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# Factors Affecting Bone Mineral Density in Elite Female Runners

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# FACTORS AFFECTING BONE MINERAL DENSITY IN ELITE FEMALE RUNNERS

By

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**APPROVAL**

FACTORS AFFECTING BONE MINERAL DENSITY
IN ELITE FEMALE RUNNERS

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## ABSTRACT

**Introduction:** The benefits of regular exercise on skeletal health have been well-documented in terms of stimulation of bone accrual and bone maintenance. Medium-impact sports activities such as running have been demonstrated to exert site-specific enhancement of bone mass in the lower appendicular skeleton. However, elite female runners engaged in high intensity training and sports activity may also be at risk of amenorrhea and low bone mineral density (BMD) resulting from inadequate caloric intake.

**Purpose:** To investigate the effect of intensive exercise and maintenance of adequate caloric intake on BMD in a group of elite female runners.

**Methods:** This study represents a secondary assessment of existing data that were obtained between 1994 and 2009. Using dual-energy X-ray absorptiometry, a group of elite runners (n=11) in this study was screened in the Laboratory for Elite Athlete Performance at Georgia State University. This was a longitudinal study in which three sequential measurements of BMD as well as fat and lean tissue body composition of each athlete took place. The average interval between measurements was 1.1 years and 2.6 years respectively. Regional BMD measurements for head, arms, legs, trunk, ribs, pelvis, and spine were assessed, as well as the value for total body BMD. The study participants also received dietary counseling emphasizing daily caloric balance and adequate calcium intake.

**Results:** The average age of the runners increased from 24.59 (±4.41) to 28.14 (±5.94) years over the study. This was accompanied by an increase in body mass (54.98±3.54 to 56.11±4.07 kg), while height remained constant. The average body mass index (BMI) of the subjects increased from 19.34 to 19.71 kg/m$^2$, largely due to an increase in total percent body fat (13.97±2.96% to 16.01±4.28%). Average regional and total BMD values increased over the study period and increases were between 2 and 4%. At the baseline, a majority of subjects (n=7) had a BMI>19 kg/m$^2$, while a sub-group of runners (n=4) had a BMI<19 kg/m$^2$. Mean trunk, pelvis and spine BMD parameters for the two BMI groups were significantly different ($p$<0.05), with reduced BMD values in the lower BMI sub-group. The average *T*-scores associated with arm BMD were considerably lower than *T*-scores associated with leg BMD values in the runners. The average *T*-scores for leg BMD values were almost two standard deviations higher than leg BMD values for a reference population at peak bone mass. Two subjects were osteopenic, resulting in an 18% prevalence rate of osteopenia in the group of runners.

**Conclusions:** The majority of elite runners in this study exhibited a positive trend in BMD parameters. This was reflected as increased total as well as regional BMD values. Increased body mass in addition to the activity of running positively contributed to bone mass via a weight-bearing effect. Increased adipose tissue may also have been a source of endocrine hormones such as estrogen and leptin, which exert a positive effect on bone accrual.

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| | |
|---|---|
| ATP | Adenosine triphosphate |
| ANOVA | Analysis of variance |
| BMC | Bone mineral content |
| BMD | Bone mineral density |
| BMI | Body mass index |
| $Ca^2+$ | Calcium ions |
| DNA | Deoxyribonucleic acid |
| DRI | Dietary reference intake |
| DXA | Dual X-ray absorptiometry |
| $dyn/cm^2$ | Dyne per square centimeter |
| EA | Energy availability |
| EGF | Epidermal growth factor |
| EI | Dietary energy intake |
| FFM | Fat free mass |
| FN | Femoral neck |
| g | grams |
| GH | Growth hormone |
| IGF-I | Insulin-like growth factor I |
| IRB | Institutional review board |
| IU | International units |
| kg | Kilograms |
| LBM | Lean body mass |
| mRNA | Messenger ribonucleic acid |
| µg/d | Micrograms per day |
| mg/d | Milligrams per day |
| mg/dl | Milligrams per deciliter |
| nm | Nanometers |
| nmol/l | Nanomoles per liter |
| NTX | N-Telopeptide |
| 25[OH]D | 25-Hydroxyvitamin D |
| $1,25[OH]D_2$ | 1,25-Dihydroxyvitamin D |
| OPG | Osteoprotegerin |
| pg/ml | Picograms per milliliter |
| pmol | Picomoles |
| RANK | Receptor activator of nuclear factor kappa B |
| RANKL | Receptor activator of nuclear factor kappa B ligand |
| SD | Standard deviation |
| SPSS | Statistical package for the social sciences |
| TB | Total body |
| TH | Thyroid hormone |
| US | United States |
| yr | Years |

# CHAPTER I

## INTRODUCTION

Studies have shown that exercise has a positive effect on bone health (1). In addition, it is apparent that the greatest accrual of bone mineral density (BMD) occurs during the early adolescent years, and this may lead to a positive advantage in bone strength during adulthood, when a reduction in bone density becomes prevalent (2). Severe depletion of bone tissue may lead to the degenerative condition, osteoporosis, which is a major and debilitating disease of aging (2). Osteoporosis is associated with increased fragility and risk of fracture at skeletal sites such as the hip, spine and wrist (3). In 2002, the direct cost of health expenditure on osteoporotic fractures was estimated at 17.5 million US dollars and this is expected to increase as the population ages (3).

Physical activity increases the length, width, and mineral content of bones during childhood and adolescence, as a result of complex torsional interactions between bone and muscle tissue (2). These positive effects on bone accrual are greater in trained athletes, where a correlation has been noted between medium and high impact sports activity and BMD levels, provided adequate caloric and calcium intakes are maintained (4). Athletes participating in medium and high-impact sports such as running and gymnastics respectively, were shown to have higher BMD values at all measurement sites tested, compared to sedentary controls ($p<0.001$) (5).

The positive effect of sustained weight-bearing exercise on bone accrual in female athletes may however be negated if the "Female Athlete Triad" is present (6). This syndrome is characterized by disordered eating, amenorrhea and osteoporosis and may be present in female athletes participating in elite level sports. Intensive exercise accompanied by inadequate caloric intake may result in hormonal imbalance and concurrent menstrual irregularities (6). Estrogen is a vital component of positive bone development. Therefore, the hypoestrogenimia of amenorrhea may result in osteopenia and if the eating disorder is prolonged, osteoporosis. Eating disorders have been particularly noted in female athletes engaged in sports such as endurance running, where slight stature is an advantage. In fact, various studies have estimated the prevalence of eating disorders at between 15% and 65% in women athletes participating in sports where thinness is a factor (6).

The importance of adequate caloric intake, as well as the recommended amount of daily calcium and vitamin D in preserving bone health is well known. For women aged 19-30 years with regular menstrual cycles, the Dietary Reference Intake for calcium is 1,000 mg daily, while 1,500 mg calcium is recommended for women with amenorrhea (6). Female athletes participating in intense training and sports activities may underestimate the importance of balanced nutrition in maintaining general as well as skeletal health (6).

The group of elite runners in this study was screened longitudinally for BMD, as well as fat and lean tissue body composition, over a period of approximately two years. The study participants also received dietary counseling emphasizing daily caloric balance and adequate calcium intake. Based on literature precedent, it was of interest to analyze

whether there was a correlation between BMD, fat and lean tissue mass data, and whether sustained medium-impact exercise and adequate caloric intake influenced these values.

## PROBLEM

Elite female runners engaged in high intensity training and sports activity may be at risk of amenorrhea and low BMD as a consequence of inadequate caloric intake.

## RESEARCH OBJECTIVE

The objective of this study was to investigate the effect of intensive exercise and maintenance of adequate caloric intake on BMD measurements in a group of elite female runners.

## HYPOTHESES

**$H_0$:** High level exercise and adequate caloric intake has a beneficial effect on the BMD of a group of elite female runners.

**$H_1$:** High level exercise and adequate caloric intake has no beneficial effect on the BMD of a group of elite female runners.

# CHAPTER II

# REVIEW OF LITERATURE

## INTRODUCTION

There is a considerable amount of published literature supporting the influence of exercise, diet, and hormones on the bone mineral density (BMD) of individuals, both female and male (1). These factors interact in a complex interrelated manner in supporting bone growth and maintenance. This literature review will describe some of the current knowledge related to factors affecting female bone mineral density, with a particular emphasis on premenopausal individuals.

## EXERCISE AND BONE DENSITY

### Normal Bone Development

The human skeleton fulfills a number of functions, including providing structural support to the body and facilitating movement in conjunction with the attached muscles (7). The adult human skeleton is comprised of predominantly two types of bone, which are structurally dissimilar: dense cortical bone (80%) and honeycomb-like trabecular bone (20%). Long bones of the appendicular skeleton, such as the humerus, radius, ulna, femur, tibia and fibula comprise a hollow shaft or diaphysis of cortical bone which surrounds the inner marrow space. The ends of the long bone, the metaphysis and epiphysis, are capped with spongy trabecular bone (7). On the other hand, the vertebrae

of the axial skeleton contain predominantly trabecular bone, a spongy network of trabecular plates and rods.

The fundamental units of bone structure are osteons, and it is at this level that dynamic changes occur in skeletal structure (7). The skeleton is in a constant state of flux, with a dynamic interplay between the process of modeling during bone growth and development, and remodeling during bone repair and aging (2). Typically, bone growth occurs on the outer or periosteal surface of bone, leading to increasing bone width (7). On the other hand, the inner or endosteal surface of bone is the site of remodeling activity (7). Factors such as age, physical activity, adequate nutrition and hormonal status may all affect the overall balance between the modeling and remodeling processes (2).

**Mechanotransduction in Bone**

Current research suggests that osteocytes, the most numerous bone cells, act as biological sensors responding to mechanical stimulation of bone tissue (8). Specifically, imposition of a load on skeletal tissue results in pressure gradients and movement of interstitial fluid, which transmits shear stress to responsive areas of the osteocytes (8). Osteocytes, which are located in the interior of bone tissue, communicate with osteoblasts (modeling) and osteoclasts (remodeling) via cellular dendritic processes which permeate bone tissue (9). Close cell-to-cell contact is established between osteocytes and responsive cells via gap junctions which are essential for the transmission of mechanical signals in bone (9). Connexins, such as Cx43, an integral membrane protein, are present in gap junctions and allow the passage of metabolites and signaling molecules between cells and the interstitial fluid in bone (10). Factors released by osteocyte dendritic

processes following mechano- stimulation include prostaglandin $PGE_2$, adenosine triphosphate (ATP) and nitric oxide (10).

The signaling cascade resulting from mechanical stimulation of osteocytes is complex and only partially understood. An *in vitro* study using murine MLO-Y4/MC3T3 cells, which mimics an *in vivo* osteocyte/osteoblast system, has shed some light on interactions between these bone cells (10). A cellular co-culture was established with osteocytes and osteoblasts physically separated but which allowed fluid flow through the equivalent of a gap junction between the cells. Exposure of the osteocytes to physiological levels of fluid shear (4.4 dyn/$cm^2$) resulted in increased alkaline phosphatase activity and initiation of a calcium ($Ca^{2+}$) wave signal in the shielded osteoblasts. The presence of a gap junction-like contact between osteocytes and osteoblasts was essential for the transmission of the mechanical stimulus to osteoblasts (10).

There is also experimental evidence that mechanical loading inhibits the process of osteoclastogenesis through regulation of the two factors, receptor activator of nuclear factor kappa B ligand (RANKL) and osteoprotegerin (OPG) (11). The gene product OPG acts as a "decoy" receptor binding to RANKL and blocking maturation of osteoclasts. Mechanical loading in bone tissue has been shown to decrease the RANKL/OPG mRNA ratio, and therefore exert a protective effect on bone tissue (11).

**Biomechanical Aspects of Bone Development**

In healthy individuals, there is a direct relationship between an individual's muscle mass, as estimated by lean body mass (LBM) and bone mass or bone mineral content (BMC) (12). This positive relationship between LBM and BMC is particularly evident

during dynamic growth periods, such as childhood and early adolescence (12). In fact, contracting muscle imposes a larger strain on bone than gravity, and is the major stimulus promoting accumulation of bone mass and bone strength (13).

The mechanostat theory of bone development postulates that an increase in muscle mass precedes bone growth in puberty (12). In a longitudinal Canadian study which included 68 adolescent girls, the mean peak accrual of LBM occurred at age 12.19 years, which was followed six months later by accrual of mean peak BMC at 12.69 years. This lag in peak bone development occurred both at the whole body level, as well as in the upper and lower limbs.

Inherent in the mechanostat theory of muscle-bone interaction is the concept that bones adapt to the strain imposed by muscle contraction (14). Within normal limits, when strain thresholds in bone are exceeded, modeling occurs, resulting in an increase in periosteal growth and a net accrual of BMC. Body weight also increases with age and contributes to the load applied to bone by muscle contraction (15). As a result of a balance between modeling and remodeling, mechanical stress on bone results in changes in bone shape and size (16).

Maximal muscle force applied to bone is usually measured indirectly and provides an estimate for the amount of strain applied to bone, as well as changes in bone mineral content (15). For example, a group of 37 women (age 18-26 years) was subjected to five months of isokinetic concentric or eccentric resistance training applied to the non-dominant arm or leg (17). Concentric resistance training results in shortening of muscles during contraction, while eccentric resistance training results in lengthening of muscles during contraction. Both forms of resistance training allow the application of high

muscular loads, and consequently, increased skeletal loads. As a result of the asymmetric loads applied, an increase in LBM as well as BMD in the trained limbs was noted ($p<0.001$) for both forms of resistance training.

In addition to providing strength and support, the skeleton also has the property of lightness, allowing mobility (18). It is notable that although bones may differ greatly in shape and size, the BMC of longer, wider or narrower cross-sections of bone may not differ greatly in the amount of bone mineral present. For example, smaller bone cross sections may contain more bone mineral to avoid fragility. Conversely, wider cross-sections, may contain less bone mineral relative to size to preserve lightness. Yet, both smaller and larger bones may have the same volumetric bone density measurement. Bone shape, size and mass distribution is achieved by appositional growth or modeling on the periosteal surface balanced by endosteal remodeling. In addition to the influence of LBM on bone development, genetic factors delineating bone growth are also apparent.

**Exercise and Bone Mass during Childhood and Early Adulthood**

There is considerable support for the suggestion that "senile osteoporosis is a pediatric disease" (19). This statement relates to the observation that adult peak bone density may be enhanced by physical activity or sports participation during pre-puberty and early adolescence (20). During the pubertal growth spurt period (11-13 years in girls and 12-14 years in boys), approximately 25% of the adult BMC is reached (19). This amount of bone accrual approximates the proportion of bone loss during post-menopause (21). In addition, during the four perimenarcheal years in girls, 47% of the mass of the future adult skeleton is accumulated, and the introduction of regular physical activity during this

period may result in later protection against osteopenia or osteoporosis. Small increments of BMD during childhood may be significant in later years. In fact, epidemiological studies show that a decrease in BMD by one standard deviation unit (SD) doubles bone fracture risk in adult females.

The accumulation of bone mass during childhood and early adolescence is dependent on simultaneous development of skeletal muscle, which exerts forces on the attached bone, resulting in bone development (19). Weight-bearing activities such as running and jumping exert intermittent compression of the growth plates at the ends of long bones and stimulate bone growth (19). In the case of girls, rapid bone growth occurs predominantly in the pre-pubertal period resulting in an increase of bone size, strength and structure (21). Both endocortical and periosteal expansion takes place, with a minimal increase of BMC after the onset of puberty in girls (19). The pre-pubertal years in girls therefore offer a window of opportunity during which the introduction of regular weight-bearing activity may stimulate bone growth over and above normal daily activity (21).

Several recent reviews have analyzed the evidence corroborating the benefits of the early introduction of regular physical activity in children (22). In general, early pubertal study populations (Tanner I-III) reported increases in bone parameters over a six-month period compared to inactive controls ($p<0.05$) (22). Although the short-term benefits of physical activity are well documented, the exact duration and intensity required to produce maximal effect is unclear in this population group (19).

In order to estimate the type and duration of physical activity required to promote bone development, a Swedish Pediatric Osteoporosis Prevention study followed a group of 53 pre-pubertal girls (7-9 years) who performed regular physical exercise for one year

(21). The intervention group participated in a daily school-based exercise program involving 40 minutes of indoor and outdoor physical activity, including ball games, running and jumping. The authors wished to utilize typical activities enjoyed by young children in order to minimize dropout rates. The control group for this study was an age-matched group at a second school, exposed to 60 minutes of physical activity per week. The authors of this study analyzed BMC and BMD data pertaining to total body (TB), lumbar spine (L2-L4 vertebrae), third lumbar vertebra (L3), femoral neck (FN) and leg. The girls in the intervention group experienced an average increase of 2.8% and 3.1% in BMD ($p<0.001$) in the lumbar spine and L3 vertebrae respectively, compared to the control group (21). An additional advantage seen in the intervention group was a 2.9% gain in bone width at L3 compared with controls. This study suggests that physical activity in young children may contribute to BMC and BMD, which may protect against future fracture risk.

Another longitudinal study investigated the longer-term results of the early introduction of physical activity in a Canadian population (23). The authors accessed bone parameters from the University of Saskatchewan's Pediatric Bone Mineral Accrual Study (1991-1997; n=154, 82 females and 72 males, 8 to 15 years). The bone parameters of the study group were subsequently re-evaluated in young adulthood (2002-2006; 23-30 yr). In the case of young females, individuals who were physically active in childhood remained more active in young adulthood. In addition, analysis of BMC of young adult females showed an increase of 8.6% and 9.5% ($p<0.05$) in BMC at the total hip (TH) and femoral neck (FN) respectively, compared with inactive controls. Given that hip

fractures are particularly prevalent and costly in osteoporotic patients, any strengthening effect on bone conferred in childhood and early adulthood is to be encouraged (24).

**Types of Sports and Bone Density**

Studies involving recreational as well as elite athletes have provided valuable information regarding the relationship between types of sports activities and bone mineral data, due to the repetitive and dedicated nature of lean tissue and skeletal system stimulation in these individuals (25). Observations among athletes support the fact that the skeleton adapts to the magnitude and frequency of the load applied to it (26). In the first instance, increase in muscle size as a result of physical activity results in a corresponding increase in bone mass and size, in part determined by genetic factors including body mass (25). A study illustrating the parallel increase of lean tissue mass and BMD (25) involved a group of female competitive tennis players (n=47; 8-17 years. Across the group, there was a 6-13% increase in muscle and bone parameters in the playing arm of these individuals. Interestingly, regression analysis of the data in this study revealed that increased muscle area in the playing arm accounted for only 12-16% of the variance in corresponding arm BMD values (25). This study suggests that the enhanced muscle size of the playing arm is directly attributable to increased use, since genetic, nutritional and hormonal factors apply to both arms. The additional enhancement of BMD in the playing arm may be due to the direct effect of impact loading, including vibrational type osteogenic stimulation, which has been demonstrated to enhance BMD (1).

In addition to muscle stimulus of bone tissue, the mechanical loading imposed by specific sports activities is accompanied by site-specific enhancement of BMD (27). Skeletal tissue appears to be particularly responsive to dynamic loading as opposed to static loading (28). In order to elicit a significant osteogenic response, sports-related mechanical stimuli are most effective when the threshold intensity is exceeded. In addition, strain frequency is a factor, since the biological response of bone to mechanical stimulation is most effective during sports involving brief but intermittent patterns of exertion. As a result, sports involving high-impact and odd-impact motion, such as volleyball, hurdling and squash have a more pronounced effect on BMD than lower-impact sports such as running, swimming and diving (29).

The effectiveness among disparate sports in eliciting site-specific osteogenesis was examined in a recent study (30). The authors enlisted collegiate female athletes (n=99; 20.2±1.3 years) and compared site-specific BMD data across a range of sport categories. Sports participants were engaged in weight-bearing activities such as gymnastics, softball, field hockey, soccer and cross country running, as well as non-weight-bearing activities including swimming/diving and crew. In addition to sports category, assessment of the participants' menstrual status was noted, with 23 athletes being oligomenorrheic (4-9 menstrual cycles/year) or amenorrheic (0-3 menstrual cycles/year). Regression analysis in this study showed that body mass and type of sport were general predictors of total-body, pelvic and average leg BMD. As anticipated, swimmers and divers, as participants in non-weight-bearing activities, had low site-specific BMD values compared to other categories of athletes. This was in contrast to gymnasts, who exhibited the highest site-specific BMD values, due to the osteogenic stimulus imposed by a

gymnastic training regimen. The runners who participated in this study had lower site-specific BMD values, particularly in the lumbar spine (0.988±0.118 g/cm$^2$) and pelvic regions (1.023±0.085 g/cm$^2$), than the swimmers/divers examined (lumbar spine: 1.079±0.107 g/cm$^2$; pelvis: 1.060±0.071 g/cm$^2$). The authors note that the lower BMD values for runners in this study may be related to disordered eating and consequent disruption of the menstrual cycle which may be prevalent in this athlete population, in which relatively low BMI confers a competitive advantage (30).

## Runners and Bone Density

### *Running and the Appendicular Skeleton*

The physical activity of running has been variously reported as exerting both positive as well as negative effects on skeletal health and maintenance (31). In a positive sense, running has been classified as a medium-impact athletic activity, which may confer some site-specific enhancement of BMD over non-athletic controls, provided that menstrual dysfunction is not a factor (5). A more general assessment of benefits of running among premenopausal women (age 16-68 years) concluded that running a minimum of 3 hours per week had a positive effect on BMD parameters, particularly at the proximal femur (31).

In order to evaluate the positive effects exerted by running on the skeleton, it is helpful to consider three categories of runners: sprinters, middle distance and endurance athletes (32). Sprinters are regarded as power athletes, and as such, are subject to maximal intensity training and competitive activity, involving loading at both upper and lower skeletal sites. A recent study evaluated bone mass and geometry of the tibia and

radius of sprinters middle-distance runners, and long-distance runners to assess this concept further (33). The authors found that surrogate measures of bone strength, such as tibial BMC, cortical area and polar moment of resistance were greatest in sprinters, due to both larger mechanical strain exerted by tibial muscles, as well as greater ground reaction forces experienced. Surprisingly, there were no significant differences between radial BMC parameters in sprinters and longer distance runners in this study, which may have been a function of the amount of upper body conditioning carried out by runners in this study.

Further studies on female runners have confirmed that female power athletes, including sprinters have greater BMD values at the lower limb, lumbar spine and upper limb sites, compared with female endurance athletes, where only foot and tibia/fibula BMD values were enhanced (32). These results support the prediction that ground reaction forces are greatest on the foot and leg in endurance athletes, and this is observed as enhanced BMD values in these athletes, relative to BMD values for the upper limbs (32). This observation was supported in a detailed study of BMD parameters at multiple skeletal sites in female endurance runners (30.8±5.6 years), training an average distance of 32.3±17 km per week) (34). The authors noted that the greatest site-specific enhancement of BMD in these runners was in the legs, whereas BMD was lowest in the arms, ribs and thoracic spine, which are further removed from the site of active skeletal loading.

*Distance Runners and Decreased Bone Density*

In contrast to positive site-specific effects of running on the appendicular skeleton, there are studies describing low BMD values associated with elite and sub-elite runners (35, 36). Decreased BMD values are of particular concern in adolescent runners, in whom compromised BMD at trabecular sites such as the lumbar spine might lead to an increased risk of osteopenia (36). The etiology of decreased BMD values in competitive runners is generally related to inadequate caloric intake relative to energy expenditure (37). This in turn results in central suppression of reproductive function, hypoestrogenism and decreased osteogenesis.

In one study, slenderness or low BMI as well as volume of exercise were factors negatively affecting lumbar spine BMD values in endurance runners (35). It was suggested that high weekly running distances likely created a caloric deficit which negatively affected bone formation in this athlete population. The authors reported that in addition to increased caloric intake, introduction of weekly resistance training was beneficial in increasing lumbar BMD in endurance runners.

## NUTRITION STATUS AND BONE DENSITY IN FEMALE ATHLETES

### Adequate Caloric Consumption

Female athletes engaged in sport activities favoring slight body build, low body weight or slender appearance may be at risk of chronic energy deficit which compromises reproductive and skeletal health (38). Calorie deficit is of particular concern during puberty (age 12 to 16 years in girls) when 40% of bone mineral is accumulated (38). Compromised bone development is a risk factor for stress fractures and osteopenia/osteoporosis in future years (39).

A joint position statement issued by the American Dietetic Association and the American College of Sports Medicine has addressed under-consumption of calories by athletes (40). This evidence-based statement includes guidelines for adequate carbohydrate, protein and fat consumption during training, performance and recovery periods in order to "maintain body weight, replenish glycogen stores and repair body tissue". Restriction of caloric intake by athletes may also compromise micro-nutrient intake, particularly calcium and vitamin D, which are required for skeletal health.

*Estimation of adequate energy intake relative to expenditure*

In order to understand the relationship between energy intake, exercise expenditure and essential physiological energy needs, a relatively recent concept, energy availability (EA) has been introduced (4). Energy availability has been defined as "dietary energy intake (EI) minus exercise energy expenditure", and represents the amount of energy remaining post-exercise for basic body metabolism (4). Included in the EA calculation is the value for fat free mass (FFM), since this is the tissue expending the greatest proportion of energy consumption (41).

It has been estimated that the resting metabolic rate in normal healthy young females represents an energy availability of about 30 kcal/kg FFM/day while inclusion of average daily activities raises energy availability requirements to approximately 45 kcal/kg FFM/day (4). In contrast, studies have shown that reduction of energy availability below 30 kcal/kg FFM/day in female athletes leads to suppression of menstrual function and reduced bone formation (42).

For the purpose of investigating the relationship between energy restriction and aerobic exercise expenditure, a study involving a group of eumenorrheic women (age 21.4±0.6 years) found an inverse relationship between markers of bone resorption and bone formation after five days of severe energy restriction (42). In fact, at an energy availability of 10 kcal/kg FFM/day, bone resorption and bone formation became uncoupled and procollagen urinary N-telopeptide, an index of bone resorption, increased significantly ($p<0.01$). In contrast, levels of plasma osteocalcin, a marker of bone formation, were suppressed at energy availability levels below 30 kcal/kg FFM/day ($p<0.05$) in this study group. The uncoupling effect on bone resorption and bone formation in the presence of chronic under-nutrition may have a deleterious effect on achievement of peak bone density in younger athletes (42).

*Weight maintenance and athletic performance*

Female athletes engaged in aesthetic-type sports activities and sports that emphasize slight stature are particularly prone to engage in unhealthy eating behaviors in an attempt to reduce weight or change body composition (38). This behavioral pattern is related to the perception that body weight and particularly percent body fat, is closely related to athletic performance (38). Young athletes may not realize that with an increased level of training, enhanced lean mass, which is denser, may replace fat mass and consequently, body mass may remain unchanged or even increase.

In order to discourage unhealthy eating practices and caloric restriction, which may also affect athletic performance, nutrition education is recommended, particularly with regard to daily and within-day caloric balance (43). A study involving both elite

gymnasts and runners suggested that within-day energy balance deficits (>300 kcals) might paradoxically result in increased body fat percentages in both gymnasts (r=0.508; $p$=0.001) as well as runners (r=0.461; $p$=0.041) (43). This observation may be due to an adaptive response of the human body to perceived famine and a corresponding reduction in resting metabolic rate and increase in fat storage (44).

## Female Athlete Triad in Runners

### *Inadequate caloric intake and menstrual disorders*

The Female Athlete Triad, encountered in some female athletes, is an inter-related spectrum of disorders involving decreased energy availability, altered menstrual function and compromised bone density (45). Low energy availability in these athletes may be the result of conscious altered eating patterns or inadvertent caloric deficit related to increased exercise expenditure (41). Unlike the situation of food deprivation, which induces hunger, increased exercise energy expenditure may not cause a related hunger response, resulting in an inadvertent energy deficit in affected athletes.

Chronic calorie deficit with energy availability levels below 30 kcal/kg FFM/day has been associated with disruption of the female reproductive cycle (46). This is a physiological adaptive response: by reducing energy expended on reproductive processes, the brain is spared from a glucose deficit (47). In fact, detailed studies have demonstrated that low energy availability results in disruption of the hypothalamic-pituitary-ovarian axis, resulting in drastic reduction of estrogen release (46).

A closely-monitored investigation of energy availability, intense exercise activity and hormone levels showed that at energy availability levels below 30 kcal/kg FFM/day,

there was disruption of gonadotropin-releasing hormone in the hypothalamus which resulted in consequent disruption of luteinizing-hormone pulsatility and frequency of release from the pituitary gland (47). The altered luteinizing-hormone release patterns in turn disrupted ovarian functioning and estrogen as well as progesterone release. These authors also demonstrated that low energy availability rather than the stress of exercise was associated with altered luteinizing hormone pulsatility, since control subjects receiving adequate calories (45 kcal/kg FFM/day) did not experience hormonal disruption. Studies have therefore shown that exercise *per se* does not have a negative effect on reproductive health, beyond the effect of physical activity on energy availability (47, 48).

*Menstrual disorders and decreased bone density*

There is a spectrum of menstrual disorders described in athletes diagnosed with the Female Athlete Triad, ranging from primary or secondary amenorrhea (absence of menses for 3 months or more) through oligomenorrhea, which is characterized by longer menstrual cycles (48). In the general population, prevalence of secondary amenorrhea or oligomenorrhea is estimated at 2% to 5% (46). However, in athletes engaged in sports where slight stature is significant, menstrual disorders range from 21% in runners to 61% in rhythmic gymnasts (48).

Menstrual disorders associated with chronic low energy availability in female athletes may result in increased bone turnover associated with both low body mass index (BMI) and decreased estrogen levels (49). In fact, a study of women distance runners (age 27.2±1.8 years) showed a positive correlation between BMI, osteocalcin and estradiol

levels in a group of amenorrheic runners (49). This early study suggested a link between chronic energy deficit, altered hypothalamic function and increased bone turnover in this group of amenorrheic female runners.

The presence of menstrual disorders and decreased BMD predisposes competitive female athletes to stress fractures, particularly of the tibia, foot and femur, in the case of runners (50). Stress fractures may be minimized in this athlete group by promoting adequate nutrition, both in terms of caloric consumption as well as calcium and micronutrient intake (40).

*Restoration of menstruation increases bone density*

Functional hypothalamic amenorrhea associated with the Female Athlete Triad results in lower BMD values in affected athletes (51). Treatment of the amenorrhea component of the triad with hormones, both estrogen replacement therapy as well as oral contraceptives, has proved relatively ineffective, inferring that the etiology of the menstrual disturbance is complex and involves hormones in addition to estrogen. However, an overall anabolic effect on bone was noted following weight gain in addition to restoration of menstruation in some amenorrheic individuals (52). Increases in the BMD values for both spine (4.38±7.48 %; $p<0.05$) and hip (3.77±8.80 %; $p<0.05$) were noted. A significant result in this study was the observation that weight gain in this group was accompanied by an increase in serum osteocalcin values, while a marker of bone resorption, N-telopeptide (NTX) only decreased following resumption of menses. The resultant coupling of bone modeling and remodeling accompanying weight gain and

menstrual regularity in these formerly amenorrheic individuals resembled coupled bone metabolism in eumenorrheic individuals (41).

## Calcium Intake and Bone Health

In bone tissue, calcium combines with phosphorus to form the inorganic mineral compound hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) which constitutes 70% of skeletal mass (53). The organic structural component of bone is predominantly the protein, type 1 collagen (22%). Thus both an adequate calcium and protein intake are essential for bone development and health. The physiological blood serum level of calcium is tightly regulated (8-12mg/dl) and parathyroid hormone is recruited to maintain this calcium concentration by increasing dietary calcium absorption, increasing bone resorption and decreasing calcium excretion by the kidneys. Vitamin D is directly involved in maintaining serum calcium levels and low vitamin D status may compromise skeletal health.

### *Optimal calcium intake and early adolescence*

There is considerable research supporting the importance of an adequate calcium intake during the pubertal growth spurt in adolescent females (54). During a 3- to 4-year period, beginning at an average age of 12.5 years in females, 40% of adult bone mass is accumulated (54). The dietary reference intake (DRI) for calcium in adolescent females, age 9 to 13 years is 1300 mg/d (55). Decreased calcium intake during this period has led to increased incidence of childhood bone fractures, particularly affecting individuals who avoided dairy foods (54).

*Levels of daily calcium intake for active premenstrual females*

Research studies have shown a positive linear correlation between dietary calcium intake and spinal trabecular BMD values in amenorrheic compared with eumenorrheic elite athletes, such as runners and ballet dancers (56). In addition, an association has been noted between the level of calcium intake and prevalence of stress fractures in female athletes.

Although the recommended calcium intake for the female population age group, 19 to 30 years, is 1,000 mg/d, a higher calcium intake (1,500 mg/d) has been suggested for female athletes who are oligomenorrheic or amenorrheic (56).

## Vitamin D and Bone Maintenance

*Role of vitamin D in calcium homeostasis*

Vitamin D may be formed endogenously from 7-dehydrocholesterol in the skin, following exposure to ultraviolet B radiation (wavelength 290-315 nm) (57). The vitamin may also be ingested as cholecalciferol (vitamin $D_3$) or ergocalciferol (vitamin $D_2$) in the diet (57). Vitamin D is hydroxylated at the 25-position in the liver to form 25-hydroxyvitamin D (25[OH]D), the major circulating form, which is in turn converted to the active form 1,25-dihydroxyvitamin D (1,25$[OH]_2$D), in the kidney. The 1,25-dihydroxy derivative of cholecalciferol plays an essential role in the intestinal absorption of calcium, by increasing the expression of the calcium-binding protein calbindin, which assists in the transport of calcium ions from the luminal to the basolateral surface of the enterocyte.

*Vitamin D levels in the general population and athletes*

Current assessment of dietary vitamin D intake, as well as serum 25[OH]D levels in the general population suggests that some individuals are not attaining recommended vitamin D status (58). There is no consensus on vitamin D concentrations at present, and desirable serum 25[OH]D levels in adult females range from 50 nmol/l to 75- 80 nmol/l (59). These relatively high levels of serum 25[OH]D appear to minimize secondary hyperparathyroidism (parathyroid levels >65pg/ml) and consequent bone resorption (60). There is concern that the current DRI for vitamin D (5μg/d) for women, ages 9-50 years, is inadequate to support desirable serum 25[OH]D concentrations (58).

Since vitamin D plays an essential role in calcium homeostasis and consequently skeletal health, athletes should be screened for possible vitamin D deficiency (40). Athletes in northern latitudes or indoor sports participants may be particularly at risk for inadequate vitamin D status and related skeletal issues (59).

In order to increase vitamin D levels, either limited sun exposure "twice a week between the hours of 10 a.m. and 3 p.m. on the arms and legs for 5-30 min, depending on season, latitude, and skin pigmentation" or dietary supplementation with 1,000-2,000 IU (20-40μg) vitamin D3 per day has been recommended by one author (59).

## ROLE OF HORMONES IN BONE DEVELOPMENT AND MAINTENANCE

### Pivotal role of sex steroids in bone health

The estrogen sex steroid hormones play an essential role in both the attainment as well as the maintenance of peak bone mass in premenopausal females (61). The chief estrogen hormone involved in skeletal physiology is estradiol, which is synthesized

predominantly by the ovaries and to a lesser extent by adipose tissue (62). Estradiol interacts with either α- or β-estrogen receptors present on osteoblasts or osteoclasts, acting overall to conserve bone mass (63). In the context of bone metabolism, the estrogen receptors together with their substrate estradiol, complex with particular deoxyribonucleic acid (DNA) response elements in the bone cell nucleus, controlling the transcription of relevant genes associated with bone functioning (62).

Estrogen receptor expression in bone tissue is complex, and estrogen-activated signaling sequences have been identified in osteoblasts, osteoclasts and osteocytes, as well as in the immune system (64). In addition, estrogen receptor function may be modulated in the presence of hormones such as growth hormone (GH) and thyroid hormone (TH), as well as polypeptide growth factors such as epidermal growth factor (EGF) and insulin-like growth factor-I (IGF-I) (64).

*Bone size in puberty and direct control of sex steroids*

Estrogen is involved in the growth spurt observed during puberty in adolescent females, resulting in an increase in skeletal size (65). The longitudinal growth of bones during puberty involves the complex interaction of endocrine signals including estrogen, GH, TH, IGF-I and vitamin D. These hormones and growth factors influence development, maturation and closure of the growth plate, influencing final stature.

*Anti-resorptive role of estrogen: Balance between modeling and remodeling*

The process of bone remodeling is tightly controlled in premenopausal women and involves close regulation of the anabolic activity of osteoblasts versus the bone resorptive

effects of osteoclasts (66). One of the critical regulatory factors involved in bone remodeling is the glycoprotein osteoprotegerin (OPG) secreted by osteoblasts as well as by certain immune cells (67). The protein factor OPG acts as a decoy receptor competing with the secretory protein, receptor activator of nuclear factor kappa B ligand (RANKL) for the receptor activator of nuclear factor kappa (RANK), located on immature osteoclasts (66). The activation and maturation of osteoclasts follows the interaction of the factor RANKL with the receptor RANK (67). The interaction between RANKL, RANK and OPG are currently regarded as key control areas in bone metabolism (64). Significantly, the ratio of OPG versus RANKL in osteoblasts is regulated by hormones such as estrogen, as well as by cytokines secreted by immune system T cells (66). Estrogen thus plays an essential role in maintaining bone mass by regulating osteoclast activity and rate of bone resorption (64).

In addition to the regulatory effect of estrogen on OPG expression in bone tissue, physical activity has also been noted to increase OPG levels in premenopausal women. A recent study involving premenopausal exercising women (age 24.2±1 years) reported that OPG levels were higher ($p$=0.005) in a group of exercising normally menstruating women (5.2 ±0.2pmol/l) versus exercising amenorrheic women (4.6±2pmol) (68). The authors suggest that depressed OPG expression in the amenorrheic subjects was a consequence of chronic estrogen deficiency related to hypothalamic amenorrhea in this group of women. The amenorrheic exercising group of women also had lower BMD values at the total body level as well as lumbar spine ($p$=0.015), compared with eumenorrheic subjects.

*Age-related bone loss*

The gradual cessation of estrogen secretion by the ovaries during menopause is accompanied by a more rapid decline in bone mass, particularly affecting trabecular bone (61). During the post-menopausal years, loss of the moderating effect of estrogen on bone resorption results in increased osteoclast activity on the endosteal surface of bone and consequent net bone loss. Inadequate calcium and vitamin D intake at this time may initiate secondary hyperparathyroidism, further contributing to bone resorption. Uncontrolled bone loss in post-menopausal women is associated with the risk of osteopenia, osteoporosis and bone fractures (69). However, increased weight-bearing and resistance activities in post-menopausal women have been noted to attenuate the loss of BMD.

**Other significant hormones required for bone maintenance**

In addition to the essential role played by estrogen in bone metabolism, a complex network of endocrine hormones and immune-system factors interact to sustain skeletal health (70). Some of the more important hormones involved in the development and maintenance of bone include leptin, insulin, GH, TH, and IGF-I (71).

*The role of leptin in bone health*

Studies on *in vitro* bone cell cultures have shown that the hormone leptin, secreted by adipose tissue, plays a significant role in the differentiation of osteoblasts and the suppression of osteoclastogenesis (72). Leptin receptors are expressed in peripheral

tissue such as bone cells, as well as in the central nervous system, where leptin may interact with hypothalamic and pituitary endocrine axes (71).

Studies have shown that amenorrheic athletes are prone to hypoleptinemia, which has been ameliorated by treatment with recombinant human leptin (73). In addition to increased levels of estradiol, TH and IGF-I, leptin treatment of a group of amenorrheic athletes resulted in higher levels of the markers osteocalcin and bone alkaline phosphatase ($p<0.001$) associated with bone formation (73). Bone loss in amenorrheic athletes has not typically been fully compensated by weight gain and restoration of menstruation (74). The loss of fat mass, and hence leptin-synthesizing capacity in amenorrheic athletes may be a significant factor resulting in bone loss in these individuals, suggesting possible leptin treatment (70).

## CURRENT EVIDENCE REGARDING EXERCISE AND BONE HEALTH

### Weight-bearing exercise in premenopausal women

Skeletal development is influenced by genetic disposition, physical activity, nutrition and endocrine factors (1). It is now well recognized that physical activity, particularly regular weight-bearing exercise, is a major positive contributor to bone development and maintenance (75). Although the exact nature of the biochemical signaling pathway leading to bone mineralization is not fully understood, there is considerable experimental support for bone accrual resulting from increased strain magnitude, frequency and rate of bone stimulation during weight-bearing exercise.

**Exercise recommendations throughout the lifecycle**

Regular physical activity is particularly beneficial in contributing to enhanced bone mass during the pubertal growth spurt in adolescent girls, when 40-50% of adult bone mass is accumulated (36). By maximizing bone mass during this developmental period, the prevalence of osteopenia and osteoporosis in later years may be lessened (76). The inclusion of weight-bearing exercise and resistance training in post-adolescent women has also contributed to osteogenesis and maintenance of bone health (1). While there is currently no gold standard prescription for exercise and bone health, there is experimental support for maximum osteogenesis following physical activities including multiple short loading-cycles, rather than sustained endurance training, in which there is saturation of the osteogenic response (1).

Finally, regular exercise, particularly resistance training, in post-menopausal women (age 60±5 years), has been shown to increase total and hip BMD, which is advantageous in this population group (69). Regular physical activity is therefore beneficial throughout the life cycle, and together with adequate nutrition, contributes to skeletal health (75).

# CHAPTER III
# METHODS

## SUBJECTS

The athletes in this study were eleven elite female middle- and long-distance runners, who were assessed in the Laboratory for Elite Athlete Performance at Georgia State University. This study represents a secondary assessment of existing data that were obtained between 1994 and 2009. Original data were obtained followed procedures approved by the Institutional Review Board (IRB) of Georgia State University. The current study was granted IRB approval on June 19, 2009 (Appendix A).

## ASSESSMENTS

The anthropometric parameters body mass (kg) and height (cm) were obtained on a standard physician balance beam scale. Body composition was obtained using dual-energy X-ray absorptiometry (DXA) and a LUNAR (Lunar Corporation, Madison, WI) model DPXL machine and software version 1.34 (43). Quality assurance tests were performed prior to assessment of each subject.

Bone mineral content (kg), areal BMD ($g/cm^2$), fat mass (kg) and lean mass (kg) were assessed from full-body DXA scans of the subjects. Each subject was assessed for bone parameters and soft tissue composition at baseline (series 1 data), as well as two subsequent measurements at an average interval of 1.1 years (series 2 data) and 2.6 years (series 3 data). One subject was not available for collection of series 3 data and an adjustment for unequal groups was made in statistical analysis. Subjects in this study

received nutrition-related counseling regarding daily and within-day caloric balance, as well as recommendations for daily calcium intake (43).

## STATISTICAL ANALYSIS

All data were analyzed using SPSS version 17.0. Differences between the three series of BMD and soft tissue measurements were compared using repeated-measures Model II ANOVA with Bonferroni *post hoc* test. Model II ANOVA was selected on account of the unequal sample size in series 3 (n=10). Correlations between BMD at specific sites were obtained using Spearman's rho coefficients for non-parametric data. Independent *t*-tests were used to determine differences in BMD between subject groups.

# CHAPTER IV
# RESULTS

## ANTHROPOMETRIC PARAMETERS

The subjects described in this longitudinal study were a group (n=11) of elite female runners who were assessed for bone density, as well as lean mass and fat mass parameters. The runners were assessed at baseline (series 1 measurement) and subsequently re-evaluated at approximately one year intervals (series 2 and series 3 measurements). The mean values (±SD) for age, total body mass, height, BMI, percent body fat and lean tissue mass are shown in Table 1.

**Table 1:** Anthropometric Data of Elite Runners

| | | Series 1 | | Series 2 | | Series 3 | |
|---|---|---|---|---|---|---|---|
| | | n = 11 | | n = 11 | | n = 10 | |
| | | Mean (SD) | | Mean (SD) | | Mean (SD) | |
| Anthropometric data | | | | | | | |
| | Age (years) | 24.59 | (4.41) | 25.7 | (4.44) | 28.14 | (5.94) |
| | Total Mass (kg) | 54.98 | (3.54) | 55.67 | (3.59) | 56.11 | (4.07) |
| | Height (cm) | 168.56 | (4.29) | 168.79 | (3.87) | 168.66 | (4.27) |
| | BMI ($kg/m^2$) | 19.34 | (0.97) | 19.52 | (0.82) | 19.71 | (1.19) |
| | | | | | | | |
| Body Fat (%) | | | | | | | |
| | Total Body Fat (%) | 13.97 | (2.96) | 14.57 | (3.51) | 16.01 | (4.28) |
| | Arms Fat (%) | 8.93 | (3.55) | 9.79 | (3.03) | 12.54 | (5.77) |
| | Legs Fat (%) | 16.74 | (3.73) | 17.28 | (4.05) | 18.90 | (5.31) |
| | Trunk Fat (%) | 13.35 | (2.83) | 13.95 | (3.52) | 15.16 | (3.56) |
| | | | | | | | |
| Lean Mass (g) | | | | | | | |
| | Arms | 4255.27 | (350.34) | 4248.64 | (280.06) | 4171.70 | (300.17) |
| | Legs | 15219.00 | (1391.47) | 15291.73 | (1155.66) | 15234.80 | (1372.90) |
| | Trunk | 22035.73 | (1674.92) | 22023.27 | (1392.38) | 21883.40 | (1677.41) |
| | Total | 44777.18 | (3195.65) | 44800.09 | (2598.90) | 44497.10 | (3140.59) |

The average age of the runners increased from 24.59 (±4.41) to 28.14 (±5.94) years over the duration of the study. The increase in age of the runners was accompanied by a corresponding increase in body mass (54.98±3.54 to 56.11±4.07 kg), while height parameters remained constant. The average BMI of the subjects increased from 19.34±0.97 to 19.71±1.19 kg/m$^2$ ($p$>0.05), and this change was largely due to the increase in total percent body fat (13.97±2.96% to 16.01±4.28%; $p$>0.05) since lean tissue mass remained almost constant, as shown in Table 1. In support of the percent body fat and BMI trend, the Spearman's rho coefficient for the relationship between BMI and percent body fat showed a strong positive correlation between these two parameters (r=0.573, $p$=0.07 for series 2; r=0.711, $p$=0.02 for series 3). The increase in regional and total fat mass as well as the increase in regional and total body fat per cent in the runners is depicted in Figures 1 and 2.

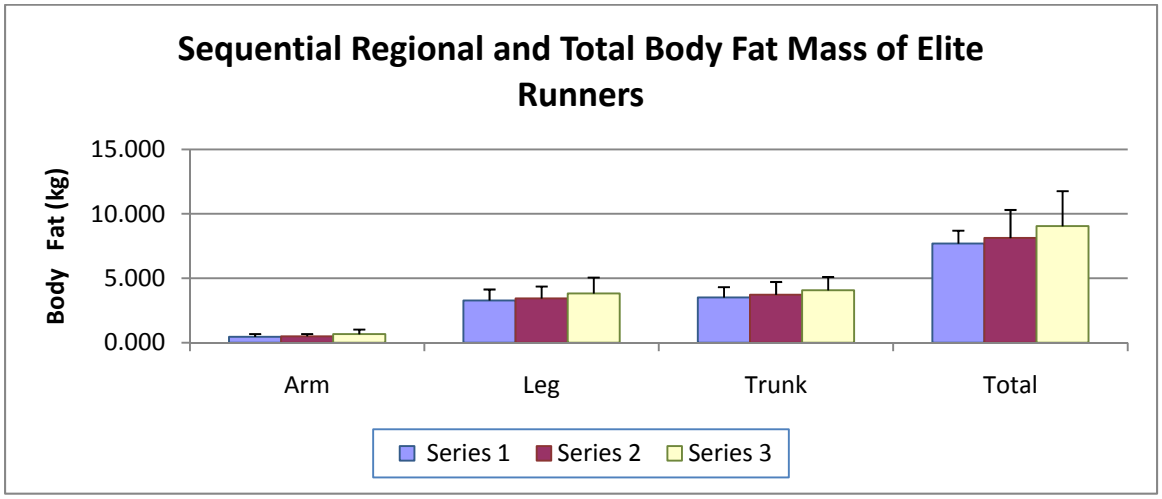


Figure 1

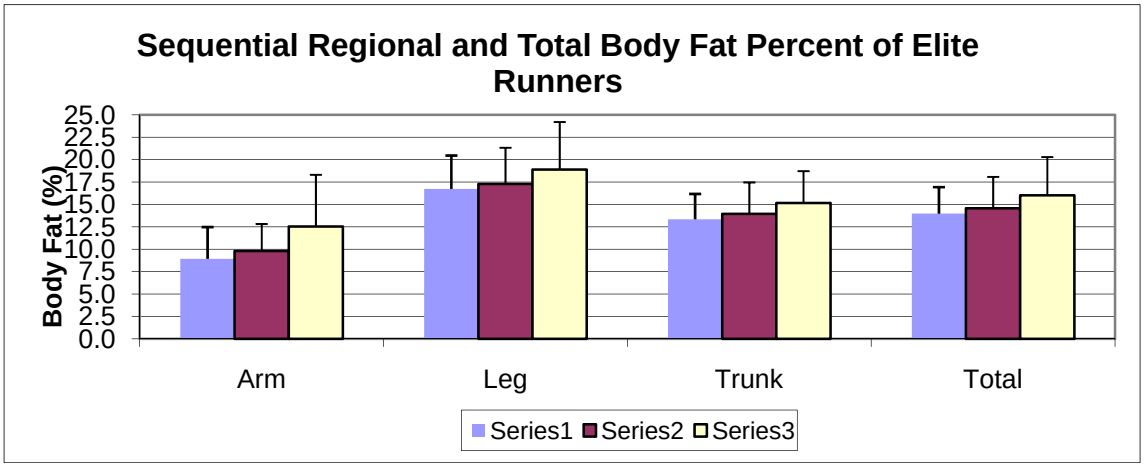


Figure 2

## BONE MINERAL DENSITY MEASUREMENTS OF RUNNERS

Measurement of BMC as well as regional and total BMD values was performed for the group of elite runners evaluated in this study. The maintenance of constant or slightly increased BMC values is shown in Figure 3 below.

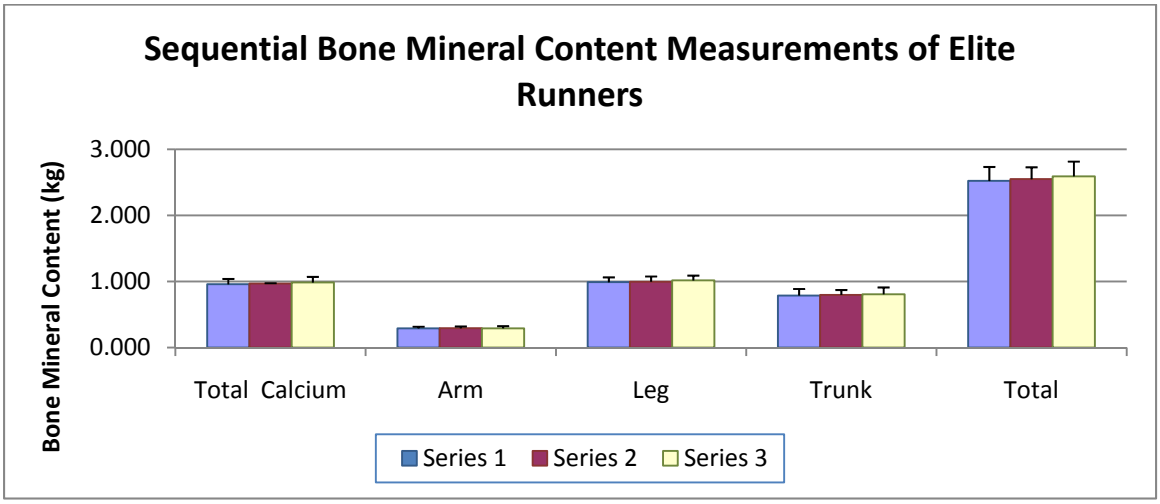


Figure 3

Total mineral calcium, leg, trunk and total BMC values showed a positive trend across series 1, 2 and 3, as shown in Figure 3.

The mean sequential areal BMD values of the elite runners are shown in Table 2. The regional measurements for head, arms, legs, trunk, ribs, pelvis and spine were assessed, as well as the value for total body BMD. Both regional and total BMD values increased over the study period. The average positive trend in regional and total BMD values is shown in Figure 4.

**Table 2:** Sequential Bone Mineral Density Measurements of Elite Runners

| | Series 1 | | Series 2 | | Series 3 | |
|---|---|---|---|---|---|---|
| | n = 11 | | n = 11 | | n = 10 | |
| BMD ($g/cm^2$) | Mean | (SD) | Mean | (SD) | Mean | (SD) |
| Head | 2.103 | (0.174) | 2.127 | (0.188) | 2.199 | (0.239) |
| Arms | 0.818 | (0.045) | 0.820 | (0.033) | 0.848 | (0.055) |
| Legs | 1.313 | (0.063) | 1.317 | (0.060) | 1.335 | (0.050) |
| Trunk | 0.938 | (0.055) | 0.943 | (0.046) | 0.956 | (0.054) |
| Ribs | 0.675 | (0.045) | 0.674 | (0.034) | 0.666 | (0.041) |
| Pelvis | 1.143 | (0.083) | 1.156 | (0.076) | 1.176 | (0.087) |
| Spine | 1.123 | (0.082) | 1.129 | (0.085) | 1.169 | (0.074) |
| Total Body | 1.165 | (0.049) | 1.172 | (0.049) | 1.197 | (0.052) |

The BMD values both within groups, as well as between groups (series 1, 2 and 3) were analyzed for variance (ANOVA) and showed no statistical significance between groups. The positive trend in BMD values over time, both regional, as well as total body BMD, is however biologically relevant, given the mean age of the elite athlete group which rose to 28.14 (±5.94) years at the end of the study period.

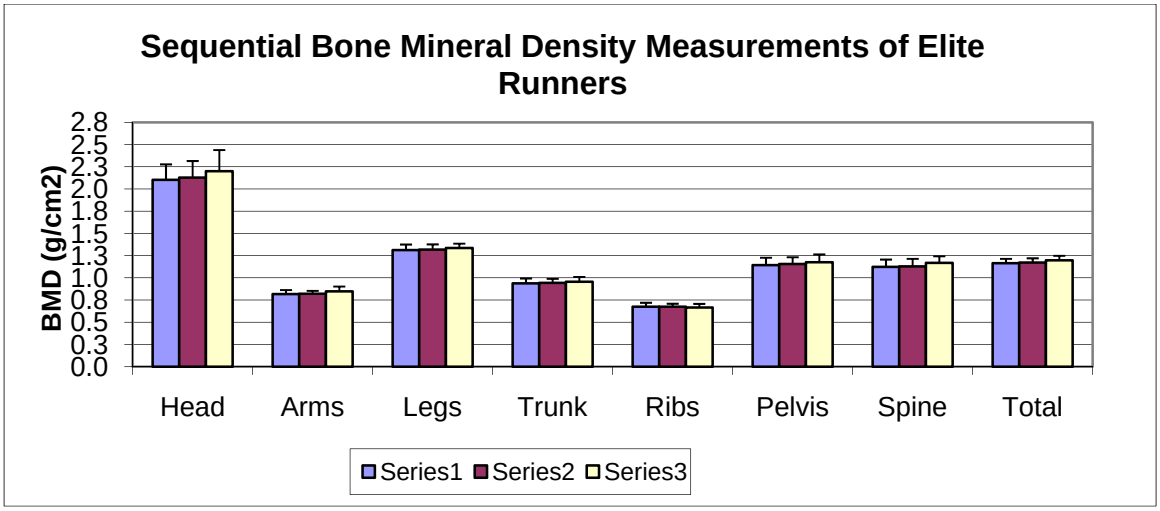


Figure 4

In order to illustrate the change in BMD parameters in the athlete group over the time course of the study, per cent change in regional and total BMD was calculated and is shown in Table 3 and Figure 5. Particularly notable were the small increases in trunk, pelvis and spine BMD values ($p$>0.05), which have previously been reported as problematic in runners (77). Although these changes in BMD were not statistically significant, the positive trend is biologically relevant.

**Table 3**: Average % Change in BMD in Elite Runners during study (g/cm$^2$)

| | S1 to S2 | S2 to S3 | S1 to S3 |
|---|---|---|---|
| Arms | 0.34% | 3.32% | 3.68% |
| Legs | 0.33% | 1.37% | 1.70% |
| Trunk | 0.51% | 1.42% | 1.94% |
| Pelvis | 1.08% | 1.78% | 2.88% |
| Spine | 0.49% | 3.52% | 4.02% |
| Total Body | 0.57% | 2.11% | 2.70% |

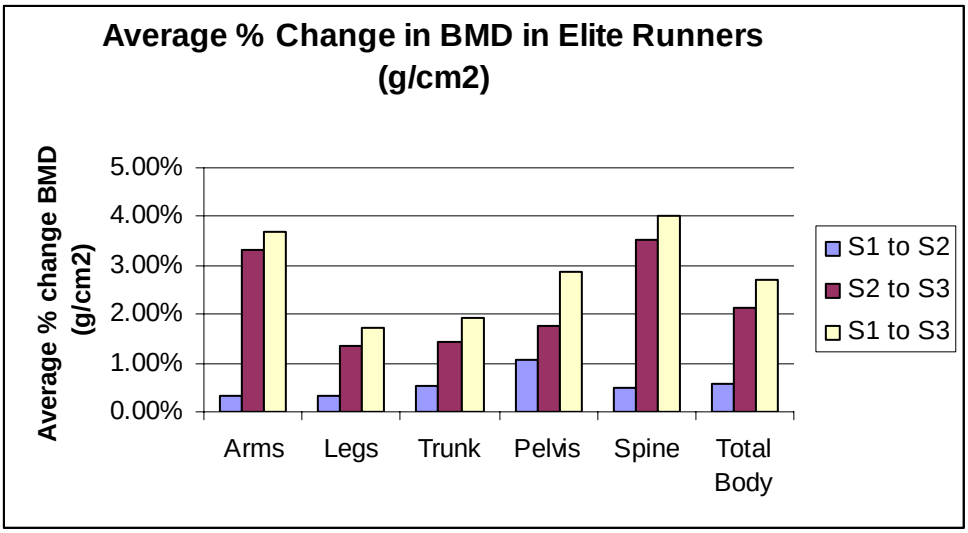


Figure 5

The percent changes in BMD values shown in Table 3 were computed from original BMD data corresponding to series 1, 2 and 3. The values described in Table 3 are averages. Since the study population was small (n=11), these changes represent a positive trend in BMD values, which is biologically relevant although not statistically significant.

Statistical analyses applied to the BMD data for the runners did, however, show some notable correlations for BMD values within the groups (series 1, 2 and 3). The BMD values for the lower body regions, notably spine, trunk and pelvis were significantly correlated within groups ($r=0.66$, $p<0.05$ to $r=0.93$, $p<0.01$), as shown in Table 4. These regions of the axial skeleton comprise predominantly trabecular bone and positive trends in BMD values in these anatomical areas are particularly relevant in a group of runners (77).

| **Table 4**: Spearman's Rho coefficients for Spine, Trunk and Pelvis in Runners | | | |
|---|---|---|---|
| | Series 1 | Series 2 | Series 3 |
| Spine/Trunk | 0.78 ($p$<0.01) | 0.78 ($p$<0.05) | 0.93 ($p$<0.01) |
| Spine/Pelvis | 0.66 ($p$<0.05) | 0.62 ($p$<0.05) | 0.93 ($p$<0.01) |
| Trunk/Pelvis | 0.92 ($p$<0.01) | 0.92 ($p$<0.01) | 0.93 ($p$<0.01) |

Further analysis of the Spearman's rho coefficients for the BMD data of the runners showed a significant correlation between the BMD values for total BMD and leg BMD in series 1, 2 and 3 measurements (r=0.70, $p$<0.05; r=0.83, $p$<0.01, and 0.78, $p$<0.01 respectively). The correlation coefficient for lean tissue mass or fat mass values and BMD values was not statistically significant in either series 1, 2 or 3.

## BONE MINERAL DENSITY VALUES AND BMI IN RUNNERS

Analysis of the baseline BMD data (series 1) associated with the runners was carried out based on the assumption of a healthy body weight corresponding to a BMI greater than 19.0 kg/m$^2$ (78). When subjected to this division, a majority of runners (n=7) had a BMI value greater than 19.0 kg/m$^2$, while the remainder of the group (n=4) had BMI values below 19.0 kg/m$^2$. Computation of an independent samples $t$-test for the equality of means between the two groups, 19.0<BMI>19.0 kg/m$^2$ showed significant differences in the BMD values at the trunk, pelvis and spine measurement sites of the runners ($p$<0.05). These regions of the axial skeleton have previously been described as subject to low BMD values in some runners (79).

## ***T*-SCORES, *Z*-SCORES AND PREVALENCE OF OSTEOPENIA IN RUNNERS**

According to current diagnostic criteria, the presence of osteopenia in an adult individual is defined by a DXA-associated *T*-score or *Z*-score between -1 and -2.5 (80). The reference population associated with *T*-scores is a gender-matched population at peak bone mass. On the other hand, *Z*-scores are gender-matched as well as age-matched. Two subjects in the present study were categorized as osteopenic, with *T*-scores less than -1 for arm, trunk, pelvis and spine. The prevalence of osteopenia in the present study population is therefore about 18%. There were no osteoporotic subjects in this study (*T*-score or *Z*-score less than -2.5) (80).

In order to assess the overall BMD status of the group of runners in the present study relative to a reference population at peak bone mass, the average *T*-scores of the group were computed, and are shown in Table 5 and Figure 6.

**Table 5:** Average T-Scores of Elite Runners

| | Series 1 | Series 2 | Series 3 |
|---|---|---|---|
| Arm | -0.34 | -0.30 | 0.09 |
| Leg | 1.74 | 1.79 | 1.99 |
| Trunk | 0.26 | 0.33 | 0.48 |
| Pelvis | 0.33 | 0.44 | 0.66 |
| Spine | -0.12 | -0.01 | 0.21 |
| Total | 0.50 | 0.58 | 0.89 |

Based on average *T*-scores, the group of runners had BMD values in the positive range, apart from small negative *T*-scores corresponding to arm and spine BMD in series 1 and 2. However, the arm and spine *T*-scores entered the positive range as BMD values

increased in series 3 as shown in Table 5. It is notable that the average *T*-scores for arm BMD were considerably lower than *T*-scores associated with leg BMD values in the runners. In fact, the average *T*-scores for leg BMD values were almost two standard deviations higher than leg BMD values for a reference population at peak bone mass.

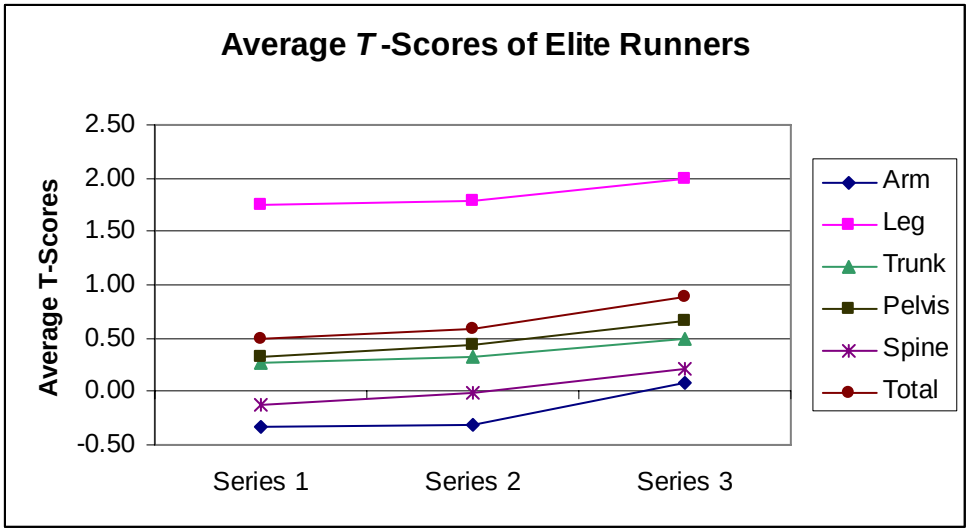


Figure 6

The average *Z*-scores associated with the study group was calculated and the data are summarized in Table 6 and Figure 7. Since the reference population for the BMD *Z*-score values is age-matched to the study group, the *Z*-scores are numerically greater than corresponding *T*-scores for the group. According to the *Z*-score assessment, only one individual was osteopenic with a *Z*-score of -1.31 for arm BMD.

| **Table 6**: Average *Z*-Scores of Elite Runners | | | |
|---|---|---|---|
| | | | |
| | Series 1 | Series 2 | Series 3 |
| Arm | -0.11 | -0.09 | 0.24 |
| Leg | 2.17 | 2.19 | 2.36 |
| Trunk | 0.95 | 1.25 | 1.13 |
| Pelvis | 1.00 | 1.08 | 1.25 |
| Spine | 0.35 | 0.36 | 0.62 |
| Total | 0.94 | 0.99 | 1.28 |

The average Z-scores shown in Table 6 suggest that the arm BMD values of the study group were low when compared to an age-matched reference population, while leg BMD values were more than 2 standard deviations greater than the reference group.

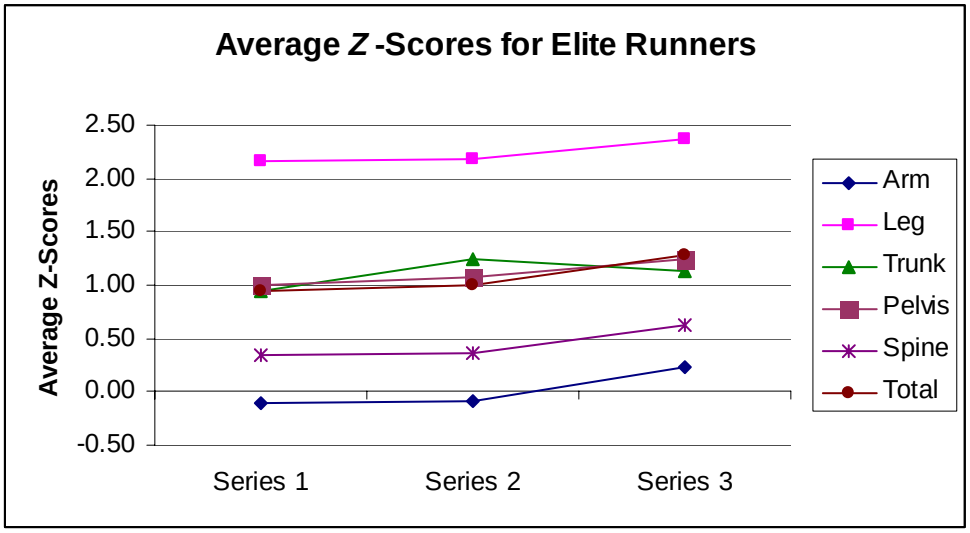


Figure 7

## OSTEOPENIC SUBJECTS AND BMI

Two subjects A and B, examined during this study were categorized as osteopenic, with *T*-scores and *Z*-scores in the negative range for arms, trunk, pelvis and spine. Osteopenia in young adults is defined by a *T*-score between -1.0 and -2.5 SD below the mean BMD value for a young adult population (81). The *T*-scores, *Z*-scores and BMI values of these two individuals was examined and is outlined below.

Subject A was osteopenic with respect to arm BMD values, as reflected by arm *T*-scores and *Z*-scores (series 1) shown in Table 7, Figure 8 and Figure 9.

**Table 7:** BMI Data, *T*-Scores and *Z*-Scores of Osteopenic Subject A

| | *T*-Score | | | *Z*-Score | | |
|---|---|---|---|---|---|---|
| | Series 1 | Series 2 | Series 3 | Series 1 | Series 2 | Series 3 |
| Arms | -1.49 | -0.74 | -0.97 | -1.31 | -0.62 | -0.78 |
| Trunk | -0.72 | -0.09 | -0.04 | -0.19 | 0.29 | 0.52 |
| Pelvis | -0.78 | 0.04 | 0.06 | -0.27 | 0.40 | 0.60 |
| Spine | -0.75 | -0.35 | -0.39 | -0.39 | -0.09 | -0.01 |
| Total | -0.26 | 0.41 | 0.40 | 0.08 | 0.65 | 0.76 |
| | | | | | | |
| BMI ($kg/m^2$) | 18.65 | 19.39 | 18.50 | 18.65 | 19.39 | 18.50 |

As subject A gained weight, as reflected in an increase in BMI from 18.65 $kg/m^2$ to 19.39 $kg/m^2$ (series 1 to series 2), regional and total *T*-score and *Z*-score values showed a positive trend, which remained almost constant for trunk, pelvis, spine and total BMD. However, the arm BMD *T*-score and *Z*-score for subject A became more negative (series 3) as the BMI value decreased to 18.50 $kg/m^2$. The trend in *T*-scores and *Z*-scores for subject A is shown in Figure 8 and Figure 9.

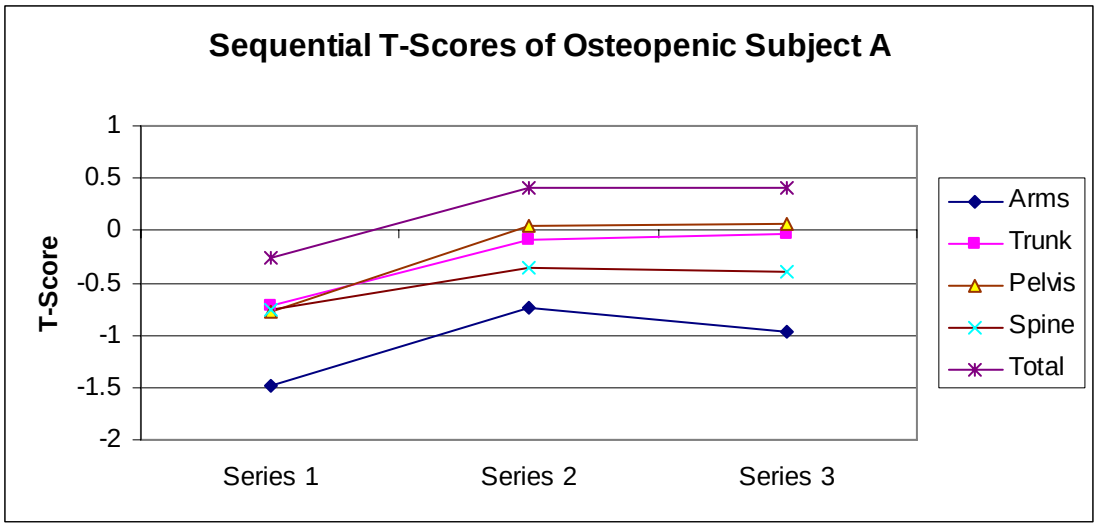


Figure 8

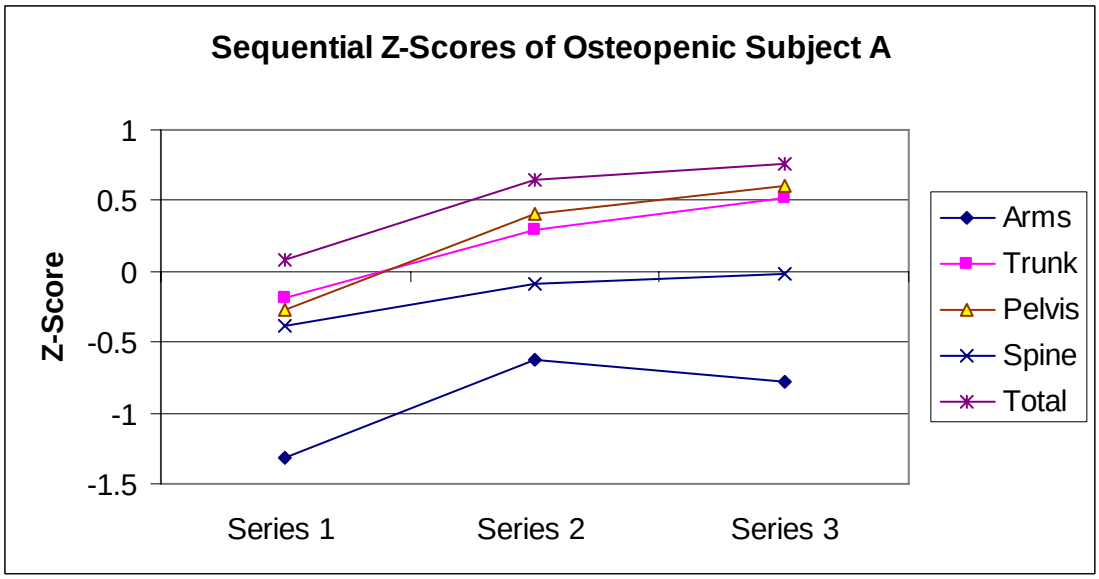


Figure 9

The trend in BMD for subject A was also computed as percent change in regional and total BMD values, and these changes are shown in Table 8 and Figure 10. The arms BMD declined by approximately 2% between series 2 and series 3. As depicted in Figure

10, the final arm BMD value (series 3) is however, approximately 6% higher than the baseline BMD measurement (series 1). Similarly, there is a positive overall trend in percent change corresponding to spine BMD values for subject A, as shown in Figure 10.

**Table 8:** % Change in BMD in Osteopenic Subject A ($g/cm^2$)

| | S1 to S2 | S2 to S3 | S1 to S3 |
|---|---|---|---|
| Arms | 8.13% | -2.29% | 5.65% |
| Trunk | 5.18% | 0.22% | 5.41% |
| Pelvis | 7.90% | 0.18% | 8.14% |
| Spine | 5.41% | -0.55% | 4.83% |
| Total | 4.89% | -0.09% | 4.80% |

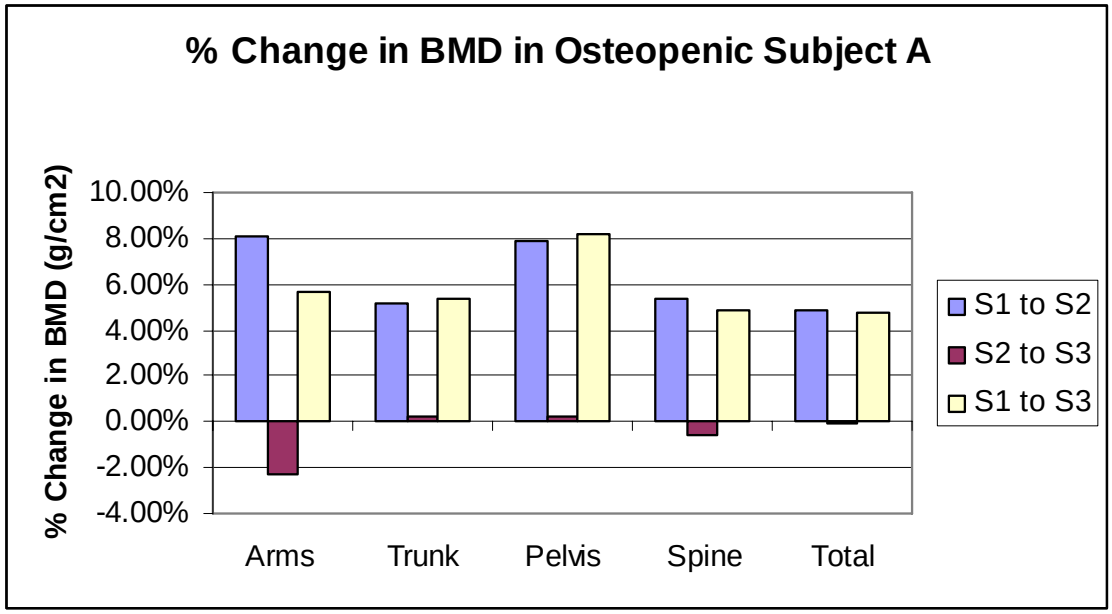


Figure 10

Subject B was osteopenic with respect to arms, trunk, pelvis and spine BMD values, as reflected by corresponding *T*-scores shown in Table 9 and Figure 11. In general, the *T*-scores for subject B became more negative over the study period suggesting possible involvement of the Female Athlete Triad (82). The *Z*-scores for subject B, which were gender- and age-matched, did not however enter the osteopenic range for subject B, as shown in Table 9 and Figure 12.

**Table 9:** BMI Data, *T*-Scores and *Z*-Scores of Osteopenic Subject B

| | T-Score | | | Z-Score | | |
|---|---|---|---|---|---|---|
| | Series 1 | Series 2 | Series 3 | Series 1 | Series 2 | Series 3 |
| Arms | -0.82 | -0.75 | -1.04 | -0.42 | -0.39 | -0.67 |
| Trunk | -1.04 | -0.67 | -0.98 | 0.20 | 0.42 | 0.17 |
| Pelvis | -1.09 | -1.01 | -1.33 | 0.11 | 0.04 | -0.22 |
| Spine | -1.20 | -0.98 | -0.71 | -0.36 | -0.24 | 0.07 |
| Total | -0.37 | -0.26 | -0.49 | 0.42 | 0.44 | 0.25 |
| | | | | | | |
| BMI ($kg/m^2$) | 17.70 | 18.58 | 18.15 | 17.70 | 18.58 | 18.15 |

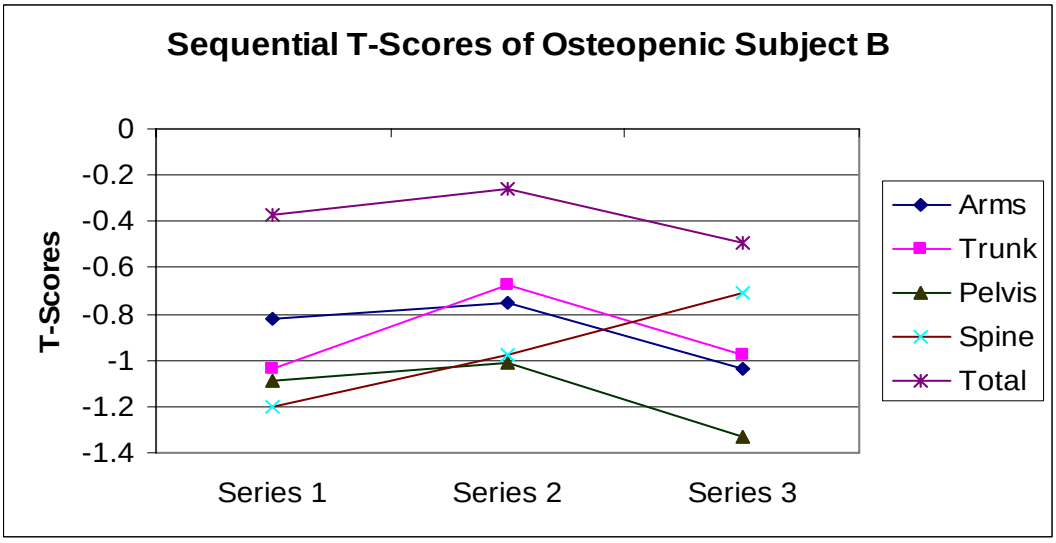


Figure 11

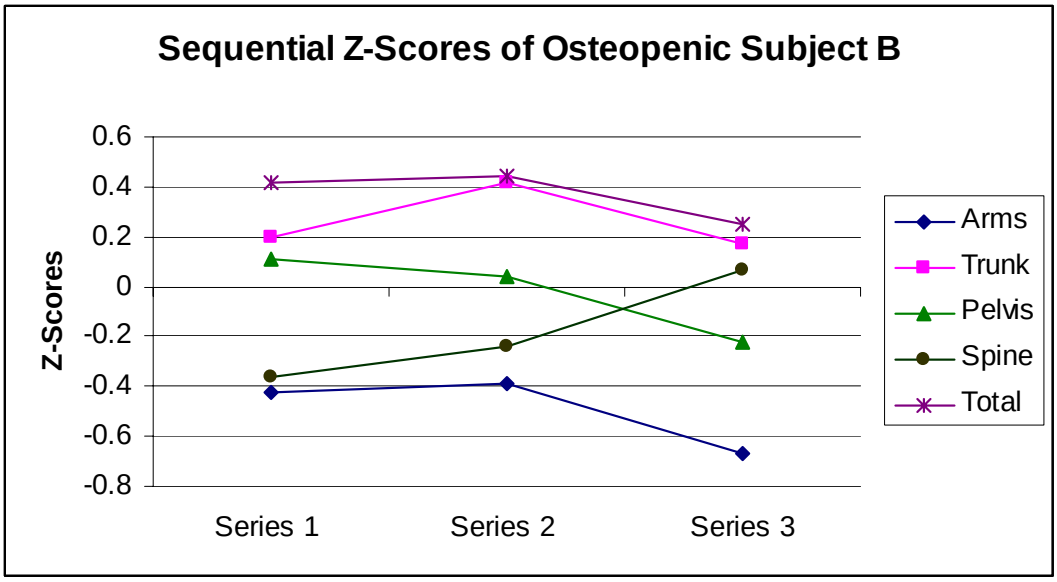


Figure 12

The trend in BMD values for subject B was also computed as per cent change in regional and total BMD values, and these changes are shown in Table 10 and Figure 13. The arms, pelvis and total BMD values for subject B declined over the duration of the study, as shown in Figure 13. Interestingly however, the spine BMD values showed a positive trend, as depicted in Figure 13.

**Table 10:** % Change in BMD in Osteopenic Subject B ($g/cm^2$)

| | S1 to S2 | S2 to S3 | S1 to S3 |
|---|---|---|---|
| Arms | 0.77% | -3.05% | -2.31% |
| Trunk | 3.19% | -2.52% | 0.59% |
| Pelvis | 0.80% | -3.17% | -2.39% |
| Spine | 3.29% | 3.79% | 7.21% |
| Total | 0.82% | -1.63% | -0.82% |

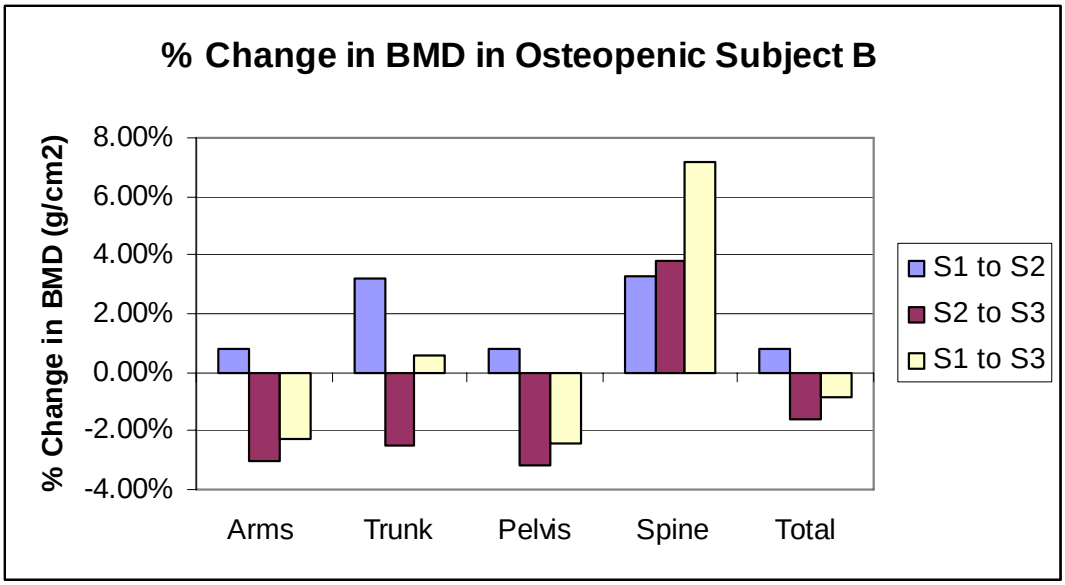
% Change in BMD in Osteopenic Subject B
% Change in BMD (g/cm2)
8.00%
6.00%
4.00%
2.00%
0.00%
-2.00%
-4.00%
Arms
Trunk
Pelvis
Spine
Total
S1 to S2
S2 to S3
S1 to S3

Figure 13

# CHAPTER V
# DISCUSSION AND CONCLUSIONS

## BONE MINERAL DENSITY AND BODY COMPOSITION IN RUNNERS

The group of elite runners assessed for this study demonstrated an overall positive trend in BMC and BMD parameters. Over an approximately two-year period, the runners showed an average 2-3% increase in total BMD, as well as positive trends in regional BMD values. The mean age of the study group at the conclusion of the study was 28.14 (± 5.94) years, which is past the age corresponding to peak bone mass in premenopausal women (78). However, different regions of the skeleton mature at slightly different rates in premenopausal women, with peak hip BMD being reached in late adolescence, and peak lumbar spine and total BMD being attained at approximately age 20 years in this population group (76). Notably, between the ages of 18 and 23 years, the amount of bone mass accrued may only be of the order of 2 to 3% (76). This is similar to the order of magnitude of bone accrual observed in the elite runners group. While the positive trend in BMD in the runners was not statistically significant, the result was however biologically relevant, since bone accrual during adolescence and early adulthood is protective against fractures in later years (78).

The average BMI of the runners increased from 19.34 (±0.97) $kg/m^2$ to 19.71 (±1.19) $kg/m^2$ over the duration of the study. Since the height of the group remained constant, the average increase in total body mass in this study group was an important contributor to the positive trend in BMD values. Studies have shown that body mass is a critical

factor associated with bone mass (83). The positive association of body mass, and therefore BMI with skeletal mass has been ascribed to the load-bearing contribution of enhanced lean mass as well as fat mass toward bone accrual. The increase in BMI of the runners during this study also suggested that the calorie intake of the group, on average, was adequate in terms of energy availability requirements for exercise as well as normal metabolic activity (41).

The increased BMI status of the runners was highly associated with an increase in per cent body fat in the group ($r=0.711$, $p=0.02$). The average per cent body fat increased from 13.97 (±2.96)% to 16.01 (±4.28)% over the duration of the study. Since lean tissue mass in the runners remained almost constant, the increase in regional and total body fat was the primary contributor to increased body mass. Both lean tissue mass as well as fat mass have been recognized as contributors to BMD status (84). However, unlike the present study, the contribution of lean tissue mass to bone mass accrual was found to be more significant in endurance-trained athletes in one study.

In support of the current study, fat mass has been reported as a significant determinant of whole body BMD in premenopausal women, not only for the weight-bearing association with bone, but also as a source of hormones such as estrogens and leptin (85). In the present study, increased fat mass may therefore have also contributed to enhanced BMD status via release of these endrocrine factors. One study has described a similar association between plasma leptin levels and lumbar BMD values in endurance-trained female athletes (86).

## RUNNERS AND SITE-SPECIFIC BMD TRENDS

The activity of running at the elite level appears to be associated with an overall positive effect on the BMD status of the present group of athletes. The average regional and total BMD values of the group showed a positive trend over the study period, with increases of 2 to 4 per cent in the appendicular and axial skeleton. Although menstrual history of the runners was not available for this study, other indicators of health status, such as increased body mass, infer that, on average, the runners were mindful of adequate caloric intake which was emphasized during dietary counseling received during the DXA screening process. Inadequate nutrition has been associated with menstrual disorders and low bone density status in elite female runners (87).

Middle- and long-distance running has been classified as a medium impact activity in terms of its overall influence on bone mass (5). However, it was observed that BMD measurements at the lumbar spine ($L_2$-$L_4$) were relatively low in athletes engaged in medium-impact sports, including running (5). It is notable in the present study that there was a positive trend in spine BMD, as well as trunk and pelvis BMD values. Spine, trunk and pelvis represent skeletal regions comprising predominantly trabecular bone, which is subject to higher turnover and bone loss than denser cortical bone (88). In one study, loss of trabecular bone accelerated in runners who were amenorrheic and therefore in a hypoestrogenic state (79).

The leg BMD values associated with the present athlete group were relatively high, as reflected in the average positive *T*-scores and *Z*-scores, which were approximately two standard deviations greater than leg BMD values of reference populations. The leg BMD values in the current study were associated with enhanced bone mass in the legs of the

runners, due to repeated leg muscle use during training and performance, as well as the impact-enhancing effect of running on leg bone mass (77). Adequate calcium intake has also been associated with increased leg BMD in runners (34). The enhanced leg BMD status suggested that this group of runners would be less prone to fracture risk related to endurance running. One study has shown that amenorrheic runners had significantly lower BMD values in the femoral and tibial sites, which led to osteoporotic stress fractures at these sites (79).

The leg BMD values of the runners were also enhanced relative to arms BMD, as reflected by the average arms *T*-scores and *Z*-scores, which were negative or low positive. The low arms BMD values were probably related to the site-specific enhancement of leg BMD associated with running (34). Additional factors which affect the appendicular skeleton in runners have been reported, including distances run per week, as well as the number of years of training (34). Whereas running increased distances per week was associated positively with arms and legs BMD values, additional years of training negatively impacted arms and lumbar spine BMD ($p<0.001$) (34). It is therefore possible that in the present group of runners, length of training was a factor negatively affecting arms BMD.

## PREVALENCE OF OSTEOPENIA IN RUNNERS

A sub-group of runners (n=4**)** was identified with BMI less than 19 kg/m$^2$. These individuals had low BMD values at the trunk, pelvis and spine measurement sites compared with the higher BMI>19 group (n=7) ($p<0.05$). However, only two individuals in the low BMI sub-group, subject A and B were identified as osteopenic, with *T*-scores

less than -1 in the arm, trunk, pelvis and spine regions. The prevalence rate of osteopenia in the entire group of runners studied (n=11) was 18%, which approximates the rate reported for osteopenia among elite athletes (89).

Subject A was initially osteopenic with respect to arms BMD, and the arms *T*-score and *Z*-score associated with this individual remained in the borderline osteopenic range. The spine *T*- score for subject A was also in the low range initially, but increased over the course of the study. However, subject B remained osteopenic throughout the study, as reflected by *T*-scores for arms and pelvis. The *T*-scores for subject B suggest the presence of the Female Athlete Triad syndrome in this athlete, which has been widely reported among elite female runners (82). It is interesting that the *Z*-scores for subject B, which are gender-matched and age-matched, are not suggestive of osteopenia. The lack of concordance of *T*-scores and *Z*-scores is disturbing in this case, since a missed diagnosis of osteopenia might lead to deterioration of bone health (80). A recent review has addressed the significance of inconsistencies in calculation and interpretation of T-scores and Z-scores related to DXA measurements (80).

The relatively low BMI values (BMI<19 $kg/m^2$) as well as the existence of osteopenia in subject A and B implies that the caloric intake of these two runners was inadequate based on energy needs related to training and performance in these individuals (41). Although the remaining two subjects in the low BMI group demonstrated relatively low BMD measurements at trabecular trunk, pelvis and spine sites, these two subjects were not osteopenic. The activity of running may have exerted a positive effect on bone accrual in these individuals (76).

## CONCLUSIONS

The group of elite runners in this study exhibited overall positive skeletal health, as reflected by increased total as well as regional BMD values. The positive trend in BMD values was associated with an increase in BMI related to increased fat mass, which contributed to bone mass via a weight-bearing effect. In addition, increased adipose tissue was a source of endocrine hormones such as estrogen and leptin, which contribute to bone accrual and maintenance.

The increase in average BMI of the runners suggests that the caloric intake of the group was mostly adequate in terms of increased sports-related energy expenditure. However, the presence of osteopenia in two of the subjects was likely the result of some measure of low energy availability due to inadvertent or deliberate low calorie intake. The early identification of osteopenia is important, since low BMD has been widely associated with runners, and may lead to stress fractures if left untreated. The need for periodic monitoring of bone density values and nutrition counseling is an important aspect of skeletal health in this group of athletes.

There were several inherent limitations in this study. The small sample size (n=11) limited the usefulness of some statistical analyses. There was also a lack of randomness in the sample, and the absence of a control group, which limits the general applicability of the results to all runners. However, this study suggests that the activity of running in a group of elite athletes contributed towards a positive effect on bone health provided that caloric intake was adequate.

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**Principal Investigator:** Dan Benardot **Current Status:** Approved

**Admin Assigned:** Susan Vogtner **Last Activity:** 06/10/2010 - Continuing Review #1 for H09538 Letter Recorded

**Committee Assigned:** COMMITTEE 1 **Original Approval Start:** 06/19/2009

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**Associated Research Personnel**

| Name | Role | Certification |
|---|---|---|
| | | CITI Training: On-line Training for the Protection of Human Research |
| | | Subjects (Approved): August 12, 2005 - August 11, 2008 >> ! This |
| Dan Benardot | PI | certification may expire before the protocol ! |
| | | CITI Refresher: On-lining Training for the Protection of Human Research |
| | | Subjects (Approved): August 9, 2008 - August 8, 2011 |
| Maureen | Student | CITI Training: On-line Training for the Protection of Human Research |
| Cartoon | PI | Subjects (Approved): January 4, 2009 - January 3, 2012 |

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