through physical activity may be important to avoid exacerbating bone loss through disuse. However, physical activity programs for anorectics have not been commonly recommended due to the therapeutic focus of caloric intake and weight restoration and the restriction of excessive activity. Moreover, the optimum exercise prescription including type, duration, and intensity of activity for anorectics has not yet been defined.

3. METHODS AND PROCEDURES

3.0 Subjects: Recruitment and Selection

3.0.0 Physical Activity

Subjects were recruited by posters, bulletins, newsletters, letters and personal contact. A recruitment booth was operated at the 1988 Manitoba Marathon. Bulletins were posted at the University of Manitoba, health/fitness clubs, athletic training centers and competition sites in Winnipeg. The Manitoba Sports Federation and individual sport associations received letters and an information package encouraging athlete participation. Provincial and local sport newsletters included recruitment information. The general information included a brief description of the research, the extent of subject participation and eligibility criteria: at least 18 years old, moderate to high training load, and non-smoker. Additional non-athletic subjects were recruited as described above and through recommendation by those currently registered in the study.

The purpose of the study, the extent of subject involvement, and exclusion criteria were reviewed upon initial contact,with prospective subjects. Female non-smokers in the age range of 18-48 years were included in the initial data collection. All postmenopausal, perimenopausal, pregnant and lactating females were also excluded from this study. The investigation process, testing sites, testing procedures and potential risks to subjects were explained. Subjects were informed of their right to deny consent or to withdraw, without prejudice, from the investigation at any time. Subjects were requested to sign a voluntary consent form (Appendix A) and to complete a brief screening survey on physical activity, menstrual history and fracture (Appendix B). Personal results and general findings of the investigation were promised to all study participants. No payment was offered for participation. However parking costs for bone densitometry appointments were reimbursed. After receipt of written consent and the preliminary survey, subjects were registered then contacted regarding further participation.

There were 175 responders. Of these, 158 were registered and underwent the first phase of the study, which was bone densitometry. Young adolescent athletes and older women whose ages were not within the selected age range of 18-48 y underwent bone mineral density measurements but these data were excluded. The age matching of control groups with the anorectics required that the data of four young subjects be included in only those data. The data on two black athletes were excluded. There were 136 subjects eligible for inclusion. However, 130 remained after 6 subjects withdrew from the study for various reasons.

The final sample size for the general investigation of physical activity and bone mineral density was 130. These subjects were included in the second phase of the study and were scheduled for an appointment which included a complete anthropometric assessment (Appendix C) and an interview during which four extensive questionnaires were completed. These were: a general medical interview (Appendix D), a menstrual history (Appendix E), a physical activity (EIA) questionnaire (Appendix F) and a dietary calcium survey (Appendix G). There were 101 subjects who completed the entire set of anthropometric measurements and of these 95 completed and returned the questionnaires.

The 130 subjects were grouped according to responses from the two surveys regarding physical activity. The athlete group (n=74) included: (i) national high performance stream athletes (ii) university athletes (iii) elite provincial athletes and (iv) master's athletes and (v) and former elite athletes. All subjects included in the athlete group maintained a high level of activity. The non-athlete group (n=56) was composed of those who were excluded from the athlete category and who recorded low to moderate past and current activity levels. Current activity levels of not more than four hours per week of regular planned activity fulfilled the low to moderate category. Past activity levels were computed and those who exceeded 200 hours of planned physical activity per year were considered athletic whereas those participating less than 100 hours per year in childhood were classified as non-athletic. Past and current activity levels provided information regarding lifetime physical activity.

3.0.1 Athletic Amenorrhea

Subjects were categorized on the basis of reported menstrual status: amenorrhea, oligomenorrhea and eumenorrhea. These categories were defined as: amenorrhea, not more than 3 menses per year and not more than 1 period in last 6 months of the study; oligomenorrhea, 3-10 menses per year and only 1 period within the last 3 months; and eumenorrhea, 10.5-13.5 menses per year and menstrual cycle of 27-35 day intervals (3-7 days menses). Menstrual dysfunction due to training or weight loss was a criterion. Use of oral contraceptives was documented and current users were initially included with the eumenorrheic group then further analysed as a separate group.

The third phase of the study required subjects to have blood drawn for sex-steroids, cortisol, prolactin, osteocalcin and parathyroid hormone assays. The sex-steroid assay results confirmed menstrual status and provided further information regarding menstrual dysfunction for those subjects who completed this phase of the investigation.

3.0.2 Anorexia Nervosa

The anorectic subjects were patients of the Eating Disorders Clinic at the Health Sciences Center, Winnipeg, Manitoba. A preliminary list of 22 Caucasian females who met the third edition Diagnostic and Statistical Manual criteria for Anorexia Nervosa (American Psychiatric Association, 1987) was formed. The essential features of anorexia nervosa are: (i) refusal to maintain a minimal body weight optimal for age and height, (ii) intense fear of gaining weight or becoming fat in spite of being underweight, (iii) a distorted body image and (iv) amenorrhea defined as the absence of more than three consecutive menstrual cycles.

All patients signed a consent form to release medical information for the purpose of research. We included patients who had undergone bone mineral density measurements (DPA). We excluded patients with less than one year duration of anorexia. Restrictive and bulimic anorectics were included in this study. The characteristics of bulimia are: recurrent

episodes of binge eating and a feeling of lack of control of eating behaviour (American Psychiatric Association, 1987).

There remained a group of 12 anorectic patients whose ages ranged from 15.6-38.5 years at the time of the skeletal assessment as described above. Three subjects were in-patients at the time of measurement. Data regarding onset and duration of eating disorder, menstrual and reproductive histories and lifestyle risk factors for osteoporosis were obtained through review of medical records.

Twelve subjects were selected, on the basis of age, from both the oligo/amenorrheic athletes and the normal controls in the larger pool of 158 subjects described above. Similarly, four younger age-matched subjects, aged 15-16 years were selected from the group of young subjects excluded from the general data analyses. Of those who were eumenorrheic and reported sedentary to low levels of current physical activity (not more than 30 minutes per day, three times per week of low intensity activity), 12 were age-matched to form the normal group. Similarly, of those elite athletes who reported amenorrhea and/or oligomenorrhea, 12 were age-matched to the anorectics to form the menstrually dysfunctional group (oligo/amenorrheic).

3.0.3 Longitudinal Study

Of the 95 subjects who had completed the second phase of the larger investigation and who lived in Winnipeg, 73 were contacted by mail and/or telephone for remeasurement on the DPA. Responses were received from approximately 40 subjects. Subjects were screened to determine if there were any changes, since their commencement in the study, in their general health, weight, diet, physical activity and medications. Primarily due to scheduling restrictions, 27 confirmed appointments but only 23 subjects completed this phase of research. Of this group, 16 were athletes and 7 were non-athletes. Due to the small sample of non-athletes, only the data for the athletes were included.

The longitudinal study was designed to investigate bone loss and compare these results with the results of the cross-sectional estimation of bone loss. Because cross-sectional studies do not yield a real measure of

bone loss or bone gain, the longitudinal research provided additional information to verify the athletes' skeletal state.

A summary of the data collection is included in Table 3.1.

Table 3.1. Summary of investigations and data collection
(The numbers in parentheses represent the data included in analyses where different from collection number.)

Cross-sectional Data	Investigation	Subjects
	General	
	Amenorrhea Survey	158
	(I) Bone densitometry	158 (130)
	(II) Anthropometry	101 (98)
	(II) Surveys	95
	(III) Assays	65 (62)
	Athletic Amenorrhea	95
	Anorexia Nervosa	12
Longitudinal Data		
	Repeated measurement	23 (14)

3.1 Experimental Design

The main investigation was cross-sectional and descriptive. The primary focus was the dependent variable of BMD and its relationship to the independent variables of age, physical activity, anthropometric indices, body composition indices, menstrual status and hormonal (particularly estrogenic) milieu, and dietary influences (particularly calcium). Cross-sectional investigation of bone mineral density over the range of ages represented by subjects is recognized as inferior to more definitive prospective studies on age-related bone loss. Time and financial constraints have limited this investigation to the cross-sectional method of examining bone loss with age. However, a small sample consisting of 23 subjects consented to undergo a repeated bone mineral density measurement by

DPA to determine any changes in bone mineral density. This sample was representative because it consisted of athletes and non-athletes who were enrolled in the larger study. Hypothetically, the longitudinal data would confirm the results of the cross-sectional data. The small number of subjects, the time between measurements and the precision of the DPA have moderated the conclusions from this study.

Due to the dependence on self-reported, historical data and large inter-individual variation in the continuous variables concerning physical activity, menstrual cycles, assays and calcium intake; several categorical variables were composed. Thus, categories such as athletic and nonathletic, eumenorrheic and oligo/amenorrheic, high and low serum estradiol and high and low calcium intake have been utilized. The use of oral contraceptives was recognized as a possible confounding factor. Therefore, such use was documented.

The investigation which compared age-matched anorectics, amenorrheic athletes and normal controls was also cross-sectional and descriptive in nature. The usual criticisms of cross-sectional investigations of non-randomization and self-selection bias are recognized. However, the estimation and attainment of a sufficient number of subjects strengthens the validity of conclusions based on these cross-sectional data. One advantage is that all subjects were selected from the same population, white Canadian premenopausal females, thus decreasing variability and increasing generalizability of results.

3.2 Procedures

3.2.0 Bone Mineral Density Measurements

Bone mineral density of the lumbar spine (L2-4) and the femoral neck (FN), Ward's triangle (WT) and trochanter (FT) were measured by dual photon absorptiometry (DP3, Lunar Radiation Corp., Wisconsin). The dual photon absorptiometry (DPA) measurements and analyses were performed by a Registered Technologist in the Nuclear Medicine Department at the St. Boniface General Hospital, 409 Tache Avenue, Winnipeg. Radiation exposure with this DP3 is approximately 10 mrems from the dual energy isotope ^{153} Gadolinium.

Precision and accuracy of this instrument were assessed previously and found to be similar to literature reports, 1% and 3% respectively (Chan, Freuhm & Lee, 1987). In preparation for this study, the author conducted a pilot study to determine the *in vivo* precision of this DPA instrument in a sample of healthy premenopausal females. This investigation of BMD sites found long term *in vivo* precision to be: L2-4= 2.0% , FN= 3.1%, WT= 3.4% and FT= 4.7%.

3.2.1 Surveys and Questionnaires

General information regarding physical activity and menstrual status was collected on the initial screening survey and by personal communication. The surveys and questionnaires were piloted in a group of 4 subjects. Minor text and format changes were made in the menstrual and general medical surveys. Extensive data were registered via questionnaires on physical activity, caloric and calcium intake, and medical/menstrual history. Subjects reported on the frequency, length, and type of early and later childhood activity. Childhood and adult activity levels and perception of fitness levels were gauged from low to high on a five point scale. A checklist of annual participation in recreational activities and organized sports collected information on the parameters of current physical activity. Where applicable, subjects completed a survey of type, intensity, frequency and periodization of training programs. The nutritional survey elicited

general dietary information regarding current and past calcium and caloric intake. Only the data on dietary calcium intake will be reported. The subjects were grouped according to past and current dietary calcium intake with high being the equivalent of 800 mg/day or more, medium being 400-799 mg/day and low being less than 400 mg/day. Calcium supplementation was recorded. The Medical/Menstrual surveys consisted of questions regarding general medical history, reproductive and menstrual history and fracture information.

3.2.2 Anthropometric Measurements

The anthropometric measurements; height, weight, body girths, skinfolds, and bone breadths were taken at the Sports and Exercise Sciences Institute at the Max Bell Center, Fort Gary Campus, the University of Manitoba. Three pilot testing sessions were completed: the last session included 4 subjects. Anthropometric measurements (Appendix F) were made in accordance with standardized methods (Ross et al, 1982) (Lohman, Roche & Martorell, 1988). Skinfolds measured were: triceps, subscapular, iliac crest, supraspinale, abdomen, anterior mid-thigh and medial calf. Girths measured with a metal tape were: head, neck, mid-brachium, forearm, wrist, chest, waist, gluteal, upper thigh, mid-thigh and calf. Selected girth and corresponding skinfold measurements provided indices of muscularity (Martin, Spenst, Drinkwater & Clarys, 1990). Bone breadths measured by calipers were: distal humerus, wrist, distal femur and ankle. Weight was measured on a calibrated electronic scale. Each measurement except weight was made twice by the same observer: a third measurement was made if the difference between the first two values exceeded predetermined values. Means of these measurements were used for all analyses. Height and weight as reported at the time of bone densitometry were used for those subjects who did not complete the extensive anthropometric data collection (n= 35).

Several measures were derived or estimated from the data collected by anthropometry. Body mass index (BMI) was derived by weight (kg) divided by height (m), squared. A waist to hip ratio, an indicator of fat distribution, was determined from the body circumference measurements

(Kissebah et al, 1982). An estimate of body density was derived from skinfold measurements by a previously reported method (Jackson, Pollock & Ward, 1980). The per cent body fat was calculated according to the Siri equation (Siri, 1961). The sum of six skinfolds, which included the thigh measurement, was also used as a measure of fatness. The estimate of muscle mass was determined by a previously described procedure (Martin et al, 1990).

3.2.3 Blood Samples and Assays

A total of twenty milliliters (20mL) of blood were drawn into four glass serum separator tubes by venipuncture of the antecubital vein without use of a tourniquet. Blood samples (mid-follicular phase; days 7-9) were taken between 0800-0830 am after an overnight fast, in a non-exercised state, by a Registered Nurse at the Endocrinology and Metabolism Clinic (Health Sciences Center, 700 William Avenue, Winnipeg). Amenorrheic subjects' blood samples were taken randomly. For these subjects, the dates of last normal cycle and resumption of next menses were recorded. Samples were collected according to the Health Sciences Center's Endocrinology and Chemistry Laboratories protocols. Aliquots of serum were stored at the Endocrinology and Metabolism Laboratory at -20° C until analysed. The PTH and calcium panel assays run by the Chemistry Laboratory were not batch-processed and the analyses were done as samples were submitted. The Clinical Chemistry assays were: parathyroid hormone [Allegro® Intact PTH, Nichols institute Diagnostics, San Juan Capistrano, California, CA 92675) and a calcium panel assay of total calcium, an ionized calcium, albumin, phosphate, and creatinine. The inclusion of the calcium panel assays permitted the parathyroid hormone assay to include an interpretive report. Analysis of calcium panel assays was by standard automated analysis on a Hitachi 717 computer. The laboratory reported inter-assay coefficient of variation to be less than 5% for the calcium panel assay. The inter-assay coefficients of variation for two PTH levels were approximately 10% (50 pg/mL) and 6-7% (150 pg/mL).

The endocrine assays were performed at the Endocrinology and Metabolism Laboratory by a research technician. The serum specimens were analysed for: estradiol (E2), estrone (E1), luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin (PRL), cortisol (C) and osteocalcin. Total serum estradiol concentration was determined by commercially purchased solid phase double-antibody ^{125}I-radioimmunoassay kits [DPC Diagnostic Products Corp., Los Angeles, CA 90045]. Total serum estrone concentration was determined by an in-house extraction method ^{3}H-radioimmunoassay. Total serum LH, FSH and PRL concentrations were determined by commercially purchased solid phase fluoroimmunoassay (FIA) kits [DELFIA®; Wallac Oy, Turku, Finland SF-20101]. Total serum Cortisol was determined by commercially purchased solid phase ^{125}I-radioimmunoassay (RIA) kits [Coat-a Count®; DPC Diagnostic Products Corp., Los Angeles, CA 90045]. Total serum Osteocalcin concentration was determined by commercially purchased solid phase ^{125}I-radioimmunoassay purchased kits [Incstar®; Incstar Corp., Stillwater, MN, 55082; and BTI®, Biomedical Technologies Incorporation, Stoughton, MASS. 02072].

All assays were run in duplicate and each assay was compared to kit or laboratory quality control samples. There were five assay series for E1, three for Cortisol and two assay series each for E2, PRL, LH, and FSH. There were two assay series for the Incstar® osteocalcin assay which were repeated due to poor quality control. There was one series for the BTI® osteocalcin assay. The osteocalcin assays were not successful for undetermined reasons although sample preparation is a likely factor. Thus, the osteocalcin data will be excluded from the study. For all other analyses except E2, the intra-assay coefficients of variation were within laboratory standards of 4-6%. Inter-assay variation generally ranged from 6-10% with no significant differences noted over the time of the assay nor over the past year. The E2 assay showed an inter-assay variation of 22.8% for low levels (114 pmol/L) and 10.2% for high levels (940 pmol/L).

3.3 Ethics

This study received approval from the University of Manitoba's Faculty of Medicine Committee on the Use of Human Subjects in Research. Written consent for participation in this study was obtained from all subjects. The access to medical information was approved by the Director of Research and was conducted in accordance with the research policies of the Health Sciences Centre.

3.4 Statistical Analyses

Statistical analyses were performed using StatView SE and SuperAnova 1.1 software (Abacus, Berkeley, California). All levels of significance were based on two-tailed tests, except where indicated and probability values below 0.05 were considered significant.

Means and standard errors were calculated for the anthropometric measurements, body mass index, % body fat, bone mineral density measurements and the assay data. Unpaired t-tests (two tailed) were utilized to determine if there were any significant differences between the means of the athletic and the control group. Linear regression analyses were performed to investigate the relationship between age and bone loss. An analysis of covariance (Statistics Advisory Bureau, University of Manitoba) was used to test if the interaction of age and bone loss was significantly different between groups. In the analyses of data concerning bone mineral density and other independent variables the parametric tests included: unpaired t-tests, one-way analysis of variance, two-factor analysis of variance and linear regression analyses. Chi-square analysis and Spearman rank correlations were used to determine the relationships between categorical variables.

Means and standard errors for anthropometric measures, body mass index and bone mineral density values were calculated for the anorectic patients and the two comparison groups. Differences between groups were determined by analysis of variance and, where significance was indicated, the Scheffé or Fisher post-hoc tests were employed to determine significant differences between means.

3.5 Presentation of Results

The results and discussion are presented in the next three chapters. Chapter 4 includes the results of the general cross-sectional data on age and activity. This section investigates the model of bone loss in healthy premenopausal women and the parameters of that model: site, onset, and rate of bone loss. The results of the variable, physical activity, are then presented. Bone mineral density, models of bone loss, anthropometry, fractures and dietary calcium are compared in non-athletes and athletes. Results of the longitudinal study of BMD in athletes concludes the results segment. A discussion concerning the preceding results completes the fourth chapter.

Chapter 5 presents and discusses the results of the cross-sectional data on menstrual status and bone mineral density. Also groups defined by menstrual status and athletic status are compared regarding menarche, parity, oral contraceptive use, fracture history, anthropometry and dietary calcium. A section of assay results follows. A discussion of the results pertaining to menstrual status and reproductive history completes this chapter.

The sixth chapter includes the results and discussion of the investigation of bone mineral density in anorectics, oligo/amenorrheic athletes and normal controls. The discussion comments on the pattern and degree of bone loss, fractures, duration of the disease and peak bone mass in anorexia nervosa and athletic amenorrhea.

4. AGING AND PHYSICAL ACTIVITY: RESULTS AND DISCUSSION

4.0 Results

4.0.0 Description of subjects

The subjects were 130 healthy, normal premenopausal women aged 19-47 years. These subject data are displayed in Table 4.1.

Table 4.1. General and anthropometric characteristics of 130 healthy premenopausal female subjects

Variable	Mean ± S.E.
Age (y)	31.1± 0.7
Height (cm)	164.5 ± 0.66
Weight (kg)	57.9± 0.62
Body Mass Index (kg/m^2)	21.4± 0.22
Body Fat (%) (n=98)	20.7± 0.64
Sum of 6 Skinfolds (mm) (n=98)	92.3±4.2
Waist to Hip Ratio (n=98)	0.73±0.004

4.0.1 Bone Mineral Density

The BMD values of the subjects are presented in Table 4.2. The lumbar spine values are similar. The femoral values are significantly higher ($p<0.0002$) than the published norms for 248, 30-39 year-old, women in the United States {1.26 (L2-L4), 0.99 (FN), 0.91(WT), 0.80(FT), g/cm^2 }(Mazess et al, 1987a).

Table 4.2 BMD data of normal premenopausal female subjects aged 19-47y, expressed as mean±SE

	BMD (g/cm^2)	Number
Lumbar spine (L2-L4)	1.25±0.01	130
Femoral neck	1.04±0.01	128
Ward's triangle	0.96±0.01	127
Trochanter	0.88±0.01	128

4.0.2 Age

Age and bone mineral density were investigated in 130 subjects by least squares linear regression analysis and found that the relationship was not significant at the lumbar spine (Figure 4.1) but was significant at all three femoral sites: FN, $p<0.0001$ (Figure 4.2); WT, $p<0.0001$ (Figure 4.3); FT, $p<0.05$ (Figure 4.4).

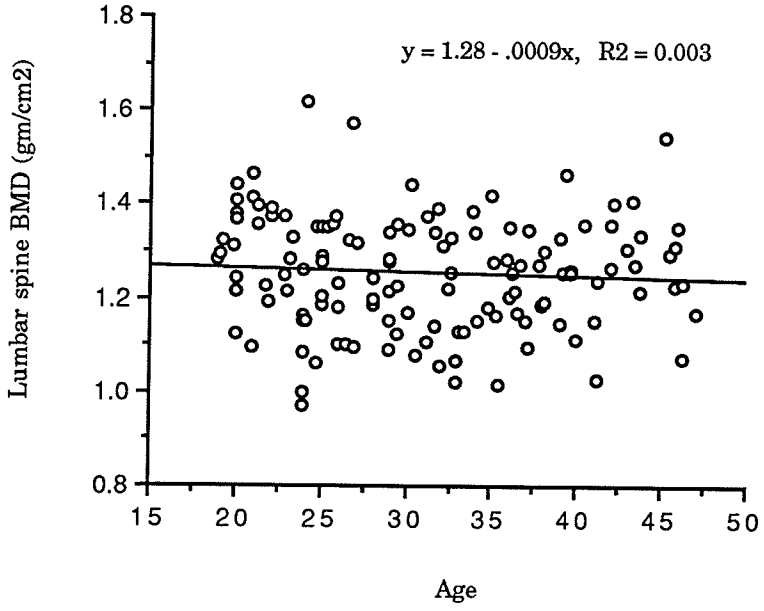

Figure 4.1 The relationship of lumbar spine bone mineral density and age in 130 healthy premenopausal females

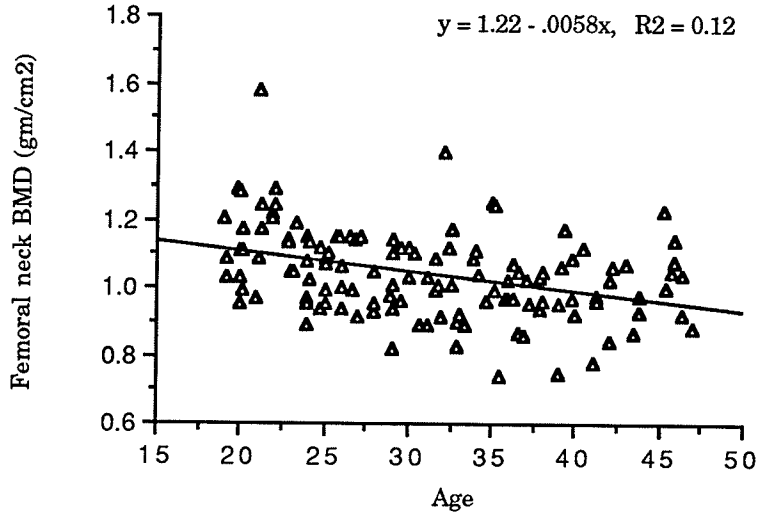

Figure 4.2 The relationship of femoral neck bone mineral density and age in 128 healthy premenopausal females

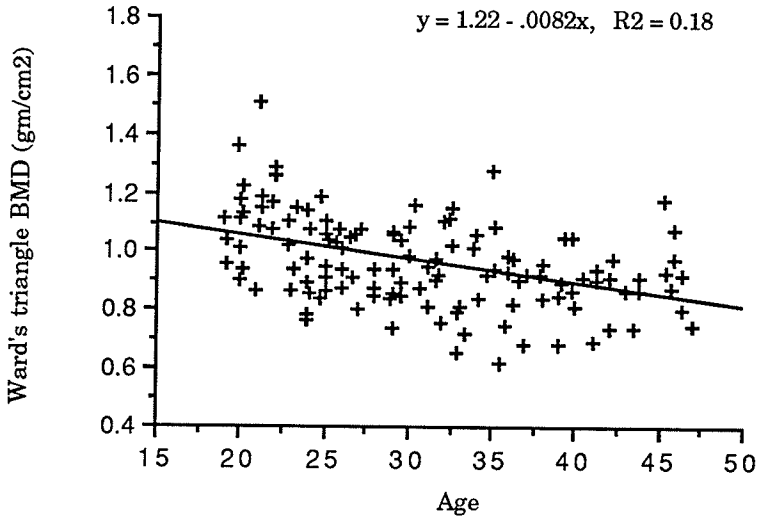

Figure 4.3 The relationship of Ward's triangle bone mineral density and age in 127 healthy premenopausal females

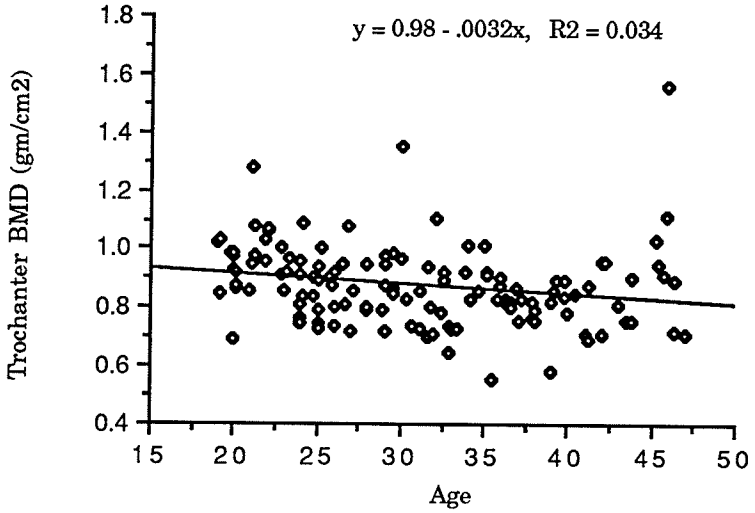

Figure 4.4 The relationship of trochanteric bone mineral density and age in 128 healthy premenopausal females

A second order polynomial regression showed results similar to linear regression analyses with no significance at the lumbar spine and significance at all femoral sites: FN, $p<0.0001$; WT, $p<0.0001$; FT, $p<0.001$.

Subjects were then regrouped by age: 21.0 or less, 21.1-24.9, 25.0-29.9, 30-34.9, 35-39.9, and 40 or more years. BMD measurements are presented in Table 4.3. There were no significant differences between age groups in height and weight.

Table 4.3 BMD of healthy premenopausal women by age intervals (mean±SE)

	<21y (n=14)	21-24 (n=20)	25-29 (n=28)	30-34 (n=24)	35-39 (n=23)	40 + (n=20)
L2-L4	1.31±0.03	1.24±0.04	1.25±0.02	1.23±0.03	1.24±0.02	1.27±0.03
FN	1.14±0.04	1.10±0.03	1.03±0.02	1.03±0.03	0.99±0.02	0.99±0.03
WT	1.10±0.05	1.03±0.04	0.96±0.02	0.95±0.03	0.89±0.03	0.89±0.03
FT	0.95±0.04	0.93±0.02	0.87±0.02	0.86±0.03	0.82±0.02	0.88±0.05

There were no significant differences in L2-4 BMD among these age groups (Figure 4.5). At all femoral sites, BMD decreased as the mean age increased. An analysis of variance of FN BMD and age groups was significant, $p<0.0007$ (Figure 4.6). A post-hoc Scheffé test showed that the BMD was significantly lower in both the two older groups compared to the youngest group. The same was true of the WT site as the analysis of variance was significant ($p<0.0001$) and the post-hoc Scheffé test indicated results identical to the FN differences (Figure 4.7). At the FT, the analysis of variance was significant ($p<0.05$) but post-hoc analyses showed no significant differences between groups (Figure 4.8).

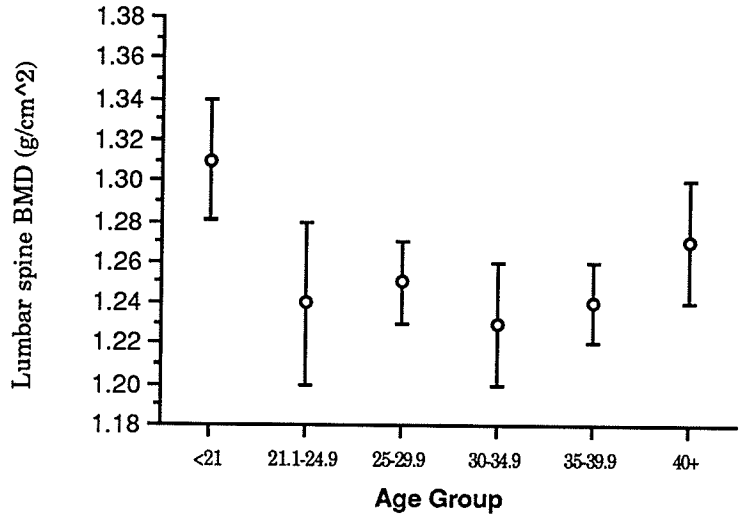

Figure 4.5 Lumbar spine BMD in six age groups of healthy premenopausal women (mean ±SE)

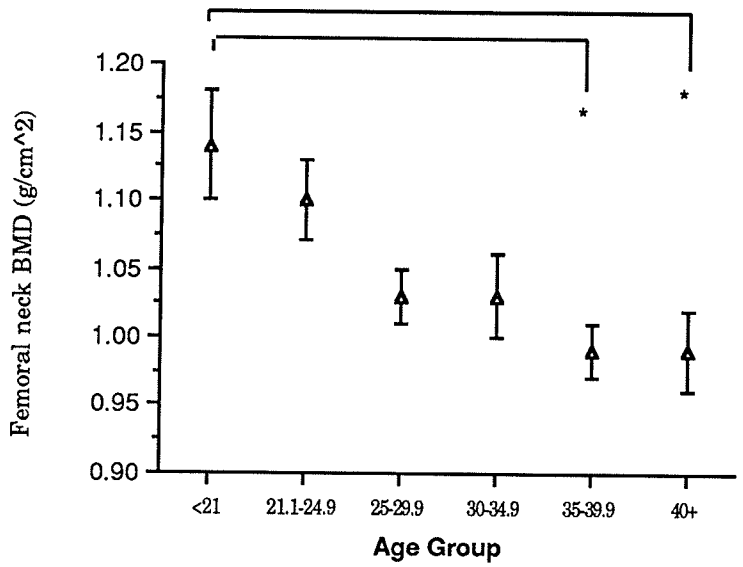

Figure 4.6 Femoral neck BMD in six age groups of healthy premenopausal women (mean ±SE)
* denotes significant difference between groups connected by brackets

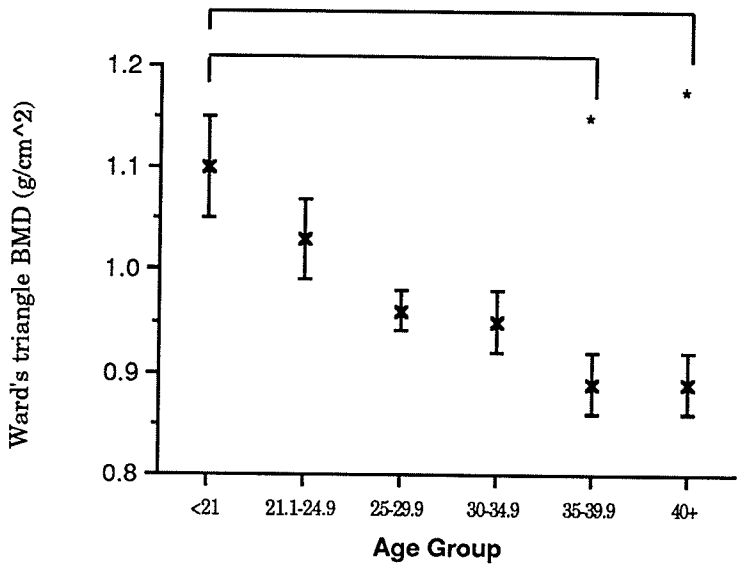

Figure 4.7 Ward's triangle BMD in six age groups of healthy premenopausal women (mean ±SE)
* denotes significant difference between groups connected by brackets

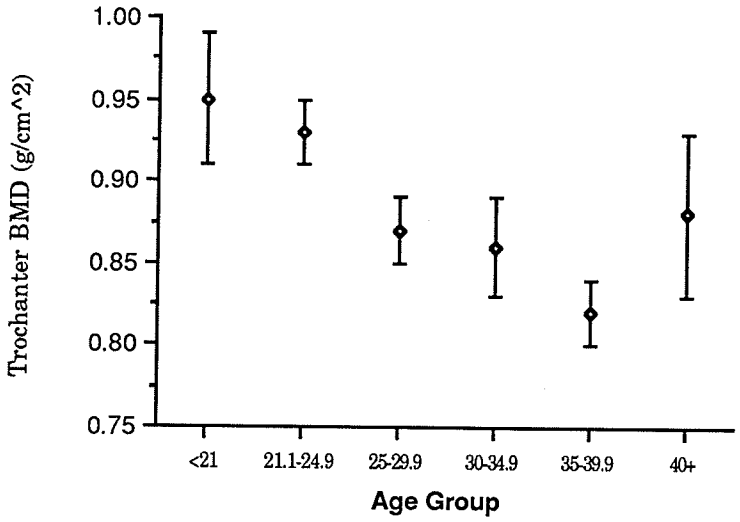

Figure 4.8 Trochanteric BMD in six age groups of healthy premenopausal women (mean ±SE)

4.0.3 Height and Weight

A least squares regression analysis was used to determine the relationship of height and weight to BMD in all regularly menstruating subjects (n=101). These correlation coefficients and significance levels are presented in Table 4.4.

Multiple regression equations showed that weight was the most significant predictor of BMD at all sites ($p<0.05$) except at the FT where the p-value bordered on significance ($p=0.06$). Thus, the variance in BMD at these sites explained by height is not independent of the variance explained by weight since height and weight are significantly correlated.

Table 4.4 The significance of height, weight and body mass index as predictor variables of BMD in healthy premenopausal women

Site	Height	Weight	Height/Weight	BMI
L2-L4	r=0.04, n.s.	r=0.21, p<0.05	r=0.22, n.s.	r=0.18, n.s.
FN	r=0.27, p<0.01	r=0.29, p<0.005	r=0.34, n.s.	r=0.08, n.s.
WT	r=0.19, p<0.06	r=0.29, p<0.005	r=0.30, n.s.	r=0.14, n.s.
FT	r=0.07, n.s.	r=0.20, p<0.05	r=0.20, n.s.	r=0.15, n.s.

A stepwise regression analysis of age, height and weight on BMD was calculated for each site. Variables were entered into the regression equation if an F value of 4.0 was reached. Weight, the only significant variable entered (F=9.9), explained 27% of the variability at the lumbar spine. Age (F=17.8) and weight (F=15) were both significant variables at the femoral neck with age accounting for 35% of the variability and weight an additional 9%. At Ward's triangle, age (F=26.9) and weight (F=20.2) were again significant predictors of BMD. Together these variables explained approximately 50% of the variation

with 42% related to age. At the trochanter, weight explained 22% of the variation and was the only significant variable (F=6.5).

4.0.4 Body Mass Index

An index of body ponderosity (BMI) was calculated from height and weight (n=101). Bone mineral density measurements at all sites were positively but not significantly related to BMI (Table 4.4).

4.0.5 Body Girths and Skinfold Measurements

Waist to hip ratio Waist and hip circumferences were used to calculate a waist to hip ratio (WHR), a commonly-used measure of the body's fat distribution. The WHR for 98 subjects was 0.72±0.004 (mean ±SE). There was no significant relationship between bone mineral density measurement at any site and waist to hip ratio.

Skinfolds No single skinfold measurement was significantly related to bone mineral density at any site. The sum of six skinfolds was not significantly related to BMD at any site. The skinfold measurements were used to predict body fat percentage.

Per cent body fat The per cent body fat was not significantly related to BMD at any site. The relationship between per cent body fat and the waist to hip ratio was significant ($r=0.25$, $p<0.05$). Per cent body fat and Body Mass Index were significant related ($r=0.73$, $p<0.0001$).

Muscularity Indices of muscularity for the triceps, calf and mid-thigh were calculated from selected body girth and skinfold measurements. The results of the analyses of the relationships between forearm girth and the indices of muscularity to BMD are presented in Table 4.5. The relationship between bone mineral density and the triceps muscularity index was significant at L2-L4 ($r=0.22$, $p<0.05$) and approached significance

at FN (r=0.19, p<0.06). The correlation between the thigh muscularity index was significant only at the FN (r=0.23, p<0.05). The muscularity index of the calf was not significantly related to any bone mineral density measurement.

Table 4.5 The relationship of BMD to forearm girth and muscularity indices of the triceps, mid-thigh and calf

Site	Forearm	Triceps	Mid-thigh	Calf
L2-L4	r=0.15, n.s.	r=0.22, p<0.05	r=0.15, n.s.	r=0.04, n.s.
FN	r=0.11, n.s.	r-0.19, p<0.06	r=0.23, p<0.05	r=0.04, n.s.
WT	r=0.13, n.s.	r=0.14, n.s.	r=0.14, n.s.	r=0.02, n.s.
FT	r=0.09, n.s.	r=0.13, n.s.	r=0.07, n.s.	r=0.0003, n.s.

An estimate of muscle mass was calculated according to an equation derived from male cadaver data (Martin et al, 1990). The present subjects' muscle mass estimates ranged from 19.5-42.0 kg with a mean of 29.2± 0.4. The relationship between muscle mass and weight was analysed by linear regression and showed that the two variables were highly correlated (p<0.0001) with muscle mass explaining 42% of variation in weight.

Linear regression analyses illustrated that the muscle mass estimate was positively correlated to BMD: L2-L4, r=0.20, n.s., FN, r=0.30, p<0.05, WT, r=0.20, p<0.001 and FT, r=0.10, n.s. These correlations were similar at L2-L4 and FN as those reported for weight (Table 4.4). However, weight was clearly a better predictor than muscle mass of BMD at the WT and FT. Thus, an estimate of total muscle mass is generally not as highly correlated with BMD as is weight. However, regional muscularity was shown to provide a good indication of regional BMD as in: the triceps muscularity index to L2-L4 and the thigh muscularity index to the FN.

4.1 Age and Activity Results

On the basis of reported past and current activity levels, subjects were categorized as athletic (n=74) or nonathletic (n=56). The athletes participated in a wide variety of sports and activities which were primarily aerobic. Although athletes declared the following sport specialties, many were active in several sports: 'aerobics'(n=23), endurance running (n=11), cycling (n=4), track (n=5), team sports (n=3), cross-training activities (n=13), dance (n=1) and swimming (n=1). Several athletes were trained in activities which selectively load the spine, such as gymnastics, rowing and weight training (n=8). A mixed program of aerobic activities and weight training was reported by (n=5) athletes. All of these subjects had specific training schedules which chronicled high levels of activity.

4.1.0 Anthropometric Measurements

The anthropometric data for the athletes and non-athletes is presented in Table 4.6. There were no significant differences between athletes and non-athletes in age, height, weight, BMI and waist to hip ratio.

Per cent body fat The difference between percent body fat of the athletes and the non-athletes was significant ($p<0.0001$). Similarly, the athletes had a significantly lower sum of 6 skinfolds ($p<0.0001$).

Muscularity The estimate of total muscle mass was significantly greater in athletes ($p<0.02$).

Waist to hip ratio In the athletes, the waist to hip ratio was not significantly related to the % body fat ($r=0.21, p<0.073$) however, this relationship was highly significant in the non-athlete group ($r=0.61$, $p<0.0001$).

Table 4.6 General and anthropometric data of athletes and non-athletes, expressed as mean±SE

	Athletes	Non-athletes	p value
	n=74	n=56	
Age (y)	29.9± 0.9	32.6± 1.0	n.s.
Height (cm)	165.2± 0.8	163.5± 1.0	n.s.
Weight (kg)	57.6± 0.7	58.4± 1.0	n.s.
Body Mass Index (kg/m^2)	21.2± 0.2	21.9± 0.4	n.s.
Body Fat (%)	18.3± 0.6	24.6± 1.1	p<0.0001
Sum of 6 Skinfolds (mm)	77.6±3.1	116.6±8.3	p<0.0001
Waist to Hip ratio	0.726±0.004 (61)	0.727±0.008(36)	n.s.
Muscle mass (kg)	30.0±0.6 (61)	27.8±0.6 (36)	p<0.02

4.1.1 Bone Density Measurements

The athletes had significantly higher BMD than the non-athletes at all four sites (Table 4.7). The values for the non-athletes are not significantly different from the published norms for 248, 30-39 year-old, women in the United States {1.26 (L2-L4), 0.99 (FN), 0.91(WT), 0.80(FT), g/cm^2 }(Mazess et al, 1987a). Compared to the non-athletes, the athletes' BMD was 7-9% higher at the proximal femur sites and 3.8% higher at the lumbar site.

Table 4.7 BMD (g/cm^2) of the athlete and non-athlete groups, expressed as mean±SE

Site	Athletes	Non-athletes	p Value
L2-L4	1.28 ± 0.01 n=74	1.23 ± 0.02 n=56	p<0.05
Femoral neck	1.07 ± 0.01 n=74	0.99 ± 0.02 n=54	p<0.001
Ward's Triangle	1.00 ± 0.02 n=73	0.92 ± 0.02 n=54	p<0.05
Trochanter	0.91 ± 0.01 n=74	0.83 ± 0.02 n=54	p<0.001

4.1.2 Age and Bone Mineral Density

The cross-sectional data were analysed to clarify the relationship between age and site-specific BMD loss. At the L2-L4 region in non-athletes and athletes, the slopes of the regression lines were not significantly different from zero. At all three femoral sites, the non-athletes showed a significant decline of BMD with age, ($p \leq 0.06$ at FT) whereas the athletes' BMD loss was significant only at WT (Table 4.8).

Table 4.8 Regression line data (intercept and slope) of the relationship of bone mineral density (y) and age (x) in premenopausal athletes and non-athletes

Site	Athletes	p value	Non-athletes	p value
L2-L4	1.25-0.004x	n.s	1.26-0.001x	n.s
Femoral neck	1.17-0.003x	n.s.	1.23-0.007x	<0.001
Ward's triangle	1.15-0.005x	<0.05	1.26-0.01x	<0.0001
Trochanter	0.94-0.001x	n.s.	0.97-0.004x	≤0.06

At the FN, the athletes' rate of bone loss was 0.003 g/cm^2/y which was found to be non-significant. The non-athletes' rate of bone loss, 0.007 g/cm^2/y was significant ($p<0.005$) (Figure 4.8). The non-athletes' rate of bone loss at the FN was significantly greater than that of the athletes ($p=0.007$).

At WT, both groups' rates of losses were significant: athletes' 0.005 g/cm^2/y and non-athletes' 0.010 g/cm^2/y. The non-athletes' WT rate of bone loss was greater than that of the athletes but the difference was found not to be significant.

At the FT site, the athletes' rate of bone loss 0.001 g/cm^2/y and the non-athletes' rate of bone loss 0.004 g/cm^2/y were non-significant. However significance was narrowly missed in the non-athletes' group ($p \leq 0.06$). The non-athletes' rate of loss was not significantly greater than the athletes' rate of loss at the trochanter.

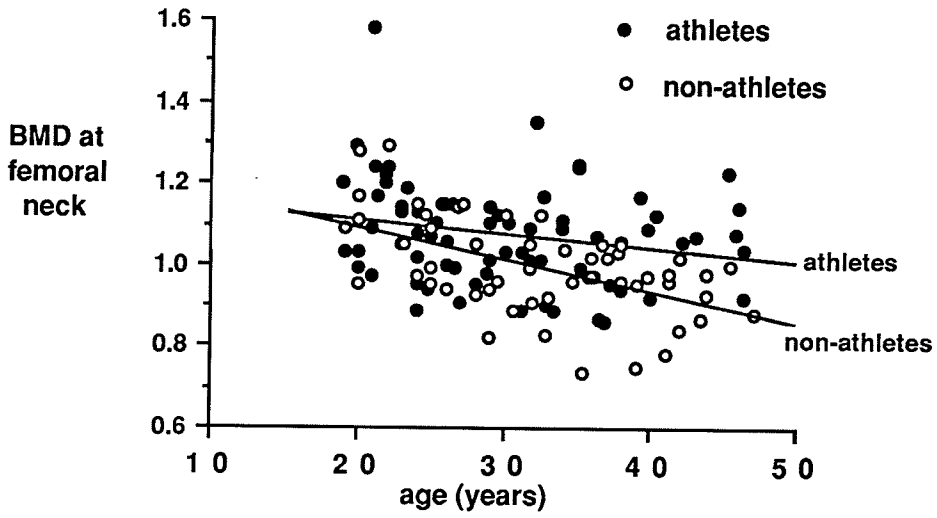

Figure 4.9 Bone mineral density (g/cm^2) at the femoral neck versus age for athletes and non-athletes.
Regression line data for athletes: y=1.17-0.003x, n.s. and Non-athletes: y=1.23-0.007x, p<0.001. The slopes of these two lines are significantly different from each other, (p=0.007).

4.2 Fractures and Stress Fractures Results

The fracture histories of athletes and non-athletes were compared. There were no significant differences between groups for incidence of fractures or stress fractures. The mean number of fractures per subject reported by the groups was: athletes, 0.48±0.11 and non-athletes 0.51±0.15. Subjects reported all lifetime fractures in this category.

The stress or fatigue fractures were reported separately. Five athletes reported one stress fracture and 4 reported two stress fractures. Only one of these subjects had noticeably low BMD. There were only two non-athletes who reported a single stress fracture. The prevalence of stress fractures was higher in the athletes but did not reach significance by Chi-square analysis. Among the athletes, 16.36% reported 1 or 2 stress fractures whereas only 5.41% of non-athletes reported only 1 fracture.

4.3 Dietary Survey Results

The dietary survey, designed to estimate dietary calcium intake, was completed by 95 subjects. This questionnaire elicited information regarding consumption of calcium, fibre, protein, caffeine, alcohol and special dietary regimes. The survey results showed that 9 non-athletes and 7 athletes had used calcium supplements. The supplementation was included in the daily total calcium intake. The mean dietary calcium intake was 600±28 with a range of 140-1600 mg/day.

There was no significant difference between the calcium intake consumed by the athlete and non-athlete groups.

Subsequently, subjects were categorized by high, medium or low calcium intake. This grouping was done for both current and past dietary calcium intake. Those subjects who reported the consumption of 400-799 mg/d were allocated to the medium group. Low and high categories were respectively below and above this criterion. This resulted in the following groups for current calcium consumption: high, n=44, medium, n=30 and low, n=20.

The current high calcium intake group consistently had the highest bone mineral density. The difference between group means was significant at FN and WT ($p<0.01$). The post-hoc Scheffé test revealed that there were significant differences between the means of the low and high categories at FN and WT.

A similar analysis of past calcium intake which regrouped subjects accordingly: high, n=47, medium, n=37 and low, n=9; found similar results. The differences between the group means were significant at FN and WT ($p<0.05$), with past high calcium intake showing the highest BMD at all sites. Only the difference between the medium and high group means was significant employing a Scheffé test ($p<0.05$).

Those subjects who fit the high category, both currently and historically were included in the 'lifetime high' calcium intake group. As only 4 subjects were categorized as 'lifetime low' due to chronic and current low calcium intake, all those not fitting the criteria for the lifetime high category were included in a "low/medium" group.

The differences in BMD between the two calcium groups in the non-athletes were significant at all sites (Table 4.9). In the athletes, only the FT BMD was not significantly different between the calcium groups. Thus, a strong association exists between habitually high calcium consumption and higher BMD.

A two factor analysis examined the effects of lifetime calcium intake and physical activity on BMD (Table 4.9). There were significant differences in BMD between the lifetime high and low group at L2-L4, FN and WT ($p<0.0001$).

The interaction effect of activity status and lifetime calcium was not significant at any site. Of the four groups, the BMD at all four sites was lowest in the low/medium calcium non-athlete group and highest in the high calcium athlete group.

Table 4.9 BMD (mean±SE, g/cm^2) and habitual dietary calcium intake in non-athletes, athletes and all subjects

	LOW/MEDIUM			HIGH		
	Non-athletes (22)	Athletes (38)	**Overall**	Non-athletes (15)	Athletes (20)	**Overall**
L2-L4	1.19±0.02 ^{a}	1.26±0.02 ^{c}	**1.23 ±0.02***	1.29±0.03 ^{a}	1.32±0.02 ^{c}	**1.31± 0.02***
FN	0.96±0.02 ^{a}	1.05±0.02 ^{d}	**1.02± 0.02***	1.07±0.03 ^{a}	1.12±0.02 ^{d}	**1.09± 0.02***
W T	0.86±0.02 ^{a}	0.97±0.02 ^{c}	**0.93 ±0.02***	1.01±0.04 ^{a}	1.04±0.03 ^{c}	**1.03±0.03***
FT	0.80±0.02 ^{b}	0.91±0.02	**0.87±0.02**	0.87±0.03 ^{b}	0.92±0.02	**0.90±0.02**

* significant differences between overall high and low, $p<0.0001$

^{a} significant differences between non-athlete groups, $p<0.01$

^{b} significant differences between non-athlete groups, $p<0.05$

^{c} significant differences between athlete groups, $p<0.05$,n=19 high calcium athlete group

^{d} nonsignificant, $p\leq0.07$

FT, WT & FT; n=14 non-athlete high calcium group : WT; n=19 high calcium athlete group

4.4 Repeated Measurements Results

A group of 16 athletes underwent a repeated bone mineral density measurement session. Two of these athletes had less than 6 months elapse between measurements and their data were therefore excluded from analyses. In the remaining 14 athletes, there was a minimum interval of 9 months between measurements with the time between measurements being 360± 11 days (mean±S.E.). There was no change in height or weight measurements from the first to the last measurement. The athletes reported no major changes in activity, diet or lifestyle factors. The range and mean age of the athletes was 20.9-40.7 and 29.2±1.9 y (mean±S.E.).

Nine were not currently using oral contraceptives. In this group, 5 stated no previous use of oral contraceptives and the 4 past users had terminated use 4-15 years prior to the study. The 5 users of oral contraceptives reported a duration of use from 18-120 months with the median use being 74 months.

The data analysis showed that bone gain was evident at all four sites. The annual rates of bone gain were: L2-L4 =0.041, FN =0.026, WT =0.018 and FT =0.026 g/cm^2. Two batteries of t-tests evaluated the significance of these measured rates of bone gain.

The first tests compared the longitudinal bone gains to the rates of bone loss derived from this investigation's cross-sectional data. The rates of bone gain were significantly different from the athletes' cross-sectional annual rates of bone loss at L2-L4 ($p<0.001$) and neared significance at FT ($p\leq0.06$). Compared to the non-athletes' cross-sectional rates of bone loss per year, the longitudinal rates of bone gain were significant at L2-L4 ($p<0.002$), FT ($p<0.05$) and tending towards significance at FN ($p\leq0.08$).

Secondly, the longitudinal bone loss gains were calculated as percentages and tested against the lowest of the range rates of bone loss suggested by Riggs and Melton (Riggs et al, 1986b), 0.03-0.05% per year. The bone gain in the athletes was significantly different at L2-L4 ($p<0.005$) and FT ($p<0.05$).

4.5 Discussion

The subjects were 130 normal, healthy premenopausal women aged 19-47 years. The age, height and weight of this sample were 31.1± 7.8 y, 164.5±.66 cm and 57.9± 7.1 kg (mean ± S.D.). Compared to the Canadian Standardized Test of Fitness norms, the height of these 20-29 and 30-39 year old subjects matched the 54th and 57.5th percentiles, respectively (Anonymous, 1986). Similarly, the weight for these two age groups was comparable to the 57.5th and 45th percentiles (Anonymous, 1986).

The BMI was 21.4± 2.5 units and body fat was 20.7± 6.3% (n=98). Both of these measures indicate that these subjects are within normal ranges. BMI was similar to the 52nd and 67th per centiles for the 20-29 and 30-39 y age groups (Anonymous, 1986). Body fat in normal women of these age groups is estimated to be approximately 20 and 22% (Durnin & Womersley, 1974).

The mean bone mineral density measurements: L2-L4 1.25±0.01, FN 1.04±0.01, WT 0.96±0.01, and FT 0.88±0.01 g/cm^2 were comparable to the most recent norms for 30-34 year old women: L2-L4 1.29±0.014, FN 1.00±0.002, WT 0.94±0.002 and FT 0.81±0.001 g/cm^2 (Mazess et al, 1991). With regrouping, the BMD values of the present study were similar, with slightly lower L2-L4 values and higher femoral values, to American normative data of BMD by 5-y intervals (Mazess et al, 1991).

The subjects in this study are thus representative of two groups of premenopausal Canadian women. The results from the data of the very active athletes and moderately active non-athletes should have a high degree of generalizability.

4.5.0 Bone Loss and Aging

Lumbar spine Our finding that the lumbar spine site does not show an age-associated diminution in premenopausal subjects confirms the results of recent investigations (Elliott et al, 1990; Mazess et al, 1991; Mazess et al, 1987a; Schaadt et al, 1988) but is contrary to earlier data (Mazess, 1982) (Riggs et al, 1981; Riggs et al, 1986b).

Laitinen's cross-sectional study of 186 premenopausal and 71 postmenopausal women with BMD measured by DEXA showed the highest L2-L4 BMD in the 31-35y females (Laitinen, Välimäki & Keto, 1991). Unlike Rodin's (1990) results, these data did not show a peak in lumbar bone mass in the third decade nor in the early fourth decade (Laitinen et al, 1991). In the data presented here, the youngest age group of 21 year olds had the highest L2-4 BMD and in all other age groups, BMD was lower. Schaadt and Bohr's data (1988) showed that bone loss at the L2-L4 site occurred mainly at menopause. Similarly, in this investigation, there was no evidence of a significant age-related decrease at the lumbar spine in either athletes, non-athletes or overall. Thus, although the present results do not show an extended phase of consolidation for the lumbar spine, these do confirm the maintenance of vertebral bone mass until menopause.

Proximal femur In the non-athlete group there was a significant inverse relationship between age and BMD at the three sites of the proximal femur. This age-related decrement in BMD was significant at only the Ward's triangle region in the athlete group.

Over all subjects, the BMD at the three sites of the proximal femur showed that the 21 and under age group had the highest value and thereafter there was an obvious trend of declining BMD. However, the differences were only significant at FT and WT when contrasting this youngest group to the two age groups after 35 years. Schaadt and Bohr (1988) stated that at the FN there was a significant linear decline from young adulthood to old age and the seventh decade signalled significant bone loss at the midshaft femoral site.

Rodin's data showed no post-adolescent peak in femoral neck BMD and an annual 0.4% rate of loss began in the late 20's (Rodin et al, 1990). In comparison, a higher annual rate of femoral neck bone loss was found in the non-athlete group, 0.7% and an attenuated, non-significant rate in the athletes, 0.3%. With like age categories, the present data showed similar results to Rodin's study in that the youngest group had the highest BMD and the femoral sites showed progressive decrements with age.

Elliot and colleagues (1990) also reported that bone loss at the hip was evident prior to menopause and continued throughout adult life with significant age-related BMD reduction at all three hip sites particularly Ward's triangle.

Laitinen (1991) recently confirmed early adulthood femoral bone loss in a cross-sectional study of 186 premenopausal and 71 postmenopasal women with BMD measured by DEXA. In premenopausal women, peak femoral BMD was approximately 20 years and thereafter a linear dimunition occurred at FN and WT. However, this bone loss was only significant postmenopausally.

In the present study, the effects of age were evident at the highly cortical sites of the proximal femur as opposed to the trabecular vertebral site. However, the femoral regions exhibited a hierarchy of susceptibility which may be dependent upon the proportion of trabecular bone. The Ward's triangle region, the femoral area of highest trabecular content, showed the greatest rate of loss in both groups and the only significant relationship with age for the athletes and non-athletes. This finding may appear contradictory to the finding of no vertebral loss; however site specificity may be the principal factor determining bone loss. The Ward's triangle differs from the lumbar vertebrae in density, composition, architecture and the effects of environmental factors. The Ward's triangle and the trochanter region show the highest variance in skeletal BMD measurements (Mazess et al, 1990a). Mazess (1990) stated that Ward's triangle, a low density area which is more responsive to aging and disease, shows the earliest and highest degree of bone loss.

The results of the present study support the hypothesis that involutional bone loss, prior to menopause is primarily at the proximal femur and that the estrogen deficiency associated with menopause may be the main cause of vertebral bone loss. In the non-athlete group, the rate of loss at the FN (0.007 g/cm^2 per year) was significantly higher than the athletes' rate of loss (0.003 g/cm^2 per year). When this FN rate of loss was calculated to age 40, it signified decrements of 16% for the non-athletes and 7% for the athletes. The calculated loss for the non-athletes is more extreme than Mazess' estimate of a 10% decrease per decade in spinal density in

women prior to 50 years and a 10% BMD decrement at all three femoral neck sites prior to age 40 (Mazess et al, 1987a). The attenuated loss in the athletes supports the association between athletes and higher BMD.

The cross-sectional research design has a major limitation in that the 'rates of bone loss' derived from such data are estimates. Therefore a small longitudinal component was undertaken to confirm the results of the cross-sectional study. The athletes who underwent repeated measurements, showed that they were gaining rather than losing bone. There was no difference in age between the cross-sectional and repeated measurement athlete groups. The amount of bone gain compared to expected bone loss was substantial in absolute terms and when expressed as a percentage. Statistically, bone gain at the lumbar spine and the femoral neck was found to be significantly different from the cross-sectional rates of loss in both the non-athletes and athletes. There were consistent, significant differences at L2-L4 and FN. The prospective data confirmed the conclusion from the cross-sectional research that physical activity attenuates bone loss in premenopausal females.

The measured BMD gain may have been partly due to the non-random subject selection and the small sample size. With repeated measurements, both biological and technical factors may be sources of variation in BMD (Wasnich et al, 1989). Operator technical errors were minimized by employing the same technician to perform and analyse scans.

A technical factor, which may account for a portion of the variation is the drift due to isotope decay. It has been reported that an old source consistently overestimated vertebral BMD with no systematic effect at the hip (Shipp, Berger, Deehr & Dawson-Hughes, 1988). However, this drift was corrected by a revised software edition (Wahner, 1989).

In this investigation, the DPA instrument was calibrated daily and precision of a phantom spine was reported to be 1% (Chan et al, 1987). The source was not changed during the data collection. There was no evidence of drift in from the daily calibration procedures. Thus, isotope decay or source change was an unlikely contributor to variation.

All final measurements of the longitudinal investigation were taken approximately six months after the completion of the the cross-sectional data

collection. The final measures were all taken in one six week period in the late autumn, approximately one year after the first measurement.

Thus, it appears that, in aging premenopausal women, the pattern of bone loss is initiated early in the third decade at the femoral regions and lumbar spine loss is not evident until menopause. At these specific sites, cortical bone loss precedes trabecular bone loss and there is greater cortical involvement. This bone loss pattern may not be solely due to aging itself but other factors such as body weight and physical activity may be influential.

4.5.1 Anthropometry and Bone Mass

In this analysis, an attempt was made to distinguish between those anthropometric variables, such as weight, height, BMI, % body fat and muscularity which have been reported to predict BMD. Weight appears to be an important determinant of BMD (Mazess et al, 1990a; Slemenda et al, 1990b; Stevenson et al, 1989). More specifically, weight has been directly and significantly correlated with L2-4 and FN BMD (Stevenson et al, 1989). Mazess and Barden (1991) estimated the influence of body weight on spinal, femoral and radial BMD to be approximately 0.2-0.3%/kg increase.

In analyses of the present data, weight consistently demonstrated a direct and substantial relationship to BMD. In stepwise regression analyses, weight and age were significant determinants of BMD at FN and WT whereas weight alone predicted a significant portion of variation of L2-L4 and FT BMD. Height, BMI, waist to hip ratio (WHR) and % body fat as single variables were not significant predictors of BMD.

Muscularity may be another predictor of BMD and these data confirmed evidence of the influence of muscle mass on BMD. The triceps muscularity index was significant at only L2-L4, approximating significance at FN and the thigh muscularity index was significant at only the FN. These relationships may be partly explained by the principle of the specificity of loading bones which includes both direct and indirect mechanisms. It is likely that both the direct effect of muscle pulling on bone and the indirect effect of general limb loading increase both muscle mass and BMD. An estimate of total muscle mass was related to all sites,

demonstrating significance at all sites except the trochanter. This is an interesting finding because regional muscle mass may provide a more reliable method than weight by which the effects of physical activity on bone can be evaluated.

The estimate of total muscle mass and weight are inter-related factors with 42% of the variation of weight explained by muscle mass. A forward stepwise regression analysis of all 4 BMD sites separated the effects of weight and muscle mass. At all sites except the trochanter, weight was the only variable entered into the equations : L2-L4, r=0.20, F= 3.96; FN, r=0.32, F= 11.09; and WT, r=0.26, F= 6.72. At all these sites, the contribution of muscle mass other than that explained through its relationship to weight, were non-significant.

4.5.2 Physical Activity

The athlete and non-athlete groups were similar except that the athletes were significantly less fat according to both % body fat and sum of six skinfolds. The means for weight in subjects with complete anthropometry (n=97) were 58.7 ±1.12 and 57.6±0.79, the non-athletes being significantly heavier ($p<0.0001$). Analyses of covariance with % body fat as the covariate, showed that there was a significant difference in weight and waist to hip ratio between the two groups. The adjusted means for weight were non-athletes 56.43 ±1.05 and athletes 59.02±0.77, the athletes tending to be significantly heavier ($p\leq0.05$). There was no significant interaction effect of athlete status and % body fat revealing that the relationship of body fat to weight did not differ between the two groups. These results suggest that although body weight in athletes was less than in non-athletes, this situation was reversed when controlling for % body fat. This means that the fat free mass of the athletes was greater than the non-athletes. This may be an important distinction in relation to loading of bones.

The athletes had a significantly greater estimate of muscle mass than the non-athletes, 30.0±0.6 vs 27.8±0.6 kg ($p<0.05$). This increased muscularity in athletes may partially explain their significantly greater BMD. The relationship of weight to muscle mass was significant in both the athlete ($p<0.0001$) and non-athlete groups ($p<0.01$). However, the proportion

of variation in weight explained by muscle mass in the athletes is 65% compared to 23% in the non-athletes.

The effects of weight and muscle mass on BMD were analysed. Using only these two variables, a forward stepwise regression analysis determined that weight was the significant variable at all sites except the lumbar spine: FN, r=0.41 F=7.04; WT, r=0.39 F=6.16 and FT, r=0.38 F=5.75. Muscle mass was not entered into the equation for any bone site. In contrast, an analysis of the athlete group showed that at all sites except the FN, neither weight nor muscle mass were entered into the equation. At the FN, weight was the only significant variable.

There is a strong relationship between weight and BMD. The major component of weight as it relates to promotion of bone density is muscle mass. As neither weight nor muscle mass are significant predictor variables of athlete BMD, other factors concerning the dynamic application of weight and muscle mass to the skeleton must be considered.

The distribution of body fat has recently been recognized as an important predictor of health status. The classic android ratio has been associated with increased incidence of cardiovascular and diabetes disease. The waist to hip ratio of females, normally lower than males has been regarded as a function of estrogenic action. Thus, the patterning of body fat as it relates to athletic status and menstrual status is an interesting largely uninvestigated topic. The waist to hip mean was significantly ($p<0.0001$) lower in the athletes than non-athletes when controlling for percentage of body fat. The relationship of per cent body fat to waist to hip ratio ($p\leq0.0507$) and their interactive effect ($p<0.0001$) were also significant. Thus, a common slope does not explain the relationship of body fat and body fat distribution in the athletes and the non-athletes.

4.5.3 Peak Bone Mass

To further examine the hypothesis that peak bone mass (PBM) may be established earlier than routinely reported (Gilsanz et al, 1988a), these cross-sectional data were stratified into two age groups: late adolescent (19-21 y) and early adult (25-35y). These sub-groups were selected to be as similar as possible to the ages of the subjects investigated in Gilsanz's study (Gilsanz et al, 1988a). Only 52 women met the age criteria for the older group and this group had a mean age similar to Gilsanz's group (29.5±3 vs. 30.3 y respectively). The 14 youngest subjects were included in the late adolescent group which was older than Gilsanz's adolescent group (20±.6 vs. 15.9 y respectively). There were no significant differences in the anthropometric or BMD measurements between the 6 non-athletes and 8 athletes in the late adolescent group. The early adult group was comprised of 22 non-athletes and 30 athletes and there were no significant differences in mean age, height and weight between these two subgroups. However, there were significantly higher BMD values at FN and FT in the athletes compared to non-athletes in this 25-35 age group.

The adult BMD was significantly lower than the adolescent group at all femoral sites: FN ($p<0.01$), WT ($p<0.001$) and FT ($p<0.05$). This difference was also observed at the lumbar spine but did not attain significance ($p\leq0.054$). A further analysis of groups by athletic status showed that the adolescent's BMD was significantly higher than the adult non-athletes' at all sites ($p<0.001$ to $p<0.05$). There was a very different outcome when the adolescents' BMD was compared to BMD in the adult athletes: only the WT BMD was significantly higher ($p<0.05$).

Although there was a small number of young subjects and there was an imbalance of athletes, this supplementary analysis suggests that PBM may be achieved earlier than the fourth decade and that physical activity may provide a method by which PBM is increased and maintained.

4.5.4 Dietary Calcium

The survey collected data which addressed some of the main points regarding dietary calcium. Almost one half of these subjects reported current calcium intake over the 800 mg/d RDA. These data confirm that a threshold for dietary calcium may exist at approximately 800 mg/d as the high intake group, which met this criterion for both current and past use had consistently higher BMD than the low/moderate designation. The results showed that there are significant differences in BMD at all sites but the FT between habitual high and low/moderate calcium consumers.

Although the athletes and non-athletes did not report significantly different dietary calcium intake, the data analysis revealed that the athlete BMD was higher than the non-athlete BMD in both low and high calcium categories. The higher BMD's were generally associated with high calcium intake as opposed to athletic status. The interaction effect of physical activity and calcium was not significant. However, the highest BMD's at all sites were observed in the high calcium athletes' group.

Several authors have alluded to a dietary calcium level below which significant adverse effects are seen (Mazess et al, 1991; Sowers et al, 1985; Stevenson et al, 1989). The importance of calcium in the growth and development phase has been accepted though not conclusively proven. Matkovic's latest study of calcium intake in young girls did not provide evidence of a critical role for calcium in bone health of youth (Matkovic et al, 1990). However, Cumming's (1990) review concluded that dietary calcium is an important factor in bone mass.

Generally, the use of dietary surveys is acknowledged as being vulnerable to error. The calcium intake survey of this investigation was recommended for use in American female college athletes. Although the survey is appropriate, it is retrospective and investigates dietary calcium with minor consideration of other dietary elements which may influence calcium absorption and/or excretion. Thus, the survey results are presented as an estimate of habitual calcium consumption. The results presented herein of past dietary calcium intake and BMD signified that high consumption was associated with high BMD at all sites. These data

also show that 9 subjects reported a low childhood intake in contrast to 20 in the low category of current calcium intake. This confirms that post-adolescent females generally decrease calcium intake. According to a recent report, calcium was the only nutrient intake which showed a significant decline in females from 20-24y to 35-40y (Mazess et al, 1991). In view of the positive association of dietary calcium and BMD shown herein and elsewhere, ensuring the threshold dietary intake may help prevent osteoporosis. Thus, the reductions of dietary calcium in young adult females warrant renewed and innovative preventative health measures.

5. ATHLETIC AMENORRHEA: RESULTS AND DISCUSSION

5.0 Menstrual and Reproductive History Results

5.0.0 Description of Subjects

The survey information from the amenorrhea and the menstrual questionnaires was used to categorize subjects as eumenorrheic, oligomenorrheic and amenorrheic. The number of missed menses in all subjects ranged from 0 to 174 months. Subjects reported amenorrhea due to pubertal irregularity, cessation of oral contraceptives, pregnancy and lactation and menopause.

Others reported that amenorrhea was related to stress, weight loss and/or intense training. Only this latter group were included and classified as amenorrheic or oligomenorrheic as defined in Chapter 1. From information reported on the surveys, most subjects with menstrual dysfunction did not solely exhibit amenorrhea or oligomenorrhea as they were defined at the initiation of this study. There was substantial intra- and inter-individual variation in menstrual cycle dysfunction. The amenorrheic and oligomenorrheic group were combined because most subjects with menstrual dysfunction could not be defined as both current and past amenorrheics. Thus, the oligo/amenorrheic category was employed to include the spectrum of chronic menstrual dysfunctions reported by athletes and non-athletes. Thus, the oligo/amenorrheic group was not a homogeneous category but heterogeneous with menstrual irregularity ranging from mild symptoms to chronic amenorrhea.

Current users of birth control pills were included in the eumenorrheic category for the initial analyses then analysed separately.

The description of subjects categorized by menstrual status is presented in Table 5.1. Per cent body fat was significantly lower in the oligo/amenorrheics than the eumenorrheics. The estimate of muscle mass was not significantly different between oligo/amenorrheics and eumenorrheics.

Table 5.1 General and anthropometric data of eumenorrheic and oligo/amenorrheic subjects, expressed as mean±SE.

	Eumenorrheic	Oligo/amenorrheic	p value
	n= 66	n=29	
Age (y)	32.8±1.0	30.8±1.4	n.s.
Height (cm)	165.3±1.0	164.1±1.1	n.s.
Weight (kg)	59.1±0.9	55.9±1.0	p<0.05
Body Mass Index (kg/m^2)	21.6±0.3	20.7±0.3	n.s.
Body Fat (%) (93)	22.2±0.8	17.3±0.8	p<0.001
Sum 6 Skinfolds (mm)(93)	101.5±5.7	72.8±4.0	p<0.002
Waist to Hip ratio (93)	0.727±0.005	0.724±0.005	n.s.
Muscle mass (kg) (92)	29.1±0.6	29.0±0.7	n.s.

5.0.1 Menstrual Status and Physical Activity

In the subjects (n=95), there were 29 reporting oligo/amenorrheic status considering solely those reasons concerned with training, stress and weight loss (Table 5.2).

Table 5.2 The distribution of subjects into categories based on information from a physical activity questionnaire and a menstrual history

	Non-athletes	Athletes	Totals
Eumenorrheic	31 (32.7%)	35 (36.8%)	66 (69.5%)
Oligo/amenorrheic	5 (5.3%)	24 (25.2%)	29 (30.5%)
Totals	36 (39%)	59 (61%)	95

The prevalence of menstrual dysfunction, defined here as oligo/amenorrhea was significantly greater in the athlete group (40.7%) compared to the non-athlete group (13.9%) ($p<0.01$). The non-athlete's group included 5 oligo/amenorrheic; only 1 was currently amenorrheic. Of the 24 oligo/amenorrheic athletes, 6 were currently amenorrheic and 2 were chronic amenorrheics.

The regularly menstruating subjects reported that the interval between menstrual cycles was 27.3±0.33 days and the length of menses was 4.6±0.12 days (mean±S.E.). In the regularly menstruating group, 43 subjects reported a lifetime total of 10.5±1.9 missed menstrual cycles due to training, stress or weight loss. The entire oligomenorrheic group missed 19.3±5.7 menstrual cycles and the oligomenorrheic athletes reported 19.8±7.4 missed menstrual cycles. Thus, the oligo/amenorrheic group reported almost twice the frequency of absent menses than the regular group which was two years older.

The general anthropometric data in the four groups are presented in Table 5.3. There were no significant differences in age, height, weight or body mass index although the weight of the oligo/amenorrheic non-athlete was observably lower.

Table 5.3 Age, height, weight and body mass index (mean±SE) of athletes and non-athletes categorized by menstrual history

	Oligo/amenorrheic Athlete (24)	Eumenorrheic Athlete (35)	Oligo/amenorrheic Non-athlete (5)	Eumenorrheic Non-athlete (31)
Age (y)	29.3±1.6	31.8±1.4	33.1±1.8	33.9±1.4
Height	164.9±1.2	165.8±1.4	159.9±2.3	164.7±1.4
Weight (kg)	56.5±1.2	58.2±1.1	52.8±1.6	60.0±1.5
Body mass index	20.7±0.3	21.2±0.3	20.7±0.8	22.2±0.6

5.0.2 Bone Mineral Density

Bone mineral density at all sites was higher, but not significantly so, in the eumenorrheic subjects than in the oligo/amenorrheic subjects. The bone mineral density results considering the factors of activity and menstrual history are presented in Table 5.4.

Table 5.4 Bone mineral density results (mean±SE) of athletes and non-athletes categorized by menstrual history

	Eumenorrheic Athlete (35)	Oligo/amenorrheic Athlete (24)	Eumenorrheic Non-athlete (31)	Oligo/amenorrheic Non-athlete (5)
L2-L4	1.30±0.02	1.25±0.03	1.23±0.02 ^{b}	1.19±0.04 ^{a}
FN	1.10±0.02	1.04±0.02 ^{a}	1.01±0.02 ^{c}	0.96±0.04 ^{b}
W T	1.02±0.02	0.96±0.03 ^{a}	0.94±0.03 ^{b} *	0.82±0.03 $^{c\,d}$ *
FT	0.94±0.02	0.87±0.02 ^{a}	0.83±0.02 ^{c}	0.82±0.03 ^{a}

Significantly different from Eumenorrheic athletes: ^{a} *p<0.05,* ^{b} *p<0.01,* ^{c} *p<0.001.*
Significantly different from Oligo/amenorrheic athletes: ^{d} *p<0.05*
Difference almost significant * *p=0.053*

A trend was evident for all sites which showed that athletes, eumenorrheic followed by oligo/amenorrheic had higher bone mineral density than eumenorrheic and oligo/amenorrheic non-athletes. A series of one-tailed t-tests determined that the eumenorrheic athletes had significantly higher BMD than other subject groups at all sites, except the L2-L4 of the oligo/amenorrheic athlete . (Figures 5.1, 5.2, 5.3, 5.4). A two-factor analysis of variance determined no significant interactive effect of activity and menstrual categorization at any bone mineral density measurement site.

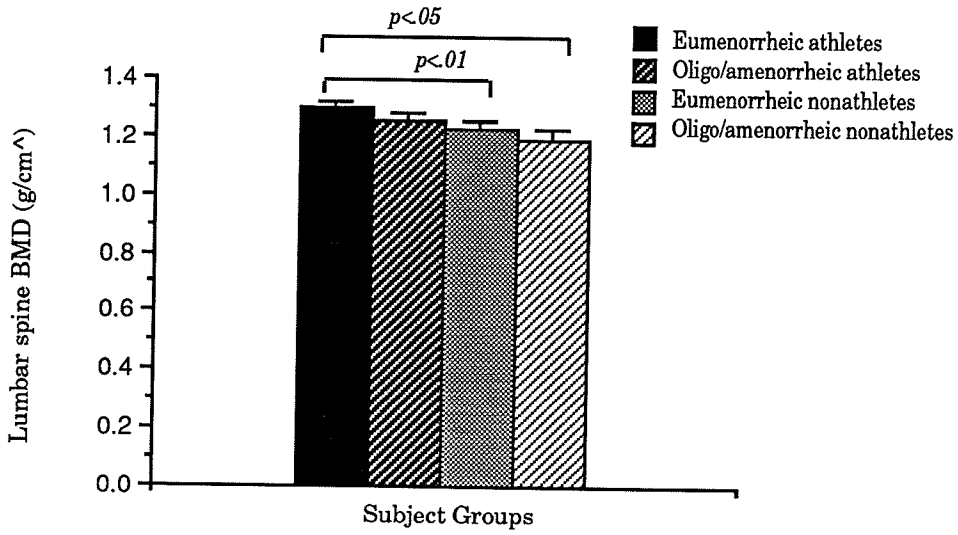

Figure 5.1 Lumbar spine bone mineral density of subjects grouped by menstrual history and athletic status (mean ± SE)

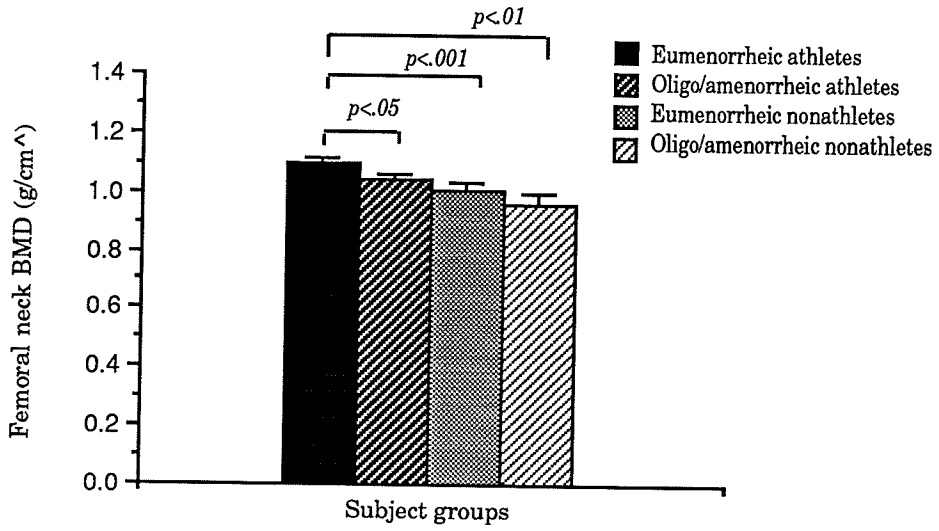

Figure 5.2 Femoral neck bone mineral density of subjects grouped by menstrual history and athletic status (mean ± SE)

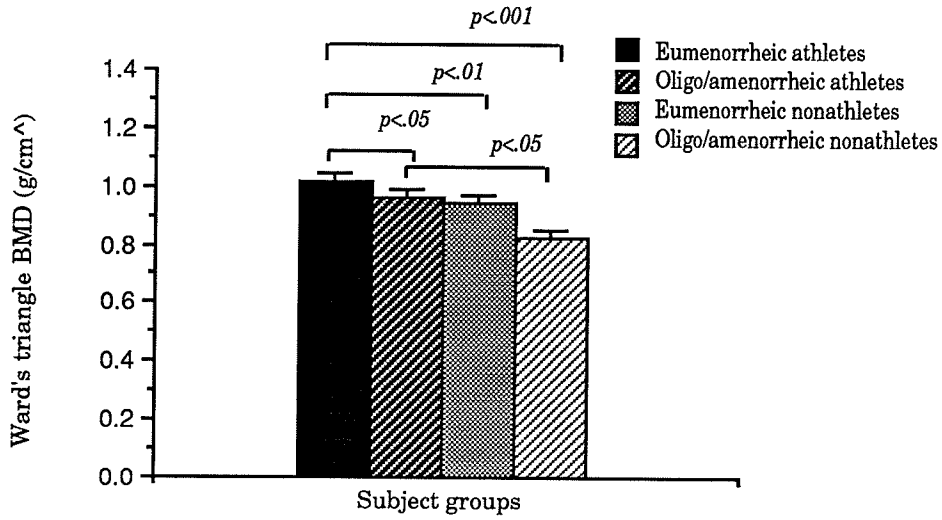

Figure 5.3 Ward's triangle bone mineral density of subjects grouped by menstrual history and athletic status (mean ± SE)

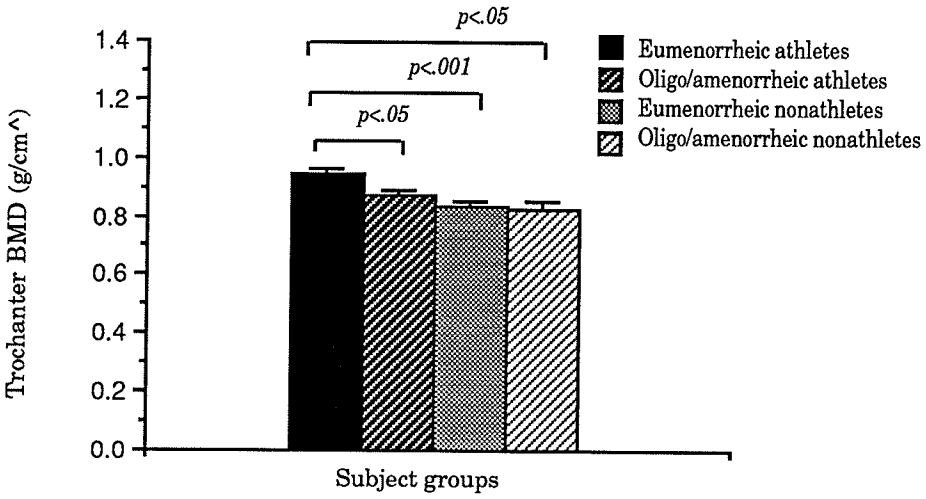

Figure 5.4 Trochanter bone mineral density of subjects grouped by menstrual history and athletic status (mean ± SE)

Linear regression analysis of the number of missed menstrual cycles and bone mineral density at all sites was not significant in the oligo/amenorrheic group or the oligo/amenorrheic athletes.

An analysis of covariance in which menstrual category was a factor and age the regressor, found no significant interactive effect of these two variables on bone mineral density at any site. At the lumbar spine and the trochanter, neither variable had a significant effect. However, at the other sites there were significant effects of: a) menstrual group; FN, $p<0.07$ and WT, $p<0.05$ and b) age; FN, $p\leq0.0008$ and WT, $p\leq0.0001$. The negative relationship of femoral BMD with age was shown in the larger cross-sectional data set. The present analysis showed age again to be inversely related to BMD at the FN and WT with a similar separate effect of oligo/amenorrhea.

5.0.3 Menarche

The age at menarche reported by 94 subjects was 12.9±0.15. The 58 athletes reported a significantly later age at menarche (13.2±0.21) than the 36 non-athletes (12.5±0.17). There was an inverse significant relationship between the age at menarche and current per cent body fat ($r=0.21, p<0.05$). This suggests that those with an earlier menarche were fatter as adults. An unpaired t-test of adult per cent body fat in those reporting an early menarche (<12 y, n=30) compared to a late menarche group (≥13.8 y, n=23) was significant ($p<0.05$, one-tail t-test): the respective values were 21.7±1.2% and 18.7±1.1% body fat.

A two factor analysis of athletic status and menarche revealed that the athletes with delayed menarche had the lowest body fat, ~17.8%.

The relationship between bone mineral density and age at menarche was analysed by linear regression. At all sites, the relationship was inverse but the correlation coefficients were low and not significant.

5.0.4 Parity

There was no significant difference between athletes and non-athletes in the number of pregnancies. The relationship between parity (0.8±0.13) and BMD was investigated over all subjects (n=92). The Spearman correlation coefficient was significant at the Ward's triangle site ($r=-0.25$, $p<0.05$). At all other sites, the correlation coefficients were also negative but not significant.

5.0.5 Oral Contraceptive Use

The effect of oral contraceptive use on BMD was investigated as use of these hormones is common and the effect on athletic and nonathletic populations has not been determined. Specifically, the effect of oral contraceptives as a possible confounding factor in the higher BMD of the athlete group required clarification.

In the 95 subjects completing both the general medical history and the menstrual history, there were 15 who documented never using oral contraceptives and 58 replied that they had used oral contraceptives. Thus approximately 80% of responders indicated use. The particulars of this use were described by 45 subjects and the duration of use was 59.5± 44, 1-216 months (mean±S.D., range).

The relation between the duration of oral contraceptive use and BMD was not significant at any site. The linear regression correlation coefficients were: L2-L4 $r=-0.06$, FN, $r=-0.013$, WT, $r=-0.16$, FT, $r=-0.081$.

In the non-athlete group, there were responses confirming 3 never users and 23 users of oral contraceptives. In the athlete group, responses indicated 12 never users and 35 users. The duration of use for the non-athletes and the athletes respectively was; 65.5±8.9, 3-132 and 54.7±10.1, 1-216 months (mean±S.E., range). There was no significant difference between groups regarding the duration of oral contraceptive use nor age at start of usage, approximately 20 years.

In the non-athletes, the former users indicated no use for 9.6±1.1, 1-24y (mean ±S.E., range) while the athletes reported former use ending 7.8±1.3y before entry to the study.

An analysis of former use by menstrual dysfunction groups indicated that there were 27 eumenorrheics who reported 8.9±1.3, 1-24y (mean ±S.E., range) since usage. Eight oligo/amenorrheics reported 6.1±1.4, 2-12y since oral contraceptive use.

An initial analysis categorized use as follows: no use, current use, use terminated 0-2 years prior to study, use terminated 2-4 years prior to study, and use terminated more than 4 years prior to study. There were no significant differences between these groups in BMD at any site.

Consequently, the effect of oral contraceptive use on bone mineral density was investigated for the group of 15 never users and 58 users. Analyses by unpaired t-tests showed no significant differences at all sites except for Ward's triangle where the non-users' higher BMD reached significance ($p<0.05$).

Table 5.5 Bone mineral density results (mean±SE) of athletes and non-athletes categorized by use and non-use of oral contraceptives (OC)

	Non-athlete Non-user OC (3)	Non-athlete User OC (23)	Athlete Non-user (12)	Athlete User OC (35)
L2-L4	1.26±0.06	1.24±0.02	1.25±0.03	1.29±0.02
FN	1.18±0.11 [a]	1.01±0.02	1.10±0.03	1.08±0.02
W T	1.08±0.13	0.93±0.03	1.04±0.03	0.99±0.02
F T	0.92±0.09	0.84±0.02	0.92±0.02	0.92±0.03

Significantly different from Non-athletes User [a] *$p<0.05$,*

A series of unpaired t-tests of the four groups found little evidence of the influence of oral contraceptives on BMD. There were no significant differences between users and non-users in the athletes. In non-athletes, only the FN site was significantly higher in non-users than users ($p<0.05$). (Table 5.5) This determination of significance may be due to the small number of subjects in the former group, the large error variation or the number of t-tests.

A series of two-factor analyses of variance was used to examine the influences of oral contraceptive use and athletic status on bone mineral density. Oral contraceptive use had a significant, inverse effect on BMD at the femoral neck and Ward's triangle ($p<0.05$). Although the athletes had consistently higher BMD, there were no significant differences between the athlete and non-athlete groups in this analysis. The trend of BMD from high to low at FN and WT was: athlete non-user, athlete user, non-athlete non-user and non-athlete user (Table 5.5).

A similar trend existed at L2-L4 and FT although there were no significant differences in BMD between users and non-users of oral contraceptives. This trend was identical to that of the FN and WT except that the athlete user showed the highest BMD. The interaction effect of non-athlete status and oral contraceptive use neared significance only at the femoral neck ($p\leq0.0567$).

These results suggest that the use of oral contraceptives was not a contributing factor to the higher BMD of athletes compared to non-athletes. The non-athlete group using oral contraceptives consistently showed the lowest BMD.

5.0.6 Stress Fractures

A two-factor analysis determined that there was a non-significant trend to an increased number of stress fractures in athletes compared to the non-athletes ($p<0.07$). The higher prevalence of stress fractures in the oligo/amenorrheic athletes (38%) compared to the eumenorrheic athletes (22%) was found to be not significant. In non-athletes, the prevalence of stress fractures was 0% in oligo/amenorrheics and 6% in eumenorrheics.

5.0.7 Anthropometry

Height and weight Linear regression equations were calculated for the BMD relationship to height and weight. In the regularly menstruating non-athletes (n=51), weight was positively related to BMD at all femoral

sites. The correlation coefficients and levels of significance were: FN ($r=0.35$, $p<0.05$), WT ($r=0.35$, $p<0.05$) and FT ($r=0.38$, $p<0.01$).

The relationship of weight to BMD in the regularly menstruating athletes ($n=50$) was positive at all sites and significant at L2-4 ($r=0.28$, $p<0.05$), FN ($r=0.32$, $p<0.05$) and WT ($r=0.29$, $p<0.05$). Height was not a significant contributor to BMD except for the FN site in the athlete group ($r=.29$, $p<0.05$).

Multiple regression equations showed that height and weight together were significant predictors of bone mass at the FN ($r=0.35$, $p<0.05$) in the athletes (Table 5.6). In the non-athletes, the regressions of height and weight on BMD at the femoral sites were significant at FN ($r=0.37$, $p<0.05$) and FT ($r=0.43$,$p<0.05$) and bordered on significance at the WT ($r=0.35$, $p<0.052$). Only weight made a significant contribution to these regression equations.

Table 5.6. Multiple regression equations and significance of height (cm) and weight (kg) as predictor variables of bone mineral density

	Regression Equation	Height (p)[a]	Weight (p)	r	Ht+Wt (p)
Athletes (50)					
L2-L4	=1.331-0.003Ht+0.006Wt	n.s.	<0.05	0.31	n.s.
FN	=.403+0.003Ht+0.004Wt	n.s.	n.s.	0.35	<0.05
W T	=.538+0.001Ht+0.005Wt	n.s.	n.s.	0.29	n.s.
FT	=.418+0.003Ht-0.001Wt	n.s.	n.s.	0.17	n.s.
Non-athletes (51)					
L2-L4	=1.235-0.001Ht+0.003Wt	n.s.	n.s.	0.20	n.s.
FN	=.42+0.002Ht+0.005Wt	n.s.	<0.05	0.37	<0.05
W T	=.453+0.001Ht+0.006Wt	n.s.	<0.05	0.35	<0.052
FT	=.977-0.004Ht+0.007Wt	n.s.	<0.01	0.43	<0.01

[a] Levels of significance for height and weight are based on multiple regression models which include both coefficients.

Age When only eumenorrheic subjects were included in the analysis of age and BMD, the second-order polynomial regressions were significant at only the femoral sites.

A multiple regression equation with age, height, weight, triceps and thigh muscularity indices was formulated for all regularly menstruating subjects (n=70). The correlation coefficients and level of significance were: L2-L4 (r=0.31, n.s.), FN (r=0.46, p<0.01), WT (r=0.46, p<0.01) and FT (r=0.21, n.s.).

Waist to hip ratio The waist to hip ratio was examined in the menstrual groups. After controlling for body fat, the adjusted means were oligo/amenorrheics; 0.718±0.01 and eumenorrheics; 0.724±0.01. There was no significant difference between these means. There was also no significant difference between the adjusted means for the activity and menstrual category groups (Table 5.7).

Body composition Selected body composition variables are presented according to menstrual and activity designation in Table 5.7. The estimate of total muscle mass was significantly different between only the eumenorrheic athlete and eumenorrheic non-athlete groups.

Table 5.7 Body fat, sum of six skinfolds and muscle mass estimate results (mean±SE) of athletes and non-athletes categorized by menstrual history

	Oligo/amenorrheic Athlete (24)	Eumenorrheic Athlete (35)	Oligo/amenorrheic Non-athlete (4)	Eumenorrheic Non-athlete (30)
Waist to hip ratio	0.715±0.01	0.725±0.01	0.724±0.02	0.708 ±0.01
% body fat	16.9±0.87 $^{a\,b}$	19.4±0.8 $^{a\,c}$	19.8±1.84	25.43±1.31 $^{b\,c}$
Sum 6 SF (mm)	70.54±4.28 ^{b}	83.28±4.45 ^{d}	86.25±10.17	122.67±9.84 $^{b\,d}$
Muscle mass (kg)	29.37±0.74	30.13±0.84 ^{a}	26.51±0.57	27.92±0.61 ^{a}

Levels of significance ^{a} $p<0.05$, $^{b\,c}$ $p<0.0001$, ^{d} $p<0.001$

A series of two factor analyses examined anthropometric variables and the activity and menstrual history factors. Only per cent body fat was significantly different both between activity groups ($p<0.01$) and between menstrual status groups ($p<0.05$) but the interaction effect was not significant.

Further analyses by one-tailed t-tests examined the differences in per cent body fat in the four groups. These results showed a trend for body fat to be lower in athletes particularly in oligo/amenorrheic athletes. The oligo/amenorrheic athletes had a significantly lower percentage of body fat than the athlete and non-athlete eumenorrheics. The sum of six skinfold results confirmed this observation of increased fatness with non-athlete and eumenorrheic status (Table 5.7).

5.0.8 Dietary Calcium

The oligo/amenorrheic subjects' data were excluded from this analysis of BMD and dietary calcium. There were no significant differences in BMD between the high and low/moderate calcium consumption in athletes. The non-athletes who were in the high calcium group showed significantly higher at all sites except FT where $p \leq 0.06$ (Table 5.8).

A two factor analysis regarding physical activity and lifetime calcium intake was employed over regularly menstruating subjects. There were no significant differences in any of the BMD means between the two calcium groups (Table 5.8). As shown previously, at all femoral sites, the athletes, overall, had significantly higher BMD and L2-L4 difference neared significance ($p \leq 0.06$). The interaction effect of calcium and physical activity was significant at all four BMD sites ($p < 0.05$). It is interesting to note that this analysis which controlled for eumenorrheic status, did not show the significant differences revealed in the previous analysis of BMD in high and low calcium groups overall subjects (Table 4.9).

Table 5.8 BMD (mean, g/cm2) and habitual dietary calcium intake in eumenorrheic non-athletes, athletes and all subjects

	LOW/MODERATE			HIGH		
	Non-athletes (17)	Athletes (23)	**Overall**	Non-athletes (14)	Athletes (11)	**Overall**
L2-L4	1.19±0.03 [b]	1.30±0.03	**1.25±0.02**	1.29±0.03 [b]	1.28±0.03	**1.28±0.02**
FN	0.96±0.02 [a]	1.11±0.02	**1.04±0.02**	1.07±0.03 [a]	1.09±0.04	**1.08±0.02**
W T	0.88±0.03 [a]	1.03±0.02	**0.97±0.02**	1.01±0.04 [a]	1.00±0.04	**1.01±0.03**
FT	0.80±0.03 [c]	0.97±0.03	**0.90±0.03**	0.87±0.03 [c]	0.90±0.03	**0.88±0.02**

a significant differences between non-athlete groups, $p < 0.01$

b significant differences between non-athlete groups, $p < 0.05$

c nonsignificant, $p \leq 0.06$

5.1 Assay Results

5.1.0 Endocrine Assays

The blood sample consisted of one mid-follicular early morning fasting sample. Samples from 65 subjects within the 19-47 year age range were assayed. Four subjects with high FSH values were excluded from further analysis of assay data as they were suspected of being menopausal. Assay results for fifteen subjects currently taking oral contraceptives were excluded from this analysis. Former use of oral contraceptives, described in section 5.05, noted that use had ceased at least 1 year prior to assay data collection. The remaining individual values were generally within normal limits as defined by laboratory reference ranges except for the following: 4 high cortisol, 3 high prolactin and 9 low estradiol levels. The results of the assays for the 46 subjects are presented in Table 5.9.

Table 5.9 Endocrine assay results for premenopausal subjects, expressed as mean±SE

	Reference range	Subject results n=46	Subject range
Cortisol (nmol/L)	150-700	526.7±25.6	250-1150
Estradiol (pmol/L)	70-450	170.9±17.5	20-720
Estrone (pmol/L)	na	546.0±24.3	124-1153
FSH (IU/L)	1.3-7.5	4.88±0.23	0.4-8.7
LH (IU/L)	3.2-9.6	5.62±0.53	0.4-20
Prolactin (μg/L)	<15	8.43±1.05	1-50

Reference ranges: (Anonymous, 1989a)
na:Laboratory reference range not available

The results according to two methods of subject grouping are displayed subsequently. In Table 5.10, the hormonal data for athletes and non-athletes are presented. All mean results are within normal reference ranges. Significantly lower values for serum estradiol, estrone and LH were observed in the athletes.

Table 5.10 Endocrine assay results categorized according to activity groups, expressed as mean±SE

	Athletes n=27	Non-athletes n=19	p value
Cortisol (nmol/L)	531.1±31.9	520.5±43.2	n.s.
Estradiol (pmol/L)	139.0±15.0	216.1±34.5	p<0.05
Estrone (pmol/L)	503.4±25.7	606.6±43.3	p<0.05
FSH (IU/L)	4.89±0.33	4.86±0.30	n.s.
LH (IU/L)	4.61±0.39	7.05±1.09	p<0.05
Prolactin (μg/L)	9.21±1.72	7.33±0.71	n.s.

Assay results according to menstrual status as defined by survey information are presented in Table 5.11. There were no significant differences between groups. However, serum estradiol (-31%), LH (-18%) and estrone (-17%) were observably lower in the oligo/amenorrheic group. Cortisol (+5%), FSH(+11%) and prolactin (+39%) were higher in the oligo/amenorrheic group.

Table 5.11 Endocrine assay results of oligo/amenorrheic and eumenorrheic groups, expressed as mean±SE

	Oligo/amenorrheic n=11	Eumenorrheic n=35	p value
Cortisol (nmol/L)	547.3±26.1	521.1±32.7	n.s.
Estradiol (pmol/L)	129.7±26.8	187.1±20.9	n.s.
Estrone (pmol/L)	474.7±32.6	569.4±29.6	n.s.
FSH (IU/L)	5.24±0.52	4.73±0.26	n.s.
LH (IU/L)	4.75±0.44	5.81±0.68	n.s.
Prolactin (μg/L)	10.64±4.0	7.63±0.59	n.s.

A two factor analysis showed no significant differences in cortisol, estradiol, estrone, FSH, LH, and prolactin between groups based on activity

and menstrual dysfunction. The interaction effect of these two factors was not significant. However, this trend was observed for estradiol: oligo/amenorrheic athlete <oligo/amenorrheic non-athlete <eumenorrheic athlete <eumenorrheic non-athlete. Estrone and LH demonstrated a similar pattern.

Although the results for the 9 oligo/amenorrheic athletes were within normal laboratory ranges, the following were observed: an intermediate cortisol level (570 nmol/L), low LH (4.87 IU/L), low estradiol (129.3 pmol/L), low estrone (485.9 pmol/L), and high prolactin (11.6 μg/L).

The relationships between selected assay results and BMD values were investigated. There were no significant relationships between BMD at any site and estradiol, estrone, cortisol, and prolactin assay results.

The relationships between estrogens and selected anthropometric measures were examined. There was no significant relationship between BMI and estradiol ($r=0.06$) or estrone ($r=0.22$). Neither estradiol nor estrone was significantly related to per cent body fat.

The inverse relationship of estradiol to WHR was significant without adjusting for body fat ($p<0.05$). When per cent body fat was controlled, this relationship failed to reach significance. The adjusted WHR means of athletes and non-athletes were not significantly different.

5.1.1 Clinical Chemistry Assays

All assay results were within respective laboratory reference ranges except for: 2 subjects with slightly decreased total calcium (2.04 and 2.07 mmol/L), 2 subjects with decreased total calcium (1.72 and 1.79 mmol/L), 1 subject with elevated PTH (82 ng/L) and 1 subject with slightly elevated total calcium (2.71 mmol/L) and elevated creatinine (129 mmol/L). The results are presented in Tables 5.12 and 5.13. There were no significant differences between groups according to athletic status or menstrual history.

Table 5.12 Calcium panel assay results of athletes and non-athletes, expressed as mean±SE

	Reference range	Athletes n=26	Non-athletes n=18	p value
Total calcium (mmol/L)	2.10-2.60	2.24±0.03	2.22±0.02	n.s.
Ionized calcium(mmol/L)	1.17-1.33	1.25±0.01	1.23±0.01	n.s.
Albumin (g/L)	35-50	41.5±0.49	40.9±0.62	n.s.
Phosphate (mmol/L)	0.81-1.45	1.22±0.03	1.18±0.03	n.s.
Creatinine (μmol/L)	70-110	60.1±8.93	66.5±8.72	n.s.
PTH (ng/L)	<65	34.3±2.79 (19)	36.8±3.37	n.s.

Table 5.13 Calcium panel assay results for oligo/amenorrheic and eumenorrheic groups, expressed as mean±SE

	Reference range	Oligo/amenorrheic n=11	Eumenorrheic n=35	p value
Total calcium (mmol/L)	2.10-2.60	2.23±0.03	2.22±0.02	n.s.
Ionized calcium(mmol/L)	1.17-1.33	1.24±0.02	1.24±0.01	n.s.
Albumin (g/L)	35-50	41.2±0.62	41.0±0.51	n.s.
Phosphate (mmol/L)	0.81-1.45	1.23±0.02	1.17±0.02	n.s.
Creatinine (μmol/L)	70-110	57.7±13.9	65.4±6.98	n.s.
PTH (ng/L)	< 65	39.1±7.4 (9)	35.2±1.8 (29)	n.s.

5.1.12 Oral Contraceptive Usage

The assay results for the 15 subjects, 6 non-athletes and 9 athletes who were using oral contraceptives at the time of blood sampling are presented in Table 5.14. These subjects had used oral contraceptives for 60.6±14.4 months beginning at the age of 21.0±3.6y (mean±S.D.). The types of oral contraceptives used were: Triquilar 21, Triphasil 21, Ortho 777, Ortho Novum (unspecified), Minovral, Premarin and Provera.

Table 5.14 Endocrine and clinical chemistry assay results for subjects currently using oral contraceptives, expressed as mean±SE

	Reference range	Subject results n=15	Subject range
Cortisol (nmol/L)	150-700	864.7±75.9	490-1400
Estradiol (pmol/L)	70-450	49.8±15.5	20-245
Estrone (pmol/L)	na	398.3±27.8	182-552
FSH (IU/L)	1.3-7.5	3.51±0.53	0.4-6.2
LH (IU/L)	3.2-9.6	3.86±0.79	0.4-10
Prolactin (μg/L)	<15	7.7±1.38	1-50
Total calcium (mmol/L)	2.10-2.60	2.18±0.03	1.96-2.41
Ionized calcium(mmol/L)	1.17-1.33	1.24±0.01	1.18-1.31
Albumin (g/L)	35-50	39.3±0.77	33-45
Phosphate (mmol/L)	0.81-1.45	1.25±0.04	0.98-1.52
Creatinine (μmol/L)	70-110	55.1±12.1	65.4±6.98
PTH (ng/L)	< 65	29.0±7.4 (11)	17-43

The assay results for the 15 users of oral contraceptives were compared to the results of the 35 eumenorrheic subjects (Table 5.11). The oral contraceptive users showed significantly higher cortisol ($p<0.0001$); significantly lower estradiol ($p<0.001$), FSH ($p<0.05$), estrone ($p<0.001$) and albumin ($p<0.05$); and observably lower LH ($p\leq0.09$) and PTH ($p\leq0.09$).

5.2 Discussion

The estimated prevalence of oligo/amenorrhea among the general population has been reported to be 5% (Shangold, 1985). Baker (1981) reported that menstrual dysfunction prevalence ranged from 0-50% in various groups. The subjects in this study showed a higher prevalence of menstrual dysfunction which may be due to the non-randomized selection and to the variable definitions of menstrual dysfunction. Approximately 30% of these subjects reported the absence of at least one menstrual cycle. Among the athletes, the loss of menstrual cycles was more common and occurred with greater frequency than in the non-athlete group. Those subjects meeting the criteria for inclusion to the oligo/amenorrheic group reported approximately 20 months of missed menses. The prevalence of 40.7% in the athletes was significantly higher than 13.9% of the non-athletes.

The elite athletes included in this study represented a variety of sports with varying prevalences of exercise-induced menstrual dysfunction. Shangold (1985) reported that the prevalence of amenorrhea was 25% in runners, 50% in competitive runners and 12% in swimmers and cyclists. Although the stress of physical training is a recognized contributor, body composition remains a central, unresolved factor in athletic amenorrhea.

Compared to the eumenorrheic subjects, the oligo/amenorrheics weighed significantly less and were significantly less fat as determined by per cent body fat and sum of six skinfolds. Although muscle mass was similar, when body weight was considered, 52% of the oligo/amenorrheics' body weight was accounted for by muscle mass compared to the eumenorrheics' 49%. Further analysis illustrated that the eumenorrheic athlete group had the highest muscle mass value followed by the oligo/amenorrheic athletes, the eumenorrheic non-athletes and the oligo/amenorrheic non-athletes. Thus, the athletes weighed less, had more muscle mass and were less fat than the non-athletes.

The promotion of bone mass and higher bone densities have been associated with physical activity. However, excessive physical activity can be detrimental to bone directly through unusually high loads of strain

and/or indirectly through athletic amenorrhea. The rarefaction of bones in amenorrheic athletes has been well documented for the lumbar spine and forearm (Cann et al, 1984; Drinkwater et al, 1984; Lindberg et al, 1984; Linell, Stager, Blue & Robertshaw, 1984; Warren et al, 1986). This investigation featured measurements of the spine and proximal femur. Bone mineral density was higher in the eumenorrheic subjects than the oligo/amenorrheics but these differences were not significant. When subjects were grouped by both athletic and menstrual status, the eumenorrheic athletes had the highest bone mineral density at all sites. The oligo/amenorrheic athletes had the second highest BMD measurements which were reduced from the eumenorrheic athletes' values to the following extent: L2-L4 3.85%, FN 5.45%, WT 5.88%, FT 7.45%. The differences at all three femoral sites were significant.

This order of BMD, from high to low, was consistent at all sites: eumenorrheic athletes, oligo/amenorrheic athletes, eumenorrheic non-athletes, oligo/amenorrheic non-athletes. Thus, menstrual status and physical activity are both important for optimal skeletal health. However, the interaction effect was not significant. Because the oligo/amenorrheic athlete maintained intermediate BMD values, the effect of physical activity appeared to be paramount. However, the literature suggested that estrogen is probably the critical factor and once a certain level is assured other factors may be permitted to operate on bone. These data offer support for this theory. The BMD values of the oligo/amenorrheic athletes were higher than the two non-athlete groups but the differences reached significance only at the WT. Thus, the oligo/amenorrheic athletes maintained very little of the skeletal increments displayed by their eumenorrheic counterparts. More severe menstrual dysfunction, chronic amenorrhea results in more dramatic, perhaps irreversible osteopenia (Cann, Martin & Jaffe, 1985b). Consequently, the present data confirm a spectrum of menstrual dysfunctions and document the moderate but obvious skeletal effects of intermittent amenorrhea and oligomenorrhea. BMD at the lumbar spine was moderately lower whereas the proximal femur was significantly lower than eumenorrheic athletes.

The athletes reported a higher incidence of fractures than non-athletes with the oligo/amenorrheics' fracture rate exceeding that of the eumenorrheic athletes. Although this difference was not significant, the trend of increased fractures with menstrual dysfunction has been documented by others (Schwartz et al, 1981; Warren et al, 1986). The incidence of stress fractures, 38% in these oligo/amenorrheic athletes was less than previously reported for amenorrheic runners, 49% (Lindberg et al, 1984), 54% (Marcus et al, 1985) and 72%(Nelson et al, 1987). This moderate fracture rate may be due to the less extreme osteopenia in this study's subjects.

The determination of factors which might be predictive of BMD has been well-documented for postmenopausal women. Age is one variable which has been used in simple and multiple regressions to predict BMD. The data included in Chapter 4 demonstrated that in young premenopausal women, there is not a significant age-related bone loss at the lumbar spine. There was, however, a significant effect of age on femoral bone loss. This loss of bone mass was attenuated by physical activity.

In this chapter, the relationship between age and femoral BMD in the eumenorrheic subjects was best explained by quadratic polynomial regressions which were significant all three sites. There was no relationship between age and lumbar BMD. There was no significant interactive effect between age and menstrual category at any site. These results support the initial conclusions that lumbar spine rarefaction is preceded by decreases in femoral bone mass, which begins early in the third decade.

Aside from age, derived muscle mass and muscularity indices of body segments may be reliable indicators of BMD. However, height and weight are more often used as predictors of BMD. Laitinen (1991) found a significant positive correlation between body weight and BMD, measured by DEXA. The relation of body habitus to BMD has most often been cited in postmenopausal women as in Stevenson's study where the weight and BMD relationship was evident for the postmenopausal but not the premenopausal subjects (Stevenson et al, 1989).

Slemenda (1990b) also examined anthropometric variables and BMD pre- and postmenopausal women. Frame size, muscularity and adiposity

were together only able to predict 40-45% of the variation in bone mass. Therefore, the utility of anthropometric characteristics lies in the identification of skeletal risk factors. Obesity has been related to higher BMD in postmenopausal women: Slemenda reported that the subscapular skinfold thickness consistently predicted BMD at all three femoral sites, particularly in older women. The present data analysis found no relation between the subscapular skinfold measurement and BMD at any site. According to Slemenda and this investigation, waist to hip ratio was not significantly associated with BMD.

Frame size, determined by biacromial width was not measured in this study but Slemenda determined that this was an important predictor in young females. Muscularity was associated with higher bone mass, particularly L2-L4 and FT in pre- and postmenopausal women, as indicated by an estimate of calf muscularity (Slemenda et al, 1990b).

The present analyses of eumenorrheic subjects determined weight and height to be predictor variables of BMD, with weight being the significant variable. These equations explained significant proportions of the variability, 35-43%, at all three proximal femur sites in the non-athlete group. However, in the athletes, these variables only achieved significance at the FN, accounting for 35% of the BMD variability. This suggests that in athletes, where body weight and body fat are reduced, other factors such as muscle mass and dynamic loading must be considered. The BMD hierarchy of: eumenorrheic athletes, oligo/amenorrheic athletes, eumenorrheic non-athletes, oligo/amenorrheic non-athletes is identical to that of muscle mass. This underscores the important contribution of skeletal muscles to the mechanical loading of bones. Moreover, the relationship between regional muscle mass indices and BMD suggest that physical exertion, along with systemic effects on BMD, has potent localized effects. Investigations of physical activity in the promotion of bone mass should reveal exercise prescriptives in the near future.

Physical activity was determined to be an important variable in the promotion and maintenance of bone mass, but evident from the literature is the primary role of euestrogenism. The endocrine assays revealed that the athletes had low estradiol, estrone and LH, similar to the endocrine

characteristics of amenorrhea (Shangold, 1985). This endocrine pattern was also revealed by the comparison of the oligo/amenorrheic and eumenorrheics assay results. The oligo/amenorrheics had depressed estradiol, estrone, LH and FSH with elevated cortisol and prolactin. These differences, although not significant, confirmed Laatikainen's work except for the opposite direction of prolactin (Laatikainen et al, 1986).

The strong association between high cortisol and low estradiol supports Ding's research and suggests the involvement of stress-related mechanisms in athletic amenorrhea (Ding, Sheckter, Drinkwater, Soules & Bremner, 1988). Changes in metabolic clearance and secretion rates have been a suggested rationale for high cortisol levels in weight loss (Henley & Vaitukaitis, 1985). However, involvement of endogenous opioids and disruption of the hypothalamic-pituitary-adrenal axis have also been hypothesized (Casper, 1990). Hypercortisolism has been implicated in decreased BMD (Ding et al, 1988; Newman et al, 1989).

In the present subjects, neither cortisol nor estradiol values were related to BMD values. A relationship between estradiol and BMD was reported by one group (Nelson et al, 1987) while no correlation was found by another group (Drinkwater et al, 1984). Also, the amount of bone loss in athletes has been related to the duration of amenorrhea (Cann et al, 1985b). In these data, there was no significant relationship between missed menstrual cycles and BMD.

Chronically low estrogen levels or duration of amenorrhea should illustrate a strong association with low BMD. It is not surprising that these data, which consisted of one sample from subjects at various stages of menstrual dysfunctions, did not show a strong relationship with BMD. Also, because the subjects in this study were generally not chronic amenorrheics and were experiencing intermittent menses, their estrogen levels may have been sufficient to avoid significant osteopenia. However, there was a variable degree of hypoestrogenism which did result in decreased BMD. Also, recent research confirms that mild cycle abnormalities may result in substantial bone loss in young women (Johnston & Longcope, 1990; Prior, Vigna, Schechter & Burgess, 1990). The mechanism of bone loss associated with shortened luteal phase and anovulatory cycles may depend upon decreased levels of estrogen,

progesterone and/or androgens. Progesterone has been suggested as a key hormone in bone metabolism (Prior, 1990; Prior et al, 1990).

Dhuper's group (1990) compiled an estrogen index of several variables which have been related to BMD. Variables such as age at menarche, Tanner age, menstrual cycles, estradiol levels and oral contraceptive use were scored to determine estrogen exposure. Similarly, several of these variables were included in this investigation to determine their impact on bone.

Over all subjects, menarche occurred at 12.9±.15 y. This value is normal by North American standards, agreeing with the menarcheal age recently reported by Dhuper and colleagues, 12.9±1.3 y (Dhuper et al, 1990). As expected, the athletes reported a significantly later age at menarche, 13.2±.21y than the non-athletes 12.5±.17 (Baker et al, 1988).

Rosenthal (1989) reported that a later menarche meant lower bone mineral density. The present analysis showed evidence of an inverse relationship between menarche and bone mineral density. All correlation coefficients were positive but low and significance was not attained. In her review, Shangold (1990) also discussed late menarche and low BMD and suggested that if menarche had not occurred by 16y, then a complete medical examination was warranted.

A connection between body fatness as an adult and early menarche was determined to be significant. Those who reported an earlier menarche were fatter as adults. Moreover, the athletes who reported a late menarche had the lowest per cent body fat of the adult groups.

Other events in reproductive history such as pregnancy and lactation have been postulated to influence bone mass. It had been supposed that parity might have a positive effect on the skeleton due to increased weight-bearing and metabolic changes (Stevenson et al, 1989). Hreshchyshyn (1988a) reported that there was no association between lumbar spine BMD and parity but femoral neck BMD decreased 1.1% per live-birth. Laitinen (1991) reported a weak positive relation between number of pregnancies and BMD at FT and L2-L4 in premenopausal women. Others have shown no connection between parity and bone mineral density (McCulloch et al, 1990).

In the present data, the relationship between parity and BMD was significant only at WT, where an increased number of pregnancies was associated with reduced BMD. The mechanism for this reduction is unclear. The WT BMD should be responsive to the increased loading of pregnancy. However, physical exertion may in fact be decreased during pregnancy such that cumulative loading as well as loading through different planes is reduced. Perhaps the extra demands for calcium during pregnancy and lactation are more readily available from the WT which has been denoted as a site sensitive to change. The literature has stated that the during lactation the maternal skeletal calcium deficit of pregnancy is replaced. This reversal may be incomplete at WT due to erosion of the complex trabecular arrangement.

This finding of reduced WT BMD with parity contrasts with Stevenson's (1989) report on postmenopausal women that there was a negative effect of nulliparity at the lumbar spine. However, for premenopausal women, Stevenson's results showed low but negative correlations between gravidity and BMD at all sites, reaching significance at the trochanteric region. Thus, the present results of a negative relation in premenopausal women between number of pregnancies and BMD at all sites support Stevenson's and Hreshchyshyn's findings. Parity and lactation, which was not analysed here, may have differential effects on the selected sites of measurement.

It was expected that the women who used oral contraceptives might have greater BMD as estrogen replacement therapy is widely accepted for its positive effects on skeletal health in postmenopausal women. Interestingly, Stevenson (1989) reported that the relation of oral contraceptive use to BMD was evident in postmenopausal women but not in premenopausal women. Details of the types of oral contraceptives were not reported. Likewise in Laitinen's paper (1991), the specifics of oral contraceptive use were not included with the report of a positive effect of use on FN and WT BMD. However, the younger age of the oral contraceptive users was a confounding variable and when controlled, only the FN remained significantly higher in the user group (Laitinen et al, 1991). The correlation coefficients reported by Laitinen were positive at all femoral sites but negative at the L2-L4 site (r=-0.0643), which was confirmed by the present data (r=-0.064). Rodin's group

reported no significant effect of combined oral contraceptive use on bone mineral density and pooled users and non-users in a large cross-sectional investigation (Rodin, Fogelman & Chapman, 1987; Rodin et al, 1990). Other investigators have also reported no significant effect of oral contraceptives on BMD (Sowers et al, 1985) (Elliott et al, 1990) (Davee et al, 1990) (Hreshchyshyn et al, 1988a). The most recent study of oral contraceptive use found no association with BMD at any site and a two year prospective study showed no relationship to change in BMD (Mazess et al, 1991).

The present data showed no significant effect of oral contraceptive use on BMD overall except at the WT where users had a lower BMD value. Further analysis of athlete and non-athlete groups showed that oral contraceptive use was associated with lower BMD particularly in the non-athletes at the femoral neck and Ward's triangle sites. In conclusion, these data showed that the higher BMD in the athletes was not related to the athletes' use of oral contraceptives. Moreover, oral contraceptive use and non-athlete status were associated with reduced bone mass.

The assays of the 15 subjects who were currently taking oral contraceptives were noteworthy in several respects. The oral contraceptive users showed significantly higher cortisol, significantly lower estradiol, FSH, estrone and observably lower LH ($p \leq 0.09$) and PTH ($p \leq 0.09$). This endocrine profile, although determined from a single mid-follicular phase sample and normal for an oral contraceptive user, is similar to the endocrine patterns reported for amenorrheics, oligo/amenorrheics and athletes. However, the serum levels of estrogen and progestin, not measured here, do not reveal the optimum level or dose of synthetic or natural oral contraceptive required to effect bone remodelling. Free cortisol is likely not different as the increased cortisol is the result of increased cortisol binding globulin. The trend to lower PTH may be a secondary response to the increased flux of calcium from the skeleton because of the reduced protective effect associated with lower estradiol.

The possibility was suggested by Shangold (1990) that athletes using oral contraceptives, without which they might be hypoestrogenic, may maintain bone density. According to Shangold, a minimum level of estrogen, 220 pmol/l or 60 pg/ml, is required to maintain bone density.

However, the concept of an adequate, bone-sparing level of estradiol has been debated by Stevenson who suggests that direct measurements of bone is the best indication of bone preservation (Utian, 1990). Shangold (1990) advised that estrogen replacement therapy should be initiated in amenorrheic athletes after six months because after 3 years, the effects of amenorrhea were considered to be irreversible. Emans (1990) suggested that earlier and more aggressive therapy, in the form of 0.625 mg of conjugated estrogen (Premarin) days 1-21 and medroxyprogesterone acetate (Provera) 10 mg days 12-21 each calendar month was required in hypoestrogenic young females. It was also noted that the hypoestrogenic female may require more calcium than euestrogenic females to simply maintain zero calcium balance and thereby protect the skeleton (Shangold et al, 1990).

The oral contraceptives used by the subjects in this study were varied and consisted of either sequential or continuous combined therapy of estrogen and progestogen. The type of oral contraceptive which may have the most positive effects on the skeleton in postmenopausal women has not been resolved. Progestogens, added to estrogen replacement therapy to decrease the risk of cancer, may help bone conservation (Whitehead, Hillard & Crook, 1990). Further research is required to investigate the effect or lack of effect of different types of oral contraceptives on bone mass in premenopausal women.

Generally, the results of the calcium panel assay were normal when analysed by activity or by menstrual status. There were no significant differences in any variable. No significant differences in dietary calcium existed between athletes and non-athletes overall. As reported in Chapter 4, there were significant differences between L2-L4, FT and WT BMD with higher BMD's in the high calcium group. A subsequent data analysis of only eumenorrheic subjects did not determine the same results. There was a non-significant trend toward higher BMD in the high calcium group. Thus, when only eumenorrheic subjects were included in the data analysis, the postulated effect of dietary calcium on BMD was attenuated. The lack of a significant relation between dietary calcium and BMD in premenopausal females has been reported by others (McCulloch et al, 1990; Stevenson et al, 1989). The recent literature confirms that the effect of

calcium may well be positive but fail to reach significance due to a number of factors (Avioli & Heaney, 1991; Cumming, 1990). Avioli and Heaney (1991) have stated that seemingly low correlations, even if not statistically significant should not be misinterpreted as trivial. In premenopausal women, the factors which influence BMD are highly inter-related and each assumes a variable portion of the variation in BMD (Avioli et al, 1991). This suggests that dietary calcium plays a secondary role to estrogen and physical activity in the maintenance of bone mass.

6. ANOREXIA NERVOSA: RESULTS AND DISCUSSION

6.0 Results

6.0.0 Description of Subjects

The clinical data of the anorectic patients are presented in Table 6.1. Five patients were also classified as Bulimia Nervosa according to the DSM III criteria (American Psychiatric Association, 1987). All patients had histories of menstrual dysfunction with the duration of secondary oligo/amenorrhea ranging from 1-10 years. One patient's history documented a hysterectomy at age 20, slight tobacco use and suspected alcohol abuse. Another two patients reported tobacco use and short term oral contraceptive use. Only one patient was taking oral contraceptives at the time of the BMD assessment. One subject reported a chronic regimen of Vitamin C, multivitamins and calcium. Excessive physical activity was documented for 5 patients. One exercise abuser daily undertook more than 5 hours of physical activity.

Age, height, weight and BMI data for the anorectic patients and the age-matched controls: the oligo/amenorrheic athletes and eumenorrheic normal subjects are presented in Table 6.2. The normal controls were selected from the non-athletes: 3 were currently taking oral contraceptives and 1 subject indicated past use. The oligo/amenorrheic athletes were subjects who were, due to physical activity, currently experiencing menstrual dysfunction as defined in Chapter 1. Among the oligo/amenorrheics, 6 subjects listed previous use of oral contraceptives.

No significant differences existed between the mean age or height of the groups. The anorectics' mean weight was significantly lower than the normal group. BMI (kg/cm^2) was significantly lower in the anorectic (17.3±0.9) in comparison to the normal controls. The oligo/amenorrheic mean BMI (19.6± 0.5) was an intermediate value, not significantly different from either the anorectic or the normal controls (22.1±0.9).

The oligo/amenorrheic athlete group documented serious disturbances in menstrual cyclicity with 7 athletes reporting several years of prolonged secondary amenorrhea and in 3 of these subjects, estradiol levels were recently assessed as low or undetectable.

Table 6.1 Clinical data on anorexia nervosa patients

	Diagnosis	Duration AN/BN (y)	Age at BMD (y)	Height (m)	Weight (k)	BMI (wt/ht^2)	OA Duration (y)
01	AN/BN	15	38.5	1.52	45.9	19.87	10.0
02	AN/BN	5	18.1	1.70	59.1	20.45	3.0
03	AN	6	27.1	1.54	36.0	15.18	7.0
04	AN/BN	11	24.2	1.82	52.7	15.91	10.0
05	AN/BN	6	28.3	1.70	40.0	13.84	3.0
06	AN	6	19.2	1.58	49.7	19.91	5.5
07	AN	7	23.6	1.65	37.2	13.66	7.0
08	AN	6	23.5	1.60	38.0	14.84	4.0
09	AN	1	16.3	1.63	51.0	19.20	1.0
10	AN	3	20.6	1.57	52.3	21.22	7.0
11	AN/BN	1.5	15.6	1.57	50.0	20.28	2.5
12	AN	6	25.9	1.67	36.4	13.05	6.0
Mean ± S.D.		6.12±3.8	23.41±6.3	1.63±0.08	45.7±7.9	17.28±3.1	5.5±2.9

AN/BN, Anorexia nervosa/Bulimia nervosa; **OA,** Oligo/amenorrhea

Table 6.2 Characteristics of anorexia nervosa patients and comparison groups (mean±SE)

	Anorectic	OA Athlete	Normal
Number	12	12	12
Age (y)	23.4± 1.8	24.3± 1.7	24.7± 2.1
Height (cm)	162.9± 2.4	164.1± 1.4	164.9± 2.5
Weight (k)	45.7± 2.3 *	52.9± 1.5	59.7± 2.2 *
Body Mass Index	17.3± 0.9 *	19.6± 0.5	22.1± 0.9 *

Level of significance * $p < 0.05$, AN vs N.
OA= Oligo/amenorrhea, AN=Anorectic, N=Normal

6.0.1 Bone Mineral Density

The anorectic patients had significantly lower mean BMD values than the other groups at all sites except for the oligo/amenorrheics' Ward's triangle (Table 6.3). Analyses showed significant high negative correlations between the duration of anorexia nervosa and bone mineral density at all sites. The anorectic patients' individual BMD measurements are represented in Figures 6.1 and 6.2. The anorectics' Ward's triangle bone mineral density was significantly different from the oligo/amenorrheic WT when using the less robust Fisher test, ($p<0.05$). There was a tendency for the oligo/amenorrheic group to have lower BMD than the normal group however these differences were not statistically significant.

Table 6.3 Bone mineral density measurements (g/cm^2) of anorexia nervosa , oligo/amenorrheic athletes and normal controls (mean ± SE)

BMD (g/cm^2)	Anorectic	OA Athlete	Normal
Lumbar Spine	0.97±0.05 * †	1.18±0.03 †	1.29±0.03 *
Femoral Neck	0.80±0.05 * †	1.03±0.05 †	1.08±0.04 *
Ward's triangle	0.74±0.06 * †	0.95±0.06 †	1.05±0.05 *
Trochanter	0.62±0.05 * †	0.87±0.04 †	0.87±0.03 *

OA, Oligo/amenorrhea
Level of significance * $p<0.05$, Anorectic vs Normal ; † Anorectic vs Oligo/amenorrheic

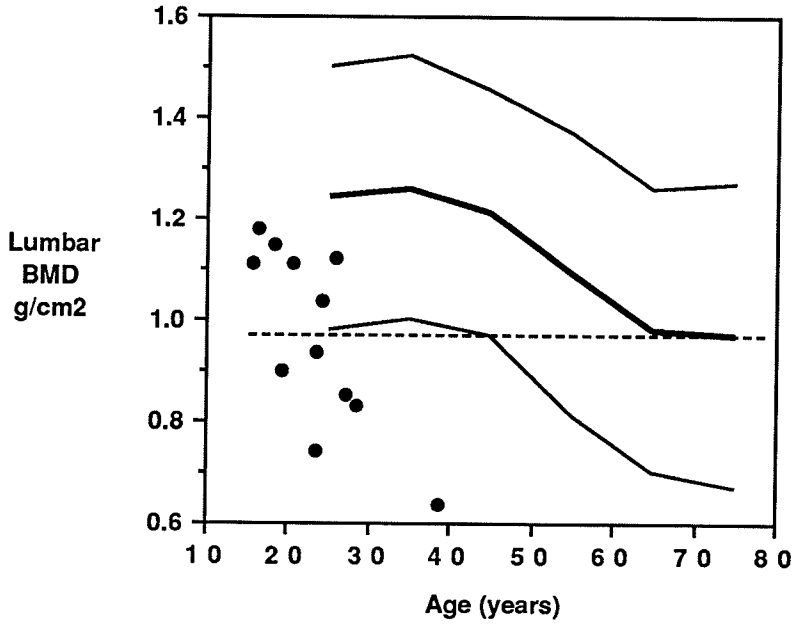

Figure 6.1 The lumbar bone mineral density of anorectic subjects (--- mean) compared to distribution for normal women (mean ± 2 SD) (Mazess et al, 1987a)

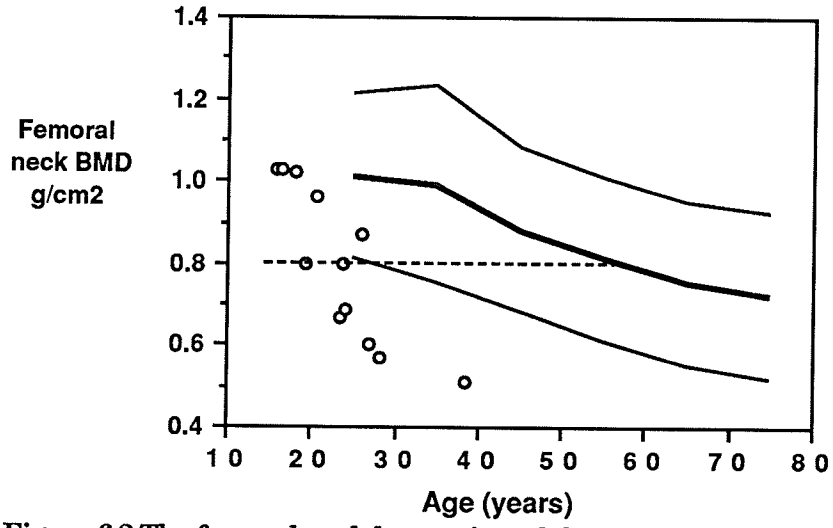

Figure 6.2 The femoral neck bone mineral density of anorectic subjects (--- mean) compared to distribution for normal women (mean ± 2 SD) (Mazess et al, 1987a)

6.1 Discussion

6.1.0 Lumbar Spine

In the investigation of the lumbar site, the BMD of the anorectic group, 0.97±0.05 g/cm^2 (mean±S.E.), was significantly lower than either of the two other groups (Table 6.3). The oligo/amenorrheic athlete mean (1.18±0.03) was also significantly lower than that of the normal controls (1.29± 0.03) when using a Fisher test. The very low anorectic value was more than two standard deviations below the normal control mean. Newton-John and Morgan (1970) introduced the concept of a fracture threshold where bone mass was reduced to two and a half standard deviations below the normal for young women. Although a gradient of risk approach is currently favored, a fracture threshold of two standard deviations below the normal mean values for BMD is a useful marker (Mazess et al, 1987a; Riggs et al, 1982a; Wahner, 1989). The lumbar spine data were compared to normal mean values compiled by Mazess and the mean BMD of the anorectic group corresponded to the mean BMD of a 75 year old woman (Mazess, Barden & Green, 1987b)(Figure 6.1). The value for the anorectic group was less than either of the suggested fracture thresholds of 0.98 g/cm^2 (Riggs et al, 1981; Wahner, 1989) or 1.00 g/cm^2 (Cummings, Kelsey & Nevitt, 1985; Mazess et al, 1987a; Melton III, Wahner, Richelson, O'Fallon & Riggs, 1986). While bone mineral density is the primary determinant of bone strength, the prediction of fractures is difficult as the architecture of bone and other individual aspects of fracture are involved. Nonetheless, the threshold concept indicates that the anorectic group, particularly those 6 patients who have a L2-4 BMD below this level, are currently predisposed to fractures.

These results agree with the body of research which affirms low spinal BMD in anorexia nervosa (Bachrach et al, 1990; Baum et al, 1987; Davies et al, 1990b; Joyce et al, 1990; Treasure et al, 1986; Treasure et al, 1987; Warren, 1985).

6.1.1 Proximal Femur

The anorectic subjects' BMD values were: femoral neck 0.80 ±0.05 , Ward's triangle 0.74 ±0.06, trochanter 0.62 ±0.05; results are expressed as g/cm^2, mean ±S.E.(Table 6.3). These mean values are more than two standard deviations below the normal control means at all sites except for WT where the loss narrowly misses this distinction. In comparison to the regression line for bone loss with age in normal women (Mazess et al, 1987a), the mean BMD values at the femoral neck for the anorectic patients were similar to that of a 60 year old female (Figure 6.2). Five of the anorectic group have a FN BMD which is below the fracture threshold of 0.75 g/cm^2 (Mazess et al, 1987a). This is an important finding because hip fractures represent the most serious manifestation of osteoporosis. The amount and the site of osteoporosis found in the anorectic patients predisposes to both of the two types of hip fractures, femoral neck and trochanteric.

6.1.2 Pattern of Bone Loss

These data contribute important information relating to two facets of low bone density in anorexia nervosa. First, a comparison of BMD in the anorectics and normal controls shows dramatic differences; L2-4=24.8%, FN=25.9%, WT=29.5%, FT=28.7%. These decrements are much greater than the initial investigations in this field which generally reported 10-20% losses and also greater than more recent reports which utilized DPA or DPX. Secondly, most studies demonstrated that trabecular bone was particularly vulnerable in anorexia though limited evidence indicated that there may be concomitant involvement of cortical bone (Bachrach et al, 1990; Crosby et al, 1985; Davies et al, 1990a; Rigotti et al, 1984). At the highly trabecular lumbar spine, these measurements reflected losses of 24.8% which confirm that trabecular bone is compromised by anorexia nervosa. However, at the predominantly cortical femoral neck region a 25.9% reduction in bone mineral density compared to controls indicates that cortical bone is also vulnerable in anorexia nervosa.

Treasure et al (1986) confirmed that the differential pattern of bone loss did not reflect the proportions of trabecular bone. In that investigation, the femoral neck was most affected, then the lumbar spine and there was negligible loss at the radius. The magnitude of the losses at FN, WT and FT compared to the L2-L4 site indicated that both types of bone are reduced in anorexia nervosa. Our data confirm that the bone loss associated with anorexia does not preferentially affect trabecular bone. Rather, bone loss is generalized with involvement of both trabecular and cortical bone at both axial and appendicular sites. The mechanisms of this bone loss are site-specific and an initial short term accelerated phase of trabecular bone loss, similar to the menopausal bone loss pattern may occur. If cortical bone is slower to manifest bone loss, the fact that these subjects have a comparable level of impairment at a primarily cortical bone site, the femoral neck, underscores the severity of skeletal involvement.

6.1.3 Fracture Predisposition

In this investigation several patients suffered from chronic low back pain but only one patient had radiological evidence of fracture. This patient showed evidence of a healed femoral neck fracture and a sclerotic zone from the symphysis pubis to the pubic ramus. Her BMD measurements were the second lowest of the group at the hip and third lowest at the spine.

6.1.4 Duration of Anorexia Nervosa

A longer duration of anorexia appears to be predictive of the severity of bone loss. For these data, there was a significant high negative correlation between the duration of anorexia nervosa and bone mineral density at all sites. The relationship between menstrual dysfunction and bone mineral density was not significant but is likely a critical factor as other investigators have found this relationship to be significant. Similarly, the time since menopause and duration of amenorrhea have been correlated with the degree of bone loss. Estrogen deficiency is likely the common factor which yields similar states of skeletal rarefication in eating disorders, athletic amenorrhea and menopause. Estrogen levels were not measured in this study but 7 of the anorectic patients had recent chart entries of low or undetectable serum levels of estradiol. The menstrual status of the athlete group was more similar to the anorectic patients than the normals.

The anorectic patients were clearly underweight for their height with their mean BMI being lower than either of the two other groups, but only significantly lower than the normal controls. As expected, the L2-4 and FN BMD in the anorectics displayed a positive correlation to BMI.

The oligo/amenorrheic group demonstrated intermediate values for both BMI and BMD at L2-L4 and WT. This differs from data presented in Table 5.4. At the mainly cortical sites of the FN and the FT, the oligo/amenorrheic values were closer to or equalled the normal mean values. An explanation may be that the athletes benefitted from an intermediate level of estrogen protection, compared to the anorectics, which attenuated bone loss. However, this group of oligo/amenorrheic athletes had more severe menstrual dysfunction than the larger pool of oligo/amenorrheic athletes. Thus, the predominantly trabecular sites which are particularly sensitive to estrogen deficiency, reflected intermediary losses compared to the anorectics which were not evident in Chapter 5's comparison to non-athletes. An alternative hypothesis is that the mechanical loading regime of physical activity, along with higher BMI may have attenuated the athletes' bone loss, particularly the cortical bone of

the proximal femur. These postulated effects of estrogen and activity may operate synergistically.

6.1.5 Anorexia Nervosa and Peak Bone Mass

The attainment of peak bone mass may be a critical factor in the young anorectic patient's long term fracture risk. The putative consolidation phase of bone occurring from approximately 18 to 30 years may also be impaired by anorexia nervosa. Eight of these anorectic patients could be categorized as juvenile anorectics in that the age at onset was less than 18 years. Moreover, 3 of these were 13 years of age at onset. Thus, 75 % of the anorectic patients were in the high risk group for significant deficiencies of peak bone mass, through attenuated growth and development as well as impaired consolidation of bone. This group contained 3 subjects whose early age at onset of 13 years may have exacerbated the osteoporotic process through the peripubertal skeletal delay described by Ayer's group (Ayers et al, 1984). The failure to augment bone during the modelling phase implies that the anorectic would be at increased risk for fracture. The short term fracture risk for anorectics is considerably increased as bone mass is the most important factor in bone strength. The failure to attain peak bone mass may represent a permanent irreversible deficit of bone which regardless of the consolidation phase would predispose to increased long term fracture risk.

7. SUMMARY AND CONCLUSIONS

7.0 Summary

The maintenance of ideal skeletal mass depends upon the establishment of an optimum amount of bone during the growth and development phases, the maximization of the consolidation phase and the minimization of subsequent bone loss. The rate and pattern of bone loss in premenopausal female athletes and their non-athletic counterparts were examined. The limitations of this study are that the data are cross-sectional and that self-reported retrospective and current histories of physical activity were utilized.

The literature review indicated that the information regarding skeletal health in premenopausal women was gathered from many varied and not current sources and was largely derived from results of investigations of postmenopausal osteoporosis. Many discrepancies exist in the literature concerning bone growth, development and involution. The time of PBM is controversial and no one model of bone gain and loss has been agreed upon.

Recent investigations have utilized biomedical instruments with improved safety and increased accuracy and precision. New measurement capabilities have permitted the measurement of clinically more important and more informative sites. It is now clear that there are differences between the initiation and the rates of loss throughout the skeleton. Also, earlier models of the age-related rate and pattern of bone loss may have overlooked the complexities of bone loss at specific sites.

Although the reports from investigations featuring postmenopausal BMD have directed studies in younger age groups, the perspective and the population needs are quite different. In premenopausal women, the emphasis is to delineate the factors which are crucial to the primary prevention of osteoporosis.

The main factors, estrogen, physical activity and dietary calcium, which are suspected to influence BMD, have not been clarified for the normal premenopausal woman. There is some controversy regarding each

one of these factors which is in part due to kinds of studies conducted in premenopausal women. For the most part, the research scope has been either too broad, including too many variables, or too narrow, examining one special population. The paradox surrounding physical activity is a good example of such research. Originally, physical activity has been associated with increased bone mass. However, dramatic reductions in BMD have been observed in amenorrheic athletes. Obviously, there is a need for definitive, prospective studies which will investigate the effects of different modes of exercise on BMD. Likewise, longitudinal research is necessary to investigate other important lifestyle factors influencing bone mass in premenopausal women. However, a cross-sectional study, even with its limitations, may also make valuable contributions to this investigative area.

This study proposed to study selected modulators of BMD with reference to an updated model of bone loss. The results have confirmed a model of bone loss in premenopausal women which postulates that bone loss occurs differentially at two skeletal sites. These data demonstrate that bone loss at the lumbar spine does not begin before menopause. Bone loss in the proximal femur begins shortly after linear growth ceases, in the third decade of life.

This age-related loss may be reduced or prevented by regular strenuous physical activity. Results suggest that the the effect of activity is to attenuate the rate of bone loss at the femoral neck, a highly cortical site with a trabecular bone component which may be particularly responsive to multidirectional loading. This attenuation may be due to increased formation or a modification in resorption and formation such that bone mass is augmented. It was the intent to test this hypothesis by assaying osteocalcin, marker of bone formation. However, these assay results were not reportable.

Thus, physical activity may play an major role in the maintenance of cortical bone of the lower appendicular skeleton but it may be even more important to the promotion of bone accretion at maturity. High levels of physical activity throughout early life may be a major determinant in the maximization of peak bone mass. Declining levels of physical activity with increasing age have been implicated in the process of involutional bone

loss. Predictably, the positive influences of habitual physical activity, increased peak bone mass and maintenance of adult bone mass, would have significant impact on osteoporotic fractures.

Paradoxically, strenuous physical activity can result in osteopenia. Exercise-induced menstrual dysfunction, characterized by hypoestrogenism has been noted as the primary mechanism of bone loss. The oligo/amenorrheic subjects in this investigation were not chronically amenorrheic and experienced intermittent short term interruption of menses. Forty per cent of the athletes demonstrated oligo/amenorrhea compared to approximately 14% in the non-athlete group.

Previous reports of chronic amenorrhea in athletes have shown severe reductions in vertebral bone. However, there was evidence that the lumbar spine bone mineral density was maintained in the menstrually dysfunctional group in this study. The oligo/amenorrheics in this study demonstrated moderate but non-significant reductions in bone mineral density at the femur. The bone mineral density values were graduated and although the oligo/amenorrheic athletes were second highest next to the eumenorrheic athletes, their measurements were numerically closer to the eumenorrheic non-athletes. It is important to note that this menstrual dysfunction, though not as severe as amenorrhea had a negative influence on BMD. In the comparison of oligo/amenorrheics, anorectics and normals, the more severe menstrual dysfunction of the oligo/amenorrheic group resulted in this group having reduced BMD compared to normals. Thus, the effects of physical activity could not fully compensate for the state of menstrual dysfunction. Along with the estrogen deficiency. other factors such as decreased body fat and/or decreased body weight may have mediated this process.

The relationship between body fat and reproductive state was also investigated with regard to menarche. The role of inactivity and diet may have contributed to the trend for North American females to undergo menarche at an earlier age. The athletes reported a later menarche, confirming previous reports. It is also interesting to note that those athletes with earlier menarche were significantly fatter as adults. There was no relationship between age at menarche and bone mineral density. Although

it has been suggested that a later menarche, with a deficit in endogenous estrogen could impair bone mass, the present data do not confirm this.

The influence of diet was investigated only with respect to dietary calcium intake and the severe caloric deprivation of the anorectic subjects. The level chosen for the group of high dietary calcium corresponded with the suggested calcium threshold value of 800 mg/day. The calcium data, although a rudimentary assessment, showed no significant relationship of lifetime dietary calcium on bone mineral density in premenopausal women. However, there was a trend towards higher bone mass in the subjects reporting consistent, high dietary calcium. These findings support the idea of a threshold level below which bone mass may be reduced. There was an interactive effect of athletic status and high dietary calcium.

Low BMD, particularly at the spine, has been reported in anorectics. These results also showed that BMD in the anorexia nervosa group was decreased at all sites and comprised a critical amount of bone. The mean values for the lumbar spine 0.97 g/cm^2 and the femoral neck 0.80 g/cm^2 are at or near fracture threshold values and are similar to the spine of a 75 year old and the hip of a 60 year old woman. These extreme losses of bone represent a significant risk for osteoporotic fractures and indicate that intervention is necessary. The question of reversibility of bone loss in recovered anorectics has not been answered by the literature. Studies have not defined what is meant by reversibility and the recovery phases were short, approximately one to two years. However, with a return to normal weight and recovery of menses, it is still possible that BMD may not ever reverse to norm values. Whether bone is capable of recovery may depend upon the age at onset and duration of anorexia, the severity of bone loss and age during the recovery phase.

The menstrual status of the age-matched oligo/amenorrheic athlete control group was more similar to the anorectic patients than the normal controls. Correspondingly, the oligo/amenorrheic athletes' mean lumbar spine bone mineral density was significantly lower than that of the normal controls. Thus, along with the anorectic and the amenorrheic, the oligomenorrheic athlete is also at risk for lower bone mineral density. The degree of skeletal impairment in athletes, although moderate in the larger

group of oligo/amenorrheic subjects is still a cause for concern. It is obvious from the literature and the present results that the degree of menstrual impairment largely determines the degree of skeletal osteopenia. The issue of reversibility of bone loss must also be addressed in this group. Hypothetically, similar conditions regarding timing and severity of bone loss as well as the capability of response at the bone unit may determine whether bone mass will be restored to normal.

The hierarchical order of bone mineral density measurements illustrated that the beneficial effects of mechanical loading may be completely nullified by prolonged menstrual dysfunction and low body weight. The low body weight and menstrual dysfunction in the anorectic patients resulted in extreme skeletal rarefaction. The female athlete may be highly susceptible to eating disorders at both the clinical and sub-clinical levels and this may contribute to the osteopenia which has been diagnosed as exercise-induced menstrual dysfunction.

Thus, physical activity, menstrual state and eating disorders are highly inter-related and critically involved in the skeletal health of the premenopausal woman. Each of these factors represents a spectrum of states which likely affect bone directly and indirectly, through the estrogen state. Together the results from this investigation underscore the importance of maintenance of normal menstrual cycles and a normal body habitus.

The effects of parity and oral contraceptive use on BMD require further investigation. The present results showed that the use of oral contraceptives did not have a positive influence on BMD particularly in athletes. The data on non-athletes' BMD and the endocrine assays of current oral contraceptive users indicate that oral contraceptive use may have a negative influence on BMD. Adequate estrogen dosage for the maintenance of BMD is a key consideration as the efficacy of estrogen replacement therapy has been proved in postmenopausal women. The determination of adequate dosage requires further research which considers, among other factors, inter-individual variation.

The anthropometric factors, which have been used to predict bone mass in postmenopausal women, are not as reliable in physically active

premenopausal females. However, the investigation of these characteristics is not without purpose because positive risk factors have been identified. Age proved to be unable to predict a significant proportion of BMD in athletes. The effects of body size, adiposity and muscularity indices are inter-related and difficult to separate. However, in premenopausal women the relationship of adiposity to BMD is not as evident as in postmenopausal women. Weight has an important influence on the skeleton and this is largely due to the lean tissue component. A positive influence of muscularity on BMD was found in premenopausal women. This finding, taken with evidence from the literature indicate that physical activity and muscularity could play a greater role in the prevention of osteoporosis in postmenopausal women. To date, the emphasis in the literature on body habitus in postmenopausal women has listed a slenderness as a risk factor for osteoporosis whereas obesity is associated with maintained bone mass. Although the role of fat cells in maintaining estrone levels and thereby bone mass has been well-documented, decreased fatness induced by activity might be more detrimental to bone mass in postmenopausal women. In premenopausal females, maintenance of optimal bone mass was associated with a lean, muscular body habitus within a normal range of body weights. The role of physical inactivity with aging and its effects on anthropometric variables and bone mass requires further investigation.

In summary, this study proposed to investigate bone gain and loss in premenopausal women. A model has been proposed and in addition several important factors have been characterized regarding their impact on BMD. Body weight, estrogen status, physical activity and dietary calcium were investigated as the main influential variables. The extreme states of anorexia nervosa and athletic amenorrhea reflect extreme reductions in BMD. It is not clear if these reductions are reversible. Prolonged moderate deviations, below optimal standards or levels, of body weight, estrogenism, physical activity, and dietary calcium may result in moderate to severe skeletal dimunition.

7.1 Conclusions and Recommendations

The present investigation supports the following conclusions:

1. Bone loss with aging begins at the proximal femur at the beginning of the third decade.

2. The lumbar spine does not show evidence of significant bone loss prior to the fifth decade.

3. Strenuous physical activity is associated with higher bone mineral density in normal premenopausal women.

4. Physical activity may attenuate the age-related bone loss at the proximal femur in females.

5. Exercise-induced menstrual dysfunction has negative effects on the skeleton in proportion to the degree of amenorrhea and oligomenorrhea.

6. Anorexia nervosa results in severe osteopenia.

7. Menstrual state, physical activity and nutrition are critical to optimum skeletal mass and menstrual state is likely the primary factor.

8. Oral contraceptive use does not promote significant increments in the bone mass. In non-athletes, the use of oral contraceptives may have a negative influence on bone mass.

9. Dietary calcium at the current recommended intakes, is associated with high bone mineral density when accompanied by normal menstrual state and physical activity.

The following are topics which require investigation: the age at peak bone mass, the potential amount of bone mass accretion during consolidation, factors affecting peak bone mass, factors influencing the

maintenance of optimal bone mass, the rates and patterns of involutional bone loss and factors modifying bone loss. It is important that these critical components of bone health be prospectively investigated. Such research would direct therapeutic and more importantly, formulate prophylactic alternatives.

The effects of physical activity, menstrual dysfunction and eating disorders have been identified in this study as important factors in premenopausal skeletal health. It is recommended that future investigations attempt to further clarify the scope and relative contribution of genetics, mechanical loading, endocrine factors and nutrition. It is further recommended that future research investigate the effects of a variety of modes of exercise through intervention studies in younger females and premenopausal women. Bone mineral density screening of chronically amenorrheic athletes and those athletes exhibiting signs of "athletica nervosa" is recommended. Bone mass studies regarding specified and non-specified eating disorders in adolescent female athletes should be initiated.

To date, the focus of osteoporosis has been reactive. The prevention of inordinate bone loss should be a key strategy against osteoporosis. Current instrumentation permits the measurement and monitoring of bone loss with machines which are non-invasive, safe, accurate, precise, cost effective and have important applications in all populations which are susceptible to osteoporosis. Measurements of bone mineral density are recommended to screen the anorectic patient and the amenorrheic athlete for determination of bone loss and to monitor the effects of therapy. The bone mineral screening of anorexia nervosa patients may also be useful to impress upon these individuals that the obsession with thinness increases their individual osteoporotic risk. Athletes may be persuaded to modify training regimes and/or increase body weight to avoid injury.

If osteoporosis is determined, the question of therapy arises. Reasonable measures of therapy should focus on weight restoration, adequate calcium intake and exclusion of negative risk factors. Combined therapy of estrogen and progestin should be investigated for those at high skeletal risk. Prospective investigations must be initiated to evaluate the

reversibility of bone loss in amenorrheics and anorectics. The utility of physical activity in the treatment of anorexia nervosa also needs further research.

Based on the results of this investigation, the premenopausal woman is encouraged to maintain a normal body weight for height and participate in regular, vigorous physical activity so that the BMD and muscularity are enhanced. Physical activity and diet, which includes a recommended intake of dietary calcium, should be adjusted to ensure maintenance of normal menstrual cycles.

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APPENDICES

APPENDIX A

Sport & Exercise Sciences Research Institute
The University of Manitoba, Winnipeg
Canada R3T 2N2
(204) 474-8629
Alan Martin, Ph.D., Director

CONSENT FORM

I have read the description of the study and understand the measurement procedures involved.

I also consent to the release of those portions of my medical records relating to bone fracture to Dr. Alan Martin, the Director of the study.

I understand that I may withdraw from the study at any time without prejudice.

All information will be kept confidential.

_____ _____

Date Participant

_____ _____

Parent/Guardian's Signature Witness
(if subject is less than 18 years)

APPENDIX B

AMENORRHEA QUESTIONNAIRE

Elite female athletes often experience unusual gynecological histories. Some athletes may be chronically amenorrhea while others may have occasional or no irregular periods. We would like to examine the relationship between menstrual status and bone density in female athletes. This initial questionnaire will provide us with some general information upon entrance to this study. Your cooperation in completing this is greatly appreciated.

1. My first period occurred at the age of (Circle one):

less than 10 years	10	11
12	13	14
15	16	17
18 or older	has not begun yet	

2. My period is presently (Circle one):
   a) REGULAR (a period every 25-35 days)
   b) IRREGULAR

3. Have you ever missed any periods or been amenorrhea?

   YES_____ NO_____

   If yes, WHEN? presently_____ or in the past_____or both_____

   If yes, how many months were missed?_____

4. My normal menstrual cycle occurs every_____days and my menstrual flow lasts for _____days.

5. Do your periods change with changes in your training regime?

   YES_____ NO_____

6. Are you presently taking any estrogen supplements? (this includes estrogen or birth control pills)

   YES_____ NO_____

7. Have you ever had a stress fracture? YES_____ NO_____

   If yes, when?_____ which bone?_____

   If yes, was it diagnosed by X-rays? YES_____ NO_____

   If yes, was it diagnosed by bone scan? YES_____ NO_____

8. Have you ever had any other type of major musculoskeletal injury?

   If so what type and when? _____

   _____

9. The SPORT I participate in:_____

   The SPECIFIC ACTIVITY I participate in:_____

10. Comments: please provide any other information you feel is important on the back of this sheet.

NAME_____

ADDRESS_____

POSTAL CODE_____

TELEPHONE home_____ work_____

APPENDIX C

Sport & Exercise Sciences Research Institute

University of Manitoba

Anthropometric Proforma

Name_____ ID#_____

(last) (first & initial)

Birth Date _____/_____/_____ Measurement Date _____/_____/_____

(month / day / year) (month / day / year)

Measured by _____

Body Size:

height [stature] (cm)..... |_____•__|_____•__|_____•__||_____•__|

weight (kg)..... |_____•__|_____•__|_____•__||_____•__|

Skinfolds (mm):

biceps..... |_____•__|_____•__|_____•__||_____•__|

triceps..... |_____•__|_____•__|_____•__||_____•__|

subscapular..... |_____•__|_____•__|_____•__||_____•__|

iliac crest..... |_____•__|_____•__|_____•__||_____•__|

abdominal/umbilical (vertical)..... |_____•__|_____•__|_____•__||_____•__|

front thigh..... |_____•__|_____•__|_____•__||_____•__|

medial calf..... |_____•__|_____•__|_____•__||_____•__|

Girths (cm):

arm..... |_____•__|_____•__|_____•__||_____•__|

forearm..... |_____•__|_____•__|_____•__||_____•__|

wrist..... |_____•__|_____•__|_____•__||_____•__|

chest..... |_____•__|_____•__|_____•__||_____•__|

waist..... |_____•__|_____•__|_____•__||_____•__|

gluteal..... |_____•__|_____•__|_____•__||_____•__|

upper thigh..... |_____•__|_____•__|_____•__||_____•__|

mid thigh..... |_____•__|_____•__|_____•__||_____•__|

calf..... |_____•__|_____•__|_____•__||_____•__|

ankle..... |_____•__|_____•__|_____•__||_____•__|

neck..... |_____•__|_____•__|_____•__||_____•__|

head..... |_____•__|_____•__|_____•__||_____•__|

Breadths (cm):

humerus..... |_____•__|_____•__|_____•__||_____•__|

wrist..... |_____•__|_____•__|_____•__||_____•__|

femur..... |_____•__|_____•__|_____•__||_____•__|

ankle..... |_____•__|_____•__|_____•__||_____•__|

APPENDIX D

GENERAL MEDICAL INTERVIEW

1. In the last 6 months, have you seen a doctor? _____yes _____no

2. If yes, why did you go to the doctor? _____

_____.

3. In the last 6 months, have you had any injuries that caused you to go to the doctor?

_____yes _____no

4. If yes, what was the nature of the injury?

_____

_____

_____

5. In the last 6 months, have you been hospitalized? ____yes ____no

6. If yes, why? _____.

7. In the last 6 months, have you been sick with fever or in bed?

____yes ____no

8. Are you taking any medications? _____yes _____no

9. If yes, what are they? _____.

10. Do you know why you are taking this medicine? ____yes ____no

11. Have you had any problems with your bones such as a fracture, a stress fracture or shin splints?

____yes ____no

12. If any, how many stress fractures have you had? _____

13. If any, how many other fractures have you had? _____

14. Please fill in: Type and location of fracture Age at fracture

_____ _____

_____ _____

_____ _____

15. Did you have a bone scan to diagnose any of these fractures?

_____yes _____no (when_____)

16. Have you ever been told that you have scoliosis? _____yes _____no

17. If yes, when was it diagnosed? _____

18. What degree of curvature was diagnosed? _____

19. Have you had X-rays for scoliosis?

_____yes _____no (when_____)

20. List the doctors treating you for:

Scoliosis: Doctor's name _____

address _____

phone number _____

Fractures including stress fractures:

Doctor's name _____

address _____

phone number _____

21. Do we have permission to contact the doctor(s) to obtain medical information?

_____yes _____no

22. Do we have permission to contact the doctor(s) to look at X-rays?

_____yes _____no

APPENDIX E

Name_____

Date _____

Menstrual History

1. Have you had your first menstrual period? _____yes _____no

2. How old were you when you had your first period? _____

3. What year was it? _____ What month was it? _____

4. Do you menstruate regularly? _____yes _____no

5. How many cycles do you have in a year? _____

6. When was your last period? _____

7. How many days are there from the start on one period to the start of the next period?

_____

8. How many days does your menstrual flow last? _____

9. Have you ever missed a period? _____yes _____no

If you have <u>never missed</u> a menstrual period and answered "<u>no</u>" to question #9 please continue at question #19.

If you answered "<u>yes</u>" to #9 please continue to answer questions 10-18:

10. At what age did you skip a period? _____

11. For how long have you missed menstruating? _____

12. At this time, how tall were you? (height) _____

13. At this time, how much did you weigh? (weight) _____

14. Had you recently lost weight? _____yes _____no

15. If you had lost weight, how many pounds had you lost? _____

16. Were you dieting to lose weight at the same time you missed your menstrual period(s)?

_____yes _____no

17. Were you under stress at the time you missed your menstrual period(s)?

_____yes _____no

18. If yes, what kind of events were causing this stress? _____

_____

_____

_____.

19. Can you readily predict the onset of your period? (check one)

very easily _____ somewhat _____ not at all _____

20. When is your next period likely to occur? _____

21. **Please complete the following menstrual calendar.**

Put a (0) in every box where you are certain that you missed a menstrual period. Be sure to leave blank all those months that you are certain that you did menstruate.

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC
1974												
1975												
1976												
1977												
1978												
1979												
1980												
1981												
1982												
1983												
1984												
1985												
1986												
1987												
1988												
1989												

22. Do you now take or have you ever taken any hormones?

_____yes _____no

23. If yes, which hormones were taken and how old were you?

_____

_____.

24. Have you ever been pregnant? _____yes _____no

25. List pregnancies and outcome by year:

_____

_____

_____

26. How old were you when you grew the most? _____

27. How much did you grow in that time? _____ (inches)

28. In the last 6 months, have you gained or lost weight?

_____yes _____no

29. If yes, how much weight?

gained_____(pounds)

lost_____(pounds)

30. What has been your lowest adult (since 16 years) weight? _____

31. How long ago was this? _____yrs _____mos

32. How long did you maintain this low weight? _____yrs _____mos

33. What has been your highest (excluding pregnancy) weight? _____

34. How long ago was this? _____yrs _____mos

35. How long did you maintain this weight? _____yrs _____mos

36. Have you ever had secretion of milk from your breasts? (other than nursing)

_____yes _____no

37. Have you ever had unusual growth of hair on your body?

_____yes _____no

Please specify _____.

APPENDIX F

EIA QUESTIONNAIRE

ID Number:_____

Today's Date:____/____/____

1. NAME:_____

2. AGE:_____

3. DATE OF BIRTH:_____/_____/_____

4. OCCUPATION:_____

5. SPORT HISTORY:

Name of Sport_____

How long have you been training?_____(years).

How old were you when you started training?_____(years).

PHYSICAL ACTIVITY QUESTIONNAIRE

Because this study seeks to relate bone mineral content to physical activity level, we are asking you to give us a brief summary of your past and present activity level in home, school, job, recreational pursuits, and athletic training. We realize that it is not always easy to recall everything, but we would appreciate as complete and accurate an account as possible.

6.I. Childhood (6 - 12 years) and adolescent (13 - 18 years) activity.

1. Rate your overall level of activity as a child and adolescent (circle one).

1	2	3	4	5
very active	moderately active	active	sometimes active	seldom active

2. How would you describe the games you played most often as a child?(circle one)

1	2	3	4
mostly running jumping, climbing throwing games	games requiring some running, jumping, climbing throwing, etc.	games requiring little jumping running, climbing etc., mostly start and stop activities.	sedentary games such as board games, drawing, puzzles, etc.

3. How much television did you watch as a child and adolescent? (circle one)

1	2	3	4
0-1 hr/day	1-2 hrs/day	2-3 hrs/day	3 or more hrs/day

4. How much reading/studying did you do as a child and adolescent? (circle one)

1	2	3	4
0-1 hr/day	1-2 hrs/day	2-3 hrs/day	3 or more hrs/day

5. Did you participate in organized sports as a child and adolescent? _____If yes,

a) list the sports in which you participated_____

_____

b) how many days per week did you workout (practice)?_____

c) how many weeks per year?_____

d) approximately how long did each workout last (hours and minutes)?

_____

6. In addition to practicing a particular sport skill, were you active in any other form of regular physical training?_____.
If yes,

a) what kind of training did you do?_____

b) how many days per week?_____

c) how many weeks per year?_____

d) approximately how long did each workout last (hours and minutes)?

_____

7. During which years were you most active? (circle one)

1	2	3	4
6-9 years	9-12 years	13-15 years	15-18 years

II. Activities in the home.

How would you rate the amount of physical activity required of you while at home?

_____ practically none

_____ slight

_____ moderate

_____ active

_____ very active

III. Activities at school.

How would you rate the amount of physical activity required of you while at school?

_____ practically none

_____ slight

_____ moderate

_____ active

_____ very active

IV. Are you currently employed? If so, how many hours per week?

_____ hours per week.

How would you rate the amount of physical activity required of you while on the job?

_____ practically none

_____ slight

_____ moderate

_____ active

_____ very active

V. Current level of physical activity in competitive sports and recreational pursuits.

1. How would you rate your level of physical fitness right now:

____very low ____low ____average ____high ____very high

2. How would you rate your current level of physical activity (other than normal activities associated with home or job)?

_____ very low (0 day per week)

_____ low (1 -2 days per week)

_____ moderate (3 days per week)

_____ high (4 - 5 days per week)

_____ very high (6 - 7 days per week)

3. Over the last year in what sports /physical activities did you normally participate?

Activity	Level			Intensity					Session Duration (min)	Times Per Week	Weeks Per Year
				Low		Moder.		High			
	Organized	Recr.	Work	1	2	3	4	5			
Aerobics											
Badminton											
Basketball											
Bicycling											
Dancing (aerobic)											
Dancing (ballet, modern)											
Diving											
Fencing											
Gymnastics											
Hiking (backpack)											
Hiking (3-8 hr. walks)											

Activity	Level			Intensity					Session Duration (min)	Times per week	Weeks per yr.
				Low		Moder.		High			
	Organized	Recr.	Work	1	2	3	4	5			
Hockey (field)											
Ice skating											
Jogging/Running											
Karate/Judo											
Racquetball											
Riding(horseback)											
Roller skating											
Rowing											
Sailing(boat)											
Skiing(downhill)											
Skiing (cross country)											
Skiing (water)											
Soccer											
Squash/handball											
Swimming											
Tennis(lawn)											
Tennis (table)											
Track (field events, shot, discus, etc.)											
Volleyball (gym)											
Walking											
Weightlifting											
Work-lawn & garden (mowing, clipping,weeding)											
Work-farm, forest & construction (lifting, carrying, digging, etc.)											

VI. If you are a competitive athlete please complete the following section.

1. **Name the sport you are presently training for:** _____.

2. **Training Schedule:**

Number of hours per day _____

Number of days per week _____

Number of weeks per month _____

Number of months per year _____

Season(s) of maximal training: Circle:

Winter Spring Summer Fall

3. **Competitive Schedule:** Circle the appropriate season

Major provincial competition	winter	spring	summer	fall
Major national competition	winter	spring	summer	fall
Season of maximal competition	winter	spring	summer	fall

4. **Rest Schedule:**

Are rest days built into your weekly or monthly schedule?

_____yes _____no

How many days do you rest per month?_____

Are intervals of relatively low training intensities built into your yearly schedule?

_____yes _____no

APPENDIX G

Name: _____ Subject #:_____

Date of Birth____/____/____ Date of Interview____/____/____ Age:_____

DIET EVALUATION - FOOD FREQUENCY

Indicate the approximate number of servings of the following foods you eat each week.

	Total Servings Per Week
Group A	
Milk - whole, two percent, one percent, skim, chocolate, buttermilk (1 serving = 1 cup)	_____
Yogurt - plain, with fruit, or flavored (1 serving = 1 cup)	_____
Sardines with bones - (1 serving = 3 ounces)	_____
Group A=	_____
Group B	
Cheese - American, Brick Cheddar, Colby, Edam, Mozzarella, processed cheese (1 serving = 1" cube)	_____
Cottage cheese, ice cream, ice milk, pudding (1 serving = 1 cup)	_____
Oysters - (1 serving = 3/4 cup)	_____
Malt, shakes - (1 serving = 1 cup)	_____
Creamed soups - (1 serving = 1 cup)	_____
Group B=	_____
Group C	
Tofu - (1 serving = 3 1/2 ounces)	_____
Cooked dried beans or peas (1 serving = 1 cup)	_____
Group C=	_____

Group D

Protein - meat, poultry, fish

(1 serving = 2 ounces) Group D= _____

I - greatly - slightly - decreased - the amount of milk I consumed.

_____ before age 11
_____ between age 11 and 15
_____ between age 16 and 20
_____ between age 21 and 30
_____ between age 31 and 40
_____ over age 40
_____ I have always drunk the same amount

PLEASE ANSWER THE FOLLOWING QUESTIONS:

Do you eat a special diet? Yes_____ No_____

If yes: _____vegetarian
_____low sodium
_____low cholesterol
_____other _____
_____

Do you take a calcium supplement? _____Yes _____No

If the answer is yes, how many times a day do you take it? _____

What is the supplement name?_____

How many milligrams of calcium does it contain? _____

How many units of vitamin "D" does it contain? _____

Do you take a multivitamin/mineral supplement? _____Yes _____No

If the answer is yes, how many times a day do you take it? _____

What is the supplement name?_____

How many milligrams of calcium does it contain? _____

How many units of vitamin "D" does it contain? _____

Do you take any of the following antacids on a daily basis?
Tums, Titralac, Titralac Syrup, Bicarbonate, Alka-Seltzer-2 _____Yes _____No

If the answer if yes, how many times a day do you take it? _____

Do you take a bran or fiber supplement? _____Yes _____No

If the answer if yes, how many times a day do you take it? _____

How many grams of fiber does the supplement contain? _____

What is the supplement name?_____

How many cups of coffee do you drink each day? (circle) 1-2 3-4 5-6 7-8 8+

How many 12 ounce cans of pop containing caffeine do you drink each day?
(circle) 1-2 3-4 5-6 7-8 8+

Do you drink two or more beverages containing alcohol each day? _____Yes _____No

How many minutes do you spend outdoors each day? _____less than 15 minutes
_____16 - 30 minutes
_____30 minutes