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UNIVERSITY OF ALBERTA

Bone Mineral Density in Highly Active, Moderately Active and Sedentary University-Aged Females.

by

Rebecca Joanne Sunderland

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of

Master of Science

Faculty of Physical Education and Recreation.

Edmonton, Alberta.

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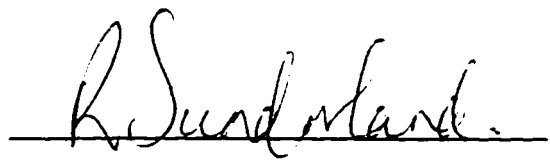

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Bone Mineral Density in Highly Active, Moderately Active and Sedentary University-Aged Females** submitted by **Rebecca Joanne Sunderland** in partial fulfillment of the requirements for the degree of **Master of Science.**

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DATED SEPT. 2/97.

DEDICATION

To my mum and dad, who taught me to follow my dreams, and despite being so far away, always had faith that I would succeed, even when I did not believe it myself. Without their constant support and encouragement I would not be where I am today.

Thanks.

ABSTRACT

The main purpose of this study was to investigate bone mineral density (BMD) in 20 highly active (HA) females [elite field hockey players; mean exercise time=14.5 h/wk], who were matched for height, weight, menarcheal age, chronological age and oral contraceptive (OC) use to 19 moderately active (MA) [recreational soccer players; mean exercise time=4.3 h/wk] and 19 sedentary (SED) [mean exercise time=0.0 h/wk] university-aged females. Dietary intake, body composition and menstrual status were also compared between the three groups.

BMD was measured using dual energy X-ray absorptiometry at the lumbar spine (L1-L4), whole body, femoral neck, Ward's triangle, distal fibula, distal tibia, distal tibia/fibula, mid fibula, mid tibia and mid tibia/fibula. Body composition was assessed using the sum of five skinfolds. Subjects also completed a 3-day dietary record, calcium food frequency questionnaire, menstrual history questionnaire and a training history questionnaire.

BMD in the HA subjects was significantly greater than the SED subjects at every site except at the mid fibula, and significantly greater than the MA subjects at the femoral neck and Ward's triangle. The MA subjects had significantly greater BMD at the femoral neck, Ward's triangle, distal tibia and distal tibia/fibula than the SED subjects.

The HA subjects were significantly leaner than the MA and SED subjects. Dietary intake was similar between all three groups. There were no differences in menstrual history or OC-use between the three groups. When BMD data were collapsed and compared according to menstrual history and OC-use there were no significant

differences in BMD between groups.

Results suggested that both volume of exercise and type of exercise led to important differences in BMD between the groups studied. 14.5 h/wk of exercise does not appear to have any detrimental effects on BMD in this group of HA subjects. 4.3 h/wk of exercise is sufficient to have an effect on BMD, and as such sedentary individuals should continue to be encouraged to exercise.

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CHAPTER 1

Introduction

Osteoporosis is a disease characterised by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk (Consensus Development Conference, 1991). With the demographic shift towards an aging population osteoporosis has become an increasing public health concern. The lifetime risk (the proportion of the population that can be expected to experience fractures over a life of average length, 78.9 years) for fractures in white women at age fifty has been estimated to be almost 40% (Melton, Chrischilles, Cooper, Lane & Riggs, 1992). Approximately 1.2 million fractures attributable to osteoporosis occur each year, resulting in an estimated $5.2 billion in direct and indirect costs (Zohman & Lieberman, 1995).

There is a growing awareness that the foundations of osteoporosis may be found early in life. Peak bone mass (PBM) attained in the adult years, and rate of bone loss as one ages are both important factors in determining who is at risk for osteoporotic fractures in their later years. To date, efforts to prevent osteoporotic fractures have focussed on either maintaining existing bone mass in adults, minimizing bone mass loss in the elderly, or augmenting bone mass gains in the young (Snow-Harter & Marcus, 1991). However, many researchers generally accept that maximizing bone mass accretion during the years leading to skeletal maturity is the best protection against osteoporosis and related fractures (Grimston, Morrison, Harder & Hanley, 1992).

PBM is determined mainly by genetic factors, but environmental factors such as calcium intake and physical activity during childhood and adolescence could play a role (Ruiz, Mandel & Garabedian, 1995). Heaney (1986) suggested that if calcium deficiency occurs during childhood or early adulthood, maximum skeletal potential may not be attained, which could predispose individuals to osteoporosis in later life. Retrospective studies which have investigated childhood dietary habits and activity patterns suggest that high calcium intake and exercise during childhood lead to greater PBM (Nieves, Golden,

Siris, Kelsey & Lindsay, 1995; Ward, Lord, Williams, Anstey & Zivanovic, 1995).

Factors that may lead to an increased rate of bone growth and an increased PBM are being investigated in the younger female population. For example, Haapasalo et al. (1994) found that in the Finnish national women's squash team those players who had started training during puberty had greater bone mineral density (BMD) compared to those who began training after puberty. This suggests that training during puberty may optimize BMD in women. As a result of some of these studies, young females are being encouraged to exercise frequently. It is hoped that increased activity in the teenage years will be beneficial in later life, delaying osteopenia, and reducing the incidence of osteoporosis.

Research on postmenopausal women has provided evidence that physical activity, estrogen replacement therapy and a diet rich in calcium may all contribute to slowing the rate of bone loss and subsequently reducing the risk of fracture (Nelson, Fisher, Dilmanian, Dallal & Evans, 1991; Pruitt, Jackson, Baitels & Lehnhard, 1992; Taaffe, Villa, Delay & Marcus, 1995). However, if physical activity and a calcium rich diet are implemented during childhood and adolescence then fractures which occur as a result of osteoporosis may potentially be even further reduced (Recker et al., 1992; Ruiz, Mandell & Garabedian, 1995).

Further research, focussing on active premenopausal women, has linked low BMD to low body weight, disordered eating and menstrual dysfunction (Yeager, Agostini, Nattiv & Drinkwater, 1993). The identification of the combination of amenorrhea, disordered eating and osteoporosis amongst adolescent and young female athletes has resulted in the identification of a disorder known as the "female athlete triad" (Yeager et al., 1993). While the presence of any one of these disorders may be detrimental to performance (Drinkwater et al., 1984; Frusztajer, Dhuper, Warren, Brooks-Gunn & Fox, 1990), a combination of all three could potentially be fatal (Yeager et al., 1993). Consequently, the focus of recent research has turned towards athletes who participate in sports such as distance running, dancing and gymnastics (Kirchner, Lewis & O'Conner, 1995; Robinson et al., 1995). Both of these groups of researchers found that

exercise was beneficial in enhancing BMD when compared to a sedentary lifestyle. They added that a condition of this was that individuals ate a balanced diet, had regular menses, and had a "normal" percent body fat (Kirchner et al., 1995; Robinson et al., 1995). The presence of amenorrhea, a poor diet and a low percentage body fat may reduce the magnitude of the benefit of exercise. Many elite athletes have been shown to have poor dietary habits, a very low percentage body fat and have irregular or absent menses (Drinkwater et al., 1984; Frusztajer et al., 1990; Kirchner et al., 1995). If these negative factors outweigh the positive benefits of exercise on bone some elite athletes may have a lower than expected BMD (Marcus et al., 1985; Robinson et al., 1995).

Although exercise is advocated for the young growing female population, it is presently unclear as to which types of sports and activities are the most beneficial, and what is the optimal duration, intensity and frequency of training. Also, it is believed that different sports may have differing effects on the skeleton (Heinonen et al., 1993; Taaffe et al., 1995). Different loading patterns seen in sports have led to the suggestion of a sport specific effect on BMD. However, only a narrow spectrum of sports has been investigated, and as a consequence, it has been difficult to suggest that participation in one sport may be more beneficial than participation in another. It has also been proposed that too much exercise when combined with amenorrhea, may in fact have detrimental effects on skeletal health, leading to lower BMDs in highly trained athletes (Drinkwater et al., 1984; Marcus et al., 1985).

Statement of the Problem

Exercise induces a complex endocrine response which may be dependent on a variety of factors such as exercise intensity, sport, body composition, body weight, nutritional status, age and psychological stress (Fruth & Worrell, 1995). The coexistence of strenuous exercise, an immature reproductive system, a low percentage body fat and insufficient energy availability have been suggested as precursors to amenorrhea, a condition that researchers have shown leads to low BMD (Arena et al., 1995; Bale et al., 1994; Drinkwater et al., 1984; Frisch & McArthur, 1974; Marcus et al., 1985; Warren,

1980).

Young women are involved in many different types of sports, but the effect of activity on BMD has only been investigated in some of these sports. For example, BMD in runners, gymnasts, dancers and figure skaters have been extensively investigated (Drinkwater et al., 1984; Frusztajer et al., 1990; Kirchner et al., 1995; Marcus et al., 1985; Nelson et al., 1986; Robinson et al., 1995). Conversely, the effects of popular sports such as field hockey and soccer on BMD are virtually unknown.

Elite athletes typically undertake an intense high volume training schedule that is both demanding and time consuming. For example, the players in the Canadian junior women's field hockey program are involved minimally in five technical, three cardiovascular fitness and two weight sessions a week, which averages about three hours of training a day, six days a week (Marshall, personal communication, October, 1996).

Recreational athletes, whether participating purely for fun or for competition, do not usually undergo the same rigorous training schedule as elite athletes. As a result they are involved in a much lower volume of training, typically two to three hours per week.

To date, there have been few studies which have compared volume of training and its effect on skeletal health in university-aged elite female athletes. Furthermore, there have been few published articles that have looked at BMD in the sports of soccer and field hockey. While the age at which PBM is achieved is still being debated (Riggs & Melton, 1986; Glastre et al., 1990; Ott, 1991; Bonjour, Theintz, Buchs, Slosman & Rizzoli, 1991), regular exercise during university years may turn out to be an important time to maximize BMD and PBM in females. The aim of the present study is to provide a snapshot account of BMD at a number of sites in active and inactive university-aged females.

Many studies have shown that elite athletes in some sports have unusually low BMDs, despite spending many hours each week exercising (Drinkwater et al., 1984; Marcus et al., 1985; Nelson et al., 1986; Robinson et al., 1995). While it is not conclusive that excessive exercising has detrimental effects on the skeleton in every sport, it is imperative that this issue is investigated across a wide spectrum of sports. As low BMD

may occur as a result of several factors, if low BMDs are found in field hockey players it may be important to implement a holistic program (to include changes in training regimen, and nutrition and female health education) to prevent future problems in these elite athletes.

Further, if exercise of a recreational nature has clear benefits to bone health it is essential that sedentary females are encouraged to become involved in recreational sports teams, community leagues and high school physical education classes.

Purpose of the Study

The primary purpose of the study was to investigate the relationship between BMD and exercise levels in university-aged females. Specifically, the following research question was addressed:

Is there a difference in site-specific BMD between highly active, moderately active, and sedentary females?

In addition, five further questions were investigated:

(A) Are there differences in body composition between highly active, moderately active, and sedentary females?

(B) Are there differences in dietary intake between highly active, moderately active, and sedentary females?

(C) Are there differences in menstrual history between highly active, moderately active, and sedentary females?

(D) Is there a difference in BMD between those subjects who are amenorrheic, those subjects who are eumenorrheic and those subjects who are using oral contraceptives (OCs)?

(E) What factors (physical characteristics, exercise pattern, dietary intake, menstrual history, body composition) are the best predictors of BMD?

In an attempt to answer all of the questions above, the following seven hypotheses were tested:

(1) Subjects who are highly active and moderately active will have a higher BMD at all sites compared with sedentary subjects.

(2) Subjects who are moderately active will have higher a BMD at all sites compared with highly active and sedentary subjects.

(3) The highly active females will be leaner and will have a lower percentage body fat than the moderately active and sedentary females.

(4) There will be no differences in dietary intake between the three groups.

(5) There will be differences in menstrual history between the three groups. The highly active subjects will have a higher incidence of menstrual dysfunction than either the moderately active or the sedentary subjects.

(6) Eumenorrheic subjects and the oral contraceptive users will have greater BMDs than the amenorrheic subjects.

(7) Physical characteristics, volume of exercise, dietary intake, menstrual history and body composition are expected to be predictors of BMD in this age group.

The above hypotheses were tested in a cross-sectional study, using 20 highly active females (elite field hockey players), 19 moderately active females (recreational soccer players), and 19 sedentary females. Each subject was measured for height, weight and body composition, and was asked to complete a menstrual history questionnaire, a 3-day dietary record, a calcium food frequency questionnaire (FFQ), a training questionnaire, and had BMD measured at the following sites: right and left tibia/fibula; right and left hip; lumbar spine (L1-L4); and whole body.

The results of this study will provide valuable additional information on female skeletal health. The sports being studied are a step away from the traditional sports that have previously been investigated, and the results of this study will be beneficial to these sport organizations as a whole, and also to the individuals who participate in these sports.

Limitations

1. The highly active and moderately active subjects were not involved in the same sport. Because of limited subject numbers in this geographic location, it was not practical to use field hockey players as the moderately active subject group. Instead, soccer players were recruited for the moderately active group, because of the similarities between the two games in terms of pitch size, seasonality, training and playing regimen and number of players on the pitch.
2. It was assumed that any differences in BMD would be due to differences in lifestyle choices that the subjects had made.
3. The results can only be generalizable to females in this age range and to the sports concerned.

It is believed that these limitations will not detract from the contribution that this research will make to the area of female skeletal health, and more specifically to the sports concerned.

Definitions

Osteoporosis is a disease characterised by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk (Consensus Development Conference, 1991).

Osteopenia is defined as a state of heightened fracture risk due to low bone mass. A level of less than two standard deviations below the mean of young normals has been suggested as being clinically significant for osteopenia (Melton et al., 1992).

Peak bone mass (PBM) is defined as the amount of bony tissue present at the end of skeletal maturation, and is an important determinant of osteoporotic fracture risk in adulthood (Bonjour, Theintz, Law, Slosman & Rizzoli, 1995).

Bone mineral content (BMC) values are given as grams of calcium hydroxyapatite.

Bone mineral density (BMD) values measured by dual energy X-ray absorptiometry (DXA) are given as areal bone density in grams/square centimetre (which

is BMC divided by the projected area of the bone).

The present study is primarily concerned with BMD. The BMC data, while important in terms of total bone mass and developmental bone mass changes, are inappropriate when making comparisons between subjects, because of the effects of height and bone length on BMC (Hassager & Christiansen, 1995). The use of BMD, which is BMC normalized for bone length, allows for a more accurate comparison of skeletal health between subjects. The majority of literature in the female athlete area focuses on BMD, and fracture thresholds are often given in terms of BMD rather than BMC. As such, details in later chapters will focus on BMD as a measurement of skeletal health rather than on BMC.

For the purposes of this study, the following activity definitions were applied:

A highly active subject was a field hockey player who had been selected to play on the Canadian junior field hockey squad. These athletes were involved in approximately ten training sessions a week, and averaged about three hours of training per day, six days per week.

A moderately active subject was a member of a soccer team which competed in the Edmonton Women's Soccer Association League. Subjects in this group performed a minimum of three one hour exercise sessions per week. Longitudinal studies that have investigated the effect of training sedentary people have shown benefits using a protocol of exercising three times a week for an hour (Friedlander, Genant, Sadowsky, Byl & Glüer, 1995; Snow-Harter, Bouxsein, Lewis Carter & Marcus, 1992).

Sedentary controls were females who were not regularly participating in any sport or exercise class, and did no more than one hour of exercise a week.

CHAPTER 2

LITERATURE REVIEW

Measurement of Bone Mineral Density

Over the last three decades there has been much effort spent on developing methods for the quantitative assessment of the skeleton that would permit early detection of osteoporosis, specifically, the calculation of present and future fracture risk. Identification of early signs of osteopenia would allow intervention and possibly the reduction of future fractures.

Non-invasive measures of bone mass have largely replaced biopsies in the diagnosis and follow-up of osteoporosis. The low radiation and high precision of recently developed non-invasive techniques has also made feasible the investigation of young children and adolescents for the non-invasive quantitative assessment of bone mass (Slosman, Rizzoli & Bonjour, 1995).

Several methods are in current use; their precision and accuracy vary, and they measure bone with different ratios of trabecular and cortical bone.

Single photon absorptiometry (SPA), which uses ^{125}I as the radioactive source, was the first of these techniques to be developed, but it was limited to the peripheral skeleton, particularly the radius. At this site the amount of soft tissue is minimal and its attenuation of radiation could be easily discriminated from that of bone. SPA requires a uniform thickness of soft tissue at the site of measurement which can be solved by immersing the forearm or heel (calcaneus) in water. Adequate fat correction must be made for measurement of obese subjects and for those subjects whose soft tissue composition changes during the period of the study (Hassager, Borg & Christiansen, 1989). Inadequate fat correction can lead to an underestimation of BMC in the forearm by up to 15% (Hassager et al.).

Dual photon absorptiometry (DPA), using ^{153}Ga as the radioactive source, was developed to measure bone mass at skeletal sites associated with osteoporotic fractures, such as the spine and the hip. DPA measures the attenuations of gamma fluxes at both 44

and 100 keV through soft tissue in the vicinity of the bone as well as the bone area under study (Slosman et al., 1995). A major limitation of DPA is that the decay of the radioactive source over time leads to a lack of precision in estimating BMD changes in the same subject. The advent of DXA in which the radioactive source is replaced by an X-ray tube that produces mono-energetic peaks of 40 and 100 keV, corrected this flaw (Roche, Heymsfield & Lohman, 1996, pp 63-78).

Theoretically DPA and DXA can measure any area of interest in the body. The values are given as BMC in grams, or as BMD which is BMC divided by the projected area of the bone in grams/square centimetre. It should be noted that BMD does not correspond to the true density of the bone which would be measured in g/cm^3 (Hassager & Christiansen, 1995).

Quantitative computerised tomography (QCT) is another technique based on X-ray attenuation. By means of a simultaneously measured external standard it allows determination of the volumetric density of bone inside a vertebral body. The instrument can use single or dual energy X-rays. Dual-energy can provide more accurate measurements, as there is better correction for soft tissue attenuation. The QCT technique can also be used for the measurement of peripheral bone (pQCT) (Slosman et al., 1995). An advantage is that it can distinguish between cortical and trabecular bone, but sophisticated calibration and careful positioning are necessary to elicit quantitative information from the QCT image (Hassager & Christiansen, 1995).

The DXA apparatus, although three to four times more expensive than that used for SPA, is no more than one third the cost of the QCT apparatus. It allows rapid data acquisition with low irradiation, typically 2-4 mRem/scan compared to 5-10 mRem/scan or 200-1000 mRem/scan for SPA and QCT, respectively. The precision is good (CV=1-3%), while the accuracy is in the same range as other techniques (Wahner & Fogelman, 1994). The limits of the DXA technique are related mainly to accuracy; there are difficulties associated with cross-calibration of different apparatus. In addition, precision can be reduced by poor quality control and inadequate operator training (Slosman, et al., 1995). However, these problems are similar whichever device is used.

The choice between these various techniques will depend on several factors, such as the site of measurement, cost effectiveness, the irradiation dose required, the precision (how close a second measurement is to a previous measurement), and accuracy (how close a measurement is to the true value) of the measurement. When all techniques above are compared in terms of cost effectiveness, precision, accuracy and irradiation for measurement of the hip and spine, DXA represents the first choice. DXA appears to be an adequate tool for measuring bone mass in the clinical setting and it represents the best technical choice for pediatric use to date (Slosman et al., 1995). It has the advantage over the other techniques of using low doses of irradiation and allowing repeated measurements at multiple sites of interest. For proper use it requires trained staff, regular quality control and verification of in-house performance. QCT is inappropriate unless true bone density of the inner part of the vertebral body is required because of high cost and irradiation (Slosman et al., 1995; Roche et al., 1996).

Bone Remodelling

Bones are made up of two basic types of osseous tissue. Compact bone is dense and looks smooth and homogenous. Spongy, or cancellous, bone is composed of small needlelike or flat pieces of bone called trabeculae and has considerable porosity. Most rapid growth and development of the skeleton occurs between early childhood and late adolescence. The amount of skeletal mass acquired during this period is one of the most important determinants for the risk of postmenopausal and involutional osteoporosis (Theintz et al., 1995).

Bone is a very dynamic and active tissue. It is continually changing due to growth and repair. In the adult skeleton, bone deposition (osteoblast cells), and bone resorption (osteoclast cells) occurs at all the bone surfaces. Together these processes constitute bone remodelling. In young healthy adults, bone mass remains constant, an indication that the rates of bone deposition and resorption are essentially equal. Bone remodelling is regulated by two control loops. One is a negative feedback hormonal mechanism; the other involves mechanical and gravitational forces acting on the skeleton. The hormonal

mechanism involves the interaction of parathyroid hormone (PTH) and calcitonin. PTH is released when blood levels of ionic calcium decrease which enhances calcium release from the bone matrix by stimulating osteoclastic activity and bone resorption. As blood concentrations of calcium increase the stimulus for PTH release decreases. Calcitonin is secreted when blood calcium levels rise; it inhibits bone resorption. This causes calcium salts to be deposited in the matrix, effectively reducing blood calcium levels. The hormonal loop acts to maintain blood calcium homeostasis, rather than skeletal strength or well-being (Marieb, 1989).

In addition to this, estrogen helps to protect skeletal health in regularly menstruating women (Shangold, Rebar, Wentz & Schiff, 1990). Although the exact mechanism of how estrogen increases skeletal density is still unclear, it is believed that part of its effect is to suppress bone resorption (Carbon, 1992) and to maintain the remodelling balance between osteoblastic and osteoclastic activity (Turner, Riggs & Spelsberg, 1994). Low concentrations of estrogen receptors have been found in osteoblasts and osteoclasts. It is possible that the binding of estrogen to receptors in osteoblasts regulates osteoclast function indirectly, which would add a second layer of regulation to the direct regulation of osteoclasts by estrogen (Turner et al., 1994). Alternatively or additionally, estrogen, produced during both the follicular and luteal phases of the menstrual cycle increases the efficiency of intestinal absorption of calcium, and suppresses urinary calcium losses (Heaney, Recker & Saville, 1978). Whatever the mechanism, a disruption to the hypothalamic-pituitary-ovarian axis, which results in reduced levels of estradiol, will likely lead to increased bone resorption and bone remodelling and ultimately a reduction in bone mass (Prior, Vigna, Schlechter & Burgess, 1990).

The second mechanism for regulating bone remodelling involves the response of bone to mechanical stress (muscle pull) and gravity. Unlike the hormonal mechanism, this set of controls serves the needs of the skeleton itself, keeping the bones strong where the stressors are acting. Wolff (1986) defined a static relationship between the stress trajectory and the trabecular architecture of bone. He stated that bone grows or remodels

in response to the forces and stressors placed on it (Wolff's Law). For example, most long bones are stressed by bending, a combination of compression and tension forces acting at opposite sides of the shaft. But since both forces are minimal toward the centre of the bone it can be "hollowed out" for lightness without jeopardy. Wolff's Law also helps explain other facts; for example, long bones are thickest midway along the shaft, curved bones are thickest where they are most likely to buckle and large bony projections occur where heavy active muscles are attached (Marieb, 1989).

Frost's (1987) mechanostat theory was an attempt to devise a dynamic relationship between the form of bone and its mechanical environment. Frost suggested that bone tissue possesses a minimum effective strain threshold. Mechanical stimuli such as exercise could cause strain levels above the threshold value that would influence bone remodelling. According to this scenario appropriate changes in bone remodelling would occur until the strain level sensed by the bone would no longer exceed the threshold level. An appropriate change in bone remodelling could include a suppression of new remodelling sites which would result in the sparing of bone tissue. Fewer remodelling sites would favour bone maintenance.

However, if bone remodelling is unable to keep up with the strain in the bone, stress fractures may develop (Sallis & Jones, 1991). A stress fracture is a partial or complete fracture of the bone due to its inability to withstand rhythmic nonviolent stress applied repeatedly in a submaximal manner (Barrow & Saha, 1988). Stress fractures occur much more in female athletes than in their male counterparts. Possible causes are gait differences and unfavourable biomechanics of the female pelvis (Sallis & Jones). Equally important causes may be sudden changes in running surface (Sallis & Jones) and a high incidence of amenorrhea (Drinkwater et al., 1984).

Skeletal Growth and Development

Peak bone mass (PBM) is the maximum bone mass achieved at the time at which skeletal growth and consolidation are complete (Bonjour et al., 1995). The issue of PBM is attracting attention at present because it is believed to be a key identification of risk for

osteoporosis. People who have low PBM may be predisposed to osteoporosis. Identification of the age at which PBM is achieved will allow for the development of age appropriate interventions designed to increase PBM. It has been repeatedly stated that PBM is not achieved until the third or fourth decade of life (Ott, 1991; Riggs & Melton, 1986). However, other studies (Bonjour et al., 1991; Glastre et al., 1990; Theintz et al., 1992) have lead present researchers to suspect that PBM in most people may be achieved towards the end of the second decade. While genetic influences play an important role in early skeletal growth, it is unclear at present whether bone gains occurring after adolescence in young adulthood are modifiable by non-genetic, lifestyle factors.

Bonjour et al. (1991) assessed bone mass in 207 nine to eighteen year old Caucasian subjects. BMD and BMC was measured by dual energy X-ray absorption (DXA) at the lumbar spine (L2-L4), femoral neck and femoral mid-shaft (this last site being taken as a cortical weight-bearing site). The values obtained were related to age and pubertal status. In addition, these values were related to PBM values in a concomitantly determined group of young healthy adults (N=44, aged 20-35 years). In males compared to females, there was a marked age-related delay in vertebral BMD or BMC increase. But when all subjects were stratified according to pubertal stage, males had greater BMD than females at all sites and at all stages. At the end of the rapid growth spurt, trends for higher mean values in males were observed for vertebral BMC, femoral neck and femoral shaft BMD, but no sex difference was observed for vertebral BMD. In females, but not in males, a dramatic reduction in bone mass growth was observed after 15 years of age, particularly for vertebral BMD and BMC, and femoral neck BMD. This reduction occurred in the second to fourth year after menarche. When compared to mean values for PBM in a group of 20-35 year old female subjects, the authors found that the 14-15 year old females had achieved 99.2%, 105.1%, 94.1% and 97.6 % of these values for vertebral, femoral neck and femoral shaft BMD, and vertebral BMC, respectively. This suggests that the generally accepted notion of bone mass continuing to accumulate substantially at all skeletal sites until the fourth decade may be in doubt.

A year later the same research group (Theintz et al., 1992) produced results that

indicated that in a cohort of healthy females with apparently adequate intakes of energy and calcium, the rate of bone mass accumulation slows drastically by 16 years of age in both the lumbar spine and the femoral neck. DXA was used to measure BMD at the lumbar spine (L2-L4) and the femoral neck in 100 healthy males and 98 healthy female adolescents. Mean daily energy and calcium intake, as measured by a trained dietician using the technique of a five day dietary diary, were found to be in the normal range for that age. Sexual maturity was rated using criteria from Tanner (1962), and modified to divide the stage five girls into four sub-groups based on the number of years since menarche. In females the increment rate of BMD was particularly pronounced over a three year period from 11 to 14 years of age (L2-L4=0.080$\pm$0.007 and femoral neck=0.041$\pm$0.007 g/cm^2/yr.). This rate of increment decreased after 16 years and/or 2 years after menarche (L2-L4=0.008$\pm$0.006 and femoral neck=0.008$\pm$0.006 g/cm^2/yr.). In males, the steepest increment was from 13-17 years (L2-L4=0.075$\pm$0.006 and femoral neck=0.037$\pm$0.008 g/cm^2/yr). Again the rate of increment decreased after age 17, but unlike the females, it still remained significant between 17 and 20 years at the lumbar spine (L2-L4=0.024$\pm$0.007). These data add to the growing body of literature which supports the notion of PBM being achieved before the fourth decade of life.

A similar study by Rubin et al. (1993) measured BMD at the distal radius by single photon absorption (SPA), and at the lumbar spine (L2-L4) by dual photon absorption (DPA), in 299 healthy white children of both sexes, aged 6 to 18 years. Pubertal staging was performed using the method of Tanner. A 4-day prospective food record of all foods and beverages consumed was obtained for each subject. In addition, each subject completed a physical activity questionnaire, allowing an energy expenditure score to be calculated. The peripheral BMD at the radius had a relatively constant rise with age in both sexes up until 15 years, when a steeper increase was observed in males. The BMD of the lumbar spine had a gradual linear increase with age in both sexes until puberty, followed by accelerated increments in both sexes during puberty. The most rapid increase in axial BMD in the girls was from 10 to 15 years of age, with a marked slowing thereafter. In boys, the most rapid increase began much later, at 13 years, with the

steepest increase at 15 and 16 years of age and no slowing until 17 years of age. Multiple regression equations indicated that 76% of the variance in radial BMD was explained by height, weight, pubertal stage and age, while at the lumbar spine 80% of the variance was explained by weight and pubertal stage. In this study, many of the changes in BMD at the radius and lumbar spine can be accounted for by standard clinical measures of growth and development, including body weight, height and stage of pubertal development, which are largely genetically determined. In agreement with other studies (Bonjour et al., 1991; Theintz et al., 1992), Rubin et al. (1993) also reported the age-related delay of increasing lumbar BMD in male subjects compared to female subjects. This is most likely due to puberty occurring at a later age in males as compared to females. The presumed hormonal effect of puberty was stronger in determining spinal BMD, a predominantly trabecular site, in comparison to peripheral BMD at the radius, a predominantly cortical site.

Ruiz et al. (1995) measured BMD at the lumbar spine (L1-L4) and at the upper femur using DXA in 151 healthy children and adolescents, age 7-15.3 years. The data were analysed in terms of the height, weight, sexual maturation, spontaneous calcium intake and physical activity level of the children. Pubertal stage was determined using Tanner's criteria, and calcium intake was assessed using a semi-quantitative food frequency questionnaire checked by comparison with the results of weekly calcium intake analyses. The results showed that BMD at the spine increased with pubertal maturation, from 0.68 g/cm^2 to 0.92 g/cm^2 between Tanner stage 1 and 5. No sex differences were observed when children were grouped according to maturation stage, with the exception of femoral density being greater in boys at the prepubertal stage ($p=0.001$). Multiple regression analyses showed that body weight and Tanner stage were determinants of BMD. Sixty-three percent of subjects had calcium levels below 1000 mg/day. Of these, 26 subjects had low vertebral BMD (z score below -1), while of the 50 subjects who consumed over 1000 mg/day of calcium only 2 had low vertebral BMD. Dietary calcium was an independent determinant of BMD, especially before puberty in the vertebral site ($p=0.02$). This influence of calcium may have been unmasked because so many of the children had low calcium intakes. Multiple regression analysis revealed that the weekly

duration of sports activity also positively influenced both vertebral (ß=0.009, p<0.001) and femoral (ß=0.006, p=0.01) sites, especially in girls and during puberty. The authors concluded that pubertal maturation plays an important role in BMD, supporting earlier work by Bonjour et al. (1991) and Rubin et al. (1993).

Gilsanz et al. (1988) collected data on children aged 2-18 years, using QCT, in order to determine the effect of puberty on bone density during skeletal growth. Subjects (n=101, 58 male and 45 female) were abdominal trauma patients at Los Angeles Children's Hospital. However, because of the inappropriateness of a physical examination to determine actual individual sexual maturation at the time of acute trauma, classification was based on patient age. Girls and boys who were younger than 9 and 9.5 years respectively, were classified as prepubertal; while girls and boys older than 13.4 and 13.7 years respectively, were classified as pubertal. Those children in between were classified as having indeterminate pubertal status. Compared with prepubertal children, pubertal adolescents had significantly higher trabecular bone density and more compact bone in the spine (p<0.001). The results of multiple regression analyses showed that, as a set, the variables of height, weight, surface area and body mass index failed to significantly predict trabecular vertebral density among either the pubertal or prepubertal children. After accounting for puberty, there was no correlation between vertebral bone density and age. This contradicts the findings of Bonjour et al., (1991), Theintz et al., (1992) and Rubin et al., (1993). The authors suggest that this may have occurred because of the inappropriate division of pubertal status. Because the vertebral density of boys and girls was similar the results of this study indicate that sexual differences in the frequency of osteoporosis may be explained by a different rate of bone loss, since women and men seem to have approximately equal bone density at the time of skeletal maturity.

Faulkner et al. (1993) derived normative values for total BMC and total body BMD in 234 children, male and female aged 8-16 years of age. Bone measurements were made utilising DXA (Hologic QDR-2000). Age groups were constructed based on the midpoint values of decimal ages, and there was a significant age effect at all sites (p< 0.001); that is, both BMD and BMC for the total body increased significantly with age,

however, pubertal stage was not assessed. There were no significant gender differences in BMC and BMD at any sites, although there was a tendency for boys to have higher values. This is in agreement with the work of Gilsanz et al. (1988), who also found no significant difference in the BMD at any site between male and female subjects.

Further normative data were collected by Proesmans et al. (1994) who measured lumbar spine (L2-L4), and total body BMD and BMC by DPA in 97 subjects aged 3-14 years, none of whom had reached puberty. Total body BMC varied from 236 g in the youngest subject, to 1830 g in the oldest child. Total body BMD ranged from 0.606 g/cm^2 in the youngest subject to 0.961 g/cm^2 in the oldest subject, an increase of 59% over the age range. Total body BMC and BMD correlated highly with age, height and weight ($r=0.83-0.96$, $p<0.001$). BMC and BMD of the lumbar spine also exhibited similar correlations ($r=0.89-0.93$, $p<0.001$ and $r=0.79-0.81$, $p<0.001$, for BMC and BMD, respectively). Again, there were no gender differences in BMD and BMC, but it is unclear as to how the authors determined the pubertal status of the children. Other authors (Rubin et al., 1993; Theintz et al., 1995) have clearly shown that pubertal stage can have a very dramatic affect on the BMD, and lack of accounting for stage of puberty may lead to erroneous results.

In one of the few studies investigating bone growth in a slightly older population, Recker et al. (1992) examined whether there was any increase in BMD after the cessation of linear growth in healthy white women during early adult life. One hundred and eighty-four healthy white college-aged women (18 -26 years) completed this five year longitudinal study. BMD and BMC were measured at the spine (L2-L4) by DPA and at the forearm by SPA every six months. Total body bone mineral level was measured twice during the study. Subjects were asked to keep a 7-day dietary record during the week prior to each 6 month visit, and were questioned on family history of osteoporosis, oral contraceptive use and their menstrual cycle at each visit. Physical activity was measured in arbitrary units over four days with a small, lightweight electronic accelerometer worn on the waist. The estimated age when mineral acquisition ceased ranged from 28.3 years to 29.5 years at the sites studied. This analysis was performed on

the 156 women who were seen at least three times, with an average length of investigation of 3.4 years. For total body bone mass, 65 women were measured, but only twice, giving only two data points, over an average time span of 2.8 years. From their results the authors calculated that the median gain in bone mass for the third decade of life, expressed as a percentage, was 4.8% for forearm BMC, 5.9% for lumbar BMC, 6.8% for lumbar BMD and 12.5% for total body BMD ($p < 0.0001$ that the population median is different from zero in all cases). By both bivariate and multiple regression analyses the rate of gain of bone density of the spine was negatively correlated with age and positively correlated with the calcium/protein intake ratio. Bivariate analysis also revealed that use of oral contraceptives was associated with greater gain in total body BMD ($r=0.31$, $p=0.01$). The authors concluded that there are gains in bone mass in healthy young women during the third decade of life. Also, the gain in bone mass was enhanced by an increased self-selected calcium intake and increased self-selected physical activity. However, despite the length of the study being five years, average participation time was 3.4 years, and in some cases not all the 184 subjects were involved in all aspects of data collection. Many of the women entered the study when they were aged 20-21, thus the data on when mineral acquisition ceased and the rate of bone mineral increase over the third decade have been extrapolated considerably. Further, it is possible that bone gain does not follow a linear pattern which may lead to the authors making quite exaggerated claims on bone gain.

Summary

Many studies have measured both BMD and BMC cross-sectionally over a wide range of ages in order to determine normal values for specific age groups. Unfortunately, due to the time and practicality, there have been few studies published that have looked longitudinally across populations.

The rate of bone accumulation seems to slow quite considerably after puberty (Theintz et al., 1992), although it still maintains a slow increase at the beginning of the third decade. It is possible that accretion continues after this but remains unnoticed

because techniques are unable to detect such small increments.

The importance of achieving maximal PBM is not in any doubt. Puberty seems to play a crucial role in BMD (Bonjour et al., 1991; Theintz et al., 1992; Rubin et al., 1993), along with other variables such as weight, height and sex, factors which are all largely genetic. Further, both Rubin et al. (1993) and Ruiz, Mandell & Garebedian (1995) found evidence to support that activity and calcium intake could also have a positive affect on BMD during adolescence. Haapasalo et al. (1994) supported this claim by finding that Finnish national level squash players who began training before or during puberty had greater BMD in the arm compared to those players who began training after puberty. This suggests that BMD is susceptible to external factors in addition to genetics during adolescence.

Genetic Influence on Bone Mineral Density

Theoretically, inheritance could affect the development of osteoporosis in two ways. Genetic factors could influence peak BMD achieved at maturity, and subjects with genetically determined low BMD might then be more susceptible to developing osteoporosis after entering the period of age-related bone loss. Alternatively, or in conjunction with the above, genetic factors may influence the rate of bone loss (Smith, Nance, Kang, Christian & Johnston, 1973). Under this latter model, subjects with a genetically determined accelerated bone loss would be more susceptible to osteoporosis. Most of the work focussing on the genetic aspect of skeletal bone density has involved twin studies (Pocock et al., 1987; Slemanda, Christian, Williams, Norton & Johnston, 1991), with some authors attributing up to 80% of BMD to genetics (Politzer & Anderson, 1989). Other studies have focussed on familial lines, looking at mother-daughter bone health (McKay, Bailey, Wilkinson & Houston, 1993). However, small sample sizes and methodological shortcomings, including lack of instrument precision, combining of pre- and postmenopausal daughters with their postmenopausal mothers and the selection of primary bone sites for measurement have hindered the interpretation of these studies (McKay et al., 1993).

Analyses of twin studies assumes that intrapair variance of monozygotic (MZ) and hence genetically identical twins is due to environmental factors and measurement error, while intrapair variance in dizygotic (DZ) twins is additionally affected by genetic factors. It is assumed that common environmental factors are shared to a similar extent between MZ and DZ twins, however this could be seriously undermined if each twin lives in a different geographical or cultural area. Comparison of the correlation between two MZ twins with the correlation between two DZ twins can therefore provide a means of determining the genetic contribution to observed variation in BMD. For any trait, such as BMD, the demonstration of a significant covariance (and hence a correlation) between MZ and DZ twin pairs is consistent with a significant genetic determinant in that trait (Pocock et al., 1987).

In an early study, Smith et al. (1973) evaluated possible genetic determinants of bone mass with the premise that inheritance of bone mass could be of etiological importance in osteoporosis. Bone mass was measured in the right radius by SPA in 71 juvenile and 80 adult like-sexed twin pairs (ages not reported). The variance of intrapair differences of bone mass in MZ juvenile twins (male=20, female=28) was 0.0013 compared to 0.0052 in the DZ twins (male=12, female=11). For the adult twin pairs (all male) the variance of intrapair differences in bone mass was 0.0069 for MZ and 0.0137 for DZ twins. The significantly larger variation in intrapair differences in DZ twins vs. MZ twins ($p<0.001$ vs. $p<0.025$) indicates that these traits have significant genetic determinants. These intrapair differences were found to increase with age, suggesting that genetic-environmental interaction also contributes to the observed variation in bone mass. Unfortunately all the adult twin pairs in this study were male, which prevents any conclusions being reached regarding genetic-environmental interactions before and after menopause. Also, because of the lack of significant difference between the male and female juvenile subjects, they were analysed together, despite the males having a higher BMD at the site of measurement than the females. No ages were reported for the male and female subjects. The DZ juvenile twins were older than the MZ twins (mean=11.3 vs. 12.1 years), and hence some of the variation could be explained because of the advanced

maturation of the DZ twins. Also, no mention was made of pubertal stage, both of which are known to effect BMD.

In a similar, more recent study, Pocock et al. (1987) measured BMD at the lumbar spine (L2-L4) and femoral neck by DPA, and at the distal radius and ulna by SPA, in 38 MZ (male=6, female=32) and 27 DZ (male=1, female=26) twin pairs, aged 24 to 75 years. BMD was significantly more highly correlated in MZ twins than DZ twins for the spine ($r=0.92$ vs. 0.36, $p<0.001$), femoral neck ($r=0.73$ vs. 0.33, $p<0.005$) and BMC of the forearm ($r=0.71$ vs. 0.50, $p<0.08$), which is consistent with significant genetic contributions to bone mass at all sites. The lesser genetic contribution to bone mass at the proximal femur and distal forearm compared with the spine suggests that environmental factors may be of greater importance in the aetiology of osteopenia of the hip and wrist. The apparent greater contribution of genetic factors in determining forearm bone mass in premenopausal women, compared with the group as a whole ($r=0.88$, $n=19$ vs. $r=0.71$, $n=38$) for MZ twin pairs suggests that environmental factors are of increasing importance after menopause and/or with advancing age at this site. These latter two factors suggest a greater potential for lifestyle intervention, such as initiation of weight bearing exercise and dietary changes, in achieving a reduction in fractures of the hip and forearm, especially in postmenopausal women. In this study the male and female pairs were grouped together and also the ages of the subjects ranged from 24-75 years for MZ and 24-65 years for DZ twin pairs. This may add suspicion to the results because men and women have different patterns of bone loss, and older individuals have had more time to be susceptible to environmental effects.

In a larger sample of 124 MZ and 47 DZ female twin pairs, Slemander et al. (1991) evaluated bone mass at the radius, lumbar spine and hip in an attempt to reevaluate the twin model. Again, at all skeletal sites, MZ intraclass correlations exceeded DZ correlations for both pre- and postmenopausal women, yielding highly significant ($p<0.01$) estimates of heritability for bone mass. They also compared MZ twins younger than 45 to those who were older than 45 and found significantly greater ($p<0.01$) MZ variability at the radius and spine in the older pairs, although the reason for

choosing this age as a cut-off point was unclear. These correlations were seen to decrease with age, supporting the findings of Smith et al. (1973), indicating that the importance of genetic effects diminishes with age. Slemanda et al. (1991) stated that there were important failures in the assumptions of the twin model, notably, the greater MZ environmental similarity and the probability of gene interaction. This can lead to heritability estimates that are probably too high, particularly in older twin pairs who may have had a longer exposure to differing geographical and/or cultural lifestyles than younger twin pairs.

McKay et al. (1993) studied familial resemblance of BMD at the anteroposterior lumbar spine and the proximal femur, using DXA, in 41 mother-daughter (MD), 42 mother-son (MS), 24 mother-grandmother (MG) pairs and 18 mother-daughter-grandmother (MDG) triads. Children were placed into one of three maturity categories based on an assessment of secondary sex characteristics and growth velocities. Two sets of z scores were derived for the children based on either their chronological age or their maturation status. For all three regions of the proximal femur and for the total anteroposterior spine the correlations between z score values were similar and significant ($p<0.05$) between the MD and MG pairs, ranging from 0.41 to 0.57. In general, the familial correlations improved when maturity based z scores were used for comparison, indicating that pubertal stage is a better determinant of BMD than age, which agrees with the work of Bonjour et al. (1991) and Rubin et al. (1993). Compared to their mothers, the late-pubescent girls and boys had 115 and 123% more BMD, respectively, at the region of the trochanter and neck of the proximal femur, whereas at the spine these groups averaged 92% and 85%, respectively, of their mothers' values. Three generation comparisons demonstrated a strong familial resemblance in BMD, supporting the genetic contribution to BMD. However, a possible alternative may be that in some instances common environmental effects resulting from grandmothers, mothers and/or children living together in the same residence could enhance the supposed genetic effects. However, the authors point out that differences in generational lifestyle, such as diet and exercise trends, may also affect BMD values between parents and children.

In a study to determine whether daughters of postmenopausal osteoporotic mothers have lower bone mass than women of the same age, Seeman et al. (1989) measured BMC of the lumbar spine (L2-L4), and femoral neck and mid-shaft using DPA. Twenty-five postmenopausal women with osteoporotic compression fractures and 32 of their premenopausal daughters, in addition to 20 normal postmenopausal women and 22 of their premenopausal daughters, participated in the study. As compared to normal women, women with osteoporosis had lower BMC in the lumbar spine, femoral neck, and mid-femoral shaft by 33, 24 and 15% respectively ($p<0.001$). As compared with normal premenopausal women, the daughters of women with osteoporosis had lower BMC at these sites by 7, 5 and 3% respectively. The authors concluded that daughters of women with osteoporosis have reduced bone mass in the lumbar spine and the femoral neck. If the low bone mass found in patients with osteoporosis was due to excessive bone loss, their daughters' bone mass would be expected to be normal. If, however, the lower bone mass found in patients with osteoporosis was due to an attainment of a low PBM, their daughter's bone mass would be expected to be closer to the mean of the control subjects, than that of their mothers. Because the latter is the case in this study, for some of the premenopausal women a genetic predisposition to a lower PBM rather than excessive bone loss is likely to put them at greater risk for osteoporosis. This contrasts with the conclusions made by Gilsanz et al. (1988), who stated that the incidence of osteoporosis was due to an increased bone loss in women compared to men, rather than a reduced PBM. However, in that study, the children were not assessed for sexual maturation and it is unclear as to whether skeletal maturity had been reached. In this study (Seeman et al.), the mothers with osteoporosis were shorter ($p=0.005$), were older ($p=0.08$) and weighed less ($p=0.07$) compared to those mothers without osteoporosis. Unfortunately the control mothers were matched for age at menopause (48.1 years) rather than for number of years since menopause. As a result of this the controls had experienced a fewer number of years since menopause than the mothers with osteoporosis (10.1 vs. 19.8 years) which could explain why the osteoporotic mothers had bone densities that were well below "normal". It is expected that a genetic predisposition to both a lower PBM and to accelerated bone

loss is common in many osteoporotic patients (Seeman et al, 1989).

Summary

A genetic predisposition to either a low PBM or an accelerated bone loss can put women at a greater risk for osteoporosis. BMD has been shown to possess a high heritability, but the amount appears to be under debate. Many twin studies have shown similar correlations in BMD values (r=0.71-0.92 for MZ pairs and r=0.36-0.50 for DZ pairs), which indicates a strong genetic component, which some say may be up to 80% (Politzer & Anderson, 1989).

In contrast, some studies (Pocock et al., 1987; Smith et al., 1973) have shown that BMD is influenced by external factors as one ages, indicating that the genetic influence on BMD is less predominant in older populations. This is positive news for older adults who may want to improve their bone health.

Effects of Exercise on Bone Mineral Density

The area of skeletal health in the female athlete is a relatively new topic, with the surge in research beginning in the 1980's. The early work of Rubin & Lanyon (1984) provided the foundation for which most research has since been based. They devised the following four concepts regarding exercise and bone:

(1) Mechanical loading through physical exercise is a positive influence on BMD.

(2) A lack of physical exercise is a negative influence on BMD.

(3) Bone mass is maintained at appropriate levels to afford structural competence for functional loading.

(4) The positive influence of exercise on bone can be attenuated by environmental conditions including the hormonal and nutritional status of the individual.

In addition to providing support to these early statements of Rubin & Lanyon (1984), the studies below also help to expand the concepts with regard to the type of

exercise that would be most beneficial. Appendix A1 contains a summary of studies which investigated the effects of different types of exercise on the skeleton.

Kirchner et al. (1995) examined the relationship between BMD and physical activity, diet, and menstrual history in a group of college gymnasts and a group of sedentary controls (who had a mean age of 20 years). The BMD of the gymnasts was significantly greater (all $p<0.0001$) than the controls at the lumbar spine (1.202 vs. 0.979 g/cm^2), total proximal femur (1.147 vs. 0.901 g/cm^2), femoral neck (1.091 vs. 0.845 g/cm^2), Ward's triangle (1.043 vs. 0.766 g/cm^2) and whole body (1.151 vs. 1.027 g/cm^2). The gymnasts had a lower calcium and calorie intake than the Recommended Dietary Allowance (RDA), and also consumed a lower calorie intake than controls. More gymnasts than controls (59 vs. 24%) reported an interruption to their menstrual cycle since menarche. However, interruption was defined as the number of times a subject had absent menses for a period of only three months. Also, despite gymnasts reporting a lower use of OCs, (38% vs. 61%), the length of time they were used was for longer duration than the controls. The authors concluded that the negative influences of inadequate dietary calcium and amenorrhea on skeletal health were overridden by the unique mechanical forces that are generated during gymnastics training, and that these forces lead to the gymnasts' high BMD.

Robinson et al. (1995) investigated BMD and menstrual history/status in 60 females (collegiate gymnasts, runners and non-athletic controls), whose mean age was 20 years. Bone mineral density was measured at the lumbar spine (L2-L4), and at the proximal femur by DXA. Subjects also completed a 4-day dietary record. There was no difference in calcium intake between the groups. There was no difference in BMD due to amenorrhea, or eumenorrhea, although there was a trend for regularly menstruating athletes to have slightly higher values. Gymnasts had a higher BMD than the runners and the controls at the lumbar spine (1.17 vs. 0.98 vs. 1.11 g/cm^2), femoral neck (1.09 vs. 0.88 vs. 0.97 g/cm^2), and whole body (1.11 vs. 1.04 vs. 1.07 g/cm^2), despite having a greater prevalence of oligomenorrhea/amenorrhea. They concluded that the mechanical forces generated from high impact loading and muscular contraction during gymnastics

training have a powerful osteogenic effect which appears to counteract the increased bone resorption shown to result from amenorrhea, supporting the work of Kirchner et al. (1995). A further surprise was that the runners had significantly lower BMD at all sites compared with the controls (lumbar spine, 0.98 vs. 1.11 g/cm^2, $p<0.0001$, femoral neck, 0.88 vs. 0.97 g/cm^2, $p<0.0001$, and whole body, 1.04 vs. 1.11 g/cm^2, $p<0.01$), despite the fact they were running an average of at least 30 miles per week, and that they also had an earlier age at menarche and were older than the gymnasts and controls. This supports the hypothesis that for elite athletes in some sports too much exercise may in fact be detrimental to their BMD. The authors noted that the controls were regularly menstruating and were not sedentary and some did perform up to three hours of exercise per week. The fact that the controls had greater BMD than the runners suggests that moderate levels of exercise may be more beneficial to skeletal health than higher levels of exercise.

Moderate exercise was also advocated in a review paper written by Sharpe & Freeman (1993). They were particularly concerned about the increased risk of fracture in young female anorexia nervosa (AN) patients who were exercising heavily and had very low spinal BMD. The authors found a dramatic increase in fractures in subjects who had a spinal bone density below 1 g/cm^2. This could be particularly dangerous in the case of AN subjects, many of whom have a spinal BMD less than 1 g/cm^2 .

Joyce, Warren, Humphries, Smith & Coon (1990) measured BMD in 33 eating disordered patients (AN, bulimia nervosa and eating disorder not otherwise specified) at the radius by SPA, and at the lumbar spine and femur by DPA. A fourth group of women (age and number not reported) acted as controls. Subjects were also assessed for menstrual status and activity levels. All three eating disordered sub-groups had decreased BMD ($p<0.005$) at the femur and lumbar spine compared to the controls. Dividing the subjects into activity levels of <1 hour a week, 1-6 hours a week and >6 hours a week, they discovered that those subjects who exercised moderately (1-6 hours a week) had greater BMD than either those women who exercised less or those women who exercised more. This suggests that moderate exercise has a protective effect on the skeleton

whereas minimal or strenuous exercise is detrimental. However, the number of subjects in each group was small and may have had an effect on the outcome. It is unclear as to how the control group were selected, as the authors report no information on their physical characteristics or on the amount of exercise they performed each week.

In another study (Rigotti, Nussbaum, Herzog & Neer, 1984) investigating eating disordered patients, radial BMD was measured by DPA in 18 anorexia nervosa (AN) patients and 28 controls. BMD was significantly lower in the anorectic patients than the controls (0.64 vs. 0.72 g/cm^2). Within the anorectic group, those patients who exercised vigorously more than three times a week had a higher BMD than those patients who were sedentary (0.72 vs. 0.61 g/cm^2). But the active AN subjects had no higher BMD than either the active or sedentary control subjects (0.73 and 0.71 g/cm^2). However, the active patients were younger, heavier, and had a shorter duration of AN and amenorrhea than the less active patients, therefore potentially confounding the association between activity and bone density.

Wolman, Faulmann, Clark, Hesp, & Harries (1991) investigated the effects of sporting activity and menstrual status on the bone mineral density of the femoral mid-shaft, which is predominantly cortical bone. Sixty-seven elite athletes (21 runners, 36 rowers and 10 dancers) participated in the study, along with 13 eumenorrheic sedentary controls. The BMD (measured by DPA) of the runners was significantly higher than the rowers, dancers and the controls (1.51 g/cm^2 vs. 1.43, 1.39 and 1.40 g/cm^2, respectively). When all the subjects were divided based on menstrual status, there was no significant difference in BMD when it was compared between the amenorrheic, eumenorrheic and OC-taking athletes (1.45 vs. 1.45 vs. 1.46 g/cm^2). The runners performed intense weight bearing exercise, running up to 70 miles per week. This produced considerable cyclical loading of the lower body. The dancers also performed weight bearing exercise, but much of their work consisted of slow movements involving coordinating flexibility and balance, with less than 10% involving jumping. The amount of cyclical loading was therefore much less. Rowing is chiefly a non-weight bearing activity. Although weight training of the legs forms part of the training, the degree of cyclical loading is much less

than in running. The rowers in this study were heavier and taller than the dancers, which may explain why their BMD's were greater then those of the dancers, despite less cyclical loading. These results suggest that the intense cyclical loading involved in running may produce an anabolic effect on bone mineralisation in excess of moderate loading, such as that seen in rowing. Although this study supported the idea of activity increasing bone mineral density, it failed to show that cortical bone in the femoral mid-shaft is affected by low estrogen status. Unfortunately, the duration of training and low estrogen status of these athletes was not reported in the study. Both of these factors may explain why no difference was found between the groups at the femoral mid-shaft. However, cortical bone has a much slower rate of turnover compared to trabecular bone, and so any changes, whether environmental, hormonal or mechanical, will take a longer time to become evident.

Comparison of BMD in the dominant and non-dominant arm in a group of athletes allows researchers to examine the effects of training on BMD without needing to account for genetics, diet, or menstrual status, as both limbs have been influenced by all the same factors with the exception of exercise. Haapasalo et al. (1994) compared BMD and BMC in the dominant and non-dominant arm of 19 Finnish female national squash players (mean age=25 years), with 19 healthy age-matched female controls. DXA was used to measure BMD at six sites in the upper extremity and also the right calcaneus. Daily calcium intake was assessed using a prospective 7 day questionnaire of consumed food. There was no difference in calcium intake between the groups (810 mg/day vs. 813 mg/day for athletes and controls, respectively). Athletes had significantly higher BMD and BMC than controls at all sites, (eg. BMD at the distal radius was 0.428 g/cm^2 and 0.388 g/cm^2 for each group, respectively). In the proximal humerus the difference in BMD and BMC between the dominant arm and the non-dominant arm was 15.6 and 17.8%, respectively for the athletes, while for controls, these values were smaller (1.7 % and 4.1 %) for BMD and BMC. They found that the number of years playing had the strongest correlation with bone parameters (r=0.632-0.685). More importantly, when those athletes were divided into those who started training before menarche and those

who began training after menarche, an important difference was noted. The side-to-side difference was 20.6-24.0% in those who began training before menarche, while it was less (7.7-11.1%) in those who began training after menarche. This could be an important finding for deciding when is the best age to be exercising in order to gain the maximum benefit for the skeleton. These findings support the site specific approach (Rubin & Lanyon, 1984), that is, that the skeleton adapts to weight bearing at specific sites. They also suggest that during puberty, the time of steepest bone growth, environmental effects may have a more powerful stimuli on bone growth than at other times.

Tsuji et al. (1995) also found data to support the benefit of exercise in unilateral loading of tennis players. DXA was used to measure radial BMD in the dominant arm and non-dominant arm of a group of 12 female tennis players (mean age 21 years). They also found a significant positive correlation between BMD and grip strength and between radial BMD and body weight. A higher BMD was found in the bones of the dominant hand than the non-dominant hand (0.705 vs. 0.661 g/cm^2 respectively, $p<0.05$), which supports the findings of Haapasalo et al. (1994).

In a longitudinal study, Snow-Harter et al. (1992) investigated the effects of an eight month exercise program on a group of 20 year old women. The women were randomly assigned to either a resistive training group, an aerobic training group or to a control group. In total 31 people completed the study. Competitive athletes were not eligible to participate in the study and none of the subjects belonged to an athletic team. At the start of the study, mean BMD measured by DXA for the women was 1.11 g/cm^2 at the lumbar spine (L2-L4), and 0.85 g/cm^2 at Ward's triangle. Eight months of training saw no significant increases in BMD between the groups, but there was a trend for the exercise groups, both weight training and jogging, to have increased their BMD compared to the controls. The short length of this study, the small number of finishers, (there was a drop out rate of 40%), and the fact that all the women were encouraged to continue doing any activity or exercise may have lead to a lack of significant findings. The authors concluded that eight months of supervised progressive training in either running or resistive exercise modestly increases lumbar spine BMD in young

women. This supports the cross-sectional studies of Kirchner et al. (1995) and Robinson et al. (1995), who found that athletes had greater BMD than controls.

Nelson et al. (1991) assigned 36 postmenopausal women according to preference to either a one-year walking program or a control group. In a double-blind fashion the women were given either a calcium supplement (831 mg/d) or a placebo (41 mg/d of calcium). Lumbar spine BMD, which is about 60% trabecular bone, was analysed by CT and shown to increase by 0.5% after a year in the exercising group, while in the sedentary group it was shown to be reduced by 7% ($p=0.02$). The femoral neck, which contains about 43% trabecular bone, was analysed by DPA, and showed a 2% increase in those subjects taking the calcium supplement, and a 1.1% decrease in those women who were taking the placebo pill ($p=0.001$). Exercise or calcium had no effect on the lumbar spine when measured by DPA, on the distal radius (measured by SPA), or on total body calcium measured by in vivo neutron activation. They concluded that the varying proportions of trabecular and cortical bone from one site to another suggest that high dietary calcium and exercise may preferentially alter bone density at different sites. This study also highlights problems with using different techniques to measure BMD.

In two further papers, BMD data was collected on other sports in addition to running and gymnastics. Taaffe et al. (1995) examined the role of skeletal loading patterns on BMD. They compared eumenorrheic swimmers and gymnasts (who were all members of National Collegiate Athletic Association teams), and a group of non-athletic controls. Gymnasts had the greatest BMD, as measured by DXA, in the femoral neck (1.117 g/cm^2). This was significantly greater ($p=0.0001$) than both the swimmers (0.875 g/cm^2) and the controls (0.974 g/cm^2), and remained significant when corrected for weight and bone size. The swimmers had significantly lower BMD than controls. This paper compares two extremes of the loading continuum: the high loading of gymnastics, that has been previously investigated (Kirchner et al., 1995; Robinson et al., 1995), and the no loading of the swimmers. The results supported the hypothesis that the swimmers would have a lower BMD than the gymnasts, because of the lack of weight bearing placed on the back and femoral neck during swimming.

In an attempt to broaden the data on females participating in sports, Heinonen et al. (1993) compared BMD measured by DXA at seven different sites in female orienteers, cross-country-skiers, cyclists and weight-lifters. One hundred and five Finnish competitive athletes who had trained for a minimum of four years, and had a mean age of 23 years old were recruited for this study. The weight-lifters had significantly higher (9-26%, $p<0.001$) weight adjusted BMD in the lumbar spine, femoral neck, tibia and distal radius, than the other athlete groups, and the control group. The skiers and orienteers had similar BMD in the lower limbs, but values were lower than for the weight-lifters. The cyclists had the lowest BMD at all sites, except for the radius. The authors suggested that the high BMD in the weight lifters was a result of the generation of reaction forces during weight lifting, which is about 18-36 times the body weight. Therefore the increase in BMD is due to the mechanical loading of the skeleton. Similarly, the forces generated by body weight in skiing and running lead to increased lower limb BMD in these groups compared to the cyclists. Cycling does not include vertical weight bearing activity. The low BMD of the cyclists suggests that gravity plays an important role in the process of bone mineralization. The BMD of the radius in the cyclists was 5% higher than for the runners and the skiers. This could be due to the fact that in the cycling position, the weight of the upper body is supported by the hands. Only three out of the 105 athletes were amenorrheic, and their BMD data was no different from the rest of their sporting groups. However, 19 out of the 25 controls were using OCs, a far higher percentage than the other groups, which may enhance BMD. This contraceptive use, and the activity level of the controls, which was about five times a week, may explain why the BMD of the controls was no different than the athletes. The authors concluded that the differences in BMD at different sites between groups were consistent with the specificity of the training stimulus of the studied sport. Different loading patterns that are specific to sports, will lead to BMD patterns that are also specific to that sport.

There have only been two published articles which have measured BMD in female soccer players. The first, by Lee et al. (1995), compared contralateral, regional and total body BMD in college athletes in the sports of volleyball, soccer, basketball and

swimming with moderately active and sedentary control subjects. All subjects were eumenorrheic and had a mean age of 19.48 years. BMD was measured using DXA at the lumbar spine (L2-L4), proximal femur, right and left arms and legs, and whole body. (Values for the spine, whole body and femoral neck for all groups are given in Appendix A1). The volleyball and basketball players had significantly greater leg and arm measurements than the other groups, while contralateral comparisons revealed significantly greater right arm measurements for all groups, except for the swimmers. Volleyball players and basketball players had significantly higher total body and lumbar spine BMD values than the swimmers, and the moderate and sedentary control subjects. Basketball players had significantly higher BMD at the proximal femur than the swimmers and the moderate and sedentary control subjects. The authors concluded that results of the study show site-specific differences in BMD associated with specific sports programs. However, the soccer players in this study were training for 7 hours per week during the season (which was three months) and training only one hour a week during the off-season. In contrast, the other athletes were training 17 to 21 h/wk in season (5 to 7 months of the year) and 16 to 20 h/wk the rest of the year. If impact exercise provides such a stimulus for increases in BMD just the difference in number of hours per week of training would account for the differences in BMD between the groups, regardless of sport type. In addition, the moderate control group was exercising for three hours a week and had a mean oxygen uptake that was very similar to the soccer players. It would have been useful if the authors had documented the length of time each subject had been participating in their chosen sport. The basketball and volleyball players were significantly taller than the other groups. The volleyball players were significantly heavier than the soccer players, swimmers and moderately active control subjects. Both a greater height and a greater weight could lead to greater BMD. The authors also found that 39% of the athletes and 40% of the control subjects used OC, but there was no correlation between OC use and BMD at any site.

The second study by Alfredson, Nordström & Lorentzon (1996) focussed specifically on female soccer players, with a mean age of 20.9 years. BMD was measured

at the lumbar spine (L1-L4), proximal femur, whole femur, humerus and tibia using DXA. The soccer players had significantly higher BMD at the lumber spine (10.7%), femoral neck (13.7%), Ward's triangle (19.6%), nondominant femur and humerus (8.2 and 8.0% respectively), distal femur (12.6%) and proximal tibia (12.0%) than a group of weight and height matched nonactive females (aged 25.0 years). Muscle strength of the thigh was not correlated with BMD at any of the local or distant bone sites in the soccer players, however BMD at the hip and femur was correlated with muscle strength of the hamstrings and quadriceps in the control subjects. The soccer players in this study had been participating at an elite level for a mean of 5.2 years and practised 6 h/wk for the 42 week season, and 3 h/wk in the off-season. It is unfortunate that in this study the control subjects weren't matched for age with the soccer players. It is possible that some of the control subjects had achieved peak bone mass and had already started to lose bone mass, which would lead to lower BMD in this group. It may also have been useful for the authors to document dietary intake and menstrual history.

Summary

Some research has shown that moderate exercise can be beneficial in enhancing BMD. However, points of contention remain as to what type of exercise provides the most benefit, and what is the optimal volume of training. It seems that while some forms of exercise, notably gymnastics (Kirchner et al., 1995; Robinson et al., 1995), lead to large gains in BMD compared to sedentary subjects, other forms of exercise such as running (Robinson et al., 1995), cycling (Heinonen et al., 1993) and swimming (Taaffe et al., 1995) have lead to smaller gains in BMD compared to sedentary controls. Reasons for this have included poor selection of sedentary subjects, lack of weight bearing (loading) involvement in the sport and the presence of amenorrhea. The first of these problems may be corrected by better subject selection criteria. However, the amount and type of weight bearing activity is defined by each specific sport, suggesting that differences in BMD could be sport specific. The effect of amenorrhea will be discussed in the following section, and although the causes of amenorrhea remain disputed it is likely that heavy and

long training and energy availability may be prime suspects. To summarize in the words of Rubin & Lanyon (1984), the positive influence of exercise on bone can be attenuated by environmental conditions including the hormonal and nutritional status of the individual.

Effects of Menstrual Status on Bone Mineral Density

Estrogen helps to protect the skeletal health in regularly menstruating women (Shangold et al., 1990). Women who are amenorrheic (have absent menses) do not have this protective effect of estrogen.

The normal reproductive cycle in females is maintained by gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrogen and progesterone. GnRH, produced in the hypothalamus, is transported to the anterior pituitary and is necessary for the synthesis of LH and FSH. FSH and LH act at the ovaries and are responsible for estrogen and progesterone production. Estrogen and progesterone then exert a positive and a negative feedback mechanism to the pituitary which helps to maintain the delicate cycle, resulting in ovulation. Normal cyclic ovulation is therefore dependent on hypothalamic release of GnRH, an intact pituitary response to GnRH through the release of FSH and LH, the response of the ovaries to FSH and LH to produce estrogen and progesterone, and an intact feedback mechanism that allows the hypothalamic-pituitary-ovarian axis to remain balanced (Fruth & Worrel, 1995).

Regular menses are dependent on accurately timed pulses of GnRH. Pulse intervals of 60 to 90 minutes result in the steady pulsed release of FSH and increasing pulses of LH from the pituitary gland during the follicular phase of the cycle. Appropriate estrogen secretion and formation of an ovarian follicle is dependent on the correct ratio of FSH to LH with the mid-cycle surge in LH resulting in ovulation. The remaining corpus luteum secretes progesterone and a luteal phase of at least ten days follows in preparation for fertilization and pregnancy. Alternatively, the corpus luteum degenerates and menses ensues some 28 days after the hormonal interplay began (Carbon, 1992). A disruption at

any point in the sequence will result in menstrual disturbance (Fruth & Worrel, 1995). The changes in athletic amenorrhea are currently believed to be due to an abnormally decreased rate of pulsatile GnRH secretion. Pituitary FSH secretion is decreased and the LH surge is inadequate both in amplitude and frequency. Ovarian function becomes depressed with lower estradiol secretion and an inadequate luteal phase. Ultimately the LH surge may become ineffective in stimulating ovulation and the anovulatory cycle becomes prolonged (Carbon, 1992). A disruption at any point in the sequence will result in menstrual disturbance (Fruth & Worrel, 1995).

On the basis of potency and quantity, estradiol-17ß is the most important of the class of compounds called estrogens, and is the primary form of estrogen found in the blood plasma (Fruth & Worrel, 1995). Estradiol is secreted and synthesized primarily by the ovarian follicle and the corpus luteum. Concentrations of estradiol change throughout the menstrual cycle. In the early follicular phase, estradiol is secreted at a rate of 60 to 170 μg/day. During the latter half of the follicular phase, this is increased to 400 to 800 μg/day. As a result, plasma estradiol increases to 25 to 40 ng/dl (LaBarberra, 1996).

Authors who have examined blood profiles in amenorrheic athletes found extremely low or undetectable levels of circulating estradiol (Drinkwater et al., 1984; Drinkwater et al., 1986; Drinkwater et al., 1990; Marcus et al., 1985; Nelson et al., 1986; and Wolman et al., 1990). However, in sedentary controls and eumenorrheic athletes, all these studies demonstrated normal or near normal levels of estradiol.

The cause of menstrual dysfunction in athletes is unclear although changes in body weight, body composition, training, diet and emotional stress have all been proposed (Bale, 1994). It is likely that a combination of some or all of these factors indirectly lead to menstrual dysfunction. It is believed that hormonal suppression at the hypothalamic level is the primary cause of athletic amenorrhea (Fruth & Worrel, 1995). Of further concern are problems associated with menstrual dysfunction, which are not related to athletic participation, i.e. pituitary tumours, thyroid dysfunction, polycystic ovary disease and premature ovarian failure (Shangold et al, 1990). Although exercise promotes bone formation and retards resorption, in many individuals it does not

compensate for estrogen deficiency (Drinkwater et al., 1984; Marcus et al., 1985; Nelson et al., 1986). It also does not reduce the risk of the above conditions occurring.

At present it seems unclear as to what percentage of female athletes are amenorrheic, although values of 3.4 to 66% have been recorded (Loucks & Horvarth, 1985; Nattiv, Agostini, Drinkwater & Yeager, 1994; Sallis & Jones, 1991; Shangold et al., 1990). In comparison, the incidence of amenorrhea amongst the general female population has been reported as 2-5% (Loucks & Horvarth, 1985; Nattiv et al., 1994). The risk of osteoporosis in hypoestrogenic amenorrheic athletes is real and proportionally much greater than in the general population. As such, the development of educational programs should be initiated.

In a study to investigate whether the cause of amenorrhea had any effect on BMD, Jones, Ravnikar, Tulchinsky & Schiff (1985) compared BMD in three groups of hypoestrogenic women. Radial bone density was measured by DPA in 39 women who had developed secondary amenorrhea due to either athletics, weight loss or premature menopause, and in 25 age matched controls who had regular menses. BMD in the athletes was 0.738 g/cm^2 which was not significantly different from the controls. However, both the athletes and the controls had significantly higher radial BMD than either those women who had premature menopause or the women who were amenorrheic due to weight loss. The radial site is predominantly cortical bone and may not respond as quickly to hormonal changes and the effects of exercise as other sites. It is surprising that a weight bearing site, such as the femoral neck or lumbar spine was not also measured for this study, especially because the athletes were running distances of more than 30 miles each week. Linear regression analysis of this data showed a significant negative correlation between length of amenorrhea and bone density ($r=-0.506$, $p<0.01$), indicating that even in the athletes a long duration of amenorrhea may be detrimental to bone health.

Drinkwater et al. (1984) compared bone mineral density in 14 amenorrheic and 14 eumenorrheic athletes, who were matched for age, height, weight, and length of training. The only difference between the groups was the number of miles run per week, 41.8 vs. 24.9, for the amenorrheic and eumenorrheic groups, respectively. They found that BMD

was higher in the lumbar spine (L1-L4) of the eumenorrheic athletes compared to the amenorrheic athletes, (1.30 g/cm^2 vs. 1.12 g/cm^2), but the BMD of the radius was the same in both groups. They explained that no difference in BMD would be expected at the radius because there is no loading at this point. They suggested that the decreased BMD of the lumbar vertebrae in the amenorrheic group was probably due to the effects of amenorrhea, which is possibly linked to the higher amount of physical activity in this group. There was also no difference in calcium intake, body fat and age at menarche between the groups.

In a follow-up study 15 months later, Drinkwater et al. (1986) analysed 9 of the original 14 amenorrheic runners, after some had resumed their menses, and 9 of the original 14 eumenorrheic runners . Vertebral BMD had significantly increased ($p<0.01$) by 6.3% to 1.19 g/cm^2 in those athletes who had resumed their menses, but had decreased by 3.4% to 1.08 g/cm^2 in those who were still amenorrheic. The eumenorrheic women maintained their BMD at 1.369 g/cm^2, which was still significantly ($P<0.05$) greater than those women who had resumed their menses. Although it was a positive sign that bone density may be regained after resuming menses, this large increase may be due to the fact that the previously amenorrheic runners had low density in the spine. The findings should be examined with caution, especially as there were only 9 women in the cyclic group, 7 women in the "resumed menses" group and only two in the "remained amenorrheic" group. They concluded that the resumption of menses in the primary amenorrheic athletes was a primary factor for the significant increase in BMD.

The early work of Drinkwater et al. (1984) was supported by Nelson et al. (1986) who examined diet and bone status in a group of 17 eumenorrheic and 11 amenorrheic athletes (mean ages=29.2 and 25.2 years, respectively). Subjects submitted 3-day dietary records, volunteered blood samples and underwent BMD analysis of the lumbar spine (L1-L4) by DPA, and the radius by SPA. The eumenorrheic athletes had a greater BMD at the spine compared to their amenorrheic peers (1.196 vs. 1.099 g/cm^2) but there was no difference between groups at the radius. Physical characteristics and training regimen were similar for both groups, however amenorrheic athletes consumed significantly lower

amounts of energy, carbohydrate and fat than their eumenorrheic peers, and 82% ate less than the RDA for protein. There was no significant difference in calcium intake between the groups, due to the large variation in the diet of the amenorrheic athletes. They concluded that when weight bearing exercise and a low energy diet are associated with amenorrhea, the accretion of a large bone mass in young, trained women is not favoured. This is in sharp contrast to the works of Kirchner et al. (1995) and Robinson et al. (1995) who found that gymnasts had higher BMDs than runners and controls despite a higher incidence of menstrual dysfunction.

Marcus et al. (1985) documented training intensity, body composition, and endocrine features in a group of elite female distance runners (n=17, mean age=21.9 years). The group was divided into two, those women who were amenorrheic (absent menses for at least twelve months), and those who were cyclic (10-12 menses a year). BMD was measured in both groups at the lumbar spine (L1-L2) by CT, and at the radius by SPA. Radial bone density was similar for both groups, but the amenorrheic women had a lower BMD at the spine than those women who were regularly menstruating (151 g/cm^3 vs. 182 g/cm^3, $p<0.02$). The amenorrheic women were younger and lighter than their counterparts, but had the same age at menarche and had the same training regimen (93 km a week). However, whereas the cyclic women began training on average 5 years after menarche, those who were amenorrheic began 0.9 years after menarche. A 3-day dietary record indicated a mean calorie intake of 1715 vs. 1272 kcal for the cyclic and amenorrheic women, respectively, but large standard deviations prevented significance from being reached. In addition, 50% of the amenorrheic women and 40% of the cyclic women were not consuming two thirds of the recommended allowances for calcium. This supports the work of Frusztajer et al. (1990), who found that a greater proportion of dancers, compared to controls, were consuming less than 85% RDA for calories, and avoiding eating high fat dairy foods. However, calcium intake was not recorded specifically in this study. Marcus et al. (1985) concluded that intense exercise may reduce the impact of amenorrhea on bone mass; however, runners remain at a high risk for exercise related fractures.

Lloyd et al. (1986) collected information regarding physical characteristics, health history, menstrual history, running history and injuries from over 300 women involved in a national 10 km foot race, a local 10 km foot race and collegiate athletes from a variety of sporting fields. The women were grouped into those who had a continuous training program and those who had interrupted their program due to injury. (An injury was classed as any musculoskeletal ailment attributed to running that caused the runner to interrupt her program and/or seek medical help). The two groups were similar except that those women in the interrupted group were younger and had been training for more years. Women who had interrupted their training due to injury were more likely to have absent or irregular menses and were less likely to have used oral contraceptives. In the analysis of the collegiate athletes the frequency of stress fractures in women with irregular menses was nearly four times greater than those who were cyclic, 15 vs. 4%. The 10 km runners who had interrupted their program had been running longer, ran more miles per week and had a greater incidence of irregular or absent menses. Lloyd et al. (1986) concluded that premenopausal women who have absent or irregular menses while engaging in vigorous physical activity programs are at an increased risk of musculoskeletal injury.

Lloyd et al.'s (1986) findings were supported later by the research of Barrow & Saha (1988), who classified 240 collegiate female distance runners according to menstrual status. Very irregular was classified as 0-5 menses a year, irregular was 6-9 menses a year and regular was classified as 10-12 menses a year. They found that stress fractures occurred in 49% of those with very irregular menses, 39% of those with irregular menses, and 29% of those with regular menses. Those with "very irregular" menses ran more miles per week, but had been running for the same number of years as the other groups, suggesting that high intensity training may lead to irregular menses and a higher incidence of fractures. They also found that those runners who had never used oral contraceptives were more than twice as likely to have a stress fracture compared to runners who had used oral contraceptives for more than a year. In summary, they concluded that those women athletes who have irregular or absent menses and are not using oral contraceptives are at increased risk for stress fractures (Lloyd et al, 1986).

In contrast, Wolman et al. (1991) found no significant difference in BMD at the femoral mid-shaft between amenorrheic (n=25), eumenorrheic (n=27) and oral contraceptive-taking (n=15) athletes aged 20 to 30 years old. BMD values were 1.45 g/cm^2 for both the amenorrheic and eumenorrheic athletes, and 1.46 g/cm^2 for the oral contraceptive taking athletes, which were not significantly different (p=0.38) from controls (n=13). However, the authors defined amenorrheic as having one or fewer periods within the last six months, whereas other studies define amenorrhea as no periods within the past year (Drinkwater et al., 1984). This could lead to a lack of significant findings between the amenorrheic athletes and the other groups. Unfortunately, fracture history was not reported in this study.

Athletes who are amenorrheic are very similar in hormone status to patients who suffer from anorexia nervosa (Bale, 1994). Many anorexia nervosa (AN) patients have reduced food intake and some participate in vigorous activity in order to try and conform to their ideal image (Rigotti et al., 1984).

Seeman, Szmukler, Formica, Tsalamandris & Mestrovic (1992) measured BMD in 65 patients with AN and 52 premenopausal healthy women at the lumbar spine (L2-L4), hip and the total body using DXA. The patients had significantly lower BMD at all sites (lumbar spine=1.05 g/cm^2 and femoral neck=0.91 g/cm^2, $p<0.001$) compared to controls, following adjustments for age and height. Those patients with primary amenorrhea had lower BMD than those who had secondary amenorrhea. Those patients who had used oral contraceptives had a higher BMD than those who had not, but were still lower than the controls. Oral contraceptive use was an independent predictor of bone density at the lumbar spine (r^2=0.36-0.56, p=0.02), and at the femoral neck (r^2=0.31-0.52, p=0.02). Bone density at the lumbar spine (r=-0.53, p=0.02) and at the femoral neck (r=-0.49, p=0.01) diminished with increasing duration of amenorrhea, supporting the findings of Jones et al. (1985). Using linear regression, 47% of the variance at the spine and 45% of the variance at the femoral neck was explained by the duration of amenorrhea and fat free mass combined. Those patients who exercised hard for more than three times a week had significantly greater BMD at the femoral neck than those who did not exercise (0.97

vs 0.85 g/cm^2, $p<0.05$), but this value was still less than controls (1.02 g/cm^2). The negative affect of amenorrhea on BMD was compensated for by the exercise undertaken by the AN patients, yet it still wasn't enough to bring their BMD up to that of the control subjects. A possible explanation for this is the low dietary intake in the AN patients, but this is unknown in this study as dietary intake was not recorded. It is also unclear whether the control subjects in this study performed any exercise, which may have elevated their BMD values above normal.

Summary

Studies have shown that amenorrhea can have negative effects on BMD (Drinkwater et al. 1984; Drinkwater et al., 1986; Jones et al., 1985; Marcus et al., 1985). In some cases the positive effect of exercise (mechanical stimulus) on the skeleton appears to outweigh the negative effect of amenorrhea (hormonal stimulus) (Marcus et al., 1985; Seeman et al., 1992; Wolman et al., 1991). Gymnasts have been shown to have a greater BMD than sedentary subjects, despite exhibiting a higher prevalence of menstrual dysfunction (Kirchner et al., 1995; Robinson et al., 1995), while runners and swimmers have been shown to have lower BMDs than sedentary subjects (Robinson et al., 1995; Taaffe et al., 1995). It seems that while amount of training may lead to differences in BMD, the sport specific type of training and its link with hypoestrogenism also appears to play a role. Further investigation is necessary in other sports to see how athletes in these sports respond to training.

The best way to avoid a low BMD would be to prevent or reduce the occurrence of amenorrhea amongst female athletes. Sallis & Jones (1991) suggested that amenorrheic individuals should be advised to reduce the intensity and frequency of training to a level where menses can resume. At present, there have been no adequate studies to show what would be the optimal level at which to train and retain normal menses. Additionally, dietary intake, energy expenditure and body composition should be investigated in individuals with amenorrhea to see whether changes in these factors would promote better health and performance. It is likely that any changes which have to be made in

relation to training regimen or diet are dependent not only on the genetic make up of the individual, but also on the sport in which she is involved. Appendix A2 contains a brief comparison of the articles concerned with amenorrhea and BMD.

Influence of Calcium Intake on Bone Mineral Density

A two year comparison of the effects of estrogen therapy and calcium supplementation was undertaken by Riis, Thomsen & Christiansen (1987). They investigated the effects of calcium (2000 mg/d) and estrogen supplementation (3 mg/d 17 ß-estradiol) in a group of postmenopausal women (n=43, mean age=50.5 years) in a double blind design. They measured BMC of the forearms by SPA and BMD of the total body and spine (L2-L4) by DPA every three months. BMC in the forearm and total body remained constant over the two year period in the estrogen group, but decreased significantly ($p<0.001$) in the calcium group and the placebo group by 4-8%. In the calcium treated group, there was an observation toward a slower loss of compact bone in the proximal forearm and total skeleton compared to the placebo group. This data suggests that calcium supplementation in the dosage used was not as effective as estrogen therapy for the prevention of early postmenopausal bone loss, but it is better than nothing. Dietary records were not assessed in this study, which is unfortunate, but the rate of bone formation was measured by radioimmunoassay in an attempt to determine calcium status. However, in the calcium and placebo groups, there was no difference between bone remodelling throughout the study. No account of activity was taken in the study.

In contrast, Nelson et al. (1991) found a 2% increase in BMD at the femoral neck when analysed by DPA in 18 postmenopausal women (mean age=60 years) who had taken a calcium supplement for 1 year compared to those who had not. Calcium dosage was 831 mg/d, however 9 of the subjects participated in a one year walking program which could have falsely elevated the results.

Frusztajer et al. (1990) compared the incidence of stress fractures, diet, history of eating disorders, menstrual history and bone density in a group of ballet dancers. A group of 10 dancers with stress fractures were matched for age, height and weight with 10

dancers without stress fractures and 10 controls who also had not had stress fractures. BMD was measured at the radius by SPA and at the spine (L1-L4) and the first metatarsal using DPA. Nutrient intake was measured using both a 2-day dietary history and a semi-quantitative food frequency questionnaire. Dietary measures revealed that in the stress fractures group, a higher percentage were consuming less than 85% of the RDA for all nutrients and were avoiding high fat dairy products compared to the other two groups. It is possible that the dietary measures underestimated food intake, and in this study no data were given regarding calcium intake. It is likely though, that due to a low caloric consumption, all other food groups were ingested in much lower amounts. There were no significant differences between the groups regarding BMD of any site measured and in the incidence of amenorrhea. The stress fractures group showed a trend towards a higher incidence of eating disorders and a higher level of dieting, as measured by the EAT-26 questionnaire and by interview, although this failed to reach significance. This could be due to small numbers in each group, or because the three groups were matched for age and height/weight ratio which resulted in the control group exhibiting poor dietary habits and a relatively high prevalence of eating disorders, both of which would be expected to lead to similar BMD. The most salient differences noted in this study were the restrictive dietary patterns and eating disorders in the stress fractures group. The authors were surprised that these abnormalities were not reflected in the bone density studies, which may indicate that factors other than diet play a more crucial role in increasing or decreasing BMD.

In a study comparing the benefits to BMD of exercise or stretching, calcium supplements or a placebo were also given in a double blind manner. Friedlander et al. (1995) randomly assigned women, mean age 29 years, to either an exercise program involving weight training and aerobic activity or to a stretching program for two years. Sixty-three women completed the study, which had an attrition rate of 50%. BMD was measured by QCT at the spine, SPA at the calcaneus and DPA at the hip and spine. The exercising group showed significant gains in bone mass compared to the stretching group (spine, 2.5%, femoral neck, 2.4% and calcaneus, 6.4%). The calcium intervention had no

detectable positive effects . However this could be due to the differing amount of calcium given to each subject, ranging from 400-1500 mg/day, and the fact that the group were not calcium deficient initially.

In one of the few longitudinal studies, Welton et al. (1994) investigated bone mass and calcium intake in males (n=84) and females (n=98) over a 15 year period. Subjects entered the study when they were 13 years old, and were assessed for BMD, diet and activity over the following fifteen years. A modification of the cross-check dietary history interview was used to assess the individual food intake, and calcium was calculated from the results of this instrument. Multiple regression analysis showed that only the amount of weight bearing activity and body weight made a significant contribution to the final model of lumbar (L2-L4) BMD at age 27 years. Calcium intake appeared not to be a significant predictor of BMD in both sexes for the three periods, ages 13-17, 13-22 and 13-28 years.

Kristinsson, Valdimarsson, Steingrimsdottir & Sigurdsson (1994) investigated the relationship between calcium intake and BMD in the forearms of 13 and 15 year old girls (n=139). Dietary calcium was assessed using a simple food frequency questionnaire similar to the one developed by Angus, Sambrook, Pocock & Eisman (1989). SPA was used to measure BMD and BMC at the distal and ultra-distal radius in both forearms. Univariate analysis showed no significant correlation between calcium intake and BMC or BMD for either age group. After adjustment for menarcheal age and weight in the older group, calcium intake was found to be significantly correlated to distal and ultra distal BMD in both the dominant and non-dominant forearms ($r=0.44$, $p<0.05$). The authors concluded that their study supported the hypothesis that a threshold effect of calcium on BMD might exist at 1000-1200 mg/d.

Nieves et al. (1995) also found a positive relationship between calcium intake and BMD at the hip in a retrospective study. They evaluated 139 white females, aged 30-39 years for BMD and diet. A food frequency questionnaire was used to assess dietary intake in the year prior to the study and for when they were aged 13-17 years old. BMD was measured at the spine, hip and forearm by both DXA and DPA. Current dietary calcium

was modestly related to hip BMD (ß=0.077, p=0.074). When fibre intake was added to the multiple regression model the association between calcium and hip BMD was strengthened (ß=0.101, p=0.037). This would be expected because fibre interferes with calcium absorption. In the teenage diet phosphorus and calcium were related to hip BMD. A higher lifetime calcium intake was associated with a higher hip BMD compared with a low lifetime calcium intake. However, the retrospective nature of this study casts doubts on the accuracy of the dietary assessments. Despite claims about the use of food frequency questionnaires, some of the women in the study will have had to recall their diet from 26 years previously!

Trabecular bone density of the lumbar spine (L2-L4) was measured by computed tomography in 67 elite female dancers, runners and rowers (aged 20-30 years old) in a study by Wolman, Clark, McNally, Harries & Reeve (1992). Dietary calcium was also assessed by use of a questionnaire. Trabecular bone density was significantly lower ($P<0.0001$) in amenorrheic athletes (168 mg/cm^3) than in their eumenorrheic (211 mg/cm^3), or oral contraceptive-taking (215 mg/cm^3) peers. They found a linear relationship between calcium intake and spinal bone density for all three groups, however the correlation between calcium intake and BMD is not quoted in the paper. Formation of regression equations were similar for all three groups, suggesting that for every 100 mg of calcium bone density would increase by 3.9 mg/cm^3. If this relationship is causal the authors estimate that the amenorrheic athletes would have to increase their calcium intake by 1200 mg a day to reach the trabecular bone density of the eumenorrheic athletes. However, the relationship between calcium intake and BMD is thought to be a threshold model, so increasing calcium intake to high amounts may not be the solution to a low BMD.

Kanders, Dempsay & Lindsay (1988) examined the relationship between calcium intake, mechanical stress and bone density. Sixty eumenorrheic women aged 25-34 years old were measured for BMD at the radius by SPA and measured at the spine by DPA. Calcium intake was calculated from a 6-day dietary record and a 24 hour dietary recall. Vertebral BMD was positively related to activity but was not significantly related to

calcium intake. Elimination of the effects of activity disclosed a significant correlation between total calcium and vertebral BMD ($r=0.36$, $p<0.05$). Closer examination of the relationship suggested that vertebral BMD did not appear to increase with calcium intakes above 800-1000 mg/d, implying a threshold effect. Radial BMD was not significantly related to calcium intake.

Summary

There are little conclusive data regarding the benefits of calcium intake on BMD, with some studies (Nelson et al., 1991; Nieves et al., 1995) showing that increased calcium leads to greater BMD, while other studies have shown no effect for calcium (Friedlander et al., 1995; Welton et al., 1994). A summary of all the studies mentioned in this section can be found in Appendix A3. Further, some studies are suggesting a threshold effect of calcium (Kanders et al., 1988; Wolman et al., 1992). In these studies, BMD increased in relation to increased intakes of calcium up to about 1000 mg/d. Intakes of calcium above this value may have no additional effect on increasing BMD. Because doubt still exists as to the benefit of calcium on BMD it is recommended that all individuals try to achieve the RNI of calcium.

Measurement of Dietary Intake

There are generally four methods which are frequently used for the measurement of dietary intake: dietary history, food frequency questionnaires, 24-hour dietary recall and multiple day food records. The following is a brief review of each of these methods, including the strengths and weaknesses of each method, and the suggested populations for which each method is most suitable.

Dietary history is used to ascertain a person's typical food intake in which many details about characteristics of foods usually consumed are assessed. In addition, the frequency and amount of food consumed is also recorded. The original idea came from Burke & Stuart (1938), who included a 3-day dietary record, a food list and frequency questionnaire and a detailed interview to determine usual dietary habits. Since then, their

ideas have been modified and adapted according to the population under study, and today many dietary history techniques have been automated to save time and money (Thompson & Byers, 1994). The major strength of the dietary history method is that it assesses usual meal patterns and details of food intake, rather than intakes over a short period of time or frequency of consumption of specific foods. However, interviews require the respondents to make subjective judgements on usual foods and the amounts eaten which may be difficult for some people (Thompson & Byers, 1994). Also, the approach requires highly trained interviewers, and Burke & Stuart (1938) cautioned that the results should be interpreted as relative rather than absolute values.

Food frequency questionnaires (FFQs) require respondents to report their usual frequency of consumption of each food from a list of foods for a specific period. Many FFQs are simple and respondents can complete them alone, or over the telephone or in an interview. Unfortunately, many details of dietary intake are not measured and quantification of intake is not as accurate as with recalls or records (Thompson & Byers, 1994). Inaccuracies result from an incomplete listing of all possible foods, from errors in frequency estimation, and from errors in estimation of usual serving sizes (Young & Nestle, 1995; Thompson & Byers, 1994). The FFQ has become a common way to estimate usual dietary intake because the costs of data collection and processing and the respondent burden are typically much lower for the FFQ than for dietary records or dietary recall (Thompson & Byers, 1994). FFQs are also much better suited to ranking individuals according to food or nutrient intake than for estimating levels of intake (Young & Nestle, 1995; Thompson & Byers, 1994).

FFQs can also be designed for the determination of specific nutrients. Angus et al. (1989) devised a 24-item FFQ for the determination of calcium based on responses from a weighed 4-day food record which was completed by 147 women. The foods were selected based on the percentage of calcium that they provided to an average diet. This questionnaire was validated against a 4-day weighed dietary record by a group of 54 Caucasian women aged 29-72 years. The FFQ was found to correlate well ($r=0.79$, $p<0.001$) with data from a 4-day weighed record. However, absence of some fruits,

vegetables and cereal products, each of which contain small amounts of calcium, means that values of calcium calculated from this method may be conservative. The authors concluded that the data from a short, simple questionnaire can be used to rank individuals according to adequacy of calcium intake. The advantages of this questionnaire are that it is self-administered, and requires little time from the researcher to enter and then code the data.

Musgrave, Giambalvo, Leclerc, Cook & Rosen (1989) developed a 53-item FFQ to assess calcium intake and validated it with a group of 26 perimenopausal women. The calcium intake estimated from the questionnaire correlated ($r=0.73$ in winter and $r=0.84$ in summer) with the estimated amount from 4-day records. Less than five minutes were required to calculate the daily calcium consumption from the questionnaire. However, it is unclear as to how the 53 foods for the questionnaire were selected, and why other age groups of women were not measured.

Block et al. (1986) devised a questionnaire containing 100 food groups based on the data from the Second National Health And Nutrition Examination Survey (NHANES II). The food items selected represented 90% of the total intake for energy and 17 nutrients, and supplied 96% of the total intake of calcium. The authors found good correlations ($r>0.70$) between the FFQ and dietary records. Unfortunately, this questionnaire is interview administered and although more detailed information is obtained, it takes much more time to complete this questionnaire compared to the two previously mentioned FFQ's.

In the 24-hour recall method, the respondent attends a personal interview and is asked to remember and report all the foods and beverages consumed in the preceding 24 hours. A well trained interviewer is essential in administering a 24-hour recall because much of the dietary information is collected by asking probing questions. The interview lasts only 20 minutes and hence more people are liable to consent to it, thereby making the results likely to be more representative of the population (Block, 1982; Thompson & Byers, 1994). Also, because of the retrospective nature of this method, it has less potential than a multiple-day record to affect dietary behaviour, and the immediacy of the

recall period means that respondents are generally able to recall most of their dietary intake (Thompson & Byers). The major problem with a 24-hour recall is that an individual's diet varies from day to day. Therefore, one cannot apply data from a 24-hour recall to characterize an individual's usual intake (Thompson & Byers, 1994). Balough, Kahn & Medalie (1971) found day to day variation within individuals to be very high. They concluded that in order to have a 95% probability of being within $\pm$ 20% of a person's true year long mean for calories, it would be necessary to obtain at least four 24-hour recalls for the least variable half of the population. The recall of one day's intake is of interest only because of the implicit assumption that a single day is somewhat representative of a usual pattern of intake (Block, 1982). Data from a single 24-hour recall should not be used to estimate the proportion of the population that has adequate or inadequate diets, but may be used to describe the average dietary intake of a group (Block, 1982).

A multiple-day dietary record asks the respondent to record all foods and beverages, the amount and method of cooking, and brand of food (if possible) over a certain period of time. The record is typically no more than three or four days. Recording periods of more than seven consecutive days are usually unsatisfactory because of respondent fatigue. Gersovitz, Madden & Smiciklas-Wright (1978) showed that there was a significant increase in incomplete records as more days of records were kept. They serrupticiously weighed the meals of their subjects for lunch over a seven day period, and the group means agreed well with the known values. For individual data, however, regression analysis revealed a significant association of actual and recorded foods during the first couple of days, but suggested that the accuracy of recording deteriorates in the last three days of a seven day period. In this study the numbers were small and the respondents were elderly men (mean age=71.7 years).

Food records are often regarded as the gold standard against which other dietary assessment methods are compared (Jain, Howe & Rohan, 1996). This is because the dietary record method has the potential for providing quantitatively accurate information on the foods consumed during the recording period. The immediate record of food

consumed should allow fuller food description and provide an accurate portion size compared to 24-hour recall (Thompson & Byers, 1994). However, it demands a high degree of cooperation on the part of the subjects and the number who could be induced to participate may be a small and under representative sample (Block, 1982).

One problem with food records is that the prospective nature of the diary can affect dietary practices of the individuals participating in the study (Thompson & Byers, 1994). Under-reporting of foods is probably a result of a combination of incomplete recording and the impact of the recording process on dietary choices. Validity may be limited to group mean values since individual records may become unreliable after the first few days (Block, 1982).

The dietary record method appears to be more suited to the present study than other methods. Such a method should give reasonably accurate measurement of actual intake, and an average taken from a multiple day record is more representative than a single day.

Summary

All methods of measuring dietary intake have limitations. These range from poor recording methods of the subjects (eg, in the multiple day dietary record) to inaccurate recall of foods consumed (eg, in the FFQ, history and 24-h recall methods).

The dietary record method is seen as the gold standard for measuring food intake. Three days of recording provides the researcher with sufficient information to calculate the individual's total calories, protein, fat and carbohydrate intake, while being brief enough to maximize compliance. Unfortunately, micronutrient analysis is not possible from a 3-d record because daily intake of these nutrients is highly variable. To compensate for this drawback, a FFQ can be used that specifically measures a particular nutrient. A FFQ is an ideal way to measure dietary intake of a nutrient, such as calcium, because it is easy to understand by the subject, takes little time to complete, is easy to code into a computer, and provides the researcher with sufficient information to rank the individual according to calcium consumption.

Conclusions

In conclusion, it appears that skeletal growth is at its most rapid during the years around puberty. After this stage bone accretion slows considerably, but it is unclear whether this means PBM has been reached. It is likely that PBM may be reached toward the beginning of the third decade of life.

Research has shown that exercise is beneficial to BMD, although many studies are cross-sectional in nature. Some studies have specifically compared different types of exercise to try to determine which sport or exercise provides the greatest benefits to the skeleton. High impact activity, such as gymnastics, has been shown to provide greater benefits than a low impact activity such as running. Activities involving loading, such as weightlifting, have been shown to lead to greater gains in BMD than activities in which there is no loading, such as swimming, or in activities where body weight is supported, such as in cycling. However, it remains unclear as to the optimal amount and type of exercise that is required for maximal BMD gains in the skeleton.

Many studies have shown that amenorrhea can have deleterious effects on BMD in athletes, and in the general population. The causes of amenorrhea remain unclear, but heavy training regimen, poor diet, low percent body fat, energy availability, stress of competition and performance have been postulated as causes in athletes.

It is difficult to determine the major factors required to maximize BMD. Whether participation in one activity is better than in another is unclear, yet it is known that in some sports the negative hormonal stimuli and the positive mechanical stimuli seem to balance each other out, while in other sports the negative hormonal stimuli override the positive mechanical stimuli. It appears that while volume of training has an effect on BMD, the strong sport specificity of these effects suggests that investigation into a variety of different sports is warranted.

Research regarding the benefits of calcium seems equivocal, with some studies (Kristinsson et al., 1994; Nelson et al., 1991) showing that low dietary calcium intake may be detrimental to bone health, while others (Welton et al., 1994; Friedlander et al., 1995) find no negative effects of low calcium intake. It also seems plausible that effects

of calcium are different in different stages of life, and as yet these have not been defined. Some studies (Wolman et al., 1992; Kanders et al., 1988) have supported the idea of a threshold effect. Increasing dietary calcium leads to concomitant increases in BMD up to a value of about 1000 mg/d. However, dietary calcium intake above 1000 mg/d does not lead to further increments in BMD. It would therefore be wise to suggest that people try to consume the RNI of calcium, until conclusive research has been formulated.

It seems that previous research has primarily concentrated only on athletes who are involved in individual sports. A large proportion of women are involved in team sports, yet in the literature there is an under representation of information from athletes involved in team sports. Field hockey is the second most popular sport in the world, but it gleans very little mention in the literature. This research proposes to examine BMD in athletes involved in team sports.

CHAPTER 3

METHODS AND PROCEDURES

Subjects

Fifty-eight females, aged 17 to 23 years were recruited to participate in the study: 20 highly active (HA) subjects (elite field hockey players), 19 moderately active (MA) subjects (recreational soccer players) and 19 sedentary (SED) subjects (controls). The study was explained to all subjects and informed consent (Appendix B) was obtained prior to any participation. The MA and SED subjects were matched to the HA subjects for height, weight and menarcheal age. This was done by asking all prospective subjects in the moderately active and sedentary groups to complete a preliminary questionnaire (Appendix C). Of 35 MA subjects who volunteered for the study, 19 were matched to the HA subjects. Similarly, 19 SED subjects were selected from a possible pool of 28 volunteers. An effort was also made to match subjects for age and oral contraceptive (OC) exposure. All subjects recruited were at least 17 years old and they were all post-pubertal. All subjects were non-smokers and had no medical problems known to affect bone metabolism.

Ethics approval was obtained, prior to data collection, from the Ethics Committee, Faculty of Physical Education and Recreation, and also from the Radiation Safety Committee, at the University of Alberta Hospitals.

Field hockey players were recruited through Field Hockey Canada. Approval was sought from the Field Hockey Canada Sport Sciences Committee before the commencement of the study. The field hockey players from the Canadian junior squad were approached to participate in the study while they were at a training camp in Edmonton. Players were excluded if they were not exercising for a minimum of 10 h/wk and had not been training with the team for at least two years.

Recreational soccer players were selected based on convenience and permission. Subjects were recruited from five teams playing in the Edmonton Women's Soccer Association League. Recreational athletes had all been participating at this level for at

least two years and were exercising for at least 3 h/wk. Players who were exercising for more than 7 h/wk were excluded from the study.

Sedentary control subjects were volunteers who were recruited from class talks at the University of Alberta. Sedentary subjects were not involved in any regular exercise classes and did no more than one hour of exercise per week. All subjects, except two, had maintained this level of activity for at least five years prior to the start of the study. Subjects were first, second or third year students, from a wide background of courses, such as, humanities, nutrition, business, English and education.

General Exclusion Criteria

BMD is affected by OCs, medications and disease. Any subjects with medical disorders believed to affect skeletal health, such as hypoparathyroidism and hyperparathyroidism, were excluded from the study. Any subjects who presently smoked, or who had smoked in the past were excluded from the study. Subjects who had or were taking oral contraceptives were allowed to participate in the study, in an attempt to increase subject numbers. An attempt was made to match OC use between the groups to counteract these effects. Statistical analysis was used to determine if there was any difference between OC using subjects and other subjects in the same group.

Study Design

A cross-sectional study with an *ex post facto* design was used to test the hypotheses. The study was explained to the field hockey team, soccer teams and classes at the University of Alberta. All field hockey players who were interested in participating in the study and who met the inclusion criteria were scheduled for measurement of BMD. Potential subjects in the MA and SED groups who were interested in participating in the study and who met the inclusion criteria were asked to complete a preliminary questionnaire. Subjects who were matched to the HA group were scheduled for measurement of BMD. Those subjects who were not matched were excused from participating in the study. Subjects had their BMD measured once, by DXA, at the

following sites: right and left tibia/fibula, right and left hip, lumbar spine (L1-L4) and the whole body. On the same day the subjects were measured for weight, height and body composition. Subjects were also asked to complete a menstrual history questionnaire (Appendix D), a training history questionnaire (Appendix E), and a calcium FFQ (modified from Angus et al., 1989) (Appendix F).

On the day of the BMD measurement, subjects received clear instructions on maintaining an accurate 3-day dietary record (Appendix G). Subjects were asked to complete the record and then either return it directly, or mail it back to the researcher, who then encoded the data for computer analysis. A 3-day diary was chosen because it has been shown to be one of the most reliable and detailed measures of dietary intake (Thompson & Byers, 1994; Block, 1982).

Measures

BMD and BMC were measured by DXA, using a Hologic QDR-4500$_{TM}$ X-ray bone densitometer. A brief overview of the Hologic QDR-4500$_{TM}$ X-ray bone densitometer is given in the Appendix H.

BMD and BMC were measured in the left and right hip (femoral neck and Ward's triangle), lumbar spine (L1-L4) and the whole body. Six sites in the left and right lower leg were also measured (distal fibula, distal tibia, distal tibia/fibula, mid fibula, mid tibia and mid tibia/fibula). The lower leg was divided into ninths between the lateral malleolus and lateral condyle. The distal site was the lower three ninths of this section. The mid site was the middle ninth plus the two ninths below it (Figure 1). (There was one ninth overlap between the distal and mid sites). BMC is computed as the mass equivalent (in g) per scanned segment (eg, for the entire skeleton or the lumbar vertebrae) or the mass equivalent for a standard scan length (eg, for the lower limb or hip). BMD is BMC normalized for the projected scan area (segment length x bone width), resulting in areal bone density in units of g/cm^2 of calcium hydroxyapatite.

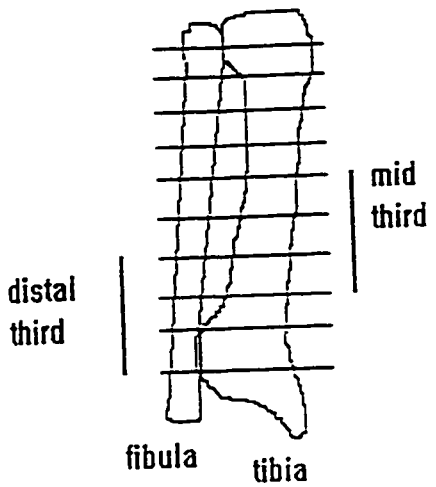

Figure 1. Anterior view of the right lower leg indicating the sites of measurement.

The sites of BMD measurement have been chosen because of their relevance to fracture sites. The site of measurement for future fracture identification has been debated. The forearm is simpler and less expensive to measure than the spine. But as spinal compression fractures are one of the most common osteoporotic fractures, many advocate spinal measurement. Also, fractures at the hip can be very debilitating, with many sufferers being put into nursing homes and losing their independence.

The incidence of lower limb stress fractures in young elite female field hockey players has been estimated to be between 19 % (junior level) and 30 % (senior) (Marshall, personal communication, August, 1996). The incidence of tibial fractures is high in these populations, which may in part be due to the nature of the game, the extent of training, and changing training surfaces. There are no data in the literature regarding the incidence of stress fractures in female soccer players, however Lawson, Aajducka &

McQueen (1995) in a study of 225 sports related fractures found that 50% of them were in soccer players, more than in rugby and skiing. They also found that playing soccer on artificial surfaces increased the likelihood of fracture fivefold, and of the total fractures, over 40% were in the tibia. The authors did note that many of the fractures could be related to the contact nature that develops in many team sports.

Detection of low BMD at these sites in youth will allow individuals to make lifestyle changes to compensate for such a reduction. Measurement of BMD at common fracture sites is of more relevance to the athletes, coaches and team doctors, than at other places of the skeleton. Both right and left limbs were measured to avoid any unknown or chosen dominance of one limb over the other.

Height was measured as the distance from the floor to the vertex with the subject in bare feet and standing erect with heels and back against the wall. Height was recorded once and measured to the nearest 0.5 cm.

Weight was measured by beam balance scale with the subject in minimal clothing and shoes removed. Weight was recorded to the nearest 0.1 kg and was measured once.

The sum of five skinfolds (tricep, bicep, subscapular, iliac crest and calf) was used to estimate body composition. Skinfolds were measured according to the procedures outlined in the Canadian Standard Test of Fitness (CSTF) manual (1986). The sum of five skinfolds was compared to Canadian population normative data (CSTF, 1986). Percent body fat was derived from skinfold measurements using the formulae of Siri (1961) and Durnin & Womersley (1974).

Whole body percent fat was also calculated from the DXA scan which uses the attenuation of energy from soft-tissue phantoms of known density to calibrate the apparatus, and subsequently to determine subject fat mass. Percent fat is then simply calculated as the proportion of fat mass in the whole body mass.

Dietary intake was measured using a 3-day dietary record. A computer software program (Food Processor Plus, version 6.01) was used to calculate average daily intakes of total energy, and grams of fat, carbohydrate and protein. On the day of BMD measurement, subjects were given clear instructions on how to complete a 3-day dietary

record, including information on portion size, brand foods, and home made recipes. Subjects were asked to record everything they ate and drank over a three day period, which was to include one weekend day and two weekdays. Field hockey subjects were asked to mail the completed 3-day dietary record back to the researcher in prepaid envelopes that were provided. All other subjects returned the completed dietary records directly to the researcher. The researcher then conferred individually with each subject to check for inaccuracies and omissions in the diaries.

For measurement of micronutrients such as calcium, a 3-day record is insufficient. To compensate for this limitation, a FFQ designed specifically to measure calcium intake was used (Angus et al., 1989). This FFQ was developed using information from 147 4-day weighed food records. The FFQ was validated against a 4-day weighed food record completed by 54 women aged 29-72 years. Calcium intake from the FFQ was correlated ($r=0.79$) with calcium intake from the 4-day food record. Subjects completed the FFQ on the same day they were measured for BMD.

Menstrual status was established using a modified classification system based on the work of Drinkwater, Breumner & Chesnut (1990). Subjects were asked to report whether their cycles were (1) always regular, (2) usually regular but had irregularities in the past, (3) usually irregular but were regular in the past, (4) usually regular but were amenorrheic in the past and (5) never regular. Definitions of irregular and amenorrheic cycles were given on the questionnaire (Appendix D).

The training history questionnaire (Appendix E) was used to help quantify activity, length and type of training. Details on the form included the approximate number of hours spent each day in each type of activity (eg. weights, technical practice and aerobic training). Subjects were also asked about bone injuries that had prevented them from participating in physical activity.

Data Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS). Multiple one-tailed t-tests using the Bonferroni method were used to compare

BMC and BMD data between the SED and the MA subjects, the SED and the HA subjects and the MA and the HA subjects.

Similarly, multiple one-tailed t-tests using the Bonferroni method were used to compare dietary intake, menstrual history and body composition data between the SED and the MA subjects, the SED and the HA subjects and the MA and the HA subjects.

In statistical tests where data were compared between SED, MA and HA subject groups, values were classified as significant at an alpha level of < 0.017 (after consideration of the Bonferroni correction at an alpha level of 0.05).

The subject data were collapsed according to menstrual status. Differences between groups 1, 2, 3, 4, and 5 were investigated by multiple one-tailed t-tests using an alpha level of ≤ 0.05. Differences between the subjects currently using OCs and the subjects who had never used OCs were also investigated by multiple one-tailed t-tests using an alpha level ≤ 0.05 as significant.

A correlation matrix was constructed for the entire subject sample to determine the relationship between the variables. Values were classed as significant at an alpha level ≤ 0.05.

Multiple regression was used to generate prediction equations for BMD for the entire subject sample.

Three of the highly active subjects failed to return dietary records, calcium FFQ's, menstrual history questionnaires and the training history questionnaire. Mean values of these variables were calculated from those subjects who did return packages.

CHAPTER 4

Results

Subject Characteristics

The descriptive characteristics of the subjects are given in Table 1. The highly active subjects (HA) and the moderately active subjects (MA) were of similar ages. The sedentary subjects (SED) were significantly older than both the HA ($t = 5.78$: $p<0.017$) and the MA ($t = 4.28$: $p<0.017$) groups. Height, weight and age of menarche were not significantly different. However, the SED group had a greater gynecological age than the HA group ($t = 3.61$: $p<0.017$) and the MA group ($t = 2.80$: $p<0.017$).

Table 1. Group descriptives (mean ± SD)

	n	age (y)	height (cm)	weight (kg)	age at menarche (y)	gynecological age (y)	exercise (h/wk)*
HA	20	19.1 ± 0.9	165.3 ± 4.6	62.4 ± 9.5	13.1 ± 1.2	6.0 ± 1.4	14.5 ± 4.1
MA	19	19.3 ± 1.2	165.1 ± 5.4	61.9 ± 9.9	13.3 ± 1.3	6.0 ± 2.0	4.3 ± 1.0 c
SED	19	21.0 ± 1.2 ab	164.8 ± 8.9	58.3 ± 9.2	13.7 ± 0.9	7.3 ± 1.0 ab	0.0 ± 0.0 ab

* n=17 for HA.

a - SED significantly different ($p<0.017$) from HA.

b - SED significantly different ($p<0.017$) from MA.

c - MA significantly different ($p<0.017$) from HA.

There was a significant difference in the number of hours of exercise each group performed per week ($p<0.017$ in all cases). Subjects in the HA group were exercising for a mean of 14.5 h/wk, and had been this active for an average of four years (range from

2.5 years to 7 years). The subjects in the MA group were exercising for a mean of 4.3 h/wk, and had been this active for an average of 4.5 years (range from 2 to 9 years). All of the subjects in the SED group were inactive at the time of the study. Two of the control subjects had been active on an irregular basis two years prior to the start of the study. Three other subjects in this group had been regularly active until they were 16 years old, while the remaining subjects had been inactive their whole life.

Bone mineral density

The HA subjects had significantly higher BMC and BMD than the SED subjects at the lumbar spine ($t=2.90$: $p<0.017$, and $t=3.07$: $p<0.017$, respectively for BMC and BMD) (Table 2a, Figure 2 and Table 2b). The HA subjects also had greater ($t=3.30$: $p<0.017$) whole body BMC and BMD ($t=4.12$: $p<0.017$) than the SED subjects. There were no significant differences in BMC or BMD between the MA subjects and the SED subjects and the MA subjects and the HA subjects at either the whole body or the lumbar spine.

HA subjects had higher BMC and BMD than the SED subjects at both the femoral neck ($t=6.26$: $p<0.017$ and 8.02: $p<0.017$) and Ward's triangle ($t=5.82$: $p<0.017$ and $t=7.49$: $p<0.017$ for BMC and BMD, respectively). MA subjects also had significantly greater BMC and BMD than the SED subjects at the femoral neck ($t=3.59$: $p<0.017$ and $t=4.42$: $p<0.017$) and at Ward's triangle ($t=3.45$: $p<0.017$ and $t=4.45$: $p<0.017$, for BMC and BMD respectively).

The only significant differences between the HA subjects and the MA subjects were at the hip. The HA subjects had higher femoral neck BMC ($t=2.63$: $p<0.017$), femoral neck BMD ($t=3.67$: $p<0.017$), and Ward's triangle BMD ($t=2.69$: $p<0.017$) than MA subjects.

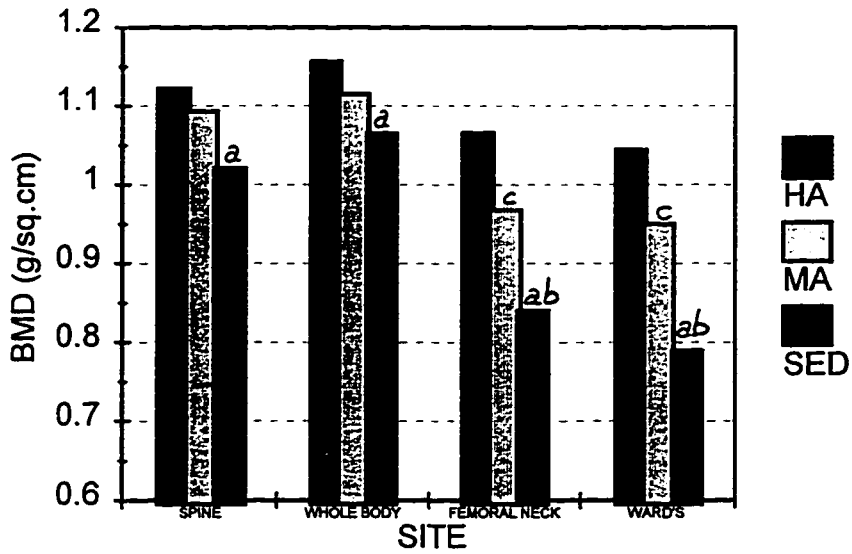

Figure 2. Bone mineral density (BMD) for each group (*from left to right*, spine, whole body, femoral neck and Ward's triangle).

a - SED significantly different ($p<0.017$) from HA.

b - SED significantly different ($p<0.017$) from MA.

c - MA significantly different ($p<0.017$) from HA.

Table 2a. Bone mineral content (BMC) of the spine, whole body, femoral neck and Ward's triangle in g (mean $\pm$ SD).

	Spine	Whole Body	Femoral Neck	Ward's Triangle
HA	69.165 $\pm$ 9.94	2370.607 $\pm$ 275.37	5.224 $\pm$ 0.59	1.232 $\pm$ 0.17
MA	63.704 $\pm$ 6.95	2215.652 $\pm$ 219.66	4.727 $\pm$ 0.58 c	1.106 $\pm$ 0.16
SED	58.788 $\pm$ 12.22 a	2045.283 $\pm$ 335.77 a	4.050 $\pm$ 0.58 ab	0.928 $\pm$ 0.15 ab

a - SED significantly different ($p<0.017$) from HA.

b - SED significantly different ($p<0.017$) from MA.

c - MA significantly different ($p<0.017$) from HA

Table 2b. Bone mineral density (BMD) of the spine, whole body, femoral neck and Ward's triangle in g/cm^2 (mean $\pm$ SD).

	Spine	Whole Body	Femoral Neck	Ward's Triangle
HA	1.122 $\pm$ 0.08	1.157 $\pm$ 0.07	1.066 $\pm$ 0.08	1.045 $\pm$ 0.11
MA	1.093 $\pm$ 0.09	1.115 $\pm$ 0.06	0.968 $\pm$ 0.08 c	0.950 $\pm$ 0.12 c
SED	1.021 $\pm$ 0.12 a	1.065 $\pm$ 0.07 a	0.840 $\pm$ 0.10 ab	0.790 $\pm$ 0.11 ab

a - SED significantly different ($p<0.017$) from HA.

b - SED significantly different ($p<0.017$) from MA.

c - MA significantly different ($p<0.017$) from HA.

There were no statistically significant differences in lower leg BMC between the three groups (Table 3a). However, the MA subjects had the greatest BMC at all sites except at the mid tibia (where HA subjects had the greatest BMC). The SED subjects had the lowest BMC at all sites.

Table 3a. Bone mineral content (BMC) of the lower leg in g (mean $\pm$ SD).

	HA	MA	SED
Distal fibula	9.817 $\pm$ 1.43	9.993 $\pm$ 1.48	9.486$\pm$ 1.782
Distal tibia	31.385 $\pm$ 3.71	31.399 $\pm$ 4.59	27.950 $\pm$ 5.84
Distal tibia/fibula	41.182 $\pm$ 4.79	41.391 $\pm$ 5.76	38.592 $\pm$ 9.92
Mid fibula	10.079 $\pm$ 1.58	10.364 $\pm$ 1.63	9.964 $\pm$ 1.60
Mid tibia	36.420 $\pm$ 3.68	36.128 $\pm$ 4.43	32.949 $\pm$ 5.81
Mid tibia/fibula	46.452 $\pm$ 4.60	46.488 $\pm$ 5.57	43.060 $\pm$ 6.64

Table 3b. Bone mineral density (BMD) of the lower leg in g/cm^2 (mean $\pm$ SD).

	HA	MA	SED
Distal fibula	0.780 $\pm$ 0.05	0.745 $\pm$ 0.06	0.714 $\pm$ 0.06 a
Distal tibia	1.064 $\pm$ 0.06	1.049 $\pm$ 0.09	0.944 $\pm$ 0.10 ab
Distal tibia/fibula	0.978 $\pm$ 0.05	0.955 $\pm$ 0.08	0.873 $\pm$ 0.09 ab
Mid fibula	0.792 $\pm$ 0.06	0.759 $\pm$ 0.07	0.751 $\pm$ 0.06
Mid tibia	1.428 $\pm$ 0.09	1.399 $\pm$ 0.11	1.327 $\pm$ 0.09 a
Mid tibia/fibula	1.215 $\pm$ 0.07	1.177 $\pm$ 0.08	1.123 $\pm$ 0.08 a

a - SED significantly different ($p<0.017$) from HA.

b - SED significantly different ($p<0.017$) from MA.

Similar to sites at the hip, spine and whole body, BMD in the lower leg was greatest in the HA subjects, followed by the MA subjects and was smallest in the SED subjects (Table 3b). The HA subjects had statistically significantly greater BMD than the SED subjects at the distal fibula ($t=3.77$: $p<0.017$), distal tibia ($t=4.69$: $p<0.017$), distal tibia/fibula ($t=4.78$: $p<0.017$), mid tibia ($t=3.57$: $p<0.017$) and mid tibia/fibula ($t=3.96$: $p<0.017$). The MA subjects had significantly greater BMD than the SED subjects at the distal tibia ($t=3.49$: $p<0.017$) and at the distal tibia/fibula ($t=3.18$: $p<0.017$). There was no significant differences in BMD between MA subjects and HA subjects at any site in the lower leg.

Body Composition

The HA subjects were statistically significantly leaner (sum of five skinfolds [SOS]=58.0 $\pm$ 10.5 mm) compared to either the MA group (70.4 $\pm$ 18.7 mm, $t=2.53$: $p<0.017$) and the SED group (76.2 $\pm$ 16.3 mm, $t=4.13$: $p<0.017$) (Figure 4). These mean values correspond to the 60th, 35th and 25th percentiles (for the HA, MA and SED subject groups, respectively) of Canadian population norms (CSTF, 1986).

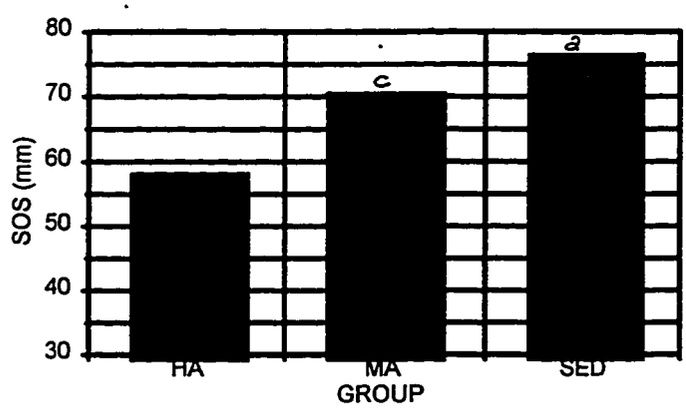

Figure 3. Mean sum of five (tricep, bicep, subscapular, iliac crest and medial calf) skinfolds (SOS) for each group.

a - SED significantly different ($p<0.017$) from HA.

c - MA significantly different ($p<0.017$) from HA.

The DXA body composition results confirm the results from the skinfold measurements. The HA subjects had significantly lower percent body fat than the MA subjects ($t=3.94$: $p<0.017$) and the SED subjects ($t=7.24$: $p<0.017$). Values were 21.8 ± 2.8, 27.3 ± 5.3 and $30.5 \pm 4.5\%$, for HA, MA and SED, respectively. Percent body fat calculated from the skinfolds measurements were 24.8 ± 2.5, 27.6 ± 3.4 and $29.3 \pm 3.3\%$ for the HA, MA and SED groups, respectively.

Dietary Analyses

The HA subject group had a significantly higher carbohydrate intake than the SED subject group ($t=2.77$: $p<0.017$). There were no other statistically significant differences in dietary intake of calories, fat, protein and calcium between the HA , MA, and SED subjects (Table 4). However, when examining trends, HA subjects had the highest caloric, protein and calcium intakes of the three groups.

Table 4. Average Daily Dietary intakes (mean ± SD).

	Calories	Carbohydrate (g)	Fat (g)	Protein (g)	Calciumϕ (mg)
HA*	2184 ± 543	345 ± 103	60 ± 16	80 ± 22	1191 ± 432
MA	1863 ± 377	277 ± 65	55 ± 19	69 ± 17	1054 ± 438
SED	1872 ± 467	261 ± 76 a	64 ± 20	70 ± 19	1068 ± 693

* n=17 for HA.

ϕ Calcium measured by the calcium FFQ.

a - SED significantly different ($p<0.017$) from HA.

The HA subjects had a higher percent caloric intake from carbohydrates than the MA subjects and the SED subjects (61.3%, 59.1% and 54.3%, respectively for the three groups) (Figure 4). These differences reached significance between the HA and SED groups ($t=3.47$: $p<0.017$) and between the MA and SED groups ($t=2.53$: $p<0.017$). Percent of calories from fat was 24.0%, 25.3% and 29.9% for the HA, MA and SED subjects, respectively. These differences reached significance between the HA and SED subject groups ($t=3.15$: $p<0.017$) and the MA and SED subject groups ($t=2.64$: $p<0.017$). There was no significant difference between percent calories from protein, which was approximately 14.5% for each group.

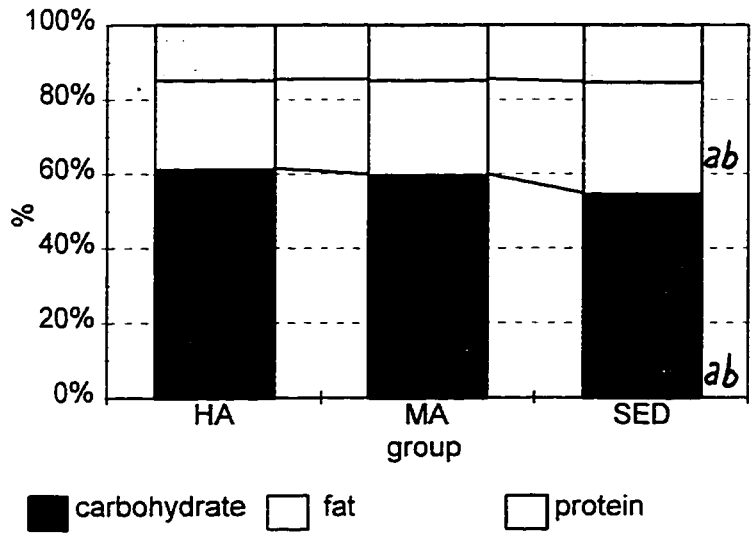

Figure 4. Mean caloric distribution from carbohydrate, fat and protein.

a - SED significantly different ($p<0.017$) from HA.

b - SED significantly different ($p<0.017$) from MA.

Menstrual History/OC-use

The gynaecological age of the SED subjects was statistically greater than the HA subjects and the MA subjects (Table 1). Most of the subjects in the study (96%) reported having regular menses (at intervals of 25-35 days) at the time of the study. There were no statistically significant differences between incidence of amenorrhea/oligomenorrhea between the three groups, despite differing amounts of exercise. Eleven (55%) HA subjects, 13 (68%) MA subjects and 14 (74%) SED subjects reported experiencing irregular cycles in the past. Of these subjects, one HA subject reported 4 months of amenorrhea, one MA subject reported 16 months of amenorrhea, and three SED subjects reported one year, seven months and six months of amenorrhea. Stress, travel, changes in diet and changes in exercise patterns were reasons reported by subjects to be associated with irregularities in their cycles.

Six (30%) of the HA subjects, seven (37%) of the MA subjects and ten (50%) of the SED subjects were currently using OCs, with duration of pill use ranging from 8 to 48

months in the HA group, 6 to 36 months in the MA group, and 6 to 48 months in the SED group. Additionally, two HA subjects, two MA subjects and one SED subject reported that although they currently were not using OCs, they had used them in the past for durations ranging from six to eighteen months

When data were grouped according to OC use, 23 subjects were currently using OCs, 30 subjects had never used OCs and 5 subjects had used OCs in the past but were currently not using them. The data from these five subjects were not used in the analysis on OC use. Although BMC and BMD tended to be higher in the OC group compared to the non-OC group at many sites (Table 5), there were no statistically significant differences between the two groups.

When data were analysed according to the classification system of Drinkwater et al. (1990), 20 subjects reported always having regular cycles (group 1), 28 subjects reported having regular cycles but having had irregularities in the past (group 2), two subjects reported that they usually had irregular cycles (group 3) and five subjects reported that they had regular cycles with an incidence of amenorrhea in the past (group 4). No statistically significant differences were found at any site in terms of BMC or BMD between these four groups (Tables 6a and 6b). When the subjects were divided into those subjects who always had regular cycles (n=20, group 1) and those subjects who had episodes of irregularity/amenorrhea (n=35, group 5), there was a trend for higher spinal BMD, and higher BMC and BMD at the whole body, femoral neck and Ward's triangle in group 1 compared to group 5. Differences failed to reach significance at any site.

Table 5. Bone mineral content (BMC) in g and bone mineral density (BMD) in g/cm^2 according to OC use (mean ± SD).

	OC USE			
	NO (n=30)		YES (n=23)	
SITE	BMC	BMD	BMC	BMD
spine	63.235 ± 10.56	1.079 ± 0.11	63.470 ± 10.57	1.073 ± 0.10
whole body	2180.480 ± 294.39	1.106 ± 0.07	2207.604 ± 316.84	1.113 ± 0.08
femoral neck	4.667 ± 0.79	0.958 ± 0.13	4.582 ± 0.69	0.939 ± 0.13
Ward's triangle	1.102 ± 0.22	0.936 ± 0.15	1.034 ± 0.18	0.892 ± 0.14
Distal fibula	9.445 ± 1.360	0.738 ± 0.06	9.909 ± 1.63	0.747 ± 0.06
Distal tibia	29.791 ± 5.16	1.079 ± 0.10	30.254 ± 4.78	1.017 ± 0.10
Distal tibia/fibula	39.968 ± 7.92	0.933 ± 0.09	40.146 ± 6.17	0.933 ± 0.08
Mid fibula	9.8053 ± 1.44	0.763 ± 0.07	10.237 ± 1.52	0.764 ± 0.07
Mid tibia	34.853 ± 4.88	1.392 ± 0.10	35.142 ± 5.00	1.373 ± 0.10
Mid tibia/fibula	44.592 ± 5.76	1.178 ± 0.09	45.544 ± 5.53	1.171 ± 0.08

Table 6a. Bone mineral content (BMC) in g according to menstrual categories (mean ± SD).

SITE	group 1 (n=20)	group 2 (n=28)	group 3 (n=2)	group 4 (n=5)	group 5 (n=35)
spine	61.746 ± 7.49	63.366 ± 9.89	60.746 ± 7.49	70.656 ± 21.96	64.230 ± 11.92
whole body	2191.776 ± 279.40	2179.850 ± 276.91	2092.735 ± 217.49	2281.848 ± 502.52	2189.443 ± 306.39
femoral neck	4.640 ± 0.70	4.595 ± 0.66	4.168 ± 0.53	4.894 ± 1.26	4.613 ± 0.75
Ward's triangle	1.111 ± 0.22	1.039 ± 0.17	1.055 ± 0.12	1.124 ± 0.29	1.052 ± 0.18
distal fibula	9.504 ± 1.38	9.672 ± 1.55	9.195 ± 0.84	10.440 ± 2.25	9.754 ± 1.62
distal tibia	29.352 ± 4.58	30.055 ± 4.64	27.385 ± 5.44	33.704 ± 7.79	30.424 ± 5.23
distal tibia/fibula	38.838 ± 5.70	39.724 ± 5.92	36.575 ± 6.29	48.544 ± 13.87	40.804 ± 7.90
mid fibula	9.819 ± 1.33	10.115 ± 1.64	9.570 ± 1.53	10.626 ± 2.22	10.157 ±1.69
mid tibia	34.293 ± 5.04	35.105 ± 4.52	33.280 ± 5.87	38.356 ± 6.76	35.465 ± 4.93
mid tibia/fibula	44.359 ± 5.36	45.1459 ± 5.659	42.850 ± 7.40	48.780 ± 8.27	45.531 ± 6.10

Group 1 = always regular cycles; Group 2 = usually regular, with irregular cycles in the past; Group 3 = usually irregular, with regular cycles in the past; Group 4 = usually regular, with amenorrheic cycles in the past; Group 5 = groups 2 + 3 + 4.

Table 6b. Bone mineral density (BMD) in g/cm^2 according to menstrual categories (mean ± SD).

SITE	group 1 (n=20)	group 2 (n=28)	group 3 (n=2)	group 4 (n=5)	group 5 (n=35)
spine	1.093 ± 0.11	1.057 ± 0.09	1.055 ± 0.03	1.101 ± 0.20	1.063 ± 0.11
whole body	1.116 ± 0.08	1.100 ± 0.07	1.105 ± 0.05	1.119 ± 0.11	1.103 ± 0.07
femoral neck	0.959 ± 0.13	0.944 ± 0.12	0.914 ± 0.07	0.973 ± 0.17	0.946 ± 0.12
Ward's triangle	0.947 ± 0.16	0.893 ± 0.13	0.902 ± 0.08	0.945 ± 0.22	0.901 ± 0.14
distal fibula	0.740 ± 0.06	0.744 ± 0.06	0.703 ± 0.03	0.762 ± 0.07	0.744 ± 0.06
distal tibia	1.006 ± 0.10	1.017 ± 0.10	0.999 ± 0.17	1.055 ± 0.11	1.021 ± 0.10
distal tibia/fibula	0.924 ± 0.09	0.933 ± 0.08	0.903 ± 0.12	0.967 ± 0.10	0.937 ± 0.09
mid fibula	0.761 ± 0.07	0.765 ± 0.06	0.715 ± 0.02	0.803 ± 0.09	0.768 ± 0.07
mid tibia	1.380 ± 0.12	1.388 ± 0.10	1.370 ± 0.18	1.405 ± 0.11	1.389 ± 0.10
mid tibia/fibula	1.169 ± 0.09	1.170 ± 0.80	1.135 ± 0.11	1.2094 ± 0.109	1.174 ± 0.09

Group 1 = always regular cycles; Group 2 = usually regular, with irregular cycles in the past; Group 3 = usually irregular, with regular cycles in the past; Group 4 = usually regular, with amenorrheic cycles in the past; Group 5 = groups 2 + 3 + 4.

Correlations for BMD

A correlation matrix for BMD was generated using the independent variables of age (y), height (m), weight (kg), SOS (mm), months of OC use, age at menarche, number of years since menarche, number of hours of exercise per week and calcium intake (mg) for the entire subject sample (Table 7).

Table 7. Correlation coefficients for bone mineral density (BMD) for the entire sample (n=58).

SITE	Age	Height	Weight	E2	M	Ex	Ca	OC	SOS
spine	-.23	.41*	.46*	-.11	-.15	.29*	.32*	.10	-.16
whole body	-.25	.36*	.46*	-.05	-.27*	.37*	.17	-.05	-.26*
femoral neck	-.42*	.30*	.42*	-.21	-.17	.63*	.10	-.11	-.41*
Ward's	-.44*	.30*	.35*	-.20	-.21	.55*	.13	-.13	-.44*
distal fibula	-.15	.30*	.43*	-.02	-.15	.30*	.07	.03	-.24
distal tibia	-.26*	.27*	.56*	-.11	-.16	.39*	.11	-.06	-.15
distal tibia/ fibula	-.26	.30*	.57*	-.10	-.17	.40*	.10	-.06	-.18
mid fibula	-.02	.12	.27*	.15	-.27*	.17	.05	-.12	-.18
mid tibia	-.14	-.05	.42*	.03	-.24	.42*	-.14	-.13	-.06
mid tibia/ fibula	-.19	-.01	.42*	.01	-.28*	.45*	-.11	-.14	-.14

E2=years since menarche, M=age at menarche, Ex=amount of exercise (h/wk), Ca=calcium intake, OC=months of OC use, SOS=sum of five skinfolds.

* significant ($p < 0.05$).

Weight was significantly positively correlated with BMD at every site, the number of hours of exercise each week was positively correlated with BMD at every site except the mid fibula and height was significantly positively correlated with BMD at every site except at the mid section of the lower leg. Both sites at the hip were correlated negatively with age ($p<0.05$). Calcium intake was found to be only significantly positively correlated with BMD at the spine. SOS was significantly negatively correlated with BMD at the whole body, and at the femoral neck and Ward's triangle. Age at menarche was negatively correlated with BMD at every site, but only reached statistical significance at the whole body, mid fibula and mid tibia/fibula. OC use and gynecological age were not significantly correlated with BMD at any site.

Prediction Equations

The variables of age, height, weight, SOS, number of years since menarche, number of months of OC exposure, calcium intake and number of hours of exercise per week were entered into multiple stepwise regression to generate prediction equations for the entire subject sample (Table 8).

All sites had strong r-values, (r^2 ranged from 0.43-0.62), with the exception of the mid fibula. Weight and SOS were found to be predictors of BMD at every site. Height contributed to improved equation strength at the distal and mid tibia and tibia/fibula. The number of hours of exercise per week made a contribution to both sites at the hip, while the number of years since menarche made contributions at the mid tibia and mid tibia/fibula sites. Calcium intake was found to only contribute to the lumbar spine equation.

Table 8. Prediction equations for bone mineral density (BMD) at all sites for the entire sample (n=58).

BMD (g/cm^2)	r^2
spine = 0.741418 + 0.008022 (W) - 0.003084 (SOS) + 0.000054 (Ca)	0.47
whole body = 0.906689 + 0.006298 (W) - 0.002604 (SOS)	0.49
femoral neck = 0.645708 + 0.005917 (Ex) + 0.009459 (W) - 0.004368 (SOS)	0.61
Ward's triangle = 0.639256 + 0.004883 (Ex) + 0.010662 (W) - 0.005690 (SOS)	0.61
distal fibula = 0.591036 + 0.004777 (W) - 0.001981 (SOS)	0.43
distal tibia = 1.292707 - 0.004391 (H) + 0.011337 (W) - 0.003510 (SOS)	0.58
distal tibia/fibula = 1.125133 - 0.003440 (H) + 0.009722 (W) - 0.003137 (SOS)	0.62
mid fibula = 0.663354 + 0.003446 (W) - 0.001459 (SOS)	0.19
mid tibia = 2.431994 + 0.012747 (E2) - 0.010138 (H) + 0.012589 (W) - 0.003279 (SOS)	0.52
mid tibia/fibula = 1.997016 + 0.009674 (E2) - 0.007962 (H) + 0.010488 (W) - 0.003122 (SOS)	0.56

W=weight (kg), H=height (m), SOS in (mm), Ex=exercise (h/wk), Ca=calcium intake (mg) and E2=years since menarche.

CHAPTER 5

Discussion

There were no statistically significant differences found between subject groups for height, weight, and age at menarche, validating the matching strategy that was used. It has been suggested that these variables play an important role in the development of BMD patterns (Drinkwater et al., 1990; Bonjour et al., 1991; Henderson et al., 1995) and hence, should be controlled. The SED subjects were chronologically older, and subsequently, gynecologically older than both the HA and MA subject groups. This resulted in the SED subjects having a greater number of years of estrogen exposure compared to both the HA and MA subject groups. While an attempt to match age between subject groups was made to try to control these effects, it proved difficult to find sedentary subjects who were interested in volunteering for the study who were in the desired age range.

Bone Mineral Density

It was hypothesised that the subjects who participated in regular physical activity would have greater BMD at all sites compared to sedentary subjects. The results of the present study partially support this hypothesis. The HA subject group had significantly higher BMD at every site (except the mid fibula) compared to the SED subject group, agreeing with the works of other authors (Rubin & Lanyon, 1984; Nelson et al., 1991; Robinson et al., 1995). The MA subjects had significantly greater BMD compared to SED subjects at the femoral neck, Ward's triangle and at the distal tibia and distal tibia/fibula. The HA subjects had significantly higher BMD than the MA subjects at the femoral neck and Ward's triangle.

The HA subjects also had significantly greater BMC at the lumbar spine, whole body, femoral neck and Ward's triangle compared to the SED subjects and had significantly greater BMC than the MA subjects at the femoral neck. The MA subjects had significantly greater BMC than the SED subjects at the femoral neck and Ward's

triangle. BMC is influenced by both height and bone length. These data imply differences in BMC between the HA, MA and SED subject groups, despite no differences in height. More accurate comparisons in skeletal health between subject groups can be made when the BMC data are normalized for bone size. As such the following discussion will focus primarily on BMD (which is BMC divided by the projected area of the bone).

The HA subjects, who exercise on a regular basis, tended to have higher BMD at all sites compared to the control subjects, despite the control subjects being chronologically and gynecologically older. While no other data have been published on field hockey players, the number of hours of training per week is similar to elite athletes in other sports which have been investigated (runners [Drinkwater et al., 1984; Rencken et al., 1996], gymnasts [Kirchner et al., 1995], cyclists, cross-country skiers, orienteers [Heinonen et al., 1993] and basketball players, volleyball players and swimmers [Lee et al., 1995]). Yet, in contrast to some of these reports, for the field hockey players in the present study, the amount of exercise they performed did not appear to have detrimental effects on BMD. While the possibility exists that only those subjects who were genetically predisposed to higher BMD have chosen to play field hockey (the HA group), or have succeeded to this level in this sport, it is unlikely.

The SED subjects were two years older and had two more years of estrogen exposure than the MA subjects, yet the MA subjects had higher BMD than the SED subjects at some sites. This suggests that while activity has been proposed to increase BMD, the amount (4.5 h/wk) and the type of activity done by the MA subjects in this study was only sufficient to increase BMD at the hip, distal tibia and distal tibia/fibula. Interestingly, there were no differences reported in BMD at the spine and for the whole body. Perhaps regularly playing soccer leads to increases in BMD at sites where biomechanical stresses are imparted, namely at the hip and lower leg, due to the kicking, tackling and running action of the game. In contrast, the upper portion of the body in soccer players undergoes little stress and hence BMD of the MA subjects would not be expected to be any different than BMD in SED subjects.

Gymnasts have been found to have bone densities of 1.090 to 1.117 g/cm^2 at the

femoral neck (Kirchner et al., 1995; Taaffe et al., 1995; Robinson et al., 1995). These values are slightly higher than the values reported for the HA subjects in the present study. In comparison to the data from gymnasts reported by Kirchner et al. (1995) (see Appendix A1), the HA subject group had similar BMDs to these gymnasts at Ward's triangle, slightly higher values than the gymnasts for whole body BMD, but much lower BMD values than the gymnasts at the lumbar spine. The runners in the study by Robinson et al. (1995), had bone densities considerably lower than the HA subjects in the present study at the lumbar spine, femoral neck and whole body (see Appendix A1). In contrast, the value for the HA subjects at the lumbar spine was considerably lower than the value for the spine (1.30 g/cm^2) reported for a group of runners in the study by Drinkwater et al. (1984). However, the runners in the latter study were much older than any of the groups in the present study.

There are no published data on BMD in field hockey players. However, there have been two studies published that have investigated BMD of soccer players. In a study by Alfredson et al. (1996), female soccer players (mean age=20.9 years) who trained for six hours per week had bone densities of 1.21, 1.35, 1.16 and 1.16 g/cm^2 at the whole body, lumbar spine, femoral neck and Ward's triangle, respectively. These values are all greater than the values found for the soccer players in the present study. However, subjects in the present study performed less hours of exercise per week (4.3 h/wk vs. 6.0 h/wk) than the soccer players studied by Alfredson et al.

Lee et al. (1995) found BMD's of 1.20, 1.16, and 1.16 g/cm^2 at the whole body, femoral neck and Ward's triangle, respectively, in a group of female soccer players (mean age=19.5 years). Again, these values are greater than the results for the soccer players in the present study. Unfortunately the lumbar spine was investigated at L2-L4 only and cannot be compared with the results of the present study. The lower BMDs of the soccer players in the present study compared to these earlier studies may be a result of the amount of activity the athletes performed. Soccer players in the present study were involved at a recreational level (4.34 h/wk), while in the studies of Lee et al. and Alfredson et al. (1996) the subjects were classed as elite competitive athletes (performing

7.0 and 6.0 h/wk, respectively).

In contrast, it is surprising that the field hockey players in the present study had lower BMDs in comparison to the soccer subjects in the studies of Lee et al. (1995) and Alfredson et al. (1996), despite all athletes being classified as elite. The field hockey players exercised for an average of 14.5 h/wk, which is twice the number of hours compared to the subjects in the study of Lee et al. (7 h/wk) and Alfredson et al. (6 h/wk). Regardless of this finding, the results from the present study do support the idea of a sports specific response of BMD and the theory that highly active individuals in certain sports do have higher BMDs than sedentary individuals, agreeing with the works of others (Alfredson et al., 1996; Haapasalo et al., 1994; Heinonen et al., 1993; Kirchner et al., 1995; Robinson et al., 1995; Taaffe et al., 1995).

A possible explanation for differences in BMD values between studies may be related to measurement technique. The present study used DXA to investigate BMD, while some studies have used DPA (Drinkwater et al., 1984; Nelson et al., 1986). Additionally, Lee et al., (1995) and Alfredson et al., (1996) although using DXA, used the Lunar model while the present study used the Hologic version, preventing accurate numerical comparisons between studies.

While unlikely, an alternative explanation could be that Canadians in general are predisposed to lower BMD than other nationalities. It is well known that African-American women have higher BMDs than Caucasian women, who have higher BMDs than Oriental women (Looker et al., 1995; Bhudhikanok et al., 1996). However, there are no comparisons of BMD among Caucasian women of differing nationalities in the literature. A further possibility is that athletes in other studies have been participating in their chosen sport for a longer number of years. In the study by Alfredson et al. (1996), the soccer players had been competing at a high level for a mean of 5.2 years, slightly longer than the number of years of the subjects in the present study, and perhaps this difference caused them to have a greater BMD.

Haapasalo et al. (1994) found that those athletes who had started training before or during puberty had greater BMD's than athletes who started training after puberty.

While this issue was not investigated or documented during the present study, it is likely that some of the subjects began training in their chosen sport after puberty as athletes were in their late teens or early twenties and some had only been playing their sport for two years. This issue was also not documented in the studies by Lee et al. (1996) and Alfredson et al. (1996).

Both Kirchner et al. (1995) and Robinson et al. (1995) concluded that the unique mechanical forces that are generated in gymnastics provide a large stimulus on BMD which may override other environmental effects. In field hockey and soccer, the lower extremities are subject to high ground reaction forces. Fast runs in different directions, landing from jumps (primarily in soccer) and starts and stops produce ground reaction forces three to six times the body weight (Alfredson et al., 1996). This impact may have lead to the higher BMD at almost all sites in the field hockey players, and in some sites in the soccer players, compared to the sedentary subjects. The increased number of hours of exercise per week performed by the field hockey players as compared to the soccer players may have produced an even greater stimulus on the skeleton of the field hockey players.

Significant differences between all three groups were found in BMD at both the femoral neck and Ward's triangle, which may suggest that the type of exercise involved in field hockey and soccer plays a larger role at the hip than at the spine or whole body. While the femoral neck, Ward's triangle and lumbar vertebrae consist of a greater proportion of trabecular bone, the whole body is predominantly cortical bone. Trabecular bone responds to both environmental and mechanical stimuli in a much quicker fashion than cortical bone due to its larger surface area, which may explain why these differences between the three subject groups were not seen in the whole body measures. Additionally, in field hockey there is a lot of movement at the hip due to running, and the low posture that is necessary to play the game, and in soccer, the hip flexors play an important role in the kicking action for the ball. In both sports the hip and knee region are both subject to compressive, bending and shear forces that produce high strain levels and possibly a higher osteogenic stimulus than at other sites (Alfredson et al., 1996).

Biomechanically, these aspects of the games of soccer and field hockey could cause greater BMD at the hip compared to less significant differences that were seen between the groups at the spine.

The mid tibia and fibula contain a much greater portion of cortical bone than the other sites that were measured, and while differences did exist between subject groups, the failure to reach statistical significance could be due to cortical bone taking a longer time to respond to environmental changes than trabecular bone. Also, the fibula is a non-weight bearing bone, and thus it is not surprising that there was no difference between groups at this site. Clearer statistical differences were noticed at the distal sites of the tibia and fibula, which contain much more trabecular bone than the mid sites. The number of hours of impact exercise which these subject groups performed may explain why there are higher values in the active subjects compared to the SED subject group.

The sports of field hockey and soccer were selected in the present study because of their similar nature. Both games involve the same number of players, the same indoor and outdoor season, and similar pitch sizes and training programs. The only notable difference between the sports is skill specific - the soccer players do a lot more jumping (impact) and resistance work (kicking the ball), and have a greater weight on their nondominant leg while kicking the ball, while the field hockey players play in a much less upright position. Consequently, these skills may lead to a lack of significant difference in lower body BMDs between the soccer players and the field hockey players.

There have been few studies that have measured BMD in the tibia and/or fibula. This is an area of the skeleton where stress fractures frequently occur, but where the mechanical loading of weight-bearing sports might provide an osteogenic stimulus (Lawson et al., 1995). It would be expected that the lower limbs of the athletes in the present study undergo a lot of strain due to the nature of the sports involved compared to non-impact sports. The results from the present study tend to support this statement, as the field hockey players have significantly greater BMD at all sites in the lower leg (except the mid fibula) than the SED subjects, and the soccer players have higher BMD's than the SED subjects at the distal tibia and distal tibia/fibula.

Myburgh, Bachrach, Lewis, Kent & Marcus (1993) found the BMD of the tibia to be 1.110 ± 0.075 g/cm^2 in a group of eumenorrheic runners, who had a mean age of 27.9 years. In an earlier study, Drinkwater et al. (1990) found values of 1.56 ± 0.04 and 0.94 ± 0.03 g/cm^2 for the BMD of the tibia and fibula, respectively, in a group of eumenorrheic women (mean age=30.0 years). In another study, Rencken, Chesnut & Drinkwater (1996), found BMD in the tibia and fibula to be greater in eumenorrheic runners compared to amenorrheic runners, but they did not provide the values. In a younger group of women (mean age =18 years), Henderson et al. (1995) found the BMD of the tibia/fibula to be 0.699±0.083 g/cm^2. However, the women in this study undertook varying amounts of physical activity.

The values mentioned above are considerably different to the values reported in the present study (where ranges of 0.780-1.428, 0.745-1.399 and 0.714-1.327 g/cm^2 were found for BMD at lower limb sites in the HA, MA and SED subject groups, respectively). This may partially be due to an older mean age of the subjects in each of the above studies. More importantly, each of the studies measured BMD at a different location on the tibia and/or fibula, which makes it impossible to compare data at these sites. Myburgh et al. (1993) measured a site at the distal third of the tibia from the medial malleolus to the tibial tuberosity, while the studies by Drinkwater et al. (1990) and Rencken et al. (1996) measured the tibia and fibula at a more proximal location, midway between the lateral condyle and the lateral malleolus. In the study by Henderson et al. (1995) the distal tibia/fibula was measured at a site 8 mm proximal to the talocrural joint space, while during the present study, the sites in the lower leg were chosen at the distal third and mid third from the lateral malleolus to the lateral condyle.

While the present study provides BMC and BMD data from a number of sites in females of this age group, it is imperative that in the future, a common site of measurement is chosen for the lower leg, so that accurate comparisons amongst data can be achieved.

Hypothesis 2 stated that the moderately active subjects would have a higher BMD at all sites than the highly active subjects. The results from this study do not support this

hypothesis.

This hypothesis was proposed because some elite athletes have been shown to have increased incidence of menstrual dysfunction (Drinkwater et al., 1984; Nelson et al., 1986; Marcus et al., 1985; Robinson et al., 1995). Amenorrhea has been linked to low BMDs in athletes in these studies. As mentioned previously, although the exact mechanism is unclear, estrogen is closely linked to skeletal health. Those subjects who experience oligomenorrhea/amenorrhea, have been shown to have low levels of circulating estradiol (Drinkwater et al., 1984; Drinkwater et al., 1986; Drinkwater et al., 1990; Marcus et al., 1985; Nelson et al., 1986; Wolman et al., 1990) and low BMD's. Reasons proposed for the development of menstrual dysfunction in athletes are broad, ranging from low caloric intake, increased stress, to low body weight, low percent body fat, energy availability and sudden changes in training patterns in terms of duration, intensity and frequency (Arena et al., 1995; Bale, 1994; Carbon, 1992; Warren, 1980). It is likely that many of these factors are closely linked, and that not just one factor is the sole cause of amenorrhea.

In the present study there were no differences found in menstrual status between the HA, MA and SED subject groups. Caloric intake was similar for all three groups, agreeing with the findings of Loucks (1989), and closely matched the Canadian RNI. While Warren (1980) suggested the concept of energy drain existing in young female athletes, which may lead to menstrual dysfunction, this was not evident in the HA subjects in the present study.

The fact that there was no difference in menstrual history between the three groups, and that the HA subjects did not have a higher propensity to menstrual disruption may suggest that their present lifestyle does not cause detrimental menstrual disturbances in this group of elite athletes. However, Loucks Mortola, Girton & Yen (1989) found alterations in the hypothalamic-pituitary-ovarian axis in a group of completely asymptomatic runners (regular cycles). The group had reduced LH pulse frequency, increased LH pulse amplitude and reduced FSH response to GnRH administration and diminished luteal function (reduced luteal phase and a longer follicular phase). There was

no evidence of phasic elevations of urinary metabolites of estrogen and progesterone (estrone-glucuronide and pregnanediol-glucuronide) which suggests anovulation with zero follicular or luteal development. Yet, all these subjects had regular menstrual cycles.

The HA subjects in the present study had similar diets and calcium intake to the other subject groups, and also had similar menstrual history. Therefore, if a reduced caloric and calcium intake, a low percent body fat and menstrual dysfunction are indirect precursors to low BMD, it would be unlikely that low BMDs would be evident in the HA subject group. Percent body fat from DXA was significantly less in the HA subjects (21.8 %) compared to the MA subjects (27.2 %) and the SED subjects (30.5 %). In a similar manner, percent fat calculated from skinfold measurements was lower in the HA subjects (24.8%) than in the MA subjects (27.6%) and the SED subjects (29.4%), supporting the data from DXA. While these data indicate a lower percent body fat in the HA group compared to the MA and SED subjects, it is unlikely that this alone could cause adverse effects on BMD.

The HA subjects trained on average 14.5 hours a week, while the MA subjects exercised for a mean of 4.34 hours each week. It would appear that the number of hours of exercise performed each week is not a trigger factor for low BMD in the sport of field hockey. From a health promotion aspect it is positive to see that performing light impact exercise for 4.34 h/wk brings about changes in BMD at the hip and distal tibia and distal tibia/fibula in females of this age group, and as such it is important that sedentary individuals are encouraged to begin an exercise program. The hip is a primary fracture site in osteoporosis, and increasing BMD while individuals are young may be a way of postponing or preventing fractures at this site in the later years.

Body Composition

It was hypothesised that the athletic groups would be leaner and have a lower percentage body fat than the sedentary subjects. The results from this study partially support this hypothesis. The HA subjects had a significantly lower percentage body fat and SOS than the MA subjects and the SED subjects, but there was no statistical

difference between the MA subjects and the SED subjects. It is surprising that MA subjects did not have a lower fat percentage, considering that they were regularly active, however, they were only competing at a recreational level.

The field hockey players, soccer players and sedentary subjects had SOS values corresponding to the 60th, 35th and 25th percentile respectively, when compared to normative data from the Canadian population (CSTF, 1986). These values indicate that the field hockey players are leaner than the soccer players, who are slightly leaner than the control subjects. The results from the DXA scans support this, calculating values of 21.8, 27.2 and 30.5 % body fat for each group, respectively. Despite reports of DXA both over estimating (Gutin et al., 1996; Ogle et al., 1995) and under estimating percent body fat (Goran, Driscoll, Johnson, Nagy & Hunter et al., 1996) in females compared to skinfold measurements, percent body fat from DXA and percent body fat calculated from skinfolds has been found to be strongly correlated (r^2=0.61 to 0.65) (Goran et al., 1996; Gutin et al., 1996; Ogle et al., 1995). In the present study percent body fat from DXA and percent body fat calculated from the skinfolds measurements were very similar for both the MA (27.2 vs. 27.6%, respectively) and SED (30.5 vs. 29.4%, respectively) subject groups. DXA slightly underestimated percent body fat in the HA subject group compared to the percent body fat calculated from the skinfolds measurements (21.8 vs. 24.8%, respectively). Studies have shown that regular exercise reduces percent body fat in children (Cassell, Benedict & Specker, 1996), premenopausal women (Hetland, Haarbo & Christiansen, 1995) and in postmenopausal women (Ready et al., 1996). The results in the present study are in agreement with other authors who suggest that subjects who exercise regularly have a lower fat mass than sedentary subjects.

In a review article by Davis & Brewer (1992), female soccer players on representative teams had percent body fat values ranging from 19.7 to 22.0% as measured by skinfolds. The collegiate level soccer players in the study by Lee et al. (1995) were found to have 20.4% body fat using DXA. The soccer players in the present study had a relatively higher percent body fat (27.2%). However, the athletes in the studies by Davis & Brewer and Lee et al. were elite, older and did twice as many training hours as the

recreational soccer players in the present study. The body composition of the field hockey players in the present study was similar to the subjects in these previously mentioned studies. This may be expected because of the similarity in the games, type and amount of training, and level of competition between the groups.

As a comparison, other studies have shown percent body fat values of 26.6% in soccer players (Alfredson et al., 1996), 21.6% in orienteers, 22.5% in cross country skiers, 25.3% in cyclists, 25.7% in weight lifters (Heinonen et al., 1993); 11% to 16.8% in runners (Marcus et al., 1985; Robinson et al. 1995) and 17.0 to 17.7% in gymnasts (Kirchner et al., 1995; Robinson et al., 1995).The value of 21.8 % body fat reported by DXA for HA subjects in the present study indicates that individuals with varying levels of body fat can, and do, succeed to high levels in some sports, without disruptions to their menstrual cycle. Field hockey and soccer are not aesthetic sports, and in some ways a strong body is considered an asset to performance because of the physical nature of the game. And although a high percent body fat is not desirable in any individual, it would seem that low percent body fat, as seen in many runners, gymnasts and dancers, is not necessary to reach elite levels in field hockey. There appears to be quite a range in percent body fat in elite athletes across a wide spectrum of sports. The field hockey players in the present study fall right into the middle of this range, while the soccer players have values at the upper end of this range. This suggests that perhaps you do not need to be ultra thin in order to succeed at a high level of competitive sports.

Dietary Analyses

It was hypothesized that there would be no difference in dietary intake between the three subject groups. The Canadian RNI for caloric intake is 2100 kcal/d for females 19-24 years old (Health and Welfare Canada, 1990), with increased caloric intake suggested with increasing exercise levels. There was little difference between the groups in absolute dietary intake in terms of calories, protein, fat and calcium intake, supporting the above hypothesis, however, the HA subjects did report consuming significantly more carbohydrates than the SED group. Other studies have also found that athletic females

this age have similar dietary intakes to sedentary subject groups (Lee et al., 1996; Robinson et al., 1995; Drinkwater et al., 1984; Nelson et al., 1986). However, the HA and MA subjects obtained a significantly greater percentage of their calories from carbohydrates and a significantly lower percentage of their calories from fat compared to the SED subjects.

Although it is advisable for highly active females to increase their caloric intake to counteract their increased energy expenditure (Health & Welfare, Canada, 1990), Loucks (1989) stated that in many females dietary intake does not match energy expenditure. Despite the HA subject group performing a large number of hours of exercise each week, their caloric intake was not significantly higher than either the MA or the SED groups. However, there was a large range of caloric intake within the HA group which could have prevented statistical significance being reached.

The HA subjects in the present study had similar caloric intakes to both the MA subject and SED subjects, despite having much higher energy expenditures. While it is unclear how these athletes can maintain such an energy imbalance, perhaps it is possible that these elite athletes have developed a more efficient mechanism of utilising substrates for energy production. Myerson et al. (1991) found a reduced resting metabolic rate in a group of female runners, and concluded that this reduction in the individuals' resting metabolic rate and the incidence of amenorrhea in athletes could be part of an adaptive response to conserve energy and maintain stable weight in response to increased caloric demands that are not compensated for by increased caloric intake.

It is speculated that as the requirements of the body to remain more energy efficient become more stringent, reproductive function may be initially compromised and later sacrificed to minimize energy loss (Cumming, Wheeler & Harber, 1994).

In support of these theories, Loucks & Heath (1994) found evidence that women who consumed a restricted diet had reduced LH pulsatility and increased LH amplitude. If caloric deprivation were to be maintained for a long period of time, these findings would be indicative of menstrual dysfunction and anovulatory cycles. This supports the hypothesis that LH pulsatility is closely linked to energy availability in women. While it

is not suggested that the HA subjects in the present study are calorie deprived, Warren (1980) did suggest the concept of energy drain existing in young female athletes, where rather than reduced energy intake, increased energy expenditure may lead to some athletes exhibiting similar symptoms to malnourished individuals (one of which is menstrual dysfunction). Although menstrual dysfunction was not found in the HA group, it is possible that the questionnaire used was not sensitive enough to detect problems. Measures such as LH amplitude and pulsatility would give a more accurate determination of menstrual status, but were not taken in the present study.

Intake of protein, fat and carbohydrate is recommended to be 15%, 30% and 55%, respectively, of caloric intake (Health and Welfare Canada, 1990). The mean percent values for the SED subject group corresponded closely with these guidelines. However, both the HA and MA subject groups had a higher percent caloric intake from carbohydrates, and a lower percent caloric intake from fats compared to these guidelines. It is surprising that despite the SED subject group meeting the guidelines for healthy eating for percent caloric intake of carbohydrate, protein and fat, and reporting a lower caloric intake than is recommended, that they should have such a high percent body fat. According to Health and Welfare Canada (CSTF, 1986) the mean value for percent fat for this group falls into the estimated health risk zone established by trends in morbidity and mortality data.

Food records are often regarded as the gold standard against which other dietary assessment methods are compared (Jain et al., 1996). The 3-day dietary record method was chosen because it has been shown to give a reasonably accurate measurement of actual intake, while minimizing respondent fatigue and incomplete recording (Gersovitz et al., 1978). The immediate recording of food consumed should allow full food description and provide an accurate portion size (Thompson & Byers, 1994), but it demands a high degree of cooperation on the part of subjects (Block, 1982).

One problem with food records is that the prospective nature of the diary can affect dietary practices of the participating individuals (Thompson & Byers, 1994). Even though full instructions were given to the subjects on how to use the diaries, and subjects

were asked to keep to their normal diet, it is possible that subjects in this study under-reported foods, or changed their usual dietary habits for the recording period. Sedentary subjects have been found to be more inclined than the active subjects to under-report foods which they believe are "unhealthy" or socially unacceptable (Hebert, Clemow, Pbert, Ockene & Ockene, 1995). Therefore, analysis of these diaries may not give a true reflection of the normal dietary practices in a sedentary group of subjects.

Mean calcium intake for all groups was well above the Canadian RNI of 700 mg/d (Health and Welfare Canada, 1990). Values ranged from 415 mg/d to 3232 mg/d among the 58 subjects. Two HA subjects, 5 MA subjects and 8 SED subjects reported consuming below the RNI. BMC and BMD were lower (but not significantly so), at all sites in this group of 15 subjects compared to those subjects who maintained calcium intakes above the RNI. While full instructions were given to subjects about completing the FFQ, and answers were checked, it may be that this instrument is not sensitive enough to detect differences in calcium intake in Canadian females of this age group. Marcus et al. (1987), while finding no difference between athletes and non-athletes in terms of caloric intake, found that 40% of the athletes in the study were consuming less than the RDA for calcium. In contrast, many studies have found little difference in calcium intake between athletic groups and sedentary groups, and between eumenorrheic and amenorrheic athletes (Haapasalo et al., 1994; Robinson et al., 1995; Drinkwater et al., 1984; Nelson et al., 1986).

There was considerable variability in dietary and calcium intake amongst subjects within each group, which is likely to be one of the reasons why the data failed to reach significance.

Menstrual History / Oral Contraceptive Use

Hypothesis 5 proposed that the HA subjects would have a higher propensity to menstrual disturbances than the MA subjects and SED subjects. The present study failed to find any difference in menstrual history between the groups and does not support this hypothesis. In fact, the subjects in the SED group reported a higher incidence of

menstrual irregularity and amenorrhea than the HA group, although this did not reach significance. In other studies (Drinkwater et al., 1984; Nelson et al., 1986; Marcus et al., 1985), runners who covered a greater number of miles each week had a higher incidence of menstrual dysfunction than runners who ran fewer miles each week. Both Kirchner et al. (1995) and Robinson et al. (1995) found that elite gymnasts had a higher propensity to oligomenorrhea and amenorrhea than sedentary subjects. In still further studies (Alfredson et al., 1996; Lee et al., 1995; Heinonen et al., 1994) only athletes who met the inclusion criteria of having a regular menstrual cycle were allowed to participate. In the present study an effort was not made to select subjects who were eumenorrheic or to match subjects on menstrual histories, yet the amount of exercise undertaken by these elite HA subjects does not seem to have affected their menstrual cycles. Perhaps the variation in training patterns (cardiovascular, strength, flexibility and skill specific training) may have prevented any disruption to their cycles.

In the present study, menstrual status was established by self-report, and as such may not be as accurate as other techniques, namely hormonal measures, of identifying abnormalities in the menstrual cycle. Subjects were asked to assign themselves to a menstrual category, but according to Loucks et al. (1989), reproductive hormone assays are the only measure to detect changes in hormones which may not be evident in changes in the menstrual cycle.

The SED subjects had significantly lower BMD than the HA subjects (at all sites except the mid fibula) and the MA subjects (at the hip and distal tibia and tibia/fibula) and also were gynecologically older. Additionally, there was a tendency for the HA and MA subjects to have higher BMC than the SED subjects. Other studies have also found that sedentary subjects who were gynecologically older than active subjects did not have higher BMDs than the active subjects, despite having more years of estrogen exposure (Wolman et al., 1991; Heinonen et al., 1993; Haapasalo et al., 1994). This suggests that regular physical activity may have more of a benefit on the skeleton than the number of years since menarche. This was confirmed by the results in the correlation matrices. There were no significant correlations between BMD at any site and gynecological age,

while exercise was significantly correlated with BMD at all sites except the mid fibula for the entire subject group.

Many studies indicate that irregular/absent menses leads to decreases in BMD (Drinkwater et al., 1984; Jones et al., 1985; Nelson et al., 1986; Marcus et al., 1987 and Seeman et al., 1992). When the menstrual data from the present study was categorized according to the classification of Drinkwater et al. (1990), those subjects who were always regular had higher BMD at the spine, whole body and hip than those subjects who had disruptions to their cycles in the past, though these differences did not reach significance at any site.

There were no significant differences in BMC and BMD between the regularly menstruating subjects (group 1) and those subjects who reported episodes of amenorrhea (group 4). Five (8.6%) of the subjects in the study reported an incidence of amenorrhea, which is a higher frequency than has been reported in the normal population (Loucks &Hovarth, 1985, Nattiv et al., 1994). Both Jones et al. (1985) and Seeman et al. (1992) found a negative correlation between BMD and length of amenorrhea. In studies which have shown amenorrheic athletes to have lower BMD than eumenorrheic athletes, the length of amenorrhea of these subjects was considerably longer (40 to 50 months) than any of the subjects in the present study (Drinkwater et al. 1984, Nelson et al., 1986, Marcus et al. 1987). In this study, one subject reported an 18 month period of amenorrhea, while the other four subjects reported a six month episode of amenorrhea, which may not have been a sufficient length of time to effect the skeleton. It may be possible that the amount of exercise being done by these subjects is sufficient to override the negative effect of hypoestrogenism. According to Loucks (1990), while luteal suppression (menstrual dysfunction) may be an intermediate condition between menstrual regularity and amenorrhea in athletes, alternatively it could represent an endpoint of a successful acclimation to exercise training. Gymnasts have been found to have higher BMDs than eumenorrheic athletes and control subjects despite an increased prevalence of menstrual dysfunction (Kirchner et al., 1995; Robinson et al., 1995). This suggests that in some sports, even when menstrual function is compromised the body may be able to

maintain and even increase BMD.

Lack of significant differences in BMC and BMD between menstrual categories may also be attributed to the fact that all subjects had a regular cycle at the time of the study. Subjects were asked to self-report their own menstrual history and assign themselves to one of the menstrual categories, which may have lead to inconsistency among subjects. In the future, a more detailed menstrual history questionnaire may be more appropriate. However, other studies (Heinonen et al. 1993) have found similar results.

It was hypothesised that the eumenorrheic subjects and OC using subjects would have a higher BMD than amenorrheic subjects. It proved difficult to test this hypothesis, because 96% of the subjects had a regular cycle at the time of the study, plus only five of the subjects had experienced periods of amenorrhea. Instead, a comparison was made between those subjects who were currently using OCs with those subjects who had never used OCs. A further comparison was made between those subjects who admitted always having a regular cycle and those subjects who had experienced irregular/ amenorrheic cycles in the past. The findings of the present study indicated that there were no significant differences in BMC and BMD between the groups at any site.

The use of OC was not highly correlated with bone density at any of the measured sites. This supports the findings of Lee et al. (1995) who found no correlation between BMD at any site and OC use in a group of collegiate athletes, and Wolman et al. (1991) who found that there was no relation between OC use and BMD at the femoral mid-shaft. This is in contrast to the findings of Seeman et al. (1992), who found BMD at the spine, femoral neck, Ward's triangle and trochanter to be highly correlated with OC use (r=0.63-0.66). A later study by Wolman et al. (1992), which analysed lumbar spine BMD with CT, found OC athletes to have a slightly higher BMD than non-OC using athletes, (215 vs. 211 mg/cm^3), but the difference was not significant. Similar findings occurred in the present study, where OC using subjects had higher BMDs than non-OC using subjects at some sites, but not at the level of significance. In two further studies (Lloyd et al., 1986; Barrow and Saha, 1988) those athletes who had used OCs had a decreased incidence of

stress fractures than those athletes who had never used OCs.

The lack of significant difference between the OC and non-OC groups could be due to the wide range in the length of OC exposure between the subjects. Additionally, some subjects had only used OCs for 6 months, while other subjects had used OC for 4 years, and the short duration of OC exposure may not be sufficient time to allow a benefit to be revealed in the skeleton. While the present study did not document specific types of OC pills regarding their estrogen and progesterone composition, it could be possible that different OC pills have different effects on BMD. The considerable variability within the group and the young age of the subjects in this study may have confounded the effects of OC use.

Correlations

Many significant correlations were seen between the independent variables and BMD in the entire subject sample. The highest variance accounted for was 40% between femoral neck BMD and exercise. Thus, approximately 50-70% of the variance in these relationships remains unexplained.

Body weight was a significant predictor for current BMD at all sites. A possible explanation for this is that in activities where body weight is supported, such as field hockey and soccer, the skeleton responds to the greater mechanical stress by increasing mass. Drinkwater et al. (1990) found weight to be a significant predictor of BMD at the lumbar spine, femoral neck, tibia and fibula in an older group of eumenorrheic female runners. Henderson et al. (1995) also found weight to be a predictor of BMD at the spine, femoral neck, and distal tibia/fibula in a group of 18 year old females of varying activity levels.

The rate of change in weight is highly correlated with the change in BMD during childhood and adolescence. Many studies have shown that changes in BMD at various sites (radius, femoral neck and lumbar spine) can be accounted for by standard clinical measures of growth and development, including body weight, height and stage of pubertal development, all of which are largely genetically determined (Rubins et al.,

1993; Proesmans et al., 1994; Ruiz, Mandell & Garabedian, 1995). In contrast, Gilsanz et al. (1988) found that height, weight, surface area and BMI failed to significantly predict trabecular vertebral BMD in either pubertal or prepubertal children.

Age was significantly negatively correlated with BMD at the hip and distal tibia and was weakly correlated with BMD at all other sites, suggesting that BMD decreases with aging. This supports the findings of Recker et al. (1992), who also found age to be negatively correlated with BMD at the spine in a study of women aged 18-26 yrs. In both the present study and the work of Recker et al. (1992) many of the subjects were in their third decade and it could be possible that some of the subjects had already reached their peak bone mass and bone mass accretion has ceased.

Height has also been found to be a predictor of BMD in subjects who are still growing (Bonjour et al., 1991; Glastre et al., 1990). In the present study height was significantly correlated with BMD at all sites except those of the mid lower leg, and it was found to be a predictor of BMD at some sites in the lower leg.

Unlike the results from the present study, where SOS was significantly negatively correlated with whole body, femoral neck and Ward's triangle BMD, other authors have found little relationship between SOS and BMD (Henderson et al., 1995). The present study also found SOS to contribute to prediction equations for BMD at all sites for the whole subject group. Differing methods of body composition analysis and varying ages of subjects may have lead to a lack of significant findings in previous studies. Also, other studies have not included SOS in regression equations. In older women body fat has been correlated with BMD (Reid et al., 1992; Hassager et al., 1989; Lindsay et al., 1992). SOS tends to decrease as exercise levels increase, which may confound the two variables, however exercise was still a valuable predictor of BMD at Ward's triangle and the femoral neck in addition to SOS.

The number of years since menarche provided a predictor of BMD at some sites in the lower leg. Drinkwater et al. (1990) found that age at menarche was a predictor of BMD at the fibula. They also found menstrual history to be a predictor of BMD at the spine and femoral neck. Nelson et al., (1991) concluded that calcium and exercise

preferentially affected BMD at the hip and spine, so perhaps estrogen exposure may preferentially effect BMD at differing sites due to the different composition of bone at these sites.

Calcium was significantly correlated with BMD at the spine only. Despite numerous previous investigations of the effect of calcium intake on BMD no consistent relations have been identified. Kristinsson et al. (1994) found calcium intake to be significantly correlated with BMD in the distal and ultra distal forearm, after adjustment for menarcheal age and weight in a group of 15 year old girls. Neives et al. (1995) found that current calcium intake was moderately correlated with hip BMD, and that a higher lifetime calcium intake was associated with a higher hip BMD compared to a lower lifetime calcium intake. Nelson et al. (1991) found calcium to preferentially enhance BMD at the hip in postmenopausal women. Wolman et al. (1992) found a linear relationship between calcium intake and spinal BMD in athletes in their mid twenties, suggesting that for every 100mg of calcium, BMD would increase by 3.9 mg/cm^3. Kanders et al., (1988) found a significant correlation between total calcium and vertebral BMD once activity levels had been removed ($r=0.36$, $p<0.05$) in 25-34 year old women. They suggested that vertebral BMD did not appear to increase with calcium intakes above 800-1000 mg/d. It is unclear why calcium isn't an important predictor at other sites, but perhaps the other variables have a stronger influence on BMD at these other sites. Nelson et al., (1991) concluded that high dietary calcium may preferentially alter bone density at different sites due to the varying proportions of trabecular and cortical bone found at the lumber spine and the hip.

Exercise was shown to be a strong predictor of BMD in the hip. Many other studies have demonstrated an important link between BMD and activity. Robinson et al. (1995), Kirchner et al. (1995), Taaffe et al. (1995) and Heinonen et al. (1994) all found that athletes had higher BMD than sedentary subjects. In the present study, BMD at all sites, except the mid fibula, was correlated with number of hours of exercise per week.

Ruiz, Mandell & Garabedian (1995) found that weekly duration of sports activities positively influenced both vertebral and femoral BMD in children and

adolescents. Welton et al. (1994) found in a longitudinal study that only the amount of weight bearing activity and body weight made a significant contribution to a final model of lumbar BMD at age 27. Drinkwater et al. (1990) found the number of hours of exercise per week to be a predictor of femoral neck and tibial BMD in a group of female runners who were older than the subjects in the present study. As previously mentioned, the reason for exercise only predicting BMD at both sites at the hip could be related to the biomechanical forces that are generated at this site in performing the types of exercise examined in the present study.

Prediction Equations

Both equations for BMD at the hip had high r^2 values (61% for the femoral neck and 61% for Ward's triangle), indicating that the variables measured in this study appear to have a role in BMD at the hip. Particularly interesting is the contribution that the number of hours of exercise per week makes to the equations at these sites. As mentioned previously, the stresses generated in both field hockey and soccer may primarily act at the hip due to the nature of the games. The BMD data (Table 2b and Figure 2) support this statement. Exercise does not contribute to the equations at the other sites, suggesting that there may be a sport specific effect occurring at the hip. For the entire subject sample, with the exception of the mid fibula, 43 to 62% of the variance in BMD at all sites was accounted for by some combination of the variables of age, height, weight, number of years since menarche, SOS, calcium intake, and number of hours of exercise per week.

While it is difficult to determine what other possible variables could be entered to improve equation strength, it is likely that the genetic component contributes considerably to the unexplained variance in BMD (Smith et al., 1973; Politzer & Anderson, 1989; Pocock et al., 1987; Slemanda et al., 1991). Additionally, variables that were not investigated in the present study, such as muscle strength, and hormonal measures may also strengthen the equations.

These equations will be useful in allowing the estimation of BMD in university-aged females who are deemed at high risk of developing osteoporosis, particularly those

individuals who have a family history of osteoporosis, or those individuals who are suffering with a condition that is known to effect skeletal health. Values for the variables in the equation (age, height, weight, number of years since menarche, calcium intake, SOS and number of hours of exercise per week) require little time and minimal inconvenience to collect both on the part of the individual and on the part of the professional who is asked to perform the analysis. As the cost of a DXA scan for measurement of BMD (and body composition) may be prohibitive in some cases, the use of the equations can provide a simple first hand guide to individuals' skeletal health at minimal expense. A DXA scan can then be recommended in the event that the BMD values calculated from the equations are suspiciously low.

CHAPTER 6

Summary

The primary purpose of this study was to investigate the relationship between BMD and exercise in university-aged females. The results from this study provide some evidence to support hypothesis 1, that regularly active subjects would have higher BMDs than sedentary subjects. The HA subjects had significantly higher BMD at the lumbar spine, whole body, femoral neck, Ward's triangle, distal fibula, distal tibia, tibia/fibula, mid tibia and mid tibia/fibula compared with the SED subjects. The MA subjects also had significantly higher BMD than the SED subjects at the femoral neck, Ward's triangle, distal tibia and distal tibia/fibula, sites which may have been stressed due to the nature of the actions involved in playing soccer. It appears that 4.3 h/wk of physical activity can make a difference in BMD at specific sites, depending on the types of exercise performed.

It was expected that those subjects who exercised regularly would have a higher BMD than sedentary subjects (Robinson et al., 1995; Kirchner et al., 1995; Haapasalo et al., 1994; Heinonen et al., 1993; Wolman et al., 1991). Even though the SED subjects were older chronologically and gynecologically than the HA subjects, they still had lower BMDs at all sites except the mid fibula. Hypothesis 2 stated that the MA subjects would have higher BMDs than the HA subjects. There was no evidence found to support this hypothesis, and in fact, the HA subjects had significantly higher BMD at the femoral neck and Ward's triangle compared with the MA subjects. The HA and MA subjects were matched for age, height, weight, and age of menarche. They consumed similar diets and had similar menstrual patterns. The main difference between these two groups was the weekly amount of exercise. The HA subjects performed an average of 14.5 h/wk while the MA subjects performed only 4.3 h/wk. As exercise has been shown to be an important determinant of BMD it was not surprising that the HA subjects had higher BMDs than the MA subjects at the femoral neck and Ward's triangle when these other variables were controlled for. The lack of difference at the other sites could be due to a slower response of the bone at these sites to mechanical stressors and may be due to the type of

mechanical stress associated with the activities.

The MA subjects had greater BMD than the SED subjects at the hip and distal tibia and distal tibia/fibula. This suggests that 4.3 hours of exercise (soccer) per week is sufficient duration of training to show differences in BMD at these sites. As the hip is a primary fracture site in osteoporotic individuals increasing physical activity levels in sedentary subjects while they are young may be a beneficial method to reduce the incidence of hip fractures in this population when they are older.

It was hypothesized that the active subjects would be leaner than the sedentary subjects. The HA subjects in the present study had a lower SOS and a lower percentage body fat than the MA subjects and the SED subjects, however other studies have found some elite athletes to have much lower percent body fat (11% in runners [Marcus et al., 1985]; 14.7% in runners and 15.6% in gymnasts [Robinson et al., 1995]) than subjects in the present study and also to have a higher incidence of menstrual disturbances. Field hockey and soccer are not aesthetic sports, and in some ways a strong body is considered to be an asset to performance because of the physical nature of the game. Although a high percent body fat is not desirable in any individual, it would seem that the relatively low percent body fat seen in many runners, gymnasts and dancers is not necessary to reach the elite levels in field hockey. The value of 21.8% body fat reported in the HA subjects in the present study indicates that individuals with varying levels of body fat can, and do, succeed to high levels in some sports.

It was hypothesized that there would be no difference in dietary intake between the three subject groups. The HA subjects consumed significantly more grams of carbohydrates than the SED subjects, but there was no difference in caloric, fat, protein or calcium intake between the groups. Part of the reason for this is no doubt related to the large variability in dietary intake and calcium intake among all individuals. But, the mean values for all subject groups were in the ranges reported by Health & Welfare Canada (1995) for good health. However, the SED subjects consumed a significantly lower percentage of calories from carbohydrate and a significantly higher percentage of calories from fat compared to both the HA and MA subject groups. Although it is suggested that

athletes should increase their caloric intake to counteract their increased energy expenditure, the HA subjects did not consume significantly more calories than the two less active subject groups.

Hypothesis 5 was rejected because the HA subjects did not have a higher propensity to menstrual dysfunction than the MA subjects or the SED subjects. There was also no evidence found to support hypothesis 6, which stated that the OC using and eumenorrheic subjects would have higher BMDs than amenorrheic subjects.

Those subjects who had regular menstrual cycles had slightly higher BMDs at the spine, whole body, femoral neck and Ward's triangle than the group with menstrual dysfunctions, but these differences were not statistically significant. Those subjects who were using OCs had higher BMDs at the spine, whole body and femoral neck compared to those subjects who had never used OCs, but these differences did not reach statistical significance at any site.

Reasons proposed for the development of menstrual dysfunction in athletes are broad, ranging from low caloric intake and increased stress, to low body weight, low percent body fat and sudden changes in training patterns in terms of duration, intensity and frequency (Bale, 1994; Arena et al., 1995; Carbon, 1992). The similar diet of the HA subjects to other subject groups and their relatively high percent body fat may explain why they did not have a higher propensity to menstrual dysfunction as reported in other research.

The fact that there were no differences in menstrual history between the three groups, and that the HA subjects didn't have a higher propensity to menstrual disruption may suggest that their present lifestyle does not cause detrimental menstrual disturbances in this group of elite athletes. While both low diet and low percent body fat have been highlighted as factors that could lead to menstrual disturbances, the HA subjects do not appear to be at risk from either of these factors. Their body fat, while lower than the other subject groups is not especially low, and their dietary and calcium intakes are in the normal range for Canadian females of their age. However, it is possible that blood hormone measures could have elucidated problems in the HA group. These were not done

in the present study.

The findings regarding OC use were equivocal. There was wide variability in OC use, and thus, results did not reach statistical significance.

Height, weight and the number of hours of exercise each week were positively correlated with BMD at almost all sites. Age and SOS were significantly negatively correlated with BMD at some sites, suggesting that aging and fatness may contribute to lower BMD. However, in all cases a large portion of the variance remains unexplained, but is likely related to the contribution of genetics to BMD. Weight and SOS played an important role in predicting BMD at all sites of measurement. Depending on the site, other variables which contributed to improved equation strength were height, calcium intake, number of years since menarche and number of hours of exercise per week. The sport specific theory was evident at both hip sites as the number of hours of exercise per week contributed to equation strength in these areas. OC use and age did not increase equation strength. It is important to note that in females of this age group, the environmental effects of calcium intake and exercise do play a role in BMD. As a result, individuals do have a choice in improving their BMD.

From the coach-athlete point of view, these results have a positive note. Although some studies have suggested that elite athletes may have reduced BMD, accompanied by menstrual dysfunction, this may not be the case for all sports. All of the HA subjects reported having regular menses. It is suggested that the training regime of these athletes is not too heavy or intensive as to cause detrimental effects on the bone structure. It would appear that the incidence of fractures within the squad may be largely due to the nature of the game, the competitiveness of the athletes and the training facilities, rather than due to a low bone mass as a result of extensive training.

Directions for the Future

It would be valuable to follow these individuals over the coming years to see how their bone density changes. Activity levels and lifestyle changes could be closely monitored over this time period, so that more definite conclusions could be drawn

regarding bone density. A longitudinal study would allow us to more accurately determine when peak mass is achieved in these subjects, and would give an idea as to whether or not some of these athletes have already reached peak bone mass.

It is important to establish training schedules that, while optimizing performance do not compromise female athlete health. For the field hockey athletes in the present study their demanding training schedule of 14.5 h/wk in addition to their other obligations such as study, employment and social aspects, does not appear to have caused detrimental skeletal effects. Many of the field hockey athletes reported accompanying skill specific training and game play with additional physiological work-outs, involving cardiovascular running and biking, flexibility and weights. Fewer of the soccer players were involved in this additional training, however, more soccer players reported being involved in other types of sport, such as squash, volleyball and basketball.

Details regarding lifetime physical activity, type of OC used and when they were used, and when training was started in relation to menarche would enhance future work in this area.

While it was not convenient in the present study to investigate muscle strength other studies have shown muscle strength of the knee flexors and extensors (Friedlander et al., 1995) and of the trunk flexors (Henderson et al., 1995) to be highly correlated with bone density at certain sites. It would be interesting to investigate muscle strength and it's correlation with BMD, especially hamstrings, quadriceps and hip flexors in the group of athletes studied. It would also be interesting to examine arm strength and radius BMD in field hockey players because of the involvement of carrying and using the stick.

Loucks et al. (1989) found disturbances in reproductive hormones in a group of athletes who had regular menstrual cycles. Without these hormonal assays, all athletes would be classed as regularly menstruating. It is possible that some of the HA subjects in the present study also may have had unidentifiable menstrual disturbances. While impractical during the present study, it would be more accurate in the future if blood work was used to help classify and validate menstrual status.

The results from this study provide another piece of evidence to add to the body

of literature which suggests that females who are sedentary should begin to exercise on a regular basis.

The results of this study also provide valuable additional information on female skeletal health. The sports studied are not the traditional sports that have previously been investigated, and the results of this study will be beneficial to these sports organizations as a whole, and also to the individuals who participate in them. Further investigation will be needed to define the optimal intensity, frequency and duration of training that will lead to maximal BMD levels, without adverse affects.

REFERENCES

Alfredson, H., Nordström, P. & Lorentzon, R. (1996). Total and regional bone mass in female soccer players. Calcified Tissue International, 59, 438-442.

Angus, R.M., Sambrook., P.N., Pocock, N.A. & Eisman, J.A. (1989). A simple method for assessing calcium intake in Caucasian women. Journal of the American Dietetic Association, 89, 209-214.

Arena, B., Maffulli, N., Maffulli, F. & Morleo, M.A. (1995). Reproductive hormones and menstrual changes with exercise in female athletes. Sports Medicine, 19(4), 278-287.

Bale, P. (1994). Body composition and menstrual irregularities in female athletes. Sports Medicine, 17(6), 347-352

Balough, M., Kahn, H. & Medalie, J.H. (1971). Random repeat 24-hour dietary recalls. American Journal of Clinical Nutrition, 21, 304-310.

Barrow, G.W. & Saha, S. (1988). Menstrual irregularity and stress fractures in collegiate female distance runners. The American Journal of Sports Medicine, 16(8), 209-215.

Bhudhikanok, G.S., Wang, M.C., Eckert, K., Matkin, C., Marcus, R. & Bachrach, L.K. (1996). Differences in bone mineral in young Asian and Caucasian Americans may reflect differences in bone size. Journal of bone and Mineral Research, 11(10), 1545-1556.

Block, G. (1982). A review of validations of dietary assessment methods. American Journal of Epidemiology, 115(4), 492-505.

Block, G., Hartman, A.M., Dresser, C.M., Carroll, M.D., Gannon, J. & Gardner, L. (1986). A data-based approach to diet questionnaire design and testing. American Journal of Epidemiology, 124, 453-469.

Bonjour, J.-P., Theintz, G., Buchs, B., Slosman., D & Rizzoli, R. (1991). Critical years and stages of puberty for spinal and femoral bone mass accumulation during adolescence. Journal of Clinical Endocrinology and Metabolism, 73, 555-563.

Bonjour, J.-P., Theintz, G., Law, F., Slosman., D & Rizzoli, R. (1995). Peak bone mass: facts and uncertainties. Archives de Pediatrie, 2(5), 460-468.

Bouchard, C., Shephard, R.J. & Stephens, T. (1992). Physical activity, fitness and health. Champaign, Il: Human Kinetics.

Burke, B.S. & Stuart, H.C. (1938). A method of diet analysis. Journal of Pediatrics, 12, 493-503.

Cassell, C., Benedict, M. & Specker, B. (1996). Bone mineral density in elite 7-to-9-yr-old female gymnasts and swimmers. Medicine and Science in Sports and Exercise, 28 (10), 1243-1246.

Carbon, R.J. (1992). Exercise, amenorrhea and the skeleton. British Medical Bulletin, 48 (3), 546-560.

Cohen, J. (1988). Statistical power analysis for the behavioral sciences, (2nd ed.). Hillsdale, NJ: Erlbaum.

Consensus Development Conference. (1991). Osteoporosis. American Journal of Medicine, 90, 107-110.

Cumming, D.C., Wheeler, G.D. & Harber, V.J. (1994). Physical activity, nutrition and reproduction. Annals of the New York Academy of Sciences, 709, 55-76.

Davis, J.A. & Brewer, J. (1992). Applied physiology of female soccer players. Sports Medicine, 16(3), 180-189.

Drinkwater, B.L., Breumner, B. & Chesnut III, C.H. (1990). Menstrual history as a determinant of current bone density in young athletes. Journal of the American Medical Association, 263, 545-548.

Drinkwater, B.L., Nilson, K., Chesnut III, C.H., Bremner, W.J., Shainholtz, S. & Southworth, M.B. (1984). Bone mineral content of amenorrheic and eumenorrheic athletes. New England Journal of Medicine, 311, 277-281.

Drinkwater, B.L., Nilson, K., Ott, S. & Chesnut III, C.H. (1986). Bone mineral density after the resumption of menses in amenorrheic athletes. Journal of the American Medical Association, 256(3), 380-382.

Durnin, J.V.G.A. & Womersley, J. (1974). Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. British Journal of Nutrition, 32, 22-97.

Fitness and Amateur Sport Canada (1986). Canadian standardized test of fitness: operations manual (3rd ed.). Ottawa: Health and Welfare Canada.

Frisch, R.E. & McArthur, J.W. (1974). Menstrual cycles: fatness as a determinant of minimal weight for height necessary for their maintenance and onset. Science, 185, 949-950.

Frost, H.M., (1987). The mechanostat: A proposed pathogenic mechanism of osteoporosis and the bone mass effects of mechanical and non-mechanical agents. Bone and Mineral, 2, 73-85.

Frusztajer, N.T., Dhuper, S., Warren, M.P., Brooks-Gunn, J. & Fox, R.P. (1990). Nutrition and the incidence of stress fractures in ballet dancers. American Journal of Clinical Nutrition, 51, 779-783.

Fruth, S.J. & Worrell, T.W. (1995). Factors associated with menstrual irregularities and decreased bone mineral density in female athletes. Journal of Orthopaedic and Sports Physical Therapy, 22(1), 26-38.

Galle, P.C., Freeman, E.W., Galle, M.G., Huggins, G.R. & Sondheimer, S.T. (1983). Physiologic and psychologic profiles in a survey of women runners. Fertility and Sterility, 39, 633-639.

Gersovitz, M., Madden, J.P. & Smiciklas-Wright, H. (1978). Validity of the 24-hour dietary recall and seven-day record for group comparisons. Journal of the American Dietetic Association, 73, 48-55.

Gilsanz, V., Gibbens, G.T., Roe, T.F., Carlson, M., Senac, M.O., Boechat, M.I., Huang, H.K., Schulz, E.E., Libanti, C.R & Cann, C.C. (1988). Vertebral bone density in children: effect of puberty. Radiology, 166, 847-850.

Glastre, C., Braillon, P., David, L., Cochat, P., Meunier, P.J. & Delmas P.D. (1990). Measurement of bone mineral density of the lumbar spine by dual energy X-ray absorptiometry in normal children: correlations with growth parameters. Journal of Clinical Endocrinology and Metabolism, 70, 1330-1333.

Goran, M.I., Driscoll, P., Johnson, R., Nagy, T.R. & Hunter, G. (1996). Cross-calibration of body-composition techniques against dual-energy X-ray absorptiometry in young children. American Journal of Clinical Nutrition, 63(3), 299-305.

Gutin, B., Litaker, M., Islam, S., Manos, T., Smith, C. & Treiber, F. (1996). Body-composition measurements in 9-11-y-old children by dual-energy X-ray absorptiometry, skinfold-thickness measurements, and bioimpedance analysis. American Journal of Clinical Nutrition, 63(3), 287-292.

Grimston, S.K., Morrison, K., Harder, J.A. & Hanley, D.A. (1992). Bone mineral density during puberty in Western Canadian children. Bone and Mineral, 19, 85-96.

Haapasalo, H., Kannus, P., Sievänen, H., Heinonen, A., Oja, P. & Vuori, I. (1994). Long-term unilateral loading and bone mineral density and content in female squash players. Calcified Tissue International, 54 (4), 249-255.

Hassager, C., Borg, J. & Christiansen, C. (1989). Measurement of the subcutaneous fat in the distal forearm by single photon absorptiometry. Metabolism, 38, 159-165.

Hassager, C. & Christiansen, C. (1989). Influence of soft tissue body composition on bone mass and metabolism. Bone, 10, 415-419.

Hassager, C. & Christiansen, C. (1995). Measurement of bone mineral density. Calcified Tissue International, 57, 1-5.

Heaney, R.P. (1986). Calcium, bone health and osteoporosis. In: W.A. Peck (Ed.) Bone and Mineral Research/4. New York: Elsevier Science Publishers: pp 255-301.

Heaney, R.P., Recker, R.R. & Saville, P.D. (1978). Menopausal changes in calcium balance performance. Journal of Laboratory Clinical Medicine, 92, 953-963.

Hebert, J.R., Clemow, L., Pbert, L., Ockene, I.S. & Ockene, J.K. (1995). Social desirability bias in dietary self-report may compromise the validity of dietary intake measures. International Journal of Epidemiology, 24(2), 389-398.

Heinonen, A., Oja, P., Kannus, P., Sievänen, H., Märittäri, A. & Vuori, I. (1993). Bone mineral density of female athletes in different sports. Bone and Mineral, 23, 1-14.

Henderson, N.K., Price, R.I., Cole, J.H., Gutteridge, D.H. & Bhagat, C.I. (1995). Bone density in young women is associated with body weight and muscle strength but not dietary intakes. Journal of Bone and Mineral Research, 10 (3), 384-393.

Hetland, M.L., Haarbo, J. & Christiansen, C. (1995). Body composition and serum lipids in female runners: influence of exercise level and menstrual bleeding patterns. European Journal of Clinical Investigations, 25, 553-558.

Jain, M., Howe, G.R. & Rohan, T. (1996). Dietary assessment in epidemiology: comparison of a food frequency and a diet history questionnaire with a 7-day food record. American Journal of Epidemiology, 143(9), 963-960.

Jones, K.P., Ravnikar, V.A., Tulchinsky, D. & Schiff, I. (1985). Comparison of bone density in amenorrheic women due to athletics, weight loss and premature menopause. Obstetrics and Gynecology, 66(1), 5-7.

Joyce, J.M., Warren, D.L., Humphries, L.L., Smith, A.J. & Coon, J.S. (1990). Osteoporosis in women with eating disorders: comparison of physical parameters, exercise and menstrual status with SPD and DPA evaluation. Journal of Nuclear Medicine, 31(3), 5-331.

Kirchner, E.M., Lewis, R.D. & O'Connor, P.J. (1995). Bone mineral density and dietary intake of female college gymnasts. Medicine and Science in Sports and Exercise, 27 (4), 543-549.

LaBarbera, R.A. (1996). The female reproductive system. In: N. Sperelakis & R.O. Banks (Eds.) Essentials of physiology (2nd ed.). New York: Little, Brown & Company: pp 621-637.

Lawson, G.M., Aajducka, C. & McQueen, M.M. (1995). Sports fractures of the distal radius-epidemiology and outcomes. Injury, 26(1), 33-36.

Lee, E.J., Long, K.A., Risser, W.L., Poindexter, H.B.W., Gibbons, W.E. & Goldzieher, J. (1995). Variations in bone status of contralateral and regional sites in young athletic women. Medicine and Science in Sports and Exercise, 27(10), 1354-1361.

Lindsay, R., Cosman, F., Herrington, B. & Himmelstein, S. (1992). Bone mass and body composition in normal women. Journal of Bone and Mineral Research, 7, 55-63.

Lohman, T.G. (1996). Dual energy X-ray absorptiometry. In: A.F. Roche, S.B. Heymsfield & T.G. Lohman (Eds.) Human body composition. Champaign, IL: Human Kinetics: pp 63-78.

Looker, A.C., Wahner, H.W., Dunn, W.L., Calvo, M.S., Harris, T.B., Heyse, S.P., Johnston, C.C. Jr. & Lindsay, R.L. (1995). Proximal femur levels of US adults. Osteoporosis International, 5(5), 389-409.

Loucks, A.B. (1990). Effects of exercise training on the menstrual cycle: existence and mechanisms. Medicine and Science in Sports and Exercise, 22(3), 275-280.

Loucks, A.B. & Heath, E.M. (1994). Dietary restriction reduces luteinizing hormone (LH) pulse frequency during waking hours and increases LH pulse amplitude during sleep in young menstruating women. Journal of Clinical Endocrinology and Metabolism, 78, 910-915.

Loucks, A.B. & Hovarth, S.M. (1985). Athletic amenorrhea: a review. Medicine and Science in Sports and Exercise, 17(1), 56-72.

Loucks, A.B., Mortola, J.F., Girton, L. & Yen, S.S.C. (1989). Alterations in the hypothalamic-pituitary-ovarian and hypothalamic-pituitary-adrenal axes in athletic women. Journal of Clinical Endocrinology and Metabolism, 68, 402-411.

Marcus, R., Cann, C, Madvig, P., Minkof, P., Goddard, M., Bayer, M., Martin, M., Gaudiani, L., Haskell, W. & Genant, H. (1985). Menstrual function and bone mass in elite women distance runners. Annals of internal medicine, 102, 158-163.

Marieb, E.N. (1989). Human anatomy and physiology. California: The Benjamin/Cummings Publishing Company, Inc.

McKay, H.A., Bailey, D.A., Wilkinson, A.A. & Houston, C.S. (1993). Familial comparison of bone mineral density at the proximal femur and lumbar spine. Bone and Mineral, 24, 95-107.

Melton, L.J., Chrischilles, E.A., Cooper, G., Lane, A.W. & Riggs, B.L. (1992). Perspective: How many women have Osteoporosis? Journal of Bone and Mineral Research, 7, 1005-1010.

Minister of Supply and Services. (1990). Nutrition Recommendations. The Report of the Scientific Review Committee. Ottawa: Health and Welfare Canada.

Musgrave, K.O., Giambalvo, L., Leclerc, H.L., Cook, R.A. & Rosen, C.J. (1989). Validation of a quantitative food frequency questionnaire for rapid assessment of dietary calcium intake. Journal of the American Dietetic Association, 89, 1484-1488.

Myburgh, K.H., Bachrach, L.K., Lewis, B, Kent, K. & Marcus, R. (1993).Bone mineral density at the axial and appendicular sites in amenorrheic athletes. Medicine and Science in Sports and Exercise, 25, 1197-1202.

Myerson, M., Gutin, B., Warren, M.P., May, M.T., Contento, I., Lee, M., Pi-Sunyer, F.X., Pierson, R.N., Jr, & Brooks-Gunn, J. (1991). Resting metabolic rate and energy balance in amenorrheic and eumenorrheic runners. Medicine and Science in Sports and Exercise, 23(1), 17-22.

Nattiv, A., Agostini, R., Drinkwater, B. & Yeager, K. (1994). The female athlete triad. Clinics in Sports Medicine, 13(2), 405-418.

Nelson, M.E., Fisher, E.C., Catsos, P.D., Meredith, C.N., Turksoy, R.N. & Evans, W.J. (1986). Diet and bone status in amenorrheic runners. American Journal of Clinical Nutrition, 43, 910-916.

Nelson, M.E., Fisher, E.C., Dalmanian, F.E., Dallal, G.E. & Evans, W.J. (1991). A 1-y walking program and increased dietary calcium in postmenopausal women: effects on bone. American Journal of Clinical Nutrition, 53, 1304-1311.

Ogle, G.D., Allen, J.R., Humphries, I.R., Lu, P.W., Briody, J.N., Morley, K., Howman-Giles, R. & Cowell, C.T. (1995). American Journal of Clinical Nutrition, 61(4), 746-753.

Ott, S.M. (1991). Bone mineral density in adolescents. New England Journal of Medicine, 325, 1646-1647.

Pocock, N.A., Eisman, J.A., Hopper, J.L., Yeates, M.G., Sambrook, P.N. & Ebert, S. (1987). Genetic determinants of bone mass in adults. Journal of Clinical Investigations, 80, 706-710.

Prior, J.C., Vigna, Y.M., Schlechter, M.T. & Burgess, A.E. (1990). Spinal bone loss and ovulatory disturbances. New England Journal of Medicine, 323, 1221-1227.

Proesmans, W., Goos, G., Emma, F., Geusens, P., Nijs, J. & Dequeker, J. (1994). Total bone mineral mass measured with dual photon absorptiometry in healthy children. European Journal of Pediatrics, 153, 807-812.

Pruitt, L.A., Jackson, R.D., Baitels, R.L. & Lehnhard, H.J. (1992). Weight training effects on bone mineral density in early postmenopausal women. Journal of Bone and Mineral Research, 7(2), 179-185.

Ready, A.E., Naimark, B., Ducas, J., Sawatzky, J.V., Boreskie, S.L., Drinkwater, D.T. & Oosterveen, S. (1996). Influence on walking volume on health benefits in women post-menopause. Medicine and Science in Sports and Exercise, 28 (9), 1097-1105.

Recker, R.R., Davies, K.M., Hinders, S.M., Heaney, R.P., Stegman, M.R. & Kimmel, D.B. (1992). Bone gain in young adult women. Journal of the American Medical Association, 268, 2403-2408.

Reid, I.R., Plank, L.D. & Evans, M.C. (1992). Fat mass is an important determinant of whole body bone density in premenopausal women but not in men. Journal of Clinical Endocrinology and Metabolism, 75, 779-782.

Rencken, M.L., Chesnut III, C.H. & Drinkwater, B.L. (1996). Bone density at multiple skeletal sites in amenorrheic athletes. Journal of the American Medical Association, 276 (3), 238-240.

Riggs, B.L., Melton, L.J. III. (1986). Involutional osteoporosis. New England Journal of Medicine, 314, 1676-1686.

Rigotti, N.A., Nussbaum, S.R., Herzog, D.B. & Neer, R.M. (1984). Osteoporosis in women with anorexia nervosa. New England Journal of Medicine, 311, 1601-1606.

Riis, B., Thomsen, K. & Christiansen, C. (1987). Does calcium supplementation prevent postmenopausal bone loss? New England Journal of Medicine, 316(4), 173-177.

Robinson, T.L., Snow-Harter, C., Taaffe, D.R., Gillis, D., Shaw, J. & Marcus, R. (1995). Gymnasts exhibit higher bone mass than runners despite similar prevalence of amenorrhea. Journal of Bone and Mineral Research, 10 (1), 26-35.

Rubin, C.T. & Lanyon, L.E. (1984). Regulation of bone formation by applied dynamic loads. Journal of Bone and Joint Surgery [America], 66, 397-402.

Rubin, K., Schirduan, V., Gendreau, P., Sarfarazi, M., Mendola, R. & Dalsky, G. (1993). Predictors of axial and peripheral bone mineral density in healthy children and adolescents, with special attention to the role of puberty. Journal of Pediatrics, 123, 863-870.

Ruiz, J.C., Mandel, C. & Garabedian, M. (1995). Influence of spontaneous calcium intake and physical exercise on the vertebral and femoral bone mineral density of children and adolescents. Journal of Bone and Mineral Research, 10(5), 675-681.

Sallis, R.E. & Jones, K. (1991). Stress fractures in athletes. Postgraduate Medicine, 89(6), 185-192.

Seeman, E., Hopper, J.L., Bach, L.A., Cooper, M.E., Parkinson, E., McKay, J. & Jerums, G. (1989). Reduced bone mass in daughters of women with osteoporosis. New England Journal of Medicine, 320, 554-558.

Seeman, E., Szmukler, G.I., Formica, C., Tsalamandris, C. & Mestrovic, R. (1992). Osteoporosis in anorexia nervosa: The influence of peak bone density, bone loss, oral contraceptive use, and exercise. Journal of Bone and Mineral Research, 7(12), 1467-1474.

Sharpe, C.W. & Freeman, C.P.L. (1993). The medical complications of anorexia nervosa. British Journal of Psychiatry, 162, 452-462.

Siri, W.E. (1961). Body composition from fluid space and density: analysis of methods. In: J. Brozek & A. Henshel (Eds.). Techniques for measuring body composition. Washington, D.C.: National Academy of Sciences. pp223-244.

Slemanda, C.W., Christian, J.C., Williams, C.J., Norton, J.A., Johnston, Jr, C.C. (1991). Genetic determinants of bone mass in adult women: a reevaluation of the twin model and the potential importance of gene interaction on heritability estimates. Journal of Bone and Mineral Research, 6(6), 561-567.

Slosman, D.O., Rizzoli, R. & Bonjour, J-P. (1995). Bone absorptiometry: a critical appraisal of various methods. Acta Paediatrica, (Suppl.. 411), 9-11.

Smith, D.M., Nance, W.E., Kang, K.W., Christian, J.C. & Johnston, Jr, C.C. (1973). Genetic factors in determining bone mass. The Journal of Clinical Investigation, 52, 2800-2808.

Snow-Harter, C., Bouxsein, M.L., Lewis, B.T., Carter, D.R. & Marcus, R. (1992). Effects of resistance and endurance exercise on bone mineral status of young women: a randomized exercise intervention trial. Journal of Bone and Mineral Research, 7(7), 761-769.

Snow-Harter, C. & Marcus, R. (1991). Exercise, bone mineral density and osteoporosis. In: J.O. Holloszy (Ed.). Exercise and sport sciences reviews. Baltimore: Williams and Wilkins: pp 351-388.

Taaffe, D.R., Snow-Harter, C., Connolly, D.A., Robinson, T.L., Brown, M.D. & Marcus, R. (1995). Differential effects of swimming versus weight bearing activity on bone mineral status of eumenorrheic athletes. Journal of Bone and Mineral Research, 10 (4), 586-593.

Taaffe, D.R., Villa, M.L., Delay, R. & Marcus, R. (1995). Maximal muscle strength of elderly women is not influenced by oestrogen status. Age and Ageing, 24, 329-333.

Tanner, J.M. (1962). Growth at adolescence, (2nd ed.). Oxford: Blackwell.

Theintz, G., Buchs, B., Rizzoli, R., Slosman, D., Clavien, H., Sizoninko, P.C. & Bonjour, J.P. (1992). Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. Journal of Clinical Endocrinology and Metabolism, 75, 1060-1065.

Thompson, F.E. & Byers, T. (1994). Dietary assessment resource manual. Journal of Nutrition, 124, 2245S-2317S.

Tsuji, S., Tsunoda, S., Yata, H., Katsukawa, F., Orushi, S. & Yamazaki, H. (1995). Relation between grip strength and radial bone mineral density in young athletes. Archives of Physical Medicine and Rehabilitation, 76, 234-238.

Turner, R.T., Riggs, B.L. & Spelsberg, T.C. (1994). Skeletal effects of estrogen. Endocrine Reviews, 15(3), 275-300.

Wahner, H.W. & Fogelman, I. (1994). The evaluation of osteoporosis: dual-energy X-ray absorptiometry in clinical practice. In: I. Fogelman (Ed.) Metabolic bone disease. Cambridge: Martin Dunitz Ltd.: pp 141-148.

Ward, J.A., Lord, S.R., Williams, P., Anstey. K. & Zivanovic, E. (1995). Physiologic, health and lifestyle factors associated with femoral neck bone density in older women. Bone, 16 (Suppl.. 4), 373S-378S.

Warren, M.P. (1980). The effects of exercise on pubertal progression and reproductive function in girls. Journal of Clinical Endocrinology and Metabolism, 51, 1150-1157.

Welton, D.C., Kemper, H.C.G., Post, G.B., Van Mechelen, W., Twisk, J., Lips, P. & Teule, G.J. (1994). Weight-bearing activity during youth is a more important factor for peak bone mass than calcium intake. Journal of Bone and Mineral Research, 9(7), 1089-1096.

Wolff, J. (1986). The law of bone remodelling. Translated by P. Maquet and R Furlong. Berlin: Springer-Verlag.

Wolman, R.L., Clark, P., McNally, E., Harries, M.G. & Reeve, S. (1992). Dietary calcium as a statistical determinant of spinal bone density in amenorrheic and oestrogen-replete athletes. Bone and Mineral, 17, 415-423.

Wolman, R.L., Faulmann, L., Clark, P., Hesp, R & Harries, M.C. (1991). Different training patterns and bone mineral density in the femoral shaft in elite female athletes. Annals of Rheumatic Diseases, 50, 487-489.

Yeager, K.K., Agostini, R., Nattiv, A. & Drinkwater, B. (1993). The female athlete triad: disordered eating, amenorrhea and osteoporosis. Medicine and Science in Sports and Exercise, 25(7), 775-779.

Young, L.R. & Nestle, M. (1995). Portion sizes in dietary assessment: issues and policy implications. Nutrition Reviews, 53(6), 149-158.

Zohman, G.L. & Lieberman, J.R. (1995). Perioperative aspects of hip fracture. Guidelines for interventions that will impact prevalence and outcome. American Journal of Orthopedics, 24(9), 666-671.

APPENDIX A1

LITERATURE SUMMARY TABLE

EFFECTS OF EXERCISE ON BONE MINERAL DENSITY

TABLE A1
Comparison of studies to measure BMD and Exercise.

AUTHOR	N	AGE (years)	TECHNIQUE	SITE	VALUE (g/cm^2 unless otherwise stated)	COMMENTS
Rigotti et al. (1986)	18 AN patients 28 controls	25±5 27±5	SPA	distal radius	0.64 0.72	The active AN patients (>3 x week) had a greater bone density than the less active AN (<3 x week) (P<0.001) by self report.
Joyce et al. (1990)	33 eating disorder patients unknown # of controls	20-53 20-29	SPA DPA	distal radius femoral neck trochanter Wards triangle	data not reported dat not reported data not reported data not reported	The eating disorder group had evidence of decreased BMD. In the AN subgroup, exercise was related to BMD. Moderate exercise (1-6 hr/wk) had a protective effect and strenuous exercise (>6 hr/wk) being detrimental.
Nelson et al. (1991)	18 active 18 inactive (postmenopausal women)	60.2±1.1	SPA QCT γ neutron activation DPA	distal radius L1-L3 total body L2-L4 femoral neck	(active, inactive) 0.637, 0.612 101.1, 74.1 (g/cm^3) 20.5, 21.0 (mol) 1.170, 1.082 0.827, 1.082	Half of the women in the study participated in a 1-yr walking program. There was an increase of 0.5% in the active women and a decrease of 7% in the sedentary women as measured by QCT. Exercise may preferentially alter BMD at different sites.

Wolman et al. (1991)	21 runners 36 rowers 16 dancers 15 controls	26.0 24.7 22.8 28.4	DPA	femoral midshaft	(runners, rowers, dancers, controls) 1.51, 1.43, 1.39, 1.40	Runners had the greatest BMD. BMD was not related to estrogen status.
Snow-Harter et al. (1992)	12 weight training 10 running 8 controls	19.9	DXA	L2-L4 femoral neck trochanter Wards triangle	(weight training, running, controls) 1.14, 1.13, 1.08 0.97, 0.94, 0.93 0.81, 0.75, 0.75 0.88, 0.83, 0.80	8 month training program, lead to increases in BMD ($p<0.05$) in both the runners and the weight trainers. There was no change in the BMD of control subjects, and no changes at the femur.
Heinonen et al. (1993)	30 orienteers 28 cross country skiers 29 cyclists 18 weightlifters 25 controls	23.3±3.1 21.3±3.2 24.0±5.7 24.6±4.6 22.6±2.8	DXA	L2-L4 femoral neck proximal tibia distal femur, patella calcaneus, distal radius	(orienteers, skiers, cyclists, weightlifters, controls) 1.068, 1.072, 1.067, 1.230, 1.071 1.000, 1.035, 0.963, 1.082, 0.983 1.151, 1.139, 1.094, 1.234, 1.104	Weightlifters had the greatest BMD at all sites. The difference in BMD was explained by the difference in loading between the sports.
Haapasalo et al. (1994)	19 squash players 19 controls	25.4±4.4 25.4±3.9	DXA	proximal humerus humeral shaft radial shaft ulnar shaft distal radius distal ulna	(squash players, controls) 0.907, 0.833 1.145, 1.042 0.634, 0.630 0.590, 0.582 0.428, 0.388 0.338, 0.312	Players had higher ($P<0.001$) BMD in the playing arm compared to the other arm. The difference between dominant and non-dominant arm was greater in players than in controls ($P<0.01$-$P<0.001$)

Kirchner, Lewis & O'Connor (1995)	26 gymnasts 26 controls	19.7±0.2 20.0±0.2	DXA	L1-L4 proximal femur femoral neck Wards triangle	(gymnasts, controls) 1.202, 0.979 1.147, 0.901 1.091, 0.845 1.043. 0.766	Gymnasts had significantly higher (P<0.0001) BMD at all sites, despite inadequate calcium intake and a higher propensity to interruption of menses.
Robinson et al. (1995)	21 gymnasts 20 runners 19 controls	19.5±1.6 22.0±2.6 19.2±1.6	DXA	L2-L4 proximal femur whole body	(gymnasts, runners, controls) 1.172, 0.986, 1.111 1.086, 0.882, 0.974 1.110, 1.043, 1.092	Gymnasts had higher BMD despite a greater prevalence of menstrual dysfunction.
Taaffe et al. (1995)	13 gymnasts 26 swimmers 19 controls	19.3±1.2 19.2±2.1 19.2±1.6	DXA	femoral neck trochanter	(gymnasts, swimmers, controls) 1.117, 0.875, 0.974 0.898, 0.748, 0.784	There was no difference between the groups at the lumbar spine L2-L4 and whole body BMD. The difference in BMD was explained by the lack of loading involved in swimming.
Tsuji et al. (1995)	16 basketball 12 tennis	19.9±1.9 21.1±1.0	DXA	distal radius mid-radius	(basketball, tennis) 0.462, 0.481 0.764, 0.705	Found that radial BMD at both sites was strongly correlated to grip strength.

Lee et al. (1995)	7 basketball 11 volleyball 7 swimmers 9 soccer 17 mod controls 11 sed controls	19.5±1.8	DXA	total body lumbar spine (L2-L4) femoral neck trochanter Ward's triangle	(volleyball, basketball, soccer, swimmers, mod, sed) 1.23, 1.24, 1.20, 1.13, 1.13, 1.15 1.38, 1.33, 1.24, 1.20, 1.18, 1.17 1.17, 1.26, 1.16, 0.99, 1.01, 1.05 0.97, 1.03, 0.96, 0.83, 0.79, 0.83 1.08, 1.23, 1.16, 1.00, 1.17, 1.15	VB, BB had higher BMD at total body & spine than SW, MOD & SED. BB higher BMD at Ward's, femoral neck & trochanter than SW, MOD & SED. The findings show site-specific differences in BMD associated with selected sports' programs.
Alfredson et al. (1996)	16 soccer 13 controls	20.9±2.2 25.0±2.4	DXA	total body lumbar spine (L1-L4) femoral neck trochanter Ward's triangle	(soccer, control) 1.21, 1.14 1.35, 1.22 1.16, 1.02 0.98, 0.87 1.16, 0.97	Soccer had significantly higher BMD at the lumbar spine, femoral neck, Ward's triangle, nondominant femur & humerus, distal femur, proximal tibia. Muscle strength in the thigh is not related to bone mass in the soccer players.

APPENDIX A2

LITERATURE SUMMARY TABLE

EFFECTS OF MENSTRUAL STATUS ON BONE MINERAL DENSITY

TABLE A2
Comparison of Studies Measuring the Effects of Amenorrhea on Bone Mineral Density.

AUTHORS	N	AGE (years)	DEFINITION OF AMENORRHEA	AMENORRHEA (months)	BMD (g/cm^2, site)	COMMENTS
Jones et al. (1985)	8 athletic amenorrhea 20 amenorrhea due to weight loss 11 premature menopause 25 eumenorrheic	26.5±5.0 24.3±3.7 32.5±8.3 28.9±4.3	All subjects have an absence of menses for at least 12 months.	33±23 43±22 83±61 0	0.738 0.672 0.616 0.726 Distal radius by SPA	There was no significant difference between he athletes and the controls, but the women with amenorrhea due to premature menopause and weight loss had significantly less BMD than controls ($P<0.001$, $P<0.05$, respectively). There was a positive correlation between number of months of amenorrhea and a decreased BMD ($r=0.506$, $P<0.001$).
Drinkwater et al. (1984)	14 amenorrheic 14 eumenorrheic	24.9±1.3 25.5±1.4	One or less menses per year.	41.7±7.4 0	1.12 1.30 Vertebral BMD by DPA	Amenorrheic athletes had significantly less BMD than eumenorrheic. Only difference between groups was in mile run per week, (41.8 vs. 24.9 for amenorrheic and eumenorrheic respectively).

Drinkwater et al. (1986)	7 regained menses 7 eumenorrheic 2 remained amenorrheic	27.9±2.0 28.3±2.4 Data not reported	One or less menses per year.	40.4 0 data not reported	Data not reported	Over 15.5 months the regained menses group showed a 6.3% increase in vertebral BMD, the eumenorrheic group had a 0.3% decrease and the remained amenorrheic group had a 3.4%decrease. The authors concluded that the resumption of menses was a primary factor for the significant increase in BMD in the former amenorrheic athletes.
Nelson et al. (1986)	11 amenorrheic 17 eumenorrheic	25.2±1.47 29.2±1.23	No menses in the last 12 months.	24.9±3.8	1.099 1.196 Vertebral BMD by DPA	Eumenorrheic BMD was significantly higher than amenorrheic ($p<0.02$). BMD was positively correlated with estradiol levels ($r=0.50$, $p=0.008$).
Marcus et al. (1985)	11 amenorrheic 6 eumenorrheic	20.0±0.4 23.8±0.7	No menses in the last 12 months.	54±7.2 0	151 mg/cm^3 182 mg/cm^3 L1-L2 by QCT	Eumenorrheic BMD was significantly higher than amenorrheic ($P<0.02$). The authors stated that intense exercise may reduce the impact of amenorrhea on bone mass.
Wolman et al. (1991)	25 amenorrheic athletes 27 eumenorrheic athletes 15 OC using athletes 13 eumenorrheic controls	20.7-27.5 28.4	One or less menses in the past 6 months.	Data not reported.	1.45 1.45 1.46 1.40 Mid-femur by DPA	Found no significant difference in BMD based on menstrual status. However no indication of length of amenorrhea is given in the paper.

Seeman et al. (1992)	12 primary amenorrhea 37 secondary amenorrhea 16 OC users 52 eumenorrheic controls	20.1±1.2 24.4±1.4 27.6±1.9 27.8±0.9	Data not reported	Data not reported	DXA from spine, femoral neck, Wards triangle, trochanter). 0.88, 0.80, 0.78, 0.70 (primary amenorrhea) 1.06, 0.92, 0.86, 0.71 (secondary amenorrhea) 1.14, 0.94, 0.86, 0.71 (OC users) 1.27, 1.02, 0.99, 0.86 (controls).	BMD was highly correlated with oral contraceptive use (r=0.63-r=0.66), and negatively correlated with amenorrhea (r=-0.53 to r=-0.49).

APPENDIX A3

LITERATURE SUMMARY TABLE

INFLUENCE OF CALCIUM INTAKE ON BONE MINERAL DENSITY

TABLE 3
Comparison of studies that have measured calcium.

AUTHOR	N	AGE (years)	METHOD	CALCIUM (mg/day)	COMMENTS
Welton et al. (1994)	84 male 98 female	27.1±0.79 27.1±0.75	FFQ (measured calcium at 13-17, 13-21 & 13-27 y)	1435 1204	Calcium intake was not a significant predictor of BMD in the periods, 13-17, 13-21 &13-27 for both sexes.
Wolman et al. (1992)	25 amenorrheic 27 eumenorrheic 15 OC users	24.2 25.1 25.2	Questionnaire	778 686 728	There was a linear relationship between calcium and trabecular spine BMD in all three groups. At all levels of calcium, BMD was lower in the amenorrheic athletes. For every 100 mg increase in calcium, BMD increased by 3.9 mg/cm^3.
Kristinssen et al. (1994)	80 females 82 females	13 15	FFQ based on dairy products	1293±452 1082±382	Calcium was significantly correlated with BMD in the older group after adjustment for menarcheal age and weight (r=0.24, P<0.05).
Kirchner et al. (1995)	26 gymnasts 26 controls	19.7±0.2 20.0±0.2	FFQ FFQ modified for calcium (age 13-18 years)	683±58, 752±63 747±132, 615±76	There was a negative correlation (r=-0.40, P<0.005) between current calcium and lumbar BMD in the gymnasts. Explained by excess activity of gymnasts.
Ruiz et al. (1995)	151 male & female	7-15.3	Semiquantitative FFQ	810 (range 157-2033)	51-71% of subjects had calcium intakes below the RDA of 10000 mg/d. Calcium was an independent determinant of BMD at the vertebral site (P=0.02) especially before puberty.

Angus et al. (1988)	88 pre-menopausal 71 post-menopausal	23-75	4-d record (with digital weigh scale) Semiquantitative FFQ (combined)	738±32 698±37	65% of the premenopausal women and 83% of the postmenopausal women failed to meet the Australian RDA for calcium. Postmenopausal women who consumed 600 ml of milk per day up to age 20 had significantly higher mean forearm BMD than those who consumed less than 300 ml of milk per day (P<0.05). No significant correlation between current calcium intake and BMD for both groups.
Nieves et al. (1995)	139	30-39	FFQ (94 questions) for present and ages 13-17 7-d record	1159±520 1608±769	All subjects met the RDA for current calcium. This was moderately related to femoral neck and trochanteric BMD(ß=0.08, P=0.074). A higher lifetime calcium was associated with higher hip BMD.
Drinkwater et al. (1984)	14 amenorrheic 14 eumenorrheic	24.9±1.3 25.5±1.4	3-d record	960±98 1100±153	The 3-d record showed no significant difference between the two groups.
Nelson et al. (1986)	11 amenorrheic 17 eumenorrheic	25.2±1.47 29.2±1.23	3-d record	886±312 1150±117	No significant difference between the two groups. 55% of the amenorrheic and 35% of the eumenorrheic athletes consumed less then the US RDA for calcium. Abnormal eating behaviours may be associated with amenorrhea.
Robinson et al. (1995)	21 gymnasts 20 runners 19 controls	19.5±1.0 22.0±2.6 19.2±1.6	4-d record	1041±271 923±347 816±293	There was no significant difference between the three groups. Lumbar BMD was correlated (r=0.46, P=0.05) with calcium intake in the runners. There were no correlations for gymnasts and controls.
Nelson et al. (1991)	18 high calcium 18 low calcium	60.2	7-d record	1462±53 761±51	After one year of high calcium there was an increase of 2.0% in femoral neck BMD. The low calcium group displayed a decrease of 1.1% after a year (P=0.001).

Kanders et al. (1988)	60 women	25-34	24-h recall with food models 6-d record (combined for 7-d)	871±41	Women who consumed more than 800 mg/d had significantly greater vertebral and radial BMD than those who consumed less than 800 mg/d ($r=0.36$, $p<0.02$) when exercise has been removed from the equation.
Frusztajer et al. (1990)	10 stress fractures 10 no fractures 10 controls	20.5±3.9	2-d dietary history (2 24-h recalls) Semiquantitative FFQ	data not reported data not reported	The stress fractures group showed a significant ($P<0.05$) greater tendency to restrict food intake, and a greater avoidance of high-fat dairy foods.

APPENDIX B

SUBJECT CONSENT FORM

UNIVERSITY OF ALBERTA
Department of Physical Education and Recreation

SUBJECT CONSENT
Bone Mineral Density and Physical Activity in Females

INVESTIGATORS
Rebecca Sunderland & Dr. Dru Marshall

I _____ (Subject's name) am giving my consent to participate in this research study. In so doing, I understand fully all the following statements:

1. The information to be collected includes: age, date of birth, height, weight, skinfolds (tricep, bicep, subscapular, iliac crest and calf), a menstrual history questionnaire, an x-ray of 3 sites (shin, hip and lower back), a training history questionnaire, a 3-day dietary record and a calcium food frequency questionnaire. The time required for my participation in the study is about 1.5 hours (excluding the dietary record).

2. Immediate benefits that I will realize include: an increased awareness regarding osteoporosis, nutritional habits and knowledge of current body composition.

3. I agree that I am voluntarily participating in the study as it is described. I understand that I have the right to withdraw from the study without any penalty at any time. I understand that there is no financial remuneration for participating in this study.

4. I have been informed of the possible benefits of my participation in this research project and understand that there is virtually no risk or danger associated with my participation.

5. I expect to have my confidentiality fully protected during the time of my participation in this project, in the future and in any published results.

6. I understand that should I have any questions related to any part of my participation in this project, my questions will be answered fully and to my total satisfaction.

7. I understand that I will be given a copy of this consent form after I have signed it.

8. I hereby make available to Rebecca Sunderland and Dr. Dru Marshall all results obtained as a consequence of my participation in this project, whether these results are in individual or group form.

9. I further certify that all procedures in which I will be involved have been fully explained to me. I hereby declare that I am totally satisfied with these explanations.

_____	_____
Subject's name (print)	Subject's signature
_____	_____
Witness name (print)	Witness signature
_____	_____
Investigator's name (print)	Investigator's signature

PLEASE CONTACT REBECCA SUNDERLAND (403) 434-1513 OR DR. DRU MARSHALL (403) 492-1035 IF YOU HAVE ANY QUESTIONS ABOUT THE STUDY

APPENDIX C

PRELIMINARY SCREENING QUESTIONNAIRE

PRELIMINARY SCREENING QUESTIONNAIRE

Name: ____________________

Telephone #: ____________

ID #: ____________

✂ -

ID #:

AGE: ____________ Years ____________ Months

HEIGHT: ____________ cm

WEIGHT: ____________ Kg

How old were you when you had your first period? If possible, please give months and years.

____________ Years. ____________ Months.

Have you ever smoked? YES / NO

Do you currently smoke? YES / NO

Have you ever been told by a doctor/physician that you suffer from any condition that affects your skeleton/bones? YES / NO

Have you ever used oral contraceptives? YES / NO

Are you currently using oral contraceptives? YES / NO

If you answered YES to either of the previous two questions, please give the length of time that you have been using oral contraceptives for:

____________ Years and ____________ Months.

Thank you.

APPENDIX D

MENSTRUAL HISTORY QUESTIONNAIRE

Menstrual Cycle Information

ID# _____
Date _____
Date of Birth _____ Age _____

Please try to answer the questions below as accurately as you can. Please answer all the questions.

1. At what age did you have your first menstrual period? State your age in years and months. For example, 12 years, 3 months (if months are unknown, please state years only).
   YEAR _____ MONTHS _____

2. Is your current cycle regular? (ie., about every 25-35 days?)
   YES _____ No _____

3. Date of day 1(onset of flow) of your last menstrual period _____

4. Has your cycle ever been amenorrheic? (Less than two periods a year or no period in the last six months).
   YES _____ NO _____

   If YES, please give the duration of amenorrhea (in months or years). _____

5. Other than during the few months following your first period (when your cycle was getting established) has your cycle ever been irregular (3-6 periods a year at intervals greater than 36 days)?
   YES _____ NO _____

6. Please indicate below which pattern best describes your menstrual cycle occurrence throughout your life since the onset of your first period.
   Always regular _____
   Currently regular with intervals of irregular in the past _____
   Currently regular with intervals of amenorrhea in the past _____
   Currently irregular with intervals of regularity/irregularity in the past _____
   Never regular _____

7. Does the pattern that you selected in the previous question repeat itself? For example, are you regular during some months of the year and irregular during others?
   YES _____ NO _____

8. Can you identify events that appear to influence your menstrual cycle pattern? List the event and state how it alters the pattern.

EVENT	CHANGE IN PATTERN	CHANGE IN FLOW
eg. Hard training in summer	irregular	lighter

9. How many periods do you usually have in a year? _____

10. What is the interval of days between your periods? Indicate the number of days between day 1 (onset of flow) of a period and day 1 of the subsequent period

_____

11. On average, how many days does your period last? _____

12. Have you experienced a significant weight loss or gain in the last 12 months?
YES NO
(Specify weight loss or gain, and time involved)_____
_____

13. Have you ever used oral contraceptive pills?
YES _____ Are you currently using them? _____
How old were you when you first started to use them? _____
How long (in months or years) did you or have you been taking the pill for? _____
NO _____

14. Is there a history of osteoporosis in your family?
YES _____ NO _____ DON'T KNOW _____

If YES, could you give the relationship of the person to you (ie grandmother) and any other information that you know about (ie fractures, age of person etc).

PERSON	OTHER DETAILS
_____	_____
_____	_____
_____	_____

APPENDIX E
TRAINING HISTORY QUESTIONNAIRE

TRAINING HISTORY QUESTIONNAIRE

Date: _____

ID# _____
Date of Birth: _____
Age: _____

1) How many hours of exercise do you perform each week? Please give the AVERAGE number of hours per week.

2) Write down the activity that you spend the greatest amount of time participating in. (eg. Field hockey, soccer, walking etc.). Please give one activity only.

The remaining questions refer to the activity that you have just written down in question two.

3) How many hours per week do you spend participating in this activity? (If relevant. please include the number of hours spent training and playing for this activity).

4) How many months or years (excluding time off for vacation/injury/illness) have you maintained this number of hours of physical activity per week?

5) Do you spend any additional time participating in activities (such as weight training or aerobic training) in order to **benefit your performance in the activity indicated in question two**?

Please specify, eg. Weights - 2 hours per week.

ACTIVITY	TIME (# hours/week)
_____	_____
_____	_____
_____	_____
_____	_____

6) Have you ever had any illness, injury or condition that has prevented you from continuing this level of activity?

YES NO

If YES, please list the injury, the date it happened/was diagnosed, and how long it prevented you from participating in physical activity.

INJURY (# WEEKS)	DATE	ABSENCE FROM ACTIVITY

7) Do you participate in any other physical activity in addition to the activity mentioned in question two? (For example, exercise classes, intramural sports).

YES NO

If YES, please specify the activity and the number of hours spent participating in it every week, eg. Basketball - 1 hour/week.

ACTIVITY	TIME (# hours/week)

APPENDIX F

CALCIUM FOOD FREQUENCY QUESTIONNAIRE

INSTRUCTIONS FOR THE FREQUENCY QUESTIONNAIRE FOR CALCIUM INTAKE

You will be recording your intake of a list of foods and drinks on the form provided. It is important that you record all food items as accurately as possible.

You are asked to record foods eaten either daily or weekly. Please read each food carefully so you are aware whether you must record by the day or by the week. The first five questions require a daily amount. The rest require a weekly amount.

Please answer the questions according to an AVERAGE daily or an AVERAGE weekly consumption.

Most of the questions give a specific portion size (amount) of food/drink. Please try to answer the questions according to the portion size listed. For example, if you drink 1 litre of milk per day, put a number 1 in the amount eaten box. If you drink 3/4 pint of milk a day, put a 3 in the 1/4 pint column. If you eat 3 scoops of ice cream per week, write 1.5 in the amount eaten column.

What if I regularly eat or drink a different portion size to that which is given on the form?
If this is the case, please write the portion size legibly in the food column and then write the number of portions per day/week in the amount eaten column. For example, if you drink a 330 ml bottle of orange juice three times a week, write 330 ml next to 'orange juice' and a '3' in the amount eaten column.

And finally, if you eat vitamin tablets, please list the brand and if possible the amount of calcium per pill.

The questionnaire will take you about ten minutes to complete.
Thank you for your time.

FOOD FREQUENCY QUESTIONNAIRE FOR CALCIUM	ID#	Date:
Please record your intake of the following foods.		
	Amount	Ca (mg)
	Eaten	day week
How much of the following foods do you usually eat EACH DAY?		
MILK		P
What type of milk do you usually drink? (please circle your answer).		L
		E
Whole : 1% : 2% : Skim.		A
		S
How many cups of tea/coffee with milk do you drink each day?		E
How much milk do you usually add to breakfast cereal? (1/2 cup=75ml).		D
		O
How much milk in total do you usually use each day? (tick your answer).		
1.5 pints (1litre)		N
1.0 pints (600 ml)		O
1/2 pint (300 ml)		T
1/4 pint (150 ml)		
less than 150 ml		W
		R
BREAD		I
How many slices of bread do you usually eat each day? (1 slice=25g).		T
White bread		E
Wholemeal bread		
		I
How many bagels do you usually eat each day?		N
Plain		
wholemeal		T
Other		H
		I
How much of the following foods do you usually eat EACH WEEK?		S
YOGURT		C
Natural (1 small carton = 200ml)		O
Fruit (1 small carton = 200 ml)		L
		U
CHEESE		M
Hard/Tasty Cheeses		N
1 thin slice or 1" cube (30g)		
4 thin slices (1/4 lb) (120g)		
8 thin slices (1/2 lb) (240g)		
16 thin slices (1 lb) (480g)		

How much of the following foods do you usually eat EACH WEEK?		
Soft/Cream/Cottage Cheeses		
1 tablespoon (30g)		
1 small carton (250g)		
1 large carton (480g)		
		P
What type of cheese(s) do you usually buy?		L
_____		E
		A
EGGS		S
1 large (60g)		E
1 medium (45g)		
		D
FISH		O
Tinned Salmon 1/2 cup (120g)		
Tinned sardines 4-5 (60g)		N
Prawns/Shrimps 3-4 (100g)		O
Scallops 5-6 (90g)		T
White fish 1 medium filet (100g)		
		W
CEREAL FOODS		R
Muesli 3 tablespoons (60g)		I
All Bran cereal 2 tablespoons (10g)		T
Sweet biscuits/Crackers 1 (15g)		E
Chocolate Biscuits 1 (15g)		
Plain cake 1 slice (40g)		I
		N
FRUITS/VEGETABLES/NUTS		
Spinach/Silverbeet 1/3 cup (60g)		T
Dried fruits 1 tablespoon (15g)		H
Peanuts 18-20 nuts (15g)		I
		S
BEVERAGES		
White wine 1 glass (100ml)		C
Red wine 1 glass (100ml)		O
Beer 1 glass (200ml)		L
		U
Coffee (regular) 1 cup		M
Coffee (decaf.) 1 cup		N
Tea (regular) 1 cup		
Iced tea 1 glass (200ml)		

How much of the following foods do you usually eat EACH WEEK?		PLEASE
COLA		
1 large glass (200ml)		
1 can (355ml)		DO
What type of cola do you usually drink? (please circle answer).		
Regular : Diet : Caffeine free		NOT
MISCELLANEOUS		
Chocolate 4 squares (20g)		
Orange juice 1 large glass (200ml)		WRITE
Calais 1 large glass (200ml)		
Do you take any calcium/multivitamin tablets?		
[please specify brand and number taken]		IN
_____		
Do you take any antacids or indigestion tablets?		
[please specify brand and number taken]		THIS
_____		
THANK YOU FOR YOUR HELP		COLUMN

APPENDIX G
3-DAY DIETARY RECORD

INSTRUCTIONS FOR THREE DAY DIETARY RECORD

WHAT DID YOU EAT ???

You will be recording your daily intake of food and fluids for 3 consecutive days. They must be a Sunday/Monday/Tuesday combination.

It is imperative that you record EVERYTHING that you eat and drink (water, vitamin/mineral pills as well!!!). In addition, you must be as ACCURATE as possible when determining the amount (volume or weight) of the food and drink you are recording. This may be difficult for those of you who have your food prepared and served by someone else, but try to be as accurate as possible. Use measuring cups/spoons and weigh scales whenever possible.

HINTS FOR RECORDING DIETARY INTAKE

ACCURACY

1. Accurate Measurement: Read the weights or volumes of foods or drinks from packages. Example: milk carton, juice box, chocolate bar, potato chips. A "fistful" of meat = 100 gm., "fistful" veggies = 1 cup, 1 cheese single = 1 oz.

2. Method of Cooking: Indicate how your food was cooked. Example: fried, steamed, baked, broiled, etc.

3. "Extras": Don't forget the EXTRAS. Example: ketchup, mustard, mayonnaise, gravy, or butter.

4. Food Types: Be specific about TYPES of food/drink. Example: cheddar cheese, 2% milk, margarine or butter. Whenever possible, identify brand names of the foods.

5. Cooked or Dry Measurement: Indicate whether the food measurement is of "cooked" or "dry". Example: chicken weight before or after cooked.

6. Specific Parts: Indicate the exact part of the food you ate or what was removed Before eating. Example: chicken (white or dark, bone in or out, skin or skinless) baked potato (skin or skinless), ground beef (lean, extra lean, or regular).

7 Labels: Whenever possible, attach the nutritional information label from the container (box/can/bag). This will help identify specific brand food nutrients. If you can't remove the label, copy the information onto a piece of paper.

BEVERAGES

8. TEA and COFFEE should be included along with the cream, milk, and sugar you add.

9. Don't forget WATER.

10. Yes, you do have to record BEER and ALCOHOL as well.....!!

PREPARED OR RESTAURANT MEALS

11. Use PORTION PAKS whenever possible. Example: salad dressing, butter, jams, peanut butter, cheese. It is easier to quantify the volume of these foods... 1 portion pak = 1 tablespoon.

12. Fast Foods: Include FAST FOOD items by name. Example: McDonald's, Pizza Hut, Wendy's. Be sure to indicate whether you had a small, medium or large size.

13. Recipes: Record the AMOUNT/VOLUME of ingredients, the number of servings the entire recipe makes and how many servings you ate.

14. Restaurant Meals: When you eat at a restaurant (other than a fast food place, eg. Earl's), record the name of the meal you ate, list the different ingredients on your plate and list the quantities of each.

TAKE THE RECORDING BOOK WITH YOU AT ALL TIMES...IT'S EASIER TO RECORD WHAT YOU'RE EATING!!

DIETARY INTAKE RECORD

Name: _____

Telephone: _____

Date of Birth: ____ ____ ____
(Month) (Day) (Year)

Record Dates: ____ ____ to ____ ____
(Month/Day) (Month/Day)

Directions For Daily Menu

The purpose of this study is to discover everthing you consume during a three day period. It is important to record all foods and beverages – from a full course family dinner at home to a quick cup of coffee at work. Before you begin to record in your diary, however, please read the following directions and examine closely the sample day:

There is a section for every day.

The day is broken into 6 consumption periods:

Morning Meal
Midmorning Snack

Midday Meal
Afternoon Snack

Evening Meal
Evening Snack

Foods and beverages consumed away from home – at work, at a restaurant, or when visiting friends – are just as important as those eaten at home. Therefore, it is important that your record your entries as soon after eating as possible. The following entries should be included in your recording:

1. **Menu Item Column:** Enter in this column all foods, beverages, etc. consumed during the meal or snack. If your family eats two kinds of cereals or has several different types of sandwiches for example, please record the correct type.

   Enter in the same block as the menu item all toppings or additives used on the menu item at the time of eating (syrups, gravies, butter, milk, sugar, etc.). Please be specific in your entries – maple syrup, 2% lowfat milk, grape jelly, etc.

2. **Unit of Measure Column:** For every menu item and every topping or additive, enter in this column either the word "number", "cup", "ounce", "teaspoon", or "tablespoon". Not only the menu item, but the topping or additive as well, must have its own unit of measure.

3. **Number of Units Column:** In this area, record the number of units consumed. Include the amount of the menu item and the amount of toppings or additives consumed. An estimate of the unit is satisfactory. Actual measuring is unnecessary unless the exact weight, eg. meat, is known.

4. **Description Column:** For ever menu item please include in this column:

   - the brand (if known)
   - the type and flavour (if applicable)
     i.e. homemade, strawberry waffles
   - the method of cooking (if applicable)
     i.e. scrambled, baked, fried

   It is not necessary to describe the toppings or additives, only the menu item.

5. **"Where Eaten" Category:** Items consumed away from home are just as important as those items consumed at home. All consumption should be recorded. It is also important, at the end of each meal, to check where that meal was consumed.

   For example at the morning meal, one of the three categories below must be checked.
   Eaten at home
   Eaten away from home
   Did not eat

6. **Daily Check:** After you have finished your recording for the day go back over your entries and make sure that for every entry (every menu item, and topping or additive), there is an appropriate unit of measure and the corresponding numbers are given. Also check to see that at the end of each meal, the appropriate category is checked.

What you eat and drink everyday is important and your entry should be as accurate as possible.

Thank you for your participation and co-operation in helping to produce a detailed quality study. Please examine carefully the sample day before beginning.

ADDITIONAL INFORMATION

		MENU ITEM	UNIT OF MEAS. Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	DESCRIPTION OF MENU ITEM Brand Name	Type of Flavour	Method of Cooking
MORNING MEAL	Menu Item	eggs	number	3	Dunland		scrambled
	Toppings or Additives	ketchup	tablespoon	2			
	Menu Item	sausage links	number	2	Schneiders	sausage	fried
	Toppings or Additives						
	Menu Item	whole milk	cup	1	Silverwood		
	Toppings or Additives	choc mix	tablespoon	2			
	Menu Item	corn flakes	cup	1	Kellogg	corn flakes	
	Toppings or Additives	whole milk	cup	1/2			
		sugar	tsp.	1			
	Menu Item	banana	no.	1			
	Toppings or Additives						
	Menu Item	multi vitamin	number	1	One-A-Day		
	Toppings or Additives						
	Mark (X) One Category	Eaten at Your Home		X			
		Eaten Away From Your Home					
		Did Not Eat					

Sample Day

	MENU ITEM Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating	UNIT OF MEAS. Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	DESCRIPTION OF MENU ITEM Brand	Type of Flavour	Method of Cooking
MORNING MEAL	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Mark (X) One Category	Eaten at Your Home				
		Eaten Away From Your Home				
		Did Not Eat				

Day One

	MENU ITEM Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating	UNIT OF MEAS. Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	DESCRIPTION OF MENU ITEM Brand	Type of Flavour	Method of Cooking
EVENING SNACK	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Mark (X) One Category	Eaten at Your Home				
		Eaten Away From Your Home				
		Did Not Eat				

Day Three

	MENU ITEM Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating	UNIT OF MEAS. Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	DESCRIPTION OF MENU ITEM Brand	Type of Flavour	Method of Cooking
MIDDAY MEAL	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Mark (X) One Category	Eaten at Your Home				
		Eaten Away From Your Home				
		Did Not Eat				

Day One

	MENU ITEM Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating	UNIT OF MEAS. Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	DESCRIPTION OF MENU ITEM Brand	Type of Flavour	Method of Cooking
AFTERNOON SNACK	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Mark (X) One Category	Eaten at Your Home				
		Eaten Away From Your Home				

Day Three

	MENU ITEM Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating	UNIT OF MEAS. Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	DESCRIPTION OF MENU ITEM Brand	Type of Flavour	Method of Cooking
EVENING MEAL	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Mark (X) One Category	Eaten at Your Home				
		Eaten Away From Your Home				
		Did Not Eat				

Day One

	MENU ITEM Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating	UNIT OF MEAS. Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	DESCRIPTION OF MENU ITEM Brand	Type of Flavour	Method of Cooking
MIDMORNING SNACK	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Mark (X) One Category	Eaten at Your Home				
		Eaten Away from Home				

Day Three

	MENU ITEM Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating	UNIT OF MEAS. Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	DESCRIPTION OF MENU ITEM Brand	Type of Flavour	Method of Cooking
MORNING MEAL	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Mark (X) One Category	Eaten at Your Home				
		Eaten Away From Your Home				
		Did Not Eat				

Day Two

	MENU ITEM Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating	UNIT OF MEAS. Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	DESCRIPTION OF MENU ITEM Brand	Type of Flavour	Method of Cooking
EVENING SNACK	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Mark (X) One Category	Eaten at Your Home				
		Eaten Away From Your Home				
		Did Not Eat				

Day Two

	MENU ITEM Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating		UNIT OF MEAS. Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	DESCRIPTION OF MENU ITEM Brand	Type of Flavour	Method of Cooking
EVENING MEAL	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Mark (X) One Category	Eaten at Your Home					
		Eaten Away From Your Home					
		Did Not Eat					

Day Two

	MENU ITEM Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating		UNIT OF MEAS. Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	DESCRIPTION OF MENU ITEM Brand	Type of Flavour	Method of Cooking
MID MORNING SNACK	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Mark (X) One Category	Eaten at Your Home					
		Eaten Away From Your Home					
		Did Not Eat					

Day Two

	MENU ITEM	UNIT OF MEAS.		DESCRIPTION OF MENU ITEM		
	Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating	Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	Brand	Type or Flavour	Method of Cooking
EVENING MEAL	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Mark (X) One Category	Eaten at Your Home				
		Eaten Away From Your Home				
		Did Not Eat				

Day Three

	MENU ITEM	UNIT OF MEAS.		DESCRIPTION OF MENU ITEM		
	Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating	Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	Brand	Type or Flavour	Method of Cooking
MID-MORNING SNACK	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Mark (X) One Category	Eaten at Your Home				
		Eaten Away From Your Home				
		Did Not Eat				

Day One

	MENU ITEM Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating	UNIT OF MEAS. Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	DESCRIPTION OF MENU ITEM Brand	Type of Flavour	Method of Cooking
MIDDAY MEAL	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Mark (X) One Category	Eaten at Your Home				
		Eaten Away From Your Home				
		Did Not Eat				

Day Three

	MENU ITEM Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating	UNIT OF MEAS. Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	DESCRIPTION OF MENU ITEM Brand	Type of Flavour	Method of Cooking
AFTERNOON SNACK	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Mark (X) One Category	Eaten at Your Home				
		Eaten Away From Your Home				
		Did Not Eat				

Day One

	MENU ITEM Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating	UNIT OF MEAS. Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	DESCRIPTION OF MENU ITEM Brand	Type of Flavour	Method of Cooking
MORNING MEAL	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Mark (X) One Category	Eaten at Your Home				
		Eaten Away From Your Home				
		Did Not Eat				

Day Three

	MENU ITEM Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating	UNIT OF MEAS. Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	DESCRIPTION OF MENU ITEM Brand	Type of Flavour	Method of Cooking
EVENING SNACK	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Mark (X) One Category	Eaten at Your Home				
		Eaten Away From Your Home				
		Did Not Eat				

Day One

	MENU ITEM Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating	UNIT OF MEAS. Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	DESCRIPTION OF MENU ITEM Brand	Type of Flavour	Method of Cooking
EVENING MEAL	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Mark (X) One Category	Eaten at Your Home				
		Eaten Away From Your Home				
		Did Not Eat				

Day Two

	MENU ITEM Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating	UNIT OF MEAS. Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	DESCRIPTION OF MENU ITEM Brand	Type of Flavour	Method of Cooking
MIDMORNING SNACK	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Menu Item					
	Toppings or Additives					
	Mark (X) One Category	Eaten at Your Home				
		Eaten Away From Your Home				
		Did Not Eat				

Day Two

APPENDIX H

OVERVIEW OF HOLOGIC QDR-4500$_{TM}$ BONE DENSITOMETER

BONE MINERAL DENSITY
System Overview

Bone mineral density will be assessed using Dual Energy X-ray Absorptiometry, (DXA), measured by a Hologic QDR®4500 X-ray Bone Densitometer, at three sites: (1) the lumbar vertebrae (L2-L4), (2) the hip, and (3) the lower leg.

This system images and estimates BMC and BMD of selected areas of the body, or of the entire skeleton. It does so using X-rays of two different energy levels; this dual energy scheme allows soft tissue within the selected area to be subtracted out, leaving bone to be imaged and estimated.

The QDR 4500 employs a patented Automatic Internal Reference System, which continuously calibrates the machine to eliminate the effects of variations in temperature and tube flux. No daily calibration is required, however, the daily scanning of a quality control phantom is required to provide assurance that the system is functioning correctly, and to aid in the detection of any long-term drift. The operator is not required to select X-ray technique factors as these are selected and fixed by the Hologic 4500.

Procedure

The technique will be performed according to the manufacturer's protocol and will use Hologic software. The patient lies face up on the table, and with the aid of a cross-hair laser, the operator positions the scanning arm over the region of interest. After entering the patient data and selecting the type and size of scan needed, the operator initiates the scan with a single keystroke.

BMC results are expressed in grams of calcium hydroxyapatite, and BMD is reported in grams/cm^2 of the same compound.

Radiation Dose

DXA allows for a rapid, accurate and highly reproducible assessment of BMC and BMD with a low radiation exposure. Although it is impossible to ascertain with any degree of certainty the risk incurred by an individual exposed to low levels of radiation, it is the

opinion of radiation experts that low levels of radiation can be considered harmless. Under standard operating conditions, the patient entrance dose is less than 35 mR (0.35 mSv), which is approximately the same exposure of a standard chest X-ray. Leakage radiation at one metre is less than 1.0 mR/hour, or approximately one percent of the limit specified in 21 CFR 1020.30 (k). No additional shielding is necessary for patient, operator or room, and the QDR 4500 can be placed in any convenient non-shielded examination room.