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EFFECT OF PHYSICAL ACTIVITY ON BONE MINERAL ACCRETION
IN ADOLESCENT FEMALES:
A FOUR YEAR LONGITUDINAL STUDY

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
The Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

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ABSTRACT

The weight-bearing of physical activity (PA) is often promoted to increase bone health. One goal of this study was to design a five-year retrospective method of measuring PA across a spectrum of normal activities appropriate for use an in ongoing longitudinal study of bone mineral accretion in adolescent females. Additionally, we modeled the contribution of PA in bone mineral accretion over four years comparing time since menarche and age models. The relationship of athletic menstrual disturbances to bone mineral accretion and the potential role of leptin in predicting these menstrual disturbances were also evaluated.

The modified questionnaire was sent to 314 subjects who had completed the first four years of the study (1992-1996). Test-retest studies were completed on 26 of the 267 respondents to document reliability. Questionnaire Spearman coefficients ranged from r=0.587 to r=0.928 with only the 1992-93 values below r=0.84. Questionnaire validity was measured by correlation with biannual percent body fat and METs/kg/day. Each reported activity was assigned a weight-bearing value using the published body weight impacts of a few activities and all reported activities categorized. The annual and mean osteogenic indicators (Ost) were derived using these values.
Annual and average Ost consistently appeared as significant predictors of bone density. The skeletal benefits gained from participating in rigorous sporting events were not linear as Ost was best modeled as a quadratic variable in the total body models. We predicted the additional annual bone mineral accretion for subjects participating in 300, 8000 and 13000 annual Ost to be 1, 26.3 and 42.8 grams of bone mineral, respectively. This conferred a 10% advantage for the 13000 Ost subject over inactive subjects considering normal accretion (40-400 grams). We did not identify bone mineral accretion disadvantages in athletes with menstrual irregularities. Leptin was less predictive than percent body fat when athletic menstrual disruption was modeled with logistic regression.
Dedicated to Balance in Life and the Future
ACKNOWLEDGMENTS

I am grateful to Jamie for her commitment to make this chapter of my life possible with her relentless emotional and financial support.

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<td>DXA</td>
<td>dual x-ray absorptiometry</td>
</tr>
<tr>
<td>BMC</td>
<td>bone mineral content (measured in grams)</td>
</tr>
<tr>
<td>BA</td>
<td>bone area (measured in cm$^2$)</td>
</tr>
<tr>
<td>BMD</td>
<td>bone mineral density (measured in grams/cm$^2$), may also be referred to as bone mineral areal density</td>
</tr>
<tr>
<td>TB</td>
<td>total body</td>
</tr>
<tr>
<td>UD</td>
<td>ultra distal radius site (primarily trabecular)</td>
</tr>
<tr>
<td>33% distal</td>
<td>most distal third of the radius (primarily cortical)</td>
</tr>
<tr>
<td>Ward's triangle</td>
<td>area in proximal femur commonly measured as trabecular site, may be early response area, thus early predictor for hip fracture</td>
</tr>
<tr>
<td>femoral neck</td>
<td>area between trochanters and head of femur commonly measured as cortical site, area of devastating hip fractures</td>
</tr>
<tr>
<td>trochanter</td>
<td>bony protuberance on the outside of hip for great muscle attachment</td>
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<tr>
<td>Volumetric density</td>
<td>three dimensional evaluation of bone mineral (g/cm$^3$), independent of size</td>
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<td>BMI</td>
<td>body mass index as calculated with weight and height to index obesity wt (kg)/ht$^2$ (m)</td>
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<td>Ost</td>
<td>units to represent osteogenic intensity of physical activity</td>
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eumenorrheic (eu) at least 10 menstrual cycles per year, at least 5-6 per 6 months
amenorrheic less than 3 menstrual cycles per year and none in past 6 months
oligomenorrheic 3-8 menstrual cycles per year or cycles are longer than 38 days long 2-4 menstrual cycles in the past six months
* amenorrheic and oligomenorrheic subjects will be grouped together as menstrual dysfunction group (dys)
time since menarche number months before or after the date of menarche, this can be calculated for each visit and plotted as a time (x) variable
bone mineral matrix of hydroxyapatite crystals that give bone structure (assumed to be 38% calcium)
osteoblasts cells responsible for making new bone
osteoclasts cells responsible for bone resorption
cortical bone compact, dense bone typical of that found in the shaft of long bones
trabecular bone bone arranged in a network of trabeculae to offer maximum support with less weight, typical of that found in the end of long bones or in the vertebral bodies
periosteum outside lining of bone
endosteum inside lining of bones, lines medullary canal
epiphysis growth plate area and end of bone
diaphysis shaft of long bones
metaphysis area where bone widens between epiphysis and diaphysis
CHAPTER 1

INTRODUCTION

It is commonly accepted that increased physical activity enhances many aspects of health, including bone health (bone density). This widely accepted positive relationship arises from numerous cross-sectional studies evaluating the bone density of athletes or physically active individuals in comparison with their less active or sedentary counterparts (1-15). To the contrary, studies which do not find strong associations between physical activity and bone health indicators cite more contributory factors as the determinants or predictors of bone density, such as genetics, body weight, hormonal status or nutrition (calcium intake) (16-21). To further cloud the picture, a few laboratories have associated very high levels of training with a decreased bone mineral density (10, 22). Actually, there are few longitudinal studies of significant length to determine the role of exercise and physical activity (PA) in growth, development and bone mineral acquisition (23-25). Many 2-3 year longitudinal studies that looked at the plausible effect of physical activity on bone used primarily adult populations and excluded adolescent subjects. The remodeling of adult bones should be viewed differently than the growth and modeling of the pubescent skeleton (26, 27). Current
literature suggests that the effects of PA may be heightened during bone growth and skeletal development (bone modeling) (28-34).

1.1 Osteoporosis

The burgeoning disease process of osteoporosis continues to plague many post-menopausal women (type I) and the ever-increasing geriatric segment of our population (type II). Type I osteoporosis describes the onset of bone loss in the immediate post-menopausal time period when the female estrogen levels are waning. Type II osteoporosis describes the clinical syndrome associated with the geriatric bone loss as it affects males and females after the sixth decade. Osteoporosis affects 28 million individuals and is projected to require 60 billion dollars annually in medical costs by the year 2020 (35). Osteoporosis brings with it many physical disabilities, psychological strains and financial burdens for the afflicted patients such as the inability to perform housecleaning chores, enjoy social environments and activities, or adhere to fixed income budgets (36). The rising costs of health care and judgments of third party managed-care payers promise to exacerbate the social and financial strains.

Current management of overt osteoporosis includes efforts to slow bone loss or restore bone mineral after loss, but these interventions would be unnecessary if the disease could be better prevented. Primary prevention of involutional osteoporosis calls for maximal bone mineral accretion (peak bone mass) during the developmental years to offset menopausal and aging losses (4, 34, 37-47). Attainment of peak bone mass is thought to be achieved when an individual maintains an internal environment which promotes attainment of the
full genetic (skeletal) potential and is defined as the highest level of bone mass achieved as a result of normal growth (48, 49). Literature suggests that the menopausal bone loss is similar in women so that those who have more bone before menopause will have more bone throughout the aging process (50, 51).

Research has focused on identifying the amount of bone loss or bone threshold which marks significant fracture risk so that preventing attainment of that critically low level would decrease the relative risk of fractures (52, 53). Osteoporosis in females is usually defined as bone mineral density (BMD) 2.5 standard deviations below the level considered normal in young, adult women (54). This deficient level of bone mineral has been shown to significantly increase the risk of debilitating hip, wrist or spine fractures (26, 39, 44, 55-61). Riggs contends that for every one standard deviation decrease in bone density there is a two-fold increase in fracture risk (62). Melton et al recently revisited similar criteria for fracture prediction in men (53).

Attainment of adequate peak bone mass would allow for normal menopausal and aging losses without allowing the individual to reach the threshold associated with the increased fracture risk (49). It is imperative to further define the roles of exercise and physical activity during adolescence as modifiable environmental factors which may help prevent osteoporosis through achievement of greater peak bone mass.

1.2 On-going Peak Bone Mass Study

The current study began in 1992 to study the influential factors which determine peak bone mass in adolescent females through puberty. The study
was designed as a double-blind placebo calcium intervention trial and has included bone density (DXA), biological specimens (urine, feces, blood), dietary and physical activity reports and many anthropometrical parameters. The calcium intervention subjects have been followed every six months while a group of subjects with high habitual calcium intake have been followed annually. The primary investigator has not been satisfied with the previous measurement of energy expenditure as a reflection of PA using the Bouchard tool and initiated this project to investigate other methods of evaluating PA.

1.3 **Project Goals**

The main objective of this project was to adopt or create a five year retrospective questionnaire and methodology to adequately evaluate the relationship between physical activity and bone mineral status after four years of study in adolescent females. Current literature suggests that higher impact activities are more osteogenic, so it was desirable to create a scoring system to reflect the impact nature or osteogenic potential of the activities. The need for a scoring system that works across a variety of mixed activities and incorporates the time spent with the impact characteristics of the activity was the main goal of this questionnaire and method. Comparison of this scoring system with the more traditional measures of time, metabolic equivalents (METs) and athlete/non-athlete categories was to be carried out using the same historical information. The newly collected information would be compared with the physical activity information already collected in this four-year study. Proper documentation of the questionnaire/impact method included completion of
reliability and validity studies. As with any questionnaire, some subjects chose not to participate, and the non-respondents were compared with the respondents to help ensure random participation.

Due to the double-blind nature of the ongoing study, it was not possible to evaluate thoroughly the potential role or bias with regard to calcium intake. The mean calcium intakes of subjects in the high and low physical activity categories would be compared to ensure differences were not significant. Thorough evaluation of calcium intake was not a primary objective of this project but was necessary to try to minimize bias and limitations.

Another goal of this project was to evaluate four years of longitudinal data to assess the influence of physical activity and sports on skeletal development in pubertal females. Numerous cross-sectional studies have found exercise to have a positive influence on bone in adults, but there are very few longitudinal studies more than two years long to adequately address the influence of physical activity in female adolescents. Cross-sectional studies of athletes commonly identify greater bone mineral density in athletes compared with non-athletic controls, but these studies are limited because they are not able to measure the bone density in the subjects before participation. It is plausible that subjects with greater skeletal endowment choose to participate in sport more often than their skeletal inferiors. This project was to allow for identification of athletes from non-athletes. Within this athlete/non-athlete analysis, we also wanted to compare four-year athletes with athletes who appeared to initiate sport after the first year of the study (three-year athletes) to examine the potential bias issue.
Traditional longitudinal studies of bone mass have evaluated subjects over time using age as a covariate. Recent research indicates that the evaluation coinciding with puberty may reveal the most information about bone growth and modeling in adolescents. In females, it is reasonable to define the time around puberty with menarche. It was a goal of this project to compare modeling across time since menarche with the traditional modeling across age. Both the age and time since menarche models were to allow for the normal increases and decreases (not a linear function) of very rapid growth and bone mineralization. Additionally, we wished to look for a potential heightened effect of exercise in the time period around menarche. These objectives will be reflected in the time since menarche model.

This project lends itself to evaluation of athletic menstrual issues due to the retrospective collection of sport activity data. This objective was to compare athletes who had normal menstrual cycles over three years with athletes who had experienced abnormal menstruation as an assumed result of their training. The notion that athletic amenorrhea is associated with low levels of body fat is common, and the new leptin research has associated low levels of leptin with amenorrheic athletes. The final objective of this study was to see if the levels of leptin (and other energy status variables), as measured annually, were associated with menstrual status of the four-year athletes during puberty.
1.4 **The Questions for This Project**

1) Did the adapted historical tool appear to collect valid and reliable information in these subjects?

2) Did subjects who reported higher levels of physical activity or sport participation have higher rates of change in bone mass measurements? Were the impact characteristics and the time spent in sports important to the change in bone mass? Were these differences present before initiation of sport activity?

3) What impact did participation in higher levels of physical activity have on bone measures with respect to menarche? Were the responses to exercise heightened around the onset of menarche?

4) Did athletes with menstrual disturbances or late menarche differ in bone mass change from eumenorrheic, normal menarche athletes?

5) Is menstrual dysfunction (secondary a/oligomenorrhea) related to serum leptin or were other energy status variables more important predictors?
CHAPTER 2

LITERATURE REVIEW

2.1 Historical Perspective

Wolff’s Law (1892) is often cited as the foundation for bone change in response to mechanical stresses. It is less well known that Wolff formulated his opinions on the work of Culman and Mayer (1867) which identified the organization of trabecular bone. Bassett acknowledges this history and quotes the most elementary form of Wolff's Law in that “a bone becomes adapted during its growth to the functional forces acting upon it” (63). It has long been recognized that bone will respond in form to the functional need, but the mechanism of this adaptation has yet to be explained and has been the source of speculation and theory throughout the years. In the interest of early exercise physiology (1933), Steinhaus theorized on the potentially positive effects of exercise on bone mass development (64). The study of exercise-induced changes in bone mass has developed since the work of these early pioneers and continues to grow with more interest in efforts to prevent osteoporosis.

Industrialization and technological advances have seen concomitant increases in the prevalence of osteoporosis. This has encouraged researchers to hypothesize that the modern lack of physical demand on the body (skeleton) has
discouraged the bone strength and development so beneficial to our ancestors (65, 66). Cordain has reviewed the evolutionary aspects of our skeletal and physiological development (65). Cranial capacity, metabolic needs and bone characteristics through the eras (according to archaeological evidence) were included in this review of pongids and hominids up through current day humans. Increased cranial capacity of humans was well demonstrated by the evolution of the tools used in activities of daily living - grindstones to computers. At the same time, human metabolic demands and the width of the long bones of the lower extremities have decreased contributing to more chronic diseases such as obesity and bone fragility. According to Myers, children spend an average of 150-180 minutes per day in front of the computer or television (67). If decreased activity patterns are considered a risk factor for osteoporosis and distract from attainment of peak bone mass, physical activity promotion initiatives such as those initiated by the National Coalition for Promoting Physical Activity should be better funded to discourage such sedentary lifestyles and impending disease (68).

Studies of the relationship of physical activity to bone density have theorized on the potential mechanisms responsible for such an osteogenic effect. Early in the 1960's there seemed to be a tremendous amount of effort poured into identifying the piezoelectric response of bone crystals to mechanical stresses, and Bassett was responsible for much of this work (63). A more modern list of the mechanisms potentially responsible for exercise-induced osteogenesis includes: prostaglandin-2 release, microstreaming potentials, increases in blood flow, piezoelectricity, microdamage and hormonal mediation (69). It is worthy to note
that many of these same mechanotransduction ideas are found throughout the literature and are not novel. Frost has proposed a paradigm for bone response to mechanical usage. This historically important model will be further elaborated in the "Mechanical Usage" section (27, 70-82). Identification of the mechanism(s) responsible for bone stimulation would help identify the activity characteristics most likely to permit development of peak bone mass and play a preventive role in osteoporosis.

2.2 Animal Studies

Animal models have the distinct advantage of allowing certain manipulations of the feeding, activity restriction or promotion, and measurement of actual bone strain, strength and composition which most human subjects committees and ethical researchers would not permit. An obvious disadvantage in looking at animal models is the inability to predict if the changes identified in the animals would be similar in humans under the same circumstances. Many intervention studies have been premised on early animal models as outlined in approximate chronological order.

Saville et al's 1969 study of a bipedal rat model was an early demonstration of the potential positive effects treadmill running could have on the weight-bearing skeleton (83). The rats ran 2000 meters per day for 5, 10, 15 or 20 sessions, and the bones were evaluated for density and strength. The rats running the most sessions had an increased density and breaking strength leading to the conclusion that bone response to increasing levels of exercise was
positive. It is worthy to note the relatively moderate amounts of exercise performed by the animals and the exclusion of an over-exercise group.

Hert and co-workers are commonly cited throughout the animal literature for their work with a rabbit model of the tibia (84-88). Hert et al developed dynamic and static models of bone strain through artificial loading and found that increased bone density and strength were the product of dynamic and intermittent strains. Technological limitations did not allow for measurement of the strains engendered within the bone to define the stress characteristics. The artificial strain procedure included drilling and pinning the bone and applying lateral stresses (unusual distribution) to the bone, but all stresses were considered within physiological range. Hert's work served as a foundation for many animal models incorporating artificial strains.

Chamay looked at bone response to static or dynamic strains in a dog model (84). The adult canine model was developed by anatomically altering the front leg by resecting part of the radius to create a dynamic model of new strain on the ulna. The static overload (a one-time large strain applied to one group of animals) failed to show the significant bone hypertrophy seen in the dynamic model. These authors also concluded bone was more responsive to dynamic strains and supported the piezoelectric theory.

Recognizing the need to measure the strain within bone in these artificial strain studies, Lanyon and Smith documented an in vivo technique using rosette strain gauges (89). Many of the animal models where the primary interest was the effect of certain strain magnitudes, rates or cycles employed this measurement technique. Churches, Howlett and Wards were responsible for the
early demonstration that the procedure for drilling and pinning bones in preparation for artificial and controlled strains was fairly innocuous to the bone away from the site of procedure (85). They were able to show that there was very little osteogenic or osteolytic effect of the procedure on the sheep bones. O'Connor and Lanyon used this same in vivo procedure to investigate the response of the sheep radius to artificial strains by altering strain rate and magnitude (86). They also used the strain gauges to document normal strain curves and compare them with the artificial loads. By multiple linear regression, they concluded that the best explanation for the observed increase in geometry (via fluorescent labeling) was the ratio between the release of strain rate during walking and the release of strain rate in the artificially induced strains. The above work lended itself to the continuing idea that novel and dynamic strains are most osteogenic.

The commonly cited work of Lance Lanyon, in conjunction with Clinton Rubin, using an avian model has also suggested the osteogenic characteristics of exercise. They continued to use artificial strains as seen in the other animal models and measured the strains with rosette strain gauges. The 1984 rooster study demonstrated 36 cycles (72 seconds total) of novel strain per day was sufficient to induce osteogenesis (87). Using male turkeys in 1985, they demonstrated that periosteal hypertrophy is linear to the strain magnitude when the strain distribution is unique (88). Calcium nutrition and disuse were examined in a 1986 hen turkey study where they demonstrated that the artificial strains were barely able to inhibit the effect of calcium deficiency on bone seen in the control animals (90). This hen model was significant for using female
animals and unique in that they monitored the physiological degree of calcium deficiency by measuring the calcium content of the eggshell. The work of Rubin and Lanyon continued to support the osteogenic nature of novel, dynamic strains. Additionally, they defined the peak strain rates and magnitudes associated with osteogenesis in response to artificial loading.

Using a less invasive approach, Matsuda et al worked with an avian model more consistent with the human exercise condition (91). Young Leghorn roosters were run on a treadmill to look for the effect of exercise in a growth model. The birds ran 35 to 45 minutes each of 5 days per week for 5 or 9 weeks at an intensity equivalent to 70-80% of measured maximal oxygen consumption. Bones were measured in vitro by computerized morphometry and three point bending studies. The runners had an increased cortical area and decreased medullary area indicating an increased periosteal apposition and decreased endocortical resorption. When the bones were tested for failure characteristics, the runner bones had increased stiffness and more abrupt failures. The authors concluded that exercise at the frequency and intensity used in this model demonstrated a negative effect on the quality of growing bone.

Other animal models have made use of the treadmill to demonstrate various relationships of walking or running to bone health. Tommerup et al used adult female swine to demonstrate the local effects exercise has on the skeleton (92). They demonstrated increased bone formation of the weight-bearing femora, but failed to see any changes in the ribs. Biewener et al used a more reasonable running regime with Leghorn roosters compared to that of Matsuda et al (28). The chickens ran 15 minutes per day for five days a week at an intensity, which
calculated out to be only 2500 cycles of loading. The cortical thickness increased by 26% and the calculated second moment of inertia increased by 40% after 8 weeks of running, then returned to values more similar to baseline. The study demonstrated the positive, adaptive and transient nature of growing bones in response to a more reasonable regime of treadmill running. Wheeler et al used a young rat model to look at exercise intensity and duration (93). Reasonable exercise seemed to benefit the skeleton while overtraining suppressed periosteal and epiphyseal growth. These treadmill studies have looked at the spectrum of exercise intensities and durations.

Two most recent studies have addressed the loading nature of osteogenic responses to physical activity. A Japanese study by Umemura et al most directly introduces the subject of interest- impact of exercise (94). The rats in this study were positioned in a box on an electrical grid and encouraged (by electrical stimulus) to jump up to the top of the box. The rats held on to the top of the box with the forelimbs to avoid landing on the electrical stimulus. After each jump, the rat was repositioned before another jump stimulus so that the number of jumps per day was the exercise variable. The osteogenic results seen from jumping far outweighed the positive results seen in most animal treadmill models. The rats in this study were not permitted to land, and it would have been interesting to see if there would be any further differences with landing. The loading of jumping up was very osteogenic in this rat model. Mosley and Lanyon have reported similar findings in an artificial loading study performed on growing male rats (95). The left ulnae of the test animals were loaded artificially with low, moderate or high strain rates for 1200 cycles per day for a
total of 8 days in two four day sequences. The rats were dosed with calcein to evaluate histological changes under the microscope. The authors demonstrated periosteal load-related changes over the entire ulna. Because the ulna has a slight curvature, the authors were able to substantiate an increased mineralization on the tension side of the bone, which was accompanied by an activation of osteoblastic activity on the compression surface as well. Strain rate has been shown to be important in the mechanotransduction pathway in animal models.

This short review of some of the key animal studies provides a chronological framework for human studies as well. Many of the early studies of exercise in human bone health were running models. After Rubin and Lanyon helped identify the responsive nature of bone to strain magnitude and rate, the human studies that followed developed the idea that the unique characteristics of the sport may be more important than the time or energy spent in the sport. Additionally, animal models have demonstrated the difference in the modeling of the growing skeleton and the more moderate response of the mature skeleton to exercise. Bone modeling responses suggested in this literature include increased periosteal expansion with decreased endosteal apposition (91), transient increases in cortical thickness (28), periosteal and epiphyseal growth suppression (28) and an increased mineralization by osteoblastic stimulation (95).

2.3 Human Studies

Physical activity as a modality to increase bone mass is not a novel idea, but the exact contribution and mechanism have not been well defined. Bone
mass is reflective of multiple endogenous and exogenous skeletal effectors. The
genetic and hormonal contributions to skeletal health are good examples of
endogenous factors even though the hormonal controls may be altered
exogenously. The nutrition and physical activity variables represent exogenous
and modifiable effectors. These factors are likely to vary within different
populations. Cultural practices and social norms influence the passing of genetic
material, hormonal evaluation and treatment, the types and quantities of foods
and supplements consumed and the acceptable and necessary physical activity
patterns and practices. Much of the literature attempts to define the role of
skeletal effectors in various populations with respect to race, gender, age,
physical activity level or athletic activity status or type.

Growing female adolescents are the primary focus of this project and will
be the primary focus of this human literature review. Studies conducted among
young and aging adults are included to help demonstrate the importance of the
response the growing skeleton may have to exercise. Additionally, the studies
among adults provide a historical perspective to this review. The framework for
review of the human studies will be based on the design and age of the target
population as follows:

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Studies of some of the important hormonal issues in athletes will be reviewed separately, namely the calcitrophic hormones, estrogen and leptin.

In addition to looking at the relationships between bone mineral content and physical activity, it is important to consider how physical activity has been evaluated. As will become evident, many cross-sectional studies identifying a higher bone mass in athletes have associated the impact characteristics of the sport as the osteogenic stimulus. Longitudinal evaluations of physical activity have rarely included the impact characteristics of physical activity. The methods for collection of physical activity information are reviewed as closely as the relationships identified.

2.3.1 Cross-sectional studies:

2.3.1.1 growing individuals

It is important to remember the bone and estrogen differences between senescence and adolescence. Aging bone is likely marked by increased remodeling concomitant to changes in (decreased) mechanical stresses, while the adolescent bone is responding to (increased) mechanical stresses with modeling and consolidation. Menopause for females is marked by a rapid decrease in estrogen and waning reproductive functions while the adolescent is experiencing the opposite- rapid increase in estrogen and viable reproductive functions.

Much of the work in youth was designed to identify norms for growth within a certain population or to set the normative framework as new methodologies have become available. The methodology of choice in the early
1970's was single photon absorptiometry of the forearm. Studies like those of Krabbe et al and Bonjour et al were important to demonstrate normal bone biology (96, 97). Boys and girls were similar before puberty, girls experienced the adolescent growth spurt well before boys and the growth spurt dissociates bone mineral density (BMD) from height. Age, weight and height are commonly recognized as strong correlates with bone density measures (8, 20, 23, 39, 98-114). Additionally, many of the bone correlates are also highly correlated to each other or collinear (112, 115-121). Studies such as those by Hui et al provide insight into the technological limitations of large sample (n=1774), wide age range, multi-center studies and acknowledge bone mass as a function of bone width at any age (122). As is typical, Hui et al were unable to identify if the differences were true differences or if they were due to a difference in machines and/or operators, selection bias (geographical) or dietary/physical activity differences. The single photon absorptiometer cross-sectional studies helped define skeletal growth norms as well as the limitations of bone densitometry, but have failed to incorporate physical activity as a covariate.

More recent studies employing single photon absorptiometry (SPA) for the forearm and occasionally dual photon absorptiometry (DPA) to look at the spine or hip incorporated PA into the densitometry studies of normal children. DePriester et al looked at the forearm (SPA) of 420 four to ten year old subjects (98). They noticed the cortical width and area (volume) to be greater in boys and hypothesized it to be a proxy for increased muscle mass and exercise (though they did not evaluate either within their study). Slemenda et al used SPA and DPA techniques and measured physical activity from a questionnaire adapted
from the National Children and Youth Fitness Study (9). The study included 59 twin pairs of 5 to 14 year old subjects. Total hours per week of weight-bearing physical activity strongly correlated with bone density measures and the active children had an estimated 5-10% increased bone mass. Children reporting biking and swimming had lower correlation coefficients, but this was proposed to be due to the more frequent reporting for that activity in younger children. Karen Rubin et al looked at SPA of the radius and DPA scans of the spine in 299 six to eighteen-year old subjects (112). Using regression analysis, they found height, pubertal stage, weight and age to best predict radial bone mineral density. The model that predicted lumbar bone mineral density was limited to the pubertal stage and weight as the independent variables. Addition of energy expenditure in kilocalories to the lumbar model was significant (p=0.036), but small (1%). Rubin’s group concluded physical activity affected the skeleton in a site dependent mode since it appeared to influence the axial (central) skeleton and not the forearm.

With the advent of dual x-ray absorptiometry (DXA) in the late 1980’s, the need for normative data was revisited. Glastre et al used DXA to evaluate the spinal density in 135 children (1-15 years old) (102). The DXA results confirmed SPA findings that the largest bone changes happen in adolescence and occur earlier in girls than boys. Height, weight, body surface area and bone age were all highly correlated with BMD, and they found the largest skeletal changes in females occurred in pubertal stage 4. Bonjour et al also used the DXA (spine and femur) to look at growth in 9-18-year old subjects (97). They also found the 12-15-year old age time frame to be critical in female bone development and
determined that females reach peak bone mass at the femoral neck and spine at 14-15 years of age. The findings of this study supported the growth spurt dissociation from height as previously discussed with Krabbe’s work. Bonjour et al used unique categories within pubertal status 5 to help evaluate the changes across time since menarche. Bonjour et al wanted normative data and intentionally excluded subjects exercising more than 10 hours per week. The 1992 work of Lloyd et al used 10-14-year old female subjects and evaluated the time period around menarche (106). They used the whole body DXA scan to demonstrate body weight as the highest correlate to bone density measurements. In regression modeling, they found body mass index, height and pubertal score to be the best predictors of whole body bone mineral content (BMC) and BMD. In trying to sort out the hormonal influences of puberty, his group developed an integrated estrogen scoring system, which included pubertal stage, menarche, menstrual frequency, breast diameter and urinary estrogen components. Breast diameter was found to be a stronger correlate to total body bone mineral content (TBBMC) and total body bone mineral density (TBBMD) than the integrated estrogen exposure score, and the final regression included pubertal stage (pubic) as a significantly predictive variable. Physical activity was not evaluated, but they did analyze dietary data. The results from this same sample of girls were reported as the first whole body normative data as well (123). Faulkner et al reported similar data for Canadian children (99). The 8-16-year old boys (n=110) and girls (n=124) were similar except boys had greater BMD of the head and upper extremity while females had greater BMD of the pelvis. The relationship of height, weight and age was again highlighted and the collinearity noted. Lean
body mass was a significant predictor of bone mass and authors suggested this to support the notion that muscular activity enhances bone density. In a follow-up analysis of these data, they compared the dominant extremities to the non-dominant extremities finding a greater BMD in the dominant upper extremity and no differences in the legs (124). As is usual with intra-individual studies, these authors attributed the differences in arm BMD to increased physical activity and loading of the dominant arm. Turner et al used the DXA to establish hip and spine norms in 138 high school girls (16.4 ±0.34 years old) (125). Weight, height, physical activity and calcium intake were shown to have a positive correlation with BMD. Physical activity was measured by a modification of a standard New Zealand ranking tool and was determined to explain 4% and 5% of the femoral neck and trochanteric bone mineral variability (respectively). Turner chose to also compare the spine and hip data with 20-25-year old normal females (young adult normative data) and concluded the 16-year olds had reached peak bone mass at the hip, but not the spine. Matkovic et al used SPA of the radius and DXA of the whole body, spine and hip to evaluate the 265 females in a 1994 study to identify the timing of peak bone mass in female subjects 8 to 50 years old (126). From the cross-sectional analysis of this wide age range, they found the hip and spine sites to reach maturity earlier than the forearm and head. The period of consolidation was identified to be site specific and occur 1-7 years after cessation of linear growth. Again, it was noted that most bone mineral gains during growth are due to increased volume and not necessarily increased BMD. Though many of the cross-sectional studies find similar results, it is interesting to note the differences and novelties.
Ruiz et al looked at BMD and BMC as well as the Z-scores in their 7-15-year old sample of 151 subjects (113). They accounted for exercise as an annual average in three categories: usual, 1-3 hours per week, or 3-12 hours per week. Though body weight was the main predictor for the hip and spine sites, the vertebral prediction included pubertal stage and physical activity. Haapasalo et al used 330 Finnish females spanning 7-47 years old (103). Confirming the earlier results of others, they concluded peak bone mass is attained by 18-21 years of age and the spine accrues bone mineral through age 21-23. They evaluated bone mineral by BMAD (bone mineral apparent density) which was BMC corrected for estimated bone volume. BMAD was used to account for the bias of height and weight (size issue) of the subjects. In 1996, Moro et al reported on the bone differences in young subjects with various ethnic backgrounds (n=375) (118). They demonstrated that the ethnic differences in adult cross-sectional studies come about in the peak bone mass years. Moro et al included evaluation of the subjects for average hours of weight-bearing activity, calcium intake and pubertal stage score. Collinearity issues were described, and the need to define the biomechanical impact of weight-bearing activity was recognized. The whole body scan was used to calculate cortical cross-sectional area and bone strength indices of the femur using a mid-femoral software package. Should this software turn out to predict these variables with great accuracy, it could be valuable to the study of exercise effects on bone. Goulding et al have begun looking at the geometry of the proximal femur by evaluating the femur axis length (FAL) and the bone width at the femoral neck (FW) (127). From the study of 200 three- to sixteen-year old females, they concluded that the FW was more influenced than
the FAL by weight-bearing activity. The working hypothesis was that increased nutrition before puberty or increased growth hormone would stimulate linear growth to increase FAL without increasing FW and that this may lead to a predisposition to hip fracture later in life. Goulding et al cited other researchers (Flicker et al) who have used geometrical data similar to this measure (FAL) termed HAL (hip axis length). Goulding et al did not find a significant correlation of sport activity or calcium nutrition to FAL or FW, and FAL was highly associated with height. The baseline data of the current subjects were analyzed in a cross-sectional fashion and published by Ilich et al (128). The original 456 females were stratified by energy expenditure (as predicted from the Bouchard grid) and corrected for BMI (kcal/BMI). The total body BMD, radial BMD and lean body mass were highest in the upper quartile for physical activity. The criterion \( p \) (Cp) model selection method was used to help overcome the collinearity issues in formulating prediction models. The final regression equations were:

- Total body BMD: total bone area, body fat, lean mass, stature, skeletal age, dietary calcium
- Radius BMD: radial bone area, body fat, lean mass, skeletal age, dietary calcium
- TB and radial BMC: bone area, body fat, lean mass, calcium intake, skeletal age

*Bone area was forced into each model to correct for bone size.

The wealth of cross sectional studies in the literature that have examined athletes is likely responsible for the prevalent belief that increased activity promotes increased bone density. It has become common to select athletes from sports with different characteristics and training regimes to identify the quality
and quantity of exercise most osteogenic to bone. Slemenda et al studied female
figure skaters to highlight site-specific bone mass effects of skating in twenty-two
growing and young adult subjects (10-23 years old) and 22 controls (10). The
regression models corrected for age and weight to evaluate the effect of skating
on the arms, spine, legs and pelvis. Skating appeared to have a positive (higher
BMD) influence on the legs and pelvis. A trend towards lower bone mineral
density in skaters who trained more hours per week (overtraining) was also
identified. The skaters had a greater incidence of menstrual disturbances than
the controls, but Slemenda et al suggested the weight bearing nature of the sport
offset the skeletal disadvantages of menstrual irregularities as the skaters
consistently had higher bone mineral densities than the controls. Grimston et al
also approached the impact-loading characteristics of sports in a study of 10-16
year old males and females (129). The subjects were matched based on gender,
body weight, and pubertal stage so the designed comparison was limited to
participation in impact-loading sports versus swimming. Impact-loading sports
included running, gymnastics, tumbling and dance and were purported to have
a landing impact greater than three times the body weight. Competitive
involvement was required and was defined as participating for at least sixty
minutes three times per week. Dietary data were collected via two 3-day dietary
records and analyzed via Nutritionist III. An activity questionnaire was used to
assess year round activity patterns. The authors evaluated the leisure time and
training activity patterns to find no differences with exception of swimming vs.
impact-loading sport activity. The femoral neck BMD of the impact-loading
group had significantly higher BMD than that of the swimmers while the lumbar
spine BMD failed to demonstrate differences. The results varied slightly when the subjects were broken into groups by gender, but the gender analysis further contributed to low statistical power as the number of subjects was limited. The study provided impetus to challenge the osteogenic role (or lack of) swimming has on the growing skeleton. Cassell et al also compared gymnasts and swimmers, but studied 7-9 year old “elite” female athletes (14). The swimmers trained an average of 4.7 hours per week while the gymnasts averaged 13.9 hours per week, and all athletes had year round training regimes. As is typical of swimming comparison studies, the swimmers were leaner (more lean body mass (LBM)) and taller than controls or gymnasts. The relationship (slope) between total body BMD and body weight was heightened for the gymnasts compared with swimmers or controls as identified with ANCOVA and interaction between body weight and total body BMD. Dietary differences were not significant due to high variability, but it was noticeable that the gymnasts averaged 781 mg calcium (+73) intake per day and the swimmers averaged 1139 mg (+127) per day. Haapasalo et al studied 91 seven- to seventeen-year-old national level female tennis players who trained for tennis year round (130). They compared these athletes with 58 loosely defined controls. All subjects were categorized by “Tanner Staging” I-V and were analyzed within pubertal categories. Bone mass measures were taken at the proximal humerus, mid-humeral shaft and distal radius of both upper extremities as well as the lumbar spine. Isometric muscular strength was measured for flexion, extension and grip of the forearm musculature. The study demonstrated a difference in relative (nondominant to dominant) bone mineral density in the arms of players corresponding to the
third pubertal stage. The lumbar spine of players was also increased, but this finding was more apparent in the 4th and 5th stages of pubertal development. There were no differences identified between the non-dominant arms of players and controls, which further supported the site specificity of mechanical loading on the dominant extremity. The activity-induced bone accumulation in the third and fourth stages of puberty led the authors to continue their suspicion that physical activity in the period just prior to menarche is more beneficial to the skeleton than during other periods of development. This study provides impetus for evaluating the effects of exercise in adolescents across menarcheal time, not just pubertal stage.

In conclusion, previous cross-sectional studies carry many common themes, which should be taken into consideration for bone density research in adolescents. Females experience the adolescent growth spurt earlier than males and reach adult peak bone mass (PBM) as they near the end of the second decade (103). Specifically, the hip appears mature by 15-16 years of age (40, 97, 126) while the consensus is not as strong for PBM of the lumbar spine (97, 103, 126). There is a common dissociation of height and peak height velocity in mid adolescence, but age, height, weight and body surface area are consistently strong predictors of bone mass and are collinear. Bone size and shape need to be evaluated when using methodologies that measure bone mineral areal density (g/cm²). The use of (peripheral) quantitative computerized tomography (pQCT) in bone research should help overcome the potential bias of SPA, DPA and DXA technologies. It is likely necessary to evaluate bone using variables such as bone mineral apparent density (g/cm³) or radiogrammetry. Menarche marks a major
event in female adolescents, and the research has shown that bone changes may best be evaluated relative to menarche. Menarche marks the rapid closure of epiphyses ending long bone growth. Many studies omit measurement of physical activity, and when it is measured, the reported correlates are not consistent. The skeletal influence of sports other than gymnastics, skating, swimming and tennis have not been evaluated in growing athletes (such as volleyball, basketball, softball and soccer). Additionally, cross sectional studies are unable to evaluate or control for bone mass before the individuals undertook sporting activity leading to a potential bias in the results. The results of these cross-sectional studies should be confirmed with longitudinal evaluation.

23.12 young adult

The relationship of physical activity to bone density has been more consistently reported in young adults using many of the same methodologies discussed above to measure bone and employing more objective measures of physical activity. A study by Aloia et al evaluated the spine with DPA, the radius with SPA and the total body calcium with neutron activation analysis (131). Physical activity was measured using a large-scale integrated (LSI) monitor and physical activity was found to be significantly correlated with spine density and total body calcium. Kanders et al used slightly younger adults than Aloia et al measuring the spine with DPA and physical activity by the Minnesota Leisure Time Physical Activity Questionnaire (MLTPAQ) and pedometer (132). The lumbar spine BMD was positively correlated to daily energy expenditure, and according to regression prediction, there was an increase of 1.2% vertebral
BMD for every increment of 100 kcal energy expenditure. In another study, Halouia and Anderson tried to create a lifetime physical activity and calcium intake score for 20-50-year old female subjects (133). The low calcium, sedentary group had the lowest radial bone as measured by SPA. The results of this study also suggested there were no additional skeletal benefits beyond intermediate levels of calcium intake (intermediate = 500 mg/d) when adjusted for physical activity level. Fehily et al reported the density of non-dominant forearms and lifestyle factors in 371 of 581 original supplementation trial subjects where the original study was completed 14 years before the bone density measures (100). Early menarche and retrospective sports participation at age 12 was positively associated with bone density in the young adult women (20-23 yo), but there were no bone density differences between previously supplemented or unsupplemented subjects.

The 1994 study of Rico et al used a small (n=50) sample of young men and women (age 26±6 years) intentionally excluding those that “practiced sports regularly” (134). The study was unique because the (distal 4%) radial bone density was measured with computerized tomography (CT) which enabled the cortical and trabecular contributions to bone mass to be identified in a volumetric measure (mg/cm³). The study design measured both upper extremities to identify the potential influence of the difference in mechanical stimulus to the dominant and nondominant extremities. Total radial BMD and cortical BMD were significantly higher in the dominant arm as would be expected according to previous bilateral studies (135-137). Conversely, there was no significant difference in the amount of trabecular bone (BMD) between the arms. These
results suggest that chronic and consistent mechanical stimulation of bone appears to affect cortical bone more than trabecular bone. Reid et al used 99 premenopausal females to demonstrate that size-corrected BMD in non-exercising adults is most associated with fat mass (119). Regular exercise dissociated this statistical relationship in those subjects identified as exercisers based on expending more than 140 kJ/kg/day (33.3 kcal/kg/d) as assessed by a seven-day recall. By using various criteria to group subjects, this study demonstrated how important it may be to inspect the various gradations of exercise as the calorie expenditure used to determine the "exercise" group was not robust. Reid et al also addressed the issue of size dependence of DXA measurements and suggested that correcting areal BMD by height could help overcome the bias of two-dimensional measurements. Torgerson et al studied the lifestyle, environmental and health factors of 45-49-year old pre-menopausal women (138). Physical activity was categorized (from questionnaire) into >2 hours, 1-2 hours or less than 1 hour of activity per week, and 32.8% of the subjects exercised more than two hours per week. All femoral site regression equations included body weight, physical activity and history of wrist fracture as significant predictors of bone mineral density. The lumbar spine BMD prediction equations included weight, height, wrist fracture history and antacid use, and excluded current physical activity. A later study by Ho et al used 21-40-year old Chinese women (n=297) to investigate the relationship of the intensity and impact of weight-bearing activity to hip and spine BMD (39). The female subjects were categorized into two age groups (21-30 and 31-40). Intensity of physical activity was categorized by tertiles (upper, mid and lower) according to
MLTPAQ for the past twelve months, and the impact (weight-bearing) was categorized according to a method by Nieves et al (139). Physical activity was more associated with higher bone mass in the younger women and seemed to influence spine BMD more than hip BMD. This connotes an increased trabecular density in direct opposition to the CT work of Rico et al. Inclusion of a 73 item food frequency questionnaire in this study allowed the researchers to look for the interaction between calcium intake and physical activity with reference to bone mass. They were able to demonstrate higher bone densities in women in the highest tertiles for physical activity in combination with calcium intake suggesting an additive relationship of calcium intake with physical activity.

Studies of running and swimming adult athletes were popular in the late 1970s and 1980s. Aloia et al studied the effects of marathon running on the skeleton and found that male marathoners had an increased bone mass when compared with sedentary non-runners (3). This landmark study led to Aloia’s follow-up intervention studies in post-menopausal women and acknowledged the potential for a pre-selection or bias issue when studying athletes. Kirk et al studied female long distance runners with subjects matched with controls based on age (6). The runners and controls were primarily of two age groups (25-35 yo and 55-65 yo) and the bone mineral was evaluated via SPA (radius) and QCT (spine). The younger runners tended to have higher BMD than the older runners or controls, but the authors noted that the younger runners had higher serum concentrations of 1,25-dihydroxycholecalciferol (active vitamin D). They surmised the increased BMD may have been due to more sunlight exposure as the younger group ran outside more often. Orwell et al also studied more
mature athletes in a 1989 study of Master's swimmers (140). The study of 58 men and 41 women included athletes older than 40 years who swam regularly (at least 3 hours per week for past three years) and were participating in a National Swimming Championship. The study design included a control group of 78 men and 41 women chosen for the same inclusion criteria with the exception that none were exercisers. The radial (SPA) and spinal (CT) bone mineral content and bone widths were measured and the subjects completed food diaries or interviews. The male swimmers had greater vertebral and radial BMD than controls and older subjects. This difference was more marked in the spine. The data did not support any association of swimming with enhanced bone mineral density for the females. These early studies typified the nature of cross-sectional research on athletes.

The popularity of dual photon absorptiometry measurement of various adult athletes types was highlighted in 1990. Davee et al studied bone mineral density of the spine in 27 college women chosen for normal body weight, no smoking and eumenorhheic menstrual status (141). Subjects were grouped according to exercise training profiles elicited by questionnaire. The groups were sedentary, aerobic exercisers (>2.5 hours/week) and muscle builders who supplemented similar aerobic training with at least 1 hour of weight training per week. Dietary assessment was completed using a self-administered diet history, 24-hour recall and 4-day food records. Nutritionist III was used with the food records for assessment of daily intakes. Resting energy expenditure was measured by indirect calorimetry, and serum samples were analyzed for prolactin, 25- and 1,25-dihydroxyvitamin D, cortisol and insulin-like growth
factor-1 (IGF-1). Women who included weight training in their workout regime had significantly greater spinal bone mineral density than women did in the aerobic or sedentary groups. Interestingly, the aerobic group had a lower (insignificant) mean BMD than the controls. One out of nine subjects in the aerobic group swam 8.5 hours per week and had a very low spinal density. The swimming was hypothesized as a potential cause for this osteopenia. The difference in these groups was not attributed to dietary calcium or serum vitamin D metabolites. Rather, IGF-1 was significantly elevated in the weight-training group and was hypothesized to be the osteogenic factor. Risser et al investigated eumenorrheic college-aged females who played volleyball, basketball and swimming (n=29) as well as non-athletes (n=13) (8). The evaluation included spine (DPA) and calcaneus (SPA) bone densities, retrospective college and high school activity assessment by questionnaire, three 24-hour dietary recalls and various anthropometric measures. Athletes were varsity participants in Division I programs and had been athletes in high school while the controls were selected based on exercising less than three hours per week throughout high school and college. It was necessary to adjust for height and weight in the analysis, and the results indicated that volleyball and basketball athletes had greater calcaneal density than swimmers and controls. The swimmers had significantly lower spine mineral density than all other groups while the volleyball players had increased spinal density compared with control subjects. The authors suggested the changes to be related to the impact characteristics of the respective sports. Cross-sectional data from the study of athletes using dual photon absorptiometry
has supported the notion that the impact characteristics of exercise are important to evaluate in the analysis of the influence of physical activity on bone health.

Recent dual x-ray absorptiometry cross-sectional studies have also supported the role of exercise-specific impact on osteogenesis. A study by Conroy, though it excluded females, demonstrated the osteogenic nature of Olympic-style weight lifting (142). The subjects were twenty-five elite, male, adolescent (age 17.4±1.4 yrs.) weight lifters and 11 age-matched controls. The study was designed to measure the weight lifters' strength in the snatch, clean and jerk, and the squat lifts for correlation analysis with bone density measures. DXA measures were taken for the spine and hip. The adolescent weight lifters had significantly greater bone densities than age-matched controls and reference young adults for all anatomical sites evaluated. The correlation between the strength measures and the bone mineral densities were significant and the coefficients ranged from 0.39 for the squat with Ward's triangle to 0.73 for the snatch lift with the spinal bone mineral density. Additionally, the bone densities of the young weight lifters were significantly greater than young adult reference values. Tsuzuku has recently reported similar results using male power lifters (143). Heinonen's group also investigated the specificity of sport training on various anatomical sites according to the exercise impact, but in female athletes (144). The young adult athletes included 30 orienteers, 29 cyclists, 18 weight lifters, 28 cross-country skiers and 25 moderately active reference subjects. The researchers collected data on training history, calcium intake, menstrual status, VO₂ max (by sport specific measures), maximal isometric strength tests and DXA bone density. Scanned sites in this specificity study included lumbar spine,
femoral neck, distal femur, patella, proximal tibia, calcaneus and the distal radius of the dominant arm. The orienteers and skiers had significantly greater aerobic capacity (VO₂ max) than the cyclists which were greater than the referents or the weight lifters. The weight lifters were the only athlete group to consistently have significantly elevated (weight-adjusted) BMD as well as strength values. The graphic representation of the differences between the various groups of athletes with respect to the referent group was most persuasive that weight training is osteogenic at most anatomical sites (calcaneus excluded). The cyclists demonstrated relatively low BMD values for the lumbar spine and lower extremities purported due to the non-weight-bearing nature of the sport. The smaller differences between the referents and the aerobic athletes were likely due to having very active referents according to the authors. Fehling et al studied college-aged volleyball players (n=8) and gymnasts (n=13) to represent impact-loading sports and compared them with a sport more typical of active-loading (swimming, n=7) as well as control subjects (n=17) (101). All athletes participated in training year round while the control subjects were selected based on less than one hour of “training” per week. All subjects were eumenorrheic with the exception of the gymnasts who represented the spectrum of menstrual categories. The volleyball group had a higher (5-19%) mean BMD at most sites when compared with the controls or swimmers. The volleyball group also had higher BMD in the lower extremities when compared with the gymnastics group, but the gymnastics group had 11% higher bone density in the upper extremities than the volleyball players. The study reported no significant differences between the swimming and control groups at any of the measured sites.
Taaffe et al compared swimmers, gymnasts and non-athletic controls (13). All subjects were college (Division I) athletes and were categorized as eumenorrheic. The whole body and spinal bone mineral densities were similar between groups until the smaller body size of the gymnasts was considered. After controlling for body mass, the spinal, femoral and arm BMD of the gymnasts exceeded that of the swimmers and controls. The authors concluded that the skeletal stimulus associated with the intensive weight-bearing of gymnastics was responsible for these differences and that non-weight-bearing training "confers no beneficial skeletal effects on the bone mass in young women". Alfredson et al studied female volleyball players and inactive controls suggesting the nature of the impact of exercise to promote bone mineral accretion (145). Bone density and muscular strength of thirteen Division I volleyball players (mean age 20.9) were compared with thirteen controls (mean age 25). The increased BMD of multiple sites was reported for the volleyball players ranging from a 6.1% advantage for the whole body to 18.8% advantage in the trochanteric region. There were no differences between groups in the strength of the quadriceps and hamstrings as measured by isokinetic dynamometry. According to this literature, the weight-bearing nature and impact-loading nature of a sport is likely beneficial to the specific skeletal sites enduring the loads.

Other recent studies have continued to demonstrate the importance of sport impact and weight-bearing exercise by selecting various athletes for comparison. Many of these studies also address the possibility of a "steal phenomenon" whereby mechanically loaded sites of the skeleton have an
increased density while the unloaded sites experience a decreased density from (in theory) redistributing bone mineral to the loaded sites. The net effect of redistribution would not be detectable using whole body measures, but would be identified via multiple site studies. Karlsson et al suggested this phenomenon in a study to determine the sport specific influences on the skeleton of 19-68-year old male and female ballet dancers (active or retired) (146). The 42 professional dancers were compared with 42 age- and sex-matched controls. The main differences in the BMD of dancers compared with controls were lower skull BMD in male dancers and lower arm BMD in the female dancers. After correcting for body mass index, the hips and legs of the female dancers and femoral neck of the male dancers were significantly more dense than controls. There was no apparent relationship between training intensity (time spent) and bone mineral density. The authors presented evidence that retired dancers seemed to lose bone mineral at a faster rate than aging controls, but it was possible that some of the findings may have been related to hormonal aberrations which were not appreciated in the study. The results did support the local effect of exercise on the skeleton and supported the steal phenomena. Heinonen et al included athletes from squash (n=18), aerobic dance (n=27), and speed skating (n=14), along with active (n=25) and sedentary referents (n=25) in a study of college-aged females (147). The bone density of the spine, femur, patella, tibia, calcaneus and radius were measured (DXA), and the training history, calcium intake, menstrual status, VO$_2$ max and various muscle strengths were evaluated. Groups were analyzed using ANCOVA controlling for body weight. When compared with the sedentary reference group, the squash players
had a significantly increased mean BMD at each site measured. The femoral neck, proximal tibial and calcaneal sites of the aerobic dancers were also elevated, but the distal radial site was significantly lower than for any other group. The speed skaters demonstrated an increased density in the distal femoral site, likely a reflection of the closed-chain and lateral nature of the sport. There were no identified significant differences between the physically active and sedentary referent groups which suggested an activity threshold necessary for bone to reap the benefits of exercise. In the discussion, the authors questioned the steal phenomenon as an explanation for the lower BMD in the forearm of the aerobic dance group. These studies continue to support skeletal site specificity of exercise, but challenge the whole body benefit so commonly proposed.

Duppe et al have studied active junior, senior and retired Swedish football (soccer) players to demonstrate the effect of sport at different life stages and competitive levels (148). The active players were recruited from three teams of different national categories so that the intensity of the highest (senior) level was comparable to professional play and the other levels represented less intense or less rigorous play. The retired players were 40 years old (mean) with the controls matched for age for the active and retired groups of players. The ANCOVA adjusted for age, weight and BMI identified a difference between active players and controls, but failed to support a difference between the various levels of active play. The senior players tended to have higher BMD differences than controls compared with the junior player/control differences. The retired players also demonstrated greater bone mass than controls in spite of
current physical activity levels similar to that of the controls. The greatest differences between players and controls were noticed at the hip (10.5-11.1%) followed by the lumbar spine (4.8%) then whole body (3.5%) measures.

Much of the previously discussed literature has identified the skeletal benefits of exercise in lower extremity sites. Many of the early exercise models focused on upper extremity athletes such as baseball or tennis players so that the dominant and non-dominant arms may be used to demonstrate larger differences in athletes (intra-individual) compared with non-athletes (135-137). Typical of this comparison idea, Haapasalo et al chose to look at the playing arms of female squash players (compared with the non-playing arms) as well as compare these athletes with non-athletes (149). By measuring six sites in bilateral upper extremities, they defined significant lean and bone tissue hypertrophy in the playing arms of the players when compared with controls. The side to side differences (dominant to non-dominant) between extremities was 11.5% for players compared with 2.7% in controls. When they compared athletes participating in squash before menarche with those who began playing after menarche, the differences were even greater. Athletes beginning sport before menarche had 20-24% more bone mineral than controls while those beginning sport after menarche showed 7-11% advantages. Sport specific effects on bone may be better evaluated with respect to menarche as well as menstrual status (to be discussed later). As one would expect, the more years spent in the sport, the greater the bone mass, but how much of the change may be due to menarcheal timing in combination with the sport? The Haapasalo et al study previously discussed in the growing individual section also supports the evaluation of time
since menarche (130). Upper extremity densitometry studies should likely evaluate the sport specific demands and control for time spent in those activities.

Ashizawa et al was among the first to report pQCT technology used to demonstrate bone mass differences in athletes (150). They looked at 17 tennis players with the usual intra-individual differences at the mid-radial and distal radial sites. The data supported the periosteal expansion of cortical bone as well as the thickening of trabecular bone. Contrary to previous studies of cortical bone, Ashizawa also detected a significant increase in endosteal circumference accompanied with a slight, but significant, decrease in volumetric density of the dominant upper extremity. Delineation of trabecular and cortical contributions to bone mineral density will be advantageous to clarifying the role of exercise in bone mineral density.

The young adult and adult athlete literature demonstrates numerous important points to consider in the analysis of bone density and physical activity data. Calcium intake is important to evaluate (control for) as it may interact with physical activity to promote bone density (151). Most evaluation of physical activity has used questionnaires or activity monitors to derive categories based specifically on the samples studied (tertiles or quartiles). Cortical bone is thought to be more positively influenced by physical activity, while the dose of exercise (frequency and intensity) which might enhance trabecular bone is still the subject of debate. It is difficult to sort out the effect of exercise on bone in different age groups because of the typical differences in physical activity levels and the timing differences in bone maturational processes between individuals (modeling, consolidation and remodeling). Various anatomical sites have been
commonly selected for evaluation for the risk of fracture, convenience of measurement and the supposed composition (cortical and trabecular) of bone at those sites. A wider variety of sports and activity levels have been explored in the adult literature.

2.3.1.3 Cross-sectional studies: aging adult

Many researchers have chosen to study the effects of physical activity in post-menopausal women and elderly subjects with the most immediate risk of injury secondary to osteoporosis. There are historically important studies that were completed in the 1980's. Oyster et al used 40 apparently healthy women ages 60-69 to evaluate cortical and total bone diameter via x-rays of the hand (radiogrammetry) (152). The subjects filled out a physical activity profile for three days, and the investigators estimated oxygen utilization from the profile as the evaluation variable for physical activity. Body size was considered in the statistical evaluation using body mass index, height, weight, and total metacarpal diameter. Only those women who had not taken estrogen replacement for the five years previous to the study were included. The final regression equation determined by linear-model analysis included activity, weight, age, estrogen use and total bone diameter as significant predictors. The ten subjects with the highest physical activity level were compared with those in the lowest quartile, and they found the more active women to have significantly increased metacarpal diameter and greater variability than the more sedentary group. Chow et al used 50-59-year old women measuring bone mineral (calcium) by neutron activation analysis and physical activity with a five-year history
questionnaire (153). Fitness of the subjects was measured with VO$_2$ max and strength measures under the assumption that fitness reflects level of activity. Total body calcium was positively correlated to fitness (as categorized by VO$_2$) and the more fit women had slower bone loss compared with the less fit subjects. All subjects in this study averaged 0.5-1 grams of calcium ingestion per day, and none was on exogenous estrogen. This is one of a few studies demonstrating a significant positive relationship between bone (total body calcium) and oxygen uptake capacity. Pocock et al also chose to measure VO$_2$ max in their study of 84 adult (20-75 yo) women (154). The skeletal sites measured (and method utilized) were the forearm (SPA), the spine and femur (DPA). Fitness (VO$_2$ max) was a stronger and more significant predictor of femoral neck density than either height or weight and accounted for 23% of the femoral neck BMD variation. These findings are unusual in that height and weight are repeatedly significant predictors for weight-bearing sites. Astrom et al used a case-control design to look at 58 hip fracture patients and age-matched controls (all 60-70 yo) (155). Physical activity was gauged by a telephone interview to develop a scoring system that accounted for raising children, sporting activity, professional work, mode of transportation, and level of household technology. In the final analysis, the hip fracture patients had been less active than the controls during their 15-45 year age period of life (using retrospective data). Among the conclusions, the authors stated they should have controlled for a few activities of daily living, such as running water, sewage and carrying firewood. These 1980's studies strongly supported the positive influence of physical activity in bone health in aging women.
More recently, Cheng et al studied 108 women 50-60 years old chosen according to habitual physical activity and education levels (156). Contrary to the results of Chow or Pocock, Cheng reported no significant correlation of VO\textsubscript{2} max with BMD. However, when the subjects were categorized according to average weekly hours of physical activity (0-3 hrs/wk, 4-8 hrs/wk and >9 hrs/wk), the calcaneal density of the group with less physical activity was significantly lower than the other two groups. Evaluation of weekly participation in vigorous physical activity demonstrated that those subjects reporting a frequency of more than two times per week had an increased density compared with less active subjects. This study also measured isometric quadriceps strength as a surrogate of total body muscle strength and reported a significant correlation between leg extension force, BMD of the calcaneus and the level of physical activity. Kyllonen et al measured isometric back extensor strength as a physical activity indicator in their study of 78 women (104). The femoral neck density was best predicted (regression) by body weight and isometric back extensor strength. The authors noted the collinearity of weight and strength. The 1995 study of Greendale et al used men and women over the age of 50 years (157). Known as the Rancho Bernardo study, it included 1014 women and 689 men with a mean age of 73 years. The radius was measured via SPA while the hip and spine densities were determined with DXA methodology. They reported that current physical activity was associated with bone density of the hip in a dose-related pattern and lifetime physical activity was also associated with hip BMD. The authors estimated this increased BMD due to exercise to translate to a 50% decreased fracture risk in the highest physical
activity group. The influence of physical activity in the multi-center European Vertebral Osteoporosis Study (EVOS) was reported by Silman et al (158). This large study included 36 centers from 19 European countries and included men and women ages 50-79. The primary focus of this report was to examine the role of physical activity in vertebral deformity based on standard evaluation of thoraco-lumbar x-rays. Of the 14,261 total subjects, 809 men and 884 women were identified with one or more spinal deformities. Physical activity was gauged with a historical questionnaire referencing the 15-25, 25-50 and >50-year old time frames. The increased physical activity level earlier in life was associated with a 50-70% increased risk of vertebral deformity in men. The historical physical activity of the women did not increase or decrease the odds ratio of having a vertebral fracture. On the other hand, the authors reported the current walking or cycling habits of the women to be associated with a reduced risk of deformity after adjusting for age, BMI, smoking, center and retrospective activity levels. Even though the odds ratio was 0.8 and the 95% confidence interval was 0.7-1.0, the authors concluded bicycling and walking had a significant protective effect for women.

There are few cross-sectional sport or athlete studies that were exclusively performed with aging adults. Many of the studies reviewed with the young adult literature also included aging adults (6,146, 140). Kirk et al compared young runners with 55-65-year old runners and found the normal age-related differences. Orwo\Ulf failed to find swimming-related increments in bone density in the 41 women Master's swimmers while there were increases in the aging male swimmers. Karlsson included adults dancers up to 68 years old and contended
that the aging dancers lost bone faster than controls, but they did not control for hormonal status. These studies were reviewed with the young adult literature because the subjects were primarily young adults.

It seems clear from the above studies among young adult athletes that sports participants have an increased bone mineral density. It seems less clear how much physical activity is necessary to maintain those gains and if those increments are carried into the post-menopausal years as a protective factor against fracture. Etherington et al approached the long-term benefits of exercise-induced bone mass advantages (15). Female ex-elite athletes (67 runners, 16 tennis players) aged 40-65 were compared with a large sample of age-matched controls (n=585). Controls were categorized according to the Allied Dunbar National Fitness Survey into one of three activity groups (active, inconsistent or sedentary) and all subjects were scanned for lumbar spine, femoral neck and forearm densities. The bone densities at the femoral neck and lumbar spine were consistent with a dose-dependent response according to exercise level. Even though some of the athletes had retired from sport while the others still participated, there was no identifiable difference in bone loss between the active and retired athletes suggesting the bone mass advantages of the athletes will be carried into old age. The results of this study were consistent with the peak bone mass concept and suggested that bone gained early in life may help enhance bone mass throughout the aging process. Ryan et al has recently reported on aging women athletes to demonstrate that athletes and controls experience similar age-related bone losses (159). These results are contrary to the early findings of Karlsson et al who proposed aging athletes lose bone mineral faster.
than controls (146). The potential protection afforded to athletes, according to studies like Etherington et al and Ryan et al, strengthen the motivation to identify the positive and modifiable influences in attainment of peak bone mass during adolescence.

Approaching physical activity with retrospective data usually supports the potential bone influence the adolescent and young adult years may project into the later years. However, the promotion of physical activity in adolescents as a deterrent of senile or post-menopausal osteoporosis should be premised on long term follow-up studies from adolescence through senescence.

2.3.2 Longitudinal studies

2.3.2.1 growing individuals

Relative to cross-sectional studies, the literature provides scant longitudinal evidence for a firm role for exercise in normal skeletal growth and development. Many of the longitudinal analyses performed on children and adolescents were designed as calcium intervention trials to determine the pro-skeletal effects of better than usual calcium consumption (160-163).

Only few calcium intervention trials have included assessment of physical activity. Lee et al studied seven year old males (n=87) and females (n=75) through a primary school located in an area of China where low calcium intake is the norm (<300 mg/d) (164). Cherry-flavored Tums served as the calcium supplement six days per week over the 18 months of the study. One teacher was responsible for administering the supplement to children (four classrooms) as a routine part of the day after school breakfast. Physical activity was measured
with a modified Bouchard tool where activity intensities were rated from 1-7 (usual Bouchard is 1-9) for each fifteen minute period throughout the day, and the hours of 4-7 level activities were averaged. There were no differences between the levels (time spent) of physical activity for respective supplementation groups so physical activity was not further evaluated. The increased bone mineral gains of the radius in the supplemented group compared with the gains in the control group were 18.2% for BMC and 50% BMD. Cadogan et al provided one pint of milk as the supplement in a study of 80 adolescent females (mean 12.2 yo) (165). Physical activity was measured by questionnaire and reported as kJ/kg/day. There were no differences in physical activity between the milk and control groups, which led to the conclusion that physical activity did not affect the outcome of the study.

The most relevant longitudinal studies of adolescent females where physical activity was evaluated fail to support physical activity as a significant and firm contributor in bone development. Katzman et al studied 12 females (9-21 years old) from a previous cross-sectional study sample for two years (23). Sites measured included the radius, spine, hip and whole body. Physical activity was categorized into three groups according to self-report by the number of thirty minute exercise sessions per week (>3, 2-3 or <2 sessions per week). The underlying assumption in using these categories in the analysis was that the subjects were able to discriminate between 60 minutes, 60-90 minutes and over 90 minutes of habitual exercise per week over the two-year study. Pubertal stage, age, height, and weight were identified as significant predictors of bone mineral status at various sites and in various combinations. Age was modeled as
a quadratic variable (age & age²) and they evaluated BMC, BMD as well as an area-corrected BMAD (bone mineral apparent density in g/cm³). The authors concluded that most of the whole-body bone mineral gain in adolescence reflected bone expansion. The data did not support a predictive role for dietary calcium intake or habitual physical activity in the bone mineral accretion of this sample. Johnston et al also used a combination of methodologies to measure bone density in their longitudinal analysis (166). They studied 45 pairs of 6-14-year old identical twins (males and females) over a 3-year time period (also designed as a calcium supplementation trial). The radial measures were taken by SPA while the spinal and femoral measures were derived from DPA technique. Physical activity was measured every six months by self-report as hours of weight-bearing physical activity. There were no differences in the level of physical activity between the supplementation and control groups, and the authors stated that the effect of calcium supplementation was independent of the level of physical activity. These two studies among adolescents are the longest (2 and 3 years) densitometric studies reported in the literature.

The remainder of longitudinal studies where physical activity was recorded were shorter and studied both males and females. Theintz et al sampled 198 nine- to nineteen-year old youths to gain insight into the growth patterns of the spine and femur over a one-year period (167). This is likely the same sample of as reported by Bonjour in 1991, and the sample intentionally omitted subjects with more than 10 hours of “intensive practice of physical exercise for several years”. The subjects were grouped by age and pubertal stage where the fifth stage for females was further delineated into a, b, c, d (1, 1-2, 2-4
and >4 years) to represent time since menarche. The authors focused on expressing gain in bone mass as a function of age and sex and performed two-sided Student's t-tests for paired comparisons. It did not appear that the role of physical activity was addressed outside of the exclusion criteria. The "pubertal loop model" was the focus of this report where the annual change in BMC (lumbar) was plotted against the annual change in height by pubertal stage to produce a predictable "loop" pattern in males and females. Kroger et al studied a smaller sample of 7-20-year old males and females (n=28 & 37 respectively) (168). BMC, BMD and bone size of the lumbar vertebra and the femur were measured by DXA. The subjects were categorized according to three levels of physical activity (no PA outside school, active in sports / physical activity at least 3 hours per week, or active in sports over 5 hours per week). In addition to the BMC and BMD standard measures, this group also corrected for bone size by deriving the apparent or volumetric density (BMAD). In females, the main increase in BMC before menarche was due to increases in bone size while the maximum increase in bone density (volumetric) occurred at menarche. There was a trend for the highest PA category to have higher gains in BMD and volumetric BMD, while the regression analyses (controlled for weight, height and age) yielded similar results. Both of these one-year longitudinal analyses which aim to identify growth patterns only measured the hip and lumbar spine.

Theintz et al studied adolescent female athletes (gymnasts and swimmers) for 2-3.7 years with measurements occurring every 5-7 months throughout the study (169). Densitometry was not performed in this study, but the skeletal ages (Greulich-Pyle method) and growth patterns were recorded. The leg length
(growth) of the gymnasts appeared to be stunted (2 standard deviations below normal) when the sitting and standing heights were compared. The peak height velocity curve as plotted across bone age also demonstrated a detrimental pattern for gymnasts when compared with the plots of children with normal levels of physical activity. The conclusions included a suggested threshold for participation in exercise of this nature to be 15-18 hours per week.

Many of the longitudinal studies have acknowledged the importance of the pubertal stage and menarcheal status of the female, but none have evaluated the changes across puberty. Since menarche seems to mark a short duration of very rapid, but slowing increase in bone mineral, it is likely important to evaluate bone change over time with reference to this biological event.

2.3.2.2 Longitudinal studies: adults

More objective methodologies for evaluating physical activity have been used in a few of the longitudinal adult studies. It may be important to think about how these methodologies would impact data gathered on adolescents. The adult skeleton has a different physiology compared to the adolescent and the response to exercise is possibly different.

Mazess and Barden studied bone mass change in 300 women ages 20-39 years over a two-year period (20). The physical activity was measured by CalTrac and with a "conventional pedometer" and categorized according to counts. The data were not able to support an association for physical activity and BMD. Recker et al studied 18-26-year old subjects to gather some similar CalTrac data as they measured the physical activity of the subjects for four days
every six months over a four-year study period (170). The data from their 156 subjects demonstrated a small and significant positive correlation between physical activity and the rate of change in spinal bone mineral density (biannual DPA measurements). The CalTrac monitors in this latter study were worn on the subject's wrist rather than on the waist where they are usually worn. Welton et al used a unique experimental design to study 84 males and 98 females between 1977 and 1991 (25). Between the ages of 13-17, the subjects were followed annually then again at ages 21 and 27. Average weekly hours of physical activity was obtained by questionnaire, converted to METs of weight-bearing activity then categorized accordingly into 4-7, 7-10 or >10 METs per usual week. There was no consistent influence of physical activity on the bone density in females and body weight was found to be the best predictor. In contrast, physical activity was the best overall predictor in males. Some clinicians have been of the opinion that evaluating physical activity via mechanical means, such as CalTrac, may bypass the subjectivity of self-reports, but as the above studies demonstrate, there are issues of how the data may be collected most accurately and how to handle the collected data. The study of Welton et al is one of the few reported in the bone literature to convert physical activity to METs, a standard exercise measure of sport intensity in terms of metabolic costs. Many of the mechanical device studies have evaluated the counts registered and have not considered the intensity of the exercise.

Studies of adult athletes in the longitudinal literature are also fairly short in duration. Bennell et al studied young adult men and women across a twelve-month study period (test-retest) to compare power and endurance athletes from
track and field and controls (171). Menstrual, dietary and physical activity data were collected via questionnaire, and the whole body and regional DXA data were collected. Female athletes had a later menarche than controls (2 years later), and the endurance athletes reported more frequent menstrual disturbances than power athletes or controls. Athletes tended to demonstrate similar bone density results in comparison with the controls before adjusting for height and weight. After controlling for height and weight, the athletes had higher BMC than the controls, and power athletes had greater spinal BMD than endurance athletes. In general, the athletes demonstrated a greater bone gain over the year of the study than the controls. The power athletes demonstrated this at every site while the endurance athletes demonstrated this at mostly lower extremity sites, not the lumbar spine and upper extremity sites. The region-specific changes were much greater than what could be accounted for within the total body changes, which led the authors to conclude that exercise influenced the pattern of bone distribution in the skeleton. This is similar to the steal phenomena previously mentioned and supported Rubin and Lanyon’s theory that strain magnitude is more osteogenic than strain frequency. Taaffe et al studied a pool of athletes including gymnasts, runners, swimmers, and controls (172). The study included two cohorts of 18-29-year old athletes where one cohort was studied for 8 months (gymnasts, runners and controls) and the other for 12 months (gymnasts, swimmers and controls). Data for most of the subjects had been reported previously as cross-sectional analyses (13, 173). In agreement with the cross-sectional literature, the gymnasts had a superior baseline BMD of the lumbar spine and femoral neck compared with the other athletes and
controls (when adjusted for body size). The gymnasts continued to accumulate (gain) bone mineral at the lumbar spine and femoral neck while the runners, swimmers and controls commonly lost bone mineral at the lumbar spine and femoral neck. The data for the site-specific gains and losses lead to the conclusion that the type of training influenced the pattern of distribution as the changes were not always parallel. In the 8-month cohort, the total body measurement demonstrated gains across all groups despite lumbar and femoral neck decreases while the controls were the only group in the 12-month cohort to demonstrate whole body losses.

Most bone studies that evaluate the longitudinal contribution of physical activity categorize the level of activity based on time spent in weight-bearing activity or assume a linear relationship between physical activity and bone mineral. Michel et al have studied the lumbar bone mass of subjects older than 50 years using computed tomography techniques (174). The two-year longitudinal study was conducted using male and female runners as well as controls with the runners being volunteers from the local Fifty-Plus Runner's Association. Exercise was measured in minutes per week and found to have only a moderate ($r=0.36$) association with spinal bone density. When the analysis was performed using the data from women exercising at a moderate level, the correlation was 0.91. A most important contribution of this study was the modeling of exercise as a quadratic variable and identifying lower spinal bone mass in runners with the most vigorous exercise levels. The authors called this group "over-exercisers" and identified these less positive changes in four of the twelve women runners. This same research group has identified similar
evidence in a previous cross-sectional analysis (175). The results of Slemenda et al and Theintz et al as discussed in the growing individual literature also supported detrimental skeletal effects of too much exercise (10, 169).

The literature that covers longitudinal studies among adults does suggest that the adult skeleton may continue to accrue bone mineral up to the third decade. Longitudinal studies on athletes support increased bone mineral density (when the data are adjusted for body size) and gain rates in athletes compared with controls, but the athlete studies fail to address the potential for bias. It is possible that the athletes have a greater bone density before sport participation. The changes in the bone mass of athletes observed in longitudinal studies do not appear to be as dramatic as the differences in bone mass described in cross-sectional studies, but sport performed over a long period of time may confirm the larger differences found in cross-sectional analyses.

2.3.3 Clinical exercise trials:

2.3.3.1 growing individuals

There are a few intervention studies using exercise conducted among adolescent females. Eliakim et al capitalized on the unique opportunity to study 15-17-year old female and male adolescents (176, 177). The subjects were students enrolled in a five-week summer anatomy and physiology class. Pre-testing consisted of a mid-thigh magnetic resonance imaging (MRI), VO₂ max prediction by bicycle ergometry and blood samples for hormonal analysis. All participants attended traditional class for two hours then half would work on the computer while the other half underwent endurance-type training. The short
duration of the study would not warrant bone density measures, but the blood samples were analyzed for growth hormone, growth hormone binding protein, IGF-1, insulin-like growth factor binding proteins 1-5 (IGFBP-1-5), osteocalcin and estradiol. The results may be questionable due to a low number of subjects agreeing to provide blood samples (6 control, 8 exercise), but the main findings could be important to exercise physiology and injury prevention. It appeared that all subjects in the intervention group decreased serum IGF-1 and IGFBP-5 and increased serum osteocalcin levels. The decreased IGF findings could indicate an initial catabolic response to the training while the osteocalcin rise might indicate stimulation of osteoblastic (bone formation) activity and/or increased bone remodeling. Morris et al studied a younger group of subjects in a school setting (109). They ran a 10-month intervention trial on 71 nine- to ten-year old females. Pre-testing included spine, femur and whole body DXA, Cybex II peak torque for the knee and shoulder and hand (grip) dynamometry. The intervention sessions occurred three times each week for thirty minutes each session and included a variety of activities as led by a physical education teacher before school. Physical activity outside of the intervention was accounted for by using the Godin questionnaire then categorized according to METs. The groups were nonrandomized, but the intervention group did demonstrate an increased lean body mass, BMC and BMD over the course of the intervention compared with the controls. Height, weight, lean body mass, and shoulder strength were all positively associated with bone mass. Bradney et al also used the school setting to study pre-pubertal males (30). The twenty intervention subjects from one school exercised 30 minutes, three times per week for thirty-two weeks in
addition to regular physical education classes and were compared to a matched group from a second school. The changes in BMC and BMD of the intervention group were almost double the changes of the controls. Other growth indices indicated greater growth in the exercise group as well. The noted increase in cortical thickness in the exercise group was due to decreased endocortical diameter and not periosteal apposition. Witzke et al reported a nine-month intervention trial with 14-year old females (n=25) and controls (n=28) (178). The training program was preceded by DXA of the femur and whole body as well as leg strength testing. The training regime consisted of plyometric and weight training for 30-45 minutes three times each week. The authors were able to demonstrate changes significantly different from zero, but were unable to clearly demonstrate differences between the intervention and control subjects. The intervention subjects had a greater change in BMD for all sites, but this only reached statistical significance at the greater trochanter. The gains in leg strength of the intervention group were twice that of the controls. Plyometrics and weight training should be two of the more osteogenic activities available if the impact forces are the important characteristics of exercise affecting the skeleton.

2.3.3.2 Clinical exercise trials: young adults

Gleeson et al studied a wide age range of females (23-46 yo) in a twelve-month Nautilus (resistance training) program (179). The subjects were allowed to choose whether to be in intervention or control groups and were matched for age so that there were 34 exercisers and 38 controls. Nautilus intervention was performed 30 minutes three times per week. Subjects were also provided with
500 mg of supplemental calcium per day to ensure and control for adequate calcium availability. Despite choosing exercise or no exercise groups, the 49% subject drop out rate exemplifies the nature of recruitment and retention problems for these studies. The results showed a significant increase in the lumbar spine density in the exercise group while the controls lost lumbar bone mineral. In contrast, Rockwell et al demonstrated that weight training, using Eagle Cybex training equipment (pneumatic style resistance), was potentially detrimental to the 10 exercise subjects compared with the 7 controls in their study (19). Bone density at the hip and spine was measured by DPA, and blood was analyzed for serum calcium, osteocalcin and parathyroid hormone (PTH) at baseline, and after 4.5 and 9 months of intervention. The exercises were performed twice each week for 45 minutes each session at an intensity consistent with 70% 1 RM. Subjects were supplemented with 500 mg calcium and 200 IU vitamin D₃. Despite similar age and initial lumbar spine density, the exercised subjects had a significant decrease in lumbar bone mineral density and serum calcium with an associated increase in PTH. The greatest rate of loss occurred in the initial 4.5 months of the program. This weight-training study clouds the picture as to the effects of exercise on the adult skeleton, but the control for dietary habits and physical activity outside of the study was poor as was the number of subjects. A younger group of college-aged females (19.9 yo) were the subjects of study for a weight training / running study for Snow-Harter et al (24). The eight-month study began with 52 subjects, which dwindled to 31 by the study's end. Subjects were randomly assigned to running, weight training or control groups. Athletes were intentionally excluded from the study, and the
participants were asked how many hours per week and weeks per year they exercised. This information was transformed to a weekly average and physical activity was assigned into one of three groups as a way to control for activity outside the study (≤1, 2-3 or >3 hours per week). Subjects in the running and weight training groups had significantly increased spinal BMD when compared with control subjects, but the groups did not differ in hip BMD changes. This study was likely one of the better-controlled intervention studies. These three studies were likely too short to identify the long-term nature of the skeletal effects of exercise.

Other research groups have used other forms of exercise to help identify the relationship of physical activity to bone density. Bassey et al used female subjects (n=27) in a partial cross over design to demonstrate the anabolic effects of "high-impact" exercise over a twelve month period (180). The subjects were in their late 20's or early 30's and calcium intake was brought up to 1 gram per day as a nutritional control. All subjects (exercise and placebo) attended a one-hour exercise session per week and were given home exercises. The exercise intervention group was different in that the class and home exercises included jumping and high-impact movements compared with the more mild nature of the class and home exercises for the control group. After six months of exercise, the higher impact group increased density at the trochanter by 3.4%, although many of the other skeletal sites (radial, spine, other femoral) demonstrated slight decreases. Control subjects crossed over to the high-impact group at 6 months and demonstrated similar increases (+4.1%) over the last six months of the study. The higher impact activity slowed the normal loss of bone mineral in the femoral
neck and Ward's sites when compared with the lower impact exercises. The 1995 study by Friedlander et al also supported the slowing of bone loss with exercise (181). The two-year study of 20-35-year old females enrolled 127 subjects with a 50% drop out rate to complete the data for only 63 of the initial subjects. Measurements included QCT of the (trabecular) spine, SPA of the calcaneus, DXA of spine and femur, Cybex strength testing of trunk and knee musculature as well as completion of the MLTPAQ to control for previous and extra-study exercise levels. The intervention included various combinations of calcium, placebo, exercise and stretching for three one-hour sessions weekly. It took two years for the bone mineral gains in the exercise group to be significantly greater (trend after one year) compared with controls, and no calcium supplementation effect was identified.

2.3.3.3 Clinical exercise trials: aging adults

As the threat and onset of blatant osteoporosis become more evident for females with advancing age, so does the bulk of intervention literature identifying the efficacy of exercise in staving off the disease process. Most of the early osteoporosis prevention and treatment studies focused on the perimenopausal life stage as it is the time when rapid bone loss begins.

The well-cited intervention reported by Aloia in 1978 studied 9 exercise and 9 control subjects for 11-13 months (182). The women were 53 ± 5.6 years old and exercised three times per week for an hour each time. Total body neutron activation analysis was used to measure total body calcium along with SPA of the radius. The exercise group increased total body calcium while the
control group lost. This is considered a landmark study in humans. In another historically important study, Smith et al completed a 36-month intervention with 65-95-year old subjects (n=80) (183). The design included groups for exercise (3x/week, 30 minutes each), supplement (750 mg Ca, 400 IU vit. D), combination of exercise and supplement and controls. Exercise intensity was set relative to the estimated oxygen capacity of the subjects and was described as “light-to-mild (1.5-3.0 METs)”. Bone measurements of the non-dominant forearm (SPA) were taken every 4 months, and the changes in the radius were analyzed. The exercise and supplement groups both improved BMC and BMD compared with the control group while the combination treatment group did not improve. The authors noted that despite random group assignment of the volunteer participants, there were a greater number of older subjects in the combination group. This may have attributed to the lack of change. Interestingly, the bone width of the supplemented group decreased as the BMC increased which led to an even greater BMD in that group. Krolner et al used subjects with a history of distal forearm fracture (184). They studied 31 subjects between the ages of 50 and 73 over an eight-month time span. The spine and radial sites were measured via DPA at baseline, 4 months and after 8 months. Intervention exercises included walking, running, calisthenics and ‘ball games’ and subjects were allotted to the groups based on the convenience of living closer to the study facility. The exercise subjects demonstrated a significant improvement in the lumbar spine while the controls exhibited a normal age-related decrease. Both groups decreased in the radial measures, but the loss tended to be slower in the exercise group.
Chow, Harrison and Notarius randomized 48 female volunteers (50-62 yo) into three groups to compare the effects of two different exercise programs on bone mass (185). Aerobic capacity was determined by a graded exercise test performed on the treadmill to voluntary fatigue. Total body calcium (TBCa) was measured via neutron activation analysis (reproducibility within 5%) at baseline and after twelve months of structured exercise programs. One exercise group participated in aerobic exercise three times per week while the second exercise group participated in aerobics and strengthening exercises with the same frequency. Both of the exercise groups demonstrated significant increases in total body calcium while the controls showed a decrease. The group that incorporated weight training had a more significant increase, but the differences between the exercise groups were not statistically significant. The statistical analysis also found a positive correlation between the increase in TBCa and the improvement in maximal oxygen uptake. Thirty-five 55-70-year old females served as the subjects for study by Dalsky et al (186). This is one of the rare intervention studies to address detraining issues. The previously sedentary volunteers were permitted to select participation in the exercise or control group. The exercise programs lasted 9 and 22 months (short and longer term). The subjects serving in the 9-month group were re-measured at 22 months to determine the effect of 13 months of detraining. Measurements included fitness testing (VO₂ max), food record evaluation and DPA of the spine. Initially, exercise subjects performed 50-60 minutes of weight-bearing exercise (60-70% VO₂ max on treadmill) three times per week. In accordance with good progression, stair-climbing (30-40 minutes) was added at month 3 and some
subjects continued the treadmill for 20-25 minutes in addition to the steps with increased intensity (70-90%). Some additional non-weight-bearing exercises such as rowing, cycling, bench pressing were used to improve program interest and compliance. All subjects were supplemented with calcium (total intake 1500mg) and vitamin D. The short-term exercise group improved density of the spine by 5.2% while the control group decreased 1.4%. Results were similar in the long-term groups. After detraining periods, the former exercise subjects had not maintained these bone mass improvements. Dalsky surmised that the critical element in bone adaptation to exercise was the stress or intensity of the weight-bearing exercise. Sinaki et al contributed to this theory as they studied the effect of 24 months of non-loading exercises on the lumbar spine of 49-65-year old women (n=65) (18). The home exercise program was designed for the back extensors and muscle strength was measured every three months by strain gauge dynamometry. The muscle contractions (ten) were performed daily with 30% of the maximal weight lifted as backpack resistance. Physical activity outside the program was evaluated by questionnaire, and the spine density was measured every six months by DPA. The rate of change was significantly negative for both the control and exercise groups. There was no difference between groups despite clear gains in muscle strength in the exercise group. The authors offered the submaximal and non-weight-bearing nature of the exercises and subject non-compliance as potential reasons for their negative findings.

Grove and Londeree compared three levels of activity- sedentary, low impact and high impact in their one-year randomized design study (187). Subjects were screened and matched carefully to ensure reasonable homogeneity
of the sample. Blood samples, aerobic capacity, body fat and nutritional analyses were performed to ensure evaluation of potential bias. Lumbar spine density was evaluated at baseline, 6 and 12 months by DPA. The weight-bearing impact of the exercises was determined in a pilot study where various motions were analyzed on force plate to identify if they constituted more than twice the body weight (2x BW). Low impact exercises were defined as less than 2x BW. The exercise groups performed their respective programs three times per week for an hour each time where there was warm up, twenty minutes of intervention and a cool down phase. The control group lost a significant amount of bone mineral while the exercise groups demonstrated similar maintenance in BMD. The authors concluded that the threshold for bone maintenance may be lower than 1.5x BW while the stimulus required for significant gain in a similar time period may need to be higher than those performed in this study. Additionally, since the results did not demonstrate significant gains in maximal oxygen capacity, the authors felt the intensity of exercise may not have been comparable to other studies. Kohrt et al also explored the impact of exercise as compared with muscle contraction forces (188). The intervention study of 60- to 74-year old women was 11 months long and included 39 subjects divided into ground reaction forces or joint reaction forces exercise groups or sedentary control. Exercise for both groups was similar for the first two months to help establish a baseline fitness level to prevent injury. Subjects participated at least 3 times and up to five times per week. The exercise programs were somewhat individualized and were progressive in nature. The ground reaction subjects did activities like walking, jogging and stair climbing whereas the joint reaction subjects performed rowing,
and weight training (mostly Nautilus). Maximal aerobic power, muscle strength, body composition and BMD of the femur, lumbar spine, radius and total body were measured at three month intervals by graded treadmill walking, Cybex II dynamometry and DXA respectively. The modes of exercise produced expected results in muscle strength and aerobic power. Both exercise groups improved BMD of the whole body, lumbar spine and Ward’s area of the proximal femur. Additionally, the ground reaction forces group significantly improved BMD of the femoral neck. They were able to demonstrate that those subjects with the lowest baseline values were able to gain the most, an exercise physiology concept commonly termed the “Principle of Initial Values” (189). This study supports exercise as osteogenic, but did not support a difference between the muscular contraction and pounding characteristics of exercise.

Given the sweat investment and injury risk that go along with the modest bone improvements typically seen in exercise programs for adults, some new work should be included in this discussion on the mechanical nature of the most efficacious interventions. Recker et al presented preliminary data on a mechanical vibrating intervention where the subjects stood on mechanical device for just two ten minute periods each day designed to deliver a “barely perceptible” vibration to the subject (190). The early data demonstrated a 0.1% gain in the treatment group while the placebos lost 0.7% of the lumbar bone mineral measured. These results were found in a small group (40 subjects) of women who had been post-menopausal for 3-8 years and had completed six months of the intervention study.
Intervention studies on post-menopausal subjects have helped to identify the likely nature of the exercises that have the potential to be most osteogenic. While the early works of Aloia, Smith and Krolner helped identify the positive nature of exercise on the skeleton, researchers such as Chow, Dalsky, Grove and Kohrt have helped delineate the nature of those exercises. Application of these studies to the young adult and adolescent skeletons should be encouraged in the quest to prevent osteoporosis.

2.4 Hormonal Effect of Exercise

The hormonal milieu of the human body directs the anabolic and catabolic processes and ultimate state of health. Identification of the relationships between hormonal changes and aspects of health, such as bone health, may provide insight into the mechanisms of the influence of exercise and how the physiological machinery fits together. Ironically, examination of the changes in blood levels of parathyroid hormone (PTH), calcitonin, estradiol, calcitriol and other endocrine factors in bone health lead to a more obscured understanding of the commonly accepted osteogenic effect of exercise.

2.4.1 PTH, calcitriol, and calcitonin

PTH, calcitriol and calcitonin are the main serum calcium-regulating hormones. In general, PTH is released from the parathyroid glands in response to a decreased serum calcium level and it stimulates bone resorption, kidney calcium preservation and calcitriol synthesis. Calcitonin, which is secreted from the parafollicular (c) cells of the thyroid gland, is antagonistic to the effects of
PTH with respect to calcium. High serum calcium stimulates the release of calcitonin and inhibits the osteoclastic resorption of bone and may slightly increase urinary disposal of calcium. The kidney's activation of calcidiol to the active calcitriol is dependent on PTH. Calcitriol, in turn, helps overcome low serum calcium by increasing intestinal calcium absorption and bone resorption.

Aloia et al studied the exercise-induced changes in the calcium- and bone-regulating hormones (191). They measured the responses to a progressive twenty minute bicycle ergometer exercise bout in 16 male volunteers (mean age 31 and untrained). Among the serum changes identified after five minutes of exercise, was an increased concentration of total calcium and calcium ions, but Aloia attributed this increase to plasma volume shifts due to a concomitant and linear increase in albumin and hematocrit. After ten minutes of ergometry, PTH was suppressed while calcitonin increased. PTH was at its lowest point after 15 minutes of exercise. Aloia and others in this era believed the skeleton to be the calcium source to support exercise and muscle contraction, thus, demonstration that the skeleton was protected by an increased calcitonin was an important protective design of the exercising body. Does this protection weaken after fifteen minutes of exercise or are longer exercise bouts necessary? As lactic acid was an area of focus in the mid-eighties, Aloia was trying to evaluate if these hormonal responses could be attributed to elevated lactic acid levels. He was unable to completely attribute these osteotrophic changes to exercise-induced lactic acidosis. Grimston et al published a DPA study in 1993 trying to establish a mechanistic framework for osteopenia in runners (192). This study uniquely looked at the calcitrophic hormone response to changes in serum calcium in 14
female long distance runners. Instead of evaluating spine density as the
dependent variable, it was used to group the runners into similar densities to
analyze the hormonal responses of the groups after an oral calcium load. The
authors noted that subjects with lower BMD in the spine also had lower BMD at
the femoral neck and tibia. The runners were in their early thirties, ran more
than 50 km per week, and were tested during 45 minutes of sub-maximal
treadmill running. Six control subjects were tested for comparison, but only in
the resting condition and would not agree to treadmill running. Blood was
analyzed for intact PTH, osteocalcin, vitamin D metabolites, cortisol, estradiol,
sex hormone-binding globulin, and calcitonin. The osteopenic runners had an
abnormal response to the oral calcium load compared with the higher bone
mineral density group demonstrating increased parathyroid hormone and
decreased calcitonin. The noted results of this study may help explain individual
differences and why overtraining or too much exercise may lead to less positive
bone density changes in some athletes. Klausen et al evaluated a similar
hormonal response to habitual training in nine older male marathoners (41-50
yo) (193). The study design called for 3 weeks of detraining followed by a four-
week period of retraining with evaluations at baseline, after two weeks and four
weeks of retraining. The albumin-corrected serum calcium was maintained
during detraining and dropped significantly at the end of the first two weeks of
retraining to elevate again at the four-week measurement. Serum calcitonin
followed a similar pattern while serum PTH increased gradually throughout the
detraining and retraining periods. The findings of these three studies together
tell us that acute exercise responses may influence the body's handling of
calcium and the calciotropic hormones. However, the hormonal adaptation to chronic exercise remains to be confirmed.

### 2.4.2 Athletic menstrual issues and bone density

An increased incidence of menstrual dysfunction in athletes has been consistently proposed since first reported by Erdelyi in 1965 (194). In the early 1970's, Frisch and colleagues popularized the idea that amenorrhea was secondary to a lack of body fat to support the reproductive physiological function, and this signal was mediated through the hypothalamus and reproductive hormones (195). They proposed the critical level of relative body fat needed to achieve menarche to be 17% while the amount of fat needed to maintain normal and ongoing reproductive function needed to be 22%. The predicted lean to body fat ratios and absolute amounts of fat and lean mass needed are also delineated in this report. Frisch also found in the early 1980's that beginning sport before menarche was causative for delaying menarche and that each year of pre-menarcheal training would delay menarche by 5 months (196). The Frisch body fat hypothesis is still popular as there seems to be some link between amenorrhea and body fat, but some researchers refute the specific critical body fatness level proposed by Frisch et al (197-200).

The mechanism of menstrual disruption appears to be driven by an intricate hormonal cascade. In 1980, Warren discarded the body fat theory for an energy drain theory whereby afflicted athletes chronically expended more calories than consumed (197). Obviously, a chronic energy drain would result in lower body fat, but this theory also allowed for menstrual disruption in athletes
with higher levels of body fat. The menstrual disruptions were attributed to hypoestrogenism and were again suggested to be hypothalamic in origin. Loucks and Horvath agreed with the hypothalamic origin of the amenorrhea and also refuted the critical threshold of body fat idea in exchange for a relationship between amenorrhea, training distance (in runners) and cortisol (198). Loucks further defined her stance on the hormonal milieu of amenorrheic athletes to include an interaction between the gonadal and adrenal hypothalamic-pituitary axes (201). Loucks was direct in her opposition to Frisch's opinion on athletics as the cause for late menarche and expressed it as more correct to say "the average age of menarche is later in athletes, but is not caused by athletic training" (201).

A paper by Dueck serves as an excellent review of how the energy drain hypothesis interacts with the hormonal axes (200). The interactions of the adrenal, thyroid and reproductive axes make it difficult to ascertain which hormones change first in the sequence, but low estradiol levels are usually accompanied by increased cortisol levels in many of the amenorrheic athlete studies. Dueck would contend that cortisol is released in response to physical or psychological stress and the low estradiol is a predictable result of increased cortisol levels. Figure 2.1 is a modification of the schematic presented in Dueck's review and helps visualize the hypothalamic responses to various stressors. The work of Bonen reinforces these same hormonal changes and interactions as she supported the energy drain concept in her studies of the hormonal responses to exercise in women (199). These ideas have been the most prevalent in the early amenorrhea literature to define the mechanism, and it has become accepted that the amenorrhea is hypothalamic with the cascade of hormonal events initiated by
a decreased release of gonadotropin releasing hormone (GnRH). The decreased reproductive hormones and associated changes follow the consequences of decreased GnRH. The exact mechanism for athletic amenorrhea has yet to be elucidated, but the notion that it must somehow be linked to energy status and nourishment is still as popular as the menstrual disruptions themselves.
Figure 2.1 Schematic of the hormonal impact of "stress" on the hypothalamic-pituitary-ovarian, -adrenal, and -thyroid axes. Ovarian axis: gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), and progesterone (P). Adrenal axis: corticotropin-releasing hormone (CRH), adrenocorticotrophic hormone (ACTH), and cortisol. Thyroid axis: thyrotrophin-releasing hormone (TRH), thyroid-stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4). (Adapted from Dueck et al (200))
Cann *et al* were amongst the first to publish diminished bone mineral density in amenorrheic female athletes (202). Spinal bone mass was particularly decreased in the amenorrheic subjects studied and 10 of the 11 hypothalamic amenorrheic subjects were involved in regular vigorous exercise. Drinkwater *et al* studied 14 amenorrheic and 14 eumenorrheic runners and found a significantly lower spinal density in the amenorrheic group, but there were no group differences detected in the radial densities (203). Analysis of the mileage of these athletes showed the amenorrheics running 41.8 miles per week compared with the 24.9 weekly miles of the eumenorrheics. Although the difference between daily energy intake of the groups wasn’t statistically significant, the amenorrheics averaged 1622 kcals compared with the 1965 kcals of the eumenorrheic group (ran more, ate less). Fisher *et al* also looked at this relationship in a study of runners and also found a lower spinal BMD in the amenorrheic group (16). The amenorrheic group was found to have decreased GnRH, LH, FSH, estradiol, estrone levels and increased estrone:estradiol in comparison with eumenorrheic runners. Estradiol concentration was positively correlated to bone density in all the subjects and estrogen deficiency was considered a probable cause of osteopenia in the amenorrheic group.

Multiple laboratories have identified an increased fiber intake in amenorrheic athletes and this needs further examination. Lloyd *et al* compared 11 eumenorrheic and 6 oligomenorrheic athletes with 12 eumenorrheic controls (204). Though the study failed to demonstrate a significant difference between the groups, eumenorrheic athletes had the highest bone mineral content followed by the controls then athletes with menstrual disruption. The oligomenorrheic
athletes differed from the others in an apparent better dietary intake (kcal, CHO, Ca), lower body fat, increased exercise days per week and increased fiber consumption. Buchanan et al reported a similar study and was also unable to demonstrate a significant decrease in spinal bone mineral though the mean spinal bone densities had the same pattern Lloyd described (205). The oligomenorrheic group in this study had decreased reproductive hormone levels and consumed more fiber than the other two groups (eumenorrheic and control). Dhuper et al reported on 43 dancers and controls 13-20 years old (206). Use of an “integrated estrogen exposure score” incorporated menarcheal age, developmental stage of breast, number of menstrual cycles per year, estradiol level, contraceptive, and estrogen replacement pill history. When the authors limited analysis to the older subjects (18-20 yo), the lowest estrogen scores had the lowest spine and wrist densities, the lowest body weight, weight to height ratio and consumed more fiber.

Dietary fiber could be a surrogate nutrient identifying major dietary differences, could be decreasing calcium absorption or affecting estrogen metabolism. Weaver has demonstrated a decreased calcium absorption in diets high in wheat bran (207). Both low fat and high fiber diets have been shown to reduce serum estrogen concentrations in feeding studies (208, 209), while other studies fail to support this relationship (210). Additionally, it may be that the fiber replaces calorically dense foods to satiate the athlete and further contribute to the calorie drain promoted by the higher or more frequent levels of exercise. A high fiber diet is a plausible cause of amenorrhea as well as decreased bone density; however, this requires further investigation.
Very recent work by Colin et al in rats provided some evidence that the intestinal absorption of calcium is influenced by estradiol levels (211). They ovariectomized (OVX) rats, treated them with various levels of 17-βE₂ (estradiol) and analyzed the small intestinal handling of calcium and serum vitamin D metabolites. The OVX with no estradiol group had a significant decrease in duodenal calcium absorption. If this were the case with humans, it would help explain the osteopenia associated with estrogen deficiency.

Diminished skeletal integrity due to menstrual disruption in athletes has been well defined in cross-sectional studies, but there are no longitudinal studies to identify how this develops and if it is reversible. In 1986, Drinkwater et al followed up on 9 of 14 original subjects from their 1984 study (212). Seven of the nine subjects regained menses within 1-10 months of the original measurement. The previously amenorrheic subjects gained significant bone mass compared with controls, and the two remaining amenorrheics continued to lose bone mass at the lumbar spine. Warren et al have also published an initial study demonstrating lower bone density in amenorrheic ballet dancers and a follow-up study to show the increase in bone mass with resumption of menses (120, 213). Even though the follow-up study found improvement after the return of menses, the previously amenorrheic subjects still had a lower density than the eumenorrheic dancers. Interestingly, in Warren’s 1991 study, all bone mineral differences between the amenorrheic and eumenorrheic subjects could be accounted for when age and body weight were used as covariates. Young et al found a similar explanation in the data on dancers. Despite matching for age and weight, fat mass explained the bone mineral differences between menstrual
groups (12). Drinkwater et al reported the menstrual patterns (current and previous) of 97 young athletes by ranking the patterns 1-9 according to the potential skeletal consequences with always eumenorrheic as "1" (214). Body weight and menstrual pattern explained 43% of the spinal density. Body weight became a more important predictor as the menstrual pattern score increased, and the higher consequence menstrual groups had higher mileage or more days per week participation. White et al also found the difference between amenorrheic and eumenorrheic subjects disappeared when body weight was accounted for in the final analysis (215). It appears that body weight and/or body fat is important, yet the follow up studies fail to relate the increased bone mass with increased body fat or weight. The high level of training has continued to be an underlying suggestion in all of these studies. Body weight and increased energy expenditure together may reflect long term energy drain and substantiate the theory as proposed by Warren.

Winters et al documented an inverse relationship between weekly running distance and spinal BMD and supported the change in follicular phase estrogen as the most likely cause of cyclic abnormalities (22). In the study of 20 runners and 10 controls, menstrual frequency was shown to be an inaccurate index of ovarian hormone status. This inaccuracy may explain why some studies have shown a significant difference in bone density between menstrual groups and others have not. Pearce et al used dancers to demonstrate that the longer the oligomenorrhea, the more harmful the effects on bone mineralization (216). This study supported positive local influence of dance on the weight-bearing skeletal sites and also demonstrated probable redistribution of bone mineral away from
the non-weight-bearing sites (similar to steal phenomena mentioned earlier). Myerson et al studied eumenorrheic and amenorrheic runners and controls to also suggest a redistribution of bone mineral in the runners compared with the controls (217). The amenorrheic runners had decreased bone density compared with eumenorrheic runners until body weight was added to the equation. The mean lumbar spine density in both running groups approached significance as they were lower than those for the eumenorrheic, non-exercising controls. Slemenda and Johnston demonstrated an increased incidence (40%) in menstrual disruptions in female figure skaters compared with controls (10). Despite the increased menstrual disruptions, the female skaters had higher BMD’s in the lower body sites. They also demonstrated a trend for lower BMD values among skaters who trained more. They concluded that the weight-bearing nature of figure skating compensated for the hormonal disruption. Robinson et al also supported the overriding effect of weight-bearing exercise in runners, gymnasts and controls (173). The runners and gymnasts reported similar menstrual disruptions, yet the gymnasts still measured greater bone density than the runners or eumenorrheic controls. The greater BMD in gymnasts was attributed to the 10-12 x body weight impact forces in the sport. As previously outlined, the high impact sports appear to have a positive influence on bone until the point that training disrupts the hormonal balance required for normal menstrual cycles.

Low bone density in athletes increases the risk of stress fractures as well as osteoporosis. Myburgh et al used a case/control study to look at 25 athletes with stress fractures (218). Seven out of 18 women with stress fractures had
irregular menstrual cycles while none of the controls were irregular. They also reported that the controls had a more frequent use of oral contraceptives than the injured group. The only difference in the diet was that the fracture group consumed fewer dairy products. Bone loss was more pronounced in the Ward’s area of the hip and in the lumbar spine. Bennell et al studied 45 stress fractures in 22 women and found no significant differences in lumbar spine density (171). The stress fracture athletes demonstrated a more restricted (“cautious”) eating pattern. The oligomenorrheic subjects were 6 times more likely to suffer stress fracture, but when grouped by eating pattern, the careful eaters were 8 times more likely to suffer stress fractures. Warren et al compared 13-29-year old ballet dancers with controls where half of each group was amenorrheic (120). Fourteen of the 49 dancers had stress fractures. Age at menarche was a significant predictor of a stress fracture. In a 1993 study, Myburgh et al demonstrated a 13% lower spinal BMD and a 16% lower trochanteric BMD in a group of nine amenorrheic athletes when compared with controls (219). The differences were attributed to bone loss and inadequate acquisition. Rencken et al found amenorrheics to have a decreased BMD in the neck, Ward’s, trochanter and midshaft of the femur as well as in the tibia when compared with eumenorrheic athletic controls (220). Seven of the 29 amenorrheics had a spine or hip BMD that would be consistent with a diagnosis of osteoporosis. Of the eumenorrheic runners, 15% were considered osteopenic while low BMD was present in 72% of the amenorrheics. All of the above studies suggest that timely and normal menstrual function is important to bone health and injury prevention in athletes.
2.4.3 Is leptin the missing link in athletic amenorrhea?

From the review of the literature on exercise-induced amenorrhea, many studies and review papers have failed to support the importance of body fatness with regards to menstrual abnormalities as originally proposed by Frisch and McArthur (197-200). It seems most authors contend that the athletic amenorrhea is a sign of overtraining or chronic negative energy balance, which can certainly be marked by low body fat stores (200). Investigators have long-hypothesized about some yet to be identified mediator between the fat cells and the reproductive system. With the recent discovery of leptin as the hormone secreted from the fat cell and the identification of the leptin receptors in the hypothalamus (221, 222), it is reasonable to consider leptin as the potential mediator between the fat cell and the reproductive function. Researchers have been proliferative in finding potential roles for the newly discovered hormone and a few have related leptin concentration to the reproductive functions in mice and humans (223-229). In reviewing the ideas of Frisch, it may be that the body fat levels they proposed were surrogate measures for serum leptin in their subjects. It has been shown that plasma leptin concentration is highly correlated to the absolute fat mass, but it has been challenged whether leptin is secreted more acutely as a response to positive energy balance (229-236). Could a lack of leptin secretion be a response to the chronic energy drains which in turn will inhibit the pituitary release of GnRH and LHRH from the pituitary and interfere with reproductive function? If this were the case, it would complete the cascade of events (mechanism) for athletic amenorrhea. The evidence leading to this question follows.
There are many pieces of the leptin puzzle that could help explain athletic amenorrhea in terms of serum leptin and the energy drain concept. Matkovic et al were the first to find a threshold serum leptin which was required for human females to attain menarche which demonstrated a relationship between leptin and reproductive function in our current subjects (228). Laughlin et al has documented decreased leptin levels and a lack of diurnal rhythm in amenorrheic young adult athletes (227). Bornstein et al used a cell preparation from bovine adrenal glands to demonstrate a reduced cortisol response in the presence of higher leptin levels (237). Given the increased cortisol theory as discussed in Dueck, a lower leptin input to adrenals and the brain (hypothalamus) could allow for increased cortisol and could begin the inhibitory cascade of events as in Figure 3.1. Licinio et al demonstrated human leptin levels to be inversely related to pituitary-adrenal function and suggested pulsatility of leptin to be the important biological link to the gonadal axis (238). The work of Tataranni et al further supports this leptin-reproductive relationship in female athletes (229). The levels of leptin shadowed the continuum of menstrual disruption as amenorrheic athletes had the lowest leptin, followed by anovulatory athletes and then eumenorrheic athletes. After they adjusted for body fat, there was no further association between menstrual dysfunction and leptin. They concluded leptin below 3ng/ml predisposed a female to amenorrhea while 5 ng/ml might contribute to anovulation. Kohrt et al evaluated the serum leptin levels in response to exercise training intervention in 60-72-year old females over an 11-month period (239). The study included control, hormone replacement therapy (HRT), exercise and HRT/exercise groups. The administration of HRT did not
change the serum leptin levels, so it was concluded unlikely that the sex hormones are regulatory to leptin. Instead, the exercise groups experienced reduced serum leptin levels. The mouse research of Ahima et al proposed leptin as the critical signaling link between adiposity and reproduction (223). Leptin was shown to restore fertility in hypogonadal mice and in response to starvation. The restoration of estrus in the mouse injected with leptin did not affect the body weight of the animal providing direct evidence for the critical role of leptin independent of fat mass in reproductive function. Leptin has been shown to act at the hypothalamus and may be the metabolic signal in the Frisch hypothesis linking body fat to menstrual cyclicity.

The decreased bone density in many of the amenorrheic subjects may be the result of decreased estradiol or leptin itself may have a direct role in bone metabolism. Hoggard et al have identified leptin receptors in many body tissues in mice including the spleen, testes, kidney, liver, lung and adrenal (240). The presence of leptin receptors in bone would open the possibility for leptin to have a direct effect on bone tissue as well. A two-year analysis of the current subjects by Matkovic et al has demonstrated a positive correlation between serum leptin and TBBMD, which appears to be due to increased periosteal expansion in subjects with higher leptin (228, 241). Liu et al recently identified endocortical bone formation as a response to leptin administration in ob/ob mice (242). It has yet to be identified if this endocortical and/or periosteal stimulation is the result of a direct mechanism on the bone or if it is mediated in other hormonal axes. This relationship between bone mass and leptin offers an explanatory mechanism for the protective effect of obesity on skeletal mineralization.
2.5 *Frost's Mechanostat Theory*

Dr. Harold Frost has proposed and developed a paradigm for discussion of the growth, modeling and remodeling of bone (as well as other connective tissues). Much of this work has followed Wolff’s law in that bone responds to stress. This paradigm was first introduced in 1987 (proposed in 1986) and has been seasoned with additional information as the academic conversation has followed the research (70). The development and current state of this paradigm is presented.

The *mechanostat* is the central theme to the model and is a term to describe a sensing mechanism within bone which can be effected by outside modulators to change the thresholds set within the bone. In this model, bone has a different setpoint to initiate or terminate different physiological processes of remodeling and modeling. It is common for Frost to include a glossary of terms to facilitate explanation and the same regime follows:

- **MU** mechanical usage (includes any strains applied to the skeleton)
- **MES** minimal effective strain (measured in units of microstrain or μE)
- **r** the amount of strain below which activates the remodeling process
- **m** the amount of strain above which activates the modeling process
- **p** the amount of strain above which causes "plastic" changes in bone or the bone microdamage threshold where woven bone is formed instead of normal lamellar bone

Any MU of the skeleton produces strain on the bone, which depends on the architecture and arrangement of the bone. The skeleton prefers a condition where the usual MU matches the bone properties such that strain is contained
between about 100-1000 μE. When MU is low (like bed rest) or when bone architecture far exceeds the needed strength, the mechanostat senses the mismatch and turns "on" the process of remodeling to redesign the bone to be consistent with MU. This usually results in net bone loss until the new bone arrangement matches the MU. Alternatively, when MU exceeds the bone design such that μE rises above 1000, the mechanostat senses the difference and turns the modeling switch "on" to strengthen the bone so it is able to contain the strains produced by MU. As long as strains continue to be sensed outside the thresholds, bone will respond with osteoblasts and osteoclasts until the appropriate design is attained. If strains are constantly above 3000 μE, the bone cells are unable to respond with lamellar bone formation and begin trying to correct the mismatch sensed by the mechanostat making an ineffective woven bone. The fracture threshold of bone is located near 25,000 μE, so it is easy to see that detrimental effects on bone may occur long before the fracture threshold is attained.

Frost further defines lamellar modeling as a process that strengthens (cortical) bone through formation and resorption drifts such as demonstrated in Figure 2.2. This describes the periosteal expansion to strengthen the bone accompanied (in theory) by endocortical resorption to maintain a reasonable skeletal mass and bulk. Both osteoblasts and osteoclasts are involved in the modeling process. The term minimodeling is used to apply this theory to trabecular bone. As skeletal growth occurs, there is a constant mismatch between the design of the bone and demands (MU) placed on it so the μE remains constantly high the skeleton models throughout the growth period.
Modeling is thought to be minimal in adults and applies more to bone growth and skeletal development. In theory, participation in various physical activities during the growth periods would stimulate the mechanostat even further to help build a mechanically stable skeleton at the sites stimulated.

Figure 2.2  Bone modeling for growth and adaptation to stress via modeling drifts. Adapted from Frost et al (70).
Remodeling occurs at low μE, presumably during periods of minimal MU. Remodeling is the activity of the basic multicellular unit (BMU) which follows a coupled sequence of activation-> resorption->formation (ARF). The ARF is carried out by osteoclasts being activated and removing bone followed by osteoblastic activity to replace the removed bone with an amount of bone consistent with the mechanostat signal. A complete ARF is thought to take about 4 months from activation through formation. Remodeling happens throughout life, but predominantly describes bone turnover in an adult skeleton. Frost predicts that adult humans create 3 million new BMUs per year and that one third of those may be active at any given time. Remodeling can result in a net loss of bone when the μE falls below the MESr threshold of 50-100 μE. This is termed “disuse mode” and it is characterized by resorption exceeding formation and a resultant osteopenia. Fortunately, the normal adult skeleton is usually in “conservation mode” which simply means the osteoblasts of the BMU replace the bone removed by osteoclasts with a similar amount of bone in a consistent and coupled manner. Conservation mode is not defined in terms of μE, but, intuitively, since there is no net gain or loss, it should be between 100-1000 μE which Frost also calls the “comfort zone”.

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The following schematic (Figure 2.3) attempts to represent this model in preparation for further discussion:

**Figure 2.3** Schematic of Mechanostat thresholds and bone response.

Thus mechanical usage is seen as a spectrum and the mechanostat is responsible for sensing the strain. The region where the usual highest strains lie,
according to the sensing of the mechanostat, will determine the bone response. The current and more developed theory includes caveats where effectors outside of bone can modify the scheme (78). Frost has suggested the absence of estrogen to be an important modifier of the remodeling threshold during menopause (27, 72, 76). Estrogen (estradiol) is proposed to decrease theMES thresholds so that as estrogen is removed, the remodeling threshold increases. This would induce a mismatch between bone form and strain resulting in activation of remodeling for net bone loss. Bone loss would occur until the MU matches the bone structure. This would explain the post-menopausal bone loss and it fits the osteopenia identified in the dysmenorrheic situation as well. It would stand to reason then, for the peri-menarcheal addition of estrogen, that young females might accrue bone mineral at a faster than usual pace as estrogen decreases the threshold for stimulation of modeling. This exact mechanism is the proposal of Frost’s most recent publication (243). This further justifies evaluation of physical activity based on menarcheal time.
3.1 Subjects

Initial study selection of 456 subjects was made from a pool of ~2000 middle class females in the central Ohio (Columbus) area as lists were solicited from school districts. At the study's onset, the Caucasian subjects were 8-13 years old and free of any chronic disease or medication known to affect bone metabolism. All subjects were qualified for the study based on birth date and pubertal stage two for pubertal hair or breast development. Subjects were also selected and stratified according to habitual calcium intake as measured by a food frequency for calcium (244). Three hundred and fifty-four subjects were identified as low calcium intake (<1200 mg/day) and have been followed every six months as participants in a double blind calcium supplementation trial. The remainder of the subjects (n=102) were identified as high calcium intake (>1200 mg/day) and have been evaluated annually. For this study, a sport history activity questionnaire was mailed to 314 female subjects who had completed five years of longitudinal evaluation (9 or 5 visits according to calcium group) beginning in 1992. Follow-up visits have included DXA evaluation,
anthropometry, dietary and physical activity records, and biological samples (blood, urine and stool). All methods have been approved by the Human Subjects Committee of the Ohio State University and subject consents are on file (Appendix B).

3.2 Basic Anthropometry

Standing (without shoes) and sitting heights were recorded at each visit to the nearest 0.1 cm using an Accustat Ross wall-mounted stadiometer with the subject's mandible parallel to the floor. Body weight in normal indoor clothing without shoes was measured with a Detecto upright balance scale and recorded to the nearest .1 kg. Body mass index was derived from these recorded variables.

3.3 Pubertal Stage and Menstrual History

The first form subjects were asked to complete with each visit determined the current pubertal status as well as menstrual history over the past six months (Appendix C). Pubertal status was self-assessed by each subject by looking at pictures of five stages of breast development and six stages of pubertal hair development (245, 246). The subject selected the picture which best reflected her current stage of development for each area. This form has been shown to be highly valid compared with physician ranking of the same attributes (r=0.81 breast, r=0.91 pubic hair) (247). Menstrual history included date of menarche, number of cycles in the last six months, average length of menses and average length of cycles. Because these data were recorded regularly for each subject, they provided a valid method for determining interrupted menstrual function as
well as late menarche. The specific menstrual cycle data were added to the form in the fifth visit as most of the subjects were just beginning their periods. The use of oral contraceptives was recorded for only 5 subjects towards the ninth visit. The number of subjects who used oral contraceptives and the short duration of their use did not warrant evaluation within this analysis. For this project, the charts were reviewed and checked for the menstrual history at each visit to facilitate accuracy of menstrual disruption and eumenorrheic categories.

Menstrual cycle length and number of cycles per year are commonly used to categorize disturbances. Eumenorrhea was defined by the presence of 9 or more menstrual cycles per year (more than 4 per six months). If the subject did not provide information on to the number of cycles per six months, the criteria of cycle length less than 43.5 days for eumenorrheic status was applied. Subjects with four or less cycles per six months or cycles lasting longer than 43.5 days were categorized as dysmenorrheic (oligomenorrheic combined with amenorrheic) for that six month period. When this information was converted to an annual derived variable, it meant that subjects with fewer than ten menstrual cycles per year were categorized as disrupted. The categories for each visit were combined to formulate this annual indicator to be evaluated along with the annual physical activity variables. An overall menstrual indicator was also formed such that those subjects who had always had eumenorrheic visits were considered eumenorrheic while those who had ever recorded a menstrual disturbance were lumped together as dysmenorrheic. These categories are in agreement with others performing similar analyses and the categories in these
similar studies are outlined in Appendix D. The annual and overall menstrual indicators were utilized for the analyses.

3.4 Energy Balance

Subjects were provided with blank copies of 3-day food intake records (Appendix E) and physical activity logs prior to each visit (Appendix F). It was considered ideal for these to be completed prior to the visit. The three-day food record was reviewed with the subject to ensure accuracy of recording and to provide the registered dietitian the opportunity to foresee and question any software or food portion issues. Subjects were provided with food models and serving size models when needed and appropriate. The records were entered into Nutritionist III® software for Macintosh (Hearst Corp., San Bruno, CA) and all nutrients included in the software were calculated including kilocalorie (kcal) intake.

Energy expenditure was also self-reported via the Bouchard et al tool (248). This method required the subject to record the intensity of activity for every fifteen minute time block throughout the day. The intensity choices were 1 (sleeping, lying still) through 9 (very intense), and the subjects were provided with instruction and examples of activities to typify each intensity level. The total energy expenditure was calculated by multiplying the number of intervals for each intensity level by the mean METs per kilogram a “normal” person would expend at each level of intensity, then the METs were multiplied by the body weight (kg) to yield kilocalories (kcal) expended. Prior to this project, the number of kcals expended was the only physical activity information recorded.
for each visit. Each chart was reviewed to record and utilize the valuable intensity level information lost in the previous calculation to kcals for each visit. It was possible to determine how many 15 minute intervals the subjects spent in high level activity (8 & 9) versus time spent in very minimal intensity activity (1, 2, 3 & 4). This allowed for categorizing the subjects by activity without the bias of body weight. Physical activity (Bouchard) was expressed as a categorical variable (hi, moderate or low) and in METs (continuous measure of metabolic intensity in METs/kg daily activity) for each visit. The previous use of the Bouchard tool allowed for comparison of the newly developed impact scoring with a usual method for measuring physical activity. Additionally, it served as an ongoing measure to facilitate documentation of the validity of the current tool.

Two measures of energy status were derived from the energy intake and expenditure data. An energy balance variable (energy drain) was calculated by subtracting the reported energy expenditure from the reported kcal intake so the difference represented the kcal deficit. A physical activity index was also calculated by estimating the individual basal energy needs via the Harris-Benedict equation. The kilocalories of energy expenditure according to the Bouchard divided by predicted basal needs provided for a physical activity index. These derived energy status variables were designed for analysis with the menstrual status data.
3.5 Tool Development for Historical Physical Activity

Since the Bouchard activity measurement tool depended on two or four typical days as snapshots in time, it was necessary to measure the regularity of exercise and sport throughout the year. After consideration of multiple tools designed to harness this information, the *Modifiable Adolescent Questionnaire* (MAQ) as developed by Aaron and Kriska et al was seemingly most appropriate, but the initial design of the MAQ was to be used to measure the past one year's sport and exercise (249, 250). Personal communication with Dr. Kriska about use of the MAQ modified to measure the past five years resulted in her suggestion and consideration of a historical physical activity questionnaire designed to evaluate leisure time activity in adult Pima Indians (251). Dr. Kriska suggested the Pima tool be modified to reflect time periods in childhood and adolescence such as grades 1-3, 4-6, 7-9, etc.

The MAQ for adolescents is a tool designed to gather information about time spent in leisure activity over the past year and has demonstrated acceptable reliabilities (r=0.73-0.87) for one month repeated studies (250). The validity studies for this tool correlated the past year questionnaire with four one-week questionnaires using Spearman correlations. The coefficients were r=0.55-0.67 for adolescent males and 0.73-0.83 in adolescent females. The historical tool used with the Pima Indians demonstrated test-retest rank order coefficients of 0.62-0.96 in 21-36-year olds (251). The repeatability of this same tool was less than adequate in 10-14 year old subjects with a rank order correlation coefficient of 0.31. An acceptable reliability study of this tool has yet to be established in young subjects.
After consideration of Dr. Kriska's idea, we chose to use the structure of the Pima grid incorporating annual participation as the time periods in combination with some of the advantages of the MAQ. The combination of these two tools as developed for this project will be referred to subsequently as the KA (Kriska-Aaron) tool (Appendix G). Measurement of sport participation and the annual time spent in particular sports or activities allowed us to evaluate the role of impact and weight-bearing activities in bone growth and mineral accretion. Additionally, we have adopted the one-year MAQ by Aaron and Kriska for regular use throughout the remainder of the study.

The KA tool was mailed to the subjects in December of 1997 (solicitation letters and reminders in Appendix H). Two hundred sixty-eight of the questionnaires were returned, reviewed and recorded. The annual time (hours) spent in each reported activity served as the base measure from which the other exercise variables were derived. A complete listing of leisure time and sport activities for this group was formed with 56 different activities reported. It is common for studies of particular athletes to quantify the impact of the sport in multiples of body weight, such as running 2-5 times body weight (144, 172, 188, 252), gymnastics as 10-18 times body weight (13, 172) and weight-training as 18-36 times (144) body weight. It was desirable to quantify the questionnaire responses in terms of the probable osteogenesic potential (Ost) due to weight bearing (WB) and impact of the activity.

Because we were unable to find a compendium of impact to include all of the activities reported by the subjects, another questionnaire (Appendix I) was completed by ten certified athletic trainers (raters) to categorize sports and
activities into similar groups by weight-bearing, linear, lateral cutting and jumping (or loading) characteristics. The raters were not selected according to any specific criteria, but are all employed in high school or college settings in central Ohio. The raters were asked to categorize each activity into six primary categories (NWB, WB-two legs, WB single limb, WB-cutting, WB jumping and weight-loading) and were provided with an example to typify each category. They were allowed to group each activity into more than one category to help express the mixed nature of many activities as well as allow for differences in rater opinion. The raters' responses were tallied into the six categories. The tally for each category was multiplied by a weight-bearing multiple of 0.5 (NWB), 1 (WB two legs), 4 (WB single limb), 7.5 (WB-cutting), 12 (WB jumping) and 18 (weight-loading) to represent average or relative literature values (13, 144, 172, 188, 252). These products were added across all six categories, then divided by the number of tallies posted for that sport so that the osteogenic potential (Ost) for each sport or activity was an average and weighted value according to the rater's categories and literature values. These values were discussed with and approved by an expert in gait analysis to help document relative accuracy (253). A complete listing of the reported activities and calculated Ost value for each activity are also included in Appendix J. The annual time (hours) spent in each activity was multiplied by the specific Ost value for that activity, and the annual Ost for all activities was summed as the annual Ost score for each subject.

Similarly, an annual METs tally was computed for each year to represent the metabolic demand of PA for comparison with the time and Ost values. Ainsworth et al published a detailed compendium of the METs required for
various activities (254). The METs value for each activity was taken directly from this compendium. Annual hours of time spent in each activity was multiplied by this METs value and summed across all activities to derive the annual METs. The values used to derive annual METs are also included in Appendix J. Annual METs were derived to compare the PA influence in this study with other studies, like those of Welton et al or Morris et al, which have looked at METs to indicate physical activity (25, 109). Additionally, looking at PA in terms of METs for this tool had the potential to validate with the Bouchard tool already used at each follow up visit. The derived annual hours, METs and Ost allowed for a comparison of the three methods of recording exercise from this one tool.

3.6 Tool Reliability

To test the reliability of the KA tool, a number of subjects were asked to complete a second KA questionnaire after at least one month had passed since returning the first questionnaire. The primary research associate (N.B.) asked approximately 35 subjects to complete a repeat study based on timing of visit, previous willingness and time constraints of the subjects. Twenty-six subjects have completed a repeat questionnaire for the test-retest reliability study.

3.7 Tool Validity

Validity was analyzed utilizing the complete pool of returned KA questionnaires. Methods of the longitudinal analysis have included DXA scanning every six months or year depending on the subject group. The body composition as recorded from total body scans has been used to validate the KA
responses with percent body fat as an indirect validation technique. Subjects have routinely reported physical activity every six months (or one-year) for the duration of the study utilizing the Bouchard et al grid for one weekday and one weekend day (248). Parents were usually available as surrogate reporters for younger subjects throughout the study. The Bouchard et al tool was designed to estimate energy expenditure or METs/kg of physical activity. Bratteby et al used doubly-labeled water techniques to suggest a different set of MET/kg values for use with this tool (255, 256). Direct validation of the KA-derived values with the average daily METs/kg values by both Bouchard et al and Bratteby et al were analyzed. Tool validation also considered the continuous variables for annual time, annual METs and annual Ost as well as mean annual time, METs and Ost as derived from the KA questionnaire as correlated to the percent body fat and previously recorded METs/kg/d (via Bouchard tool).

An effort was made to check the middle school and high school records for the repeated data for matching of athletic rosters or other activity records as a method to validate the more time intensive activities. The difficulty in this validation technique was the number of schools included (n=32) and the differences in the records kept at each school. Additionally, the popularity of club (versus interscholastic) sport participation made many of the records impossible to validate. The records were reviewed prior to the school visit to identify the time spent in sports expected to be consistent with interscholastic participation. The roster matching reported in the results section only refers to the validity of activities expected to be documented by the schools and excludes
minimal time activities or those not expected to be coordinated by the schools. This sort of validation helps lend support to the study but is not all-inclusive!

3.8 Bone Densitometry

Subjects were measured with a Lunar dual x-ray absorptiometer (Lunar DPX-L using 1.3q software, Madison, WI). This machine uses rare-earth (cerium) filtered x-ray sources (K-edge filters) to attain the two levels of energy needed to evaluate soft tissue from bone mineral. The pencil beam DXA machines compare the absorption of the two energy levels in soft tissue and bone to determine the amount of bone mineral per pixel. The absolute amount of mineral sensed by the machine is termed bone mineral content (BMC, in grams), while the amount of mineral per squared area is the bone mineral density (BMD, in g/cm²). The amount of soft tissue is assumed to be consistent around the bone so the attenuation by soft tissue before the beam reaches bone will be similar (in the calculation) to the soft tissue on the backside of the bone. This is a disadvantage when measuring areas such as the antero-posterior lumbar spine where individuals may carry significantly different body fat patterns. The manufacturer suggests applications to individuals whose abdominal thickness is between 15 and 30 cm. This limitation may affect longitudinal data if a subject gained significant abdominal fat between visits. Mazess describes the potential errors due to fat in more detail (257). It should be remembered that DXA has been documented to be very precise in measuring bone mineral (<1%), but measurement errors for lean body mass may be 1-2% while those for fat mass are even higher at 2-3%. Ogle has demonstrated a high correlation of the percent
body fat derived by DXA with that derived by skinfold techniques in 4-26 year old females (n=128) (258). Dual x-ray absorptiometry provides information about bone as well as other body tissues.

The subjects were measured during each follow up visit using dual x-ray absorptiometry for the whole body and non-dominant forearm scans. The hip and lumbar spine scans were incorporated into the protocol with the ninth visit. The machine, subject positioning and machine software were specific and different for the various scans (whole body, forearm, hip, anterior-posterior and lateral spine) (259). The whole body scanning technique required the subject to lay supine on the scan table while the table-bound radiation source was passed through her body to be detected through the aperture on the moving arm which passed simultaneously above her. The scans proceeded longitudinally on the table while the individual slices were perpendicular to the table. The forearm was scanned with the subject sitting next to the table with her left arm parallel to the scanning surface. The left shoulder was comfortably abducted and internally rotated so the subject was able to rest the anterior forearm on the Lunar, forearm-positioning board on the table. The use of the board helped assure the forearm was parallel to the translation of the detector arm. The hip scan was taken with the subject lying supine and the machine was positioned to begin scanning about two inches below the greater trochanter in the center of the thigh. The scanner moved from distal to proximal and the femoral shaft was parallel to the table. The A/P lumbar spine scan was taken with the subject supine and the detector arm positioned about two inches below the navel. Again, a positioning block was used (under the knees) to help assure the spine was not curved and was
parallel to the scan surface. This resulted in beginning the scan with the fifth lumbar vertebral body (L₅) then proceeding towards L₁.

The advantage of DXA was the speed of measurement and the low dosage of radiation compared with other techniques. DXA was not able to measure the three-dimensional nature of the body and this limited the ability to predict true bone density (g/cm³). It is important to evaluate the true density as BMC and, to a lesser extent, BMD are size-dependent variables. Another DXA limitation was the inability to discriminate between cortical and trabecular bone. It is customary to analyze the bone mineral at multiple sites to include those which are primarily trabecular (ultradistal radius, lumbar spine) and cortical (radial shaft, femoral neck). The Lunar DPX-L densitometer has yielded excellent bone mineral results in our laboratory as the coefficients of variation (CV) % range between 0.48 and 1.06 depending on scan site. The precision errors for body fat and lean tissue mass are 2.6% and 1.1%, respectively (128).

3.9 Leptin Assay

Blood samples were obtained annually (at the odd number visits) for measurement of various serum markers and hormones including leptin, a peptide hormone produced by adipocytes. Serum was separated from whole blood by centrifugation, decanted and stored at -80°C until assayed according to the RIA method published by Ma et al (260). This RIA incorporates ¹²⁵I tracer into the leptin standards and sample, then binds the sample leptin with rabbit antibodies and anti-rabbit immunoglobulin G (IgG) for separation and counting. This technique was always performed at Linco Research (St. Charles, MO).
percent CVs within runs measured 3.4-8.3% and between runs ranged 3.6-6.2%.
The nature of the relationships of serum leptin to body fat, bone mineral density,
menarche and diurnal variation in this group of subjects has been previously
published (228, 241, 261).

3.10 Statistics

The raw data for the four years of longitudinal study and the KA
questionnaire were loaded into SAS for Macintosh® (Release 6.12) and the
derived variables were computed using macro functions (Appendix K). The
questionnaire-reported annual time spent in each activity was calculated as well
as the annual METs and Ost values. For each annual value, the tertiles were
used to determine high, moderate and low categories. The annual scores were
averaged to determine a mean time, MET and Ost to be used as a cumulative
measure and these variables were also categorized into high, moderate and low
tertiles. This statistical management provided continuous and categorical
variables for time, MET and Ost for each year and as an average for evaluation in
the bone data.

3.10.1 Questionnaire Reliability and Validity

The test-retest study for the KA tool included using the Proc Corr
procedure to identify the Spearman coefficients of correlation for the initial and
repeated values for time, METs and Ost for each visit as well as for the mean
annual values. The same variables for each year were categorized into tertiles
and analyzed for differences utilizing the non-parametric paired sign test (using
Data Desk®). The validity of the questionnaire was also examined by determining the correlations between percent body and Bouchard METs values with the reported annual and average time, METs and Ost.

3.10.2 Questionnaire Respondents vs. Non-respondents

The respondents and non-respondents were compared using Proc GLM in SAS. The assumption of equality of variances between these groups was tested by performing an F test on the higher variance divided by the lower variance and comparing the quotient with an F distribution at the ninetieth percentile with numerator, denominator degrees of freedom (262). The annual change in TBBMC, TBBMD and total body bone area were examined as well as the cumulative values for the same variables. Use of Proc GLM in this analysis allowed for a second analysis controlling for height and weight.

3.10.3 Calcium Intake

It was important to evaluate the potential confounding effect calcium intake may have exerted on the bone parameters of participants with different activity levels. Due to the nature of the ongoing supplementation trial, the code could not be revealed, but a total calcium intake variable was evaluated by a statistician to help ensure calcium intake would not change the results. The mean total calcium intake for the high and low tertiles of mean time, MET and Ost were compared via t-tests (SAS Proc TTest) as a safeguard to ensure similar calcium intakes. Additionally, the relationship (Spearman r) between mean total
calcium intake and the continuous variables for mean time, MET and Ost were analyzed to ensure lack of a consistent correlation using Proc Corr in SAS.

3.10.4 Athlete Bias: Athletes, Non-athletes and Late-starting Athletes

SAS macros were also used to determine if a subject should be categorized as an athlete. Subjects who reported participation in aerobics, basketball, cheerleading, crew, cross-country, dance, diving, drill team, field hockey, gymnastics, jogging, lacrosse, marching band, rugby, soccer, softball, swimming, tennis, track, volleyball or water polo at a minimal 8 weeks per year and 3 hours per week were categorized as an athlete in that sport for that year. Jogging/cross-country or jogging/track athletes were categorized as cross-country or track, respectively. Weight training and plyometrics were also defined sports, but the criteria for categorization as an athlete was 4 weeks and 1.5 hours per week. Participation as an athlete was then tallied so each person had an annual category for sport, such as none, specific sport, two-sport or multiple sports. A low number of athletes participating in only one sport prohibited a valuable comparison of specific sport athletes.

The athlete categories during the first year (1992/3) and second year (1993/4) were used to evaluate the athlete bias issue. Categories for comparison included control (never categorized as an athlete), early starters (categorized as an athlete all four years) and late starters (only categorized as athletes for the last three years). Comparison of these categories at baseline, third, and ninth visits was carried out to help resolve the athlete bias issue. Performing Bartlett's test for homoscedasticity tested the assumption of equal variances amongst these
groups (pp. 269-270) (263). A one way analysis of variance (ANOVA) was performed to identify differences between the three groups. Similar to other growth studies, the total body bone mineral content and density as well as the bone area were evaluated (168). Many studies have adjusted for body weight and/or height when failing to find differences between athletes and non-athletes as athletes appear to be taller and lighter than controls (8, 10, 13, 14, 146, 171, 172). To adjust for these potential differences, the ANOVA was repeated adjusting for height and weight as covariates.

3.10.5 Bone Mineral Accretion in Active and Inactive Subjects

To evaluate the data for differences in the bone mineral accretion between active and inactive subjects over the four years, the change (Δ) variables between baseline and the ninth visits were used with Proc GLM. Dependent variables for regression modeling were ΔTBBMC, ΔTBBMD, and Δradial BMC and Δradial BMD. The cross-sectional measures of BMC and BMD of the hip (Ward’s, neck and trochanter) and BMC of the spine at visit 9 were also regressed on similar site specific change variables to determine which changes may be associated with the highest accumulation of hip and spine parameters at visit 9. To further complete the analysis, all cumulative dependent variables were also regressed cross-sectionally by visit 9 to model the important time point predictors for comparison with the change models. The pool of independent/determinant variables included height, lean body mass, fat mass, body mass index, bone area, age, time since menarche, and overall menstrual status. Each model selection process relied on the Cp criterion as the primary criteria followed by the adjusted
r-square criteria. The $C_p$ selection criteria evaluates the overall mean squared error of the full pool of candidate variables and selects combinations of variables to help decide how many variables should be in the best model. The formula SAS uses for calculating this selection index is: $C_p = \frac{SSE(p)}{MSE(k)} - \frac{n-2(p+1)}{n-2}$. For each dependent variable, the model that included (mostly) significant predictors, acceptable $C_p$ criteria and explained one of the highest amounts of variability was selected. The residuals for each selected model were plotted against other variables of interest (mean Ost, METs, time, athlete/non-athlete, mean Bouchard and Bratteby METs) to explore possible explanatory relationships. The same outcome variables were reanalyzed in the same fashion adding mean annual Ost to the pool of candidate variables. The partial r-squared values for each variable in these general and Ost models were derived utilizing the stepwise regression function in SAS.

3.10.6 Bone Mineral Accretion Modeled Across Time Since Menarche or Age

The modeling of time since menarche (tsm) was investigated using Proc Mixed. Model options included tsm as a continuous variable, a pre-post menarche model with one change point at zero, tsm as a quadratic ($tsm + tsm^2$) and the four slope model with change points at 0 and .5. Covariance structures considered included independent, unstructured, unstructured(1), autoregressive, spatial power, toeplitz and various combinations of random slopes and/or intercepts. The models appeared highly significant and the most complex model (four slopes, two change points) was chosen to help answer the questions at
hand in terms of time since menarche. (Preliminary tsm model results are contained in Appendix L).

The time since menarche four slope regression model with change points at zero and .5 years tsm allowed for separate slopes for not started (ns), just started (js), eumenorrheic (eu) and dysmenorrheic (dys) menstrual categories. The dependent variable of interest is indicated by y in the following models. The following logic was used to define the slopes and force the data to connect at zero and tsm .5 just as would be the case in real life.

\[ y = \beta_1 \text{ns} + \beta_2 \text{js} + \beta_3 \text{dys} + \beta_4 \text{eu} + \beta_5 \text{ns} \times \text{tsm} + \beta_6 \text{js} \times \text{tsm} + \beta_7 \text{dys} \times \text{tsm} + \beta_8 \text{eu} \times \text{tsm} \]

At tsm=0, ns intersects js such that

\[
\text{ns: } y = \beta_1 \text{ns} + \beta_5 \text{ns} \times \text{tsm} \\
\text{js: } y = \beta_2 \text{js} + \beta_6 \text{js} \times \text{tsm}
\]

Therefore \( \beta_1 + \beta_5 \times \text{tsm} = \beta_2 + \beta_6 \times \text{tsm} \) so \( \beta_1 = \beta_2 \)

So js can be re-written \( \beta_1 + \beta_6 \times \text{tsm} \)

At tsm = .5, js, eu and dys need to intersect such that

\[
\begin{align*}
\text{y} &= \beta_2 + \beta_6 \times .5 = \beta_2 + \beta_6 / 2 \\
\text{y} &= \beta_3 + \beta_7 \times .5 = \beta_3 + \beta_7 / 2 \\
\text{y} &= \beta_4 + \beta_8 \times .5 = \beta_4 + \beta_7 / 2
\end{align*}
\]

Therefore \( \beta_2 + \beta_6 / 2 = \beta_3 + \beta_7 / 2 = \beta_4 + \beta_7 / 2 \)

\[
\begin{align*}
\beta_3 &= \beta_2 + \beta_6 / 2 - \beta_7 / 2 = \beta_1 + (\beta_6 - \beta_7) / 2 \\
\beta_4 &= \beta_2 + \beta_6 / 2 - \beta_8 / 2 = \beta_1 + (\beta_6 - \beta_8) / 2
\end{align*}
\]

So \( \text{ns: } \beta_1 + \beta_5 \times \text{tsm} \)
Js: \( \beta_1 + \beta_6 \times \text{tsm} \)

Dys: \((\beta_1 + (\beta_6 - \beta_7)/2) + \beta_7 \times \text{tsm}\)

Eu: \((\beta_1 + (\beta_6 - \beta_8)/2) + \beta_8 \times \text{tsm}\)

Now the original equation can be rewritten as:

\[
y = \beta_1 \times \text{ns} + \beta_2 \times \text{js} + (\beta_1 + (\beta_6 - \beta_7)/2) \times \text{dys} + (\beta_1 + (\beta_6 - \beta_8)/2) \times \text{eu} + \\
\beta_5 \times \text{ns} \times \text{tsm} + \beta_6 \times \text{js} \times \text{tsm} + \beta_7 \times \text{dys} \times \text{tsm} + \beta_8 \times \text{eu} \times \text{tsm}
\]

Which means that:

\[
y = \beta_1 (\text{ns} + \text{js} + \text{dys} + \text{eu}) + \beta_5 \times \text{ns} \times \text{tsm} + \beta_6 (\text{dys}/2 + \text{eu}/2 + \text{js} \times \text{tsm}) + \beta_7 (-\text{dys}/2 + \\
\text{dys} \times \text{tsm}) + \beta_8 (-\text{eu}/2 + \text{eu} \times \text{tsm})
\]

Thus, the time since menarche variables (slopes) were derived by these functions in SAS:

```sas
ns = .;
js = .;
eu = .;
dys = .;
if (mstat = 'ns') then ns = 1;
else if (mstat ne ' ') then ns = 0;
if (mstat = 'js') then js = 1;
else if (mstat ne ' ') then js = 0;
if (mstat = 'eu') then eu = 1;
else if (mstat ne ' ') then eu = 0;
if (mstat = 'dys') then dys = 1;
else if (mstat ne ' ') then dys = 0;
ns_slope = tsm * ns;
j_s_slope = tsm * js + (dys + eu)/2;
eu_slope = tsm * eu - eu/2;
dys_slope = tsm * dys - dys/2;
```

Advantages of the four slope model included the ability to analyze the potential effect of exercise on bone changes before and after menarche as well as
evaluate for differences in menstrual status (eumenorrheic vs. dysmenorrheic). The time period for "just started" was included because of the difficulty in classifying the normality of cycles until regular menstruation was established and this helped avoid dysmenorrheic misclassification of this time period. Additionally, we wished to look at the difference in changes related to exercise close to menarche. The four slope model was used in conjunction with Proc Mixed to identify the best regression models to predict the annual change ($\Delta$) in total body BMC and radius BMC (33% site).

Age has been well demonstrated to have a quadratic relationship with bone measures thus the age models for comparison with the time since menarche models were developed as quadratic models ($\text{age} + \text{age}^2$) (23). The change in TBBMC and radius BMC variables were evaluated across age for comparison with the time since menarche models also using the advantages of Proc Mixed. Due to the collinear nature of growth data, it was important to evaluate the possible inclusion of random effects as well as fixed effects in all models. Proc Mixed allowed for designation of the most appropriate covariance structure within a repeated-measures regression framework for the time since menarche and age models.

The general approach to regression development for the time since menarche and age models to investigate the influence of exercise was standard for each developed model.

a) The initial step developed the pool of candidate variables. The entire pool considered included a linear and quadratic variable for height, lean body
mass, fat mass, body mass index, bone area and age. The annual change and quadratic of annual change for height, lean body mass, fat mass, body mass index, and bone area were also included in this initial pool. The pool was evaluated for each dependent variable using Proc Reg with the collinearity and variance inflation factor (vif) diagnostics. For example, the following variables were dropped from the candidate pool in this order for the annual \( \Delta \) total body BMC four slope tsm model to remedy very high vif's: \( ht^2, ba^2, bmi^2, age^2, lbm^2, bf^2, \) and \( \Delta ht^2 \). This left the pool of candidate variables so that each vif was less than 80. The vif evaluation was carried out for each of the other models (tsm \( \Delta \) radius BMC, age \( \Delta \) total body BMC and age \( \Delta \) radius BMC) and followed similar variable removal patterns.

b) Preliminary model selection used the forward stepwise procedure and demanded a model with acceptable \( Cp \) criterion and p values for individual variables. This preliminary model was then evaluated using Proc Mixed for the final model development.

c) The covariance structure of the preliminary model was investigated to allow the Proc Mixed model development based on the correct structure. Autoregressive, independence, spatial power (based on age), toeplitz, and unstructured covariance structures were considered and compared. Random effects were also considered for the intercept and tsm in combination and separately. The best-suited covariance structure for the time since menarche and forearm age models appeared to be the unstructured(1) option without any random effects. The full body age model was best evaluated with the Toeplitz covariance structure.
d) Proc Univariate was utilized to check the model assumptions for the final models to assure normally distributed residuals. The residuals were plotted against the predicted values to ensure reasonably equal prediction across all levels of the residual values.

e) The model was checked to assure that previously excluded variables should not enter the model by regressing (Proc GLM) and plotting the residuals against each excluded candidate variable. Variables with suspicious adjusted r-square and p values were entered into the model and evaluated similarly.

f) The residuals were regressed against each of the exercise variables as well as many nutritional variables.

g) Each model was re-evaluated including each of the exercise variables. The linear form of each exercise variable was evaluated along with the quadratic exercise variable to identify the best fit of the exercise variables.

h) The final model (including the most significant exercise variable) was again reviewed for covariance structure and normal residual distribution.

i) Variance inflation factors for the variables included in each model were reviewed with the goal that all included variables had vif<10 (265).

The "estimate" command in Proc Mixed allowed for statistical testing for differences in the slopes between the eumenorrheic and dysmenorrheic subjects as well as the other menstrual categories within this four-slope model. An Instructional Machine Language (IML) program was written to calculate where the peak in a quadratic variable occurs in the data according to the model selected.
Because Proc Mixed allows for the covariance matrix to be designated and the selected models did not include random effects, it was of interest to compare the annual change in total body BMC across menarche model using the unstructured and unstructured(1) covariance structures and Proc GLM. This analysis confirmed the competence of using Proc Mixed to formulate the models.

3.10.7 Bone Mineral Accretion in Athletic Menstrual Dysfunction

Differences in the bone parameters between eumenorrheic and dysmenorrheic and timely and late menarche athletes were investigated using Proc GLM. The overall menstrual status variable (MstatOl) indicated always eumenorrheic (0) or at least one occurrence of dysmenorrhea (1). The timely menarche indicator (menOl) was used to indicate menarche before sample mean (12.8 yrs.) or later menarche. The homoscedasticity (variances equal) assumption amongst the four groups was checked using the Bartlett's test (263). The model-building strategy was similar to the steps outlined in question two. The primary criterion of Cp regression selection was employed while limiting the subjects to those qualified as athletes for all four years. The eligible Cp models (unbiased) were identified using Proc Reg to assure use of the best model with mostly significant or near significant predictors. The pool of candidate variables included baseline variables for height, body mass index, lean body mass, body fat, bone area and age as well as change (9-1) variables for height, body mass index, lean body mass, body fat and bone area. Time since menarche at visit nine (or menOl) and the overall menstrual status indicator were also included in the pool. The adjusted r-squared criterion served as the final evaluation in the case
of similar models. Each selected model for total change (visit9-visit1) in total body and radius BMC and BMD as well as total body bone area was evaluated using Proc GLM. The cross-sectional model for the femoral neck BMC was initially evaluated with the full pool of candidate change variables, but the selected models did not utilize the change variables so they were excluded for the final model selection to be sure the \( C_p \) statistic was accurate. Instead of evaluating the hip cross-sectional model controlling for baseline values, it made more sense to use the same variable pool, but at visit 9. The time since menarche (tsm) and menstrual status (Mstat01) variables as well as the interaction between the variables were entered into each model to determine group differences.

3.10.8 Prediction of Athletic Menstrual Status (Leptin)

Logistic regression equations were formulated to model the eumenorrheic or dysmenorrheic menstrual status of the athletes (mstatath) in terms of body fat, leptin or energy drain indicators. Only data from the fifth, seventh and ninth visits were used in a repeated measures fashion for this analyses due to the incomplete nature of the menstrual data in the third visit and the evaluation excludes subjects who had not started or were just started. The logistic regression is commonly used to predict the presence of a disease or condition (eumenorrheic or dysmenorrheic) based on factors of interest or risk (body fat, percent body fat, leptin, exercise, energy drain, physical activity index).

For this analysis, menstrual status (eumenorrheic or dysmenorrheic) was categorized for the 12 months preceding the leptin sample. The menstrual categories were defined with the same criteria as outlined in section 3.2. Energy
drain (bdrain) at each visit (5, 7, 9) was calculated by subtracting the average energy expended from the Bouchard at that visit from the average energy intake reported via the food records at that visit. The physical activity index was calculated by using the same Bouchard average energy expenditure and dividing it by the estimated basal energy requirements for that subject according to the Harris-Benedict equation (bratio). The body fat (grams) and percent body fat were taken from the DXA data. Exercise (annual hours) was evaluated within these models using the time spent in activity according to the KA questionnaire.
CHAPTER 4

RESULTS

Two hundred sixty-eight subjects returned the Kriska-Aaron modified questionnaire. The grid values were entered into a SAS data file and the annual and mean annual values for physical activity (time, METs and Ost) were derived using the values recorded in Appendix J. Table 4.1 provides the ranges and means ± standard deviations of the values reported as well as the tertile cut-off values for the analogous categorical variable. The number of subjects qualifying as athletes are reported in Table 4.2.

4.1 Questionnaire Reliability and Validity

Reliability of the KA historical questionnaire was evaluated in 26 subjects who completed repeat questionnaires. Spearman coefficients of correlation were determined for annual time, METs and Ost at each visit as well as for the mean annual values. The coefficients ranged from $r=0.587$ for time at the 1992/3 year to $r=0.921$ for the mean annual time. For comparison, the same variables were analyzed as categorical tertiles utilizing the non-parametric paired sign test. Seven of fifteen tests had a p-value of 1.000 while six tests had a result of
p=0.8450. The only less desirable result was for the MET variable at the 1995/6 year where the p-value was 0.5572. These results are displayed in Table 4.3.

<table>
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<th>Year</th>
<th>Variable:</th>
<th>range</th>
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<th>standard dev.</th>
<th>low tertile</th>
<th>high tertile</th>
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<td>79.5</td>
<td>251.7</td>
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<td>93/4</td>
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<td>293</td>
<td>337</td>
<td>119</td>
<td>300</td>
</tr>
<tr>
<td>94/5</td>
<td>time</td>
<td>0-2510</td>
<td>348</td>
<td>354</td>
<td>161</td>
<td>377.5</td>
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<tr>
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<td>430</td>
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<tr>
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<td>2273</td>
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<td>1965.2</td>
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Table 4.1 Summary and tertile information for annual and four year mean time (hours), METs and Ost variables.
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<td>4</td>
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<td>5</td>
</tr>
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<td>1</td>
<td>1</td>
<td>0</td>
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<td>0</td>
<td>1</td>
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<td>3</td>
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<tr>
<td>none</td>
<td>90</td>
<td>71</td>
<td>64</td>
<td>57</td>
</tr>
<tr>
<td>softball</td>
<td>11</td>
<td>14</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>soccer</td>
<td>17</td>
<td>13</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>swimming</td>
<td>20</td>
<td>20</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>synchronized swimming</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>tennis</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>7</td>
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<tr>
<td>track</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>two sport</td>
<td>55</td>
<td>55</td>
<td>62</td>
<td>67</td>
</tr>
<tr>
<td>volleyball</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>weight training</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>cross country</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 4.2 Synopsis of numbers of various athletes per year according to criteria for this study.
Table 4.3  Test-retest correlation study for annual and mean time, METs and Ost.

The initial reported annual time, METs and Ost were plotted against the repeat test variables to allow for visualization of the reliability. The plots were completed for the 1992/3, 1993/4, 1994/5 and the 1995/6 years as the longitudinal period of interest for this project. The reliability plot of the time data is contained in Figure 4.1 while the annual Mets and Ost are plotted in Figures 4.2 and 4.3 respectively. Figure 4.4 was completed using the average annual scores for time, METs and Ost as these variables were also used in the statistical analysis.
Figure 4.1  Plots of the repeat study for time by year.
Figure 4.2  Plots of the repeat study for METs by year.
Figure 4.3  Plots of the repeat study for Ost by year.
Figure 4.4 Plots of the repeat study for mean annual time, METs and Ost.
Pearson correlations for the annual time, METs and Ost with DXA-derived percent body fat and the Bouchard-derived METs recorded along the course of the study for the 268 questionnaire respondents were utilized to examine validity of the physical activity reported on the KA questionnaire. The weak, but significant correlation coefficients between percent body fat and annual time, METs or Ost ranged between $r=-0.146$ and $r=-0.243$ (Table 4.4). The Bouchard value for each subject expressed in METs/kg/d, was an average of the Bouchard tools collected during that year of study. In the case of biannual visits, this snapshot of physical activity included four days (2 weekdays and 2 weekend days/year) while it only included two days/year for the annual visit subjects. When the KA-derived values were correlated to the Bouchard values, the correlation coefficients demonstrated the strongest relationship between the Bouchard and time or METs scores. For METs to METs analysis (Bouchard to KA), the correlation coefficients ranged from 0.3392 in 1993/4 to a stronger 0.4827 for the mean annual METs. The ability to moderately correlate the KA historical questionnaire with the longitudinal Bouchard measures strengthens the case for a valid questionnaire.

Since the KA historical tool was supposed to measure sports participation as well as leisure activity, an additional measure of validity was completed for sports roster and record matching from the schools of the repeat subjects. It was possible to check the junior and senior high school records for twenty-four of the twenty-six subjects. Viable records included athletic rosters, administrative enrollment records, coach confirmation or yearbook inclusion. Only the
Table 4.4  Validity of the KA tool when compared with percent body fat and Bouchard daily average METs.

activities expected to be school-related were included in this evaluation. For example, if a school did not offer soccer for women the year it was reported, it was not included as an expected sport, and club participation was surmised. If the subject reported participation similar to six weeks of softball for three hours each week during the ninth grade, this was not compatible with a high school level sport while it might be more acceptable and checked as a junior high sport.
It is recognized that there was a lot of subjectivity to this portion of the validity study.

There were 113 reported sports and activities expected to be found in school records from the 24 subjects reviewed. One hundred (88.5%) of the activities were confirmed with school officials via a combination of the methods mentioned above. Two of the twenty-six repeat subjects were excluded from this evaluation because the data were not accessible. One of the two subjects only reported junior high (7th & 8th grade) sports (young subject) which further decreased the likelihood that school records would have been helpful. The most common reason for the failed confirmation of 13 sports was a lack of school resources along with the possibility that participation was misreported. One subject simply reported lacrosse participation in the wrong year.

4.2 Questionnaire Respondents vs. Non-respondents

It was important to consider the differences between questionnaire respondents and non-respondents to ensure random sampling or identify potential biases. Table 4.5 outlines descriptive information for general comparison of the two groups. The 268 subjects who returned the questionnaire were compared with the 46 subjects who did not respond for annual changes in (Δ) and cumulative TBBMC, TBBMD and total body bone area (BA) across visits. The general linear model results are reported in Table 4.6 and include models controlled for body weight and height. The weight- and height-adjusted ΔTBBMC and ΔTBBMD bone parameters of the non-respondents were lower in comparison to the subjects returning the questionnaire. The final body fat and
mean Bouchard METs were utilized to determine if the physical activity levels of the non-responders were lower, but the differences were not significant. It is possible that the subjects who felt they had no sports/activities to report may have failed to return the questionnaire. This may exclude a substantial number of control or less active subjects.

<table>
<thead>
<tr>
<th>Non-responders</th>
<th>Responders</th>
</tr>
</thead>
<tbody>
<tr>
<td>n Mean Standard Deviation</td>
<td>n Mean Standard Deviation</td>
</tr>
<tr>
<td>Baseline age (years)</td>
<td>46 11.0 0.75</td>
</tr>
<tr>
<td>Baseline height (cm)</td>
<td>46 145.9 7.47</td>
</tr>
<tr>
<td>ΔHeight (cm)</td>
<td>46 4.2 1.28</td>
</tr>
<tr>
<td>Baseline weight (kg)</td>
<td>46 40.6 8.99</td>
</tr>
<tr>
<td>ΔWeight (kg)</td>
<td>46 4.2 1.40</td>
</tr>
<tr>
<td>Baseline %Body Fat</td>
<td>46 24.3 8.81</td>
</tr>
<tr>
<td>Final %Body Fat</td>
<td>46 28.9 7.10</td>
</tr>
<tr>
<td>ΔBody Fat (g)</td>
<td>46 1611.3 962.16</td>
</tr>
<tr>
<td>ΔLean Body Mass (g)</td>
<td>46 2591.0 758.74</td>
</tr>
<tr>
<td>Mean Bouchard METs</td>
<td>46 45.8 4.33</td>
</tr>
</tbody>
</table>

Table 4.5 Various descriptive information for the questionnaire non-respondents and respondents at baseline visit, ninth (final) visit and change from baseline to final(Δ) visits.
Table 4.6 Comparison of cumulative and change (9-1) TBBMC, TBBMD and bone area in questionnaire non-respondents and respondents using Proc GLM.

4.3 Calcium Intake

The mean total calcium intake of subjects falling into the high and low tertiles of mean time, MET and Ost was compared to ensure similar calcium intakes between groups. The results in Table 4.7 demonstrate the mean values, variability and insignificant differences between the high and low tertiles for mean annual time, METs and Ost. Additionally, the relationships between mean total calcium intake and the continuous variables for mean time, METs and Ost were analyzed (Table 4.8). A bar chart of mean daily calcium intake for the
grouped exercise tertiles is also provided (Figure 4.5). Due to the lack of correlation or differences between groups, the calcium intake was not examined further.

<table>
<thead>
<tr>
<th>Mean annual variable</th>
<th>Tertile</th>
<th>n</th>
<th>Calcium intake (mg)</th>
<th>T score</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>time</td>
<td>High</td>
<td>89</td>
<td>1206 ± 436*</td>
<td>0.936</td>
<td>0.3506</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>90</td>
<td>1146 ± 423</td>
<td></td>
<td></td>
</tr>
<tr>
<td>METs</td>
<td>High</td>
<td>89</td>
<td>1236 ± 444</td>
<td>1.0894</td>
<td>0.2775</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>90</td>
<td>1165 ± 427</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ost</td>
<td>High</td>
<td>89</td>
<td>1226 ± 440</td>
<td>0.9848</td>
<td>0.3261</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>90</td>
<td>1162 ± 429</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.7  T-tests between high and low mean annual time, METs and Ost tertiles for calcium intake. (*mean±SD)

<table>
<thead>
<tr>
<th>Correlation variable</th>
<th>Correlation coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean time</td>
<td>-.01832</td>
<td>0.7653</td>
</tr>
<tr>
<td>Mean METS</td>
<td>.01035</td>
<td>0.8661</td>
</tr>
<tr>
<td>Mean Ost</td>
<td>.00981</td>
<td>0.8730</td>
</tr>
</tbody>
</table>

Table 4.8  Correlation coefficients and p-values for mean calcium intake and mean time, METs and Ost values.
Figure 4.5 Mean daily calcium intake (in mg) and standard error by tertile for mean time, METs, and Ost.

4.4 Athlete Bias

The goal of this analysis was to determine if there were differences between athletes and non-athletes before initiation of activity. There were 33 controls, 142 four-year participants (early starters) and 40 late starters in the analysis. The high number of athletes in the four-year group is likely due to the lenient criteria to be categorized as an athlete. Evaluation of the between groups data in a cross-sectional fashion at the baseline, third and ninth visits,
demonstrated it was common for the control group to have larger variability than the two athletic groups, but Bartlett's test demonstrated homoscedasticity or equal variances. Table 4.9 outlines the lack of differences between the athletic groups and controls when analyzed without covariates. Table 4.10 shows similar data after the bone parameters were adjusted for height and weight to incorporate body size and obesity in the evaluation.

When the groups were compared without adjusting for height and weight, there was no significant difference between early or late starters or controls. When body size was taken into consideration, both the early and late starters had a greater TBBMC and BA than the controls at all three (1,3, and 9) visits. The TBBMD of the athletes was similar to that of controls at baseline, but demonstrated superiority at the third and ninth visits. However, the values for early and late starters were always similar regardless of the outcome variable analyzed.
<table>
<thead>
<tr>
<th>Total Body BMC</th>
<th>n</th>
<th>group mean (grams)</th>
<th>Difference</th>
<th>F value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visit 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late starters</td>
<td>40</td>
<td>1352.28±243.80</td>
<td>early-late</td>
<td>-15.58</td>
<td>1.154</td>
</tr>
<tr>
<td>Early starters</td>
<td>142</td>
<td>1336.70±210.28</td>
<td>early-control</td>
<td>59.73</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>33</td>
<td>1276.97±272.61</td>
<td>late-control</td>
<td>75.31</td>
<td></td>
</tr>
<tr>
<td>Visit 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late starters</td>
<td>40</td>
<td>1626.63±309.01</td>
<td>early-late</td>
<td>-5.25</td>
<td>1.173</td>
</tr>
<tr>
<td>Early starters</td>
<td>142</td>
<td>1621.37±285.28</td>
<td>early-control</td>
<td>84.81</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>33</td>
<td>1536.45±334.16</td>
<td>late-control</td>
<td>90.17</td>
<td></td>
</tr>
<tr>
<td>Visit 9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late starters</td>
<td>40</td>
<td>2312.75±330.64</td>
<td>early-late</td>
<td>4.76</td>
<td>0.66</td>
</tr>
<tr>
<td>Early starters</td>
<td>142</td>
<td>2317.42±325.69</td>
<td>early-control</td>
<td>75.66</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>33</td>
<td>2241.76±428.82</td>
<td>late-control</td>
<td>70.99</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total Body BMD</th>
<th>Visit 1</th>
<th>(grams/cm²)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Late starters</td>
<td>40</td>
<td>0.9021±0.05254</td>
<td>early-late</td>
<td>-0.0047</td>
<td>0.541</td>
</tr>
<tr>
<td>Early starters</td>
<td>142</td>
<td>0.8974±0.05613</td>
<td>early-control</td>
<td>0.0088</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>33</td>
<td>0.8885±0.06107</td>
<td>late-control</td>
<td>0.0136</td>
<td></td>
</tr>
<tr>
<td>Visit 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late starters</td>
<td>40</td>
<td>0.9562±0.06179</td>
<td>early-late</td>
<td>-0.0042</td>
<td>1.108</td>
</tr>
<tr>
<td>Early starters</td>
<td>142</td>
<td>0.9520±0.06941</td>
<td>early-control</td>
<td>0.0177</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>33</td>
<td>0.9343±0.06837</td>
<td>late-control</td>
<td>0.0220</td>
<td></td>
</tr>
<tr>
<td>Visit 9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late starters</td>
<td>40</td>
<td>1.0975±0.05997</td>
<td>early-late</td>
<td>0.0021</td>
<td>1.074</td>
</tr>
<tr>
<td>Early starters</td>
<td>142</td>
<td>1.0996±0.07240</td>
<td>early-control</td>
<td>0.0200</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>33</td>
<td>1.0796±0.07660</td>
<td>late-control</td>
<td>0.0179</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Bone Area</th>
<th>Visit 1</th>
<th>(cm²)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Late starters</td>
<td>40</td>
<td>1491.12±200.05</td>
<td>early-late</td>
<td>-8.07</td>
<td>1.546</td>
</tr>
<tr>
<td>Early starters</td>
<td>142</td>
<td>1483.05±162.63</td>
<td>early-control</td>
<td>57.18</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>33</td>
<td>1425.87±217.07</td>
<td>late-control</td>
<td>65.25</td>
<td></td>
</tr>
<tr>
<td>Visit 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late starters</td>
<td>40</td>
<td>1690.73±233.57</td>
<td>early-late</td>
<td>2.08</td>
<td>1.202</td>
</tr>
<tr>
<td>Early starters</td>
<td>142</td>
<td>1692.81±194.00</td>
<td>early-control</td>
<td>62.21</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>33</td>
<td>1630.59±248.12</td>
<td>late-control</td>
<td>60.13</td>
<td></td>
</tr>
<tr>
<td>Visit 9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late starters</td>
<td>40</td>
<td>2099.72±212.56</td>
<td>early-late</td>
<td>0.94</td>
<td>0.436</td>
</tr>
<tr>
<td>Early starters</td>
<td>142</td>
<td>2100.65±193.34</td>
<td>early-control</td>
<td>36.96</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>33</td>
<td>2063.69±258.97</td>
<td>late-control</td>
<td>36.03</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.9 Differences in total body bone parameters amongst early starting athletes, late starting athletes and controls at baseline, third and ninth visits. F- and p-values are the result of Proc GLM statistical procedure. (Mean±standard deviation)
Table 4.10 Proc GLM statistics and least squares means for total body BMC, BMD and bone area (controlling for height and weight) amongst early and late starting athletes and controls at baseline, third and ninth visits.

<table>
<thead>
<tr>
<th></th>
<th>F-value</th>
<th>p</th>
<th>Least Squares Means</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Body BMC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group differences</td>
<td>190.91</td>
<td>0.0001</td>
<td>1289.45 1336.39 1343.07</td>
</tr>
<tr>
<td>Visit 3</td>
<td>191.61</td>
<td>0.0001</td>
<td>1546.81 1620.95 1619.56</td>
</tr>
<tr>
<td>Visit 9</td>
<td>134.03</td>
<td>0.0001</td>
<td>2219.85 2320.35 2320.44</td>
</tr>
<tr>
<td><strong>Total Body BMD</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group differences</td>
<td>34.02</td>
<td>0.0001</td>
<td>0.8878 0.8979 0.9009</td>
</tr>
<tr>
<td>Visit 3</td>
<td>41.69</td>
<td>0.0001</td>
<td>0.9334 0.9524 0.9556</td>
</tr>
<tr>
<td>Visit 9</td>
<td>21.86</td>
<td>0.0001</td>
<td>1.0734 1.100 1.0997</td>
</tr>
<tr>
<td><strong>Bone Area</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 1</td>
<td>367.65</td>
<td>0.0001</td>
<td>1441.75 1482.84 1482.84</td>
</tr>
<tr>
<td>Visit 3</td>
<td>380.19</td>
<td>0.0001</td>
<td>1643.98 1691.53 1684.23</td>
</tr>
<tr>
<td>Visit 9</td>
<td>299.63</td>
<td>0.0001</td>
<td>2057.60 2101.48 2101.79</td>
</tr>
</tbody>
</table>

4.5 *Bone Mineral Accretion in Active and Inactive Subjects*

Another important objective of the study was to identify if the rate of change in bone mineral acquisition was different for physically active and less active participants. The analysis proceeded with the changes between the final (9th) and baseline visits (Δ) used as continuous variables. The selected general
models are presented for change from baseline to 9th visit as well as cross-sectionally at the 9th visit in Tables 4.11 and 4.12 respectively. The results of plotting the general model residuals against the exercise variables of interest (mean Ost, METs, time, athlete/non-athlete, mean Bouchard and Bratteby METs) are reported in the right-hand column of these tables. Tables 4.13 and 4.14 report the similar analyses of the same dependent variables with mean annual Ost in the pool of candidate predictor variables. The partial r-squared values are reported to help identify the relative importance of each predictor variable. Tables 4.15 and 4.16 condense this same information to identify the predictor variables for each model so the reader can more readily see the most frequently selected predictor variables.

Continuous participation in sports and activities that generate the higher Ost did predict and determine bone mineral content and density in many skeletal sites. This was particularly noticeable in the weight-bearing hip sites as the chance of making a type I error was significantly reduced.
### Table 4.11 General regression models for longitudinal analysis (change from baseline to final)

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variables</th>
<th>Estimate</th>
<th>P value</th>
<th>Partial $R^2$</th>
<th>Cp criteria</th>
<th>Adjusted $R^2$</th>
<th>Residuals Pr&lt;$\wedge$</th>
<th>Regressed against residuals</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta$Total body BMC mean = 234.662 grams</td>
<td>Intercept</td>
<td>24.3628</td>
<td>0.0001</td>
<td>0.0713</td>
<td>5.7242</td>
<td>0.8617</td>
<td>0.8503</td>
<td>Mean Ost</td>
<td>0.0019</td>
</tr>
<tr>
<td></td>
<td>$\Delta$height</td>
<td>-18.1828</td>
<td>0.0001</td>
<td>0.0199</td>
<td></td>
<td></td>
<td></td>
<td>Mean METs</td>
<td>0.0715</td>
</tr>
<tr>
<td></td>
<td>$\Delta$BMI</td>
<td>-35.2798</td>
<td>0.0001</td>
<td>0.0158</td>
<td></td>
<td></td>
<td></td>
<td>Mean time</td>
<td>0.1120</td>
</tr>
<tr>
<td></td>
<td>$\Delta$lean body mass</td>
<td>0.0291</td>
<td>0.0001</td>
<td>0.7485</td>
<td></td>
<td></td>
<td></td>
<td>Athlete 0/1</td>
<td>0.0370</td>
</tr>
<tr>
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*candidate variables included $\Delta$height, $\Delta$BMI, $\Delta$specific bone area, $\Delta$tsm @ visit 9, $\Delta$lean body mass, $\Delta$body fat, $\Delta$age @ visit 9, overall menstrual status 0/1

continued
Table 4.11

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*biased Cp>p
Table 4.12  General regression models for cross-sectional analysis at ninth visit. *candidate variables at visit nine included height, BMI, specific bone area, tsm, lean body mass, body fat, age, overall menstrual status 0/1
Table 4.12

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<th>Independent variables</th>
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<td>Bouchard 0.0309</td>
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<td>0.00001</td>
<td>0.0027</td>
<td>0.0151</td>
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<td>Bratteby 0.0466</td>
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<tr>
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<td>4.44495</td>
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<th>Cp criteria</th>
<th>Adjusted R² (top model)</th>
<th>Residuals Pr&lt;W</th>
<th>Regressed against residuals p</th>
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<tbody>
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<td>-1.1624</td>
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<td>.00013</td>
<td>0.0001</td>
<td>0.0120</td>
<td>5.23393 (5)</td>
<td>0.6959</td>
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Table 4.13  Exercise (mean Ost) inclusive regression models for longitudinal analysis (change from baseline to final)  *candidate variables included mean Ost, Δheight, ΔBMI, Δspecific bone area, tsm @ visit 9, Δlean body mass, Δbody fat, age @ visit 9, overall menstrual status 0/1

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<tr>
<th>Dependent variable</th>
<th>Independent variables</th>
<th>Estimate</th>
<th>P value</th>
<th>Partial R²</th>
<th>Cp criteria (p)</th>
<th>Adjusted R² (top model)</th>
<th>Residuals Pr &lt;W</th>
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<tr>
<td>ΔTotal Body BMC mean = 237.407 grams</td>
<td>Intercept mean Ost Δheight ΔBMI Δlean body mass Δbone area tsm @ visit 9 Δbody fat age @ visit 9</td>
<td>70.4196 0.00175 -18.0199 -354.141 0.0279 1.2657 8.0296 0.0161 -3.1214</td>
<td>0.0008 0.0001 0.0001 0.0001 0.0001 0.0001 0.0001 0.0001 0.0923</td>
<td>0.0048 0.0734 0.0163 0.0165 0.7483 0.0115 0.0047 0.0014</td>
<td>8.0137 (9) 0.8730 (top model)</td>
<td>0.9371</td>
<td>Model too biased without age @ visit 9</td>
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</table>

| ΔTotal Body BMD mean = 0.04844 g/cm³ | Intercept mean Ost ΔBMI Δlean body mass Δbone area tsm @ visit 9 Δbody fat age @ visit 9 | 0.030406 0.000004 0.000004 0.000067 0.0036 -0.00097 | 0.0485 0.0001 0.0002 0.0001 0.0001 0.1571 | 0.0066 0.2573 0.0380 0.0640 0.0043 | 6.6428 (7) 0.4294 (0.4306) | 0.6926 | Model too biased without age @ visit 9 |

| Δ33% Radius BMC mean = 0.10371 grams | Intercept mean Ost Δlean body mass Δbone area tsm @ visit 9 Δbody fat | 0.0385 0.000014 0.0000066 0.0045 -0.000021 | 0.0015 0.0002 0.0001 0.0001 0.086 | 0.0247 0.0187 0.4611 0.0104 0.0130 | 5.7981 (6) 0.51963 (0.51960) | 0.3931 | Model resolves bias in general model |

| Δ33% Radius BMD mean = 0.03250 g/cm³ | Intercept mean Ost Δheight ΔBMI tsm @ visit 9 Δbody fat | 0.016875 0.0000034 0.00269 0.00194 -0.0000387 | 0.0795 0.0001 0.0001 0.0001 0.0001 | 0.0101 0.0341 0.0738 0.0305 0.0223 | 3.6377 (6) 0.1554 (top model) | 0.4977 | continued |
Table 4.13

<table>
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<th>Independent variables</th>
<th>Estimate</th>
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<th>Cp criteria</th>
<th>Adjusted R² (top model)</th>
<th>Residuals Pr &lt;W</th>
<th>Note:</th>
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<tbody>
<tr>
<td>L₁⁺ BMC mean = 43.5125 grams</td>
<td>Intercept mean Ost Δheight Δlean body mass bone area tsm @ visit 9 age @ visit 9</td>
<td>-2.9997</td>
<td>0.0207</td>
<td>0.0062</td>
<td>6.8879 (7)</td>
<td>0.7225 (0.7235)</td>
<td>0.3782</td>
<td>Model resolves bias in general model</td>
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<tr>
<td>Neck BMC mean = 5.20517 grams</td>
<td>Intercept mean Ost Δheight ΔBMI Δlean body mass bone area tsm @ visit 9 age @ visit 9 Δbody fat</td>
<td>1.8773</td>
<td>0.0001</td>
<td>0.0297</td>
<td>8.0214 (9)</td>
<td>0.5829 (top model)</td>
<td>0.3657</td>
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<tr>
<td>Neck BMD mean = 1.01317 g/cm²</td>
<td>Intercept mean Ost Δheight ΔBMI Δlean body mass bone area tsm @ visit 9 age @ visit 9 Δbody fat</td>
<td>1.34694</td>
<td>0.0001</td>
<td>0.0551</td>
<td>8.0091 (9)</td>
<td>0.2864 (top model)</td>
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continued
Table 4.13

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<th>Partial R²</th>
<th>Cp criteria (p)</th>
<th>Adjusted R² (top model)</th>
<th>Residuals Pr &lt;W</th>
<th>Note:</th>
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<tbody>
<tr>
<td>Ward's BMC</td>
<td>Intercept, mean Ost, Δheight, ΔBMI, Δlean body mass, bone area, tsm @ visit 9, Δbody fat</td>
<td>-0.070997</td>
<td>0.0003</td>
<td>0.0200</td>
<td>6.8953 (8)</td>
<td>0.6811 (top model)</td>
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<td>mean = 3.00468 grams</td>
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<tr>
<td>Ward's BMD</td>
<td>Intercept, mean Ost, Δheight, ΔBMI, Δlean body mass, bone area, tsm @ visit 9, Δbody fat</td>
<td>0.929</td>
<td>0.0001</td>
<td>0.0502</td>
<td>6.1940 (7)</td>
<td>0.1518 (0.1520)</td>
<td>0.0538</td>
<td>Addition of age @ visit 9 or xotca does not improve residual distribution. Needs transformation.</td>
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<td>mean = 1.01854 g/cm²</td>
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<tr>
<td>Trochanter BMC</td>
<td>Intercept, mean Ost, Δheight, ΔBMI, Δlean body mass, bone area, age @ visit 9, Δbody fat</td>
<td>0.07123</td>
<td>0.0001</td>
<td>0.0170</td>
<td>8.0982 (9)</td>
<td>0.7117 (0.7119)</td>
<td>0.3247</td>
<td>Model too biased without age @ visit 9</td>
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<tr>
<td>mean = 7.84302 grams</td>
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<tr>
<td>Trochanter BMD</td>
<td>Intercept, mean Ost, Δheight, ΔBMI, Δlean body mass, Δbody fat</td>
<td>0.7917</td>
<td>0.0002</td>
<td>0.0303</td>
<td>8.2268 (6)</td>
<td>0.2250 (0.2318)</td>
<td>0.7937</td>
<td>All models biased except one with 9 variables where 4 p-values = 0.1329, 0.2793, 0.2477, 0.2085</td>
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<tr>
<td>mean = 0.86156 g/cm²</td>
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<td>Dependent variable</td>
<td>Independent variables</td>
<td>Estimate</td>
<td>P value</td>
<td>Partial R²</td>
<td>Cp criteria (p)</td>
<td>Adjusted R² (top model)</td>
<td>Residuals Pr &lt;W</td>
<td>Note:</td>
</tr>
<tr>
<td>-------------------</td>
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<tr>
<td><strong>Total Body BMC</strong></td>
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<td>-9.2569</td>
<td>0.0555</td>
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<td><strong>Total Body BMD</strong></td>
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<td>1.0614</td>
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<td>-0.00353</td>
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<tr>
<td><strong>33% Radius BMC</strong></td>
<td>intercept mean Ost height BMI bone area tsm body fat menstrual status 0/1</td>
<td>-1.4468</td>
<td>0.0000098</td>
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Table 4.14 Exercise (Mean Ost) inclusive regression models for cross-sectional analysis at visit nine. *candidate variables at visit nine included mean Ost, height, BMI, specific bone area, tsm, lean body mass, body fat, age, menstrual status 0/1
### Table 4.14

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<th>Estimate</th>
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<th>Cp criteria</th>
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<td>intercept, mean Ost, lean body mass, bone area, age</td>
<td>-12.396</td>
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<td>0.0043</td>
<td>6.5352 (6)</td>
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<td>6.5766 (8)</td>
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<td>Neck BMD mean =1.01317 g/cm²</td>
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continued
Table 4.14

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<td></td>
<td></td>
</tr>
<tr>
<td>g/cm^3</td>
<td>BMI</td>
<td>0.00381</td>
<td>0.0332</td>
<td>0.0129</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>lean body mass</td>
<td>0.000009</td>
<td>0.0001</td>
<td>0.2310</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.15  Synopsis of regression models for prediction of baseline to final changes in bone mineral accretion at multiple skeletal sites with (shaded rows) and without (unshaded rows) mean annual Ost.
Table 4.16  Synopsis of regression models for prediction of cross-sectional bone mineral accretion (visit 9) at multiple skeletal sites with (shaded rows) and without (unshaded rows) mean annual Ost.
It was common for the $C_p$-selected model containing the mean Ost variable to be exactly like the general model selected for the same site except for the addition of the Ost variable. Accounting for exercise with the mean Ost score while modeling bone density/content changes appears to account for and contribute unique information within the models. It was also notable that time since menarche was usually selected into these models while age was only selected in a few cases. From the partial r-squared values, it is easy to realize the important contribution of bone area as well as the very small contribution of exercise.

4.6 Bone Mineral Accretion Across Time Since Menarche or Age

The primary objective for this analysis was to evaluate the influence of physical activity on bone accumulation over time since menarche (tsm) as compared to models which use age or chronological time as the repeat variable. The annual changes in total body BMC and radial BMC were modeled across time since menarche as well as age. Each model includes the data for 267 subjects as one subject was missing time since menarche. The frequencies for the menstrual categories (not started, just started, eumenorrheic and dysmenorrheic) across the four years are represented in Figure 4.6. The total body BMC modeled across time since menarche is reported in Table 4.17 while the analogous model for age is contained in Table 4.19. The forearm models for time since menarche and age are likewise reported in Tables 4.21 and 4.22.
Menstrual status for 1992/3

ns = 226
js = 29
eu = 12

Menstrual status 1993/4

eu = 76
js = 54
dys = 11
ns = 126

Menstrual status 1994/5

eu = 153
dys = 30
js = 39
ns = 45

Menstrual status 1995/6

eu = 219
dys = 29
js = 7
ns = 12

Figure 4.6 Menstrual status frequency for each of the four years in the longitudinal analysis. (ns = not started, js = just started, eu = eumenorrheic, dys = dysmenorrheic)
<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>T</th>
<th>Pr&gt;lT</th>
<th>Covariance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>32.34168581</td>
<td>35.98350085</td>
<td>0.9</td>
<td>0.3696</td>
<td>Covariance</td>
</tr>
<tr>
<td>Not started slope</td>
<td>6.35334679</td>
<td>2.39428063</td>
<td>2.65</td>
<td>0.0081</td>
<td>Structure</td>
</tr>
<tr>
<td>Just started slope</td>
<td>103.6190244</td>
<td>8.45992617</td>
<td>12.25</td>
<td>0.0001</td>
<td>UN(1)</td>
</tr>
<tr>
<td>Eumenorrheic slope</td>
<td>-30.09470788</td>
<td>2.12929022</td>
<td>-14.13</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Dysmenorrheic slope</td>
<td>-37.19436771</td>
<td>3.87342536</td>
<td>-9.6</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>-1.27371012</td>
<td>0.30746423</td>
<td>-4.14</td>
<td>0.0001</td>
<td>Residuals</td>
</tr>
<tr>
<td>Body Fat (g)</td>
<td>-0.00064596</td>
<td>0.0002198</td>
<td>-2.94</td>
<td>0.0034</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>-3.24681325</td>
<td>1.31341772</td>
<td>-2.47</td>
<td>0.0136</td>
<td>p=0.5954</td>
</tr>
<tr>
<td>Bone Area (cm$^2$)</td>
<td>0.13769567</td>
<td>0.01178898</td>
<td>11.68</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>$\Delta$Lean Body Mass (g)</td>
<td>0.00544006</td>
<td>0.00089309</td>
<td>6.09</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>$\Delta$Body Fat (g)</td>
<td>0.00262728</td>
<td>0.00050887</td>
<td>5.16</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>$\Delta$Bone Area (cm$^2$)</td>
<td>1.08886049</td>
<td>0.02516519</td>
<td>43.27</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Annual Ost</td>
<td>0.00329372</td>
<td>0.000000000003</td>
<td>3.35</td>
<td>0.0009</td>
<td></td>
</tr>
<tr>
<td>Annual Ost$^2$</td>
<td>-0.00000020</td>
<td>-0.000000000002</td>
<td>-2.72</td>
<td>0.0066</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.17  Time since menarche mixed regression model for the annual change in total body bone mineral content over four years.

The $\Delta$TBBMC four-slope tsm model was best suited to a one-banded unstructured covariance ($p=0.1300$) without random effects. The Ost quadratic exercise variable fit into the general model much better than the time, METs or the Bouchard variables, and the quadratic exercise variables fit much better than the linear options. For example, the p-values for the quadratic Ost variables were 0.0009 and 0.0066 while the linear Ost variable yielded a p-value of 0.0460. The p-values for the quadratic time and METs variables were 0.0198, 0.0338 and
0.0180, 0.0393 respectively. The addition of the Ost variables into the candidate pool of variables had an overall lowering effect on the variance inflation factors of the other pool variables.

Figure 4.7 allows for comparison of the fitted model with the observed values for the annual change in total body bone mineral content. Both the actual and predicted values are plotted against time since menarche in separate graphs and the Ost tertiles are separated in the body of the graphs. It is difficult to see the small, but significant (according to the model), effect of exercise in these graphs. The plots in Figure 4.8 are also based on these same Ost tertiles, but allow the reader to see the differences in the eumenorrheic and dysmenorrheic slopes. The general pattern related to lower bone changes for the menstrual dysfunction groups are more noticeable when the reader takes into account that the moderate Ost, dysfunction points at 1.25 and 1.75 years tsm are the result of one data point each. The number of subjects taken into account for each data point in the figures to illustrate the total body and forearm time since menarche models are reported in Table 4.18. The eumenorrheic slope was estimated to be 7.1 grams higher (ΔTBBMC) than the dysmenorrheic slope (p=0.0388).

The positive estimate of the linear Ost with the negative estimate of the squared term indicates a curvilinear relationship that is linear and positive up to a certain point where it peaks and Ost becomes a negative influence. The peak for Ost was determined to be at 8114 (5400, 10,828 for 95%CI) annual Ost. Thirty-eight of the data points (19 subjects) exceeded the peak value with 18 data points (7 subjects) exceeding the upper confidence limit. A quadratic exercise variable such as this with a peak that falls within the range of data indicates that too
much exercise is less positive to the change in TBBMC. The peak for annual hours was estimated to be 979 ±209 hours while the same peak for the METs variable was estimated at 5944 (3408, 8478 for 95% CI). These values could be compared to 156 Ost, 18.8 hours or 114 METs per week when they are divided by 52 weeks in a year.

<table>
<thead>
<tr>
<th>Time Since Menarche</th>
<th>Hi Ost</th>
<th>Low Ost</th>
<th>Moderate Ost</th>
</tr>
</thead>
<tbody>
<tr>
<td>-2.75 not started</td>
<td>10</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>-2.25 not started</td>
<td>17</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>-1.75 not started</td>
<td>10</td>
<td>22</td>
<td>16</td>
</tr>
<tr>
<td>-1.25 not started</td>
<td>33</td>
<td>25</td>
<td>22</td>
</tr>
<tr>
<td>-0.75 not started</td>
<td>26</td>
<td>39</td>
<td>36</td>
</tr>
<tr>
<td>-0.375 not started</td>
<td>18</td>
<td>16</td>
<td>23</td>
</tr>
<tr>
<td>-0.125 not started</td>
<td>26</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>0.125 just started</td>
<td>23</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>0.375 just started</td>
<td>16</td>
<td>29</td>
<td>22</td>
</tr>
<tr>
<td>0.75 eumenorrheic</td>
<td>30</td>
<td>28</td>
<td>40</td>
</tr>
<tr>
<td>dysmenorrheic</td>
<td>12</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>1.25 eumenorrheic</td>
<td>33</td>
<td>36</td>
<td>37</td>
</tr>
<tr>
<td>dysmenorrheic</td>
<td>8</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>1.75 eumenorrheic</td>
<td>33</td>
<td>21</td>
<td>34</td>
</tr>
<tr>
<td>dysmenorrheic</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>2.25 eumenorrheic</td>
<td>15</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>dysmenorrheic</td>
<td>8</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>2.75 eumenorrheic</td>
<td>28</td>
<td>33</td>
<td>36</td>
</tr>
<tr>
<td>dysmenorrheic</td>
<td>5</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 4.18 Frequency of Ost categories by tsm (menstrual status) used for plotting purposes.
Figure 4.7  Observed (top) and predicted (bottom) plots of annual change in total body bone mineral content across menarche by Ost tertile.
Figure 4.8 Observed (top) and predicted (bottom) plots of annual change in total body bone mineral content across time since menarche by hi, low and moderate Ost tertile including menstrual status (eu = eumenorrheic, dys = dysmenorrheic).
The same data for total body bone mineral content (n=267) was modeled across age (quadratic) (Table 4.19). Body mass index, body fat, bone area, annual change in body mass index, annual change in lean body mass, annual change in body fat, annual change in bone area and the Ost quadratic variables formed the best predictive model for annual change in TBBMC across age. The data were best modeled with the Toeplitz covariance structure (p=0.0025), and the residuals were normally distributed for the model. Again, the quadratic Ost (p=0.0064, 0.0299) fit the model better than the linear option (p=0.0810), and the peak of this quadratic effect fell within the range of the data. The peak of the Ost effect appeared to occur at 8425 Ost (4923 and 11926 95% confidence interval). The categories and subject distribution used in plotting the total body and forearm age models are outlined in Table 4.20. The plots of annual change in TBBMC contained in Figure 4.9 demonstrate the differences in the Ost tertiles when plotted across age.
### Table 4.19
Age quadratic mixed regression model for the annual change in total body bone mineral content over four years.

| Effect                  | Estimate | Standard Error | T     | Pr>|ltl |
|-------------------------|----------|----------------|-------|------|
| Intercept               | -918.147 | 102.971        | -8.92 | 0.0001 |
| Body Mass Index (kg/m²) | 3.938    | 1.005          | 3.92  | 0.0001 |
| Body Fat (g)            | -0.00229 | 0.00049        | -4.68 | 0.0001 |
| Age (yr)                | 110.106  | 15.707         | 7.01  | 0.0001 |
| Age²                    | -4.218   | 0.586          | -7.2  | 0.0001 |
| Bone Area (cm²)         | 0.105    | 0.007          | 15.44 | 0.0001 |
| ΔBody Mass Index (kg/m²)| -5.827   | 2.770          | -2.1  | 0.0358 |
| ΔLean Body Mass (g)     | 0.005    | 0.001          | 4.52  | 0.0001 |
| ΔBone Area (cm²)        | 1.119    | 0.026          | 42.37 | 0.0001 |
| Annual Ost              | 0.003    | 0.000          | 2.73  | 0.0064 |
| Annual Ost²             | -0.000   | -0.000         | -2.18 | 0.0299 |

### Table 4.20
Frequency of Ost categories by Age used for plotting purposes.

<table>
<thead>
<tr>
<th>Age</th>
<th>Hi Ost</th>
<th>Low Ost</th>
<th>Moderate Ost</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.25</td>
<td>31</td>
<td>35</td>
<td>31</td>
</tr>
<tr>
<td>11.75</td>
<td>34</td>
<td>35</td>
<td>29</td>
</tr>
<tr>
<td>12.25</td>
<td>34</td>
<td>38</td>
<td>41</td>
</tr>
<tr>
<td>12.75</td>
<td>42</td>
<td>44</td>
<td>47</td>
</tr>
<tr>
<td>13.25</td>
<td>46</td>
<td>42</td>
<td>34</td>
</tr>
<tr>
<td>13.75</td>
<td>43</td>
<td>47</td>
<td>50</td>
</tr>
<tr>
<td>14.25</td>
<td>35</td>
<td>35</td>
<td>43</td>
</tr>
<tr>
<td>14.75</td>
<td>37</td>
<td>41</td>
<td>35</td>
</tr>
<tr>
<td>15.25</td>
<td>29</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>15.75</td>
<td>25</td>
<td>20</td>
<td>24</td>
</tr>
</tbody>
</table>
Figure 4.9  Observed (top) and predicted (bottom) plots of annual change in total body bone mineral content across age by Ost tertile.
The model to predict annual change in radius BMC across TSM is reported in Table 4.21. Similar to the total body TSM model, it was also best modeled using the unstructured(l) covariance structure (p=0.0000). Selected predictor variables included lean body mass, body fat, bone area, annual change in height, annual change in body mass index and annual change in bone area. Neither the linear nor quadratic Ost (nor other exercise) variables would stay in the model according to the p-value (p=0.1742 and p=0.4021, 0.1031 respectively). The impact characteristic Ost score for physical activity and sport did not appear to help predict the annual change in radial BMC at the primarily cortical 33% site when analyzed across menarcheal time. Figures 4.10 and 4.11 illustrate the Ost tertiles with actual and predicted values across time since menarche similar to the plots presented for the total body model with Figures 4.11 including the eumenorrheic/dysmenorrheic visualization. Even though the linear Ost variable is not statistically significant and makes the model less than desirable, the difference between the eumenorrheic and dysmenorrheic slopes was estimated to be 0.006 grams (p=0.0655).
<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>T</th>
<th>Pr&gt;</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-0.02010818</td>
<td>0.01484871</td>
<td>-1.35</td>
<td>0.1768 Covariance</td>
<td></td>
</tr>
<tr>
<td>Not started slope</td>
<td>0.01584497</td>
<td>0.00257021</td>
<td>6.16</td>
<td>0.0001 Structure</td>
<td></td>
</tr>
<tr>
<td>Just started slope</td>
<td>0.03094992</td>
<td>0.00933106</td>
<td>3.32</td>
<td>0.0010 UN(1)</td>
<td></td>
</tr>
<tr>
<td>Eumenorrheic slope</td>
<td>-0.01475274</td>
<td>0.00212119</td>
<td>-6.95</td>
<td>0.0001 p=0.0000</td>
<td></td>
</tr>
<tr>
<td>Dysmenorrheic slope</td>
<td>-0.02076861</td>
<td>0.00377825</td>
<td>-5.5</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Lean Body Mass (g)</td>
<td>0.00000130</td>
<td>0.00000035</td>
<td>3.67</td>
<td>0.0003 Residuals</td>
<td></td>
</tr>
<tr>
<td>Body Fat (g)</td>
<td>0.00000069</td>
<td>0.00000017</td>
<td>-4.05</td>
<td>0.0001 Pr&lt;.W</td>
<td></td>
</tr>
<tr>
<td>Radius Bone Area (cm2)</td>
<td>0.01395771</td>
<td>0.00655201</td>
<td>2.13</td>
<td>0.0335 p=0.7144</td>
<td></td>
</tr>
<tr>
<td>ΔHeight (cm)</td>
<td>0.00659020</td>
<td>0.00089014</td>
<td>7.4</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>ΔBody Mass Index (kg/m²)</td>
<td>0.00395117</td>
<td>0.00097781</td>
<td>4.04</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>ΔRadius Bone Area (cm²)</td>
<td>0.49188515</td>
<td>0.02429590</td>
<td>20.25</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Annual Ost</td>
<td>0.00000063</td>
<td>0.00000047</td>
<td>1.36</td>
<td>0.1742</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.21  Time since menarche mixed regression model for the annual change in radius bone mineral content over four years.
Figure 4.10 Observed (top) and predicted (bottom) plots of annual change in radial bone mineral content (33% site) across time since menarche by Ost tertile.
Figure 4.11  Observed (top) and predicted (bottom) plots of annual change in radial bone mineral content (33% site) across time since menarche by hi, low and moderate Ost including menstrual status. (eu = eumenorrheic, dys = dysmenorrheic)
When the data for the radius were modeled across age for the same subjects, the unstructured(1) covariance was selected again. The p-value of the linear Ost variable within this model (p=0.1584) was similar to the same variable in the tsm forearm model (p=.1742). The other exercise variables were also excluded from this model. Table 4.22 demonstrates the inclusion of height, body mass index, body fat, annual change in height, annual change in body fat and annual change in forearm bone area as the selected model. The Ost tertiles are plotted in Figure 4.12 to include the observed and predicted plots as in the previous models.

| Effect                      | Estimate  | Standard Error | T   | Pr>|ltl |
|-----------------------------|-----------|----------------|-----|------|
| Intercept                   | -0.88295756 | 0.09996734     | -8.83 | 0.0001 Covariance |
| Height (cm)                 | 0.00159745  | 0.00021143     | 7.56 | 0.0001 Structure |
| Body Mass Index (kg/m²)     | 0.00630832  | 0.00105276     | 5.99 | 0.0001 UN(1) |
| Body Fat (g)                | -0.00000333 | 0.00000050     | -6.62 | 0.0001 p=0.0000 |
| Age (yr)                    | 0.08551099  | 0.01519444     | 5.63 | 0.0001 |
| Age²                        | -0.00313507 | 0.00055564     | -5.64 | 0.0001 Residuals |
| ΔHeight (cm)                | 0.00784548  | 0.00075126     | 10.44 | 0.0001 Pr<W |
| ΔBody Fat (g)               | 0.00000224  | 0.00000050     | 4.52 | 0.0001 p=0.9456 |
| ΔRadius Bone Area (cm²)     | 0.50985227  | 0.02459972     | 20.73 | 0.0001 |
| Annual Ost                  | 0.00000069  | 0.00000049     | 1.41 | 0.1584 |

Table 4.22  Age quadratic mixed regression model for the annual change in radius bone mineral content over four years.
Figure 4.12  Observed (top) and predicted (bottom) plots of annual change in radius bone mineral content (33% site) across age by Ost tertile.
It was of additional interest within the ΔTBBMC tsm model to identify if there is a window of time before or after menarche where physical activity is particularly beneficial to the rate of change in bone. The interaction (product) of the Ost variable with each of the tsm slopes was tested such that: Not started slope*Ost p=0.6984, Just started slope*Ost=0.1566, Eumenorrheic slope*Ost p=0.3002 and Dysmenorrheic slope*Ost p=0.3109. This indicates that the benefit from Ost was not different for the various levels of time since menarche (menstrual status). A short investigation of the two-slope tsm model with a change point at menarche was also conducted to evaluate for an interaction of menstrual status (pre- or post-) and exercise. There were no significant interactions in this model as well. It is interesting to note on the ΔTBBMC plot across tsm (Figure 4.7) that the higher Ost group appeared to peak at rates of change similar to the lower Ost group. The higher Ost group appeared to experience this peak after menarche while the lower Ost group experienced the peak change rate just before menarche.

An investigation of the change in bone area across time since menarche was conducted to see if a difference in periosteal expansion exists as the result of exercise. This analysis was limited to the change in TBBMC dependent variable due to the previously identified lack of influence on the forearm BMC model. The general model and model which includes Ost to predict annual change in total body bone area are reported in Table 4.23. This variable was best modeled using the two-banded heterogeneous Toeplitz covariance matrix (p=0.0000) which allowed the for the variances to be predicted for each visit as well as allow for a negative correlation of -0.3445 between measures taken at consecutive
visits. The linear Ost variable demonstrated a trend (p=0.0763) to help predict the annual change in total body bone area. The small and negative estimate (-0.0006) translates to mean that the subjects with higher Ost had slightly less bone area than the less active individuals. There were no differences identified between the eumenorrheic and dysmenorrheic slopes (p=0.7571). The interaction of Ost with each of the menstrual slopes failed to identify any interaction for exercise with tsm. The actual changes in bone area are plotted for the Ost tertiles in Figure 4.13.
### Table 4.23 Mixed regression models for annual change in total body bone area across time since menarche without (top) and with (bottom) annual Ost.

| General model Intercept | Effect                        | Estimate | Standard Error | t     | Pr>|ltl |
|-------------------------|-------------------------------|----------|----------------|-------|------|
|                         | Intercept                    | 56.0319  | 19.0743        | 2.94  | 0.0036 |
|                         | Not started slope            | 17.5205  | 2.3355         | 7.5   | 0.0001 |
|                         | Just started slope           | -19.5279 | 9.4860         | -2.06 | 0.0399 |
|                         | Eumenorheic slope            | -11.9703 | 2.1492         | -5.57 | 0.0001 |
|                         | Dysmenorheic slope           | -13.6091 | 3.8492         | -3.54 | 0.0004 |
|                         | Lean body mass (g)           | 0.0015   | 0.0002         | 6.42  | 0.0001 |
|                         | Age (yr)                     | -4.3880  | 1.1016         | -3.98 | 0.0001 |
|                         | ΔHeight (cm)                 | 15.6122  | 1.0102         | 15.45 | 0.0001 |
|                         | ΔLean body mass (g)          | 0.0109   | 0.0011         | 10.32 | 0.0001 |
|                         | ΔBody fat (g)                | 0.0087   | 0.0004         | 20.39 | 0.0001 |

| General model Intercept | Effect                        | Estimate | Standard Error | t     | Pr>|ltl |
|-------------------------|-------------------------------|----------|----------------|-------|------|
|                         | Intercept                    | 51.0505  | 19.2158        | 2.66  | 0.0084 |
|                         | Not started slope            | 17.3394  | 2.3318         | 7.44  | 0.0001 |
|                         | Just started slope           | -20.2599 | 9.4788         | -2.14 | 0.0329 |
|                         | Eumenorheic slope            | -12.1939 | 2.1509         | -5.67 | 0.0001 |
|                         | Dysmenorheic slope           | -13.2250 | 3.8526         | -3.43 | 0.0006 |
|                         | Lean body mass (g)           | 0.0016   | 0.0002         | 6.65  | 0.0001 |
|                         | Age (yr)                     | -4.0987  | 1.1102         | -3.69 | 0.0002 |
|                         | ΔHeight (cm)                 | 15.6120  | 1.0083         | 15.48 | 0.0001 |
|                         | ΔLean body mass (g)          | 0.0109   | 0.0011         | 10.33 | 0.0001 |
|                         | ΔBody fat (g)                | 0.0087   | 0.0004         | 20.35 | 0.0001 |

| Annual Ost              | Intercept                    | -0.0006  | 0.0004         | -1.77 | 0.0763 |
|                         | Not started slope            | 17.5205  | 2.3355         | 7.5   | 0.0001 |
|                         | Just started slope           | -19.5279 | 9.4860         | -2.06 | 0.0399 |
|                         | Eumenorheic slope            | -11.9703 | 2.1492         | -5.57 | 0.0001 |
|                         | Dysmenorheic slope           | -13.6091 | 3.8492         | -3.54 | 0.0004 |
|                         | Lean body mass (g)           | 0.0015   | 0.0002         | 6.42  | 0.0001 |
|                         | Age (yr)                     | -4.3880  | 1.1016         | -3.98 | 0.0001 |
|                         | ΔHeight (cm)                 | 15.6122  | 1.0102         | 15.45 | 0.0001 |
|                         | ΔLean body mass (g)          | 0.0109   | 0.0011         | 10.32 | 0.0001 |
|                         | ΔBody fat (g)                | 0.0087   | 0.0004         | 20.39 | 0.0001 |
|                         | Annual Ost                   | -0.0006  | 0.0004         | -1.77 | 0.0763 |
Figure 4.13  Plots of observed annual change in total body bone area across time since menarche by Ost tertile (top) and including menstrual status (bottom). (eu = eumenorrheic, dys = dysmenorrheic)
It was of interest to compare the selected ΔTotal Body BMC mixed model with the general linear model (GLM) equivalent. The mixed models in this comparison included the unstructured and the one-banded unstructured covariance structures. Table 4.24 is provided for the GLM to mixed model option comparisons. The unstructured(1) covariance structure within the mixed model framework was chosen to best model the changes across time since menarche because the variances from one visit to the next of the outcome variable appeared different when modeled longitudinally.

We hypothesized that forcing the estimates to be fixed over the four year period of growth was likely responsible for the apparent differences of the variances and analyzed the full TBBMC model at each cross-sectional time point to see how the estimates may change at each time point. These results are displayed in Table 4.25 (top) along with the modified models after backwards stepwise removal of insignificant variables (bottom). The variables in the best prediction model at each time point were different from the full longitudinal model, but the estimate and the p-value for the Ost variables were similar.
<table>
<thead>
<tr>
<th></th>
<th>General model</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>MIXED UN</td>
<td>MIXED UN(1)</td>
<td>GLM</td>
</tr>
<tr>
<td></td>
<td>estimate</td>
<td>p</td>
<td>estimate</td>
<td>p</td>
</tr>
<tr>
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<td>37.51</td>
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<td>0.0052</td>
<td>6.08</td>
<td>0.0112</td>
</tr>
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<td>0.0001</td>
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<td>0.0001</td>
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<td>-1.38</td>
<td>0.0001</td>
</tr>
<tr>
<td>Body Fat (g)</td>
<td>-0.0007</td>
<td>0.0003</td>
<td>-0.0008</td>
<td>0.0001</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>-2.83</td>
<td>0.0315</td>
<td>-2.81</td>
<td>0.0314</td>
</tr>
<tr>
<td>Bone Area (cm²)</td>
<td>0.141</td>
<td>0.0001</td>
<td>0.144</td>
<td>0.0001</td>
</tr>
<tr>
<td>ΔLean Body Mass (g)</td>
<td>0.0055</td>
<td>0.0001</td>
<td>0.0057</td>
<td>0.0001</td>
</tr>
<tr>
<td>ΔBody Fat (g)</td>
<td>0.0027</td>
<td>0.0001</td>
<td>0.0027</td>
<td>0.0001</td>
</tr>
<tr>
<td>ΔBone Area (cm²)</td>
<td>1.085</td>
<td>0.0001</td>
<td>1.08</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

|                      | Ost inclusive model |                   |                   |                   |
|                      | independs:         | MIXED UN          | MIXED UN(1)       | GLM               |
|                      |                 | estimate   | p     | estimate          | p                 |
| Intercept            | 36.07            | 0.3118     |       | 32.34             | 0.3666            | 29.89             | 0.4078 |
| Not started slope    | 7.04             | 0.0038     |       | 6.35              | 0.0081            | 6.02              | 0.0109 |
| Just started slope   | 103.76           | 0.0001     |       | 103.62            | 0.0001            | 104.21            | 0.0001 |
| Eumenorrheic slope   | -30.09           | 0.0001     |       | -30.09            | 0.0001            | -29.88            | 0.0001 |
| Dysmenorrheic slope  | -36.92           | 0.0001     |       | -37.19            | 0.0001            | -36.89            | 0.0001 |
| Height (cm)          | -1.27            | 0.0001     |       | -1.27             | 0.0001            | -1.25             | 0.0001 |
| Body Fat (g)         | -0.0006          | 0.0080     |       | -0.0006           | 0.0034            | -0.0006           | 0.0035 |
| Age (yr)             | -3.21            | 0.0145     |       | -3.25             | 0.0136            | -3.29             | 0.0128 |
| Bone Area (cm²)      | 0.135            | 0.0001     |       | 0.138             | 0.0001            | 0.137             | 0.0001 |
| ΔLean Body Mass (g)  | 0.0053           | 0.0001     |       | 0.005            | 0.0001            | 0.0056            | 0.0001 |
| ΔBody Fat (g)        | 0.0026           | 0.0001     |       | 0.0026            | 0.0001            | 0.0026            | 0.0001 |
| ΔBone Area (cm²)     | 1.091            | 0.0001     |       | 1.089             | 0.0001            | 1.09              | 0.0001 |
| Annual Ost           | 0.0033           | 0.0009     |       | 0.0033            | 0.0009            | 0.0031            | 0.0018 |
| Annual Ost2          | -0.0000002       | 0.0067     |       | -0.0000002       | 0.0066            | -0.0000002       | 0.0107 |

For comparison:
Annual Ost (linear)

|                      |                      | MIXED UN          | MIXED UN(1)       | GLM               |
|                      |                      | estimate          | p                 | estimate          | p                 |
|                      |                      | 0.0000002         | 0.0067            | -0.0000002       | 0.0066            | -0.0000002       | 0.0107 |

Table 4.24 Final model for annual change in TBBMC modeled with Proc Mixed (unstructured and unstructured(1) covariance structures) and Proc GLM (covariance structure assumed independence).
Table 4.25  Selected and modified models for annual change in TBBMC modeled for each visit using Proc GLM. (# is drop order, shaded cells are not values from presented model)
4.7 Bone Mineral Accretion in Athletic Menstrual Dysfunction

The subjects categorized as athletes for all four years (n=141) were analyzed separately to determine if there were differences in the bone mineral accretion of eumenorrheic and dysmenorrheic athletes according to time since menarche. The variances of the groups for the variables of interest were determined to be homogeneous by Bennett's test. The initial analyses with the overall menstrual status (mstat01) and the early/late menarche (men01) variables and interaction term resulted in no significant differences due to men01, mstat01 or the interaction so the continuous tsm variable was also analyzed with mstat01 and the interaction term. It was suspected that the division of men01 and inclusion of subjects close to the cut-off may have dampened any potential effects due to time since menarche. The results for the menstrual status, tsm and interaction models are reported. It should be mentioned that the Cp selected model for each outcome variable included the covariates and tsm variable as the top model with the addition of the menstrual status variable within the top 6-12 models. The Cp statistic for the model containing both tsm and the menstrual status indicator was also within acceptable Cp criteria in each model.

The predictive model for change in total body BMC included baseline height, body mass index, bone area and change in height, lean body mass and bone area. Neither the interaction for tsm and Menstrual status or the individual variables was significant. The model is outlined in Table 4.26 and box plots in Figure 4.14 allow for visual comparison of the changes in eumenorrheic and dysmenorrheic subjects over four years.
Table 4.26 Change in total body BMC in four-year athletes according to menstrual status (Mstat) and time since menarche (tsm). (eu = eumenorrheic, dys = dysmenorrheic)

| Independent                     | Estimate | pr>|T| | p   | Standard Error |
|---------------------------------|----------|------|-----|-----|-----------------|
| Intercept                       | 161.58   | 2.69 | 0.0008 | 60.03 | Cp              |
| Baseline height (cm)            | -2.28    | -4.28| 0.0001 | 0.53  | 5.3037          |
| Baseline BMI (kg/m²)            | -1.98    | -2.76| 0.0066 | 0.71  |                 |
| Baseline bone area (cm²)        | 0.15     | 6.63 | 0.0001 | 0.023 | R Square        |
| ΔHeight (cm)                    | -9.25    | -3.12| 0.0022 | 2.96  | 0.8938          |
| ΔLean body mass (g)             | 0.009    | 2.35 | 0.0202 | 0.003 |                 |
| Δbone area (cm²)                | 1.54     | 14.99| 0.0001 | 0.103 | residuals       |
| tsm @ visit 9 (yr)              | 1.71     | 0.4  | 0.6871 | 4.24  | p=0.9774        |
| Mstat                           | eu       | -13.27| -1.37| 0.1738 | 9.7            |
|                                 | dys      | .     | .     | .     | .               |
| tsm*Mstat                       | eu       | 5.58  | 5.58 | 0.1791 | 4.13           |
|                                 | dys      | .     | .     | .     | .               |

The change in total body BMD presented a different picture. Controlling for the changes in body mass index, body fat and bone area, the interaction between tsm and menstrual status was marginally significant (p=0.0506). The results for the four-year changes are reported in Table 4.27 and the box plots of the same time period are contained in Figure 4.15. Because the interaction was marginally significant, it is helpful to plot the change in total body BMD across tsm by menstrual group to see the interaction (Figure 4.16).
Figure 4.14  Box plots of four-year change (Δ) in total body BMC in four-year athletes by overall menstrual status.

Table 4.27  Change in total body BMD in four-year athletes according to menstrual status (Mstat) and time since menarche (tsm). (eu = eumenorrheic, dys = dysmenorrheic)
The change in bone area model included baseline height, body mass index, body fat, and bone area as well as the change variables for height, lean body mass and body fat. The interaction between tsm and menstrual status was insignificant (p=0.7984). The slope of change for tsm, controlling for the other variables in the model, was significant (p=0.0469) and positive. The model is outlined in Table 4.28 and the box plots of the two groups are in Figure 4.17.

Figure 4.15  Box plots of four-year change (Δ) in total body BMD in four-year athletes by overall menstrual status.
Figure 4.16  Four-year change in total body BMD in four-year athletes across time since menarche by overall menstrual status.

Table 4.28  Change in total body Bone Area in four-year athletes according to menstrual status (Mstat) and time since menarche (tsm).  (eu = eumenorrheic, dys = dysmenorrheic)
The forearm model for BMC at the 33% radius site demonstrated a significant interaction between tsm and menstrual status \((p=0.0070)\). The model was adjusted for baseline lean body mass and the changes in height, body mass index, body fat and forearm bone area (33% radius). Table 4.29 delineates the model while Figure 4.18 shows the box plots for the four-year changes. Again, since the variables of interest demonstrated a significant interaction, the graph of forearm BMC across tsm by menstrual group in Figure 4.19 helps visualize the interaction.
Table 4.29 Change in radius BMC (33% site) in four-year athletes according to menstrual status (Mstat) and time since menarche (tsm). (eu = eumenorrheic, dys = dysmenorrheic)

The BMD of the radius demonstrated similar results to the forearm BMC model. There was a significant interaction between tsm and menstrual status (p=0.0028) as this selected model included baseline forearm bone area along with the change variables for lean body mass and body fat. This model is demonstrated in Table 4.30 and the box plots follow in Figure 4.20. The nature of the interaction is identified in Figure 4.21.
Overall Menstrual Status

Figure 4.18  Box plots of four year-change ($\Delta$) in radial BMC (33% site) in four-year athletes by menstrual status.

Figure 4.19  Four-year change in radial BMC (33% site) in four-year athletes across time since menarche by menstrual status.
Independents Estimate $pr>|T|\) p Standard Error
Intercept 0.0516 6.65 0.0001 0.0078 Cp
Baseline Bone Area (cm$^2$) -0.0095 -2.92 0.0042 0.0032 3.0403
ΔLean Body Mass (g) 0.0000036 3.97 0.0001 0.0000009
ΔBody Fat (g) -0.000001 -2.24 0.0270 0.0000005 R Square
tsm@ visit 9 (yr) -0.0027 -1.8 0.0742 0.0015 0.2016
Mstat eu -0.0118 -3.18 0.0018 0.0037
 dsys . . . . residuals
 tsm*Mstat eu 0.0048 3.05 0.0028 0.0016 p=0.7859
dsys . . . .

Table 4.30 Change in radius BMD (33% site) in four-year athletes according to menstrual status (Mstat) and time since menarche (tsm). (eu = eumenorrheic, dys = dysmenorrheic)

Figure 4.20 Box plots of four-year change (Δ) in radial BMD (33% site) in four-year athletes by overall menstrual status.
Figure 4.21  Four year change in radial BMD (33% site) in four-year athletes across time since menarche by overall menstrual status.

The BMC of the femur (neck) was also evaluated, but cross-sectionally at visit 9. The results are demonstrated in Table 4.31 and fail to demonstrate a significant interaction or main effect due to menstrual status or tsm. The box plots (Figure 4.22) demonstrate the femoral neck BMC of the menstrual groups as in previous models.
### Table 4.31
Cross-sectional analysis of the BMC of the femur (neck) in four-year athletes according to menstrual status (Mstat) and time since menarche (tsm). (eu = eumenorrheic, dys = dysmenorrheic)

|            | Estimate | pr>|IT| | p   | Standard Error |
|------------|----------|------|-----|-----|----------------|
| Intercept  | -1.43    | -2.52| 0.0127| 0.567| Cp             |
| Lean Body Mass @9 (g) | 0.000066 | 5.83 | 0.0001| 0.00001| 3.44 |
| Neck Bone area @9 (cm²) | 0.7805 | 6.54 | 0.0001| 0.119| R Square      |
| tsm @9 (yr) | 0.0441 | 0.43 | 0.6700| 0.103| residuals     |
| Mstat      |          |      |      |      |                |
|            | eu       | -0.1521| -0.57| 0.5712| 0.268| 0.5591 |
|            | dys      |        |      |      |                |
| tsm*Mstat  |          |        |      |      |                |
|            | eu       | 0.0505 | 0.45 | 0.6565| 0.113| residuals |
|            | dys      |        |      |      |                |

Figure 4.22 Box plots of the BMC of the femur (neck) at visit 9 in four-year athletes by menstrual status.
4.8 Prediction of Athletic Menstrual Dysfunction (Leptin)

Logistic regression was utilized to investigate the relationship between menstrual dysfunction and leptin / body fat for the four-year athlete subjects. The dichotomous variable for menstrual status (eumenorrheic or dysmenorrheic) was the dependent variable as the models presented predicted the likelihood that the independent variable(s) could accurately predict menstrual status. The evaluation included only data from the fifth, seventh and ninth visits where the subjects were post-menarcheal. The frequencies of the dysmenorrheic and eumenorrheic occurrences are listed in Table 4.32. General plots were included to help visualize the two menstrual groups, but the plots do not symbolize the logistic regression results.

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<tr>
<th>Visit</th>
<th>Dysmenorrheic</th>
<th>Eumenorrheic</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>6</td>
<td>38</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>71</td>
</tr>
<tr>
<td>9</td>
<td>14</td>
<td>101</td>
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</table>

Table 4.32 Frequencies of dysmenorrhea and eumenorrhea at visits 5, 7, and 9 in four-year athletes.

The first logistic regression model evaluated leptin as a univariate predictor. In predicting the probability that the athlete was eumenorrheic (mstatath=1) the prediction equation: \( \text{logit}(p) = 1.1671 + 0.0411 \times \text{leptin} \). Exponentiation of this estimate for two subjects with a difference in serum leptin...
concentration of 3 ng/ml translates to the subject with higher leptin concentration being 1.13 times more likely to be eumenorrheic with a 95% confidence interval of 0.99 to 1.3. A larger difference in serum leptin concentration of 10 ng/ml would indicate 1.5 times higher likelihood of the athlete to be eumenorrheic. The ability of leptin to predict the occurrence of athletic menstrual status was not significant, but could be considered a trend (p=0.0883). Figure 4.23 identifies the mean leptin values for each athletic menstrual group at visits five, seven and nine.

Figure 4.23 Mean serum leptin concentration at visits five, seven and nine by menstrual status in four-year athletes.
Body fat was also investigated as an independent predictor of leptin. Figure 4.24 demonstrates the group mean body fat levels. The equation to predict the probability of eumenorrheic status was \( \text{logit}(p) = 0.8291 + 0.000054 \times \text{body fat} \). The p-value for the model -2 Log Likelihood was 0.0410 while the p-value for the maximum likelihood estimate was 0.0606. The subjects with higher body fat were more likely to be eumenorrheic and where 3000 grams difference in body fat existed, the subject with higher body fat was 1.176 (0.9915, 1.394 95% CI) times more likely to be eumenorrheic. Where larger differences in body fat existed, such as 5000 grams, the subject would be 1.31 (1.105, 1.55 95%CI) times more likely to be eumenorrheic.

Figure 4.24 Mean body fat at visits five, seven and nine by menstrual status in four-year athletes.
Percent body fat as the independent variable for athletic menstrual status was a stronger predictor according to the equation: \( \text{logit}(p) = 0.1673 + 0.0563 \times \text{(%body fat)} \). The model p-value (0.0138) and the estimate p-value (0.0187) were both highly significant. Where a three percent difference in body fat existed (22% versus 25% body fat), the subject with higher relative body fat was 1.184 times more likely (1.029, 1.363) to be eumenorrheic than the subject with the lower percent body fat. Figure 4.25 contains the graphic representation of this difference.

Time spent in physical activity was also evaluated as a univariate predictor of athletic menstrual status. Though the significance of time as a predictor of menstrual status was also marginal (\( p = 0.0728 \)). The equation to describe this relationship was: \( \text{logit}(p) = 2.0020 - 0.00073 \times \text{(time)} \). This indicates that subjects with less time in activity were more likely to be eumenorrheic. The graph in Figure 4.26 helps to see the annual hours engaged in physical activity for the groups.
Figure 4.25  Mean percent body fat at visits five, seven and nine by menstrual status in four-year athletes.

The last univariate model analyzed looked at the energy drain reported by the subjects calculated using the average kilocalorie intake minus the kilocalories expended per the Bouchard tool. The balance was negative for all subjects. The equation estimated the effect from the energy drain to be -0.00008 with a probability of 0.7083. The graph (Figure 4.27) of this variable across the visits.
demonstrates the irony as the eumenorrheic group had a slightly (not significant) greater energy drain than the dysmenorrheic group. The reported Bouchard daily energy expenditure was also divided by the predicted basal kilocalories needed according to the Harris Benedict equation to form a physical activity index. This activity index was analyzed similarly as a metabolic indicator. Results from evaluation of the activity index were non-significant and similar to those of the energy drain.

![Graph](image)

**Figure 4.26** Annual time spent in physical activity at visits five, seven and nine by menstrual status in four-year athletes.
Figure 4.27  Mean energy drain at visits five, seven and nine by menstrual status in four-year athletes.

Investigation of bivariate models was conducted to see if controlling for body fat, percent body fat or exercise would contribute to the prediction of menstrual status along with leptin as the primary variable of interest. The model with leptin and percent body fat was logit(p) = 0.0395 - 0.0129(leptin) + 0.0667 (percent body fat). Neither the p-values for leptin (0.7449) or percent body fat (0.0961) were significant. Similarly, modeling body fat with leptin yielded the
equation \( \text{logit}(p) = 0.8594 + 0.00973 \text{ (leptin)} + 0.000045 \text{ (body fat)} \). Again neither p-value was significant (0.8104 and 0.3528 respectively). When leptin and time were modeled together to predict the probability that the menstrual status for the athlete was eumenorrheic, the equation \( \text{logit}(p) = 1.5593 + 0.0393 \text{ (leptin)} - 0.00069 \text{ (time)} \). The p-value for leptin and time were 0.1063 and 0.0925 respectively resulting in insignificant predictive value.

Analysis of trivariate models yielded similar results. For instance, when using leptin, percent body fat and time spent in physical activity to predict the probability that the athletic menstrual status was eumenorrheic, the p-values of the predictors were all > 0.05 (0.7543, 0.1149 and 0.1090) and the odds ratio for the model was 1.000. These results indicate that there is no predictive value in this model for menstrual status of athletes. A larger model including leptin, percent body fat, time in activity and energy drain was also investigated to find similar results. The p-values were 0.4754, 0.0648, 0.1553 and 0.5125 for these predictor variables and the odds ratio was again 1.0. The prediction of menstrual dysfunction from these larger models was not useful in this sample of female athletes.
The primary objective of this study was to adopt or develop a questionnaire and method to score the impact characteristics of physical activity in this sample of adolescent females across four years of longitudinal study. The impact characteristics of physical activity, as measured by the KA tool and Ost method, did promote bone mineral accretion during puberty in adolescent females. The Ost score, developed in this study, modeled the effect of cumulative and annual physical activity better than time spent in exercise or METs derived from the KA or other tools, such as the Bouchard grid. The KA historical tool in combination with the Ost calculation is proposed as a methodology to account for physical activity in bone mineral studies.

The other goals of this study included prediction of bone mineral accretion as it related to many physical activity issues. The best predictors of bone mineral accumulation towards peak bone mass were modeled across time since menarche rather than age alone as the time-dependent factor. Menstrual
status was an important consideration in the attainment of bone mass though the influence of estrogen-deficiency may be different athletic and non-athletic individuals. Amongst the athletes, the dysmenorrheic individuals appeared to consistently demonstrate higher bone parameters than the eumenorrheic athletes. Athletic menstrual status was not predicted well using serum leptin concentration as compared with percent body fat.

5.1 Questionnaire Reliability and Validity

The combination of the Pima Indian grid and Modifiable Adolescent questionnaire (KA tool) provided reliable and valid retrospective sport and physical activity data for the 268 subjects in this study. The activity was reported as a rate based strategy where the subjects provided the number of weeks per year and the hours of participation per week. This has been previously proposed as the best approach (266).

The repeat study demonstrated good one month reliability for the continuous and categorical variables for time, METs and Ost. It did not include extremely active subjects, but it is common for tools to demonstrate good reliability when the activity level is very strenuous (248, 249, 256, 267). The repeat study of this tool did demonstrate acceptable repeatability in less active subjects. It would have strengthened the repeat study for subjects from the entire range of the data to be included, but due to the nature of repeat subject selection, this portion of the study excluded the most active subjects.
The comparison of the Bouchard values and percent body fat with the KA questionnaire-reported values indicated reasonable validity for the tool. The range of negative correlations between percent body fat and annual time (r=-0.1462 to -0.2154) or METs (r=-0.1507 to -0.2430) spent in physical activity were similar to the values reported for other tools reported in the literature (248, 268-270). The ability to correlate the retrospective METs (KA) to the METs recorded biannually (Bouchard) strengthened the likelihood that the retrospective data were valid. The correlation coefficients ranged from r=0.3392 to r=0.5188. The ability to confirm most of the sports reported with school records also indicated accurate recall of sporting activities. The questionnaire appeared to collect valid information.

5.2 Questionnaire Respondents vs. Non-respondents

The apparent differences in subjects choosing not to return the questionnaire compared with those who did return the questionnaire was a concern. The lower bone parameters of the subjects who did not return the questionnaire could have been indicative of a more sedentary lifestyle and lack of motivation. If the subjects felt they had nothing to report, they may have chosen not to waste time completing the questionnaire. There were only 33 subjects who classified as non-athletes for all four years and only three of those subjects reported no activity at all. It is possible that the truly sedentary subjects within this study failed to respond to the questionnaire. The completion of
histories by the non-respondents before completion of the parent study may provide retrospective insight into this issue.

5.3 Statistical Analyses and Collinearity of Biological Data

Many researchers have addressed the need to adjust for the collinear nature of biological data such as these (104, 112, 115-121). To further explain collinearity, as an individual grows in height and weight, so does the lean body mass, fat mass, bone mineral mass and time (time since menarche or age). Body weight is commonly cited as a highly collinear variable to bone density measures. The decision to exclude weight (as total body weight) and evaluate the lean mass and fat mass components of total body weight was also incorporated into these models to help control the collinearity. Inclusion of weight, fat mass and lean mass would have led to more collinearity in the models as the primary components of body mass are lean and fat masses. In standard two component body composition models, total body BMC is part of the lean body mass as one component of total body mass. Separation of total body mass into fat mass and bone mineral-free lean mass allowed for body mass components to be used to predict total body BMC without the bone mineral component incorporated into the independent variables. One would expect inclusion of body mass index (BMI) in these models to increase collinearity as components of weight and height are also in the pool of variables. BMI is
frequently used as an obesity indicator and was left in the pool of variables for this purpose.

The initial model selection processes used in these studies relied on the $C_p$ criterion to identify unbiased models such that $C_p < p$ and $C_p$ was near $p$ (271). The calculation of the $C_p$ criterion was previously defined in the statistics section 3.10.5. Ilich et al used similar procedures to control for collinearity (128). Additionally, all final models were reviewed so that the variance inflation factors for included variables was less than 10, as this is considered most desirable (265). Longitudinal analyses utilizing a general linear model (Proc GLM in SAS) always looked at cumulative change from baseline to visit nine. The annual evaluation modeled across each of the four year time points was statistically analyzed using a mixed regression model (Proc Mixed in SAS) to allow for evaluation of random intercepts or slopes within the data. Proc Mixed also allowed for evaluation and manipulation of the covariance structure. These statistical procedures were undertaken to decrease multi-collinearity issues.

5.4 Calcium Intake

Calcium intake was not closely examined in this project due to the double-blind nature of the ongoing study these data were not available. However, we indirectly assessed the effect of calcium intake by looking at the relationship between mean physical activity indicators and mean calcium intake. The very small and insignificant correlations between mean calcium intake and each
exercise variable demonstrated a lack of relationship between physical activity and calcium intake. When compared between the high and low tertiles of each exercise variable, the total calcium ingested was not significantly different. Johnston (166), Katzman (23), Cadogan (165), and Lee (164) have suggested that the bone effects due to calcium nutriture are independent of the effects due to exercise. Specker et al have taken the opposite stance and previously identified an interaction between calcium intake and physical activity (151). The potential interaction between total calcium intake and physical activity were not thoroughly evaluated within this project, but the relationship will be evaluated in the final analysis after seven years of follow-up.

5.5 Athlete Bias

There is evidence that the subjects drawn to athletic activity may have greater bone mineral content and size (when adjusted for height and weight) before the initiation of sport leading to a bias not identifiable in the cross-sectional literature. Researchers, like Aloia et al (3), Claessens et al (272) and Fehling et al (101), acknowledge the potential that subjects with more dense bone structures may be drawn to sport or may experience less injury and more success in sport leading to greater participation. When the data in this study were controlled for height and weight, athletes had an increased bone mineral compared with the non-athletes for the initial, third and ninth visit. It was common for similar studies in the cross-sectional literature to adjust for body size utilizing weight, height, or body mass index to identified bone mass advantages.
for athletes (8, 13, 101, 144, 147, 172). The results of this study do not clearly resolve the athlete bias issue because the identified differences in total body BMC, bone area and radial BMC depend on consideration of height and weight. The differences between athletes and non-athletes in TBBMC and BA appeared to increase with time (i.e., greater difference after four years than at initial time point. It is not possible to differentiate the eventual differences in TBBMD as differences in the true density of bone versus the changes recognized in bone area with two dimensional densitometric analyses (like DXA). Future work with pQCT technology may help better answer the bias question. The lack of consistent differences in bone measures between the subjects who were early and late starters in sports activity warrants further investigation. It may be that those children and adolescents choosing to play sports may have had higher size-adjusted bone values than those who selected never to participate in sports.

Alternatively, this analysis did not identify the subjects' activities previous to 1992, and children who chose sport may have been more active in childhood leading into these pre-existing differences. Cassell et al have identified differences between gymnasts and swimmers at 7-9 years old when weight was also considered(14). The findings of Haapasalo et al did not support bone mineral differences due to physical activity before the third pubertal stage in the forearm and more likely in the fourth and fifth stages of puberty for the lumbar spine (130). The athlete bias limitation mentioned in so many papers may be real and explain a lot of the differences between athletes and non-athletes (3, 101, 272, 273). There needs to be more longitudinal research and at a younger age to help define if children with greater lean mass and denser bones choose to play sport
more often or experience more success in sport leading to higher participation levels.

The lenient definition of athlete in this study likely buffered these results. An athlete was defined by at least 8 weeks participation in sport per year and at least three hours of participation each of those eight weeks. Grimston et al have used similar weekly criteria (3 hours per week) in year round athletes to define "competitive" in 10-16 year olds (129). If more serious athletes (those competing year round or for at least 10 hours per week) or multiple sport athletes were compared with controls or if the criteria used to categorize athletes would have changed with age, the differences may have been even greater.

5.6 Ost and Bone Mineral Accretion in Active and Inactive Subjects

The impact and weight-bearing characteristics of physical activity and specific sports have been proposed as the osteogenic stimulus for the positive effect of exercise on bone (12-15, 33, 101, 110, 129, 142, 143, 145, 172, 178, 185, 188, 252, 274, 275). Previous to this study, the impact nature of sports had not been considered across the spectrum of usual physical activities in a longitudinal design. Many previous studies of adolescents have categorized various types of athletes with different impact sports or measured the hours or METs spent in weight-bearing physical activity. The comparison of Ost with time, METs, Bouchard and Bratteby METs and the athlete/non-athlete variable indicated that the Ost score was the best variable to use to control for physical activity in this study. The use of the Ost score as a continuous variable advantageously allowed
for the evaluation of a wide spectrum of activities and was applicable to individuals at many levels of participation.

The Ost variables were the most frequently selected exercise variables in the (Cp) prediction models. Mean annual Ost usually explained 1-3% of the model variance in the four-year change and ninth visit cross-sectional models. This varied slightly for more weight-bearing skeletal sites where annual Ost explained 5.5% in the femoral neck and trochanter BMD variables. These findings were in agreement with the 4% femoral neck and 5% trochanteric contributions of physical activity identified in the models presented by Turner et al (125). Rubin et al reported a 1% contribution of physical activity to the estimation of variance in the lumbar spine when physical activity was measured as energy expenditure. In comparing activity groups, Slemenda et al found that physically active children had 5-10% more mean bone mineral than less active children (9). In the same study, Slemenda et al noticed that biking and swimming had lower correlation coefficients than the more weight-bearing activities with the bone density measures. This could have easily been a reflection of the non-weight-bearing, non-impact nature of swimming and biking, but Slemenda et al surmised it was do to more frequent reporting of biking and swimming in younger individuals.
5.7 Ost and Bone Mineral Accretion Across Time Since Menarche

The prediction equation from the mixed model analysis of annual change in TBBMC across the four years in this study was:

\[ \Delta \text{TBBMC} = 32.34 + \text{not started} \times (6.35) + \text{just started} \times (103.62) + \text{eumenorrheic} \times (-30.09) + \text{dysmenorrheic} \times (-37.19) + \text{height} \times (-1.27) + \text{body fat} \times (-.0006) + \text{age} \times (-3.25) + \Delta \text{bone area} \times (.14) + \Delta \text{lean body mass} \times (.0054)

+ \Delta \text{body fat} \times (.0026) + \Delta \text{bone area} \times (1.09) + \text{annual Ost} \times (.0033) + \text{annual Ost}^2 \times (-.0000002) \]

This indicates that, while holding all other variables in the model at a fixed value, a subject performing 300 annual Ost increased the annual change in TBBMC by one gram of bone mineral. In contrast, a subject participating in 8000 annual Ost would have increased TBBMC by 26.3 grams, and the 13000 annual Ost participant would have reaped 42.8 grams of additional total body bone mineral in comparison with a subject with no annual Ost recorded. Translating this bone mineral into calcium accretion, this indicates that the subject with 300 Ost had an additional positive calcium balance of ~1 mg/day assuming bone mineral to be 38% calcium (1 g \times .38 = .38 grams calcium per year, .38 g/365 day = 1.04 mg/day). Similar calculations estimate the positive calcium balance to be 27 and 45 additional milligrams of calcium daily for the 8000 and 13000 Ost individuals,
respectively. In comparing these gains in TBBMC it should be remembered that the normal range of gain was about 50-400 grams of bone mineral per year or 52-416 mg calcium per day. In the case of the 13000 Ost individual, this is a 10-12% advantage with respect to the highest annual accretions (42.8/400). It will be interesting to analyze the absorption fraction and urinary excretion of calcium along with the dietary intake in the final analysis after seven years of study to speculate if there are differences between active and inactive individuals in the body's handling of calcium.

The Ost score was initially conceptualized as a method of translating the impact characteristics of many different sports or activities into one common denominator. The Ost score was supposed to be a reflection of weight bearing and pounding, which means it should be excellent for use in lower extremity models. As is demonstrated by the work of Bennell et al, sports such as track and field were most difficult to categorize because there is a great difference in the site specific demands of shot putting, long jumping, sprint or hurdle training and running the mile (171). From the works of Heinonen et al (144, 147) and other researchers (8, 13, 172), we recognize it may also be important to consider the skeletal site-specific impacts of various sports. As examples of skeletal sites with low mechanical usage or impact during specific activities, Heinonen has found decreased spine and lower extremity bone densities in cyclists (144) and decreased radial densities in women participating in aerobics (147). An example of high mechanical usage in this current study was one individual who competed as a Junior Olympian shot putter. As might be expected, this
individual had superior radial bone values (high outlier on figure 4.18) as well as a large body mass, but the overall Ost for her average annual activity was categorized as moderate due to the nature of her training. Future analyses of specific skeletal sites, such as the radius, may better incorporate an Ost score specific to the training at that site and extremity. Karlsson et al (146) and Heinonen et al (147) have suggested these site-specific differences to happen as the body redistributes the bone mineral from areas of low activity and impact to areas of high activity and impact. This has been coined the "steal phenomena" and warrants more attention in total body studies.

Davee (141) and Risser (8) have identified low bone mineral densities in swimmers. The research groups of Taaffe et al (13) and Fehling et al (101) have recorded no differences between swimmers and controls in comparative studies of various sportists. According to the Ost scale in the current study, swimming was categorized as less than 1 x body weight. It is common for sports such as swimming to incorporate weight training and running for strength and cardiovascular advantages. Similarly, some coaches may incorporate weekly swimming routines for a cross country or basketball team to rest the lower extremities. Accounting for cross training methods may also be important to this sort of research and comparison of various types of athletes. Cross training techniques have not been incorporated into this study or the previous literature. Attention to detail in reporting physical activity would allow for this type of training to be incorporated into the Ost scale.
When regression equations for total body BMC were formulated across time since menarche or age, the Ost variable was retained as one of many highly significant predictor variables and indicated a quadratic relationship to total body BMC. Negative consequences of over-exercise on the skeleton have been identified in studies of animals and humans (10, 91, 169, 174, 175). Theintz et al found decreased bone development in gymnasts practicing more than 15-18 hours per week (169). Slemenda et al found a trend for decreased bone mineral density in elite figure skaters who spent more time in training when compared with elite skaters who spent less time in training (10). Michael et al demonstrated a quadratic effect of exercise similar to the results in this study (174, 175). This study identified the peak of the positive effects of exercise to occur at about 18.8 hours of exercise per week. The peak of Ost as a positive influence on bone occurred at about 156 Ost per week, which would be equivalent, within this method, to 13.5 hours of gymnastics, 14 hours of basketball or 25 hours of tennis. The accumulation of Ost through various activities for one person may be more important than any one sport. As a hypothetical example, a high school athlete who played soccer (14 hours per week) and ran cross-country (seven hours per week) would have accumulated about 140 Ost. Any additional sporting activity may have approached the peak for the most positive (linear) effect on bone. Furthermore, the threshold for the peak effect of physical activity may change with age as was indicated in the annual break down of the larger longitudinal model (annual ΔTBBMC across tsm)(see Table 4.25). The less positive change in the subjects with very high levels of exercise may be a result of athletic
amenorrhea. This should be accounted for in the model, but more important hormonal covariates may help identify this.

Evaluation of growing females across time since menarche was advantageous to modeling the same growth across chronological time or age. It was more common for the selected models to include time since menarche as the time variable instead of age in the evaluation of the initial to final (cumulative) change models. In most cases where age was a selected variable, time since menarche was also selected into the most predictive, unbiased models. The Mixed model to predict the annual change in TBBMC across time since menarche for the four years also included age. Age is commonly used in statistical analyses to account for biological events that are not included in the pool of candidate variables. Due to the significant p value and bias of the model without age, it was decided that age should be left in the time since menarche model as a way to control for these unknown time-based events. Many of the variables, including Ost, in the four-slope tsm models were more highly significant than they were in the age models. It appeared that modeling growth and development changes across age may blunt the sharper effects identified when similar models were evaluated across time since menarche. Future analyses might consider the work of Bonjour et al in combination with the four-slope model presented in this study (97). Bonjour et al partitioned the fifth pubertal stage into four categories to mark the time since menarche (post-menarche). In addition to the not started and just started menarche categories, it might have been useful to allow for different
slopes for up to and after two years menarche as there appeared to be a pattern (as discussed in athlete section).

Many researchers have suggested increased bone area and periosteal expansion as a major predictor of bone mineral change in response to exercise (30, 98, 150, 152, 168). Bone area often surfaced as the primary predictor of total body BMC, and the model to predict bone area yielded interesting results concerning the Ost score. In predicting the annual change of total body bone area, the slope of the linear Ost variable demonstrated a trend to be a negative predictor. Subjects with higher levels of physical activity appeared to have less total body bone area (less periosteal expansion) than less active subjects. The higher BMC seen in more active subjects in combination with the decreased bone area should increase the BMD for high activity subjects even more than was identified in the BMC models. According to Frost's theory, in order for bone with less area to tolerate the same or higher strains in physically active subjects, an increased true density and/or increased endocortical apposition would be required in the absence of periosteal expansion. Bradney et al recently demonstrated similar responses to exercise in adolescent males (30). It will be interesting to evaluate the cortical thickness and three-dimensional geometry of bones in these same subjects via radiogrammetry and pQCT to look for endosteal and true density. It is possible that two dimensional densitometry analyses such as DXA may underestimate the effects of exercise on bone geometry.

The evaluation of bone parameters across time since menarche within the four slope model allowed for analysis of the effect of physical activity before, just
after and longer after the onset of menses. It has been suggested in the literature that the effects of exercise may be heightened before menarche or epiphyseal closure (32). This analysis did not identify a significantly heightened response to exercise according to menstrual status. The selected models acknowledged that the steepest slopes for bone growth occurred before and just concurrent to menarche. The inclusion eumenorrheic and dysmenorrheic partitions in the model allowed for statistical confirmation of less positive changes in total body BMC for subjects with less frequent menstrual cycles when all levels of physical activity were considered.

Reid et al suggested that regular exercise dissociates the usual correlation between fat mass and size-corrected BMD (119). Though size-corrected total body BMD was not specifically evaluated in this study, neither body fat or change in body fat was selected into the Cp selected models for cumulative change in total body BMD or BMD at visit nine when all subjects (n=267) were considered. Instead, body size (height, bone area and/or BMI) and time since menarche or overall menstrual status were identified as the best predictors. On the other hand, both body fat and annual change in body fat entered into the Mixed models, which also included all subjects, to predict total body BMC. In this BMC model, the slope for total body fat was negative (-0.0006) while the slope for annual change in body fat was positive (0.0026). When only the athletes were considered for evaluation of time since menarche and menstrual status, the cumulative change in body fat over the four year period was included as a significant predictor (covariate with slope=-0.000003) in prediction of total body
BMD, but not total body BMC. These results are not in firm agreement with those of Reid et al (119) though they did indicate opposite associations for the entire group versus athletes only. In this study, these results may have been a reflection of using a different pool of candidate variables for regression selection or the result of modeling the data with different statistical procedures (Proc GLM vs. Proc Mixed). Additionally, Reid's study was performed on adult women and this association or dissociation may be different during the developmental years. It is common for epidemiology studies to conclude fat mass is highly associated bone density, but the consumer should consider the statistical methods, variables considered and population studied as these design issues influence the results.

5.8 Bone Mineral Accretion in Athletic Menstrual Dysfunction

When only the athletes were considered longitudinally for visits 5, 7, and 9 and after the onset of menses, there was an interaction between time since menarche and overall menstrual status in the total body BMD and radius BMC and BMD models. The dysmenorrheic athletes appeared to have higher bone indices then the eumenorrheic athletes early in time since menarche in these models. Slemenda has found similar results in young elite figure skaters with menstrual irregularities (10) while Robinson et al have also reported greater bone densities despite menstrual irregularities in for eumenorrheic versus oligomenorrheic gymnasts (173). As time grew further from menarche (>2.5 years), the negative consequences of menstrual disruption became more
apparent in these athletic subjects. In this study, a similar evaluation after seven years of longitudinal study may indicate even greater differences as a function of decreased estrogen over time between the eumenorrheic and dysmenorrheic athletes. Current literature in young adults, like the studies of Drinkwater et al (203, 212), Warren et al (120) and Winters et al (22), suggest that athletes with menstrual disruptions have lower bone mineral density than their eumenorrheic counterparts. Bennell et al associated a decreased bone mineral density with oligomenorrhea as well as cautious eating patterns (276). This study did not support dietary kilocalories or derived indices (energy drain or physical activity index) as significant predictors of menstrual disruptions. The future analysis of the differences between the eumenorrheic and dysmenorrheic athletes in this study may find the skeletal disadvantages of menstrual disruption to occur well after menarche and into young adulthood.

The disparate results for the dysmenorrheic individuals in the different analyses in this study may lend support to the mechanostat hypothesis of Frost as recently addressed by Schiessl, Frost and Jee. (243). The four-slope mixed model for annual change in total body BMC, which used all levels of physical activity, indicated that the dysmenorrheic subjects experienced less positive changes than the eumenorrheic subjects. The graphs of this model indicated this happened at the time points immediately after menarche. On the other hand, when only athletes were considered, the dysmenorrheic subjects had higher bone mass than the eumenorrheic counterparts immediately after menarche in all bone parameters evaluated for this analysis. Frost contends that decreases in estrogen
increase the remodeling or modeling thresholds. It was possible that the athletically active individuals exceeded the threshold for modeling regardless of the effects of lower estrogen while the non-athletic dysmenorrheics (less activity) failed to adequately stimulate the higher thresholds and modeling processes. It would be interesting to see the negative effects in the total body BMC four slope model for the dysmenorrheics if the athletes were removed from the analysis as the higher bone values of the dysmenorrheic athletes may have masked even more negative effects in inactive subjects. Alternatively, we know that the increased estrogen associated with menarche marks the rapid decrease of bone modeling and epiphyseal closure. Bone mineral accumulation after menarche is typically associated with increased consolidation. The dysmenorrheic athletes could have still been increasing periosteal expansion compared with the eumenorrheic athletes. This was likely not the case because the model of change in total body bone area did not identify significant differences between the eumenorrheic and dysmenorrheic subjects or any interactions between Ost and any of the menstrual slopes. In fact, if the differences were significant, the dysmenorrheic athletes have less bone area than the eumenorrheic counterparts.

The total body and radius BMD and the radius BMC data indicated there was an interaction between tsm and overall menstrual status in the athletes around 2-2.5 years after menarche. Dhuper et al (206) and Pearce et al (216) have attributed these later responses to hormonal deficiencies in dancers. The dysmenorrheic athletes in the current study appeared to have a greater bone density or content than the eumenorrheic athletes until about two years after
menarche. The diagrams for total body BMD, radius BMC and BMD within this evaluation showed a consistent disadvantage and falling slope for the dysmenorrheics 2.5 years after menarche. This could have been the result of decreased consolidation due to decreased levels of serum estrogen. Use of the overall menstrual status (always eumenorrheic or at least on occurrence of disruption in four years) variable instead of the specific annual indicators may have led to this late decrease if most of the subjects experienced amenorrhea or oligomenorrhea after 2 years of normal menstruation (higher estrogen). Since the occurrences of dysmenorrhea that determined the overall categories were distributed along the time since menarche, this was not likely the case.

Menstrual status in this project was measured by self-report of menstrual frequency or cycle length every six months during the follow up visits. Winters et al have proposed that menstrual frequency should not be viewed as an accurate index of ovarian hormone status (22). The athletic menstrual disruption data would have been strengthened by hormonal measurements coinciding with the follicular and luteal phases of the cycle.

5.9 Prediction of Athletic Menstrual Status (Leptin)

Serum leptin did not predict the occurrence of eumenorrhea in athletic subjects any better than relative (%) or total grams of body fat. Again, the lenient criteria used to define athletes and non-athletes may have contributed to this indifference. Additionally, many of the subjects identified as athletes had very
high relative body fatness and were dysmenorrheic. When we designed the analysis, it was expected that choosing only athletes would find that the dysmenorrheic subjects were consistently on the leaner end of the spectrum, but this was not the case. The energy drain indicator was not a significant predictor of athletic menstrual status though it was noted that most subjects reported 600-800 kcal energy deficit. Similarly, analysis of the reported physical activity index (kilocalorie intake adjusted for the Bouchard energy expenditure) did not support the energy drain concept in prediction of menstrual dysfunction. We were not able to accurately predict the oligo- and amenorrhea reported by the subjects based on the nutritional data collected in this study.

Dueck et al suggested that cortisol, released in response to physical or psychological stressors, feeds back through the hypothalamus to decrease serum estradiol (200). Decreased serum estradiol may lead to decreased bone density and ovarian amenorrhea. Additionally, Licinio et al have suggested that leptin, as a reflection of body fatness, may affect the release of cortisol from the adrenal glands via the pituitary-adrenal axis (238). Laughlin et al demonstrated lower serum leptin concentrations and a lack of the normal diurnal rhythm of leptin in amenorrheic athletes (227). Diurnal variation as a reflection of gain in body fatness has been reported for selected subjects from this study (261), but these data were not available for all subjects in this analysis. Ideally, diurnal corticotropin-releasing hormone, adrenocortico-tropic hormone, cortisol, gonadotropin-releasing hormone, luteinizing hormone, follicle stimulating hormone, estradiol, and leptin should be measured over two to three menstrual
cycles for each subject to truly investigate the nature of these relationships. Measurement of those hormones would be time consuming and expensive in free-living subjects. The timing of measurement in relationship to the menstrual cycle would also be difficult and critical. Alternatively, the hormone leptin may have many yet to be identified roles in various body tissues which may include functions above and beyond those already identified in the adrenals (238), ovaries (224), and bone (241).

Other hormonal influences on the menarcheal changes in bone mass were not evaluated in this analysis. Goulding et al have suggested that increased growth hormone and increased kilocalorie nutrition stimulate linear growth which in turn may influence bone mass (127). Human growth hormone is one of the hormones responsible for the anabolic effects of exercise. Davee et al have demonstrated an increased IGF-1 associated with an increased BMD in weight training subjects as compared with controls (141). Future correlation analysis in this study of growth factors with the annual Ost score may suggest a mechanistic framework for the small but significant contribution of exercise to bone mass.

The overall objective of longitudinal studies such as this is to identify the important influences for attaining peak bone mass. The timing of peak bone mass is site specific and occurs as early as age 14-15 in the hip (40, 97, 126). The subjects included in this study were selected for pubertal stage similarities. The different rates of maturation of individuals and of the various skeletal sites precluded evaluation of peak bone mass for the analyses described herein. These analyses evaluated many of the pubertal years, but as demonstrated by
continued positive changes in total body bone mineral content and area, the individuals in this study were still accumulating towards peak bone mass at visit nine. The final analysis where many of the subjects will be 4-5 years post-menarche may provide additional insight into the influence of exercise in attainment of peak bone mass.

5.10 Limitations and Directions for Future Research

The main limitations of this study relate to the questionnaire, subjects choosing to return the questionnaire, calcium analysis, two-dimensional nature of DXA studies and some of the definitions used in the analyses. The questionnaire was validated with the indirect criteria of percent body fat and comparison with the Bouchard grid. A direct measure of fitness completed at each follow-up such as muscle strength measures may have strengthened the validation. The exclusion of very active subjects in the repeat study for reliability may have biased the correlation coefficients and limits the application of this tool to the very active individuals until the tool has been better evaluated in very active subjects. The differences between the respondents and non-respondents may indicate an exclusion of sedentary subjects. The analyses comparing athletes and non-athletes as well as active and inactive subjects could have benefited from the addition of more sedentary subjects. Calcium intake was screened but not fully evaluated. This will be corrected in the final analysis of the parent study. The results of DXA are limited by the two dimensional nature
of the measurement. The volumetric density may be different in very active subjects in athletes. Additionally, DXA does not differentiate between cortical and trabecular contributions to bone density. It may be helpful in studies such as this project to be able to measure true bone density, size and composition. The lenient definition of athlete in this study may not match the more rigorous definitions other researchers have used for categorizing older subjects as athletes. The definitions applied in this study were a reflection of the age group at the beginning of the study and the want to analyze recreational and competitive activities. These limitations should be kept in mind as the results of this research are considered.

Future research recommendations include a variety of research designs to investigate questions promoted throughout this project. Longitudinal studies on children at a younger age to track the bone density and sport participation decisions may help resolve the athlete bias issue. Calcium balance studies comparing active and inactive individuals may identify differences in calcium handling leading to the additional calcium accretion in active subjects. Close analysis of calcium intake will also be important in this study, as it is possible that the calcium intake is the difference between active and inactive subjects. It may help resolve the steal phenomena if studies included the analysis of physical activity in a site-specific fashion. For example, if the site of interest was the radius, the physical activity (Ost) of the forearm would be included as a covariate. Total body analyses do not allow for such specificity for exercise or bone density. The quadratic fit of the Ost for the annual change in total body bone mineral across the four years or menarche raises the curiosity about the
long term effects of very intense exercise such as that performed by adolescents participating in multiple sports in one athletic season. It is important to identify if this is an overuse effect or if it is simply the principle of diminished returns. Creation of a time since menarche model which allows for five slope to be defined so that the time periods are <-.5, -.5 to 0, 0 to .5, .5 to 2.0 and >2 years may help better analyze for a heightened effect of exercise close to menarche. This five-slope model could also partition the eumenorrheic and dysmenorrheic subjects. It would be interesting to evaluate the differences in bone mineral accretion between the active and inactive dysmenorrheic subjects within the Mixed model analysis to see if the exercise of the active individuals is making up for the lack of estrogen in bone mineral accretion. Leptin was not important to the prediction of eumenorrheic menstrual status in four-year athletes, but it might be interesting to measure the diurnal variation of leptin along with other reproductive hormones to see if it is the pulsatility of this hormone appears to effect the hypothalamic-gonadal axis.
CHAPTER 6

CONCLUSIONS

The main goal of this project was to design a bone-specific retrospective (5 year) measure of physical activity in a sample of adolescent females involved in a longitudinal calcium intervention trial. Growing females who participated in high impact physical activities (as measured by this methodology) did have an increased bone mass over the four years analyzed, according to regression analysis, compared with similar but inactive counterparts. It is likely the impact characteristics of the activities which stimulated the increased bone mass accretion in more active subjects as demonstrated by the superior fit of the Ost score in the models presented. The Ost score was a better predictor of changes in bone mass than time or METs variables.

This study also indicated a potential underlying bias in many of the cross-sectional studies of athletes and non-athletes. Subjects beginning sport after the initiation of this study had greater baseline bone mass values (when controlled for height and weight) than their non-athletic counterparts. We evaluated bone
mineral accretion across time since menarche with a unique four-slope model with two change points (0 and .5 years) to identify the influence of exercise close to menarche. This model also allowed us to compare subjects with normal and abnormal menstrual function after the onset of menarche, and we found the dysmenorrheic subjects to have a lower bone mass accretion than eumenorrheic subjects when all levels of physical activity were included. Additionally, we evaluated the relationship of bone mass accretion and menstrual status/menarche in subjects identified as athletes. Early after menarche, the dysmenorrheic athletes appeared to have increased rates of bone mass accretion when compared with eumenorrheic athletes in spite of the assumed hormonal inadequacies. This relationship often reversed with time so that the eumenorrheic athletes were gaining more bone mass than the dysmenorrheic subjects after 2-2.5 years of menstruation. We evaluated the prediction of athletic menstrual status (eumenorrheic or dysmenorrheic) with serum leptin concentrations and other energy reserve reflection. Leptin did not predict the menstrual status near as well as percent body fat. Many of the objectives of this study require further evaluation.

The quantification of physical activity using a scoring system like the Ost score allowed for evaluation of physical activity over the spectrum of normal activities in this free-living sample of adolescent females. It is important to include evaluation of recent and cumulative physical activity in bone densitometry studies. The Ost score as derived from the Kriska-Aaron tool is
suggested as a good control for the impact of exercise in large-scale epidemiological studies of bone mineral accretion in the future.
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  and bone density in premenopausal women. J Clin Endocrinol Metab 

  WG. Lack of bone accretion and amenorrhea: Evidence for a relative 

121. Young D, Hopper JL, Nowson CA, et al. Determinants of bone mass in 10- 

122. Hui SL, Johnston CC, Mazess RB. Bone mass in normal children and young 

123. Lloyd T, Eggli DF. Measurement of bone mineral content and bone density 

124. Faulkner RA, Houston CS, Bailey DA, Drinkwater DT, McKay HA, 
  Wilkinson AA. Comparison of bone mineral content and bone mineral 
  density between dominant and nondominant limbs in children 8-16 years 


239. Kohrt WM, Landt M, Birge SJ. Serum leptin levels are reduced in response to exercise training, but not hormone replacement therapy, in older women. *J Clin Endocrinol Metab* 1996;81:3980-5.


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Appendix A

Literature Review Charts
### In vitro Studies

<table>
<thead>
<tr>
<th>Primary Author</th>
<th>Model</th>
<th>Stimulation</th>
<th>Objective</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>El Haj (277)</td>
<td>Canine bone biopsy cores</td>
<td>Loaded w/ hydraulic air cylinder</td>
<td>Look for biochemical changes and site of protein synthesis</td>
<td>Increased G6PDH, osteocytes and endosteal lining cells appeared to be 1st in protein synthesis, blocked by indomethacin</td>
</tr>
<tr>
<td>Rawlinson (278)</td>
<td>Canine bone biopsy cores</td>
<td>Hydraulic strains similar to in vivo</td>
<td>Localization of PGE production and test PGE induction of changes</td>
<td>Endosteal lining cells produce PGE, PGI2 increases RNA product</td>
</tr>
<tr>
<td>Rawlinson (279)</td>
<td>Fresh rat ulnae</td>
<td>Use of ion channel blockers</td>
<td>Demonstrate ion changes preceding G6PD and PGE production</td>
<td>Ingress of calcium via ion channel precedes G6PD and PGE production</td>
</tr>
<tr>
<td>Ono (280)</td>
<td>Ceramic HAP pellets</td>
<td>rhBMP and PGEI</td>
<td>Surgical improvement of ceramic HAP implants</td>
<td>Increased osteogenesis with PGEI and rhBMP incubation of implant</td>
</tr>
</tbody>
</table>

### Animal Studies

<table>
<thead>
<tr>
<th>Primary Author</th>
<th>Species</th>
<th>Bone</th>
<th>Age</th>
<th>Exercise method</th>
<th>Exercise variable</th>
<th>Main Findings</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saville (29)</td>
<td>Rat</td>
<td>(bipedal)</td>
<td>2000 m. Run</td>
<td># of 2000 m. Run sessions</td>
<td>Increased density and increased breaking stress</td>
<td>No excessive group</td>
<td></td>
</tr>
<tr>
<td>Chamay (83)</td>
<td>Dogs</td>
<td>Ulnae</td>
<td>Adult</td>
<td>Usual weight-bearing after radial resection</td>
<td>Dynamic vs. Static overload</td>
<td>Constant dynamic strain group had most hypertrophy of ulna (piezoelectric theory)</td>
<td></td>
</tr>
<tr>
<td>Churches (84)</td>
<td>Sheep</td>
<td>Metacarpi</td>
<td>Adult</td>
<td>Artificial loading with drilling/pinning mechanism</td>
<td>Controlled loading</td>
<td>Bone not affected by procedure per se'</td>
<td>Crude procedure</td>
</tr>
<tr>
<td>O'Connor (85)</td>
<td>Sheep</td>
<td>Ulna/Radius</td>
<td>Adult</td>
<td>Artificial loading with drilling/pinning mechanism</td>
<td>Strain rate and magnitude</td>
<td>Bone more responsive to strain rate</td>
<td>No densitometry, used fluorescent labels and geometry</td>
</tr>
<tr>
<td>Woo (86)</td>
<td>Swine</td>
<td>(mini)</td>
<td>Adult 1 yr.</td>
<td>Treadmill</td>
<td>40 km/week @ 65-85%VO2 max</td>
<td>Bone response varies by site Exercise increased cortical thickness and cross-sectional area</td>
<td></td>
</tr>
<tr>
<td>Primary Author</td>
<td>Species Bone</td>
<td>Age</td>
<td>Exercise method</td>
<td>Exercise variable</td>
<td>Main Findings</td>
<td>Comments</td>
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<tr>
<td>Rubin (87)</td>
<td>Rooster Ulna</td>
<td>Adult 6 weeks</td>
<td>Artificial loading</td>
<td>Number of cycles</td>
<td>None of load groups displayed disuse remodeling, 36 cycles sufficient for osteogenesis</td>
<td>Strain was novel to this bone</td>
<td></td>
</tr>
<tr>
<td>Rubin (88)</td>
<td>Turkey Ulna</td>
<td>Adult (male) 8 weeks</td>
<td>Artificial loading</td>
<td>Strain magnitude</td>
<td>Periosteal hypertrophy linear to strain magnitude</td>
<td>Unique strain distribution</td>
<td></td>
</tr>
<tr>
<td>Lanyon (90)</td>
<td>Turkey Ulna</td>
<td>Adult (female)</td>
<td>Artificial loading</td>
<td>Dietary calcium content</td>
<td>Disuse group had highest loss, artificial strains barely inhibit loss in calcium-deficient group</td>
<td>Looked at the egg shell calcium content to monitor degree of calcium deficiency</td>
<td></td>
</tr>
<tr>
<td>Matsuda (91)</td>
<td>Leghorn rooster tarsometatarsus</td>
<td>3 wk. old 9 wk.</td>
<td>Treadmill</td>
<td>Growing model</td>
<td>Runners had less medullary area, increased cortical Runner had more abrupt failures (stiffness) in breaking</td>
<td></td>
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<tr>
<td>Tommerup (92)</td>
<td>Swine Rib and Femora</td>
<td>Adult (female)</td>
<td>Treadmill</td>
<td>Included basal, control and exercise</td>
<td>Increased modeling of femora but not ribs</td>
<td>Exercise effects are local</td>
<td></td>
</tr>
<tr>
<td>Biewener (93)</td>
<td>White leghorns (chicken)</td>
<td>2 wk old for 10 wks</td>
<td>Treadmill running</td>
<td>Changed load 2500 cycles 15'/d, 5d/wk</td>
<td>Cortical increased 26% and 2nd moment of inertia increased by 40% by 8 wks, diminished by 12 wks.</td>
<td>Adaptive nature during growth</td>
<td></td>
</tr>
<tr>
<td>Turner (94)</td>
<td>Rats</td>
<td>Adult</td>
<td>4 pt. bending loads of various forces</td>
<td>Age of animal and force of bending</td>
<td>Compared with identical previous study of younger animals</td>
<td>All animals responded with periosteal woven bone in dose fashion, older animals less responsive, MES higher for adult. Even sham (compression) responded.</td>
<td></td>
</tr>
<tr>
<td>Wheeler (95)</td>
<td>Rats</td>
<td>Young</td>
<td>Treadmill</td>
<td>Exercise intensity and duration</td>
<td>Exercise increased mineralization, suppressed endocortical resorption, high intensities may suppress periosteal and epiphyseal growth</td>
<td>Too much may be harmful</td>
<td></td>
</tr>
<tr>
<td>Primary Author</td>
<td>Species</td>
<td>Age</td>
<td>Exercise method</td>
<td>Exercise variable</td>
<td>Main Findings</td>
<td>Comments</td>
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<tr>
<td>Burr (281)</td>
<td>Human</td>
<td>Adult male</td>
<td>Infantry activities</td>
<td>Defining methodology for humans</td>
<td>Identification of principle strains in humans during physical activities similar to military recruits</td>
<td>Only one subject of two yielded &quot;useful&quot; data, demonstrates safety of strain gauge in man</td>
<td></td>
</tr>
<tr>
<td>Umemura (282)</td>
<td>Rats</td>
<td>8 weeks</td>
<td>Jumping up</td>
<td>Number of jumps per day</td>
<td>Jumping groups had increased cortical area, osteogenic response in this study magnified compared with treadmill studies</td>
<td>Rats were not allowed to land</td>
<td></td>
</tr>
<tr>
<td>Mosley (283)</td>
<td>Rats</td>
<td>43 days</td>
<td>Artificial loads</td>
<td>Strain rates</td>
<td>High strain rates are more osteogenic and anti-resorptive</td>
<td>Compressive loads in growing animals</td>
<td></td>
</tr>
</tbody>
</table>

### Human Studies

**Cross-sectional, Growing Individuals**

<table>
<thead>
<tr>
<th>Author</th>
<th>Age</th>
<th>Measure method</th>
<th>Exercise/P.A.</th>
<th>Main findings</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krabbe (9)</td>
<td>7-20 yo mixed n=301</td>
<td>SPA 125I radius</td>
<td>Not evaluated</td>
<td>Boys and girls similar before puberty</td>
<td>Growth spurt dissociates BMD and height</td>
</tr>
<tr>
<td>Landin (96)</td>
<td>4-16 yo mixed n=131</td>
<td>SPA-Americanium 241</td>
<td>Hand force evaluated</td>
<td>Reference patterns correcting for age, weight, height</td>
<td>Age, weight, height most significant predictors</td>
</tr>
<tr>
<td>Hui (97)</td>
<td>6-39 yo mixed n=1774</td>
<td>SPA-Norland</td>
<td>Not evaluated</td>
<td>Bone mass is a function of bone width at any age, Bone width was not different in females so WI females &gt; bone density than IN females</td>
<td>Two center study: Wisconsin and Indiana. WI females taller and heavier. Two machines, operators. Mean discrepancy &gt; 5 SD</td>
</tr>
<tr>
<td>Glastre (98)</td>
<td>1-15 yo mixed n=135</td>
<td>Hologic spine</td>
<td>Not evaluated</td>
<td>Largest increase ages 10-15 (PS 4), earlier in girls than boys</td>
<td>Height, weight, body surface area and bone age all highly correlated with BMD</td>
</tr>
<tr>
<td>Bonjour (99)</td>
<td>9-18 yo mixed n=98 f, 108m</td>
<td>Hologic DXA spine, femur</td>
<td>Exclusion if &gt; 10 hrs./wk.</td>
<td>12-15 yo is critical for females, females PBM at femoral neck and spine @14-15 yo</td>
<td>Growth spurt dissociation supported. Used unique subcategories for PS 5</td>
</tr>
<tr>
<td>Author</td>
<td>Age</td>
<td>Measure method</td>
<td>Exercise/P.A.</td>
<td>Main findings</td>
<td>Comments</td>
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<tr>
<td>DePriester</td>
<td>4-10 yo</td>
<td>SPA</td>
<td>Not evaluated</td>
<td>Cortical width and area greater in boys, increased volume</td>
<td>BW may be proxy for increased muscle mass and exercise</td>
</tr>
<tr>
<td></td>
<td>mixed n=420</td>
<td>33% ulna</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Slemenda</td>
<td>5-14 yo</td>
<td>SPA radius</td>
<td>Adapted from Nat.</td>
<td>Total hours of WB activity strongly correlated with bone density measures</td>
<td>Active children have 5-10% increased bone mass</td>
</tr>
<tr>
<td></td>
<td>mixed n=59</td>
<td>DPA hip, spine</td>
<td>Children and Youth</td>
<td></td>
<td>Lower correlations for swim and bike (younger bias)</td>
</tr>
<tr>
<td></td>
<td>twin pairs</td>
<td></td>
<td>Fitness Study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fehily</td>
<td>20-23 yo</td>
<td>SPA non-dominant</td>
<td>Kriska tool[Kriska,</td>
<td>Early menarche and sports at 12 yo were positively correlated to adult radial BMD in females Differences between previous intervention and control groups were very small</td>
<td>No other PA associated with BMD according to Kriska tool</td>
</tr>
<tr>
<td></td>
<td>mixed n=371</td>
<td>forearm</td>
<td>1988 #281] categorized by h/wk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lloyd</td>
<td>10-14 yo</td>
<td>Hologic DXA whole</td>
<td>Not evaluated</td>
<td>Body wt. Highest correlate with total body BMC BMD, Ht. And PS best predictors of TBBMD and TBBMC</td>
<td>Used integrated estrogen exposure score: PS, menarche, menstrual frequency and urinary estrogen</td>
</tr>
<tr>
<td></td>
<td>n=112</td>
<td>body</td>
<td></td>
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</tr>
<tr>
<td>Turner</td>
<td>HS- mean age</td>
<td>DXA</td>
<td>New Zealand</td>
<td>PA and calcium good predictors of femoral BMD</td>
<td>22% of subjects had previous arm, leg fractures 32% family hx suggestive of osteoporosis</td>
</tr>
<tr>
<td></td>
<td>16.4 yo</td>
<td>spine, femur</td>
<td>standard ranking tool</td>
<td></td>
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<tr>
<td></td>
<td>n=138</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Faulkner</td>
<td>8-16 yo</td>
<td>Hologic DXA total</td>
<td>Not evaluated</td>
<td>Boys and girls similar except: boys &gt; BMD head and upper extremity, girls &gt; BMD pelvis Legs not different, dominant arm &gt;BMD than non-dominant</td>
<td>Bone free lean tissue was good predictor of bone mass in both sexes Data support bone mineral</td>
</tr>
<tr>
<td></td>
<td>mixed n=234</td>
<td>body scan divided</td>
<td></td>
<td></td>
<td>related to muscular activity Age, ht., and wt. all related and collinearity issues</td>
</tr>
<tr>
<td></td>
<td></td>
<td>into regions</td>
<td></td>
<td></td>
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<tr>
<td>Faulkner</td>
<td>6-18 yo</td>
<td>Hologic DXA total</td>
<td>Compared dominant</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>mixed n=299</td>
<td>body scan divided</td>
<td>and non-dominant</td>
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<td></td>
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<td>into regions</td>
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<tr>
<td>Rubin</td>
<td>6-18 yo</td>
<td>SPA- distal radius</td>
<td>Recreational and</td>
<td>Age, wt., ht., PS all highly correlated with bone Exercise in L-spine model with wt. and PS</td>
<td>PA likely acts in site dependent mode</td>
</tr>
<tr>
<td></td>
<td>mixed n=299</td>
<td>DPA-spine</td>
<td>sports activity</td>
<td></td>
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<td></td>
<td></td>
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<td>converted to kcal/day</td>
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<tr>
<td>Author</td>
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<tr>
<td>Matkovic (123)</td>
<td>8-50 yo</td>
<td>SPA radius DXA whole body, spine, femur</td>
<td>Not measured</td>
<td>Identify peak bone mass by site (hip and spine early) Consolidation 1-7 yrs. after cessation of linear growth</td>
<td>Most growth changes are due to increased volume, not BMD</td>
</tr>
<tr>
<td>Ruiz (124)</td>
<td>7-15 yo</td>
<td>Hologic DXA spine, femur</td>
<td>Usual, 1-3 h/wk or 3-12 h/wk</td>
<td>Body wt. Main predictor at both sites, vertebral also included PS, Sport activity had greater influence than calcium (girls before pub.)</td>
<td>Addresses the size dependence of BMD and BMC, uses Z-scores as dependent</td>
</tr>
<tr>
<td>Goulding (125)</td>
<td>3-16 yo</td>
<td>Lunar DXA hip, whole body</td>
<td>Hours vigorous PA per week</td>
<td>Femoral width (at neck) may be more influenced by WB than by femoral axis length</td>
<td>No correlation between FAL or FW and hrs./wk. Active sporting</td>
</tr>
<tr>
<td>Haapasalo (126)</td>
<td>7-47 yo</td>
<td>Norland DXA spine, femur, radius</td>
<td>Sedentary, moderately active (2-3x/wk) or very active (strenuous sports &gt;4x/wk)</td>
<td>Peak bone mass 18-21 yo Spine continued to accrue until 21-23 yo</td>
<td>Used BMAD bone mineral apparent density (BMC+ volume) to help account for age, ht. Wt. Of subjects</td>
</tr>
<tr>
<td>Moro (127)</td>
<td>9-26 yr mixed, ethnic 97 black 97 Asian 101 white 80 hispanic</td>
<td>Hologic DXA whole body used midfemoral diaphysis software</td>
<td>Estimated average hrs. WB activity (=1xBW)</td>
<td>Long bone growth is strongly driven by mechanical stimuli associated with body mass during growth. No differences in females at puberty but black adult females had greater bone strength (also heavier)</td>
<td>Calcium/caloric intake Pubertal stage score Collinearity issues Define impact of wt. Bearing Looked at cortical cross section of femur and BSI</td>
</tr>
<tr>
<td>Illich (128)</td>
<td>10-12 yo</td>
<td>Lunar DXA whole body radius</td>
<td>Stratified by Bouchard as TEE corrected for BMI</td>
<td>Total body and radial BMD and LBM greater in the highest PA quartile</td>
<td>Greater ht. in the highest calcium intake quartile, used Cp regression to over-come collinearity</td>
</tr>
<tr>
<td>Author</td>
<td>Population</td>
<td>Method</td>
<td>Main findings</td>
<td>Comments</td>
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<tr>
<td>Aloia (39)</td>
<td>Mean age 39</td>
<td>Total body neutron activation DPA for spine SPA for radius LSI for physical activity</td>
<td>Physical activity correlates with spine density ((r=.33,p&lt;.05)) and total body calcium ((r=.50,p&lt;.01))</td>
<td>Very popular study</td>
<td></td>
</tr>
<tr>
<td>Kanders (100)</td>
<td>25-35 yo</td>
<td>DPA (spine) MLTPAQ and pedometer</td>
<td>L-spine BMD positively correlated to daily energy expenditure, calcium intake only moderately correlated</td>
<td>No OCP use, 24 h recall and 6 day record of intake. Increase vertebral BMD 1.2% for every 100 kcal spent by prediction</td>
<td></td>
</tr>
<tr>
<td>Halouia (119)</td>
<td>20-50 yo n=181</td>
<td>SPA radius (distal and mid) Dietary records and recall categorize PA by time spent</td>
<td>Low calcium, sedentary group had lowest bone, suggested may be no additional benefit beyond intermediate levels of Ca and PA</td>
<td>Tried to create lifetime PA and Ca scores</td>
<td></td>
</tr>
<tr>
<td>Fehily (131)</td>
<td>20-23 yo mixed n= 371 of 581 original supplementation subjects</td>
<td>SPA Non-dominant forearm Questionnaire for lifestyle factors</td>
<td>Male manual laborers had in cr. Bone, and early menarche and sports age 12 positive correlation in females</td>
<td>No difference in supplemented vs. Not supplemented groups.</td>
<td></td>
</tr>
<tr>
<td>Rico (132)</td>
<td>26±6 y mixed</td>
<td>CT scanning both radii</td>
<td>Total and cortical bone mass greater in dominant arm</td>
<td>Excluded physically active individuals. Built-in controls for nutrition and genetics.</td>
<td></td>
</tr>
<tr>
<td>Reid (133)</td>
<td>Premenopausal n=99</td>
<td>Lunar DXA Total body, hip and spine Exerciser or not by a energy expenditure ((140kJ/kg/d))</td>
<td>BMD associated with Wt. (fat mass in non-exercisers)</td>
<td>Corrected areal BMD by height, address collinearity issues</td>
<td></td>
</tr>
<tr>
<td>Torgerson (134)</td>
<td>45-49 yo n=2240 (1227 pre-meno)</td>
<td>Norland DXA- spine, femur PA questionnaire ((&gt;2, 1-2, &lt;1 \text{ hrs./wk.}))</td>
<td>All femoral equations included BW, PA and hx wrist fx. Exercise not included in spine.</td>
<td>32.8% subjects exercised &gt; 2 hrs. Wk.</td>
<td></td>
</tr>
<tr>
<td>Ho (138)</td>
<td>21-40 yo Chinese n=297</td>
<td>Norland DXA spine and hip MLTPAQ to categorize METs WB activity by tertiles</td>
<td>Younger subjects with higher WB score had increased BMD at spine and hip (not same in older group) High calcium and high PA seemed additive More influence at spine than hip.</td>
<td>High impact WB more osteogenic and PA greater factor during consolidation</td>
<td></td>
</tr>
<tr>
<td>Author</td>
<td>Population</td>
<td>Method</td>
<td>Main findings</td>
<td>Comments</td>
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<tr>
<td>Oyster (104)</td>
<td>60-69 yo</td>
<td>Radiographs (cortical area) PA questionnaire O2 consumption by heart rate</td>
<td>Increased energy expenditure had increased cortical bone</td>
<td></td>
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<tr>
<td></td>
<td>n=40</td>
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<td></td>
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</tr>
<tr>
<td>Chow (152)</td>
<td>50-59 yo</td>
<td>Neutron activation analysis 5 yr. History of PA Categorize current PA by strength and VO2 max</td>
<td>Total body calcium of upper thighs and trunk positively correlated to fitness (VO2 max)</td>
<td>Good retardation of loss when compared with normal.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pocock (153)</td>
<td>20-75 yo</td>
<td>SPA- forearm DPA- spine, femur Fitness by VO2 max</td>
<td>Fitness stronger and more significant predictor of femoral neck than wt. or ht.</td>
<td>Physical fitness accounts for 23% of the variation in femoral neck BMD in this wide age range.</td>
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<td></td>
<td>n=84</td>
<td></td>
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<tr>
<td>Astrom (154)</td>
<td>58 Hip fx and age-matched controls (60-70 yo)</td>
<td>Telephone interview</td>
<td>Hip fxs had been less active than controls during 15-45 yo</td>
<td>Needed to control for running water, sewage and firewood</td>
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<tr>
<td>Cheng (155)</td>
<td>50-60 yo</td>
<td>Chosen by PA level 0-3 h/wk, 4-8 h/wk, &gt; 9 h/wk SPA calcaneus (AP &amp; lateral)</td>
<td>Women participating in vigorous PA &gt; 2x/wk had incr. BMD</td>
<td>No relationship VO2 and BMD</td>
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<td></td>
<td>n=106</td>
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<tr>
<td>Kyllonen (156)</td>
<td>49-55 yo</td>
<td>DPA- spine, femoral neck back extensor and flexor maximal isometric and endurance measures bicycle ergometer VO2 max</td>
<td>Regressions: spine=weight &amp; age femoral neck= weight &amp; isometric extensor strength</td>
<td>No direct measure of PA, lot of collinearity in weight, strength and age</td>
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<tr>
<td></td>
<td>n=78</td>
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<tr>
<td>Greendale  (157)</td>
<td>Mean age 73 (&gt;50yo) mixed n=1014 f, 689 m</td>
<td>SPA- radius Hologic DXA hip, spine</td>
<td>Current PA and bone density associated with hip dose-related, lifetime PA also assoc. with hip BMD</td>
<td>Increase BMD of strenuous exercisers should decrease fx risk by 50%, intermediate benefits to moderates</td>
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<tr>
<td>Silman (158)</td>
<td>50-75 yo</td>
<td>Identified fxs with x-rays PA by questionnaire</td>
<td>Activity before age fifty important to prevent fractures especially the 25-49 yo time span</td>
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<tr>
<td></td>
<td>mixed n=36 centers x 600 subjects (EVOS)</td>
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<tr>
<td>Author</td>
<td>Sport(s)</td>
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<td>Findings</td>
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<tr>
<td>Fisher (6)</td>
<td>Runners (&gt;20 m/wk)</td>
<td>Adult (~20-30)</td>
<td>DPA spine (L1-4), SPA radius</td>
<td>Eumenorrheic runners 98% controls, amenorrheics 85% controls in lumbar spine.</td>
<td></td>
</tr>
<tr>
<td>Lloyd (8)</td>
<td>Field hockey, basketball, track and controls</td>
<td>College controls ht, wt and age matched</td>
<td>QCT spine (L1-3)</td>
<td>Eumenorrheics trend for higher BMD than controls while oligomenorrheic trend lower than controls. Estrogen and androgens make independent and additive contributions to lumbar spine.</td>
<td></td>
</tr>
<tr>
<td>Buchanan (10)</td>
<td>Field hockey, basketball, track and controls</td>
<td>18-22 yo n=30</td>
<td>QCT spine (L1-3)</td>
<td>Higher active vitamin D3 in younger runners and tendency of higher BMDs. Explained by more exposure to sunlight.</td>
<td></td>
</tr>
<tr>
<td>Kirk (12)</td>
<td>Runners and matched controls</td>
<td>25-35 yo 55-65 yo</td>
<td>QCT spine, SPA radius</td>
<td>Male swimmers had greater BMD than controls but no association for swimming and BMD in females.</td>
<td></td>
</tr>
<tr>
<td>Orwoll (13)</td>
<td>Master's swimmers</td>
<td>&gt;40 yo</td>
<td>CT spine, SPA radius</td>
<td>Muscle building incr. BMD compared to aerobic or sedentary. Aerobic slightly lower than sedentary (NS) but swimmer in aerobic group was very low</td>
<td></td>
</tr>
<tr>
<td>Davee (14)</td>
<td>Muscle-building &amp; aerobics</td>
<td>20-30 yo n=27</td>
<td>DPA- spine</td>
<td>Negative effectors: low BW, low wt, low estrogen scores, high fiber intake, older menarche</td>
<td></td>
</tr>
<tr>
<td>Dhuper (15)</td>
<td>Dancers</td>
<td>13-20 yo n=43</td>
<td>DPA- radius, spine, foot Bouchard PA Integrated estrogen score</td>
<td>Impact (VB/Bkb) athletes had increased BMD in spine and heel compared with swimmers and controls. Account for body size (ht. &amp; wt.) in analysis. Swimmers &lt; all groups @spine.</td>
<td></td>
</tr>
<tr>
<td>Risser (16)</td>
<td>Volleyball, basketball, swimming and controls</td>
<td>College athletes n=29 athletes 13 controls</td>
<td>DPA spine, SPA calcaneus</td>
<td>Wt. Lifters had increased BMD at all sites compared with controls and normals for young adult men</td>
<td></td>
</tr>
<tr>
<td>Conroy (22)</td>
<td>Wt. Lifters (males)</td>
<td>Mean age 17</td>
<td>Lunar DXA- spine, hip</td>
<td>Wt. Lifters had increased BMD at all sites compared with controls and normals for young adult men</td>
<td></td>
</tr>
<tr>
<td>Grimston (101)</td>
<td>Runner (50 km/wk)</td>
<td>Mean ages 30.3-32.9</td>
<td>DPA -spine, femoral neck, tibia</td>
<td>Grouped runners by spine density and osteopenic group had abnormal response to calcium load (incr. PTH and decr. CT)</td>
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</tr>
<tr>
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<tr>
<td>Grimston (129)</td>
<td>Impact-load &amp; Active-load (mixed)</td>
<td>10-16 yo</td>
<td>DPA-spine, femoral neck</td>
<td>Femoral neck BMD &gt; in Impact group, no correlation time in weight-bearing activity to spine or femoral neck BMD, greater differences in boys</td>
<td></td>
</tr>
<tr>
<td>Heinonen (140)</td>
<td>Orienteers, cyclists, skiers and active referents</td>
<td>Young adult n=105</td>
<td>DXA spine, femur, radius, patella, tibia, calcaneus</td>
<td>Wt. Lifters had greater BMD than many other athletes, BW correlated to BMD at each site, cyclists had relatively low BMD every site except distal radius, BBT for menstrual status.</td>
<td></td>
</tr>
</tbody>
</table>
| Karlsson (141) | Ballet dancers and controls (age, sex matched) | 19-68 yo n=42 dance 42 controls (17 m, 25 f) | Lunar DPX spine, femur, whole body
Trng hr. per week, yrs. active dance, trng. hrs. during active career, retire time, menstrual hx | Males had increased BMD in arms, females had increased BMD in legs and hips. Males had decreased head BMD (steal phenomena). Increased fat and decreased LBM concomitant with decreased BMD. |
| Slemenda (142) | Figure skaters                    | 10-23 yo n=22 skate 22 controls | Lunar DXA total body
Elite skaters only | Skaters had greater bone measures than controls.
When only consider skaters- 40% skaters were menstrually irregular, trend for lower BMD's as training increased. Demonstrate skeletal consequences of irregular MP. |
| Haapasalo (143) | Squash and controls               | 18-32 yo n=19 squash 19 controls | Norland DXA six sites of upper extremities | Significant lean and bone hypertrophy in playing extremity of players vs. Controls. Side to side differences 11.5% in players vs. 2.7% in controls. Athletes beginning before menarche had 20-24% difference compared with those beginning after menarche (7-11% difference) |
| Young (144) | Ballet dancer, eumenorrheic and anorexic controls | 16-19 yo n=44 dance 23 control 18 anorexics | Lunar DXA whole body | TBBMD in dancers similar to controls unless use weight as covariate then higher in dancers. L-spine and NWB sites similar to anorexics and lower than controls. |
| Fehling (145) | Volleyball, gymnastics, swimming & controls | College | Hologic DXA whole body
(only used year round athletes) | VB and Gymn. Had greater BMD than swimmers or controls. Gymnastics had greater BMD in both arms. N.S. -2% decrease in swimmer's femoral neck BMD. Evaluates menarche and menstrual status. |
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Heinonen</td>
<td>Squash, aerobic dance, speed skaters, active and sedentary referents</td>
<td>College n= 18 squash 27 aerobics 14 skates 25 each refs.</td>
<td>DXA spine, femur, patella, tibia, calcaneus, radius</td>
<td>Squash players had highest BMD at all sites. Physically active referents no different than sedentary. Athletes 10% higher than non-athletes, presents “steal phenomena” at distal radius.</td>
</tr>
<tr>
<td>Taafe</td>
<td>Gymnasts, swimmers and controls</td>
<td>College n=26 swim. 13 gymnasts 19 controls</td>
<td>Hologic DXA whole body, femur</td>
<td>Gymnasts greater than controls and swimmers. Earlier age of training related to later menarche. High impact loading may override compromised hormonal status. Femoral neck greater in controls than in swimmers. Gymnastics 18x BW.</td>
</tr>
<tr>
<td>Cassell</td>
<td>Gymnasts &amp; swimmers</td>
<td>7-9 yo</td>
<td>Hologic DXA total body (only used year round athletes)</td>
<td>Synchronized and speed swimmers similar BMD, increased TBBMD in gymnasts compared with swimmers or controls</td>
</tr>
<tr>
<td>Duppe</td>
<td>Soccer (3 levels) retired soccer and active controls</td>
<td>15-30 yo (sport active)</td>
<td>DXA hip, spine, whole body</td>
<td>Players had signif. incr. BMD everywhere but spine, former players retained higher BMD levels except for spine. No difference in intensity of sport (adjusted for weight, age, BMI).</td>
</tr>
<tr>
<td>Etherington</td>
<td>Ex-elite runners, tennis</td>
<td>40-65 yo</td>
<td>DXA spine, femoral neck arms in tennis players</td>
<td>Athletes had greater BMD, active controls had &gt;BMD than sedentary, benefit of exercise persisted after cessation of training</td>
</tr>
<tr>
<td>Pearce</td>
<td>Ballet and controls</td>
<td>15.4-19.6 yo n=41 dancers 46 controls</td>
<td>DXA</td>
<td>BMD negatively correlated with duration oligomenorrhea. Normal or high bone density as result of exercise before puberty. Wt. Bearing cannot offset deleterious effects of exercise-induced menstrual irregularities.</td>
</tr>
<tr>
<td>Winters</td>
<td>Runners and active controls</td>
<td>College n=20</td>
<td>DXA total body and spine</td>
<td>Lower BMD as function of distance run per week. Cyclically “normal” females demonstrated to have menstrual aberrations. Fiber inversely associated with plasma estrogen levels.</td>
</tr>
<tr>
<td>Alfredson</td>
<td>Volleyball players and controls</td>
<td>College n=13 each group</td>
<td>DXA total body, hip, spine Biodex for muscle</td>
<td>VB increased total body BMD 6.1% compared with controls (p&lt;0.01) No difference in muscle strength No correlation BMD to strength in VB, but some in controls</td>
</tr>
<tr>
<td>Author</td>
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<td>Findings</td>
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<tr>
<td>Ashizawa (205)</td>
<td>Tennis</td>
<td>18-23 yo</td>
<td>pQCT/forearms bilateral forearm model</td>
<td>Cortical increases by periosteal formation in dominant arm, slight decrease in volume, greater bone area and greater PMI. Trabecular increase by thickness or number to increase volume.</td>
</tr>
<tr>
<td>Ryan (206)</td>
<td>Swimmers, runners, triathletes, controls</td>
<td>18-69 yo n=71</td>
<td>DXA-total body, spine, hip VO₂ max by treadmill Biosamples</td>
<td>Athletes had greater spine, hip than controls, runners had higher femoral neck and Ward's BMD than swimmers Training can only reduce age-related loss</td>
</tr>
<tr>
<td>Tsuzuku (216)</td>
<td>Power lifters</td>
<td>-18-21 yo n=21 males only</td>
<td>DXA-total body, spine, hip</td>
<td>Weight-lifters had higher lean mass and bone density, high intensity loads which generate compression may be most osteogenic</td>
</tr>
</tbody>
</table>

Human Studies
Longitudinal, Growing individuals

<table>
<thead>
<tr>
<th>Author</th>
<th>Age gender</th>
<th>Length of study</th>
<th>Measure method PA criteria</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rajalakshmi (23)</td>
<td>2-6 yo mixed</td>
<td>6 months n=43</td>
<td>Radiographs to measure change in skeletal age and cortical thickness No measure of PA</td>
<td>Group supplemented with dohikla and fenugreek leaves gained the most</td>
</tr>
<tr>
<td>Katzman (160)</td>
<td>9-21 yo female (subsample of larger cross sectional)</td>
<td>2 yrs. 12 all post-menarcheal</td>
<td>SPA radius, DPA spine, DXA spine, whole body, femur PA by &gt;3, 2-3, &lt;2 30' exercise sessions per week</td>
<td>Cannot interchange DXA and DPA values. Corrected BMC with volume to yield BMAD. Pubertal stage, height and weight were significant predictors, not PA or calcium intake.</td>
</tr>
<tr>
<td>Johnston (161)</td>
<td>6-14 yo mixed identical twins</td>
<td>3 yrs. 45 pairs</td>
<td>Lunar SP-2 radius Lunar DP-3 femur, spine PA [Slemenda, 1991 #124] hrs. of wt. bearing PA</td>
<td>Study designed to look at sup-plementation. No significant difference in the levels of physical activity to evaluate.</td>
</tr>
<tr>
<td>Theintz (162)</td>
<td>9-19 yo mixed</td>
<td>1 yr. n=198</td>
<td>Hologic DXA femur, spine Hours per week excluded &gt;10 h/wk</td>
<td>Female gains in first two years after menarche Pubertal Loop Model same population as Bonjour [Bonjour, 1991 #254]</td>
</tr>
<tr>
<td>Author</td>
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<tr>
<td>Lloyd (163)</td>
<td>11.9±.5 yo females</td>
<td>18 months n=94</td>
<td>Hologic DXA spine, whole body No measure of PA</td>
<td>Calcium Citrate Malate (500 mg/d) supplemented group had 2% and 1.3% whole body and 4.7% and 2.9% lumbar spine increases in BMC and BMD over controls</td>
</tr>
<tr>
<td>Kroger (164)</td>
<td>7-20 yo mixed</td>
<td>1 yr. 37 f, 28 m</td>
<td>Lunar DXA femur, spine 3 groups PA- no activity outside school, active 2-3 hrs./wk, sports &gt; 5 hrs./wk.</td>
<td>Evaluated bone volumetric density. In females, increase bone size before menarche then increased bone density. Trend for higher BMD and BMDvol in highest PA. Age, weight and height as most significant predictors.</td>
</tr>
<tr>
<td>Lee (165)</td>
<td>7 yo 87 males 75 females</td>
<td>18 months n=187</td>
<td>SPA radius PA by Bouchard 1-7 and added up daily 4-7 to use hrs/d</td>
<td>300 mg calcium carbonate improved density in supplemented group. No difference between groups for PA so no further evaluation (mean PA 4-7 was 2.75 hrs/d)</td>
</tr>
<tr>
<td>Chan (166)</td>
<td>9-13 yo females</td>
<td>12 months n=48</td>
<td>SPA radius DXA femur, spine, whole body Exclusion criteria for participation in school team sports</td>
<td>Dairy product group (1200 mg/d) had higher gains in lumbar and total body measures</td>
</tr>
<tr>
<td>Bonjour (167)</td>
<td>7.9±1 yo females</td>
<td>12 months n=149</td>
<td>DXA radius, spine, whole body No measure of physical activity</td>
<td>Enriched breakfast and snack foods (+850 mg/d) groups had increased bone mass which was still detectable after one year of discontinuation</td>
</tr>
<tr>
<td>Cadogan (168)</td>
<td>12.2±3 yo females</td>
<td>18 months n=82</td>
<td>DXA whole body Habitual physical activity measured every 6 months</td>
<td>Milk supplemented group (1 pint/d) had greater increase in bone mineral Exercise not different between groups 40.6 (18.2) kJ/kg/d vs. 44.4(26.9) milk controls</td>
</tr>
<tr>
<td>Martin (284)</td>
<td>82 males 84 females</td>
<td>Report during 5th yr. Of ongoing</td>
<td>DXA whole body, AP spine, femur Mention in introduction measurement of PA but does use in evaluation</td>
<td>Report outlines the change in bone quality around peak height velocity curves as potentially related to forearm fracture risk (Saskatchewan Pediatric Bone Mineral Study)</td>
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### Longitudinal, Adult

<table>
<thead>
<tr>
<th>Author</th>
<th>Age/Gender</th>
<th>Length</th>
<th>n</th>
<th>Method</th>
<th>Main findings</th>
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</thead>
<tbody>
<tr>
<td>Mazess (20)</td>
<td>20-39 yo females</td>
<td>2 yrs.</td>
<td>300</td>
<td>SPA radius DPA PA by Caltrac and conventional pedometer then categorized</td>
<td>No association for physical activity and BMD. Age, height and weight were most significant predictors. Younger subjects had higher calcium intake.</td>
</tr>
<tr>
<td>Recker (25)</td>
<td>18-26 yo @ baseline females</td>
<td>4 yrs.</td>
<td>156</td>
<td>Norland SPA radius Norland DPA spine (total body early and late) measured every 6 months PA by 4 day CalTrac counts(wrist)</td>
<td>PA demonstrated small but significant positive correlation with rate of change spinal bone mineral density. Accrue bone mineral up to 28-20 years old.</td>
</tr>
<tr>
<td>Welton (169)</td>
<td>13-28 yo mixed</td>
<td>15 yrs.</td>
<td>84 m, 98 f</td>
<td>Measured annually ages 13-17 then @ 21, 27 yo PA categorized by average weekly METS WB activity (4-7, 7-10, &gt;10)</td>
<td>No steady influence of physical activity on bone density in females though it was best predictor in males. BW best predictor in females.</td>
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### Longitudinal, Athletes

<table>
<thead>
<tr>
<th>Author</th>
<th>Sport(s)</th>
<th>Age/Length of study</th>
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<tbody>
<tr>
<td>Theintz (170)</td>
<td>Gymnasts &amp; swimmers</td>
<td>12.3 yo baseline 2-3.7 yrs.</td>
<td>No densitometry Skeletal age by Greulich Pyle from x-rays of wrist</td>
<td>Stunting in leg length growth of gymnasts. Decreased PHV when plotted for bone age (-2 SD for gymnasts for normal children). Intensive training of gymnasts detrimental to growth and suggest threshold for exercise @15-18 hrs/wk.</td>
</tr>
<tr>
<td>Bennell (171)</td>
<td>Track &amp; field (power vs. endurance)</td>
<td>17-26 yo 12 mo.</td>
<td>Hologic DXA Whole body with ROI</td>
<td>Female athletes later menarche, type of exercise influences the pattern of distribution</td>
</tr>
<tr>
<td>Taaffe (172)</td>
<td>Gymnasts, runners, swimmers, non-athletes</td>
<td>18-29 yo 8 or 12 mo.</td>
<td>Hologic DXA spine, femur, whole body</td>
<td>Two cohort study where gymnasts had greater density independent of menstrual status in both cohorts. Expressed measures in BMAD (g cm(^{-3})). Gymnasts had later menarche with earlier training initiation.</td>
</tr>
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</table>
### Human Studies: Intervention, Growing Individuals

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<tr>
<th>Author</th>
<th>Age gender</th>
<th>Length of study</th>
<th>Measure method</th>
<th>Exercise/P.A.</th>
<th>Findings</th>
</tr>
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<tbody>
<tr>
<td>Eliakim (109)</td>
<td>15-17 yo females</td>
<td>5 weeks n=44</td>
<td>MRI VO2 by bicycle ergometry Pre and post blood samples</td>
<td>Intervention 2.5 hrs./d five days/wk., controls on computer</td>
<td>No change in growth hormone, decreased IGF-1 and IGF-5 with increase in osteocalcin. Acute endurance training initially catabolic before anabolic.</td>
</tr>
<tr>
<td>Morris (176)</td>
<td>9-10 yo females</td>
<td>10 months n=71 (nonrandomized)</td>
<td>Hologic DXA spine, femur, whole body Strength test by Cybex II for knee and shoulder -pk. Torque Hand grip dynamometer Intervention 3x/wk. X 30' each variety of activities PE teacher Measured PA by Godin and categorized by 3, 5 &amp; 9 Mets</td>
<td></td>
<td>Exercise group increased lean mass and BMD, BMC. Height, weight, LBM, regional lean and shoulder strength all positively associated with TBBMD and TBBMC. Fat mass and strength not associated with increased bone accrual.</td>
</tr>
<tr>
<td>Witzke (178)</td>
<td>14 yo females</td>
<td>9 months n=25 exercise 28 controls</td>
<td>DXA femur Leg strength Intervention 30-45' plyometrics and resistance training 3x/wk. with weighted vests</td>
<td></td>
<td>Showed significantly increased change from zero in exercise compared with controls in greater trochanter BMD, leg strength and medial to lateral stability.</td>
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### Intervention, Young adult

<table>
<thead>
<tr>
<th>Author</th>
<th>Age gender</th>
<th>Length of study</th>
<th>Method Intervention</th>
<th>Main findings</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gleeson (19)</td>
<td>23-46 yo (age match) females</td>
<td>12 mo. n=72 (34, 38)</td>
<td>SPA left calcaneus DPA L-spine Nautilus program 30' 3x/week</td>
<td>Significant increase L-spine density in wt. trng. group, loss in controls</td>
<td>Huge gains in muscle strength Subjects chose groups and 49% drop out rate. 500 mg Ca supplement</td>
</tr>
<tr>
<td>Rockwell (24)</td>
<td>Upper 30's females</td>
<td>9 mo. n=10 exer 7 or 9 controls</td>
<td>Lunar DPX spine, hip Eagle Cybex trng. 2x/wk 45' each session @70% 1 RM</td>
<td>Demonstrated wt. trng. as detrimental, claimed increased PTH yet albumin changes without normalizing</td>
<td>Greatest changes 0-4.5 months, 500 mg Ca with 200 IU D3/d</td>
</tr>
<tr>
<td>Author</td>
<td>Subjects, Length</td>
<td>Method</td>
<td>Main findings</td>
<td>Comments</td>
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<tr>
<td>Snow-Harter</td>
<td>19.9 yo +0.7</td>
<td>Hologic DXA spine, femur PA by</td>
<td>Increased spine BMD in runners and</td>
<td>Excluded athletes, measured pre-study exercise and demonstrated good</td>
<td></td>
</tr>
<tr>
<td>(179)</td>
<td>females</td>
<td>hrs/wk and months per year to</td>
<td>wt. trn. subjects compared with</td>
<td>randomization. All subjects accepted were eumenorrheic.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8 mo. n=52</td>
<td>get to weekly average where 51,</td>
<td>controls. No subjects gained BMD in</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>randomly run,</td>
<td>2-3 or 3 h/wk. Interventions-</td>
<td>hip. Very large standard deviations.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>wt. trn. or</td>
<td>run 70-80% MHR and increase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>10%/wk (did not attain very</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(only 31</td>
<td>high mileage) wt. trn. -14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>finished study)</td>
<td>exercises 3x/wk, began 65-70% 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>RM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bassey(180)</td>
<td>Late 20s early</td>
<td>Lunar DXA hip and spine</td>
<td>Impact group incr. 3.4% trochanter,</td>
<td>Brought Ca up to 1 g/d limited number of subjects and attrition</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30s</td>
<td>High impact vs. Cbo exercises</td>
<td>Cbo crossover @ 6 mo. and also incr.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>females</td>
<td>1 hr. Class per week plus home</td>
<td>4.1% trochanter</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 mo. n=27 total</td>
<td>exercises</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Friedlander</td>
<td>20-35 yo</td>
<td>QCT spine, SPA calcaneus,</td>
<td>Took two years for gains in</td>
<td>Exercise included a variety of activities and subjects attended 3 x 1 hr.</td>
<td></td>
</tr>
<tr>
<td>(181)</td>
<td>females</td>
<td>MLTPA prior exercise</td>
<td>exercisers to be significant (trend</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 yrs. N=127</td>
<td>Trunk and knee strength by</td>
<td>in year one), no significant effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>randomly assigned</td>
<td>Cybex @ 3 speeds and averaged</td>
<td>of calcium observed. Most subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(only 63</td>
<td>Intervention combinations of</td>
<td>lost spinal trabecular, but</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>finished study)</td>
<td>calcium, Cbo, exercise and</td>
<td>exercise group lost less.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aloia(18)</td>
<td>53.5+5.6 yo, 11-13 mo. n=18</td>
<td>Total body neutron activation</td>
<td>Exercise group increased total</td>
<td>Landmark study in humans</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>analysis and SPA radius</td>
<td>body calcium while controls lost</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exercise 3x/wk. For an hour each session</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smith(182)</td>
<td>65-95 yo, 36 mo.</td>
<td>SPA radius every 4 mo. Exercise</td>
<td>Exercise and supplement groups gained</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>was 30'3x wk. Supplement,</td>
<td>bone, combo group did not change.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>exercise, combo and control</td>
<td>Combo group had older subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Author</td>
<td>Subjects, length</td>
<td>Method</td>
<td>Main findings</td>
<td>Comments</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>---------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
</tbody>
</table>
| Krohler (183) | 50-73 yo, 8 mo.  
n=31(16 exercise)  
(used subjects with hx of Colle's fx) | DPA spine, radius  
"Moderate" training was walk, run or floor exercises | Exercisers improved lumbar values and maintained forearm while controls lost bone at both sites.                                                                                                                  | Those subjects living closer chosen for exercise group for convenience.                         |
| Chow (184)   | 50-62 yo, 12 mo.  
n=48 | Aerobic exercise (30') and weight trng.  
(10-15') 3x/wk  
Neutron activation of upper thighs and trunk | Exercise groups had increased calcium index, control group lost calcium | Lowest bone index women gained more                                                                                                              |
| Dalsky (185) | 55-70 yo, 9 & 22 mo.  
n=35 | WB exercise progressive 50-60' 3x/wk  
DPA L2-4 | Exercise group increased in 9 mo, and maintained gain, controls lost.                                                                                           | Subjects which discontinued after 9 months showed detraining                                  |
| Sinaki (186) | 49-65 yo, 24 mo.  
n=65 | DPA spine  
Strain gauge dynamometer for strength of back extensors  
PA by questionnaire  
Exercise was back extension program (prone lying) | Exercise group had increased strength but lost 1.29% spine density/yr.                                                                                           | Exercise stimulus probably not enough. No weightbearing, refutes muscle pull stimulus.        |
| Grove (187) | 49-64 yo, 12 mo.  
n=65 | DPA spine  
High and low impact groups  
3x/wk., 1 hr. each  
Low impact less than 1.5 BW | Both exercise groups maintained bone while controls lost.                                                                                                      | Did not control for HRT, matched subjects on weight and BMD before random assign to groups.  |
| Fiatarone (188) | 72-98 yo, 10 wks.  
n=63 f, 37 m  
FICSIT study | No bone density measures  
Lower extremity training (3 d/wk),  
Exceed, both or neither groups  
LSI for PA | Activity counts were 25% of sedentary young adults. No improved lean mass or muscle function.                                                                | Ten weeks not long enough for strength study, demonstrates potential for changing sedentary lifestyles. |
| Kohrt (190) | 60-74 yo, 11 mo.  
n=39 | Hologic DXA wrist, spine, femur, total body  
(5 assessments over the study)  
Cybex II for muscle strength  
VO2 for aerobic power  
Intervention groups were ground reaction forces vs. joint reaction forces, 3-5 x/wk  
30-45' | Significant increase in femoral neck BMD in femoral neck in GRF, largest increase in Ward's was in those with the most to gain, other increases in both exercise groups but NS. | Demonstrate the principle of diminished returns, supplemented calcium to bring up to 1500 mg/d, good to look at differing effects of programs on body composition. |
| Recker (285) | 3-8 yrs. post-menopausal, 12 mo.  
n=62 | DXA spine, femur, radius  
Intervention "barely perceptible" whole body vibration plate 2 x 10^7/d and pcbo | Treatment group seems to be maintaining hip and spine BMD while pcbo is losing                                                                               | Preliminary data but exciting osteogenic potential                                               |
Appendix B

Consent Form
You are asked to participate in a clinical research program to evaluate the relationship between diet and skeletal formation. We think that the best protection against osteoporosis ("brittle bone disease") later in life is to have strong bones when the skeleton reaches maturity (adulthood). We believe that the critical time for building strong bones is during early adolescence (early teenage period) when needs for skeletal minerals are the highest.

I, _______________________, hereby authorize or direct V. Matkovich, M.D., Ph.D. or associates of his choosing, to perform the following treatment: (described in general terms)

Prior to inclusion into the study I will give detailed information about my dietary habits including which type, how often and how much food I eat. I will also complete the self reported form (Tanner's rating) about my physical development (secondary sexual characteristics - breasts and pubic hair). If information from both forms meet the inclusion criteria of the study, I will be selected to participate in the study which will last 4 years.

In that case, I will continue to give the detailed information of my dietary habits, physical development, as well as of my activity level, every 6 months. Blood pressure, height and weight will be measured every six months during the four-year period, together with bone mass density at forearm and skeleton (whole body). Body fat will be measured twice a year, by the bioelectrical impedance method and dual energy X-ray absorptiometry.

I will also have X-ray of my hand taken at the beginning and at the end of the study (2 times in total), for the purpose of determining the skeletal age. A 24-hr urine collection and approximately 2 tea-spoons of blood drawn from the arm vein will be taken at the beginning of the study, and every 12 months.

I will be taking four supplemental tablets daily throughout the 4-year study period. I will not know which tablet I am taking: the one with or without calcium.

Biological parents of participating girls will be asked to participate in the bone mass measurement, only once during the whole study.
Protocol No. 90H0002

All tests and measurements will be done at The Ohio State University. To complete a dietary intake and activity level forms takes 30 minutes. The measurements which will be done on the site are as follows: blood pressure, height and weight measurements; 15 minutes, hand X-ray 10 minutes; bioelectrical impedance 5 minutes; forearm densitometry 15 minutes, and the whole body bone, fat and soft tissue measurements about 20 minutes. It takes about 1 hour of time for each visit, or 9 hours during the 4 year study period. There will be a total number of 300 participants. 24 participants will be asked (not required) to take part in a 2 week in-patient metabolic study at the University Hospital.

In addition to the above I realize that I could participate in a study entitled "Nutrition & Human Growth - Enhancing Middle School Science", in my school. I will be analyzing data collected on myself and/or on my colleagues under the supervision of my science teacher. The data collected on others will be coded so that I will not know the names of the participants. The purpose of this is to stimulate science education in the schools and therefore will help me directly.

Upon ___ MYSELF _____.

The experimental (research) portion of the treatment or procedure is:
I, along with other participants who will be selected for the study, will take by mouth four tablets per day (two in the morning and two during the evening) throughout the 4-year period. One half of the participants will be given placebo (tablet without calcium) and the other half will receive calcium supplement, and I as a participant will not be aware which tablet I will be taking. This, as I understand, is the rule of a double blind clinical trial in which I am participating. I will have my dietary interview, physical examination with anthropometry (height & weight measurements), and bone mass measurements at forearm and the whole body by non-invasive techniques done at baseline (beginning of the study) every 6 months over 4 years. Hand X-rays will be taken at the beginning and at the end of the study. I will have my blood drawn at the beginning of the study and every 12 months. I will collect five 24-hour urine samples throughout the 4-year study; every 12 months.

This is done as part of an investigation entitled:
Influence of Calcium on Bone Mass Formation During Puberty

1. Purpose of the procedure or treatment:
To find adequate prevention for osteoporosis (brittle bone disease) with dietary manipulation during puberty period.

2. Possible appropriate alternative methods of treatment.

Not to participate.
Protocol No. 90H0002

3. Discomforts and risks reasonably to be expected:

The risks of taking part in this study are minimal. The placebo tablets or the supplemental calcium tablets, has no harmful effect on human health. A high mineral diet certainly is contraindicated in patients with kidney stone disease, because it can further increase the risk of forming stones. Mineral tablets can sometimes cause mild constipation. Subjects with bone or kidney stone disease will not be included in the study. All participants will continue with their regular diets throughout the study period.

I understand that the placement of a needle in the arm vein to draw blood may cause slight discomfort and a bruise may form at the site of needle puncture as well as fainting. The total amount of blood drawn during the study will be about 50 cc (1.5 oz.). The only possible invasion of my privacy will be done during the dietary interviews and determination of sexual development (Tanner staging).

I will be exposed to the external irradiation of about 0.3x2 mRad annually, from whole body bone mass measurements and 0.1x2 mRad from forearm measurements. This is total of 0.8 mRad annually, or 0.27% of annual natural exposure. In addition, at the beginning and end of the study I will have my hand X-ray taken, and I will be exposed to 10 mRad of irradiation each time to the hand only, which is amounts to 3.3 of the total natural dose for one year. I realize that this is a minimal irradiation and far below the natural annual irradiation exposure from environment which is 300 mRad. It is equal to the irradiation received on the airplane during a single 20 min. flight, or skiing in the high mountains over the period of 1-3 days.

4. Possible benefits for subjects/society:

There will be several benefits to me, the participant: 1) physical examination, 2) dietary evaluation, 3) urine and blood tests, and 4) bone mass measurements. Society may benefit by the finding of a better prevention for osteoporosis by simple dietary manipulations. I will receive compensation after each evaluation (30$). There will be total of 9 evaluations for which I will receive $270. If I remain in the study till the end of the four year period, I will receive a special bonus. I understand that I might be asked to participate in an in-patient metabolic study for which I will receive extra compensation.

5. Anticipated duration of subject's participation:

I will participate in the study which will last 48 months with 9 visits to O.S.U.
Protocol No. 90H0002

I hereby acknowledge that Dr. V. Matkovich or his collaborator has provided information about the procedure described above, about my rights as a subject and that he/she answered all questions to my satisfaction. I understand that I may contact him/her at (614) 293-3838 should I have additional questions.

I understand that where appropriate, the U.S. Food and Drug Administration may inspect my records pertaining to this study. I understand further that the records obtained during my participation may be made available to the sponsor for this study and that the records will not contain my name or other personal identifiers. Beyond this, I understand that my participation will remain confidential.

I understand that I am free to withdraw my consent and participation in this project at any time after notifying the project director without prejudicing future care. No guarantee has been given me concerning this treatment or procedure.

In the unlikely event of physical injury resulting from participation in this study, I understand that immediate medical treatment is available at University Hospital of The Ohio State University. I understand the consent form. I sign it freely and voluntarily. A copy has been given to me.

Date: ______________________  Time: _______ A.M.

P.M.

Subject: ______________________

Signed: ______________________

(Parent or guardian)

Witness: ______________________

__________________________________________

Person Authorized to consent for subject- if required

I certify that I have personally completed all blanks in this form and explained them to the subject or his/her representative before requesting the subject or his/her representative to sign it.

Signed: ______________________

(Signature of the Project Director or Authorized Representative)

HS-028A(Rev. 4/89)
Appendix C

Menstrual history/Pubertal stage questionnaire
STAGES IN BREAST DEVELOPMENT (GIRLS)

Subject Number: [ ] [ ] [ ] [ ] [ ] [ ] [ ] [ ]
Subject Initials: [ ] [ ] [ ] [ ] [ ] [ ] [ ]
Date of Birth: [ ] [ ] [ ] [ ] [ ] [ ] [ ]
Date of Exam: [ ] [ ] [ ] [ ] [ ] [ ] [ ]

Check the stage that best represents your development.

Have you started your period? Yes No
Date when started: [ ] [ ] [ ] [ ] [ ] [ ] [ ]
First day of last period: [ ] [ ] [ ] [ ] [ ] [ ] [ ]
Duration of period (days): [ ] [ ] [ ] [ ] [ ] [ ] [ ]
Average cycle: [ ] [ ] [ ] [ ] [ ] [ ] [ ] (Days from one period to the next)
Number of periods per 6 months: [ ] [ ] [ ] [ ] [ ] [ ] [ ]

Are you on any contraceptive pills? Yes No
or other contraceptive agent or device?
Yes No

Could you be pregnant? YES__ NO__
Check the stage that best represents your development.

1

2

3

4

5

6
Appendix D

Menstrual Categories Literature
<table>
<thead>
<tr>
<th>Primary author</th>
<th>eumenorrheic</th>
<th>oligomenorrheic</th>
<th>amenorrheic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinkwater (203)</td>
<td></td>
<td></td>
<td>No more than one in twelve months</td>
</tr>
<tr>
<td>Loucks (198)</td>
<td>25-38 d</td>
<td>39-90 d</td>
<td>&gt;90 d</td>
</tr>
<tr>
<td>Fisher (16)</td>
<td>No recent changes in cycle length (&lt;+5 d)</td>
<td></td>
<td>None in 12 months</td>
</tr>
<tr>
<td>Lloyd (204)</td>
<td>11-13/year</td>
<td>3-7/year</td>
<td></td>
</tr>
<tr>
<td>Buchanan (205)</td>
<td>11-13/year</td>
<td>Missed 3-10 per year</td>
<td></td>
</tr>
<tr>
<td>Bunt (286)</td>
<td></td>
<td></td>
<td>None in previous year</td>
</tr>
<tr>
<td>De Souza (287)</td>
<td>23-33 d intervals</td>
<td></td>
<td>Absence 3 or more consecutive months</td>
</tr>
<tr>
<td>Drinkwater (214)</td>
<td>10-13/year</td>
<td>3-6/year at intervals &gt;36 days</td>
<td>No more than 2 per year, none in past 6 months</td>
</tr>
<tr>
<td>Myburgh (218, 219)</td>
<td>10-13/year</td>
<td>4-9/year</td>
<td>0-3/year</td>
</tr>
<tr>
<td>White (215)</td>
<td>9-14/year</td>
<td></td>
<td>Absent at least 3 months</td>
</tr>
<tr>
<td>Bennell (276)</td>
<td>≥9/year</td>
<td>4-8/year</td>
<td>3 or less per year</td>
</tr>
<tr>
<td>Fehling (101)</td>
<td>10-13/year</td>
<td>4-8/year</td>
<td>0-3/year</td>
</tr>
<tr>
<td>Kannus (32)</td>
<td>23-35 d cycles</td>
<td>Any irregularities</td>
<td>None in 6 months</td>
</tr>
<tr>
<td>Robinson (173)</td>
<td>&gt;10/year</td>
<td>3-6/year, &gt;36 d apart</td>
<td>1-2/year, none last 6 months</td>
</tr>
<tr>
<td>Pearce (216)</td>
<td>Regular monthly</td>
<td>6 weeks-6 months at least 2/year, no more than 8-9</td>
<td>Absent for 6 months or more</td>
</tr>
<tr>
<td>Keen (288)</td>
<td>10-13/year</td>
<td>3-6/year</td>
<td>&lt;2/year or none in 6 months</td>
</tr>
<tr>
<td>Taaffe (172)</td>
<td>10-13/year</td>
<td>4-9/year</td>
<td>1-2/year, none last 6 months</td>
</tr>
</tbody>
</table>
Appendix E

Food Records
INSTRUCTIONS FOR COMPLETING THE FOOD RECORD:

1. Record all the food you eat or drink for each day you select. Most people find it helpful to do this as soon after the meal or snack as they can.

2. Write only 1 food item on a line.

3. Describe the types of food eaten as clearly as you can. Use the samples provided on page 3 as a guide.
   - List ingredients to help describe any unusual casserole or salad.
   - Indicate whether the food is canned, fresh, frozen, or diet.
   - List the brand names of foods if you know them.

4. Describe the amounts of food you eat and drink as clearly as you can. Use the following examples as a guide.
   - Liquids - list as cups, parts of cups, or fluid ounces.
   - Meat, Fish, Cheese, Eggs - list in ounces, by number, or size. Specify if the amount given is in cooked or raw weight.
   - Fruits - list as cups, parts of cups, or by number. Include the size (diameter and/or length) of fresh fruits.
   - Vegetables - list as cups, parts of cups, or by number.

Ex: Chicken drumstick Baked, no skin 1 medium
Lean ground beef patty Broiled 1/4 lb. raw
Cheddar cheese Kraft 1 slice (4"x1"x1/8")
Banana 1 small (6 in. long)
Green Beans DelMonte 1/2 cup

*cup is equal to 8 ounces
Completing the Food Record

Bread, Rolls, Crackers - list by number or size.

Ex: Whole wheat bread Ovenjoy 1 slice
    Triscuits Nabisco 4

Cereal, Rice, Noodles, Potato - list by cups, parts of cups, size or number.

Ex: Spaghetti Cooked 1 cup

Pancakes, Waffles - list by number and size.

Ex: Betty Crocker Buttermilk Mix 2 (5" diameter)

Fats:

Margarine, butter - list by teaspoons, tablespoons, or pats.

Salad dressing, cream, cooking oil, gravy - list by teaspoons, tablespoons, or parts of cups.

Bacon, sausage - list by number of slices or links.

Sweets:

Jam, jelly, honey, sugar, syrup - list by teaspoons or tablespoons.

Candy - list number and size of bar or pieces.

Desserts:

Jello, puddings, ice cream - list as cups or parts of cups.

Cookies - list by number and size.

Pie, cake - list by number and size (length and width at longest end).

Ex: Cookie, choc. chip Mrs. Fields 1 (2 1/2" diam.)
    Strawberry ice cream Hagen Daz 1 scoop, sugar cone
    Chocolate cake with Homemade 1/10 of 9" layer cake chocolate icing

5. Describe how the food was prepared. For example: baked, broiled, fried, raw, scrambled or other. Include butter, margarine, oil, sauces, dressings, gravies, dessert toppings added in cooking or at the table.
Completing the Food Record

6. Eating out:

   Give the name of the restaurant so that we may call for more information if necessary. Describe the food items eaten as carefully as you can.

   ie: Pizza Hut Pizza, Sausage & Cheese, 1 slice of medium 4"x6"

   McDonald's Quarterpounder with cheese

7. Remember to list everything you eat or drink including gum, cough drops, pickles, catsup, tartar sauce.

8. If you take a vitamin-mineral supplement please write down how much you take and the brand name (or bring the package label with you to your appointment).
<table>
<thead>
<tr>
<th>DATE</th>
<th>KIND OF FOOD</th>
<th>HOW PREPARED OR BRAND NAME</th>
<th>AMOUNT OR SIZE OF SERVING</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/2/85</td>
<td>Cheerios</td>
<td>General Mills</td>
<td>1 cup</td>
</tr>
<tr>
<td></td>
<td>2% Milk</td>
<td>Lucerne</td>
<td>12 ounces</td>
</tr>
<tr>
<td></td>
<td>Banana</td>
<td></td>
<td>1/2 small</td>
</tr>
<tr>
<td>12:00</td>
<td>Turkey Sandwich</td>
<td>Orwheat, Wheatberry</td>
<td>2 slices</td>
</tr>
<tr>
<td></td>
<td>Bread</td>
<td>Hormel</td>
<td>3 slices</td>
</tr>
<tr>
<td></td>
<td>Turkey Breast</td>
<td>Kraft</td>
<td>1 tbsp.</td>
</tr>
<tr>
<td></td>
<td>Diet Mayonnaise</td>
<td></td>
<td>1 leaf</td>
</tr>
<tr>
<td></td>
<td>Lettuce</td>
<td></td>
<td>2 slices</td>
</tr>
<tr>
<td></td>
<td>Tomato</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fruit roll</td>
<td>Grape flavor</td>
<td>1 ounce (wrapper provided)</td>
</tr>
<tr>
<td></td>
<td>Apple juice</td>
<td></td>
<td>1 hnt (oz.)</td>
</tr>
<tr>
<td>3:45 p.m.</td>
<td>String cheese</td>
<td>Lucerne</td>
<td>1 1/2 ounce</td>
</tr>
<tr>
<td></td>
<td>Tab</td>
<td>Fritoilay</td>
<td>12 ounces</td>
</tr>
<tr>
<td></td>
<td>Corn chips</td>
<td></td>
<td>1 pkg. (1 1/2 oz.)</td>
</tr>
<tr>
<td>6:30 p.m.</td>
<td>Spaghetti</td>
<td>Mom's</td>
<td>1 cup (cooked)</td>
</tr>
<tr>
<td></td>
<td>Tomato meat sauce</td>
<td>Standy</td>
<td>3/4 cup</td>
</tr>
<tr>
<td></td>
<td>String beans</td>
<td>Iceberg</td>
<td>1/3 cup</td>
</tr>
<tr>
<td></td>
<td>Lettuce</td>
<td></td>
<td>1 cup</td>
</tr>
<tr>
<td></td>
<td>Tomato</td>
<td></td>
<td>1/2 small</td>
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<tr>
<td></td>
<td>French dressing</td>
<td>Kraft diet</td>
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</tr>
<tr>
<td></td>
<td>2% Milk</td>
<td>Lucerne</td>
<td>1 cup</td>
</tr>
<tr>
<td>8:30 p.m.</td>
<td>Ice Cream</td>
<td>Lucerne, Strawberry</td>
<td>1/2 cup</td>
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Subject ID: ___________________________
Visit No. 1: ___________________________

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<th>Kind of Food</th>
<th>How Prepared or Brand Name</th>
<th>Amount or Size of Serving</th>
<th>For Research Use</th>
<th>Do Not Fill</th>
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<th>Units</th>
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Appendix F

Bouchard physical activity questionnaire
### Activity Record

**WEEKDAY**

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<thead>
<tr>
<th>Category</th>
<th>Types of activities</th>
<th>Hour</th>
<th>0-15</th>
<th>16-30</th>
<th>31-45</th>
<th>46-60</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sleeping, resting in bed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Sitting, reading, writing, watching television</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Light activity standing, washing, cooking, brushing, unloading</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Slow walk (&lt;2.5 mi/hr), driving, so dress, to shower, etc.</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Light manual work: floor sweeping, window washing, painting, walking on tables, nurse side chores, several house chores, walking at 2.5 - 3.7 mi/hr</td>
<td></td>
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</tr>
<tr>
<td>6</td>
<td>Leisure activities and sports in a recreational environment: baseball, golf, volleyball, canoeing, rowing, archery, bowling, cycling (&gt;10 mi/hr), tennis, recreational team games at school</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>7</td>
<td>Manual work at moderate pace: loading and unloading, snow shoveling, etc.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Leisure and sport activities of higher intensity (not competitive): canoeing (&lt;1.5 mi/hr), bicycling (&gt;9.3 mi/hr), dancing, skiing, tennis, badminton, swimming, horseback riding, walking (&gt;3.7 mi/hr), fitness exercises, calisthenics, water skiing, gymnastics</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>9</td>
<td>Intense manual work, high intensity sport activities or competitions: carrying heavy loads, jogging or running (&gt;3.5 mi/hr), rowing, badminton, swimming, tennis, cross-country skiing (&gt;5.0 mi/hr), hiking and mountain climbing, orienteering, soccer, European handball, water polo</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Notes on current activity:** If you mark a number above a "*", please indicate to which activity that refers. For example, 8 = gymnastics, etc.
Appendix G

Kriska-Aaron (KA)
retrospective physical activity questionnaire
1) From the following list, circle each activity you have participated in more than ten times each year.

<table>
<thead>
<tr>
<th>Activities</th>
<th>Garden/Yard work</th>
<th>Swimming (laps)</th>
<th>Marching band</th>
<th>Gymnastics</th>
<th>Drill team/Color guard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseball</td>
<td>Hiking</td>
<td>Tennis</td>
<td>Basketball</td>
<td>Ice Skating</td>
<td>Track &amp; Field</td>
</tr>
<tr>
<td>Bicycling</td>
<td>Lacrosse</td>
<td>Volleyball</td>
<td>Bowling</td>
<td>Roller Blading</td>
<td>Weight Training</td>
</tr>
<tr>
<td>Cheerleading</td>
<td>Jogging</td>
<td>Wrestling</td>
<td>Crew</td>
<td>Snow Sking</td>
<td>Dance Class</td>
</tr>
<tr>
<td>Soccer</td>
<td>Fencing</td>
<td>Softball</td>
<td>Football/Rugby</td>
<td>Street Hockey</td>
<td>Synchronized swimming</td>
</tr>
<tr>
<td>Diving</td>
<td>Start Master</td>
<td>Pyrometrics</td>
<td>Others:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2) List your circled activities in the column on the grid labeled "Activities". If you have more than 16 activities, only list the ones you've done the most.

3) Fill in the boxes across the top to indicate what grade you were in each year. It might be easiest to enter the current school year grade and work backwards to the 1992-93 year.

4) Enter the weeks per year and average hours per week. If it is appropriate, these can be partial numbers such as 10.25 hours.
   Figure out how many weeks each year by thinking about the months first then counting the weeks for each of those months and adding them up! Try not to just multiply the whole month by 4, but try account for partial months.

5) Don't forget to respond to the physical education at the bottom of the grid for each school year!

6) Fill in the schools you attended.

Please continue by following these directions on the next page!
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td></td>
<td># weeks</td>
<td>hrs/week</td>
<td># weeks</td>
<td>hrs/week</td>
<td># weeks</td>
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</tbody>
</table>

Physical education

What schools did you attend for:

1992-93

1993-94

1994-95

1995-96

1996-97

Current school

Thank You!!
Appendix H

Solicitation and reminders
Greetings!

We are thankful for your past participation in the OSU Bone Study! You have certainly contributed to a great cause in looking for better prevention of osteoporosis. Realizing you have fulfilled your commitment by completing the 9th visit, we would like to ask you another favor. We are trying to look at the effect of physical activity and sport participation on the skeleton and have realized we need more specific information about your sports participation!

We ask that you take a minute to read the instructions and then fill it out the enclosed questionnaire. Many of you are thinking this form does not apply to you, but it does! It is important that we receive this form back even if you don't participate in any sports!

We have enclosed a small token of our appreciation for your time and attention to this questionnaire. Please, just complete the form as accurately as you can and stick it in the enclosed postage paid envelope back to the lab! If you have any questions, please call so we can assist you (593-3838).

Thank You.

Velimir Markovic, M.D., Ph.D.
Principal Investigator

Jasminka Ilich, Ph.D., R.D.
Program Coordinator

Nancy Badenhop, R.D.
Research Associate

Jackie Buell, M.S., A.T.C.
Doctoral Candidate
January 16, 1998

Dear,

We will appreciate it very much if you could fill out your sport history questionnaire that was sent to you before Christmas. We realize filling out the questionnaire will take a few minutes of your time, but the information will help us document the effect of sport and physical activity on your bones and it will complement the other physical activity logs you have turned in to us!

If you feel you have received this card in error, please call us...we have received a number of records back without the green sheet which means no name! If you seem to have misplaced your form and would be willing to get another copy to fill out, we would love to send it to you to get your sport history.

Please call us at 293-3838 if we can help or send you another sport history form!!

Jackie          Nancy          Dr. M.
March 13, 1998

Dear «Fname»,

It is still not too late and we really do need your information on your past physical activity! Please have another look at this questionnaire and take a few minutes to write down your sport history. We have received all but a few of the questionnaires back and we do not have yours (unless you’ve mailed it in the past few days!). We still have about four previously completed records which were returned without a name or identification. Please call to identify yours or complete this one and pop it in the mail today! We are proud of your contribution to the research on osteoporosis and would like to make our study as complete as possible. Please help us!

We are very willing to help you fill this out if it is confusing...
just call us at 293-3838!

I wish they'd tell us about their sports!
Appendix I

Questionnaire sent to athletic trainers
I would love to have your help and expertise in my current research. I am evaluating the Influence of sport and physical activity on bone density across adolescence. Many of the studies in the literature suggest that sports which include higher weight-bearing impacts, may provide the most positive stimulation to the developing bones. In order to evaluate the questions around this issue, I need to categorize the following sports and document the validity of the categories. I am asking if you can please help me validate the categories.

I envision the following categories (definitions):

- **non-weight-bearing**
- **weight-bearing, body weight usually supported by both legs**
- **WB, body weight commonly single limb supported, activity linear**
- **WB, requires sudden cutting, running, stopping, minimal jumping as well as above**
- **WB, requires frequent jumping and landing as well as above**
- **Weight-loaded activities where you support your own body plus additional weight in progressive manner**

Based on your knowledge of the attached sports/activities, please take a few minutes to give me your opinion of how they would fit into these categories. I welcome your comments and suggestions! The second page is provided only if you need the space!

<table>
<thead>
<tr>
<th>Non-weight-bearing</th>
<th>Weight-bearing, body weight usually supported by both legs</th>
<th>Weight-bearing, body weight commonly single limb supported, activity linear</th>
<th>Weight-bearing, requires sudden cutting, running, stopping, minimal jumping as well as above</th>
<th>Weight-bearing, requires frequent jumping and landing as well as above</th>
<th>Weight-loaded activities where you support your own body plus additional weight in progressive manner</th>
</tr>
</thead>
<tbody>
<tr>
<td>swimming</td>
<td>walking</td>
<td>jogging/running</td>
<td>softball</td>
<td>volleyball</td>
<td>weight training</td>
</tr>
</tbody>
</table>

Comments and suggestions:

Thank you for your help!

Jackie
<table>
<thead>
<tr>
<th>Sports</th>
<th>Others</th>
<th>Fixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>gymnastics</td>
<td>aqua aerobics</td>
<td>swimming</td>
</tr>
<tr>
<td>cheerlead</td>
<td>archery</td>
<td>basketball</td>
</tr>
<tr>
<td>basketball</td>
<td>bike</td>
<td>bowling</td>
</tr>
<tr>
<td>softball</td>
<td>billiards</td>
<td>canoe</td>
</tr>
<tr>
<td>soccer</td>
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<td>dance</td>
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<td>pe</td>
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<td>aerobics</td>
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Please fill out this form. Include any appropriate other sport not listed.
Appendix J

Activities reported, Ost and MET values used
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<th>Sport</th>
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<th>Ost Value</th>
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<td>11.04</td>
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<td>4.5</td>
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Appendix K

SAS Macro for derived variables
/* Current update 12/10/98
Program: derived
Purpose: Compute derived variables from original data.
Transform data from 1 record per subject to 9 records
per subject (perm.final).

Datasets Needed: perm.all
Datasets Created: perm.cross, perm.final
*/

libname perm 'Macintosh HD:Stats programs:SAS612:jbdata';

/* Calculate METS by Bouchard values */
%macro bcalc(visit);
  bouch&visit = ((5/7)*((0.26*d1&visit) + (0.38*d2&visit) + (0.57*d3&visit) +
                  (0.69*d4&visit) + (0.84*d5&visit) + (1.20*d6&visit) +
                  (1.40*d7&visit) + (1.50*d8&visit) + (2.00*d9&visit))) +
             ((2/7)*((0.26*en1&visit) + (0.38*en2&visit) + (0.57*en3&visit) +
                  (0.69*en4&visit) + (0.84*en5&visit) + (1.20*en6&visit) +
                  (1.40*en7&visit) + (1.50*en8&visit) + (2.00*en9&visit)));
  bhmet&visit = ((5/7) * (1.50*d8&visit) + (2.00*d9&visit)) +
               ((2/7) * (1.50*en8&visit) + (2.00*en9&visit));
%mend bcalc;

/* Calculate METS by Bratteby values */
%macro brcalc(visit);
  brat&visit = (5*(0.2375*d1&visit + 0.3750*d2&visit + 0.5000*d3&visit +
                   0.7000*d4&visit + 0.8250*d5&visit + 1.1000*d6&visit +
                   1.6250*d7&visit + 2.5000*d8&visit + 3.7500*d9&visit) +
             2*(0.2375*en1&visit + 0.3750*en2&visit + 0.5000*en3&visit +
                0.7000*en4&visit + 0.8250*en5&visit + 1.1000*en6&visit +
                1.6250*en7&visit + 2.5000*en8&visit + 3.7500*en9&visit))/7;
  brhmet&visit = (5*(2.5000*d8&visit + 3.7500*d9&visit) +
                   2*(2.5000*en8&visit + 3.7500*en9&visit))/7;
%mend brcalc;

/* ENERGY BALANCE. Calculate kcals needed and expended and ratio. Also calculate the energy drain. */
%macro EE(visit);
  bTEE&visit = bouch&visit^wt&visit; * kcals by bouchard;
  brTEE&visit = brat&visit^wt&visit; * kcals by bratteby;
  BEE&visit = 655.1 + (wt&visit^956) + (1.85^ht&visit) - (6.8^age&visit); * basal kcals needed Harris-Benedict;
  bratio&visit = bTEE&visit/BEE&visit; * TEE/BEE bouchard;
  brratio&visit = brTEE&visit/BEE&visit; * TEE/BEE bratteby;
  bdrain&visit = dkl&visit-bTEE&visit; * energy drain with intake and bouchard values;
  brdrain&visit = dkl&visit-brTEE&visit; * energy drain with intake and bratteby values;
%mend EE;

/* Score subjects for hi, moderate and low (to be used to categorize) according to Bouchard frequencies and current ACSM guidelines of METS. */
%macro pacat(visit, locut, hicut);
  lo&visit=((5*(d1&visit+d2&visit+d3&visit+d4&visit)) +
            (2*(en1&visit+en2&visit+en3&visit+en4&visit)))/7;
  mod&visit=((5*(d5&visit+d6&visit+d7&visit)) +
             (2*(en5&visit+en6&visit+en7&visit)))/7;
  hi&visit=((5*(d8&visit+d9&visit)) +
            (2*(en8&visit+en9&visit)))/7;
  himod&visit=mod&visit + hi&visit;
  if (hi&visit > &hicut) then pacat&visit = ' hi';
  else if ((lo&visit > &locut) & visit < 1.25) then pacat&visit = ' low';
%mend pacat;
else if ((lo\&visit ne .) & (mod&visit ne .) & (hi&visit ne .))
  then pacat&visit = 'mod';
else pacat&visit = ";
  If (hi&visit > &hicut) then pa01&visit =1;
  else if ((lo&visit > &locut) & (hi&visit < 1.25))
  then pa01&visit =0;
  else pa01&visit =;
%mend pacat;

%macro simple(visit);
  TSM&visit = age&visit- MEN;
  TSMp&visit =
    if (tsm&visit le -2.5) and (tsm&visit ne .) then tsmp&visit=-2.75 ;
    else if (tsm&visit le -2.0) and (tsm&visit ne .) then tsmp&visit=-2.25 ;
    else if (tsm&visit le -1.5) and (tsm&visit ne .) then tsmp&visit=-1.75 ;
    else if (tsm&visit le -1.0) and (tsm&visit ne .) then tsmp&visit=-1.25 ;
    else if (tsm&visit le -0.5) and (tsm&visit ne .) then tsmp&visit=-0.75 ;
    else if (tsm&visit le -0.25) and (tsm&visit ne .) then tsmp&visit=-0.25 ;
    else if (tsm&visit le 0.0) and (tsm&visit ne .) then tsmp&visit=0.25 ;
    else if (tsm&visit le 0.5) and (tsm&visit ne .) then tsmp&visit=0.75 ;
    else if (tsm&visit le 1.0) and (tsm&visit ne .) then tsmp&visit=1.25 ;
    else if (tsm&visit le 1.5) and (tsm&visit ne .) then tsmp&visit=1.75 ;
    else if (tsm&visit le 2.0) and (tsm&visit ne .) then tsmp&visit=2.25 ;
    else if (tsm&visit le 2.5) and (tsm&visit ne .) then tsmp&visit=2.75 ;
    else if (tsm&visit > 2.3) and (tsm&visit ne .) then tsmp&visit=2.75 ;
    else if (tsm&visit > 11.5) and (tsm&visit ne .) then tsmp&visit=11.25 ;
    else if (tsm&visit > 12.0) and (tsm&visit ne .) then tsmp&visit=12.75 ;
    else if (tsm&visit > 12.5) and (tsm&visit ne .) then tsmp&visit=13.25 ;
    else if (tsm&visit > 13.0) and (tsm&visit ne .) then tsmp&visit=13.75 ;
    else if (tsm&visit > 13.5) and (tsm&visit ne .) then tsmp&visit=14.25 ;
    else if (tsm&visit > 14.0) and (tsm&visit ne .) then tsmp&visit=14.75 ;
    else if (tsm&visit > 14.5) and (tsm&visit ne .) then tsmp&visit=15.25 ;
    else if (tsm&visit > 15.0) and (tsm&visit ne .) then tsmp&visit=15.75 ;

BMI&visit = wt&visit/((ht&visit/100)**2) ;
PS&visit= (psh&visit + psp&visit)/2;
BA&visit= (TBC&visit/TBD&visit);
r33BA&visit= (r33BC&visit/r33BD&visit);

%mend simple;

/*categorize menstrual status*/
%macro mens(visit);
  if (tsm&visit le 0) and (tsm&visit ne .) then mstat&visit='ns';
  else if (tsm&visit > 0) and (tsm&visit le 0.5) then mstat&visit='js';
  else if (tsm&visit > 0.5) then do;
    if (cycle&visit > 0) and (cycle&visit ge 42.5) or (MP6&visit ge 4)
    then mstat&visit='eu';
    else if (cycle&visit ge 42.5) or (MP6&visit ge 4) then mstat&visit='dys';
    end;
  else mstat&visit = ";
%mend mens;

%macro mens234(visit);
  if (tsm&visit le 0 and tsm&visit ne .) then mstat&visit='ns';
  else if (tsm&visit > 0 and tsm&visit le 0.5) then mstat&visit='js';
  else if (tsm&visit > 0.5) then mstat&visit='eu';
else mstat&visit = ";
%mend mens234;
*
%macro diffodd(var);
&var31 = (&var3 - &var1)/(age3-age1);
&var31= &var31 * &var31;
&var53 = (&var5 - &var3)/(age5-age3);
&var53= &var53 * &var53;
&var75 = (&var7 - &var5)/(age7-age5);
&var75= &var75 * &var75;
&var39 = (&var9 - &var7)/(age9-age7);
&var39= &var39 * &var39;
&var41 = (&var9 - &var1)/(age9-age1);
&var41= &var41 * &var41;
%mend diffodd;

%macro diffall(var);
&var31 = (&var3 - &var1)/(age2-age1);
&var53 = (&var5 - &var3)/(age5-age3);
&var43 = (&var4 - &var3)/(age4-age3);
&var54 = (&var5 - &var4)/(age5-age4);
&var65 = (&var6 - &var5)/(age6-age5);
&var76 = (&var7 - &var6)/(age7-age6);
&var87 = (&var8 - &var7)/(age8-age7);
&var98 = (&var9 - &var8)/(age9-age8);
%mend diffall;

/*/assign METSval and Osteoval to sports...basically intensities*/
%macro mets(num);
If sport&num='arch' then METval&num=3.5;
If sport&num='bam' then METval&num=6;
If sport&num='bike' then METval&num=5;
If sport&num='biiiar' then METval&num=2.5;
If sport&num='bowl' then METval&num=3;
If sport&num='calis' then METval&num=4.5;
If sport&num='camp' then METval&num=4;
If sport&num='canoe' then METval&num=7;
If sport&num='climb' then METval&num=9;
If sport&num='crew' then METval&num=12;
If sport&num='xc' then METval&num=9;
If sport&num='dive' then METval&num=3;
If sport&num='drill' then METval&num=4;
If sport&num='horse' then METval&num=4;
If sport&num='flack' then METval&num=8;
If sport&num='fb' then METval&num=8;
If sport&num='golf' then METval&num=5.5;
If sport&num='hike' then METval&num=4;
If sport&num='iskate' then METval&num=7;
If sport&num='jrope' then METval&num=10;
If sport&num='karate' then METval&num=10;
If sport&num='lax' then METval&num=8;
If sport&num='ligd' then METval&num=3;
If sport&num='mband' then METval&num=4;
If sport&num='nordic' then METval&num=9.5;
If sport&num='plyo' then METval&num=10;
If sport&num='polo' then METval&num=8;
If sport&num='rec' then METval&num=5;
If sport&num='rbl' then METval&num=7;
If sport&num='rskate' then METval&num=7;
If sport&num='erg' then METval&num=8.5;
If sport&num='rugby' then METval&num=10;

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If sport&num='scuba' then METval&num=7;
If sport&num='sski' then METval&num=7;
If sport&num='smasr' then METval&num=6;
If sport&num='shock' then METval&num=8;
If sport&num='synsw' then METval&num=8;
If sport&num='ten' then METval&num=7;
If sport&num='tramp' then METval&num=3.5;
If sport&num='wskl' then METval&num=6;
If sport&num='wrestl' then METval&num=6;
If sport&num='yard' then METval&num=5;
If sport&num='.' then METval&num=.;
%mend mets;

%macro osteo(num);
  If sport&num='arch' then ostval&num=1.3 ;
  If sport&num='barn' then ostval&num=6.0 ;
  If sport&num='bike' then ostval&num=2.42 ;
  If sport&num='billiar' then ostval&num=1.0;
  If sport&num='bowl' then ostval&num=3.42 ;
  If sport&num='callis' then ostval&num=4.0 ;
  If sport&num='camp' then ostval&num=1.0 ;
  If sport&num='canoe' then ostval&num=1.88;
  If sport&num='climb' then ostval&num=4.38;
  If sport&num='crew' then ostval&num=2.0 .
  If sport&num='xc' then ostval&num=3.81 ;
  If sport&num='dive' then ostval&num=5.46; 
  If sport&num='drill' then ostval&num=3.15);
  If sport&num='horse' then ostval&num=0.73; 
  If sport&num='hock' then ostval&num=6.73; 
  If sport&num='tb' then ostval&num=8.93 ;
  If sport&num='golf' then ostval&num=1.69; 
  If sport&num='hike' then ostval&num=2.50; 
  If sport&num='iskate' then ostval&num=5.29; 
  If sport&num='jorpe' then ostval&num=9.92; 
  If sport&num='karate' then ostval&num=8.0;
  If sport&num='lax' then ostval&num=7.43 ;
  If sport&num='lfrd' then ostval&num=0.54;
  If sport&num='mband' then ostval&num=3.71;
  If sport&num='nordic' then ostval&num=2.0;
  If sport&num='plyo' then ostval&num=9.91;
  If sport&num='polo' then ostval&num=0.63;
  If sport&num='recr' then ostval&num=3.58;
  If sport&num='rbl' then ostval&num=3.41 ;
  If sport&num='rskate' then ostval&num=3.32;
  If sport&num='erg' then ostval&num=0.73 ;
  If sport&num='rugby' then ostval&num=7.5;
  If sport&num='scuba' then ostval&num=0.5;
  If sport&num='ssski' then ostval&num=5.41;
  If sport&num='smasr' then ostval&num=2.25;
  If sport&num='shock' then ostval&num=7.5;
  If sport&num='synsw' then ostval&num=0.5;
  If sport&num='ten' then ostval&num=6.23 ;
  If sport&num='tramp' then ostval&num=5.65;
  If sport&num='wskl' then ostval&num=6.0 ;
  If sport&num='wrestl' then ostval&num=9.36;
  If sport&num='yard' then ostval&num=1.0 ;
  If sport&num='.' then ostval&num=.;
%mend osteo;

%macro wkcut(num);
  If sport&num='arch' then wkcut&num=.;
  If sport&num='barn' then wkcut&num=.;
If sport&num='bik' then wkcut&num=.;
If sport&num='biliiar' then wkcut&num=.;
If sport&num='bowl' then wkcut&num=.;
If sport&num='calis' then wkcut&num=.;
If sport&num='camp' then wkcut&num=.;
If sport&num='canoe' then wkcut&num=.;
If sport&num='climb' then wkcut&num=.;
If sport&num='crew' then wkcut&num=8;
If sport&num='x' then wkcut&num=8;
If sport&num='dive' then wkcut&num=8;
If sport&num='drill' then wkcut&num=8;
If sport&num='hor' then wkcut&num=.;
If sport&num='flock' then wkcut&num=8;
If sport&num='fb' then wkcut&num=.;
If sport&num='golf' then wkcut&num=.;
If sport&num='hike' then wkcut&num=.;
If sport&num='ikate' then wkcut&num=.;
If sport&num='jrope' then wkcut&num=.;
If sport&num='karate' then wkcut&num=.;
If sport&num='lax' then wkcut&num=8;
If sport&num='lfgrd' then wkcut&num=.;
If sport&num='mband' then wkcut&num=8;
If sport&num='nordic' then wkcut&num=.;
If sport&num='s' then wkcut&num=4;
If sport&num='polo' then wkcut&num=8;
If sport&num='rec' then wkcut&num=.;
If sport&num='rbl' then wkcut&num=.;
If sport&num='rakte' then wkcut&num=.;
If sport&num='erg' then wkcut&num=.;
If sport&num='ruguay' then wkcut&num=8;
If sport&num='scuba' then wkcut&num=.;
If sport&num='ski' then wkcut&num=.;
If sport&num='smastr' then wkcut&num=.;
If sport&num='shock' then wkcut&num=.;
If sport&num='synsw' then wkcut&num=8;
If sport&num='ten' then wkcut&num=8;
If sport&num='tramp' then wkcut&num=.;
If sport&num='wski' then wkcut&num=.;
If sport&num='y' then wkcut&num=.;
If sport&num='.' then wkcut&num=.;
%mend wkcut;

%macro hrct(num);
If sport&num='arch' then hrct&num=.;
If sport&num='barn' then hrct&num=.;
If sport&num='bik' then hrct&num=.;
If sport&num='biliiar' then hrct&num=.;
If sport&num='bowl' then hrct&num=.;
If sport&num='calis' then hrct&num=.;
If sport&num='camp' then hrct&num=.;
If sport&num='canoe' then hrct&num=.;
If sport&num='climb' then hrct&num=.;
If sport&num='crew' then hrct&num=3;
If sport&num='x' then hrct&num=3;
If sport&num='dive' then hrct&num=3;
If sport&num='drill' then hrct&num=3;
If sport&num='hor' then hrct&num=.;
If sport&num='flock' then hrct&num=3;
If sport&num='fb' then hrct&num=.;
If sport&num='golf' then hrct&num=.;
If sport&num='hike' then hrct&num=.;
If sport&num='iskate' then hicut&num=.;
If sport&num='jrope' then hicut&num=.;
If sport&num='karate' then hicut&num=.;
If sport&num='lax' then hicut&num=3;
If sport&num='figret' then hicut&num=.;
If sport&num='mband' then hicut&num=3;
If sport&num='nordic' then hicut&num=.;
If sport&num='polo' then hicut&num=1.5;
If sport&num='pIyo' then hicut&num=3;
If sport&num='recr' then hicut&num=.;
If sport&num='ribi' then hicut&num=.;
If sport&num='skate' then hicut&num=.;
If sport&num='erg' then hicut&num=.;
If sport&num='rugby' then hicut&num=3;
If sport&num='scuba' then hicut&num=.;
If sport&num='ski' then hicut&num=.;
If sport&num='smastr' then hicut&num=.;
If sport&num='snow' then hicut&num=.;
If sport&num='sw' then hicut&num=3;
If sport&num='swim' then hicut&num=.;
If sport&num='ten' then hicut&num=3;
If sport&num='tramp' then hicut&num=.;
If sport&num='wski' then hicut&num=.;
If sport&num='wrestl' then hicut&num=.;
If sport&num='yard' then hicut&num=.;
If sport&num=': then hicut&num=.;
%end hicut;

/* Calculate time, METS, osteo values and athlete dummy variable for each
year sport activity */
%macro sports(yr, num);
tim&yricnum=wks&yr&num*hrs&yr&num;
met&yr&num=tim&yr&num*METval&num;
ost&yr&num=tim&yr&num*ostval&num;
/* Create yes (1) no (0) dummy variable to be able to determine if each year of that sport meets criteria
for "athlete" for the traditional sports. */
if (wkcut&num ne .) then do;
  if (wks&yr&num ge wkcut&num) and (hrs&yr&num ge hrcut&num) then do;
    /* athct&yr is a spot holder for single-sport athletes so it is OK if it gets
    overwritten for multi-sport athletes */
    athct&yr=sport#;
    ath&yr&num=1;
  end;
  else if (wks&yr&num ge 0) and (hrs&yr&num ge 0) then do;
    ath&yr&num=0;
  end;
  else do;
    ath&yr&num=.;
  end;
%end if (&num ge 14) %then %do;
/* If xc athlete AND jog athlete, consider just an xc athlete (not a two sport athlete) */
if (sport&num='xc') and (ath&yr&num=1) and (ath&yr.9=1) then ath&yr.9=0;
%end;
/* Same for track and jog, just label as track athlete */
%if (&num = 13) %then %do;
if ath&yr.9=1 and ath&yr.13=1 then ath&yr.9=0 ;
%end;
%end sports;

/* Sum up the 20 sports to one score (tim, mets and ost and ath) for each
visit 3,5,7,9 intentionally omitting "PE" (#11- keep to eval reporting)*/

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%macro mysum(var, visit, year);
   &var&visit = SUM(of &var&year.1 &var&year.2 &var&year.3 &var&year.4 &var&year.5 &var&year.6 &var&year.7 &var&year.8 &var&year.9 &var&year.10 &var&year.12 &var&year.13 &var&year.14 &var&year.15 &var&year.16 &var&year.17 &var&year.18 &var&year.19 &var&year.20);
%mend mysum;

%macro athcat(visit, yr);
   If ath&visit ge 3 then athcat&visit='multiple';
   else if ath&visit=2 then athcat&visit='twosport';
   else if ath&visit=1 then athcat&visit=athct&yr;
   else if ath&visit=0 then athcat&visit='none';
   else athcat&visit = ";
   If ath&visit ge 3 then spcat&visit=3;
   else if ath&visit=2 then spcat&visit=2;
   else if ath&visit=1 then spcat&visit=1;
   else if ath&visit=0 then spcat&visit=0;
   else spcat&visit = ";
   If ath&visit>0 then ath01&visit=1;
   else if ath&visit=0 then ath01&visit=0;
   else ath01&visit = ";
%mend athcat;

/*Add macro to decipher tim, met and osteo categories by tertile cutoffs*/
%macro timcat(visit, locut, hicut);
   If ((tim&visit < &locut) & (tim&visit ne .)) then timcat&visit = low;
   Else If (tim&visit > &hicut) then timcat&visit = hi';
   Else If (tim&visit > &locut) & (tim&visit < &hicut) then timcat&visit = mod';
   Else timcat&visit = ";
   If ((tim&visit < &locut) & (tim&visit ne .)) then timOl&visit =0;
   Else If (tim&visit > &hicut) then timOl&visit = 1;
   Else timOl&visit =.;
   If tim&visit ne . then return&visit=1;
   else return&visit=0;
%mend timcat;

%macro metcat(visit, locut, hicut);
   If ((met&visit < &locut) & (met&visit ne .)) then metcat&visit = 'low';
   Else If (met&visit > &hicut) then metcat&visit = hi';
   Else If (met&visit > &locut) & (met&visit < &hicut) then metcat&visit = mod';
   Else metcat&visit = ";
   If ((met&visit < &locut) & (met&visit ne .)) then metOl&visit =0;
   Else If (met&visit > &hicut) then metOl&visit = 1;
   Else metOl&visit =.;
   If met&visit ne . then return&visit=1;
   else return&visit=0;
%mend metcat;

%macro ostcat(visit, locut, hicut);
   If ((ost&visit < &locut) & (ost&visit ne .)) then ostcat&visit = low';
   Else If (ost&visit > &hicut) then ostcat&visit = hi';
   Else If (ost&visit > &locut) & (ost&visit < &hicut) then ostcat&visit = mod';
   Else ostcat&visit = ";
   If ((ost&visit < &locut) & (ost&visit ne .)) then ostOl&visit =0;
   Else If (ost&visit > &hicut) then ostOl&visit = 1;
   Else ostOl&visit =.;
   If ost&visit ne . then return&visit=1;
   else return&visit=0;
%mend ostcat; 293
/*set up macro to calculate the mean value over the nine visits to be able to
categorize by tertiles*/
%macro x(var, locut, hicut);
  x&var=mean(of &var.l-&var.9);
  x&var.l=0;
  x&var.m=0;
  x&var.h=0;
  x&var.3=" ";
  if x&var ge &hicut then do;
    x&var.h=1;
    x&var.3="hi"
  end;
  else if x&var > &locut then do;
    x&var.m=1;
    x&var.3="md"
  end;
  else if x&var ge 0 then do;
    x&var.l=1;
    x&var.3="lo"
  end;
  else if x&var= . then do;
    x&var.l= .
    x&var.m= .
    x&var.h= .
    x&var.3=" ";
  end;
%mend x;

options nomprint;

data all;
  set perm.all;
  /*Little program to correct for data error for Steckels*/
  if id='5009' then do;
    bf7=42018;
    lbm7=51001;
    tbc7=3259;
    ba7=tbc7/tfad7;
  end;
  if id='D085' then do;
    bf7=23247;
    lbm7=46644;
    tbc7=2688;
    ba7=tbc7/tbd7;
  end;
  /*No one had started menstrual cycles at study inception*/
  mstatl="ns";
  /*Set cycle=0 or mp6=0 to missing*/
  if cycle5 = 0 then cycle5 = .
  if cycle6 = 0 then cycle6 = .
  if cycle7 = 0 then cycle7 = .
  if cycle8 = 0 then cycle8 = .
  if cycle9 = 0 then cycle9 = .
  if mp65 = 0 then mp65 = .
  if mp66 = 0 then mp66 = .
  if mp67 = 0 then mp67 = .
  if mp68 = 0 then mp68 = .
  if mp69 = 0 then mp69 = .
  /*
/* Calculate METS by Bouchard and Bratteby values */
%bcalc(1);
%bcalc(2);
%bcalc(3);
%bcalc(4);
%bcalc(5);
%bcalc(6);
%bcalc(7);
%bcalc(8);
%bcalc(9);

%brcalc(1);
%brcalc(2);
%brcalc(3);
%brcalc(4);
%brcalc(5);
%brcalc(6);
%brcalc(7);
%brcalc(8);
%brcalc(9);

%EE(1);
%EE(2);
%EE(3);
%EE(4);
%EE(5);
%EE(6);
%EE(7);
%EE(8);
%EE(9);

%pacat(1, 88.3, 4.72);
%pacat(2, 90.5, 2.9);
%pacat(3, 89.8, 4.9);
%pacat(4, 90.2, 3.5);
%pacat(5, 88.6, 4.1);
%pacat(6, 89.8, 4.3);
%pacat(7, 88.2, 4.9);
%pacat(8, 90.2, 4.1);
%pacat(9, 89.2, 4.8);

%simple(1);
%simple(2);
%simple(3);
%simple(4);
%simple(5);
%simple(6);
%simple(7);
%simple(8);
%simple(9);

%mens(5);
%mens(6);
%mens(7);
%mens(8);
%mens(9);
%mens234(2);
%mens234(3);
%mens234(4);

/* Create variables for change between visits */
%diffodd(tbd);
%diffall(tbd);
%diffodd(tbc);
%diffall(thc);
%diffodd(RudBD);
%diffall(RudBD);
%diffodd(r10bd);
%diffall(r10bd);
%diffodd(r33bd);
%diffall(r33bd);
%diffodd(rudbc);
%diffall(rudbc);
%diffodd(r10bc);
%diffall(r10bc);
%diffodd(r33bc);
%diffall(r33bc);
%diffodd(ht);
%diffall(ht);
%diffodd(siht);
%diffall(siht);
%diffodd(wt);
%diffall(wt);
%diffodd(bmi);
%diffall(bmi);
%diffodd(bf);
%diffall(bf);
%diffodd(lbm);
%diffall(lbm);
%diffodd(ba);
%diffall(ba);
%diffodd(r33ba);
%diffall(r33ba);

/*create METval and ostval and athlete cuts (wks&hrs) for each sport*/

METval1=4;
METval2=4;
METval3=7;
METval4=5;
METval5=8.5;
METval6=6;
METval7=4;
METval8=9;
METval9=8;
METval10=4.5;
METval11=4;
METval12=7;
METval13=7;
%mets(14);
%mets(15);
%mets(16);
%mets(17);
%mets(18);
%mets(19);
%mets(20);

ostval1=11.57;
ostval2=10.6;
ostval3=11.04;
ostval4=6.91;
ostval5=7.91;
ostval6=8.38;
ostval7=10.57;
ostval8=0.5;
ostval9=3.5;
ostval10=16.69;
ostval11=6.5;
ostval12=5.41;
ostval13=8.03;
%osteo(14);
%osteo(15);
%osteo(16);
%osteo(17);
%osteo(18);
%osteo(19);
%osteo(20);

wkcut1=8;
wkc put2=8;
wkc put3=8;
wkc put4=8;
wkc put5=8;
wkc put6=8;
wkc put7=8;
wkc put8=8;
wkc put9=8;
wkc put10=4;
wkc put11=.;
wkc put12=8;
wkc put13=8;
%wk cut(14);
%wk cut(15);
%wk cut(16);
%wk cut(17);
%wk cut(18);
%wk cut(19);
%wk cut(20);

hr cut1=3;
hr cut2=3;
hr cut3=3;
hr cut4=3;
hr cut5=3;
hr cut6=3;
hr cut7=3;
hr cut8=3;
hr cut9=3;
hr cut10=1.5;
hr cut11=.;
hr cut12=3;
hr cut13=3;
%hr cut(14);
%hr cut(15);
%hr cut(16);
%hr cut(17);
%hr cut(18);
%hr cut(19);
%hr cut(20);

/*Calculate the METS and osteo scores and time in each sport*/

%sports(2_3,l);
%sports(3_4,l);
%sports(4_5,l);
%sports(2_3^)
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/* Certain subjects reported NO sports... they should have 0's for the following 
   variables, not missings */
/*if (id = 'A052' or id = 'B032' or id = '5042') then do;
tim3=0; tim5=0; tim7=0; tim9=0;
met3=0; met5=0; met7=0; met9=0;
ost3=0; ost5=0; ost7=0; ost9=0;
end;
*/
%athcat(3, 2_3);
%athcat(5, 3_4);
%athcat(7, 4_5);
%athcat(9, 5_6);
%timcat(3, 795, 251.7);
%timcat(5, 119, 300);
%timcat(7, 161, 377.5);
%timcat(9, 175, 430);
%metcat(3, 530, 1700);
%metcat(5, 715, 1945);
%metcat(7, 950, 2580);
%metcat(9, 1048, 2845);
%ostcat(3, 340, 1275);
%ostcat(5, 547, 1510);
%ostcat(7, 765, 2130);
%ostcat(9, 815, 2340);
%x(totca, 950, 1355);
%x(bouch, 42.7709, 46.9167);
%x(brat, 43.85, 50.411);
/*Be able to look for "athlete bias" by when began sport.*/
if (athcat3='none') & ((athcat5='none') & (athcat7 ne '') & ((athcat9 ne 'none') & (athcat9 ne ''))
  then start= late1;
else if (athcat3 = 'none') & (athcat5 = 'none') &
  ((athcat7 ne 'none') & (athcat7 ne 'none') & (athcat9 ne 'none') & (athcat9 ne 'none') & (athcat9 ne ''))
  then start= late2;
else if ((athcat3 ne 'none') & (athcat3 ne 'none') & (athcat5 ne 'none') & (athcat5 ne '') & (athcat7 ne 'none') & (athcat7 ne '') & (athcat9 ne 'none') & (athcat9 ne 'none'))
  then start= early';
else if ((athcat3 ne 'none') & (athcat3 ne 'none') or
  ((athcat5 ne 'none') & (athcat5 ne '') or
  ((athcat7 ne 'none') & (athcat7 ne 'none') or
  ((athcat9 ne 'none') & (athcat9 ne 'none'))
  then start= 'different';
else if (athcat3= 'none') & (athcat5= 'none') &
  (athcat7= 'none') & (athcat9= 'none')
  then start= 'control';
else start=";"

/* Want to compare those who were athletes at ALL years vs those who were not
 at the beginning but were later vs those who were never athletes */
if start='control' then start012=0;
else if start=' late1' or start=' late2' then start012=2;
else if start=' early' then start012=1;
else start012=;

/*Want to be able to have athlete (1) and control (0) variables for four year comparison*/
if start='control' then xath01=0;
else if start=' early' then xath01=1;
else xath01=;

if (compl2 > 100) then compl2=100;
if (compl3 > 100) then compl3=100;
if (compl4 > 100) then compl4=100;
if (compl5 > 100) then compl5=100;
if (compl6 > 100) then compl6=100;
if (compl7 > 100) then compl7=100;
if (compl8 > 100) then compl8=100;
if (compl9 > 100) then compl9=100;

/*set return or not for long data*/
return=;
return=return3;

/*Create variable to indicate when started periods and
 if those have always been eumenorrheic*/
if (men le 12.878) & (men ne .) then men01=0;
Else if men > 12.878 then men01=1;
Else men01=;
/*same but by literature value of 14 yo and name it men01b*/
if (men le 14) & (men ne .) then men01b=0;
Else if men > 14 then men01b=1;
Else men01b=;
mstat01=0;
If mstat5='dys' or mstat6='dys' or mstat7='dys' or mstat8='dys' or mstat9='dys' then
mstat01=1;
If mstat5=" & mstat6=" & mstat7=" & mstat8=" & mstat9=" then
mstat01=";

="/want to be able to look at menstrual data annually for irregularities"/
mstat23='';
mstat45='';
mstat67='';
mstat89='';
If mstat2='dys' or mstat3='dys' then mstat23='dys';
else mstat23=mstat3;
If mstat4='dys' or mstat5='dys' then mstat45='dys';
else mstat45=mstat5;
If mstat6='dys' or mstat7='dys' then mstat67='dys';
else mstat67=mstat7;
If mstat8='dys' or mstat9='dys' then mstat89='dys';
else mstat89=mstat9;

="/ Look at the athletes only by menstrual groups in 2 X 2"/
menathnc = ' ';
if xath01 = 1 then do;
  if (men01=0 and mstat01=1) then menathnc= 'dys' ;
  else if (men01=1 and mstat01=1) then menathnc= 'eula' ;
  else if (men01=0 and mstat01=0) then menathnc= 'eu' ;
  else if (men01=1 and mstat01=0) then menathnc= 'eula' ;
end;

="/variables to look at validity with repeat data"/
bmet3=mean(of bhmet2 bhmet3);
bmet5=mean(of bhmet4 bhmet5);
bmet7=mean(of bhmet6 bhmet7);
bmet9=mean(of bhmet8 bhmet9);

brmet3=mean(of brhmet2 brhmet3);
brmet5=mean(of brhmet4 brhmet5);
brmet7=mean(of brhmet6 brhmet7);
brmet9=mean(of brhmet8 brhmet9);

btim3=mean(of himod3 himod2);
btim5=mean(of himod5 himod4);
btim7=mean(of himod7 himod6);
btim9=mean(of himod9 himod8);

bouch23=mean (of bouch2 bouch3);
bouch45=mean (of bouch4 bouch5);
bouch67=mean (of bouch6 bouch7);
bouch89=mean (of bouch8 bouch9);

brat23=mean (of brat2 brat3);
brat45=mean (of brat4 brat5);
brat67=mean (of brat6 brat7);
brat89=mean (of brat8 brat9);

="/ Want to flag those who have completed visit 9 */
include=0;
if (age9 ne .) then include=1;
run;

="/save to permanent file
data penn.cross;
set all;
/* Only include those who have completed visit 9 */
if include=1;
run;
*/

data all_long;
set perm.cross;
retain id code men menOl menOlb mstatOl include start startOl xathOl
xtim xtiml xtimm xtimh xtimt xmet xmetl xmetm xmeth xmett3
xost xostm xostt xlept xleptl xleptm xleptc xlept3
tbd91 tbc91 rudbd91 r10bd91 r33bd91 rudbc91 r10bc91 r33bc91
ht91 wt91 bmi91 b91 b1m91 return;

visit=1; compl=.; age=age1; agep=agep1; sa=sal; psb=psb1; psp=pspl; ps=ps1;
ht=ht1; siht=.; wt=wt1;
pbf=pbf1; prf=prfl; tbtis=tbtis1; bf=bf1; lbm=lbm1; tbd=tbd1; tbc=tbc1;
rudbd=rudbd1; r10bd=r10bd1; r33bd=r33bd1; rudbc=rudbc1; r10bc=r10bc1;
r33bc=r33bc1; dkl=dkl1; dprt=dprtl1; dfat=dfatl1; dch=dch1; dca=dca1;
dphos=dphos1; dna=dna1; dzn=dzn1; dmg=dmg1; dvl=dvl1; dvc=dvc1;
dfb=dfb1; dse=dse1; df=df1; dva=dva1; dve=dve1; dvk=dvk1;
uvol=uvol1; uac=uac1; uphc=uphc1; uname=uname1; ucr=ucr1; bca=bcac1;
bphc=bphc1; bprc=bprcl; bap=bap1; brcr=bcrc1; boc=bocl; bpth=bpth1;
bferr=bfer1;
lept=.; mpdur=.; cycle=.; mp6=.;
tbd_6=.; tbd_12=.; tbc_6=.; tbc_12=.;
rudbd_6=.; rudbd_12=.; r10bd_6=.; r10bd_12=.; r33bd_6=.; r33bd_12=.;
rudbc_6=.; rudbc_12=.; r10bc_6=.; r10bc_12=.; r33bc_6=.; r33bc_12=.;
ht_6=.; ht_12=.; siht_6=.; siht_12=.; wt_6=.; wt_12=.;
bmi_6=.; bmi_12=.; bf_6=.; bf_12=.; lbm_6=.; lbm_12=.;
met=.; ost=.; pacat=pacat1; atcat=;
bouch=bouch1; brat=bratl1; bouchnn=.; bratnn=.; bTEE=bTEE1; brTEE=brTEE1; BEE=EEE1;
bratio=bratio1; brratio=brrat1; bdtrain=bdtrain1; brdtrain=brdtrain1;
mstat=mstat1; tsm=tsm1; tspmp=tspm1; bmi=bnim1; lo=lon1; mod=mod1; hi=hi1; pe=.;
tota=totca1; metcat=.; ostcat=.; timcat=.;
tim=.; pa=pa01; ath=ath01.; tim01=.; met01=.; ost01=.; spcat=.; ba=ba1; ba_12=.; ba_6=.; r33ba=r33ba1; r33ba_12=.; r33ba_6=.;
bmet=.; bmet=.; bhmet=bhmet1; brhmet=brhmet1; btim=.; mstatyr=.
output;

visit=2; compl=compl2; age=age2; agep=agep2; sa=.; psb=psb2; psp=psp2; ps=ps2;
ht=ht2; siht=siht2; wt=wt2;
pbf=pbf2; prf=prf2; tbtis=tbtis2; bf=bf2; lbm=lbm2; tbd=tbd2; tbc=tbc2;
rudbd=rudbd2; r10bd=r10bd2; r33bd=r33bd2; rudbc=rudbc2; r10bc=r10bc2;
r33bc=r33bc2; dkl=dkl2; dprt=dprtl2; dfat=dfatl2; dch=dch2; dca=dca2;
dphos=dphos2; dna=dna2; dzn=dzn2; dmg=dmg2; dvl=dvl2; dvc=dvc2;
dfb=dfb2; dse=dse2; df=df2; dva=dva2; dve=dve2; dvk=.;
uvol=.; uac=.; uphc=.; uname=.; ucr=.; bcac=.;
bphc=.; bprc=.; bap=.; brcr=.; boc=.; bpth=.; bferr=.;
lept=.; mpdur=.; cycle=.; mp6=.;
tbd_6=.; tbd_12=.; tbc_6=.; tbc_12=.;
rudbd_6=.; rudbd_12=.; r10bd_6=.; r10bd_12=.; r33bd_6=.; r33bd_12=.;
rudbc_6=.; rudbc_12=.; r10bc_6=.; r10bc_12=.; r33bc_6=.; r33bc_12=.;
ht_6=.; ht_12=.; siht_6=.; siht_12=.; wt_6=.; wt_12=.;
bmi_6=.; bmi_12=.; bf_6=.; bf_12=.; lbm_6=.; lbm_12=.;
met=.; ost=.; pacat=pacat2; atcat=;
bouch=bouch2; brat=bratl2; bouchnn=.; bratnn=.; bTEE=bTEE2; brTEE=brTEE2; BEE=EEE2;
Iept=lept9; m pdur=m pdur9; cyde=qrcle9; mp6=mp69;
d_6=tfad98; *d_12=*d97; tbc_6=tbc98; tfac_12=tfac97;
radbd_6=rudbd98; rudbd_12=rudbd97; rl0bd_6=rl0bd98; rlObd_12=rl0bd97;
r33bd_6=r33bd98; r33bd_12=r33bd97;

keep id code men01 men01b mstat01 include start start012 xath01
xtotc xtotc1 xtotc2 xtotc3 xtotc4 xtotc5 xtotc6 xtotc7 xtotc8
xim xitm xitm1 xitm2 xitm3 xmet xmet1 xmet2 xmet3
xost xost1 xost2 xost3 xlept xlept1 xleptm xlept6 xlept7

post = .;
postath = .;
menath = ' ';
menathnc = menath;

run;
data all_fina;
set all_long;
post = .;

if (start012 = 1) then preath=1;
else if (start012 = 2) then preath=0;
mstatus=' ';
If mstatyr='dys' then mstatus='dys';
Else mstatus=mstat;

menath = ' ';
if xath01 = 0 then menath = 'cont';
else if xath01 = 1 then do;

if (men01=0 and mstat01=1) then menath= 'dys';
else if (men01=1 and mstat01=1) then menath= 'dys';
else if (men01=0 and mstat01=0) then menath= 'euea';
else if (men01=1 and mstat01=0) then menath = 'eula';
end;

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if menathnc = 'cont' then menathnc=' ';
menathb = ' ';
if xath01 = 0 then menathb = 'cont' ;
else if xath01 = 1 then do;
if (men01b=0 and mstat01=1) then menathb= 'dyse' ;
else if (men01b=1 and mstat01=1) then menathb= 'dys' ;
else if (men01b=0 and mstat01=0) then menathb= 'euea' ;
else if (men01b=1 and mstat01=0) then menathb= 'eula' ;
end;
menathnb = menathb;
if menathnb = 'cont' then menathnb=' ';

mstatath= .;
if xath01=1 then do;
if mstatus=' eu' then mstatath=1 ;
else if mstatus='dys' then mstatath=0;
end;
ostb = ost/1000;
ost2 = ost*ostb;
metb = met/1000;
met2 = met*metb;
timb = tim/1000;
tim2 = tim*timb;
bouc2 = bouc*bouc;
brat2 = brat*brat;
tsm2 = tsm*tsm;
ht2 = ht*ht;
ht_122 = ht_12*ht_12;
bmi2 = bmi*bmi;
bmi_122 = bmi_12*bmi_12;
lbm2 = (lbm/lbm)/1000000;
lbm_122 = (lbm_12*lbm_12)/1000000;
br2 = (br*br)/1000000;
br_122 = br_12*br_12;
ba2 = ba*ba;
ba_122 = ba_12*ba_12;
r33ba2 = r33ba*r33ba;
r3ba_122 = r33ba_12*r33ba_12;
age2 = age*age;
ns = .;
js = .;
eu = .;
dys = .;
if (mstatus = ' ns') then ns = 1;
else if (mstatus ne ' ') then ns = 0;
if (mstatus = ' js') then js = 1;
else if (mstatus ne ' ') then js = 0;
if (mstatus = ' eu') then eu = 1;
else if (mstatus ne ' ') then eu = 0;
if (mstatus = ' dys') then dys = 1;
else if (mstatus ne ' ') then dys = 0;
ns_slope = tsm*ns;
js_slope = tsm*js + (dys + eu)/2;
eu_slope = tsm*eu - eu/2;
dys_slope = tsm*dys - dys/2;
run;

/*To plug into the cross-sectional data*/
/*want to be able to look at menstrual data annually for irregularities*/
data tmpcross;
set perm.cross;

307
mstat23=';
mstat45=';
mstat67=';
mstat89=';
If mstat2='dys' or mstat3='dys' then mstat23='dys';
else mstat23=mstat3;
If mstat4='dys' or mstat5='dys' then mstat45='dys';
else mstat45=mstat5;
If mstat6='dys' or mstat7='dys' then mstat67='dys';
else mstat67=mstat7;
If mstat8='dys' or mstat9='dys' then mstat89='dys';
else mstat89=mstat9;
/* Look at the athletes only by menstrual groups in 2 X 2*/
menathnc = ' '; 
if xathOl = 1 then do;
   if (men01=0 and mstat01=1) then menathnc= 'dyse';
   else if (men01=1 and mstat01=1) then menathnc= 'dyse';
   else if (men01=0 and mstat01=0) then menathnc= 'euea';
   else if (men01=1 and mstat01=0) then menathnc= 'eula';
end;
run;

/*Program to put additional vars in perm.cross*/
%macro menslope(visit, visits);
   "annual indicator only at visits 3 5 7 9;"
   ns&visit = .; 
   js&visit = .; 
   eu&visit = .; 
   dys&visit = .; 
   ns_slop&visit = .; 
   js_slop&visit = .; 
   eu_slop&visit = .; 
   dys_slop&visit = .; 
   if (mstat&visits = ' ns') then ns&visit = 1;
   else if (mstat&visits ne ' ') then ns&visit = 0;
   if (mstat&visits = ' js') then js&visit = 1;
   else if (mstat&visits ne ' ') then js&visit = 0;
   if (mstat&visits = ' eu') then eu&visit = 1;
   else if (mstat&visits ne ' ') then eu&visit = 0;
   if (mstat&visits = ' dys') then dys&visit = 1;
   else if (mstat&visits ne ' ') then dys&visit = 0;
   ns_slop&visit = tsm&visit*ns&visit;
   js_slop&visit = tsm&visit*js&visit + (dys&visit + eu&visit)/2;
   eu_slop&visit = tsm&visit*eu&visit - eu&visit/2;
   dys_slop&visit = tsm&visit*dys&visit - dys&visit/2;
%mend menslope;
%macro squares(visit);
   ostk&visit = ost&visit/1000;
   ost2&visit = ost&visit*ost&visit;
   metk&visit = met&visit/1000;
   met2&visit = met&visit*met&visit;
   timk&visit = tim&visit/1000;
   tim2&visit = tim&visit*tim&visit;
   bouch2&visit = bouch&visit*bouch&visit;
   tsm2&visit = tsm&visit*tsm&visit;
   ht2&visit = ht&visit*ht&visit;
   bmi2&visit = bmi&visit*bmi&visit;
   lbm2m&visit = (lbm&visit*lbm&visit)/1000000;
   bf2m&visit = (bf&visit*bf&visit)/1000000;
308
data tmp;
set tmppcross;
%menslope(3, 23);
%menslope(5, 45);
%menslope(7, 67);
%menslope(9, 89);
%squares(1);
%squares(3);
%squares(5);
%squares(7);
%squares(9);
%mstath(3);
%mstath(5);
%mstath(7);
%mstath(9);
run;

data perm.cross;
set tmp;
run;
data perm.final;
set all_final;
/* Only include those who have completed visit 9 */
if include=1;
  run;
Appendix L

Time since menarche modeling results
### Modeling Time Since Menarche (tsm) in Proc Mixed

**Fixed Effects:**

<table>
<thead>
<tr>
<th>Effect</th>
<th>p-value</th>
<th>Covariance Structure</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tsm (continuous)</td>
<td>0.0001</td>
<td>random intercept</td>
<td>0.0001</td>
</tr>
<tr>
<td>Change point at zero</td>
<td>0.0001</td>
<td>independence</td>
<td></td>
</tr>
<tr>
<td>tsm*post</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change point at zero</td>
<td>0.0001</td>
<td>random intercept</td>
<td>0.0001</td>
</tr>
<tr>
<td>tsm</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tsm*post</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change point at zero</td>
<td>0.0001</td>
<td>random intercept</td>
<td>0.0001</td>
</tr>
<tr>
<td>tsm</td>
<td>0.0001</td>
<td>tsm slope</td>
<td>0.2442</td>
</tr>
<tr>
<td>tsm*post</td>
<td>0.0001</td>
<td>tsm*post-slope</td>
<td></td>
</tr>
<tr>
<td>Change point at zero</td>
<td>0.0001</td>
<td>random intercept</td>
<td>0.0001</td>
</tr>
<tr>
<td>tsm</td>
<td>0.0001</td>
<td>tsm slope</td>
<td>0.2442</td>
</tr>
<tr>
<td>tsm*post</td>
<td>0.0001</td>
<td>tsm*post-slope</td>
<td>0.6486</td>
</tr>
<tr>
<td>TSM as quadratic function</td>
<td>0.0001</td>
<td>independence</td>
<td></td>
</tr>
<tr>
<td>tsm</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tsm²</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSM as quadratic</td>
<td>0.0001</td>
<td>random intercept</td>
<td>0.0003</td>
</tr>
<tr>
<td>tsm</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tsm²</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSM as quadratic</td>
<td>0.0001</td>
<td>random intercept</td>
<td>0.0005</td>
</tr>
<tr>
<td>tsm</td>
<td>0.0001</td>
<td>tsm slope</td>
<td>0.2227</td>
</tr>
<tr>
<td>tsm²</td>
<td>0.0001</td>
<td>tsm² slope</td>
<td></td>
</tr>
<tr>
<td>TSM as quadratic</td>
<td>0.0001</td>
<td>random intercept</td>
<td>0.0005</td>
</tr>
<tr>
<td>tsm</td>
<td>0.0001</td>
<td>tsm slope</td>
<td>0.2237</td>
</tr>
<tr>
<td>tsm²</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSM as quadratic</td>
<td>0.0001</td>
<td>random intercept</td>
<td>0.0004</td>
</tr>
<tr>
<td>tsm</td>
<td>0.0001</td>
<td>Tsm² slope</td>
<td>0.3942</td>
</tr>
<tr>
<td>tsm²</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Four slope model**

<table>
<thead>
<tr>
<th>Fixed Effects:</th>
<th>ns_slope</th>
<th>js_slope</th>
<th>eu_slope</th>
<th>dys_slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ns, Js, Eu/Dys model</td>
<td>0.0001</td>
<td>0.0003</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**Covariance structure:**
- independence
- random intercept

<table>
<thead>
<tr>
<th>ns_slope</th>
<th>js_slope</th>
<th>eu_slope</th>
<th>dys_slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001</td>
<td>0.0002</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**P-value structure:**
- 0.0001
- 0.0002
- 0.0001
- 0.0001

This is the starting point for modeling TBC_12.