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Physical activity has been shown to augment bone mineral; however, in some female athletes, extremely intense training may result in menstrual cycle irregularities, leading to bone loss. Forty-four women (aged 20.3±2.1 years, mean±S.D.) completed the 8-month study: 12 gymnasts (G), 18 runners (R) and 14 controls (C). Approximately one-third of each athletic group exhibited amenorrhea and oligomenorrhea; all control subjects were eumenorrheic. The gymnasts had a significantly later age at menarche (16.0±1.5 yr) compared to the runners (14.1±1.4 yr) and controls (13.0±1.2 yr). Bone mineral density (BMD) at the lumbar spine (LS) (R<G,C), femoral neck (FN) (G>C>R) and whole body (WB) (G>R) were significantly different ($p<0.05$) among the groups at baseline. Percent change scores for BMD were as follows: G>C,R for % change in LS BMD and FN BMD; the % change in WB BMD did not differ among groups. The 2.9±2.5% change in LS BMD in the gymnasts was significantly different from zero, as was the 0.7±1.3% change in LS BMD in the controls. The 1.9±1.6% and 1.4±0.7% change in WB BMD in the runners and gymnasts (respectively) were both significantly different from zero. Menstrual cycle status had a significant effect on % change in BMD which varied depending on skeletal site and athletic group. The % change in LS BMD in the eumenorrheic runners (ER) was significantly different compared to the oligo/amenorrheic runners (OAR): 1.0±2.1% and -0.9±1.5%, respectively. The % change in LS BMD in the eumenorrheic

gymnasts (EG) was significantly greater compared to the eumenorrheic controls (EC): 3.6±2.0% and 0.7±1.3%, respectively. The % change in FN BMD was significantly different in the EG (2.7±2.8%) compared to the ER (-1.3±2.6%) and EC (-0.9±2.2%). The % change in WB BMD did not differ among the groups by menstrual cycle status. There was a tendency (non-significant due to small sample sizes) for asymptomatic eumenorrheic control subjects and gymnasts (at the LS only) with a shortened luteal phase or anovulatory cycle to exhibit less of an increase or even a decrease in BMD over time compared to normal eumenorrheic gymnasts and controls. The gymnasts had significantly greater dietary calcium:phosphorus and calcium:protein ratios compared to the runners and controls. It is hypothesized that higher impact loads on the skeleton from gymnastics training and greater calcium availability among gymnasts accounted for higher initial BMD in gymnasts and may partially explain why the gymnasts tended to improve BMD over time. Additionally, estrogen status appears to potentiate the effect of exercise on bone mineral accretion, particularly at the lumbar spine.

BONE MINERAL AND MENSTRUAL CYCLE STATUS IN COMPETITIVE FEMALE ATHLETES: A LONGITUDINAL STUDY

by

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INTRODUCTION

Regular physical activity has a beneficial effect on many systems and organs of the body, including the skeletal system. There is considerable cross-sectional evidence showing that athletes have greater bone mass compared to non-athletes (Dalen & Olsson, 1974; Dalsky, 1987,1989,1990; Huddleston et al., 1980; Lane et al., 1986; Marcus et al., 1985). In some women, however, long-term, strenuous physical activity may lead to menstrual cycle irregularities, including secondary or exercise-associated amenorrhea (Boyden et al., 1982, 1983; Prior et al., 1990). In many cases, the skeletal status of these athletic women is compromised due to the low estrogen levels which accompany amenorrhea (Marcus et al., 1985; Prior et al., 1990). Several cross-sectional studies have reported that amenorrheic athletes have a reduced bone mass compared to regularly menstruating (eumenorrheic) athletes (Cann et al., 1984; Cook et al., 1987; Drinkwater, 1984; Fisher et al., 1986; Lindberg et al., 1984; Marcus et al., 1985; Myburgh et al., 1990). It is becoming increasingly clear that reduced lumbar bone mineral density in amenorrheic athletes is evidence that regular weight-bearing exercise cannot compensate for the effect of estrogen deficiency on these women's bones (Dalsky, 1990). Women with low bone mass in premenopausal years may then be at an increased risk for osteoporosis (Cann et al., 1984; Cook et al., 1987; Drinkwater et al., 1984; Klibanski et al., 1988; Raisz, 1985). The consequences of menstrual cycle irregularities, and strenuous, long-time athletic training, leading to reduced bone mineral density, may predispose these women to osteoporosis at an earlier postmenopausal age (Cook et al., 1987). Perhaps of more immediate concern is their increased incidence of musculoskeletal injuries, specifically

stress fractures (Barrow & Saha, 1988; Cann et al., 1984; Lloyd et al., 1987; Lloyd et al., 1986; Myburgh et al., 1990).

Chronic physical activity may result, not only in secondary amenorrhea, but possibly in other menstrual cycle disturbances such as a delayed age at menarche, oligomenorrhea (i.e., sporadic menstrual cycles), or a shortened luteal phase of the menstrual cycle (from ovulation to the onset of menstruation) (Dale et al., 1979; Feicht et al., 1978; Malina et al., 1978; Shangold & Levine, 1982). In the non-symptomatic case of a shortened luteal phase, normal cycle length (28$\pm$5 days) and menses were evident in women during marathon training (Prior et al., 1982), and yet, the premenstrual (luteal) phase of the cycle was short or the women had anovulatory cycles. Thus, abnormal reproductive hormone levels in these women (i.e., decreased progesterone) may go undetected (Shangold et al., 1979). Progesterone may play an important role in bone metabolism (Prior et al., 1990), and thus, diminished production of progesterone in women with luteal phase deficiency or anovulatory (monophasic) cycles may be associated with an accelerated bone loss. Prior et al. (1990) have shown that these menstrual disturbances (i.e., shortened luteal phase and anovulatory cycles) occurred in the presence of normal estrogen levels and normal cycle intervals, and were associated with a loss of spinal bone density of 4% in 12 months. Additional research needs to be conducted to substantiate these results.

Amenorrhea and oligomenorrhea are quite prevalent in women participating in certain athletic events. Runners have a much higher incidence of sporadic or missed menstrual cycles compared to swimmers and cyclists, and even more so if they are competitive (Carli et al., 1983; Feicht et al., 1978; Fisher et al., 1986; Sanborn et al., 1982; Shangold & Levine, 1982). Estimates of menstrual cycle dysfunction range from 12% to 43% of female athletes, with a higher incidence typically reported for runners, possibly related to training distance. Study results are equivocal, however, usually because the definitions of amenorrhea and oligomenorrhea differ among studies (Highet, 1989). In a

1989 study by Cavanaugh and colleagues, menstrual cycle irregularities (defined as amenorrhea and oligomenorrhea) were found in many highly competitive women athletes: 45% of field hockey players, 67% of gymnasts, 24% of modern dancers, 17% of ballet dancers and tennis players, and 37% of track and field athletes. In any case, these researchers agree that the prevalence of amenorrhea and oligomenorrhea is much greater in college athletes compared to non-athletic college-aged women. Bachmann and Kemmann (1982) reported oligomenorrhea in approximately 11% of college students, with approximately 2.5% being amenorrheic. They attributed these menstrual cycle irregularities in non-athletic women to excessive weight loss from calorie-restricted diets and the stress associated with college life.

The etiology of menstrual cycle irregularities has not been completely defined. Various factors have been suggested, at one time or another, as playing a role in the development of these menstrual cycle irregularities. Cavanaugh et al. (1989) developed a model to predict menstrual cycle irregularity in women athletes which included the following factors: pre-training frequency of menstruation, weight, trait anxiety, age at menarche, trait anger, trait curiosity and percent body fat. These factors, in this order, accounted for 36% of the variance in athletic oligo/amenorrhea. Additional factors which have been identified to play a role in menstrual cycle irregularities include: menstrual history (i.e., prior menstrual cycle irregularity); age; poor dietary habits (i.e., low energy intake); high weekly running mileage; and physical and emotional stress, related to exercise and life in general (Dale et al., 1979; Loucks & Horvath, 1984, 1985; Malina, 1983; Schwartz et al., 1981). Malina (1988) suggests a role for "energy drain"; menstrual cycle irregularities or a delayed age at menarche may be a protective mechanism for the body (i.e., to conserve energy stores for more vital, compared to reproductive, physiological processes).

Findings from numerous studies investigating one or more of these factors proposed to affect the menstrual cycle have been equivocal. For example, many studies

have investigated the question of whether there is a threshold training level, in terms of miles run per week, above which the incidence of menstrual cycle irregularities (especially amenorrhea) increases dramatically. Several researchers have shown a positive, even linear, relationship between training mileage and the prevalence of amenorrhea (Dale et al., 1979; Drinkwater et al., 1984, 1986; Feicht et al., 1978; Sanborn et al., 1982), while others have found no such relationship (Baker et al., 1981; Barrow & Saha, 1988; Speroff & Redwine, 1980; Wakat et al., 1982). None of these studies investigated the role of intensity of training (i.e., $\%VO_{2max}$ at which these athletes trained) or training pace as possible related factors affecting the menstrual cycle.

The combined or interactive effects of exercise and female reproductive hormone status on bone mineral density have been questioned in athletes who train intensively for competition. A recent longitudinal study by Puustjarvi and colleagues (1992) showed that estrogen levels and lumbar spine BMD were reduced following a 55-week progressive running program in female dogs. These adverse effects on bone were associated with the decreased serum estrogen levels. Frost (1992) proposed a "mechanostat" theory for the role of exercise and estrogen on bone. The author described a setpoint for bone that is mediated by estrogen and mechanical stimuli (e.g., physical activity). In this model, intense mechanical usage or hyperactivity stimulates modeling drifts (i.e., uncoupled osteoclast-osteoblast processes) at bone sites which result in a net bone gain until an adaptation occurs to the increased mechanical loads and a new mechanical usage setpoint is reached. Hyperactivity lowers this setpoint, whereas the setpoint is raised by disuse. According to Frost's model, estrogen operates in the same way as mechanical usage; i.e., high or normal estrogen levels lower the modeling and remodeling setpoints whereas low estrogen levels raise them. In the latter case (i.e., in amenorrheic athletes), higher mechanical loads are then required to maintain or increase bone mass. A review by Snow-Harter and Marcus (1991) pointed out that research still needs to address certain variables such as the frequency, intensity, duration, and type of exercise, and the interactions, when

investigating its effect(s) on bone. Studies need to be controlled as much as possible for confounding variables, so that the etiology of menstrual cycle irregularities and their role in bone metabolism may be determined.

Baker and colleagues (1981) found the incidence of amenorrhea among their runners to be 39%, which is similar to that reported by other researchers (e.g., in 1979, Dale et al. found a 34% incidence). Baker's group (1981) pointed out, however, the conflicting findings from many studies on the hormonal responses to exercise. They suggested that this was due to confounding factors such as prior menstrual cycle dysfunction, prior gravidity, running mileage, weight loss and changes in percent body fat, only some of which were typically controlled for in any one research project. Ronkainen and colleagues (1985) suggested the need for more longitudinal studies to investigate the hormonal responses to a prolonged training program. This group reported seasonal training differences which resulted in varying disturbances of the menstrual cycle. They proposed that the spring season consisted of higher intensity training and hence, an increased incidence of menstrual cycle irregularities. Carli et al. (1983) noted the importance of longitudinal studies investigating the effects of training progressions on hormonal status because of the large variance in individual basal levels. These researchers found that hormone levels (including estradiol and cortisol) were modified by training, but at different times during the training season. Cross-sectional studies, therefore, would be unable to discern the temporal pattern and interaction of hormones in response to long-term exercise.

Both estrogen and progesterone levels may be affected by intensive training in competitive female athletes. Along with exercise-associated amenorrhea and oligomenorrhea (in which estrogen levels are extremely low), lack of sufficient progesterone can also be associated with strenuous exercise (Prior et al., 1990; Snow and Anderson, 1986). Progesterone acts to promote bone deposition and accelerates the remodelling process, so low levels may have a negative effect on bone mineral density. Drinkwater and colleagues (1990) showed that physically active women who had menstrual

cycle irregularities (oligo- and amenorrhea) had severely diminished plasma progesterone levels, as well as significantly lower bone mineral density in the lumbar vertebrae (L_{1-4}) and shaft of the femur compared to regularly menstruating women. This was one of the few studies that has investigated bone mineral density at the femoral neck. The proximal femur, specifically the neck of the femur, is often the site with the most potential for fracture later in life (Highet, 1989). In an earlier study, Drinkwater et al. (1984) had shown that peak progesterone concentrations for amenorrheic athletes were less than one-tenth those for eumenorrheic athletes. The researchers proposed that the decreased progesterone associated with a shortened luteal phase or anovulatory cycle may have a deleterious effect on bone. Prior et al. (1990) found that low levels of progesterone resulted in a significant reduction in spinal bone mineral density (4% in one year). This loss occurred even though the women appeared to have normal menstrual cycles, i.e., they were asymptomatic.

Psychological stress may also play a significant role in menstrual cycle irregularities and hence, could have detrimental effects on bone. Even in an non-athletic population of female college students, the prevalence of amenorrhea is 2.6%, while 11.3% reported having oligomenorrhea (Bachmann and Kemmann, 1982). These researchers concluded that college-related stress appeared to interact with weight loss and jogging to induce menstrual cycle dysfunction (i.e., missed cycles). They suggested that factors such as leaving home, separation from family, and perceived excessive pressures of school may negatively influence menstrual cycle status. High levels of subjective stress have been reported in women athletes, more so in amenorrheic runners (Schwartz et al., 1981). These researchers suggested an interactive role of stress and poor nutrition, specifically inadequate caloric intake, with exercise, which resulted in menstrual cycle disturbances. Cortisol is a hormone which appears to play an important role in the menstrual cycle irregularity and bone health relationship. Villanueva et al. (1986) found significantly elevated basal serum cortisol levels in women runners compared to sedentary controls. They proposed that this was due to a greater secretion of cortisol, rather than a decreased

peripheral metabolism (as seen in anorexic women). This was supported by findings of higher 24-hour urinary cortisol levels in the runners, a more meaningful measure of secretion than single serum measures, which are subject to pulsatile fluctuations.

Diet may also play an interactive role with exercise and menstruation on bone mineral status in athletic women. Nelson et al. (1986) found that amenorrheic runners had a significantly lower caloric intake, and carbohydrate and fat intake compared to eumenorrheic runners. The amenorrheic runners also had significantly lower lumbar vertebrae bone mineral density and estradiol levels. Surprisingly, calcium intake was not significantly different between the two groups, although the amenorrheic athletes took in less than 900 mg/day compared to 1150 mg/day for the eumenorrheic women. These authors proposed that the decreased estrogen levels and slightly lower calcium intake of the amenorrheic runners act to alter calcium metabolism. These women may have an increased requirement for calcium since low estrogen levels lead to a lower intestinal absorption of calcium and an increased urinary calcium loss. Baran and colleagues (1990) studied the effect of increased calcium intake (through dairy product supplementation) on vertebral bone mass over a 3-year period. They concluded that increasing dietary calcium in eumenorrheic women may prevent age-related bone loss, even though it did not appear to increase vertebral bone mineral density in this age group (30 to 42 years).

Due to the concern of maintaining a low body weight (i.e., for performance or aesthetic reasons), some female athletes (especially runners and gymnasts) may not follow sound and sufficient dietary practices, even if they have good nutrition knowledge (Perron & Endres, 1985). Often, the milk group and dairy products in general are neglected, and thus, calcium and Vitamin D intakes are low in these female athletes. Perron and Endres (1985) found that 92% of the female athletes questioned knew that milk was a good source of calcium, but only 12% of their diets met the recommended dietary allowance (RDA) for calcium, with the average diet containing less than 67% of the RDA. This could have a significantly negative effect on the deposition of new bone mineral in the growth years.

Nieman et al. (1989) found that eumenorrheic female marathon runners had dietary calcium intakes slightly below the RDA and their Vitamin D intake was less than two-thirds of the RDA. Deuster et al. (1986) found that the fat intake of amenorrheic runners was significantly less than their eumenorrheic counterparts. The avoidance of "fattening" dairy products may play a role in diet composition of these highly-trained runners. A study by Benardot et al. (1989) found similarly insufficient diets in competitive gymnasts. This group's diet was below the RDA for several nutrients, including calcium. In the age group of gymnasts studied (7 to 14 years), abundant calcium is required to maintain the mineral of the growing skeleton (Avioli, 1988; Marcus, 1987).

Gymnasts are a group of athletes whose training regimens differ substantially from the training of endurance runners (e.g., predominantly anaerobic versus aerobic energy systems used), but often exhibit similar profiles in terms of inadequate nutrition (Benardot & Czerwinski, 1991), slight stature and extremely low percent body fat (Barlett et al., 1984; Benardot & Czerwinski, 1991; Johnson et al., 1989; Montpetit, 1976), menstrual cycle irregularities (Loucks, 1990) and possibly, low bone mass. To date, no studies have investigated the possibility of a link between menstrual cycle irregularities, training intensity, body composition and poor nutritional habits on the bone mineral of female gymnasts compared to runners. The gymnasts do exhibit much higher levels of strength and power (Montpetit, 1976), and this may aid in stimulating bone deposition. Snow-Harter et al. (1990) have shown that muscle strength is a significant predictor of bone mineral in women at the hip and the spine, specifically the pull of the muscle attachments on the bone during muscular contraction. In fact, as early as 1970, Doyle and colleagues showed that the weight of the psoas muscle was significantly correlated to the ash weight of the third lumbar vertebrae. A heavier muscle exerts more force on the bone(s) to which it attaches, augmenting bone mass. Thus, muscular contractions strong enough to stimulate bone mass through the muscle's attachment to the skeleton may serve to offset the adverse effects of menstrual cycle irregularities, low impact/high repetition exercise training,

inadequate nutrient intake (especially protein and calcium), and extremely low percent body fat on bone mineral in female athletes. This proposition must be fully investigated.

Statement of the Problem

The purpose of this study was to evaluate factors proposed to alter bone mineral in competitive collegiate female athletes over a 9-month training period. It was hypothesized that a significant reduction in bone mass would occur over this time period in those athletes experiencing the greatest incidence of menstrual cycle irregularities. Strenuous training regimens (high intensity and volume), inadequate dietary intake of nutrients such as protein and calcium, low body weight and percent body fat, and high stress/anxiety levels were factors proposed to increase the incidence of menstrual cycle abnormalities and thus, reduce bone mass. Two groups of competitive athletes, who train very different energy/muscular systems but who both experience menstrual cycle irregularities, were investigated and compared to a non-competitive control group of college-aged women.

Research Hypotheses

It was hypothesized that over a 9-month period of time:

1) Lumbar spine bone mineral density would significantly decline in athletes with amenorrhea and oligomenorrhea compared to eumenorrheic athletes and non-athletic controls;

2) Lumbar spine bone mineral density would significantly increase in eumenorrheic gymnasts and runners compared to eumenorrheic but non-athletic controls;

3) Femoral neck bone mineral density would significantly increase in eumenorrheic gymnasts and runners compared to controls;

4) Whole body bone mineral density would significantly increase in the eumenorrheic gymnasts compared to the runners and controls;

5) Women with a shortened luteal phase would exhibit a significant reduction in lumbar spine bone mineral density compared to eumenorrheic women with a normal luteal phase length;

6) The incidence of menstrual cycle irregularities would be greater in the runners compared to the gymnasts (note: all control subjects were recruited to be eumenorrheic);

7) Training, inadequate nutrition, and psychological stress/anxiety levels would be greater in the athletes compared to controls; and

8) High training levels, inadequate nutrition, and high stress/anxiety levels would be greatest in those athletes who are amenorrheic or oligomenorrheic, compared to eumenorrheic athletes.

Operational Definitions

The independent and dependent variables were operationally defined for this study as follows:

Amenorrhea was defined as having three or less menstrual periods a year and none in the preceding six months. Oligomenorrhea was defined as having sporadic menstrual periods, between four and nine a year. Eumenorrhea was defined as having ten or more menstrual periods a year.

A shortened luteal phase length (LPL) was considered present if the luteal phase of the menstrual cycle (from ovulation to the onset of menstruation) was less than ten days. This was confirmed by monitoring the menstrual cycle daily for a full cycle, with five milliliter urine samples taken each morning, on two different occasions. Urinary progesterone metabolites were measured by enzyme-immunoassay (EIA).

Runners were defined as women, aged 18 to 30 years, who were, and had for at least the past year, been running, i.e., a minimum of four to five days per week and a mileage of at least thirty miles per week, and were training intensively for track and cross-country racing in events ranging from the 800 meters to 10,000 meters. Two groups of runners were defined according to menstrual cycle status: eumenorrheic runners (ER) and oligo/amenorrheic runners (OAR).

Gymnasts were collegiate women training for Pac-10 and NCAA competition. They had been training intensively in gymnastics for at least one year prior to the study. Two groups of gymnasts were defined according to menstrual cycle status: eumenorrheic gymnasts (EG) and oligo/amenorrheic gymnasts (OAG).

Control subjects were non-competitive female controls, 18 to 30 years of age, who participated in regular, structured physical activity less than three hours per week. All control subjects were eumenorrheic (EC).

Training intensity and volume were monitored over the course of consecutive fall, winter and spring training seasons. Training was quantified using daily activity logs and coaches' workout schedules. Intensity was determined from pace of runs and type of workout; e.g., number of "intense" sessions per week (intervals, fartlek, tempo runs, hill, races) compared to "easy" distance runs. Volume of training was determined by average weekly mileage.

Bone mineral density (BMD) was measured using a dual-energy X-ray absorptiometer (Hologic QDR-1000/W). Bone mineral content was quantified in grams of hydroxyapatite (g), and BMD in grams per square centimeter (g/cm^2), for the lumbar spine (L_{2-4}), proximal femur (specifically femoral neck) and whole body.

Psychological stress/anxiety levels were measured on a seasonal basis (i.e., on four different occasions), using the Profile of Mood States (POMS) and State-Trait Anxiety Inventory (STAI) psychological questionnaires. A total mood disturbance (TMD) score

was calculated for the POMS. State and trait anxiety scores were determined from the STAI.

Nutrient intake was measured using 4-day diet records and was considered inadequate when the diet failed to meet two-thirds of the RDA for total calories, protein, fat, carbohydrate, calcium and/or Vitamin D.

Muscle strength was measured using the Kin-Com isokinetic machine, and considered to be the gravity-corrected peak torque achieved for each muscle group tested (i.e., trunk extensors, hip abductors and adductors, elbow flexors, leg extensors).

Definitions

Other terms that may be useful to the reader were defined as follows:

Menstrual cycle irregularity or dysfunction was considered to be an absence of menstrual cycles, sporadic menstruation, an alteration in the normal menstrual cycle length (28 ± 5 days) or an alteration in the normal female reproductive hormone profile. This included amenorrhea, oligomenorrhea, a shortened luteal phase and anovulatory cycles, respectively.

Stress was defined as the level of psychological and social response to anxiety and strain experienced by these women over a 9-month training period.

Assumptions

Certain assumptions were made with regard to this research project. First, since training intensity and mileage were monitored by the subjects, using log books, it was assumed that the subjects kept exact and truthful logs. Secondly, the subjects were required to collect small urine samples (about 5 ml) on a daily basis for a complete menstrual cycle on two different occasions; thus, diligence and accuracy of measurement and recording was

required. Urine samples needed to be frozen until collected by the investigator. Thirdly, it was assumed that the subjects answered all psychological stress questionnaires truthfully. Fourthly, it was assumed that the subjects would keep their 4-day diet records (on four different occasions) honestly and completely, without altering their normal dietary habits.

Delimitations

The scope of this study was defined by several delimitations. The results are only generalizable to women runners and gymnasts who have similar characteristics as these subjects (e.g., similar seasonal training intensity, mileage, and similar age and body composition). These subjects were monitored over a 9-month training period (i.e., fall, winter and spring seasons), and thus, the results pertain to such training variations. That is, seasons (for runners) may include cross-country, indoor and outdoor track, respectively, or some other scheduled program. Menstrual cycle irregularity or dysfunction was defined in terms of amenorrhea, oligomenorrhea and luteal phase deficiency, typically resulting from intensive training (i.e., exercise-induced), so changes in the menstrual cycle as a result of other factors (e.g., hypothalamic dysfunction) were not considered when looking at the effects on bone mineral.

Limitations

Some of the limitations or shortcomings of this study resulted from the above-mentioned delimitations, or restrictions in scope. Subject selection was not random, since the runners and gymnasts were recruited from the University of Oregon and Oregon State University collegiate teams, respectively. Non-competitive female controls were college-aged women who were eumenorrheic (which is the majority of non-athletic women). Also, due to the length of this longitudinal study and the measurements required, the goal was to

recruit highly motivated and dedicated volunteers. Participants were recruited from several areas of the Willamette Valley to ensure sufficient subject numbers upon completion of the study. Even so, sample size was small. Also, since the study lasted "only" nine months, less change in bone mineral may have occurred than in a more long-term study.

LITERATURE REVIEW

This chapter will review the current literature as it relates to bone health and menstrual cycle status of competitive female athletes (runners and gymnasts) over a 9-month training period compared to non-competitive, college-aged controls. The primary concern is to minimize the risk of skeletal damage (e.g., stress fractures) and future osteoporosis in these athletes, who may be predisposed to these conditions due to menstrual cycle irregularities. Bone mineral content and bone mineral density (BMD) are dynamic measures of skeletal integrity, and can be influenced by numerous factors, and their interactions, which will be discussed in this chapter. These include:

1) bone dynamics as a function of age (e.g., remodelling);

2) general physical activity;

3) reproductive hormone status (specifically estrogen and progesterone) and menstrual cycle abnormalities such as shortened luteal phase and anovulatory cycles;

4) menstrual history, including age at menarche;

5) diet (specifically calcium, protein and total caloric intake);

6) psychological stress and anxiety; and

7) specificity of training, including mode, intensity and volume of exercise and strength of muscular contractions.

The methods used to assess bone mass and bone mineral density will also be discussed.

Osteoporosis as a Result of Menstrual Irregularity

Osteoporosis can generally be defined as a reduction in the bone mass for a given volume. In postmenopausal women, the natural loss of estrogen has been associated with an accelerated loss of bone mineral and an increased incidence of osteoporosis (Cann et al., 1984; Dalsky et al., 1988; Highet, 1989; Riggs, 1986). Premenopausal women who have

temporarily stopped menstruating and thus, have amenorrhea due in many cases to intense training (Marcus et al., 1985; Prior et al., 1990) mimic the postmenopausal response to estrogen deficiency. These women with exercise-induced amenorrhea also have an accelerated rate of bone loss due to low estrogen levels. Lack of sufficient progesterone can be exercise-induced (i.e., in cycles with a shortened luteal phase) and can also negatively affect bone metabolism, since progesterone plays a role in promoting bone formation and accelerating the remodelling process (Prior et al., 1990; Snow & Anderson, 1986).

The prevalence of menstrual irregularities in female athletes is surprisingly high -up to 44% - (Loucks, 1990; Sanborn et al., 1982; Prior et al., 1982; Shangold & Levine, 1982; Shangold et al., 1990) and the long-term consequences are certainly a concern. Runners appear to be at particular risk of developing amenorrhea. Sanborn et al. (1982) found that the prevalence of amenorrhea in runners was 25.7% compared to 12.3% for swimmers and 12.1% for cyclists. There are many factors which may predispose a female athlete to manifest menstrual cycle irregularities. Not all of these are understood and there may be some confounding variables yet to be identified. Some of these factors which have been suggested as playing a role in amenorrhea, oligomenorrhea (sporadic menstrual cycles), shortened luteal phase and/or anovulatory cycles include: prior menstrual history, age at menarche, body composition, sudden and severe weight loss, poor diet, high stress levels, type (mode) and amount (duration) of exercise, and intensity of training (Dale et al., 1979; Highet, 1989; Loucks & Horvath, 1984, 1985; Marti, 1991; Schwartz et al., 1981). Several of these factors will be the focus of this study.

Although the development of osteoporosis may be a distant future possibility in these extremely active female athletes, the competitive environment, including the coaches, parents and the athletes themselves, tends to focus on the present and immediate future (e.g., one or two years). Often, amenorrhea is accepted as a fact of intense training, and often as a blessing, (e.g., rather not have to deal with menstruation), and future implications are ignored, if, in fact, they're even known. Injuries, however, will interrupt

an athlete's training program, and if severe enough (e.g., repeated stress fractures), may end her career. It has been reported that there is an increased incidence of musculoskeletal injuries in runners who exhibit irregular menstrual cycle function (Barrow & Saha, 1988; Lloyd et al., 1986; Marti, 1991; Myburgh et al., 1990). The study by Barrow and Saha (1988) found that, along with amenorrhea, those runners with a prolonged history of oligomenorrhea were more likely to sustain a stress fracture. Lloyd and co-workers (1986) found that women with a history of menstrual irregularities had a frequency of stress fractures three to four times greater than regularly menstruating women. In a study by Myburgh et al. (1990), significantly more injured athletes (with stress fractures) had menstrual cycle irregularities than non-injured athletes. The bone mineral density in the athletes with fractures was also significantly lower than the non-injured controls in the lumbar spine and femoral neck. This study and the research of Barrow and Saha (1988) both found that the injured athletes had a lower incidence of oral contraceptive use. They suggest that estrogen, via oral contraceptive use, may be beneficial by protecting against stress fractures. This is akin to treating postmenopausal women with estrogen in order to slow the decline in bone mineral content and reduce the fracture risk (Snow-Harter and Marcus, 1991; Worley, 1981). Estrogen acts by inhibiting bone resorption (Gallagher & Nordin, 1975), apparently by acting directly on the bone and not through specific estrogen receptors (vanPaassen et al., 1978). A study by Lloyd and colleagues (1989), however, concluded that long-term use of oral contraceptives in premenopausal women (longer than five years on average) had no effect (positive or negative) on lumbar vertebrae bone mineral density. The differences in the findings of these studies may be due to variations in the dosage of estrogen in the oral contraceptives taken by the women, as well as their own endogenous estrogen levels, or the site of bone measurement (i.e., cortical versus trabecular bone).

In addition to the study by Myburgh and colleagues (1990), few researchers have studied bone mineral in the hip (proximal femur), usually looking solely at the lumbar

spine. Osteoporotic fractures of the hip can be very debilitating, and fractures of the proximal femur result in the greatest morbidity, mortality and socio-economic cost (Cummings & Nevitt, 1989; Riggs & Melton, 1986; Riggs et al., 1986). Cummings and Nevitt (1989) hypothesized a cause for hip fractures, 80% to 90% of which are due to falls in the elderly, which involves four factors. One important factor and the "last defense" against hip fracture is that the bone strength of the proximal femur is insufficient to resist the energy that is transmitted to the hip during the fall. The strength of the proximal femur is determined by its bone mineral and its architecture. Women with hip fractures have decreased mineral in the bone's cortical shell, and fewer and thinner trabeculae. Heaney (1989) stated that a fracture of the femoral neck would be primarily due to low bone mass, but the additional component of accumulated fatigue damage (from excessive stress or over-use) also contributes to the risk of fracture at this site. In fact, low bone mass may aggravate the condition Heaney (1989) defined as "fragility of fatigue damage". As bone mass is lost, the same amount of mechanical loading at the skeletal site will result in higher levels of strain on the remaining bony structures, which would result in further fatigue damage that the bone is unable to adapt to, and the cycle becomes perpetuated. If skeletal loading is increased (e.g., higher training intensity), the already weakened skeletal site is at a much greater risk of fracture. In fact, Burr and colleagues (1985) stated that fatigue damage is a local stimulant for bone remodeling; however, if the remodeling process is unable to keep pace with the level of fatigue damage at that particular skeletal site, bone fragility and subsequent fracture will occur, regardless of bone mass. Dugowson et al. (1991) reported the first case of a non-traumatic femur fracture in a premenopausal female athlete, who had a long history (eight years) of oligo/amenorrhea. These researchers proposed that this 32-year old woman's fracture was caused by severe osteoporosis secondary to her history of menstrual cycle irregularities. The concern is that this athlete (and many presumably like her) has lost bone mineral while amenorrheic that she cannot completely regain, even with the resumption of menstruation. Thus, she is at an increased

risk of osteoporotic fracture in the future. In the case of luteal phase deficiency, where the athlete is asymptomatic (i.e., apparently normal menses), irreversible damage to the bone may reduce the fracture threshold (Drinkwater et al., 1984; Prior et al., 1990) while the athlete remains oblivious and continues strenuous training.

Although it was first identified in women distance runners and still is most frequent in this group of athletes, menstrual cycle dysfunction (e.g., amenorrhea) is not just a characteristic of these women (Highet, 1989). It appears to be a function of the exercise intensity, more so than the mode of training. Sanborn et al. (1982) found, however, that runners and ballet dancers had a higher incidence of menstrual cycle changes than cyclists and swimmers. Highet (1989) proposed a role for thermodynamics in athletic amenorrhea in that the tremendous amount of heat generated from activities such as running may upset the normal hypothalamic function. Snyder et al. (1986) propose, however, that mode of training is one important variable related to exercise-induced amenorrhea. They reported a high incidence of menstrual cycle irregularity among "lightweight" rowers (weighing less than 130 pounds) but no compromised bone mass in the vertebrae of these women. They concluded that the different type of activity involved in rowing, compared to running for example, protected these women from losing bone mineral despite their low levels of estrogen. These researchers suggested that their findings support the frequent reports in the literature that exercise-induced amenorrhea is: 1) not directly related to body composition, since the amenorrheic rowers did not differ from the eumenorrheic or oligomenorrheic rowers in terms of body weight or percent fat; and 2) not related to intensity of exercise, although they based this statement on the fact that the amenorrheic rowers had been rowing competitively for the same number of years as the eumenorrheic and oligomenorrheic rowers.

Drinkwater et al. (1984) compared the training regimens of amenorrheic and eumenorrheic athletes (primarily runners) in an attempt to delineate the role of frequency, intensity and duration of training in the onset of menstrual cycle dysfunction. The two

groups did not differ in the numbers of years participating in the sport, the number of hours per day and days per week spent training, their 10-km race time or training pace. The amenorrheic athletes, however, ran significantly more miles per week than the regularly menstruating athletes. The amenorrheic runners also exhibited significantly lower estradiol and progesterone levels, and their vertebral BMD was also lower than the eumenorrheic athletes. Findings by Carli et al. (1983) concurred that the increased stress of training over the course of a competitive season is associated with menstrual cycle changes. A study by Puustjarvi and colleagues (1992) showed that long-term running in dogs (approximately 14 months of up to 40 km/day) resulted in lower bone mineral density (particularly at the lumbar spine) compared to non-running control dogs. They concluded that this was associated with a decrease in serum estrogen levels in the running dogs. Loucks (1990) suggested that a high volume of intense aerobic training has the potential to disrupt menstrual cycle function in some women, and that it is related to alterations in the hypothalamic-pituitary-adrenal axis. Luteal phase suppression may be an intermediate result in the progression from menstrual regularity to amenorrhea. More intriguing is Loucks' (1990) suggestion that luteal phase deficiency may be the endpoint of a successful acclimation to exercise training.

Bone Mineral Density

The skeleton consists of cortical and trabecular bone. Cortical, or compact, bone is located primarily in the peripheral skeleton (e.g., the shafts of long, limb bones), while trabecular, or cancellous bone makes up most of the axial skeleton (Snow-Harter & Marcus, 1991). Due to the large surface area of trabecular bone (compared to cortical bone), changes in bone mass are evident earlier and to a greater extent in the trabecular skeleton (Marcus, 1987). Several researchers (Jones et al., 1985; Marcus, 1987; Marcus et al., 1985; Parfitt, 1982; Wolman, 1990) have shown that bone turnover is much more

rapid in the highly metabolic trabecular bone sites (e.g., lumbar spine, femoral neck) compared to the more compact and less metabolically active cortical bone sites (e.g., most of the appendicular skeleton). Parfitt (1982) stated that cortical bone had a turnover rate of approximately 3% per year, while the turnover rate of trabecular bone was almost 30% per year. In fact, Ruegsegger et al. (1991) reported significantly different patterns of bone loss over two years depending on whether the skeletal site measured consisted of primarily cortical or trabecular bone. These researchers found that in a group of healthy postmenopausal women, trabecular bone density of the distal radius declined 2.8% per year, while cortical bone density of the diaphyseal site of the radius declined non-significantly (0.2%).

Changes in bone mass can result from increased activity or inactivity, as bone adapts to the functional load placed on it. Bone is constantly being remodelled, a balance between bone resorption and bone formation (Snow-Harter & Marcus, 1991). The bone adapts to weight-bearing forces, as well as to forces generated by contracting muscles. Rubin and Lanyon (1984, 1985) showed that bone mass was correlated with functional loading above a certain optimal level of strain which is necessary to maintain bone mass. Repetitive loading beyond the bone's ability to adapt, however, could lead to a weakening of the bone and eventual fracture (Carter et al., 1981). Rubin and Lanyon (1984, 1985) proposed that the magnitude of skeletal loading is more important for bone deposition and maintenance than the number of cycles or repetitions. Peak vertical forces generated at impact on the skeleton during gymnastics training may approach six to fourteen times body weight (McNitt-Gray et al., 1993; Panzer et al., 1988), while those during running are approximately two to five times body weight (Williams, 1993). Running does include more repetitions or cycles of foot strike than gymnastics, but these lower loads may not be as stimulatory to bone. Williams (1993) stated that most of the impact loading during running is transmitted through the feet and lower extremities and is dissipated as it travels up the skeleton, so that by the time these vertical forces reach the trunk (i.e., lumbar spine), they

are substantially diminished. In gymnastics training, however, strong muscular forces throughout the body and extremely high impact forces tend to stimulate more of the skeleton and to a greater extent compared to running. Panzer and colleagues (1988) measured peak vertical forces at landing from a double back somersault (common in gymnasts' floor exercises) as high as eight to fourteen times body weight. These forces were greater at the knee joint compared to the hip, although forces experienced at the hip were still extremely high, and would be indicative of the loads which the spine would be subjected to. McNitt-Gray and colleagues (1993) recently studied the peak impact forces experienced by gymnasts landing from the balance beam and uneven parallel bars (i.e., heights exceeding 1.5 meters) during dismounts. Using a force plate embedded in a normal gym mat, they measured peak vertical forces between six and ten times body weight, with the higher forces observed as a result of dismounts from higher heights (e.g., uneven parallel bars). Joint forces and peak angular velocities were highest at the ankle and knee (i.e., joints closest to the point of force application), and somewhat dissipated at the hip. At the higher heights, the degree of joint flexion on landing was increased, suggesting more muscle involvement to lessen and more widely distribute the impact forces of landing. A recent study by Grimston and colleagues (1993) investigated the effects of "impact load" training (i.e., running, dance, gymnastics and tumbling) compared to "active load" training (i.e., swimming) on bone mass in children aged 10 to 16 years, who were matched for race, gender, stage of puberty and body weight. They found that in weight-bearing activities which generated forces in the range of three to ten times body weight, femoral neck BMD was significantly augmented and there was a trend for higher lumbar spine BMD values. In comparison, the children who participated in "active load" activities which do not involve the effect of gravitational forces on the skeleton but only those resulting from muscular contractions had lower values for femoral neck and lumbar spine BMD. These data support the "mechanostat theory" of Frost (1992), which describes a setpoint for bone metabolism that is mediated by estrogen and mechanical stimuli such as physical

activity. In this model, intense mechanical usage or hyperactivity stimulates modeling drifts (uncoupled osteoclast-osteoblast processes) at bone sites which result in a net bone gain. This continues until an adaptation occurs to the increased mechanical loads and a new mechanical usage set point is reached. Hyperactivity or high levels of physical activity lowers this set point, whereas disuse (i.e., bed rest or injury which forces non-weight-bearing) raises the set point. Frost's model also proposes a role for estrogen, whereby high or even normal estrogen levels lower the modeling and remodeling set points, and low estrogen levels raise the set point and decrease modeling and remodeling. In this latter case, higher mechanical loads are then required to maintain or increase bone mass.

Two other studies have also reported a protective effect of high impact loading at specific bone sites in oligo/amenorrheic athletes (Slemenda and Johnston, 1993; Wolman et al., 1990). Slemenda and Johnston (1993) reported that female ice skaters with oligo/amenorrhea had only slightly lower (approximately 2%) bone mineral in the lower extremities (legs and pelvis) compared to eumenorrheic skaters. These authors concluded that the unique loading characteristics of ice skating (i.e., take-off and landing of jumps) generated extremely high forces, which were sufficient to compensate for their deficient reproductive hormone profile. Wolman et al. (1990) reported that women rowers with amenorrhea had higher lumbar spine BMD compared to amenorrheic runners and dancers. They concluded that the high forces generated while rowing protected the spine. Their results were confounded, however, because the rowers weighed more than the runners and dancers, and body weight, particularly lean mass, has been positively correlated with bone mineral (Snow-Harter et al., 1990).

Martin and McCulloch (1987) state that strain is the intervening variable between loading forces and bone remodelling. Cyclical change in the bone's internal strain, i.e., dynamic loading, has a strong osteogenic effect, with the maximum effect occurring with relatively few cycles. They found that the greatest osteogenic effect on bone mass and cross-sectional area resulted from high loading activities that had minimal repetitions. Bone

density appears to be more strongly affected by increased joint reaction forces exerted on it rather than the number of loading cycles (Carter, 1987; Carter et al., 1987). The orientation of the trabeculae, which make up the important structure of many of the long bones, corresponds to the stress it encounters. Carter (1987) suggested that the force of gravity is primary in stressing the bone tissue. Carter et al. (1987) proposed that variations in bone mineral density among different individuals and within specific bones of the same individual are reflective of the loading history to which the bone is exposed, and are such as to optimize a certain function. Whalen et al. (1988) also found that the forces of greater magnitude influenced bone mass to more of an extent than the number of loading cycles. Repetitive loading, even of low loads (as in distance running) may result in stress fractures as the bone is unable to adapt to this strain without sufficient recovery time. Burr et al. (1985) hypothesized that bone microdamage, such as fatigue fractures, was a strong initiator of bone remodelling. Cortical bone is constantly accumulating fatigue damage in everyday activities; thus, it follows that remodelling should be initiated by such a stimulus. The results of their studies showed that bone resorption and remodelling occurred directly in association with micro-cracks in the bone, and did not appear to be related to the stress and strain levels placed on the bone. In contrast, Lanyon (1987) did not believe that bone remodelling was a reaction to damage, but instead is more preventive in that it acts to reduce the likelihood of fatigue and monotonic failure. Functional strains are necessary to stimulate the adaptive process of bone to its load. Lanyon (1987) hypothesized that osteocytes, at first, became sensitive to the magnitude and distribution of strain within the bone matrix; then, they stimulated the action of osteoblasts and osteoclasts to respond accordingly in that area of bone in order to adapt to functional load-bearing. The findings from a recent study by Grimston et al. (1993) support the theory that the magnitude of impact loading is critical in determining the beneficial forces on bone. These researchers found that children (ages 10 to 16 years) training and competing in "impact loading" kinds of activities (e.g., sports such as running, gymnastics, tumbling and dance which produced

forces at least three times body weight) had greater BMD values for the femoral neck and lumbar spine compared to those children participating in "active loading" activities (e.g., swimming), where loading is non-weight-bearing and produced primarily through muscular contraction.

Concern seems appropriate when questioning whether exercise-associated menstrual cycle changes predispose a woman to osteoporosis by lowering the fracture threshold (Highet, 1989). Many athletes who exhibit menstrual cycle irregularities do so in their growth years, and hence, may never reach peak bone mass. Thus, even if she loses bone at the rate of normal women, since she started lower, the female athlete will always be at a level which increases her vulnerability to fractures. These fractures typically occur at sites of primarily trabecular bone, including the spine, pelvis, and proximal femur. A survey study of elite female distance runners by Clark and colleagues (1988) revealed that 72% of amenorrheic runners had sustained stress fractures in the past, with 50% reporting multiple stress fractures. This was in contrast to 37% of eumenorrheic runners experiencing a stress fracture, 9% on more than one occasion. Thus, an immediate negative effect of menstrual cycle dysfunction appears to be an increased incidence of fairly severe skeletal injury, which is not conducive to optimal running performance.

Bone remodelling is a continuous, dynamic process that occurs throughout life. Whether bone deposition and resorption are in balance (bone maintenance), deposition is greater than resorption (growth), or deposition is less than resorption (aging), two main cell types are responsible for bone turnover (Raisz & Kream, 1983). Osteoblasts synthesize new bone, while osteoclasts are responsible for bone resorption. Bone remodelling is under direct and indirect hormonal control. Vitamin D indirectly affects bone growth by regulating calcium. Estrogen and progesterone also affect bone growth. Bone remodelling is not a perfect process, even in periods of maintenance (i.e., no growth or excess strain imposed). Marcus (1987) showed that bone formation did not quite match bone resorption and thus, small bone deficits appeared at the end of each cycle. This effect may explain the

bone loss associated with aging. Mosekilde (1988) and Mosekilde and co-workers (1987) pointed out, however, that loss of bone mass with age does not fully explain the loss of bone strength as one ages. They suggested that this discrepancy resulted from changes in the micro-architecture of the trabecular bone, such that its very structure was weakening or thinning.

The age at peak bone mass is still somewhat controversial and site-specific (Snow-Harter and Marcus, 1991). It has been hypothesized by some researchers (Garn et al., 1964; Krolner and Nielsen, 1982; Recker et al., 1992) that college-aged women may still be depositing new bone on the way to building peak bone mass. Recker and colleagues (1992) found that gains in bone mass occurred up to 28 or 29 years of age, depending on the skeletal site. They also concluded that physical activity and a high dietary calcium:protein ratio exerted a positive effect on the accretion of bone mass in the third decade. Other researchers, however, have found peak bone mass to occur earlier, with a subsequent loss of mineral possibly occurring as early as the third decade at some sites. Trabecular bone loss may begin as early as the third decade (Mosekilde & Mosekilde, 1988), but an acceleration of this bone loss is evident at menopause (Aloia et al., 1985; Cann et al., 1985; Gallagher et al., 1987; Krolner & Nielsen, 1982). The first five years postmenopausal appear to be the most critical in terms of accelerated bone loss, with decreases ranging from 2% to 8% per year (Aloia et al., 1985; Cann et al., 1985; Genant et al., 1980; Krolner & Nielsen, 1982). Thereafter, bone loss continues but at a slower rate. Several factors play an integrative role in determining ultimate bone mass and the subsequent rate of bone loss. These include aging, as well as exercise, diet and reproductive hormone status. There is also a genetic component to bone. Pocock et al. (1987) found that there was a significant correlation between bone mineral at the spine, but less so at the proximal femur and forearm, for twins, suggesting a role of genetics on bone mass at these sites. The weaker association with bone mass at the proximal femur and wrist, however, suggests that environmental factors play more of a role in determining

bone mass at these sites. Thus, exercise may affect bone more at the proximal femur and distal radius than at the lumbar spine in terms of osteoporosis risk.

Physical Activity and Bone Mineral Density

As a general rule, there is agreement among researchers that the level of physical activity is strongly and positively correlated with bone mineral density. In 1980, Huddleston and colleagues showed that the radius of the dominant arm of lifetime tennis players had significantly greater cortical bone mineral than the non-dominant radius. Pirnay et al. (1987) found similar increases in bone mineral density in professional tennis players when compared to sedentary college students. These findings indicate that cortical bone may be enhanced with regular physical activity. Other studies examined the effects of exercise on various sites, involving both cortical and trabecular bones. Dalen and Olsson (1974) observed higher bone mineral density in experienced cross-country runners (as compared to sedentary individuals) when measurements were taken of the humeral head, calcaneus, radius and ulna (increases of 20%), and neck of the femur and lumbar vertebrae (increases of 10%). Lane et al. (1986) found that vertebral bone mineral density was 30% greater in life-long, middle-aged runners compared to a matched, sedentary control group. Wolman and colleagues (1990) found that rowing was even more beneficial for lumbar spine bone mineral density (compared to activities such as running). Rowing involves intensive use of the back and trunk musculature which, despite not being a weight-bearing activity, creates substantial forces generated on the lumbar spine. Williams and co-workers (1984) found that bone mineral of the os calcis, an area which is subjected to relatively high impact loads from running, was significantly increased in runners. Kirk et al. (1989) found that vertebral trabecular bone mass was higher in runners compared to sedentary controls. They proposed that this was related to the runners' superior fitness level (VO_{2max} = 48 $ml.kg^{-1}.min^{-1}$ versus 32 $ml.kg^{-1}.min^{-1}$ for the sedentary women). They did not find this

relationship in postmenopausal women. Marcus et al. (1985) also found increased axial bone mineral density in elite female distance runners, with no effect on radial (cortical) bone mineral density. Aloia et al. (1978) found that exercise may retard bone loss in postmenopausal women. Krolner et al. (1983) found similar results, but also found that the active women had a 3.5% increase in lumbar spine bone mineral over eight months, while the control group decreased 2.7%. In one of the few other studies looking at gymnastics training, Nichols and colleagues (1992) reported that gymnasts exhibited significantly higher bone mass values for the lumbar spine and femoral neck compared to control subjects. These researchers concluded that the unique type of physical training done by the gymnasts (e.g., jumping) was beneficial to their bones.

It appears, then, that the beneficial effects of physical activity may be specific to the pattern of loading. Weight-bearing exercise, such as running, positively affects trabecular, without necessarily altering cortical bone mineral density. Thus, changes may be seen in lumbar spine bone mineral density but not necessarily in whole body mineral. General, overall physical activity plays a role in increasing bone mineral density at various sites; however, specific exercise programs with regard to mode, intensity, frequency and duration have not been conclusive in recommendations for optimal benefit on bone mass (Snow-Harter & Marcus, 1991). Many studies have been prospective and cross-sectional, and the long-term implications of their findings to individuals are questionable. Typically, exercise programs that are more intense and last longer are more beneficial. Exercises which overload the musculoskeletal system sufficiently for optimal functional loading result in an enhanced osteogenic stimulus. In a study by Snow-Harter and colleagues (1992), women who participated in either a running or weight training program over an eight-month period showed an increase in lumbar spine BMD.

Whalen and colleagues (1988) suggested that the bone will adapt differently to the two main types of strain: impact loading of compressive forces (e.g., walking and running) and muscular contractions pulling on the bone (e.g., weight lifting). Both types of exercise

are beneficial, but the best combination is still unclear. Since runners and gymnasts predominantly train different energy/muscular systems, study of these contrasting groups may help to answer this question. Montpetit (1976) stressed the differences between training for gymnastics and middle- and distance running. During a gymnastics routine (ranging between 30 seconds and a minute-and-a-half), anaerobic energy is utilized for the power and strength moves being displayed. As Sale (1976) pointed out, gymnastics moves involve the ability to develop a lot of force rapidly. By increasing peak muscular force through specific training for gymnastics, improvement in performance is observed. Thus, muscle strength plays a role in the success of a gymnast, while it may actually be a hindrance in a distance runner, who needs to be as light as possible in order to run a long distance fast.

Distance runners primarily train the aerobic energy system, and some, although usually only a small minority, participate in some strength training for muscular endurance. Gymnasts, on the other hand, primarily train the anaerobic energy system. Their training entails a lot of power and strength maneuvers, and weight training is an integral part of their program. Some aerobic conditioning is also done, but usually only in the pre-season. Long-distance runners and gymnasts have similarly high proportions of their body weight as fat-free mass and thus, a very low percent body fat (Montpetit, 1976). Gymnasts, however, are much more muscular looking, while runners appear linear in terms of somatotype. Gymnasts have relatively low maximal aerobic power (e.g., 50-55 $ml.kg^{-1}.min^{-1}$) compared to other athletes of similar calibre, but their strength and power is greater than most (Sale, 1976). The effects of this profile on bone mineral has not yet been defined.

The role of muscle strength in bone metabolism is becoming more evident through recent research. Doyle et al. (1970) found a significant correlation between the ash weight of the third lumbar vertebrae and the weight of the left psoas muscle. These researchers proposed that the weight of the muscle is an important determinant of bone mass, as it

exerts force at its attachment. Sowers and colleagues (1992) found that a large muscle mass was associated with higher bone mass at the proximal femur compared to a low muscle mass and high adiposity distribution of body weight. They concluded that a high body weight was only beneficial for BMD when lean mass, such as that developed through weight training, was increased. Studies by Aloia and colleagues (1991) and Bevier et al. (1989) also supported this hypothesis. Findings from studies investigating the effects of weight training (i.e., increased strength) on lumbar spine bone mineral density have been equivocal. Sinaki et al. (1986) found a significantly positive correlation between strength of the back extensors and lumbar spine bone mineral density in postmenopausal women. Halle et al. (1990) found that strength of the trunk extensors was significantly related to both spine and proximal femur bone mineral density in postmenopausal women. Similar results were reported by Sinaki et al. (1986) and Halle et al. (1990), despite the former using measures of isometric strength (force), while the latter measured isokinetic strength (force and torque).

In premenopausal women (mean age of 36 years), Rockwell et al. (1990) found that a 9-month weight training program was too short-term to positively affect bone, even though muscle strength increased (on average by 57%). In fact, they found a decreased vertebral bone mineral density by almost 4%. A 12-month weight lifting study by Gleeson et al. (1990) found only a minimal increase in vertebral bone mineral density of 0.8%. Adams (1992) would argue that the programs in these two studies were not stressing the musculoskeletal system sufficiently, and thus, increases in bone mineral were minimal. He suggested a scientifically-based program of free weights rather than Nautilus machine weights. Snow-Harter and colleagues (1992) recently carried out a carefully planned, progressive and supervised, resistance training program, investigating the effects on bone mass in college-aged women. Subjects were randomly assigned to either of two exercise groups: jogging or weight lifting, or to a control group. After eight months, lumbar spine bone mineral density was increased in both runners (1.3%) and weight trainers (1.2%),

but not in the controls. Bone mineral density of the proximal femur did not change in any group. These researchers proposed that a more prolonged stimulus (training) period was necessary to increase the bone mineral in the hip, since this site has a greater abundance of cortical bone than the lumbar spine. They also speculated that since it took longer to progress to a sufficient stimulatory intensity (in terms of increasing bone mineral) with the weight training program, if the training period had been extended beyond eight months, more improvement may have been seen in the weight lifters compared to the runners.

Several studies have shown that muscle strength is a good predictor of bone mineral density in women, and thus, the importance of a strong stimulus via muscular contraction on bone appears evident. Snow-Harter et al. (1990) found that muscle strength could account for 15% to 20% of the total variance in bone mineral density in premenopausal women (aged 18 to 31 years). One interesting finding from this study was that muscle forces during activities applied at a distance to the spine and the hip were still able to benefit bone mineral density at these sites. For example, muscle strength of the biceps was the most robust predictor of femoral neck BMD and grip strength was the best predictor of lumbar spine and radius BMD. Pocock et al. (1989) also found that muscle strength was a significant and independent predictor of bone mineral density at the lumbar spine and proximal femur in women aged 20 to 75 years. Physical fitness, as measured by VO_{2max}, was also found to be a significant predictor of femoral neck bone mineral density in this study. Block et al. (1989) looked at bone mineral density of athletes in a non-weight-bearing sport (water polo) compared to athletes in a weight-bearing sport (weight-training) and less active controls (individuals who exercised less than three hours per week). They found that bone mineral density of the spine and the hip of the two athletic groups were not different, but they were (on average) 18% and 9% greater, respectively, than the controls. Cross-sectional area of the paraspinous muscles appeared to be the most robust predictor of bone mineral density in all subjects. They found no relationship between aerobic capacity (as measured by VO_{2max}) and bone mineral density at either the spine or the hip.

Conversely, research by Snead et al. (1989) found that, in eumenorrheic runners, peak VO_2 and muscle strength at the knee and hip joints were good predictors of bone mineral density in the lumbar spine and proximal femur. The amenorrheic and oligomenorrheic runners in this study had lower vertebral bone mass values, and surprisingly, lower trunk strength values.

Methods for Assessing Bone Mineral Density

Several non-invasive methods for assessing bone mineral density (BMD) have been developed in the last twenty years (Snow-Harter & Marcus, 1991). Among these techniques are single and dual photon absorptiometry and quantitative computed tomography. Single photon absorptiometry can be used to estimate cortical bone density, but it is a poor reflection of the central trabecular bone mass. Single photon absorptiometry is also relatively insensitive compared to dual photon absorptiometry and thus, the chance of finding differences in bone mass and BMD in various populations using single photon absorptiometry is unlikely (Highet, 1989). Dual photon absorptiometry or DEXA (dual energy X-ray absorptiometry) is more accurate because the detector recognizes all transmitted photons; thus, BMD measures can include both trabecular and cortical components of a given bone (e.g., vertebrae) (Snow-Harter & Marcus, 1991). The selection of skeletal sites to be measured for the determination of bone mineral density is critical in terms of assessing the most metabolically active (trabecular) sites which have the highest turnover rates (e.g., hip, spine). Kellie (1992), in her technical report on the measurement of bone density, reported that DEXA was preferred because it can be used to accurately measure both the predominantly cortical peripheral appendicular skeleton, and the axial skeleton which has varying proportions of cortical and trabecular bone. The Hologic QDR-1000/W system uses two X-ray beams of different energies, alternating pulses at 70 kV and 140 kV peaks. An integral-line detector measures the intensity of

transmitted photons through the particular bone site being measured (Kellie, 1992). Compared to other methods of measuring BMD (i.e., the radioisotopic sources of photons used in single and dual-energy absorptiometry), the X-ray source used in DEXA provides a greater photon flux which results in better image resolution, despite shorter scanning time.

Radiation exposure from DEXA is very minimal, only slightly above normal background radiation (QDR Insight, March, 1992), or approximately one-tenth of a standard chest X-ray. With this technique, bone mineral density values are reported as grams of mineral (calcium hydroxyapatite) per square centimeter of bone area (Mazess, 1981), and measurements are accurate to less than 1% precision error (Snow-Harter & Marcus, 1991). In QDR Insights (March, 1992), Hologic stated that the precision of hip measurements using DEXA range from 0.67% (total hip) to 1.95% (Ward's Triangle). With the Hologic QDR-1000/W bone densitometer at Oregon State University, the precision of measurement is even better, with coefficients of variation ranging from 0.38% to 0.46% for bone area, bone mineral content and bone mineral density. In the report by Kellie (1992), long-term precision of DEXA measures of BMD were 0.4% for repeated scans of the spine phantom, which was scanned daily for approximately 6 months.

Reproductive Hormones, Menstrual History and Exercise Training related to BMD

Disrupted menstrual function resulting from intense exercise training can decrease trabecular BMD and increase the risk of fracture (Cann et al., 1984; Drinkwater et al., 1984; Marcus et al., 1985; Nelson et al., 1986). Cortical bone, as measured in the radius, was not affected (Marcus et al., 1985). Jones et al. (1985) also found that cortical bone was not lost as a result of exercise-associated amenorrhea, although it was compromised in conditions of amenorrhea resulting from excessive weight loss or premature menopause. Ruegsegger and colleagues (1991) found that premenopausal women who had anorexia

nervosa (and the associated amenorrhea that accompanies this disease) exhibited significantly lower trabecular bone mineral density (reduced by as much as 50%) compared to age-matched healthy females (mean age of 24 years), even though there was no difference between the groups for cortical bone mineral density. These findings suggest that trabecular bone is highly sensitive to hormonal status (i.e., estrogen presence) and age, whereas cortical bone appears to be more robust. Wolman (1990) also concluded that cortical bone was more resistant to the effects of low estrogen compared to the more metabolically active trabecular bone. Since exercise has been shown to be osteogenic, the question arises whether it can offset the bone loss due to menstrual cycle dysfunction (such as amenorrhea and shortened luteal phase) and menopause. Martin and McCulloch (1987) pointed out that the functional mass of bone at a specific skeletal site is determined by the interaction of bone formation forces (i.e., exercise) and bone resorption forces (i.e., lack of estrogen). Puustjarvi and colleagues (1992) found that long-term exercise training (running) in female dogs resulted in lower circulating serum levels of estradiol, and a consequent decrease in bone mineral. Some of these dogs exhibited exercise-associated amenorrhea. These results strongly support the role of female reproductive hormones on bone mass, even outweighing the potential beneficial effects of weight-bearing exercise, although the running program the dogs participated in may not have involved high enough impact loading to cause an increase in bone mass. The non-weight-bearing site of the iliac crest had lower bone mineral density in the running dogs compared to the control dogs, as did the weight-bearing skeletal sites (e.g., legs, hip, radius, lumbar spine).

A study by Snead et al. (1989) showed that amenorrheic and oligomenorrheic runners had lower vertebral bone mass and related lower muscle strength scores for the trunk compared to eumenorrheic runners. Muscle strength of the leg and hip musculature were strong predictors of lumbar spine and femoral neck bone mineral density in eumenorrheic runners. A compilation of recent findings by Marti (1991) concluded that amenorrheic athletes have lower BMD (particularly vertebral) despite years of regular

exercise participation (Cann et al., 1984; Cook et al., 1987; Drinkwater et al., 1984; Lindberg et al., 1984; Linnell et al., 1984; Marcus et al., 1985). Results from the study of Marcus et al. (1985) suggested that strenuous exercise has some beneficial effect on bone mineral in amenorrheic runners, although these athletes still are at high risk for stress fractures. The trabecular bone mineral in amenorrheic athletes was reduced compared to eumenorrheic athletes, but the amenorrheic athletes still exhibited higher levels of bone mass when compared to non-athletes who were also amenorrheic. This does suggest an important role for exercise in determining bone mass. The findings of Marcus et al. (1985) are supported by Snyder's group (1986), who found that amenorrheic lightweight rowers had radial and lumbar spine bone mineral density values similar to non-athletic control subjects. Cree et al. (1991) found that those amenorrheic athletes who trained more intensively had a higher bone mineral density than those amenorrheic athletes who trained less strenuously. They proposed that other factors, including body composition and nutritional deficiencies, also played a role, not just deficiency of estrogen. A study by Lindberg and co-workers (1984) showed that 49% of the amenorrheic runners sustained stress fractures compared to none of the eumenorrheic runners or controls. These researchers found that bone mineral density of both cortical and trabecular bone was reduced in the amenorrheic runners, but the difference was more pronounced in the trabecular bone.

The durability of the female reproductive hormone profile as it is affected by exercise has been questioned by several research groups (Dale et al., 1979; Loucks, 1990; Prior et al., 1982; Sanborn et al., 1982; Shangold et al., 1990). It is fairly evident when exercise-induced oligo- or amenorrhea result from intense physical training; however, non-symptomatic changes in the menstrual cycle may occur also (Loucks, 1990; Prior et al., 1990) which can negatively impact bone as well. These shortened luteal phase or anovulatory cycles do not appear to manifest only in athletic women though (Prior et al., 1990). Barr and colleagues (1994) recently found that women with a shortened luteal phase

of the menstrual cycle had a tendency for lower bone mass at the lumbar spine, regardless of age, body mass index, percent body fat and activity level. The women who exhibited highly restrained eating patterns, however, typically had the highest incidence of shortened luteal phase cycles, even though their menstrual cycle appeared to be normal in length, with symptomatic withdrawal bleeding. An interesting note was the non-significant relationship between higher exercise levels and more restrained eating habits. The authors attempted to explain this by stating that most of the women who exercised did so as one of several conscious ways to control body weight; but other confounding variables (i.e., age, low body weight) cannot be ruled out as playing a role in these non-symptomatic menstrual cycle disturbances. For example, the mean age of the twenty-seven women in this study (Barr et al., 1994) was almost 41 years. As women approach menopause, menstrual cycle disturbances and anovulatory cycles become more prevalent. The lower body weight (although non-significant) in the women who tended to exercise more, eat less and have shortened luteal phases may also play a role. These research findings do not agree with those reported by Rogol and colleagues (1992). These researchers took a group of untrained eumenorrheic women in their 30's and subjected them to a one-year progressive exercise training program to test the durability of the reproductive axis. They found that although the luteal phase was shortened in those women who trained at more intense levels (i.e., greater than lactate threshold), it was non-significantly shorter by less than two days compared to the other women. Rogol et al. (1992) concluded that the female reproductive system is quite robust in gynecologically mature eumenorrheic women, and not affected by moderate exercise programs undertaken by most of the population. They suggested that exercise-induced amenorrhea and other menstrual cycle disturbances are probably associated with non-exercise-related variables (e.g., a younger gynecological age).

It is not known for certain the long-term effects of amenorrhea or shortened luteal phase cycles. Cann et al. (1985) showed that the rate of loss of vertebral BMD in amenorrheic runners was most rapid (about 4% per year) in the first 2 years, after which

time the bone loss was not significant (less than 0.5% per year). This response is similar to that seen at menopause. These researchers suggested that intervention (e.g., treatment with estrogen) should be started early to prevent or reverse bone loss. Wolman (1990) suggested that if the amenorrheic incidences were short-lived, BMD could at least partially be recovered if the athlete began regularly menstruating again. Those women with persistent amenorrhea, however, continued to lose bone mineral, which was less likely to be regained, and thus, they were at a higher risk of fracture and osteoporosis. Cann and colleagues (1988) emphasized the importance of prior menstrual history in assessing long-term and permanency of damage to bone. They found irreversible bone loss in athletic women whose prior amenorrhea lasted three years or longer and was untreated. Drinkwater et al. (1990) concurred that extended periods of altered menstrual cycle function may be detrimental and long-lasting with regard to lumbar spine bone mineral density. Earlier, Drinkwater and colleagues (1984, 1986) had proposed that the differential effects of estrogen levels on bone mass may be due to the proportion of trabecular and cortical bone present at that particular skeletal site, and how long the individual had been amenorrheic. Cortical bone appears to be more resistant to the effects of low levels of estrogen compared to trabecular bone; thus, bone mineral at the lumbar spine and femoral neck is more "at risk" compared to total hip or whole body bone mass, even though all skeletal sites will lose bone mineral if the amenorrheic condition is prolonged (e.g., greater than three or four years). Bachrach and colleagues (1991) studied women who had recovered from anorexia nervosa during adolescence to investigate its effect on bone mineral density, particularly during the peak bone growth years. They found that increases in weight, height and body mass index (BMI) during a 12-16-month period resulted in significant increases in whole body BMD, and a trend for greater lumbar spine BMD, although bone mass was still below the expected value for girls this age. In fact, the increased bone mass occurred with weight gain before the resumption of menstruation. The researchers proposed that estrogen may have an independent effect of bone; women who had resumed menstruation after recovery

from anorexia nervosa for a longer period of time showed the most significant increases in whole body BMD. Conversely, Lindberg et al. (1987) reported that a portion of the bone loss due to amenorrhea appears to be reversible. They found that over a 15-month period, bone mineral could be increased if runners reduced their mileage (by almost one-half), gained weight (at least 3% of body weight), were supplemented with calcium (1500 mg/day) and became eumenorrheic. The subject number in this study was very small (n=4), however, so conclusions should be tempered.

Intense physical activity may delay menarche if serious training begins before puberty (Highet, 1989). Frisch et al. (1981) reported that for each year of rigorous exercise training prior to menarche, the onset of menstruation may be delayed up to five months from the average 12.9 $\pm$ 1.2 year age of menarche in the United States. Malina (1983) supports the idea that menarche occurs later in athletes compared to the normal individual. Malina summarized numerous studies by reporting the age at menarche for female athletes; e.g., 13.6 to 15.3 years for runners, 14.5 to 15.1 years for gymnasts, with the more elite athletes being delayed more. Warren (1980) found that menarche was delayed in ballet dancers (15.4 years). Peltenburg et al. (1984) found that the age at menarche was delayed approximately one to two years in gymnasts compared to other school-aged girls (eight to fourteen years) and swimmers. They proposed that this may be due in part to the higher lean mass in the gymnasts and lighter body weight. Years of training prior to menarche did not appear to play a role in delaying menarche, since the swimmers also started intensive training at a very young age, and yet their age at menarche did not differ from the school-aged girls. Both Warren (1980) and Malina (1983) proposed that this delayed menarche is caused by an energy drain (i.e., intensive exercise, inadequate nutrition, low body weight) which modifies the hypothalamic-pituitary axis at puberty. They suggested that such an energy drain may also induce amenorrhea. Wilmore and colleagues (1992), however, disagreed with the energy conservation theory of amenorrhea. They studied elite female distance runners who were either amenorrheic or eumenorrheic and compared them to

untrained female control subjects. They found that energy intakes (based on 3-day food logs) and energy expenditures (i.e., resting metabolic rate, thermic effect of a meal and the energy cost of physical activity) did not differ among the three groups of women. These researchers concluded that there was no evidence for energy conservation, as energy intake matched fairly closely with energy expenditure. They suggested, however, that there was a possible link between disordered eating, secondary amenorrhea and low bone mineral. This was based on the findings that the runners' lumbar spine BMD tended to be lower than the controls', and that 75% of the amenorrheic runners compared to none of the eumenorrheic runners reported having disordered eating. Sanborn, Albrecht and Wagner (1987) also showed a delay in menarche in runners, but years of training prior to menarche was not associated with the delay. A delayed age at menarche has been associated with a higher incidence of exercise-associated oligo/amenorrhea (Sanborn et al., 1987; Wakat et al., 1982).

Late menarche appears to deleteriously affect the skeleton (Warren et al., 1986). The incidence of stress fractures increased in ballet dancers who had delayed menarche and was higher as age at menarche increased. This phenomenon does not appear to be limited to women, even though that is often the gender of interest. Finkelstein and colleagues (1992) reported that men who had a delayed puberty had significantly lower bone mineral at the radius and lumbar spine compared to men who underwent a normal puberty. They concluded that this osteopenic condition resulting from the delayed puberty may detrimentally affect peak bone mass acquisition, and increase the risk of osteoporotic fracture in these men. Prior menstrual cycle irregularity also seems to be a predisposing factor. Schwartz and colleagues (1980) found that over half (54.5%) of amenorrheic runners had a previous history of such problems, compared to only 15% of eumenorrheic runners. Menstrual cycle function may also be affected by parity. The incidence of amenorrhea was found to be 51% in nulliparous athletes compared to 21% for parous females (Dale et al., 1979). Baker et al. (1981) pointed out that age is also related to

menstrual cycle status. Dysfunction is more prevalent in women under 30 years of age, due possibly to their less mature hypothalamic-pituitary axis. This, coupled with the very intense training done by collegiate (scholarship) athletes, could explain the relatively high incidence of menstrual cycle irregularity in this population (approximately 18 to 23 years of age). Schwartz et al. (1981) found that amenorrheic runners had a higher incidence of history of menstrual irregularities, weighed less and had a lower percent body fat than eumenorrheic runners and non-runners. The amenorrheic runners also associated more stress with their running and had an inadequate protein intake in their diet.

Diet, Reproductive Hormone Status and BMD

Calcium is a major constituent of bone. In fact, over 99% of the body's calcium is located in the skeleton, in the form of crystalline hydroxyapatite (Avioli, 1988). The bones act as a reservoir for calcium and this pool is in dynamic equilibrium with plasma levels. Constant blood calcium levels are necessary due to its function in various physiological and biochemical processes including: neuromuscular excitability, transmission of nerve impulses, maintenance and function of cell membranes, action in enzyme reactions and hormone secretions, and blood coagulation. The homeostatic control of calcium involves Vitamin D, as well as hormones from the thyroid and parathyroid glands. Calcium "balance" in the plasma and bone depends on the amount of intestinal absorption and renal reabsorption, and the accretion and resorption from bone. Vitamin D increases absorption of dietary calcium. Estrogen also plays a role in calcium metabolism and bone. Gallagher and Nordin (1973) found that estrogen reduces bone resorption. They suggested that estrogen acts by blocking the action of parathyroid hormone on bone. This may help to explain why postmenopausal women and amenorrheic athletes have reduced levels of bone mineral, as resorption is being favored over deposition.

It is common, however, for females as early as adolescence to fail to meet the daily calcium requirement (Snow-Harter & Marcus, 1991). This could have a negative effect on peak bone mass. Halioua and Anderson (1989) suggested that an adequate calcium intake during adolescence (e.g., 1200 mg/day) will optimize the development of maximal skeletal mass, but regular weight-bearing activity is also necessary. Sentipal and colleagues (1991) also found that those girls between the ages of eight and eighteen years who had higher dietary calcium intakes exhibited higher lumbar spine bone mass. These researchers concluded that dietary calcium intakes which were close to the RDA during adolescence (1200 mg/day) may optimize peak bone mineral accretion (although this would be dependent upon genetic predispositions). DiMarco and colleagues (1992) found that the average calcium intakes in intercollegiate gymnasts were approximately 700 to 800 mg per day (below the RDA for this age group). These researchers stressed the importance of building peak bone mass during adolescence and young adulthood by encouraging higher calcium intakes from dietary sources, which are often neglected or avoided in females of these ages because they think all sources of calcium (primarily found in dairy products) are fattening (Benardot et al., 1989; Nelson et al., 1986; Nieman et al., 1989; Perron and Endres, 1985). After growth has stopped, calcium nutrition is not quite as critical, since the body has a good control mechanism for adapting to varying levels of dietary calcium. Yet, the timing of peak bone mass is still questionable; and as Recker and colleagues (1992) pointed out, calcium intake (especially in relation to protein intake) is critical up to the age of 28 to 30 years. Riggs and colleagues (1987) studied the bone mineral changes in women aged 23 to 84 years over an average of four years, and found that over a wide range of dietary calcium intakes (260 to 2000 mg/day, with a mean of approximately 900 mg/day) there was no significant relationship between calcium intake and rate of bone loss. There was a significant interaction of age and menopausal status on BMD, particularly at the lumbar spine, however, and in premenopausal women there was a trend for higher rates of

bone loss when calcium intake was low. The researchers concluded that possibly higher doses of calcium (possibly with Vitamin D supplementation) may reduce bone loss.

Calcium supplementation may not be beneficial until after 60 years of age, as the compensatory mechanisms (i.e., increased calcium absorption) become less efficient. Then, supplementation with calcium may play a substantial role in maintaining or at least minimizing the loss of bone mass (Heaney, 1988; Marcus, 1986). Baran et al. (1990) found, however, that in premenopausal women (aged 30 to 42 years) who were initially below the RDA for calcium intake, supplementation with dairy products over a 3-year period (an additional 600 mg/day) prevented the age-related vertebral bone loss. The women who supplemented their diets with calcium maintained bone mineral in the lumbar spine over the entire three years, while the non-supplemented women showed a significant loss of bone between 18 and 36 months. Picard and colleagues (1987) also concluded that dietary calcium intake between the ages of 20 and 40 years plays a significant role in determining bone mineral content in premenopausal women, particularly at the lumbar spine and distal radius sites. Higher levels of dietary calcium earlier in life will build greater bone mass and reduce the risk of osteoporosis later in life. Dawson-Hughes et al. (1987) suggested that there is a threshold level of calcium (approximately 800 mg/day) which was necessary to reduce spinal mineral loss. This was in postmenopausal women, aged 40 to 70 years.

Marcus (1987) reviewed the relationship between dietary calcium and skeletal bone mass, and concluded that women are typically in negative calcium balance due to an inadequate dietary intake. The effect of this relative lack of dietary calcium on bone mass is compounded by the excessive dietary protein intake of most women. The author gave some tentative recommendations: 1) calcium intake should be 1000 mg/day, from dietary sources, in women during the reproductive years; 2) at menopause, estrogen replacement therapy is the primary factor acting to protect bone, but calcium intake should be maintained at 1000 mg/day, and increased to 1500 mg/day if no estrogen is present; and 3) adequate

dietary calcium (at least 1200 mg/day) is very important during adolescence in order to reach peak bone mass. In accordance with this last point, Sandler et al. (1985) found that adequate milk consumption in childhood and adolescence resulted in a greater peak skeletal mass and higher bone density later in menopause. Matkovic and colleagues (1990) also found that calcium intake during adolescence was a major determinant of peak bone mass. These researchers found a significant positive correlation between calcium intake and calcium balance in adolescent girls over a 2-year period, from the ages of 14 to 16 years. They concluded that adequate or even increased calcium intake (i.e., up to 1800 mg/day) during the adolescent growth years resulted in skeletal mineral retention and an enhanced peak bone mass. In fact, Matkovic (1991) stated that the RDA for calcium should be higher than the current recommendations during adolescence since a positive calcium balance is needed to build skeletal mass to its maximal level. The author found that individuals with lower calcium intakes had lower calcium retention levels in the body, despite the relatively higher absorption efficiencies of growth occurring at this time, compared to individuals with higher calcium intakes who had higher calcium retentions. Matkovic's group (1991) reported a highly significant positive correlation between calcium intake and calcium balance ($r=0.67$). Matkovic (1991) also pointed out that calcium intake during young adulthood (up to age 30) was important because, even though the skeleton may not still be growing, bone is constantly being remodelled and consolidated during this time. In an earlier review, Marcus (1982) also suggested that the RDA for calcium was too low for pre- and postmenopausal women. In order to build peak bone mass and to minimize bone mineral loss across the lifespan, sufficient calcium must be present to deposit in the skeleton (where 99% of the total body calcium is stored). Marcus (1982) recommended that the dietary intake of calcium be increased to 1000 mg/day for adolescent and adult women, and 1500 mg/day for postmenopausal women, particularly if they do not undergo estrogen replacement therapy. In a 1979 study of bone mass in individuals living in two different geographical regions of Yugoslavia, Matkovic and colleagues found that maximal

bone density of the metacarpals was significantly higher in those individuals residing in the region where high dietary calcium (approximately 1100 mg per day) was the norm, compared to those individuals whose calcium intake was fairly low (approximately 500 mg per day). Matkovic et al. (1979) cautioned, however, that the major differences in bone mineral were established by the age of thirty, after which dietary calcium intake did not play as significant a role in maintaining bone mass or preventing fractures.

The lack of estrogen present in amenorrheic athletes and postmenopausal women has a direct effect on bone, as well as an indirect effect through its interaction with calcium. With estrogen deficiency, there is a decreased efficiency of intestinal and renal calcium absorption and resorption, thereby increasing the dietary calcium necessary to maintain homeostasis or calcium balance (Snow-Harter & Marcus, 1991). Bone resorption occurs to a greater extent than bone deposition when estrogen levels are deficient, resulting in a net loss of bone mass (Dalsky, 1990). Drinkwater and colleagues (1986) pointed out that low dietary calcium intakes are of special concern in amenorrheic athletes who have low bone mass; i.e., there may be a compounding effect of lack of estrogen (causing increased rates of bone resorption) and insufficient dietary calcium (less mineral present for deposition in the bone). In amenorrheic runners, Berning and co-workers (1985) found that calcium intake was substantially below the Recommended Dietary Allowance (RDA). Thus, their skeleton seems to be at an even higher risk of bone loss as they are deficient in the mineral calcium (from dietary sources) and the hormone estrogen, which plays an integral role in maintaining bone mineralization. Total caloric intake was also below the predicted requirement in these amenorrheic runners.

Nutritional status in female athletes is often found to be inadequate. Restricted eating is quite common among female athletes whose sport performance depends on body weight and/or aesthetics (Barr et al., 1994; Benardot et al., 1989; Nelson et al., 1986; Slavin, 1991). At a very young age, girls who are training for gymnastics are encouraged to have as low a body weight as possible, in order to maximize the strength-to-weight ratio

(Benardot and Czerwinski, 1991). Unfortunately, this all too common problem affects the health and well-being of the athlete, including her menstrual cycle, bone health and psychological outlook, which, in turn, may eventually diminish performance. If body weight is kept too low, lean mass will surely be lost and hence, strength will diminish, the opposite effect of what is desired (Benardot and Czerwinski, 1991). A long-term (for now, un-noticed) consequence of restricted eating in these athletes, often during the important growth years of adolescence, is a compromised bone mass, which could increase the risk for osteoporosis later in life (Dalsky et al., 1988; Drinkwater et al., 1984; Marcus et al., 1985; Prior et al., 1990; Riggs, 1986; Warren et al., 1983). Only recently has this issue been addressed, as researchers attempt to educate the athletes, coaches, athletic trainers and parents of these women about the negative effects of restricted eating with the aim of controlling body weight for sport. In a 1992 study by DiMarco and colleagues, nutritional analyses were performed at several times over the course of a collegiate gymnastics team's training season. At pre-, peak and post-season, total caloric intake was below the Recommended Dietary Allowance (RDA) of 2200 kilocalories for college-aged women. In fact, as the season progressed, caloric intake progressively declined so that by post-season (average intake of approximately 1500 kilocalories), it was significantly less that pre-season levels (average intake of 2100 kilocalories).

Insufficient dietary calcium is common in such athletes as gymnasts and dancers (Slavin, 1991). Even with knowledge about proper nutrition, Perron and Endres (1985) found that female athletes neglected the milk and meat groups in their diet and thus, their calcium intake was less than two-thirds of the RDA. Ninety-two percent of the high school athletes knew that milk was a good source of calcium, but only 12% of the diets met the RDA. The authors suggested that the dietary habits of female athletes were determined by the motivation to be thin and the fear that milk products are fattening. Nelson and colleagues (1986) studied the effect of diet and menstrual cycle status on female runners. They found that the amenorrheic runners had significantly lower lumbar spine BMD values

compared to the eumenorrheic runners, and there was a positive correlation between bone mineral density and estrogen levels in these women. The amenorrheic runners also had lower dietary intakes of total kilocalories, carbohydrate and fat, with a trend for lower dietary protein and calcium intakes. The mean calcium intake was 1150 mg/day for the eumenorrheic runners and 886 mg/day for the amenorrheic runners. Although the mean dietary intake of calcium met the RDA of 800 mg/day for these women (aged 25 years and older), 55% of the amenorrheic runners consumed less than the RDA for dietary calcium while 35% of the eumenorrheic runners failed to meet the RDA. These researchers concluded that the endocrine and dietary deficiencies in the amenorrheic runners interacted to affect calcium metabolism; i.e., low estrogen levels cause an increased urinary calcium loss and lower calcium absorption from the intestine, which acts to increase the requirement for calcium in these women, yet they consume less. Research by Deuster et al. (1986), studying women runners who qualified to compete in the first-ever Olympic Marathon Trials for women in 1984, also found the dietary fat intake of the amenorrheic runners to be significantly less than that of the eumenorrheic runners. They suggested that this could be one factor predisposing these athletes to menstrual cycle dysfunction. Benardot et al. (1989) found that highly competitive female gymnasts had poor dietary habits, being below the RDA for several nutrients. Specifically, both total caloric intake and dietary calcium were insufficient. They suggested that these athletes are concerned about optimal weight and levels of body fat (mean of 9.3%), and tend to make conscious food choices with this in mind.

Typically, however, problems such as delayed menarche, amenorrhea, stress fractures and later osteoporosis are the results of poor eating habits. Miller (1989) reported that this inadequate calcium intake is not solely an issue with athletes. More than one-half of the U.S. female population over the age of 12 consume less than 70% of the RDA for calcium. Restricted eating habits are fairly common among women. Barr and colleagues (1994) recently studied restricted eating and menstrual cycle status and the effects they had

on bone health. The women in this study had a wide range of activity levels (i.e., training for a marathon to less than one hour of aerobic exercise per week). The researchers found a tendency (non-significant) for the women with more restrained eating patterns appeared to exercise more and have a slightly shorter luteal phase length of the menstrual cycle. Bone mineral density of the lumbar spine was not statistically different among restrained and normal eating women however. These findings partially support those of Schweiger et al. (1988), who found that poor nutrition (in terms of low caloric intake) and high stress levels resulted in a higher incidence of menstrual cycle disturbances (defined as a shortened luteal phase). They found a strong positive correlation ($r=0.70$, $p<0.01$) between caloric intake and levels of progesterone in athletes, which led them to conclude that nutrition plays an important role in determining menstrual cycle status, even in apparently normal, asymptomatic eumenorrheic women. Menstrual cycle abnormalities such as shortened luteal phase and anovulatory cycles appeared to be present in both athletes and non-athletes alike, however, and was not related to weight, age or hours of exercise per week (Schweiger et al., 1988). Those women who consumed less kilocalories also had lower progesterone levels and usually a shortened luteal phase of the menstrual cycle. These researchers also found a non-significant positive correlation ($r=0.36$, $p>0.05$) between total caloric intake and estrogen levels. They proposed that the luteal phase of the menstrual cycle is more susceptible to disruption from inadequate caloric intake compared to estrogen production over the course of the menstrual cycle. Nonetheless, insufficient estrogen and/or progesterone levels could lead to potential bone loss (Drinkwater et al., 1984; Loucks, 1990; Prior et al., 1990).

Neiman et al. (1989) found that the caloric intake and percent of carbohydrate in the diet of female marathon runners was lower than what would be recommended for their exercise level, and that they failed to meet two-thirds of the RDA for Vitamin D. Vitamin D has a hormonal role in bone metabolism (DeLuca, 1988). It is essential for calcium absorption and acts to deposit calcium in the bone, thus possibly preventing osteoporosis.

Vitamin D is very active in the mobilization and transport of calcium, its renal reabsorption and in bone remodelling, thus helping to build a stronger skeleton. Very few foods naturally contain Vitamin D: fatty fish and oils, eggs, liver and milk. Vitamin D can also be synthesized in the skin from exposure to sunlight (DeLuca, 1988; Haddad, 1992). Food choices in female athletes (i.e., neglecting dairy products, fatty foods) may limit their dietary intake of Vitamin D (Nieman et al., 1989).

The role of diet in the menstrual cycle - bone health scenerio is complex and involves several different nutrients, alone and in combination. Dietary protein intake is one such factor. Athletes generally require a slightly greater protein intake compared to sedentary individuals, in order to offset the protein catabolism that occurs during exercise and maintain lean body mass (Tarnopolsky et al., 1988). Tarnopolsky's group (1988) used nitrogen balance studies and measures of lean body mass to determine that endurance athletes required a higher daily protein intake (approximately 1.67 times that of sedentary controls) compared to bodybuilders (1.12 times that of sedentary controls) and control subjects, who need to consume 0.8 grams of protein per kilogram of body weight. Too much protein, however, can be detrimental because it causes excessive urinary calcium excretion (Allen et al., 1979; Hegsted and Linkswiler, 1981; Robertson et al., 1979). Therefore, an important dietary variable to determine with respect to calcium balance and bone is the calcium-to-protein ratio. In 1979, Allen and colleagues studied calcium balance in young men consuming various amounts of dietary protein, while maintaining a consistent intake of dietary calcium (approximately 1400 mg per day). On the high protein diet (225 grams), there was a significant increase in urinary calcium loss and calcium balance became negative. On the lower protein diet (more normal at 75 grams per day), urinary calcium loss was not as great and calcium balance was close to zero. These researchers observed no change in intestinal absorption of calcium and thus, concluded that the effect of increased protein intake was primarily affecting the kidney's role in calcium balance (i.e., increased calcium excretion). Hegsted and Linkswiler (1981) confirmed these

findings in a group of young women who had long-term high protein intakes. In individuals with normal dietary intakes of protein (i.e, not excessive), however, the amount of protein in relation to calcium ingested (i.e., the calcium-to-protein ratio) may be more important to bone than calcium alone. Recker and colleagues (1992) found that significant increases in bone mass occurred in women in the third decade of life at the lumbar spine, forearm and whole body. These gains (over an average three-and-a-half year period) in bone mineral, particularly at the lumbar spine, were positively and significantly correlated with the calcium-to-protein ratio (and physical activity level). These researchers concluded that bone mass could be gained up to about the age of 30 years in young women, and it would be enhanced, in part, by increasing calcium intake in relation to protein intake.

Adding to the complexity of this calcium-protein interaction, however, is the role of phosphorus. Several studies investigating the effects of dietary protein on calcium balance have shown equivocal results. Allen's group (1979) proposed that high levels of protein in the diet may not increase urinary calcium excretion so much as decrease renal reabsorption of calcium. They suggested that phosphorus may play a role in that phosphorus intake was significantly lower in the subjects consuming the high protein diet. Phosphorus acts to suppress urinary calcium and therefore, dampen the hypercalciuric effect. Spencer and colleagues (1978) had found similar effects on urinary calcium when dietary protein was elevated through meat consumption. These researchers found only a small effect on calcium excretion and balance with high protein intakes given as meat, regardless of the dietary calcium intake. They even suggested that the higher phosphorus levels in this meat diet may protect individuals from mineral loss. Hegsted and colleagues (1981) also found that high phosphorus intakes in conjunction with large amounts of dietary protein acted to minimize calcium excretion, such that calcium balance was maintained if phosphorus intake was increased to counteract the effects of a high dietary protein intake. Conversely, the studies by Allen and co-workers (1979) and by Robertson et al. (1979) showed that high dietary protein at a constant calcium intake level resulted in a significant increase in urinary calcium

excretion. Robertson's group (1979) also found that urinary oxalate levels increased substantially and that the risk of kidney stones was then increased almost three-fold in this high protein-constant calcium diet. It should be noted, in terms of the role of phosphorus however, that the high protein diets in these studies (Allen et al., 1979; Robertson et al., 1979) consisted of non-animal sources, i.e., a liquid formula diet.

Along with its relationship with protein intake, dietary phosphorus can affect calcium metabolism more directly, i.e., the calcium-to-phosphorus ratio. In his 1982 review, Marcus pointed out that most studies investigating the dietary calcium-to-phosphorus ratio (in a variety of mammals) have found that a ratio of one-to-one is optimal for the skeleton. A substantial increase in phosphorus intake without a concomitant rise in calcium intake may negatively affect bone, although the findings are equivocal. The proposed mechanism is that an overabundance of dietary phosphorus, especially compared to calcium, causes a hypersecretion of parathyroid hormone, and hence, an increased rate of parathyroid-dependent bone resorption (Lutwak, 1975). In humans, the effect of a low calcium-to-phosphorus ratio (e.g., less than 0.5) may not be too much of a concern (Marcus, 1982). In fact, Heaney (1988) reported no deleterious effects of phosphorus in relation to calcium intake. Bell and colleagues (1977), however, found that by increasing the dietary phosphorus level in relation to calcium, such that the calcium-to-phosphorus ratio was less than 0.5, urinary calcium excretion was reduced, but parathyroid hormone secretion was increased. These researchers concluded that an average American diet which usually contains a lot of phosphate-containing additives may contribute to age-related bone loss. This was in comparison to a healthier diet which had a calcium-to-phosphorus ratio of approximately 0.7 to 0.8. Marcus (1982) suggested that this ratio of nutrients may not be critical to the skeleton in the adult, but may play a role during the growth years. The consumption of soft-drinks, particularly colas which contain approximately 20 mg/dL of phosphorus and no calcium (Lutwak, 1975), in place of milk in adolescents and young

adults still growing, may result in a low calcium-to-phosphorus ratio, and increased parathyroid hormone-induced bone resorption.

Effect of Stress on Menstrual Cycle Function and BMD

Stress plays an important role in the integrity of the reproductive system, the normal function of which can be disrupted by stress-induced hypothalamic dysfunction (Highet, 1989). Schwartz et al. (1980) found that more amenorrheic athletes associated running with stress, as compared to regularly menstruating runners. Often, the onset of amenorrhea could be pinpointed to a psychologically stressful event. Feicht et al. (1978) reported that amenorrheic runners considered their training to be more intense for a greater part of the year, compared to eumenorrheic athletes. Ding and colleagues (1988) found that women athletes had increased basal serum cortisol levels, a measure of physiological stress, as compared to sedentary women. Cortisol was also elevated in amenorrheic as opposed to eumenorrheic athletes. The amenorrheic athletes had decreased estradiol and progesterone concentrations, and a reduced lumbar BMD compared to the eumenorrheic women and sedentary controls. These authors suggested that increased production and secretion of cortisol may be due to the physical and emotional stress that results from their rigorous training lifestyle. They were unable to determine if cortisol acts to decrease bone mineral independent of hypo-estrogenic effects. The presence of excess glucocorticoids may result in osteoporosis. In fact, Drinkwater et al. (1984) and Prior et al. (1990) have suggested that chronically-increased cortisol levels, as seen in the amenorrheic competitive athlete, may cause long-term suppression of the hypothalamic-pituitary-gonadal axis, and irreversible bone loss. Ding and colleagues (1988) had also proposed that the increased glucocorticoid levels may contribute to menstrual cycle disturbances and also decreased bone mineral by altering either or both of the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes.

High stress and anxiety levels are all too common in some female collegiate athletes when faced with the pressures of intense training, competing well, maintaining good grades in school, and possibly trying to have a social life. In a 1988 study, Schweiger and colleagues investigated the relationships between caloric intake, menstrual cycle status and stress in female endurance-trained athletes. These researchers found a significant positive correlation between caloric intake and luteal phase levels of progesterone ($r=0.70$, $p<0.01$), and a strong negative correlation between ratings of subjective stress from family, friends and partner and luteal phase levels of progesterone ($r=-0.80$, $p<0.01$). Stress from family, friends and partner was also significantly correlated with estrogen levels ($r=-0.55$, $p<0.05$), as was stress from work and studies ($r=-0.48$, $p<0.05$). Although non-significant, the correlations between luteal phase progesterone levels and stress from work and studies ($r=-0.39$) and stress from sport ($r=-0.41$) were noteworthy. These researchers concluded that the luteal phase of the menstrual cycle in athletes was specifically prone to disruption due to both caloric deficit and excessive stress from various sources. Cavanaugh et al. (1989) found that highly competitive female athletes, including gymnasts, had very high anxiety levels, and this was related to their irregular menses. In fact, 36% of the variance in athletic menstrual irregularity could be accounted for by trait anxiety and anger, along with pre-training frequency of menses, weight, age at menarche and percent body fat. Cockerill and colleagues (1992) investigated the effect of high-intensity running training on stress levels, specifically a total mood disturbance (TMD) score based on the Profile of Mood States questionnaire. Eumenorrheic runners exhibited lower TMD scores compared to amenorrheic runners and non-runners. These researchers concluded that exercise of such intensity as results in menstrual cycle disturbances appears to be "psychologically self-defeating" in that a more negative mood (i.e., elevated tension and anxiety, depression and anger, and lowered vigor) is experienced compared to non-exercisers, even though aerobic exercise (especially running) is supposed to enhance mood (Gondola and Tuckman, 1983; Morgan, 1980; Wilson et al., 1980). It appears, then, that

amenorrheic athletes had higher stress levels compared to eumenorrheic athletes and controls, which could be both the cause and the outcome of their exercise-induced amenorrhea. These studies seem to support the hypothesis that competitive athletes are subjected to increased amounts of psychological (and physical) stress, which could then manifest in menstrual cycle dysfunction. Since a runner's training year is typically divided into different seasons (including competitive and off-seasons), it may be speculated that stress levels would fluctuate over the year, as would stress-induced menstrual cycle irregularities. Feicht and colleagues (1978) had previously found that amenorrheic runners reported higher stress-related scores as a result of their intensive training compared to eumenorrheic runners. Schwartz and co-workers (1980) concluded that more amenorrheic athletes associated running with stress compared to regularly menstruating runners. The amenorrheic runners in this study were subjected to higher levels of stress (i.e., more intense exercise) compared to the eumenorrheic runners. It is possible that this stress could be the cause and the outcome of exercise-associated amenorrhea, or as Highet (1989) proposed, hypothalamic dysfunction is influenced by stress, which might disrupt the entire reproductive system.

Summary

Measurements of bone mass and BMD can be performed precisely, and may be important in tracking bone losses resulting from menopause or premenopausal menstrual cycle aberrations. In this regard, it may also be an important tool in predicting future cases of osteoporosis. If an increased risk can be identified early, intervention strategies may be introduced. The same scenario should theoretically be applied to premenopausal, competitive athletes who experience menstrual cycle dysfunction (e.g., amenorrhea or oligomenorrhea) associated with intense, prolonged exercise training, thereby putting themselves at an increased risk of losing bone mineral. First, however, some of the major

contributing "risk factors" must be identified, so that they can either be controlled or treated. Seasonal stress levels and training intensity progressions are two major variables which could affect normal menstrual cycle function. If the menstrual cycle is disrupted, is amenorrhea the result, or luteal phase suppression? Is it possible to determine whether relative lack of estrogen or progesterone, or both, is more detrimental to bone health? Sometimes, no matter what a coach or trainer may believe and suggest to his/her athletes, the runners are simply not willing to decrease their training for the purpose of regaining normal menses. For these competitive athletes, their running career is now, and they are not considering possible future health complications (i.e., increased risk of osteoporosis) which may result from present behavior (i.e., training-induced menstrual cycle dysfunction). In such cases, hormone intervention may be beneficial (Highet, 1989), often in conjunction with calcium supplementation (if the dietary intake is lacking). As a result, losses of bone mass and decreased BMD may be controlled and minimized, and the incidence of season-ending stress fractures may be diminished.

METHODS AND PROCEDURES

The following chapter outlines the experimental methods and procedures followed in this research project. A description of the subjects includes the number of subjects participating in the study, subject selection criteria, and group assignment. In addition, the experimental protocol, including instruments and apparatus used in data collection and the experimental procedures, experimental design, and the statistical analyses are discussed.

Subjects

Women were recruited from each of the following groups, based on their activity level and sport: 1) the Oregon State University gymnastics team (n=12); 2) the University of Oregon cross-country and track teams and other competitive female distance runners (n=20); and 3) a college-aged population who were within ten pounds of their ideal body weight, did not participate in a regular exercise program (i.e., less than three hours/week), and were eumenorrheic (n=19). Of the original 51 women who volunteered to participate in the study, seven failed to complete the full nine months of data collection. Two of the runners quit the team and stopped running competitively. They were both eumenorrheic, but were taking oral contraceptives. Three of the control subjects dropped out due to time demands from school, one moved out of the area, and one disqualified herself shortly after entry into the study by starting oral contraceptive use. All gymnasts completed the study. Thus, final sample size consisted of 18 runners, 12 gymnasts and 14 controls.

All subjects were 18 to 30 years of age, healthy, did not smoke or take any medication known to affect bone metabolism (e.g., thyroid or asthma medication), except oral contraceptives, and had never been pregnant. Seven runners but no gymnasts or controls were currently taking oral contraceptive medication. The runners were women

who train seriously for middle-distance and distance events (i.e., 800 m to 10,000 m). They followed a training regimen that included fall and spring competitive seasons (e.g., cross-country and track racing, respectively), with a winter off-season. They had been training seriously for at least the previous year (most of them for at least the past five years), running a minimum of 4 to 5 days a week, an average of 30 miles per week. The gymnasts were women training for Pac-10 and NCAA competition. They had been training intensively for at least one year prior to beginning the study, and most of them had been extensively involved in gymnasts for at least seven or eight years.

This study was reviewed and approved by the Oregon State University Human Subjects Committee and by the State Department of Human Resources Radiation Control Board. All subjects gave written informed consent (refer to Appendix A).

Experimental Protocol

At the beginning of the 9-month study, subjects were asked to complete a comprehensive exercise, health and nutrition questionnaire (refer to Appendix B). This served as a preliminary screening device, and also characterized the salient features of the three groups. The study extended over a 9-month training period, beginning in the fall (early September) and finishing in June. This time frame was divided into four phases and testing was done at the following times: Phase I (baseline/pre-test) in September, Phase II in December, Phase III in March, and Phase IV (post-test) in June. For the runners, these phases corresponded to the beginning of the fall (cross-country) competitive season, the beginning of the winter off-season, the beginning of the spring (track) competitive season, and the post-test or end of the track competitive season, respectively. For the gymnasts, these phases corresponded to the beginning of the training season, the end of the pre-season training, late competitive season, and early off-season, respectively.

Each subject kept a daily exercise and activity log for the duration of the study (refer to Appendix C). For the control subjects (non-competitive women), this log documented their minimal activity (less than three hours/week of regular, structured exercise), so that it could be quantified and related to any changes in psychological profile, nutritional status, or BMD. For the athletic groups, it was critical for the runners to keep track of their daily runs, and any supplemental exercises, so that seasonal changes could be followed. The runners were asked to briefly describe each day's workout, including: type of workout (e.g., distance vs intervals), total distance (miles) run, time spent in workout, and pace of run. The coach's workout schedules were consulted to confirm or add to the information in the runners' daily logs. Training was quantified for the runners as a group and according to menstrual cycle status (eumenorrheic vs oligo/amenorrheic), on a seasonal basis and as an average over the 8-month period. Training volume was defined as the average mileage per week for fall, winter and spring seasons. Training intensity was defined as the average number of "intense" training sessions (i.e., intervals, fartlek, tempo runs, races) per week, and was also determined for fall, winter and spring seasons. The researcher was unable to quantify training intensity and volume in any meaningful way for the gymnasts, so results and discussion to follow will be for runners only.

Each subject kept track of her menstrual cycle using the daily log to chart each day of the cycle (i.e., Day 1 - onset of menses - through the last day before the onset of the next menstrual flow). Body weight was also noted on a monthly basis. Menstrual cycle function, in those women who were normally menstruating, was monitored on two different occasions (once in the Fall and once in the Spring). The subjects collected daily urine samples, beginning on the first day of menses and continuing until the onset of the next menstrual cycle (i.e., 28 $\pm$ 5 days). The subjects collected approximately 5 ml of the day's first urine void each morning in a container supplied by the investigator. The sample was immediately frozen at about -20 degrees C for later analyses, as per the method of Munro et al. (1991). At the end of the study, all urine samples were batch run for enzyme

immunoassays of pregnanediol-3-glucuronide (PdG), the major urinary progesterone metabolite, following the techniques of Munro et al. (1991). These assays were sent for analysis to Dr. W. Lasley at the Veterinary School at the University of California at Davis, where the technique of Munro et al. (1991) has been established. From the urinary hormone metabolite levels, luteal phase length was determined, in order to detect possible deficiencies (i.e., a shortened luteal phase or anovulation). The luteal phase was defined as the number of days between the peak concentration of PdG (or occurrence of ovulation, and peak estradiol concentration) and the onset of menses.

The urinary measurements of pregnanediol-3-glucuronide (PdG) are a preferred method in comparison to serum or plasma analyses (Munro et al., 1991), and have been shown to be valid and reliable. Munro et al. (1991) determined the precision of these enzyme immunoassays (EIA). For these urinary PdG measures, the intra-assay CVs for pools of high, medium and low concentrations were 5.2%, 6.9% and 11.0%, respectively. The inter-assay CVs were 5.6%, 7.8% and 13.6%, respectively. The sensitivity of the EIA for urinary PdG was < 0.05 umol/L urine. Profiles for urinary PdG assayed by EIA and radioimmunoassay (RIA) resulted in a concurrent validity correlation coefficient of 0.94 ($p<0.01$). The correlation between urinary PdG and serum progesterone was 0.94 ($p<0.01$).

Psychological stress levels of each subject were assessed on four occasions during the study (i.e., September, December, March, and June), using measures of anxiety and mood states. The Profile of Mood States (POMS) was developed by McNair, Lorr and Droppleman in 1971 to assess affective states in psychiatric outpatients. Since then, it has been used successfully to study numerous athletic groups, including runners and gymnasts (Edwards & Huston, 1984; Gondola & Tuckman, 1982, 1983; Loucks & Horvath, 1984; Morgan, 1980; Wilson et al., 1980). Gondola and Tuckman (1982) reported that the various POMS scales - depression (D), tension-anxiety (T), vigor (V), anger (A), fatigue

(F), confusion (C) - have internal reliabilities ranging from 0.84 to 0.94, and test-retest reliabilities ranging from 0.65 to 0.74.

The State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970) was also used to quantify stress and anxiety. Test-retest reliability coefficients for trait anxiety measures have been reported to range between 0.73 and 0.86 for college students and psychiatric patients, over a period of one hour to 104 days (McGlynn et al., 1983; Rule & Taylor, 1983; Spielberger, 1971). Test-retest reliability coefficients for state anxiety, a more transient measure, range from 0.40 to 0.54. Both trait and state anxiety measures show internal consistency with alpha coefficients ranging from 0.83 to 0.92 (Spielberger et al., 1970).

State and trait anxiety scores were computed for all subjects. From the POMS scores, a Total Mood Disturbance (TMD) score was calculated as a single overall estimate of affective state, using the following equation (Loucks & Horvath, 1984):

$$TMD = (T + D + A - V + F + C) / 4.$$

The three psychological stress/anxiety questionnaires were administered to the subjects on the same day, and they were instructed to follow the instructions for completing each as accurately as possible. The Profile of Mood States questionnaire asks subjects to respond to numerous adjectives based on how they have been feeling during the past week. The State Anxiety inventory asks subjects to respond to several phrases based on how they feel at that specific moment. The Trait Anxiety inventory asks subjects to respond to several phrases based on how they generally feel. See Appendix D for copies of the POMS and STAI questionnaires.

A 4-day diet record was used to quantify normal dietary intake of the following nutrients: total calories, protein, carbohydrate, fat, calcium and Vitamin D. The "Nutritionist III" computer program (N-Squared Computing, Salem, OR, 1985) was used to compute these values. For the majority of food items available for analysis with "Nutritionist III", nutrient values were based on the USDA 8th Handbook Series and the

Nutrient Data Bank. Lab analyses of nutrients from some specific manufacturer's brand name products were also included in this program's data base. The calcium-to-phosphorus and calcium-to-protein ratios were then calculated. The four days were consecutive: 2 weekdays and 2 weekend days (although these were not always the same four days of the week for each monitoring period). These diet records were kept on four different occasions, at the end of each Phase (i.e., September, December, March, and June). An example worksheet for the diet record is shown in Appendix E. The subjects were shown food models as examples of various foods and portion sizes prior to diet recording and were instructed to be as detailed and accurate as possible in their recording, in order to maximize the accuracy of dietary intake.

Bone mineral density was measured in all subjects before beginning and upon completion of the 9-month study period (i.e., September/October and May/June). A Hologic QDR-1000/W dual energy X-ray absorptiometer was used to measure bone area (in cm^2), bone mineral content (BMC, in grams) and bone mineral density (BMD, in g/cm^2) of the lumbar spine (L_{2-4}), proximal femur (specifically, the femoral neck), and the whole body. Body composition (including lean body mass, LBM, and percent body fat) was also determined from the whole body scan. The Hologic QDR-1000/W uses quantitative digital radiography (QDR), to accurately quantify bone mineral content (the amount of calcium hydroxyapatite, in grams), since it is the major component of bone mineral (Hologic Manual, 1990). The resulting image is digitized and displayed on the computer terminal. The X-ray absorption of the bone is calculated via constant comparison against a known reference, or calibration wheel (refer to the diagram in Appendix F). Radiation doses to the subjects are very low: 2.0 mR to 5.0 mR for the regional scans and less than 1.5 mR per whole body scan. Scans take 13 to 15 minutes for the whole body and 6 to 8 minutes each for the regional areas. The measurement precision error for all scans is < 1.0%. The accuracy of this procedure is between 3% and 5% (Hologic Manual, 1990).

Muscle strength was tested using the Kin-Com 500H isokinetic machine (Chattecx, Corp., Hixson, TN). Peak concentric force (in kilograms), using a gravity correction, was measured in the following muscle groups: low back extensors (erector spinae), hip abductors (gluteus medius) and hip adductors (adductor magnus, minimus, brevis), leg extensors (quadriceps) and elbow flexors (primarily biceps brachii). Isokinetic speed of testing was 15 degrees per second for the back extension exercise and 30 degrees per second for the other four tests. Isokinetic testing means that the speed of the movement was kept constant throughout the range of motion for a particular exercise. As a result, force produced by the muscles while performing a given movement can be used as a measure of strength rather than the amount of weight lifted. Previous studies at this institution using the Kin-Com have shown intraclass correlations for these measures to range from 92% to 98%, in a population of 18- to 75-year old men and women (Harter et al., 1992).

Each subject, after a thorough warm-up, performed five maximal trials of each exercise, with 30 seconds rest between trials. There was approximately five minutes rest between each exercise. Descriptions of the strength tests are given below:

Back (Extension): The subject was seated upright in a trunk positioning chair. Legs were elevated and knees bent at 70 degrees of flexion. The back was positioned at zero degrees initially (upright), with a load cell-pad combination placed on the back between each scapula (shoulder blade). A pad was placed in the lower back region to prevent anterior/posterior movement and the hips were securely positioned to prevent lateral movement. With the arms placed across the chest, when instructed, the subject moved from a slightly flexed (forward) position of -20 degrees to 15 degrees of extension. Movement speed was controlled at 15 degrees per second.

Hip Abduction: The subject was positioned horizontally on her left side facing the Kin-Com with the left leg slightly bent for support. A load cell-pad combination was placed just above with right knee and the legs were held together. The subject began with the right leg resting on top of the left, then, when instructed, abducted the right leg (moving it away

from the body) from a position of -15 degrees to 30 degrees. Movement speed was controlled at 30 degrees per second.

Hip Adduction: The subject began in the same position as in hip abduction except the right leg was abducted 45 degrees. When instructed, the subject adducted the right leg (pulling the right leg toward her left leg) from a position of 45 degrees to zero degrees. Movement speed was 30 degrees per second.

Elbow flexion: The subject sat upright in a low chair with the right arm extended from the shoulder resting on a pad. The load-cell pad combination was placed on the wrist and, when instructed, the right arm was flexed from a position of zero degrees to 110 degrees of flexion. Movement speed was controlled at 30 degrees per second.

Leg extension: The subject sat in a chair with the legs suspended and the back supported. The load-cell pad combination was placed just above the right ankle. When instructed, the subject extended the leg at the knee from a position of 85 degrees to 180 degrees. Movement speed was controlled at 30 degrees per second.

All subjects completed a progressive exercise stress test to maximum on the treadmill, in order to quantify their maximal oxygen consumption (VO_{2max}). The test to determine VO_{2max} was conducted in accordance with the American College of Sports Medicine guidelines (ACSM, 1991). The treadmill protocol was designed to have the subject reach her VO_{2max} within ten to fifteen minutes. Each subject was first familiarized with walking/running on a treadmill for five to ten minutes, depending on previous treadmill exposure. The protocol varied depending on the subject group. The control subjects warmed up at a speed of 3.0 miles per hour (mph) on the level (0% grade) for two minutes. The test began at this same speed, and increased one-half mph each minute until the subject reached a comfortable jogging speed for her (ranging between 5.0 and 6.0 mph). After this, treadmill grade increased one percent each minute until the subject reached her VO_{2max}. For the gymnasts, the warm-up speed was 3.5 mph, at 0% grade, and the test progressed by increasing the speed 0.5 mph each minute, up to 6.0 mph, and then

increasing the grade 1% per minute until the subject reached VO_{2max}. For the runners, the warm-up speed was 6.0 mph, at 0% grade, and the test progressed by increasing the speed 0.5 mph each minute, up to 9.0 mph, after which the treadmill grade increased 1% per minute until VO_{2max} was reached. The subject continued exercising until volitional exhaustion. They were considered to have reached VO_{2max} if they met more than one of the following criteria: 1) no increase in VO_2 with increasing workload, 2) reach age-predicted maximal heart rate (220 beats/min - age), and 3) respiratory exchange ratio greater than 1.10. The VO_{2max} test was performed in the first two months of the 9-month study period, and was used as a descriptor of the subject's aerobic fitness level.

A SensorMedics 2900 Metabolic Cart was used to continuously measure expired gases and to calculate VO_2 (in $l.min^{-1}$ and $ml.kg^{-1}.min^{-1}$). The subject breathed room air through a Hans Rudolph t-shaped two-way non-rebreathing valve, with the nose clipped shut, and all expired air passed through a hose to the mixing chamber in the metabolic cart, where analysis took place. Before each test, the SensorMedics 2900 Metabolic Cart was calibrated for volume, using the flowmeter and a 3-liter syringe, and for gas concentrations (mixing chamber analyzer) using a two-point gas calibration: 1) 26.32% O_2, 0% CO_2, and 2) 15.96% O_2, 3.94% CO_2. The subject wore a UNIQ CIC ProTrainer Model 8733 heart rate monitor: transmitter and wrist receiver, so that heart rate could be monitored throughout the test.

The investigator contacted each subject regularly (i.e., every one to two weeks) in an effort to minimize attrition and improve accuracy of data collection over the 8-month study period.

Experimental Design

A non-randomized between/within repeated measures design was used in this longitudinal study. The between factor was subject group: runners, gymnasts, and non-

competitive female controls. The within factor was time. The study lasted eight months, encompassing one full school year/training season. Phase I or the baseline/pre-test measures were taken in September. These included: BMD of the lumbar spine, proximal femur and whole body; muscle strength of the back, hip abductors and adductors, leg extensors, and elbow flexors; POMS and STAI questionnaires for stress/anxiety levels; 4-day diet records for nutrient analysis of total calories, protein, carbohydrate, fat, calcium, and Vitamin D; and training intensity and volume. These same measures were repeated at the completion of the study - Phase IV or post-test (in June). Bone mineral was not measured in Phases II and III, due to restrictions by Oregon State Law. Body composition and muscle strength were not measured in Phases II and III either, as significant changes were not expected in this short time period. The other measures listed above were taken in December and March. Luteal phase length was determined by measuring urinary progesterone metabolites on two different occasions (Fall and Spring) for a full menstrual cycle each time only in those women who were normally menstruating. Refer to Figure 1 for an outline of the experimental design.

Statistical Analyses

Baseline data were analyzed using a 3-way analysis of variance (ANOVA) to determine whether there were any group differences (runners vs gymnasts vs controls) among the measures taken at pre-test, including: BMD of the lumbar spine, femoral neck and whole body; body composition; muscle strength of the trunk extensors, leg extensors, hip abductors and adductors, and elbow flexors; VO_{2max}; POMS and STAI; dietary nutrients; and other descriptive characteristics of the subjects. The Statview 4.0 statistical computer program (ABACUS Concepts, Inc., Berkeley, CA) was used to perform the statistical analyses.

Since menstrual cycle status is a confounding factor when investigating bone mineral density in these female athletes, the three groups were further divided according to menstrual cycle status. Then, analyses of variance were performed comparing data among the five subject groups: eumenorrheic (ER) and oligo/amenorrheic runners (OAR), eumenorrheic (EG) and oligo/amenorrheic gymnasts (OAG), and eumenorrheic controls (EC).

Repeated measures ANOVAs were performed to investigate group differences over the 8-month training period. Either a 3 (group: runners, gymnasts, controls) or 5 (group: ER, OAR, EG, OAG, EC) x 2 (time: pre/post) between/within ANOVA with repeated measures on the second factor was used to test the hypotheses related to changes over time.

Since some of the pre-test or baseline measures were significantly different among groups to start, however, percent change scores were calculated and used in the ANOVAs. For example, percent change scores were used to test the hypotheses related to changes in bone mineral density. Percent change scores were calculated by subtracting the pre-test score from the post-test score, dividing by the pre-test score, and multiplying by 100 to get a percent. T-tests were performed on the percent change scores to determine differences from zero.

If significant main effects or interactions were found in the ANOVA results, post-hoc analysis employed either the Tukey's or Scheffe's t-test. If the sphericity assumption for repeated measures analyses was not met, especially for locating mean differences for the repeated measures factor or the interaction, the more conservative Scheffe's test, which adjusts the F-value, was used. Otherwise, a Tukey test was used (Thomas & Nelson, 1990).

This research was exploratory in nature, and limitations with so much data and a number of different analyses must be recognized: the probability of making a Type I error was increased. The practical significance of certain results, however, must be acknowledged in addition to the statistical significance, especially in this type of

exploratory research. For example, the percent change in BMD (pre-to-post) was discussed as a more meaningful term, compared to absolute BMD values which appear quite small. Percent change scores were also necessary to determine differences across time when groups varied in BMD at baseline. The probability of statistical significance was set at an alpha level of 0.05 for each analysis as a compromise, in an attempt to reduce the overall experiment-wise error (and reduce the chance of making as many Type I errors) but not exclude meaningful significance of practical use. If this was a completely randomized groups design (i.e., n=120), the power to detect a medium (0.30) effect size would be approximately 0.80 at an alpha of 0.05. In this between/within non-randomized repeated measures design, power was somewhat lower, probably 0.50 to 0.80.

Upon completion of data analysis, statistical power was determined using the Bavry (1991) "Stat-Power Version 2.2" computer program (Scientific Software International, Chicago, IL). For the repeated measures analyses, power ranged from 0.90 to 1.00 for body composition and BMD (LS, FN and WB), 0.30 to 0.61 for muscle strength (depending on the muscle group), 0.59 to 0.84 for nutrient intake (depending on the specific nutrient), and 0.31 to 0.61 for the stress/anxiety inventory scores. The statistical power for repeated measures analysis of training intensity (i.e., number of intense sessions per week) was 0.13, while the statistical power of training volume (i.e., miles run per week) was 1.00.

Statistical power was also determined for the ANOVA among the groups (3 or 5) for the numerous dependent variables and revealed the following range of values for power:

<u>3-Group ANOVAs</u>:

LS BMD 0.93 to 1.00 (% change and g/cm^2, respectively)

FN BMD 0.76 to 1.00 (% change and g/cm^2, respectively)

WB BMD 0.62 to 0.88 (% change and g/cm^2, respectively)

BMD at other skeletal sites-	0.30 to 1.00 (g/cm^2, depending on the site measured)
	0.10 to 0.86 (% change, depending on the site measured)
BMD by injury status	0.1 to 0.59 (depending on the site measured)
Muscle strength-	0.63 to 0.88 (kg, depending on the muscle group tested)
	0.10 to 0.20 (% change, depending on the muscle group)
Nutrient intake	0.15 to 0.97 (depending on the specific nutrient)
Psychological stress/anxiety	0.90 to 0.99 (depending on the inventory scored)

5-Group ANOVAs (by menstrual cycle status):

LS BMD	0.42 to 0.44 (% change and g/cm^2, respectively)
FN BMD	0.35 to 0.47 (% change and g/cm^2, respectively)
WB BMD	0.28 to 0.44 (% change and g/cm^2, respectively)
BMD at other skeletal sites-	0.44 to 0.47 (g/cm^2, depending on the site measured)
	0.34 to 0.61 (% change, depending on the site measured)
Muscle strength-	0.39 to 0.66 (kg, depending on the muscle group tested)
	0.33 to 0.55 (% change, depending on the muscle group)
Nutrient intake	0.33 to 0.59 (depending on the specific nutrient)
Training intensity and volume-	0.24 and 0.62 (ER and OAR, respectively)

SEPTEMBER	DECEMBER	MARCH	JUNE
PHASE I	PHASE II	PHASE III	PHASE IV
BASELINE/ PRE-TEST			POST-TEST/ END OF COMPETITIVE SEASON
BMD LUMBAR SPINE FEMORAL NECK WHOLE BODY			BMD L. SPINE F. NECK WHOLE BODY
BODY COMPOSITION			BODY COMP.
MUSCLE STRENGTH			MUSCLE STR.
POMS/STAI	POMS/STAI	POMS/STAI	POMS/STAI
	LUTEAL PHASE LENGTH (LPL)		LPL
TRAINING INTENSITY AND VOLUME (TI, TV)	TI AND TV	TI AND TV	TI AND TV
4-DAY DIET RECORD	DIET RECORD	DIET RECORD	DIET RECORD

Figure 1. Experimental design for the study over an 8-month training period.

RESULTS

General Characteristics

Fifty-one subjects volunteered to participate in the study: 20 competitive female runners, 12 collegiate gymnasts, and 19 normally-active college women who served as controls. Group characteristics are presented in Table 1. Frequency distributions for all dependent variables are shown in Appendix G.

Table 1. Descriptive characteristics of runners (n=20), gymnasts (n=12) and control subjects (n=19) at baseline (mean±S.D.).

	Runners	Gymnasts	Controls
Age (years) *+	21.6±2.2	19.8±1.1	19.2±1.6
Weight (kg) *+	53.0±4.5	55.7±6.4	60.2±6.5
Body Mass Index *+ (BMI)	18.9±1.2	21.8±1.5	21.9±2.6
Lean Body Mass (kg) (LBM)	43.1±3.3	44.6±4.7	44.4±4.4
LBM/Height^2 (kg/m^2) +#	15.3±0.8	17.7±1.2	16.2±1.6
% Fat *#	14.7±2.3	15.8±2.4	22.3±3.0
Age training began + (years)	15.6±2.2	7.4±1.9	

* runners significantly different from controls ($0.0001<p<0.05$)
\+ runners significantly different from gymnasts ($0.0001<p<0.05$)
\# gymnasts significantly different from controls ($0.0001<p<0.05$)

Descriptive characteristics for the groups were also analyzed according to menstrual cycle status: eumenorrheic (ER) and oligo/amenorrheic (OAR) runners, eumenorrheic (EG) and oligo/amenorrheic (OAG) gymnasts, and eumenorrheic controls (EC). Table 2 illustrates some of these significant group differences at baseline.

Table 2. Descriptive characteristics of subject groups according to menstrual cycle status, measured at baseline. The five groups are: eumenorrheic runners (ER, n=12), oligo/amenorrheic runners (OAR, n=6), eumenorrheic gymnasts (EG, n=8), oligo/amenorrheic gymnasts (OAG, n=4), and eumenorrheic controls (EC, n=14) (mean±S.D.).

	Weight (kg)	BMI	LBM	%Fat	Training Age
ER	54.7±4.2[a]	19.0±1.0[ac]	44.5±2.9	14.7±2.3[a]	16.1±2.6[cf]
OAR	49.3±3.8[bd]	18.7±1.6[bd]	40.5±2.9	14.0±2.1[bd]	14.8±1.7
EG	57.6±6.4	22.3±1.3	45.6±4.8	16.8±1.0[i]	7.3±1.6
OAG	51.9±5.0[j]	20.7±1.4	42.6±4.4	14.0±3.6[j]	7.4±2.7
EC	61.0±7.0	21.9±2.7	45.1±4.9	22.3±3.0	--------

[a] ER significantly different from EC ($0.0001<p<0.05$)
[b] OAR significantly different from EC ($0.0001<p<0.05$)
[c] ER significantly different from EG ($0.0001<p<0.05$)
[d] OAR significantly different from EG ($0.0001<p<0.05$)
[e] ER significantly different from OAR ($0.0001<p<0.05$)
[f] ER significantly different from OAG ($0.0001<p<0.05$)
[g] OAR significantly different from OAG ($0.0001<p<0.05$)
[h] EG significantly different from OAG ($0.0001<p<0.05$)
[i] EG significantly different from EC ($0.0001<p<0.05$)
[j] OAG significantly different from EC ($0.0001<p<0.05$)

Of the fifty-one subjects who began this longitudinal study, forty-four completed the eight-month study period: 18 runners, 12 gymnasts and 14 controls. Two runners quit the team shortly after the baseline data were collected, and no longer engaged in a strenuous running program. Three of the control subjects dropped out of the study due to time demands from school, one moved out of the area, and one disqualified herself from the

control group shortly after entry into the study by starting oral contraceptive use. All the gymnasts completed the study.

Results from the longitudinal data were analyzed by factorial and repeated measures analysis of variance (ANOVA). Baseline values were analyzed by factorial ANOVA. Percent change scores were analyzed by factorial ANOVA, and t-tests were used to determine changes from zero (i.e., whether the percent change in a specific dependent variable increased or decreased significantly or remained constant).

Body Composition

Repeated measures analyses revealed no significant changes in body weight among the three groups, although it increased slightly in all groups: runners increased 1.6±2.9 kg, gymnasts increased 0.6±1.0 kg, and controls increased 1.8±2.1 kg. The change in body weight over the 8-month training period was not significantly different among groups according to menstrual cycle status ($p>0.05$). All five groups increased their body weight slightly. The change in lean mass was significantly greater ($p<0.05$) in the runners compared to the gymnasts (3.5±3.8% vs 0.4±2.3%), but not compared to the controls (1.9±2.7%). When the change in lean mass was analyzed according to menstrual cycle status and group, there were no differences observed ($p>0.05$). The change in % fat was not significantly different among groups, or according to menstrual cycle status. T-tests revealed a significant increase in lean mass in the runners (1.5±1.6 kg) compared to zero ($p=0.0001$), but no change in % fat (-0.2±2.0%). The lean mass of the gymnasts remained constant (i.e., a change of 0.1±10. kg), as did their % fat (0.5±2.1%). The control subjects had a significant ($p<0.01$) increase in lean mass (0.8±1.1 kg), but did not increase % fat (0.7±1.4%).

Aerobic Capacity and Muscle Strength

At baseline, the three groups differed with respect to maximal aerobic capacity (or VO_{2max}) ($p<0.05$). The runners exhibited the highest values (58.5±6.7 ml/kg.min), followed by the gymnasts (49.5±3.0 ml/kg.min), with the controls having the lowest values for VO_{2max} (42.9±5.6 ml/kg.min). Muscle force values (peak concentric force determined in five muscle groups) are illustrated in Tables 3, 4 and 5. At baseline, the gymnasts exhibited the highest forces in all muscle groups, although it did not quite reach statistical significance ($p=0.09$) for the trunk extensors. At post-test, gymnasts continued to exhibit the highest peak muscle forces, although this only reached statistical significance for the leg extensors and elbow flexors ($p<0.05$). Due to lingering sore muscles or an injury, only 17 runners performed the hip adduction and abduction evaluations. Likewise, only ten gymnasts performed the trunk extension evaluation. Repeated measures ANOVA revealed no significant differences in muscle force values over time. T-test analyses revealed that no changes in any muscle group tested were significantly different from zero for any group of subjects.

Table 3. Baseline, post-test and percent change scores for peak muscle force (in kilograms) in runners (n=18) (mean+S.D.).

	Baseline	Post-test	% change
Trunk extension	70.8±12.6	65.8±11.2	-4.4±1.4
Leg extension	57.0±10.2	57.0±15.6	0.9±9.1
Hip abduction	34.4±5.4	37.1±8.0	2.8±6.2
Hip adduction	31.1±6.7	30.2±7.5	-1.3±7.2
Elbow flexion	13.9±2.2	14.9±1.7	0.8±2.4

Table 4. Baseline, post-test and percent change scores for peak muscle force (in kilograms) in gymnasts (n=12) (mean+S.D.).

	Baseline	Post-test	% change
Trunk extension	83.1±17.9	73.8±14.1	-11.9±19.7
Leg extension	68.9±12.2	68.6±9.1	-0.3±7.6
Hip abduction	41.6±11.2	42.1±10.4	0.4±7.3
Hip adduction	35.6±8.0	33.6±5.2	-2.0±9.3
Elbow flexion	17.2±3.0	18.5±2.5	1.4±2.8

Table 5. Baseline, post-test and percent change scores for peak muscle force (in kilograms) in controls (n=14) (mean+S.D.).

	Baseline	Post-test	% change
Trunk extension	72.7±17.2	66.0±15.7	-7.4±12.0
Leg extension	56.8±11.9	56.9±11.5	2.4±7.2
Hip abduction	37.4±7.8	38.8±10.2	2.2±7.0
Hip adduction	29.2±7.0	30.3±7.7	0.7±5.5
Elbow flexion	14.4±3.5	15.4±3.3	0.5±3.0

When peak muscle force was analyzed for group differences based on menstrual cycle status at baseline, the only significant differences were for peak force of the leg extensors ($p<0.05$) and elbow flexors ($p<0.01$). The eumenorrheic runners had lower peak leg extension force compared to the eumenorrheic gymnasts (58.2±9.4 vs 69.8±10.5 kg). The oligo/amenorrheic runners (51.8±11.5 kg) exhibited lower forces for the leg extensors compared to both the EG and the oligo/amenorrheic gymnasts (67.0±16.6 kg). The EG also exhibited higher peak leg extension force compared to the eumenorrheic control subjects (55.2±11.0 kg). The eumenorrheic runners (14.7±1.7 kg) had a lower peak elbow flexion force compared to the eumenorrheic gymnasts (18.3±2.9 kg) and the

oligo/amenorrheic gymnasts (19.1±1.7 kg). The oligo/amenorrheic runners (15.3±1.6 kg) exhibited lower peak elbow flexion forces compared to the EG and the OAG. The eumenorrheic control subjects (15.4±3.3 kg) also had lower peak elbow flexion forces compared to both the EG and OAG.

Menstrual Cycle Status

The age at menarche was significantly different among the three groups ($p=0.0001$). The gymnasts had a later age at menarche (16.0±1.5 yr) compared to the runners (14.1±1.4 yr) and controls (13.0±1.2 yr). By menstrual cycle status, significant differences persisted ($p<0.001$). The eumenorrheic runners (14.3±1.2 yr) had a significantly earlier age at menarche compared to the eumenorrheic gymnasts (15.7±1.5 yr) and the oligo/amenorrheic gymnasts (16.6±1.5 yr), and significantly later age at menarche compared to the eumenorrheic controls (13.1±1.3 yr). The oligo/amenorrheic runners (14.2±1.4 yr) had a significantly earlier age at menarche compared to the EG and OAG.. The EC had an earlier age at menarche compared to both the EG and OAG. Approximately one-third of each athletic group reported menstrual cycle irregularity, i.e., amenorrhea (AM) and oligomenorrhea (OA). Three of the runners were amenorrheic and three were oligomenorrheic. Five of the runners were currently taking oral contraceptives and had been for at least the previous year (range 1 to 4 years). Five of the runners had always had regular menstrual cycles, while the others had some history of amenorrhea and/or oligomenorrhea. One gymnast had primary amenorrhea. Three gymnasts were oligomenorrheic. None of the gymnasts or control subjects were taking oral contraceptives. Five of the gymnasts had a history of regular menstrual cycles, while the others exhibited a history of irregularity. All control subjects were eumenorrheic, with no history of oligo- or amenorrhea, having an average of 12.0±0.4 cycles per year.

Bone Mineral Density

At baseline, bone mineral density values for the femoral neck (FN), lumbar spine (LS) and whole body (WB) all were significantly different among the three subject groups ($p<0.01$). This was the case when BMD was expressed in absolute terms (g/cm^2) and as a percent of the published norms (based on the expected mean for age and gender). Because of these pre-test differences in BMD, repeated measures ANOVA was used to analyze changes over time for each group separately. Percent change scores were calculated and analyzed by factorial ANOVA. In the runners, bone mineral density did not differ according to oral contraceptive use. For example, LS BMD for those runners taking oral contraceptives was 1.008 ± 0.116 g/cm^2 compared to 1.007 ± 0.068 g/cm^2 in runners not taking oral contraceptives. For the FN BMD, oral contraceptive users measured 0.928 ± 0.115 g/cm^2 while those runners not taking oral contraceptives measured 0.909 ± 0.097 g/cm^2. Thus, the BMD data for the runners was pooled for oral contraceptive use.

At post-test, significant group differences persisted for lumbar spine, femoral neck and whole body BMD ($p<0.05$), expressed in absolute terms (g/cm^2) and as a percent of the expected norm. Significant differences were observed from one-way factorial ANOVA in the percent change scores for lumbar spine BMD (Table 6) and femoral neck BMD (Table 7) ($p<0.01$), while the percent change in whole body BMD (Table 8) did not differ among the groups ($p=0.09$). When compared to zero (t-test), LS BMD increased in the gymnasts ($p<0.01$) and the controls ($p<0.05$), but not in the runners. Although none of the percent change scores for FN BMD were significantly different from zero in any group, the increase in FN BMD approached significance for the gymnasts ($p=0.07$) and the decrease in FN BMD approached significance for the runners ($p=0.08$). The increase in WB BMD was significantly different from zero in the runners and gymnasts ($p=0.0001$), but not in the controls.

Table 6. Lumbar spine bone mineral density values (in g/cm^2 and as a percent of the expected norm) at baseline and post-test, and the percent change scores for LS BMD for the three groups of subjects (mean±S.D.).

Lumbar spine BMD

	Baseline *+	Post-test *+	% change #+
Runners			
g/cm^2	0.980±0.11	0.972±0.11	0.4±2.1%
%norm	92.2±10.5	92.0±11.2	
Gymnasts			
g/cm^2	1.180±0.13	1.225±0.13	2.9±2.5%
%norm	111.2±12.7	114.6±12.8	
Controls			
g/cm^2	1.114±0.12	1.123±0.13	0.7±1.3%
%norm	103.4±10.3	104.4±11.7	

* runners significantly different from controls ($0.0001<p<0.05$)
\+ runners significantly different from gymnasts ($0.0001<p<0.05$)
\# gymnasts significantly different from controls ($0.0001<p<0.05$)

Table 7. Femoral neck bone mineral density values (in g/cm^2 and as a percent of the expected norm) at baseline and post-test, and the percent change scores for FN BMD for the three groups of subjects (mean±S.D.).

Femoral Neck BMD

	Baseline *#+	Post-test *#+	% change +#
Runners			
g/cm^2	0.882±0.11	0.875±0.11	-1.1±2.5%
%norm	99.1±11.8	97.9±12.1	
Gymnasts			
g/cm^2	1.120±0.09	1.137±0.09	1.8±3.1%
%norm	124.9±9.8	127.2±10.4	
Controls			
g/cm^2	0.971±0.12	0.963±0.12	-0.9±2.2%
%norm	108.8±11.8	107.6±13.3	

* runners significantly different from controls ($0.0001<p<0.05$)
\+ runners significantly different from gymnasts ($0.0001<p<0.05$)
\# gymnasts significantly different from controls ($0.0001<p<0.05$)

Table 8. Whole body bone mineral density values (in g/cm^2 and as a percent of the expected norm) at baseline and post-test, and the percent change scores for WB BMD for the three groups of subjects (mean±S.D.).

Whole Body BMD

	Baseline$^+$	Post-test$^+$	% change
Runners			
g/cm^2	1.043±0.06	1.060±0.07	1.9±1.6%
%norm	94.9±5.6	96.3±6.3	
Gymnasts			
g/cm^2	1.110±0.07	1.130±0.07	1.4±0.7%
%norm	101.0±6.1	102.3±6.0	
Controls			
g/cm^2	1.092±0.06	1.110±0.06	0.8±1.7%
%norm	99.2±5.9	100.2±5.1	

* runners significantly different from controls ($0.0001<p<0.05$)
$^+$ runners significantly different from gymnasts ($0.0001<p<0.05$)
$^\#$ gymnasts significantly different from controls ($0.0001<p<0.05$)

Table 9 illustrates the baseline, post-test and percent change scores in BMD for the lumbar spine according to menstrual cycle status. By factorial ANOVA, LS BMD was significantly different among the five groups at baseline ($p<0.001$) and at post-test ($p=0.0001$), as was the percent change in LS BMD ($p<0.001$). Table 10 illustrates the baseline, post-test and percent change scores in BMD for the femoral neck according to menstrual cycle status. Baseline and post-test FN BMD values ($p<0.0001$) and percent change scores ($p<0.01$) were significantly different among the groups according to

menstrual cycle status. Table 11 illustrates the baseline, post-test and percent change scores for whole body BMD among the groups according to menstrual cycle status. At baseline and post-test, WB BMD was significantly different among the five groups ($p<0.05$). The percent change scores for WB BMD did not differ among the groups.

Table 9. Lumbar spine bone mineral density values (in g/cm^2) at baseline and post-test, and the percent change scores for LS BMD for the five groups of subjects according to menstrual cycle status: eumenorrheic runners (ER, n=12), oligo/amenorrheic runners (OAR, n=6), eumenorrheic gymnasts (EG, n=8), oligo/amenorrheic gymnasts (OAG, n=4), and eumenorrheic controls (EC, n=14) (mean±S.D.).

Lumbar spine BMD

	Baseline(g/cm^2)	Post-test(g/cm^2)	% change
ER	1.007 ± 0.10^{ac}	1.018 ± 0.10^{ac}	$1.0\pm2.1\%^{ce}$
OAR	0.902 ± 0.13^{bdg}	0.899 ± 0.14^{bdg}	$-0.9\pm1.5\%$
EG	1.209 ± 0.15	1.252 ± 0.15^{i}	$3.6\pm2.0\%^{di}$
OAG	1.131 ± 0.06	1.145 ± 0.04	$1.3\pm2.8\%$
EC	1.114 ± 0.12	1.123 ± 0.13	$0.7\pm1.3\%$

[a] ER significantly different from EC ($0.0001<p<0.05$)
[b] OAR significantly different from EC ($0.0001<p<0.05$)
[c] ER significantly different from EG ($0.0001<p<0.05$)
[d] OAR significantly different from EG ($0.0001<p<0.05$)
[e] ER significantly different from OAR ($0.0001<p<0.05$)
[f] ER significantly different from OAG ($0.0001<p<0.05$)
[g] OAR significantly different from OAG ($0.0001<p<0.05$)
[h] EG significantly different from OAG ($0.0001<p<0.05$)
[i] EG significantly different from EC ($0.0001<p<0.05$)
[j] OAG significantly different from EC ($0.0001<p<0.05$)

Table 10. Femoral neck bone mineral density values (in g/cm^2) at baseline and post-test, and the percent change scores for FN BMD for the five groups of subjects according to menstrual cycle status: eumenorrheic runners (ER, n=12), oligo/amenorrheic runners (OAR, n=6), eumenorrheic gymnasts (EG, n=8), oligo/amenorrheic gymnasts (OAG, n=4), and eumenorrheic controls (EC, n=14) (mean±S.D.).

Femoral Neck BMD

	Baseline(g/cm^2)	Post-test(g/cm^2)	% change
ER	0.918±0.11[cf]	0.906±0.10[cf]	-1.3±2.6%
OAR	0.819±0.10[bdg]	0.813±0.10[bdg]	-0.7±2.4%
EG	1.126±0.06	1.157±0.07	2.7±2.8%[cdi]
OAG	1.101±0.14	1.098±0.12	-0.2±3.0%
EC	0.971±0.12[ij]	0.963±0.12[ij]	-0.9±2.2%

[a] ER significantly different from EC ($0.0001<p<0.05$)
[b] OAR significantly different from EC ($0.0001<p<0.05$)
[c] ER significantly different from EG ($0.0001<p<0.05$)
[d] OAR significantly different from EG ($0.0001<p<0.05$)
[e] ER significantly different from OAR ($0.0001<p<0.05$)
[f] ER significantly different from OAG ($0.0001<p<0.05$)
[g] OAR significantly different from OAG ($0.0001<p<0.05$)
[h] EG significantly different from OAG ($0.0001<p<0.05$)
[i] EG significantly different from EC ($0.0001<p<0.05$)
[j] OAG significantly different from EC ($0.0001<p<0.05$)

Table 11. Whole body bone mineral density values (in g/cm^2) at baseline and post-test, and the percent change scores for WB BMD for the five groups of subjects according to menstrual cycle status: eumenorrheic runners (ER, n=12), oligo/amenorrheic runners (OAR, n=6), eumenorrheic gymnasts (EG, n=8), oligo/amenorrheic gymnasts (OAG, n=4), and eumenorrheic controls (EC, n=14) (mean±S.D.).

Whole body BMD

	Baseline(g/cm^2)	Post-test(g/cm^2)	% change
ER	1.058±0.06	1.082±0.07[c]	2.2±1.3%
OAR	1.007±0.06	1.020±0.10[bd]	1.4±2.0%
EG	1.123±0.06[cd]	1.141±0.07	1.6±0.6%
OAG	1.090±0.08	1.100±0.07	1.0±0.8%
EC	1.092±0.06[b]	1.110±0.06	0.8±1.7%

[a] ER significantly different from EC ($0.0001<p<0.05$)
[b] OAR significantly different from EC ($0.0001<p<0.05$)
[c] ER significantly different from EG ($0.0001<p<0.05$)
[d] OAR significantly different from EG ($0.0001<p<0.05$)
[e] ER significantly different from OAR ($0.0001<p<0.05$)
[f] ER significantly different from OAG ($0.0001<p<0.05$)
[g] OAR significantly different from OAG ($0.0001<p<0.05$)
[h] EG significantly different from OAG ($0.0001<p<0.05$)
[i] EG significantly different from EC ($0.0001<p<0.05$)
[j] OAG significantly different from EC ($0.0001<p<0.05$)

Bone mineral density was also determined for specific segments of the body from the whole body scan (Table 12). Using factorial ANOVA, baseline measures indicated significant group differences for certain defined body segments: left and right arm BMD values were significantly lower in the runners compared to the controls, who were lower than the gymnasts ($p<0.05$); thoracic spine and pelvis BMD was significantly lower in the

runners compared to the gymnasts and controls. The left and right leg BMD values did not differ among groups at baseline ($p>0.05$). Baseline measures for BMD at these specific sites did not differ among groups according to menstrual cycle status ($p>0.05$). At post-test, the same significant group and site differences were observed. There were no significant group differences in the percent change in BMD at any of these sites, except right leg BMD with a greater increase in the gymnasts compared to the runners and controls ($p=0.01$), and left arm BMD, with runners showing a decrease which was significantly different from the percent changes seen in the gymnasts and controls ($p=0.05$). The increase in right leg BMD in the gymnasts was also significantly different from zero ($p<0.05$). By menstrual cycle status, the only significant difference ($p<0.01$) was in the percent change in right leg BMD. The EG ($4.2\pm3.2\%$) were significantly different compared to the ER ($1.9\pm1.6\%$), the OAR ($0.2\pm2.1\%$) and the EC ($1.0\pm2.0\%$). The OAG had an intermediate, but also positive % change for the right leg BMD of $2.0\pm1.1\%$.

Table 12. Bone mineral density values (BMD, in g/cm^2) for specific segments of the body (determined from the whole body scans) at baseline and post-test and the percent change scores in BMD at these sites for the runners, gymnasts and controls (mean±S.D.).

	Runners	Gymnasts	Controls
Left arm BMD			
baseline*#+	0.786±0.04	0.912±0.05	0.830±0.05
post-test*#+	0.797±0.05	0.911±0.05	0.831±0.04
%change*+	1.7±1.9%	-0.1±3.1%	-0.3±2.8%
Right arm BMD			
baseline*#+	0.791±0.04	0.916±0.05	0.837±0.05
post-test*#+	0.976±0.05	0.915±0.05	0.838±0.04
%change	0.8±2.3%	-0.2±2.1%	-0.3±2.6%
Left leg BMD			
baseline	1.143±0.09	1.194±0.08	1.150±0.09
post-test	1.172±0.10	1.218±0.09	1.172±0.10
%change	2.5±2.7%	2.0±2.6%	1.2±2.5%
Right leg BMD			
baseline	1.161±0.09	1.207±0.07	1.146±0.09
post-test	1.174±0.10	1.250±0.09	1.169±0.10
%change+#	1.3±1.9%	3.5±2.8%	1.0±2.0%
Thoracic spine BMD			
baseline*+	0.777±0.08	0.932±0.10	0.924±0.08
post-test*+	0.772±0.08	0.909±0.08	0.900±0.09
%change	-0.6±3.0%	-2.3±2.6%	-2.7±3.6%
Pelvis BMD			
baseline*+	0.980±0.10	1.140±0.10	1.074±0.08
post-test*+	0.995±0.11	1.147±0.09	1.087±0.08
%change	1.0±2.7%	0.6±1.9%	0.8±1.8%

* runners significantly different from controls ($0.0001<p<0.05$)

\+ runners significantly different from gymnasts ($0.0001<p<0.05$)

\# gymnasts significantly different from controls ($0.0001<p<0.05$)

Of the eighteen runners who completed the 8-month study period, nine had a history of stress fracture injury (i.e., one amenorrheic and eight eumenorrheic). Although no significant differences were found ($p>0.05$) for bone mineral density based on stress fracture history, there was a non-significant trend for BMD to be lower in the injured runners compared to the non-injured runners at the following sites: whole body, lumbar spine, femoral neck, right and left legs, and pelvis. Table 13 illustrates these differences in BMD at baseline and at post-test, and also shows the percent change scores over the 8-month period for these sites. The only significant difference ($p<0.05$) in percent change scores for BMD was at the femoral neck, where the injured runners had a significantly different (and negative) percent change value for FN BMD compared to the non-injured runners.

Table 13. Bone mineral density values (BMD, in g/cm^2) for injured runners (i.e., history of stress fractures, n=9) compared to non-injured runners (n=9). Whole body, lumbar spine, femoral neck, right and left leg, and pelvis BMD values are given at baseline and post-test, along with the percent change scores (mean±S.D.).

	Injured Runners	Non-injured Runners
Whole body BMD		
baseline	1.035±0.07	1.046±0.06
post-test	1.054±0.07	1.068±0.08
%change	1.8±1.0%	2.0±2.1%
Lumbar spine BMD		
baseline	0.991±0.12	0.953±0.12
post-test	0.996±0.13	0.959±0.12
%change	0.4±2.2%	0.4±2.0%
Femoral neck BMD		
baseline	0.868±0.13	0.902±0.10
post-test	0.848±0.12	0.902±0.10
%change*	-2.2±2.0%	0.0±2.5%
Right leg BMD		
baseline	1.150±0.10	1.167±0.09
post-test	1.173±0.11	1.174±0.09
%change	2.0±1.7%	0.6±1.9%
Left leg BMD		
baseline	1.133±0.10	1.154±0.10
post-test	1.165±0.10	1.178±0.11
%change	2.8±1.5%	2.1±3.6%
Pelvis BMD		
baseline	0.983±0.11	0.985±0.10
post-test	0.996±0.11	0.993±0.12
%change	1.4±2.0%	0.6±3.3%

* significant difference between injured and non-injured groups ($p<0.05$)

Menstrual Cycle Status over time

All subjects maintained their menstrual cycle status throughout the study. Approximately one-third of each athletic group currently experienced oligo- or amenorrhea. The runners had a significantly longer history of oligo/amenorrhea ($p=0.05$) compared to the gymnasts (2.9 ± 2.8 vs 1.1 ± 1.3 yr). The number of years of regular menstrual cycles was not different between the runners and gymnasts (3.8 ± 3.6 vs 1.6 ± 2.2 yr), although it approached statistical significance ($p=0.07$). The percent of time, after menarche, that the women exhibited oligo/amenorrhea in relation to time they were eumenorrheic was not significantly different between the runners and gymnasts (approximately 46% of the time for both groups).

Bone Mineral Density and Abnormal Cycles in Eumenorrheic Women

To determine whether the eumenorrheic subjects were having normal menstrual cycles, urinary assays were performed to measure progesterone metabolites over a complete cycle, from which normal (ovulatory) cycles could be differentiated from abnormal (anovulatory or shortened luteal phase) cycles. Four of the eight eumenorrheic gymnasts collected a total of five complete menstrual cycles for analysis. One of these cycles was considered abnormal, while the other four were normal ovulatory cycles. Six of the twelve eumenorrheic runners collected nine complete menstrual cycles for analysis. Five of the cycles were abnormal (two from the same women) and four were normal (two cycles each from two women). Ten of the fourteen control subjects collected a total of 19 complete menstrual cycles for analysis. Nine of these cycles were abnormal (from five subjects), while ten cycles were normal (from the other five control subjects).

Percent change in bone mineral density was examined among these apparently eumenorrheic women. No statistically significant differences were observed in the percent change in BMD at any site between those women with normal ovulatory cycles and those with abnormal cycles (Table 14). This could be due, in part, to the low subject numbers involved. Only in the control subjects was there a trend for the women with normal cycles to have a more positive bone response at all sites compared to the women with abnormal cycles. However, for the entire group (ten women with normal cycles and ten with abnormal cycles), the percent change in lumbar spine BMD (2.0$\pm$2.8% vs 0.9$\pm$1.8%), femoral neck BMD (-0.1$\pm$2.1% vs -0.9$\pm$3.4%), and whole body BMD (1.9$\pm$1.0% vs 1.1$\pm$2.0%) was more favorable for the women with normal cycles.

Table 14. Percent change in lumbar spine (LS), femoral neck (FN) and whole body (WB) BMD for eumenorrheic runners, gymnasts and controls with normal (ovulatory) or abnormal (anovulatory and/or shortened luteal phase) menstrual cycles (mean±S.D.).

	%changeLSBMD	%changeFNBMD	%changeWBBMD
RUNNERS			
normal cycles			
(n=2)	-0.9±0.6	-2.5±2.9	2.1±1.1
abnormal cycles			
(n=4)	1.4±2.1	-1.4±3.2	2.0±1.8
GYMNASTS			
normal cycles			
(n=3)	5.5±2.2	1.1±1.2	1.6±1.0
abnormal cycles			
(n=1)	2.6±0.0	5.0±0.0	2.3±0.0
CONTROLS			
normal cycles			
(n=5)	1.0±1.1	0.1±1.9	2.0±1.2
abnormal cycles			
(n=5)	0.2±1.6	-1.7±2.9	0.2±2.1

Bone Mineral Density in Athletes according to Menstrual Cycle Status

Percent change scores for whole body, femoral neck and lumbar spine BMD were compared by ANOVA between eumenorrheic athletes (n=20) and oligo/amenorrheic athletes (i.e., runners and gymnasts combined, n=10). At the lumbar spine, the change in BMD was significantly different in the oligo/amenorrheic athletes compared to the eumenorrheic athletes (0.1±2.3% vs 2.1±2.4%). No significant differences in the change in BMD were observed between the eumenorrheic and oligo/amenorrheic athletes for the whole body (2.0±1.1% vs 1.2±1.6%), or the femoral neck (0.3±3.3% vs -0.5±2.5%). When the eumenorrheic athletes (runners and gymnasts, n=20) were compared to the eumenorrheic controls (n=14), the percent change in whole body BMD was the only variable to reach statistical significance ($p<0.05$), with the eumenorrheic athletes (2.0±1.1%) showing a greater increase than the eumenorrheic controls (0.8±1.7%). The percent change in femoral neck BMD was not significantly different between the eumenorrheic athletes and controls (0.3±3.3% vs -0.9±2.2%). There was a trend for the percent change in lumbar spine BMD to be greater in the eumenorrheic controls compared to the eumenorrheic athletes (2.1±2.4% vs 0.7±1.3%), but this did not reach statistical significance ($p=0.07$).

When the eumenorrheic athletes were divided into runners (n=12) and gymnasts (n=8) and compared to the eumenorrheic controls (n=14), the eumenorrheic runners were not different ($p>0.05$) compared to the eumenorrheic controls for the percent change in lumbar spine BMD (1.0±2.1% and 0.7±1.3%, respectively). The eumenorrheic gymnasts did have a significantly greater increase in lumbar spine BMD (3.6±2.0%) compared to the controls ($p<0.001$). The eumenorrheic gymnasts also had a significantly greater increase (2.7±2.8%, $p<0.01$) in the femoral neck BMD compared to the eumenorrheic controls (-0.9±2.2%) and the eumenorrheic runners (-1.2±2.6%). The percent change in whole body BMD was significantly greater ($p<0.05$) in the eumenorrheic runners compared to the

eumenorrheic controls (2.2±1.3% vs 0.8±1.7%), and the eumenorrheic gymnasts tended to have a greater increase in whole body BMD (1.6±0.6%) compared to the controls, but this did not reach statistical significance.

Dietary Analyses

Diet records were analyzed for the following nutrients: total kilocalories, grams of carbohydrate, fat and protein, milligrams of calcium and phosphorus, Vitamin D (in International Units, IU), milligrams of iron, calcium:phosphorus ratio and calcium:protein ratio. Intake of these nutrients was expressed in absolute terms, as well as a percent of the Recommended Dietary Allowance (RDA). Dietary composition of the major energy-providing nutrients (carbohydrate, protein and fat) was determined as a percent of the total kilocalories. Eighteen runners provided an average of 3.2 four-day diet records (range 2 to 4) and the values obtained were averaged. Twelve gymnasts provided an average of 3.4 four-day diet records (range 3 to 4), and thirteen control subjects provided an average of 3.1 four-day diet records (range 1 to 4) for analysis. The dietary composition is shown in Table 15. Analysis of variance revealed that the runners had a significantly greater percentage of total kilocalories from carbohydrate ($p<0.01$) compared to the controls. Diets of the runners and gymnasts were above the recommended level of at least 60% of total caloric intake represented by carbohydrate.

Table 15. Dietary composition of the macronutrients, carbohydrate, fat and protein, expressed as a percent of the total caloric intake for runners (n=18), gymnasts (n=12) and controls (n=13), based on the average of 4-day diet records (mean±S.D.)

	Runners	Gymnasts	Controls
% carbohydrate*	67.7±10.6	62.8±4.8	57.4±7.3
% protein	15.4±3.9	16.8±1.5	15.6±3.5
% fat*	20.1±8.3	23.2±4.5	27.5±5.1

* runners significantly different compared to controls ($p \leq 0.01$)

There were significant group differences ($p<0.05$) for % carbohydrate in the diet when compared according to menstrual cycle status (ANOVA). The eumenorrheic runners (65.9±10.4%) had a higher percent of their total kilocalories from carbohydrate compared to the eumenorrheic controls (57.4±7.3%). The oligo/amenorrheic runners (71.4±11.2%) also consumed a higher percent of their caloric intake as carbohydrate compared to the eumenorrheic gymnasts (61.6±4.4%) and the eumenorrheic controls. The oligo/amenorrheic gymnasts consumed 65.0±5.3% of their total kilocalories as carbohydrates. The percent of total caloric intake from protein was not different among groups; all three groups of subjects were above the recommended levels of 10-12% of total kilocalories for protein, or 0.8 to 1.0 gram of protein for every kilogram of body weight (g/kg). In fact, the gymnasts and controls consumed approximately 1.3 grams of protein per kilogram body weight, while the runners consumed 1.4 grams/kg. The percent of total kilocalories ingested as protein also did not differ among groups according to menstrual cycle status ($p>0.05$). The control subjects had a higher percent of fat kilocalories in their diet compared to the runners ($p=0.01$), but all groups were below the recommended levels of less than 30% of total kilocalories from fat. The percent of total kilocalories from fat was significantly different among the groups according to menstrual cycle status ($p<0.05$). The

eumenorrheic control subjects (27.5±5.1%) consumed a higher % of fat kilocalories in their diets compared to the eumenorrheic (21.0±7.8%) and oligo/amenorrheic runners (18.2±9.8%). The eumenorrheic gymnasts (24.4±4.6%) tended to consume more fat kilocalories in their diets compared to the oligo/amenorrheic gymnasts (20.8±3.6%), but this did not reach significance. This pattern was also true for the runners: the oligo/amenorrheic runners tended to have a lower % of total kilocalories from fat compared to the eumenorrheic runners.

Table 16 illustrates the average dietary analyses for each nutrient by subject group, compared to the RDA for this age group of women (where appropriate), although the RDA is not necessarily based on an athletic population. The only significant group differences were for the calcium:phosphorus and calcium:protein ratios (gymnasts had greater values for both compared to runners and controls, $p<0.01$). There is wide variability within each subject group for each nutrient.

Table 16. Nutrient analysis of averaged 4-day diet records by subject group (mean±S.D.) compared to the Recommended Dietary Allowance (RDA) for college-aged women. Carbohydrate, protein and fat are given in grams (g), calcium and phosphorus and iron in milligrams (mg), Vitamin D in International Units (IU), the calcium:phosphorus ratio (mg:mg) and the calcium:protein ratio (g:g). Subject numbers for averaged diet analyses were 18 runners, 12 gymnasts and 13 controls.

	Runners	Gymnasts	Controls	RDA
Total kilocalories	1883±647	1727±209	1941±365	2200
Carbohydrate (g)	306±93	264±28	280±65	none
Protein (g)	76±29	71±11	79±19	40-45
Fat (g)	48±34	48±15	61±13	none
Calcium (mg)	804±306	1171±224	877±368	1200
Phosphorus (mg)	1193±447	1345±226	1221±358	1200
Vitamin D (IU)	91±73	243±124	194±136	400
Iron (mg)	18±8	17±5	15±6	15
Calcium:Phosphorus[+#]	0.68±.06	0.87±.07	0.72±.12	none
Calcium:Protein[+#]	0.011±.002	0.017±.002	0.011±.003	none

[+] runners significantly different compared to gymnasts ($p<0.01$)
[#] gymnasts significantly different compared to controls ($p<0.01$)

Note: The RDA for protein is based on approximately 0.8 grams of protein per kilogram of body weight.

Each nutrient which had a recommended level in the diet was analyzed as a percent of the RDA. The only nutrient, expressed as a percent of the RDA, which was significantly different among groups ($p<0.05$) was iron. The runners had a significantly higher iron intake as a percent of the RDA (135.8±71.8%) compared to the controls (90.7±31.3%); the gymnasts had an intermediate value of 97.5±26.7%. For total caloric

intake, all groups were below the recommended dietary allowance of 2200 kilocalories per day (and more for athletes). The runners consumed 90.8±28.6% of the RDA, the gymnasts consumed only 73.0±10.9% and the controls consumed 83.5±18.6%. When analyzed by group and menstrual cycle status, there were no significant differences for the total caloric intake as a percent of the RDA ($p>0.05$); however, the eumenorrheic (71.3±13.1%) and oligo/amenorrheic (76.5±3.8%) gymnasts tended to have lower values compared to the eumenorrheic controls (83.5±18.6%), oligo/amenorrheic runners (87.8±36.4%) and eumenorrheic runners (92.4±25.6%). The two running groups exhibited substantial variation in the % RDA for total kilocalories. As mentioned earlier, all groups exceeded the RDA for protein intake: runners consumed 159.9±56.1% of the RDA, gymnasts consumed 144.5±23.9% and controls consumed 151.0±35.3%. In fact, there were no significant differences among the five groups according to menstrual cycle status for protein intake as a % of the RDA ($p>0.05$), although there was substantial within-group variability. The protein intake as a % of the RDA for each group was: ER = 165.4±56.1%, OAR = 148.9±59.7%, EG = 137.2±25.3%, OAG = 159.1±13.1%, EC = 151.0±35.3%. All three groups were below the RDA for calcium intake. In fact, the control subjects consumed only 64.9±23.6% of the RDA for calcium, which, being less than two-thirds of the RDA, defines their diets as being poor in calcium. The runners consumed only 72.5±31.0% of the RDA for calcium and the gymnasts consumed 83.8±18.5% of the RDA. There were no significant differences when comparing groups according to menstrual cycle status for calcium intake as a % of the RDA ($p>0.05$), although all five groups were below the suggested levels. In fact, the OAR (66.2±21.0%) and EC (64.9±23.6%) were below two-thirds of the RDA which is considered a poor diet in terms of calcium. The ER (75.7±35.4%), EG (80.8±19.3%) and OAG (89.9±17.7%) were also below the RDA for calcium. Phosphorus intake was close to the recommended levels for all groups: runners consumed 102.7±39.1% of the RDA for phosphorus (ER = 105.8±43.4%; OAR = 96.5±31.5%), gymnasts consumed 93.4±21.4% (EG = 88.5±19.8%; OAG =

103.2±24.0%), and controls consumed 90.4±24.5%. There were no significant differences among the five groups for phosphorus intake as a percent of the RDA ($p>0.05$). Vitamin D intake from dietary sources was equally poor (less than two-thirds of the RDA) for all five groups: runners consumed 38.9±37.4% of the RDA for Vitamin D (ER = 46.7±42.1%; OAR = 23.2±20.3%), gymnasts consumed 44.3±25.4% (EG = 39.3±17.6%; OAG = 54.2±38.0%) and controls consumed 34.7±31.5%. There were no significant differences among the groups according to menstrual cycle status for dietary iron intake, although there was a trend for OAR (153.2±91.2%) to consume a higher % of the RDA compared to the EG (86.2±18.3%) and the EC (90.7±31.3%) ($p=0.08$). Both the ER (127.1±62.7%) and the OAG (120.2±28.3%) were above the RDA for dietary iron.

When the diets of all athletes (i.e., runners and gymnasts combined, n=30) were compared to the control subjects (n=14), the dietary composition was significantly different with respect to carbohydrate and fat intake ($p<0.01$). The athletes consumed 65.7±9.0% of their total kilocalories as carbohydrate compared to 57.4±7.3% for the controls. The athletes consumed 21.3±7.1% of their total kilocalories as fat compared to 27.5±5.1% for the controls. The percent of total caloric intake as protein was similar between the two groups: 16.0±3.2% for the athletes and 15.6±3.5% for the controls. The athletes consumed 83.7±24.6% of the RDA for total kilocalories, while the controls consumed 83.5±18.6% of the RDA. Both groups of subjects exceeded the RDA for protein; the athletes consumed 153.8±46.1% of the RDA and the controls consumed 151.0±35.3% of the RDA. The controls had a poor dietary intake of calcium (defined as less than two-thirds of the RDA for calcium, i.e., < 800 mg/day), consuming only 64.9±23.6% of the RDA, while the athletes had a higher dietary intake of calcium but still only consumed 77.0±26.9% of the RDA. The athletes consumed 99.0±33.0% of the RDA for phosphorus, while the controls consumed 90.4±24.5% of the RDA. Dietary Vitamin D intake was poor in both groups; the athletes consumed only 41.1±32.7% of the RDA and the controls consumed only 34.7±31.5% of the RDA. Diets of the athletes contained

120.5±60.4% of the RDA for iron, while diets of the control subjects contained 90.7±31.3% of the RDA.

As defined above, a poor dietary intake of a specific nutrient was less than two-thirds of the RDA. The following nutrient sufficiency analysis by subject revealed that although as a group the diet may not be deficient in a specific nutrient, several individuals within each group had diets lacking in one or more of these nutrients. In terms of total caloric intake, five runners failed to meet the two-thirds of the RDA criteria. Their average total caloric intake ranged between 44.1% and 60.3% of the RDA. Two gymnasts consumed less than two-thirds of the RDA for total kilocalories. Their average total caloric intake ranged between 48.9% and 62.3% of the RDA. Three of the control subjects consumed a poor diet in terms of percent of total calories, with a range between 53.2% and 62.4% of the RDA for total kilocalories. All subjects met (and exceeded) the minimum two-thirds of the RDA for protein intake for a healthy diet. Seven of the eighteen runners consumed a poor diet in terms of calcium intake, with a range of 40.9% to 58.7% of the RDA. Two of the gymnasts had a poor diet in terms of calcium intake, 58.7% to 64.0% of the RDA. Seven of the control subjects also had a poor diet in terms of calcium intake, ranging between 37.6% and 64.8% of the RDA. Four of the runners failed to meet the two-thirds of the recommended level for a healthy diet in terms of phosphorus, with intake values ranging from 37.3% to 60.5%. One gymnast barely met the recommended level, consuming only 65.8% of the RDA for phosphorus. Two control subjects had a poor diet in terms of phosphorus intake, 56.5% to 63.7% of the RDA. All but four of the runners had a poor diet in terms of Vitamin D intake, with values for the fourteen women ranging from 3.0% to 57.4% of the RDA. Similarly, only three gymnasts exhibited Vitamin D intakes above those levels defined as poor, with the values for the other nine women ranging from 13.2% to 56.0% of the RDA. Only three of the thirteen control subjects met the minimum recommended level for Vitamin D intake, with deficient diets ranging from 3.0% to 60.8% of the RDA for Vitamin intake. Only one

runner had a poor dietary intake of iron (35.6% of the RDA), and one was marginal (66.7% of the RDA). Only one gymnast failed to meet the two-thirds RDA level for iron in terms of a healthy diet, consuming only 65.1% of the RDA. Four of the control subjects had a poor diet in terms of iron consumption, ranging from 52.4% to 65.8% of the RDA.

Dietary intakes were also analyzed with respect to menstrual cycle status in the athletes to determine whether or not the oligo/amenorrheic women exhibited poorer diets than the eumenorrheic women (defined as consuming less than two-thirds of the RDA for that particular nutrient). When all the athletes were grouped (i.e., 20 eumenorrheic and 10 oligo/amenorrheic), there were no significant differences for any of the dietary nutrients analyzed (see Table 17). The eumenorrheic athletes tended to have a higher percent of total caloric intake from fat and lower carbohydrate compared to the oligo/amenorrheic athletes, but protein was similar. Both groups of athletes were below 80% of the RDA for calcium, with the eumenorrheic athletes having slightly higher intakes. Vitamin D intake was also slightly higher in the eumenorrheic athletes, although both groups were well below the RDA. Iron intake was higher in the oligo/amenorrheic athletes, although it exceeded the RDA in both groups. When the athletes were separated into either runners or gymnasts based on menstrual cycle status (i.e., ER, OAR, EG, OAG), there were no significant differences in these dietary analyses.

Table 17. Dietary composition (i.e., total kilocalories as % carbohydrate, % fat, and % protein) and percent of the Recommended Dietary Allowance (RDA) for specific nutrients (i.e., total kilocalories (Kcal), protein, calcium, phosphorus, Vitamin D, iron) for all athletes grouped by menstrual cycle status: eumenorrheic athletes (n=20) and oligo/amenorrheic athletes (n=10) (mean±S.D.).

	Eumenorrheic athletes	Oligo/amenorrheic athletes
% Carbohydrate	64.2±8.6	68.8±9.4
% Protein	16.0±3.4	16.0±2.8
% Fat	22.4±6.8	19.2±7.7
%RDA Total Kcal	83.9±23.6	83.3±27.8
%RDA Protein	154.1±47.5	153.0±45.5
%RDA Calcium	77.8±29.5	75.6±22.3
%RDA Phosphorus	98.9±36.2	99.2±27.4
%RDA Vitamin D	43.8±34.0	35.6±31.1
%RDA Iron	110.8±53.1	140.0±71.9

Psychological Stress/Anxiety

The Profile of Mood States (POMS) and State-Trait Anxiety Inventories (STAI) were used to quantify psychological stress and anxiety levels four times over the course of the study (i.e., at baseline, in December, in March, post-test). Three scores were acquired for each testing time: a Total Mood Disturbance (TMD) score which took into account the six subscales of the POMS, a State Anxiety score, and a Trait Anxiety score. Repeated measures ANOVA revealed no significant differences for TMD, State or Trait Anxiety scores over time. By factorial ANOVA, the TMD score was significantly different among the groups only at baseline ($p<0.05$). The runners scored significantly higher than the

controls (10.8±8.5 vs 4.1±3.7), while the gymnasts had an intermediate score (6.5±7.2). All three groups, however, were well within the normal range for college-aged individuals: 10.8±7.9. State Anxiety scores were also significantly different among the three subject groups at baseline ($p<0.01$), but not in December, March or at post-test. The runners (39.0±12.2) and gymnasts (40.8±8.4) scored significantly higher on the State Anxiety inventory compared to the controls (29.2±5.1) at baseline, although the athletes did not differ from one another. All three groups were scored normally for State Anxiety when compared to college-aged females (38.8±12.0) and working females aged 19 to 39 years (36.2±11.0). Trait anxiety scores were not significantly different among the three groups, except at post-test ($p<0.05$). The runners (38.2±4.4) and gymnasts (38.6±8.7) scored higher than the controls (30.2±6.8), but all three groups were normal compared to college-aged females (40.4±10.2) and working females aged 19 to 39 years (36.2±9.5).

When all the athletes were compared to the controls on these measures of stress and anxiety, the only significant differences were found at baseline and at the post-test. The 29 athletes who completed the inventories at baseline had significantly greater scores for TMD ($p<0.05$) and State Anxiety ($p=0.001$) compared to the 14 control subjects. The athletes scored 9.1±8.2 for TMD compared to the 4.1±3.7 scored by the controls. The State Anxiety score for the athletes was 39.8±10.7 compared to a score of 29.2±5.1 for the controls. The baseline scores for Trait Anxiety approached significance ($p=0.06$) between the groups, with the athletes scoring 39.9±8.2 and the controls scoring 34.7±8.1. The scores for Trait Anxiety determined in December also approached statistical significance ($p=0.08$), with the athletes ($n=30$) scoring 39.4±7.7 and the controls ($n=12$) scoring 33.8±12.4. Trait Anxiety scores were significantly different ($p=0.01$) between the groups at the post-test. The athletes ($n=13$) scored 38.4±6.1, while the controls ($n=8$) scored lower (30.2±6.8). This is surprising, since trait anxiety is a fairly stable construct; however, small subject numbers in the post-test samples could help to explain this. When menstrual cycle status was taken into account, there were no significant differences

between eumenorrheic and oligo/amenorrheic athletes, runners or gymnasts on any stress/anxiety score at any testing time ($p>0.05$).

Training Intensity and Volume

Repeated measures analyses revealed no significant differences for average training mileage for the runners as a group or according to menstrual cycle status (i.e., regular vs irregular). Running mileage averaged 46.5±15.8 miles/week in the fall, 43.2±15.8 miles/week in the winter, and 42.8±16.5 miles/week in the spring. By menstrual cycle status, average training mileage by season is shown in Table 18. When the runners' mileage was averaged over the entire 8-month period, however (range of 20.9 to 74.4 miles per week), there was a significant difference between the regularly menstruating runners and those with menstrual cycle irregularities ($p=0.05$). The runners with regular menstrual cycles ($n=11$) averaged 38.1±15.0 miles/week (range of 20.9 to 68.2 miles per week) compared to the 54.0±11.7 miles/week run by those women with menstrual cycle irregularities ($n=5$) (range of 44.6 to 74.4 miles per week). Repeated measures analyses also revealed no significant differences for average number of intense training sessions (a measure of exercise intensity) for the runners as a group or according to menstrual cycle status (range of 1.5 to 2.7 intense training sessions per week). The runners averaged 2.4±0.3 intense sessions per week in the fall, 2.2±0.4 intense sessions per week in the winter, and 2.3±0.4 intense sessions per week in the spring. By menstrual cycle status, the average number of intense sessions per week by season is shown in Table 19. When the runners' average number of intense training sessions per week was averaged over the 8-month period, there was no significant difference between regularly menstruating runners (2.2±0.3 intense sessions per week) and those women with irregular menstrual cycles (2.4±0.3 intense sessions per week); the ranges were 1.5 to 2.4 and 2.0 to 2.7 intense training sessions per week, respectively.

Table 18. Average training mileage (miles run per week) for runners with regular (n=11) and irregular (n=5) menstrual cycles for fall, winter and spring seasons (mean+S.D.).

	Fall	Winter	Spring
Runners with *regular* menstrual cycles	42.3±16.4	37.5±14.5	38.0±16.6
Runners with *irregular* menstrual cycles	54.0±12.8	54.6±12.5	53.4±11.6

Table 19. Average number of intense training sessions per week for runners with regular (n=11) and irregular (n=5) menstrual cycles for fall, winter and spring seasons (mean+S.D.).

	Fall	Winter	Spring
Runners with *regular* menstrual cycles	2.3±0.3	2.2±0.4	2.2±0.4
Runners with *irregular* menstrual cycles	2.4±0.4	2.2±0.6	2.6±0.2

DISCUSSION

Baseline Data

The gymnasts in the present study exhibited higher bone mineral density compared to control subjects and runners, despite a similar incidence of oligo/amenorrhea. Specifically, gymnasts had greater BMD values at the lumbar spine, femoral neck and whole body compared to runners, and higher femoral neck BMD values compared to the controls. It was hypothesized that athletes would have higher BMD values compared to control subjects, and this was true for the gymnasts, but not for the runners. Despite their extremely high level of regular weight-bearing exercise, however, the runners exhibited significantly lower BMD values at the lumbar spine and femoral neck compared to the control subjects, and significantly lower BMD values for all three sites when compared to the gymnasts. Although distance running involves numerous impacts with many repetitions over a longer duration, the forces generated when running are lower than those experienced by the gymnasts. Specific loading on bone, through high impact or strong muscular contractions, may act to increase bone mineral in the gymnasts. This may be especially true at the hip (femoral neck), where the greatest impact forces would be expected.

Due to initial differences between athletic groups and expected differences in bone mineral density resulting from menstrual cycle status, athletes were separated within each group according to menstrual cycle status (i.e., number of menstrual cycles per year), in order to test the hypotheses relating to BMD changes and menstrual cycle status. All subsequent analyses used this categorization. Previous data among female athletes, particularly runners, with exercise-associated amenorrhea and oligomenorrhea have shown that bone mass of the lumbar spine and peripheral skeleton is compromised compared to eumenorrheic women (Cann et al., 1984; Drinkwater et al., 1984; Marcus et al., 1985; Myburgh et al., 1993; Nelson et al., 1986). Female athletes with oligomenorrhea also

exhibit lower lumbar spine BMD compared to eumenorrheic athletes (Drinkwater et al., 1990). The baseline data from the present study support these findings for runners, but not gymnasts. At the lumbar spine, the oligo/amenorrheic runners (OAR) in the present study had significantly lower BMD values compared to the eumenorrheic controls, the eumenorrheic gymnasts and even the oligo/amenorrheic gymnasts. Although not statistically significant, the oligo/amenorrheic runners (0.902±0.13 g/cm^2) tended to have lower lumbar spine BMD values compared to the eumenorrheic runners (1.007±0.10 g/cm^2). On the other hand, the oligo/amenorrheic gymnasts' lumbar spine BMD (1.131±0.06 g/cm^2) was not significantly different from either the eumenorrheic controls' (1.114±0.12 g/cm^2) or the eumenorrheic gymnasts' (1.209±0.15 g/cm^2). At the femoral neck, baseline BMD was significantly lower in the oligo/amenorrheic runners (0.819±0.10 g/cm^2) compared to the control subjects (0.971±0.12 g/cm^2), and although not statistically different, tended to be lower than the eumenorrheic runners (0.918±0.11 g/cm^2). Femoral neck BMD at baseline did not differ significantly between oligo/amenorrheic and eumenorrheic gymnasts; but contrary to previous studies which found that menstrual cycle irregularities compromised bone mass, femoral neck BMD of the oligo/amenorrheic gymnasts (1.101±0.14 g/cm^2) was significantly greater than that of the eumenorrheic controls. The higher impact forces generated in gymnastics training may act to offset the resorptive effects of low reproductive hormones on bone mineral in these gymnasts. These data support the "mechanostat theory" of Frost (1992), which describes a setpoint for bone metabolism that is mediated by estrogen and mechanical stimuli such as physical activity. In Frost's model, intense mechanical usage or hyperactivity stimulates modeling drifts (uncoupled osteoclast-osteoblast processes) at bone sites which result in a net bone gain. This continues until an adaptation occurs to the increased mechanical loads and a new mechanical usage set point is reached. Hyperactivity or high levels of physical activity lowers this set point, whereas disuse (i.e., bed rest or injury which forces non-weight-bearing) raises the set point. Frost's model also proposes a role for estrogen, whereby high

or even normal estrogen levels lower the modeling and remodeling set points, and low estrogen levels raise the set point and decrease modeling and remodeling. In this latter case, higher mechanical loads are then required to maintain or increase bone mass. The results from this study seem to support this theory in that the extremely high impact forces which load the skeleton in gymnastics training appear to be sufficient to overcome the higher set points expected among these oligo/amenorrheic athletes. Findings of higher BMD values in the lower extremities of female ice skaters (Slemenda and Johnston, 1993) and in the lumbar spine of rowers (Wolman et al., 1990) concur that activities which generate extremely high forces may be sufficient to compensate for the deficient reproductive hormone profile of these oligo/amenorrheic athletes. Menstrual cycle status had no significant effect on whole body BMD at baseline.

The relatively high bone mass of the gymnasts is particularly striking in view of their later age at menarche. The gymnasts' average age at menarche was significantly later (approximately 16 years of age) compared to the runners (14 years of age) and controls (13 years of age), who did not differ from one another. Typically, girls who have a delayed menarche display lower bone mass than expected due to the relative lack of reproductive hormones for a prolonged period of time (Katzman et al., 1991). Menarche was delayed in the women gymnasts in the present study most likely as a result of a very early age of training (Malina, 1983; Malina et al., 1978; Warren, 1980). The gymnasts began training at a significantly younger age (approximately 7 years of age) and pre-pubertal, compared to the runners (approximately 15.5 years of age and post-pubertal).

A delayed age at menarche has been associated with a higher incidence of exercise-related oligo- and amenorrhea (Sanborn et al., 1987; Wakat et al., 1982) which, in turn, have been associated with decreased bone mineral density or accelerated bone loss compared to women with normal reproductive hormone levels (Cann et al., 1984; Drinkwater et al., 1984; Marcus et al., 1985; Warren et al., 1986). This is not strictly a female phenomenon, as there is a male equivalent - delayed puberty. Finkelstein and

colleagues (1992) reported osteopenia in men who had experienced a delayed puberty. Both lumbar spine and radial BMD were significantly lower in these men compared to their peers who underwent a normal puberty. These authors concluded that the timing of puberty was critical in determining peak bone mass accretion during adolescence and that peak BMD is a major determinant of bone mineral density later in life.

The runners had a longer history of oligo/amenorrhea since menarche compared to the gymnasts. This is due in part to their slightly older current age, as well as their later age at onset of training and earlier age at menarche. This may partly explain the lower bone mass among even the eumenorrheic runners, since most of these women had some history of oligo/amenorrhea (Drinkwater et al., 1990). Even those runners who had always been eumenorrheic, however, exhibited lower bone mineral density values compared to gymnasts and controls. The gymnasts had a shorter history of oligo/amenorrhea, primarily due to their later age at menarche, and this appears to be less detrimental to bone. It is possible that the skeleton loses more bone mineral consequent to the loss of menstrual cycles after a fairly normal age at menarche than it would as a result of primary amenorrhea or very delayed menarche. It appears that the high intensity activity of gymnastics overrides the expected lower bone mass associated with late or delayed menarche (Finkelstein et al., 1992; Warren et al., 1986).

The greater muscle strength and lean mass per unit height (or surface area) in the gymnasts in the present study may also contribute to higher bone mass. The gymnasts had significantly greater peak muscle forces at baseline in the elbow flexors, leg extensors, and hip abductors and adductors compared to the runners and controls. Absolute lean body mass and percent body fat did not differ between the gymnasts and runners in the present study; however, in relation to height (gymnasts were significantly shorter than runners), lean mass was greater in the gymnasts. Montpetit (1976) described female gymnasts as having a somatotype with the highest ratings of mesomorphy (i.e., relative musculoskeletal development) compared to female athletes of other sports. Both muscle strength and lean

mass have been reported to be positively correlated with BMD (Aloia et al., 1991; Bevier et al., 1989; Snow-Harter et al., 1992; Sowers et al., 1992). Sowers and colleagues (1992) found that a large muscle mass was associated with higher bone mass at the proximal femur compared to a low muscle mass and high adiposity distribution of body weight. They concluded that a high body weight was only beneficial for BMD when muscle mass was high, regardless of fat mass. Aloia et al. (1991) and Bevier et al. (1989) concurred that adiposity, in and by itself, did not aid skeletal health. Since percent body fat did not differ between the runners and gymnasts in the present study, the higher lean mass (in relation to body surface area) and greater muscle strength in the gymnasts may be acting to enhance bone mineral density in combination with the high impact forces generated by their training. Muscle strength of the back, biceps, and quadriceps have been shown to be related to femoral neck and lumbar spine BMD in young women (Snow-Harter et al., 1992).

Longitudinal Data

The present study followed subjects over an eight month training period, in order to monitor bone mineral changes as a result of time, physical training, menstrual cycle status, diet and stress/anxiety. Percent change scores for bone mineral density were computed and used in the analyses since BMD differed among the groups at baseline.

Percent Change in Bone Mineral Density

The percent changes in bone mineral density over the eight-month study period varied in direction and magnitude among the groups. The gymnasts had a significant increase in lumbar spine BMD (approximately 3%) and a tendency for an increase in femoral neck BMD (approximately 2%), although with large intra-group variability, this was not a statistically different change from zero. Lumbar spine BMD of the runners

remained constant over the testing period, but femoral neck BMD tended to decline by approximately 1%, although neither LS BMD or FN BMD was a significantly different change from zero. Based on the increase in spine BMD among college-aged women observed by Recker and colleagues (1992), an apparent *loss* of bone was not expected among athletes. Although still somewhat controversial and site-specific (Snow-Harter and Marcus, 1991), it is hypothesized that college-aged women may still be depositing new bone (up to the age of 30 years) on the way to building peak bone mass (Garn et al., 1964; Krolner and Nielsen, 1982; Recker et al., 1992). Heaney (1989) proposed that fatigue damage may play a role in skeletal health. In runners logging high weekly mileage, involving continuous stress to the bones without sufficient time for remodeling to complete its cycle, may result in a net loss of bone mineral, and also a higher risk of micro-fracture (i.e., stress fractures). This may explain why the runners in the present study who had a history of stress fractures had a significantly different change in femoral neck BMD (-2.2±2.0%) compared to those runners who had never sustained a skeletal injury (0.0±2.5%). The skeleton of these women athletes is continually being stressed such that bone must adapt to the loads placed upon it or breakdown will occur (Carter et al., 1981; Marcus, 1987; Heaney, 1989). Heaney (1989) pointed out that maximal skeletal loading under extreme physical exertion ranges between 3500 and 4500 microstrain units, which is well below the estimated limits of strain for bone, 6000 to 7000 microstrain units. Despite the extremely high loads experienced by the gymnasts, no bone injuries (e.g., fractures) were observed. It may be that despite the higher impact loads experienced by the gymnasts, fewer repetitions with sufficient recovery allows bone remodelling to proceed normally.

Effect of Menstrual Cycle Status on Percent Change in BMD

Menstrual cycle status contributed to significant group differences for the percent changes in femoral neck and lumbar spine BMD. The eumenorrheic gymnasts exhibited a

significantly different percent change in FN BMD compared to the eumenorrheic runners, the oligo/amenorrheic runners and the eumenorrheic control subjects. The eumenorrheic gymnasts had the largest positive change in FN BMD, i.e., more than two-and-a-half percent (even though this was not statistically different from zero), while the oligo/amenorrheic gymnasts, all runners regardless of menstrual cycle status, and the eumenorrheic controls either had no change in FN BMD, or even tended to lose bone mineral at this site. Menstrual cycle status, however, did not appear to play a role, as both eumenorrheic and oligo/amenorrheic runners lost bone mineral at the femoral neck (approximately one percent) and did not statistically differ from one another. This could be explained, in part, by the accumulation of fatigue damage in the runners (Carter et al., 1981; Heaney, 1989). On the other hand, menstrual cycle status appears to play a role in bone mineral density changes at the femoral neck in gymnasts. While the eumenorrheic gymnasts tended to increase bone mass at this site, the oligo/amenorrheic gymnasts remained constant (or actually lost an insignificant amount). Again, this is somewhat surprising given that the eumenorrheic gymnasts already had extremely high BMD values at the femoral neck (approximately 127% of the expected mean at post-test for all gymnasts). It is speculated that the extremely high impact loading experienced in gymnastics training (approximately six to ten times body weight when landing from the balance beam or uneven parallel bars, McNitt-Gray et al., 1993) is able to augment bone mineral even further (Martin and McCulloch, 1987). According to Chamay and Tschantz (1972), constant loading on bone (in the form of static compression) does not cause hypertrophy; an overload of intermittent compressive forces on bone are required to augment bone mass. Also, since estrogen apparently has a permissive effect with exercise as it affects bone mineral (Dalsky, 1990), the two factors may interact to augment bone mineral at the femoral neck in these gymnasts. In one of the few other studies investigating gymnastics training, Nichols and colleagues (1992) reported that gymnasts exhibited significantly higher BMD values for the lumbar spine and femoral neck compared to control subjects.

These researchers concluded that the type of physical training done by the gymnasts was beneficial to their bones. They proposed that the amount of jumping and similar moves involved in gymnastics training was reflected in their higher bone mass. It is important to note, however, and somewhat surprising, that 13 of the 15 women in the Nichols et al. (1992) study were eumenorrheic. In the two women with amenorrhea, bone mineral density at the lumbar spine and femoral neck was less than that of the eumenorrheic gymnasts but still greater than the controls. The results of the present study support these findings, although a higher percentage (33%) of the gymnasts were oligo/amenorrheic.

The percent change in BMD at the lumbar spine according to menstrual cycle status was also greatest in the eumenorrheic gymnasts (approximately three-and-a-half percent). This was significantly different compared to the percent change in LS BMD for the eumenorrheic runners and controls and the oligo/amenorrheic runners. In fact, the latter group was the only one of the five defined groups with a tendency to actually lose bone mineral at the lumbar spine (approximately one percent, non-significant). Again, this seems to suggest that distance running may exacerbate already low bone mineral values at highly trabecular skeletal sites in women who have irregular or absent menstrual cycles (Cann et al., 1984; Cook et al., 1987; Drinkwater et al., 1984; Lindberg et al., 1984; Linnell et al., 1984; Nelson et al., 1986). These researchers observed this phenomenon in cross-sectional studies, not over some defined period of time as in the present study. In 1985, Cann and co-workers found loss of vertebral bone mineral in amenorrheic athletes up to 4% per year for the first two years, after which loss was only about 0.5% per year. They suggested an intervention strategy of treatment with estrogen (similar to postmenopausal women) in order to prevent this rapid bone loss. Later research by Cann et al. (1988) reported that women runners with a prolonged history of amenorrhea (i.e., for three or more years) had significantly decreased bone mineral density at the lumbar spine, regardless of whether they regained normal menstruation, compared to women runners who were eumenorrheic and had no history or less than three years of amenorrhea. Results of a study by Drinkwater

and colleagues (1990) concurred that prolonged menstrual cycle dysfunction could lead to permanent bone loss. In light of these findings and those from the present study, it becomes evident that estrogen plays a key role in bone remodelling, building peak bone mass, and maintaining a high bone mineral content over many years, certainly with the aid of an exercise intervention.

The percent change in whole body BMD did not differ among the groups according to menstrual cycle status. However, the largest (although not statistically significant) percent change in WB BMD was in the eumenorrheic runners (slightly more than a two percent increase), while the increase in WB BMD was approximately one-and-a-half percent in the eumenorrheic gymnasts. The percent change in WB BMD for the oligo/amenorrheic runners was almost one-and-a-half percent and gymnasts was almost one percent, slightly less than their eumenorrheic peers. The relative lack of estrogen in these women may have contributed to this lower than expected increase in bone mineral (Lindberg et al., 1984; Wolman, 1990). The control subjects also had no change (a non-significant increase of almost one percent) in whole body bone mineral over this eight-month time period. The fact that all groups, athletes and non-athletes, eumenorrheic and oligo/amenorrheic women alike, had an increase in whole body BMD suggests that certain skeletal sites are more or less susceptible to exercise and/or estrogen status (Jones et al., 1985; Marcus et al., 1985; Wolman, 1990). Another possible explanation could be the lower metabolic activity in the compact cortical bone which constitutes the majority of bone in the whole body analysis. Several researchers (Jones et al., 1985; Marcus, 1987; Marcus et al., 1985; Parfitt, 1982; Wolman, 1990) have shown that cortical bone has a much slower turnover rate compared to trabecular bone, and it takes longer to detect any observable changes. Cortical bone is also more stable and robust when exposed to environmental variations such as hormonal status or physical activity (Ruegsegger et al., 1991).

The runners had relatively high BMD values for right and left legs; however, these were not different from the values for gymnasts and controls in this study. Runners had significantly lower bone mineral values for the right and left arms, ribs, thoracic spine and pelvis compared to gymnasts and, at some sites, controls as well. The only regional sites which had significantly different percent change scores were left arm and right leg BMD. The left arm BMD in the runners tended to increase (1.7±1.9%), while the left arm BMD of the gymnasts (-0.1±3.1%) and controls (-0.3±2.8%) remained constant. These findings are somewhat surprising given that few of the runners did any weight training or any exercise intended to overload the upper body skeleton, while the gymnasts' training involved substantial upper body work and stress to the musculature and skeleton of the area. The percent change in right leg BMD in the gymnasts (a significant increase from zero of approximately three-and-a-half percent) was statistically different from the percent change in right leg BMD of the runners (1.3±1.9%) and controls (1.0±2.0%). It is speculated that the dominant leg experiences more of the impact load during gymnastics training, with the majority of the gymnasts being right-leg dominant (OSU athletic trainer, personal communication), while in running, both legs are subjected to a similar amount of force upon impact (i.e., at foot-strike). The runners' percent increase (approximately two-and-a-half percent) in left leg BMD was slightly greater (but non-significantly different from the other groups) compared to the gymnasts (approximately two percent) and controls (approximately one percent). The largest percent changes in bone mineral for all subjects were, in fact, at the leg sites where the majority of weight-bearing and the largest impact forces would be expected before they are dissipated as they travelled up the skeleton (McNitt-Gray et al., 1993; Williams, 1993).

Menstrual cycle status appears to be the primary explanation for the differences in magnitude of bone changes observed among groups. For example, even though all five groups according to menstrual cycle status showed an increase in right leg BMD over the eight months, the eumenorrheic gymnasts (approximately four percent increase), runners

(approximately two percent increase) and controls (approximately one percent increase) exhibited greater changes compared to the oligo/amenorrheic gymnasts (approximately two percent increase) and runners (constant), respectively. Likewise, percent changes in BMD for the right and left arms were more substantial and beneficial for the eumenorrheic runners and gymnasts compared to the oligo/amenorrheic gymnasts and controls. The three eumenorrheic groups of subjects showed a non-significant increase in total hip BMD, while the oligo/amenorrheic runners and gymnasts tended to lose bone mineral at this site. Given that this area of the hip (i.e., proximal femur) is subjected to large forces, especially during gymnastics tumbling and dismount landings (McNitt-Gray et al., 1993), the above findings suggest that lack or very low levels of estrogen is a powerful determinant of bone mass (at this site) compared to the mechanical forces from exercise. Drinkwater and colleagues (1984, 1986) have proposed that the varied responses observed at different measurement sites is largely dependent on the relative amounts of trabecular and cortical bone present at these sites, and how long the individual has had amenorrhea. Cortical bone appears to be more resilient to the effects of low estrogen compared to trabecular bone, although both areas will lose bone mineral if the amenorrheic condition is prolonged.

Effect of Exercise on Percent Change in BMD

Muscular contraction exerts a primary type of strain to which bone adapts (Whalen et al., 1988); thus, muscular building exercise should play a role in determining bone mineral density. Since gymnasts engage in weight training as an integral part of their program while runners may but typically do not weight train, this may partly explain the continued increase in BMD for the gymnasts over the course of a training season. Although none of the groups in the present study had significant changes in muscle strength over the 8-month period, the gymnasts tended to be stronger for all five muscle groups at baseline (although only the quadriceps extensors and biceps flexors were statistically significant).

These results concur with previous work which found that muscle strength is a good predictor of bone mass (Pocock et al., 1989; Snow-Harter et al., 1990; Snead et al., 1989), even if muscle strength was measured at a site distant to the bone area of interest. For example, Snow-Harter and colleagues found that muscle strength of the biceps was the best predictor of femoral neck BMD and grip strength best predicted bone mineral at the lumbar spine and radius. Snead and colleagues (1989) found a more direct relationship with muscle strength at the knee and hip joints being good predictors of lumbar spine and femoral neck BMD values, particularly in eumenorrheic runners. In this study, Snead and co-workers (1989) found that amenorrheic and oligomenorrheic runners had lower vertebral bone mass and related lower strength scores for the trunk. In contrast, in eumenorrheic runners, muscle strength of the leg and hip muscles were strong predictors of lumbar spine and femoral neck BMD.

Numerous studies have shown that athletes have higher bone mass compared to sedentary individuals (e.g., Dalen and Olsson, 1974; Huddleston et al., 1980; Nilsson and Westlin, 1971; Pocock et al., 1986; Williams et al., 1984). These studies have shown that bone mineral is augmented at those skeletal sites which are stressed during the specific exercise. For example, Huddleston and colleagues (1980) found that the cortical bone of the dominant wrist in tennis players was higher compared to that of the non-playing arm. Williams and co-workers (1984) found that bone mineral of the os calcis was increased in runners, an area which is subjected to relatively high impact loads from running. Lane et al. (1986) also found that running resulted in an increased bone mineral of the lumbar spine, although not to as great an extent as rowing did (Wolman et al., 1990). Rowing involves intensive use of the back and trunk musculature which, despite not being a weight-bearing activity, creates substantial forces generated on the lumbar spine. In the present study, we would expect to see increases in both athletic groups over the course of a training season, specific to their training regimen (i.e., what systems are being overloaded).

Changes in muscle strength as a result of physical training may improve bone mass in female athletes, since muscular contraction acts as a primary strain on bone (Whalen et al., 1988). It was expected that over the course of a training season, gymnasts would gain muscle strength, as they are able to increase the level of difficulty of their routines, i.e., perform more difficult tumbling passes which result in higher impact forces on landing (Montpetit, 1976; OSU gymnastics coaches, personal communication). In the present study, however, there were no significant changes in peak muscle force for any of the five muscle groups tested, and there were no significant group differences in the percent change in peak muscle force. There was also no consistent pattern for the percent change in any group for any muscle tested (i.e., some subjects tended to lose muscle strength in some muscles but gained in others). The lack of significant findings for the percent change in peak muscle force may be explained in a couple of ways. First, the coefficients of variations for muscle strength measures on the Kin-Com range between 5% and 8% (Snow-Harter et al., 1992). Therefore, this instrumentation is not sensitive enough to detect changes less than these values. Second, the timing of the post-testing did not correspond to the time of peak training. Due to the nature of their training (i.e., anaerobic and strength/power-based), it was expected that the gymnasts would exhibit substantial increases in peak muscle strength as the season progressed (Montpetit, 1976). Even though the gymnasts still tended to be stronger than the runners and controls at post-test, they did not show the expected improvement in muscle strength as a result of a training season. Competitive training, however, had been completed for over a month prior to the post-testing; thus, it is possible that some of the expected gains during the season had been lost due to a reduction in training. It was not expected that the runners would show an increase in muscle force at the end of the study since they had just finished their track competitive season. If any weight training is performed at all, it is typically done in the off-season (i.e., winter months) when strength is being built, along with endurance, not in the spring when

training is focussed on speed and lightness (i.e., distance runners) (U of O coaches, personal communication).

Changes in Body Composition

As with any exercise training program, changes in body composition are expected as the individual becomes more fit and trained; and in the case of the runners and gymnasts, more competition-ready. There was no significant difference in the change in body weight among the three subject groups, but all groups significantly increased body weight by approximately one to three percent and these values were significantly different from zero. The composition of the weight gain, however, differed among the groups. The significant increase in lean mass accounted for the weight change in the runners, as their percent body fat remained constant. This was most marked in the oligo/amenorrheic runners, but it did not affect their menstrual cycle status. None of the runners changed status with respect to their menstrual cycle over the eight months; thus, an increase in body weight due to an increase in lean mass did not result in normal menstruation. The slight increase in body weight in the gymnasts was due primarily to a slight increase in percent fat, although neither the increase in weight or percent fat was significantly different from zero. These results differ slightly when compared to the body composition findings of Johnson et al. (1989), who reported that both track runners and collegiate gymnasts significantly increased body density and fat-free weight, and decreased percent fat (by approximately 3%) from pre- to post-season, while maintaining a constant body weight. It should be noted, however, that these groups of athletes were measured only before and after their own respective seasons (i.e., three to six months compared to eight months in the present study), and that body composition was determined by hydrostatic weighing in the Johnston et al. (1989) study, which has a higher precision error than body composition determined from DEXA. The difference between the body composition changes in gymnasts and

runners in the present study may, in part, be due to the timing of the post-test determinations in relation to the end of the competitive season. The runners were post-tested immediately after the completion of the spring track season, when they were at their peak in terms of competitive performance. On the other hand, the gymnasts had competed at the National championships approximately one month prior to post-testing. After a week off training, the gymnasts were back in the gym working out new maneuvers and up-grading the difficulty of their routines, but the intensity level had declined somewhat (OSU coaches, personal communication). A study by Vercruyssen and Shelton (1988) also measured body composition changes over a training season in collegiate gymnasts, with data collected (based on anthropometric and skinfold measures) at pre-, mid- and post-season. Body weight decreased from pre- to mid-season (three months) and then remained stable from mid- to post-season (three months). Percent body fat, on the other hand, decreased across the whole six-month training period (from a high of 21% to a low of 13.5%). The initial percent body fat value of 21% appears to be high for competitive athletes, especially compared to those gymnasts in the present study (approximately 16% fat at pre-test), and the gymnasts in the Johnson et al. (1989) study (approximately 18% at pre-test). Measurement error may explain the differences between these studies which all used different techniques to determine body composition. Martin and Drinkwater (1991) investigated the variability in the common measurement techniques used to determine body fat (e.g., hydrostatic weighing, skinfolds), and concluded that the error in percent fat estimation was reduced if bone density was taken into account (i.e., measured, not just assuming a constant value).

Effect of Exercise Training on Menstrual Cycle Status

Intense physical training can have profound effects on a woman's reproductive hormone profile. Strenuous exercise has been implicated in conditions such as delayed age

at menarche (Frisch et al., 1981; Malina, 1983; Peltenburg et al., 1984; Sanborn et al., 1987; Warren, 1980), exercise-associated oligo- and amenorrhea (Drinkwater et al., 1984; Highet, 1989; Loucks, 1990; Sanborn et al., 1982), altering menstrual cycle luteal phase length and causing anovulatory cycles (Loucks, 1990; Prior et al., 1990; Shangold et al., 1990). Numerous groups have shown that physical training may delay the age at menarche compared to normal adolescent females (e.g., Frisch et al., 1981; Malina, 1983; Peltenburg et al., 1984; Warren, 1980). In the present study, both groups of athletes had a delayed age at menarche compared to the non-athletic control group (i.e., approximately 16 and 14 years of age for the gymnasts and runners, respectively, compared to a normal age of 13 years). To support the use of "delayed" (as opposed to "late") menarche, i.e., delayed as a result of intense athletic training early in life, the subjects were asked for their mother's age at menarche; these were all between the normal ages of 11 and 13.

It has been argued that the amount and intensity of pre-pubertal training may be one factor involved in delaying the age at menarche. The gymnasts in this study had been training (on average) since the age of seven, while the runners had begun training specifically for competitive running at the age of fourteen or fifteen. Peltenburg and colleagues (1984) found that girls aged eight to fourteen years who participated in gymnastics training had a delayed age at menarche by approximately one to two years compared to normally active schoolgirls and swimmers. The girl gymnasts were leaner than the swimmers and schoolgirls, which along with the possibility of self-selection, may have predisposed the gymnasts to a later onset of puberty. These data support the hypothesis by Frisch et al. (1981) that the initiation of the pubertal growth spurt and the onset of menarche are dependent upon attaining a critical body weight and percent fat, and are delayed in athletes. Furthermore, a delayed age at menarche has been associated with a higher incidence of exercise-associated oligo/amenorrhea (Sanborn et al., 1987; Wakat et al., 1982). In the present study, however, the incidence of oligo/amenorrhea was not

significantly higher in the gymnasts, who had a delayed age at menarche, compared to the runners.

None of the subjects in the present study experienced a change in menstrual cycle status over the eight-month training period. This is probably due to the fact that these women had been grouped according to menstrual cycle status over the past year, during which most of them had been training at the same level as during the study period. The subject's menstrual cycle status had already been established upon entry to the study, and was probably a result of prior training. It is interesting to note that the oligo/amenorrheic runners in the present study ran significantly more miles per week over the course of the entire study (averaging more than 50 miles per week) compared to the eumenorrheic runners (approximately 38 miles per week). Training intensity, based on the average number of intense sessions run per week (e.g., intervals, fartlek, tempo runs, races), however, did not differentiate the runners by menstrual cycle status. Thus, in the present study, exercise duration (at least in runners) may play a role in determining menstrual cycle status. These findings agree with those of other researchers (e.g., Malina, 1983; Puustjarvi et al., 1992; Schwartz et al., 1981; Warren, 1980).

Although it is fairly evident when exercise-associated oligo- and amenorrhea are present in female athletes, non-symptomatic changes in the menstrual cycle may also occur (Loucks, 1990; Prior et al., 1990) which may negatively impact bone. In the present study, there were eumenorrheic women (athletes and controls) who had asymptomatic menstrual cycle disturbances (i.e., shortened luteal phases and/or anovulatory cycles). It should be noted, however, that compliance for the daily urine collections was relatively poor among the eumenorrheic subjects, so data should be interpreted with caution (i.e., small subject numbers). In those eumenorrheic gymnasts (n=4), runners (n=6) and controls (n=10) who participated in this part of the data collection, there appeared to be a higher incidence of shortened luteal phase and anovulatory cycles in the runners and controls (over 40%) compared to the gymnasts (20% abnormal cycles). Still, this supports data presented by

Prior and colleagues (1982,1990) and Barr et al. (1994) that such menstrual cycle disturbances are as prevalent in apparently normal non-athletic women as in athletes, possibly due to highly restrained eating patterns among these weight-conscious women. Significant differences were not found in the present study for bone mineral density changes over time between eumenorrheic women with normal cycles and those with abnormal cycles. There was a trend, however, for the percent change values to be more favorable in those women who had normal cycles. For example, the percent change in lumbar spine and whole body BMD was approximately two percent in the eumenorrheic women with normal cycles compared to only a one percent change in the women with abnormal cycles. For the femoral neck percent change in BMD, the normal cycle women remained constant, while the women with abnormal cycles lost approximately one percent. These trends suggest that asymptomatic eumenorrheic women (whether they are athletes or not) who have anovulatory menstrual cycles or cycles with a shortened luteal phase (with the consequent lower than normal levels of progesterone), may be at risk of losing bone mineral over time (Loucks, 1990; Prior et al., 1990; Shangold et al., 1990). Larger samples are needed, however, in all these studies.

Effect of Nutrition on Menstrual Cycle Status and BMD

The role of diet in the menstrual cycle-bone health scenerio is complex and involves several different nutrients. In the present study, diet analyses were performed on four separate occasions and averaged for each nutrient, since there were no significant differences across time. Poor dietary habits are common in these weight- and body-image-conscious female athletes. The runners and gymnasts did not differ in terms of energy intake, protein or calcium intake; the calcium-to-protein ratio, however, was higher in the gymnasts compared to the runners and controls, suggesting that more of the dietary calcium is available for deposition in the bone (Allen et al., 1979; Hegsted and Linkswiler, 1981;

Marcus, 1982). Energy intake (total kilocalories) appears to be somewhat restricted when considering the large energy output of these athletes, and the calcium intake is below the Recommended Dietary Allowance of 1200 mg per day for this age group (Bernardot et al., 1989; Berning et al., 1985; Nelson et al., 1986). These findings may play a role in the high incidence (approximately one-third of each athletic group) of oligo/amenorrhea observed in these women, as well as negatively affecting their bone mineral density (at least in the oligo/amenorrheic runners).

Although there were no significant group differences for any of the macronutrients analyzed, dietary composition in terms of percentage of total kilocalories consumed as carbohydrates, fat and protein did differ. The runners had a higher percentage of total caloric intake from carbohydrates compared to the control subjects, and both the runners' and gymnasts' diets were above the recommended level of at least 60% of total calories be in the form of carbohydrates (U.S. Recommended Dietary Allowances, revised 1989). All groups met the recommended levels for fat intake (i.e., less than 30% of total kilocalories), even though the controls' diets contained the most fat. Protein intake was high in all groups (greater than 15% of total kilocalories). In the present study, all the women exceeded the RDA for protein intake, averaging 150% of the recommended level, even for athletes. An important dietary variable to determine is the calcium-to-protein ratio. In the present study, the gymnasts had significantly higher values for this ratio compared to the runners and controls, suggesting that the gymnasts had more calcium available for deposition in the skeleton in order to build bone mass (Avioli, 1988; Heaney, 1988). Athletes generally require a slightly higher protein intake compared to a sedentary individual (Tarnopolsky et al., 1988), especially runners, in order to offset protein catabolism during exercise and maintain lean body mass. Too much protein, however, can be detrimental because it causes excessive urinary calcium excretion (Allen et al., 1979; Hegsted and Linkswiler, 1981; Robertson et al., 1979). Recker and colleagues (1992) have suggested that the calcium-to-protein ratio is more important for bone than the absolute amount of calcium.

Adding to the complexity of this calcium-protein interaction, however, is the role of phosphorus. In the present study, phosphorus intake in all subjects was adequate; for example, as a group, the athletes consumed approximately 99% of the RDA, while the controls consumed approximately 90% of the RDA. Thus, even though their protein intakes were relatively high, they were not deficient in phosphorus, and calcium excretion may not have been increased. As Hegsted and colleagues (1981) pointed out, the calciuretic effect of protein may be controlled by consuming that protein in the form of foods rich in phosphorus (e.g., meat and dairy products). The added benefit of obtaining protein from dairy products is the abundance of calcium in this food group. The calcium-to-phosphorus ratio is another important measure, as it also helps determine calcium availability to the skeleton. In the present study, the calcium-to-phosphorus ratio was significantly higher in the gymnasts compared to the runners and the controls. The proposed mechanism is that an overabundance of dietary phosphorus, especially compared to calcium, causes a hypersecretion of parathyroid hormone, and hence, an increased rate of parathyroid-dependent bone resorption (Lutwak, 1975; Marcus, 1982). In humans, the effect of a low calcium-to-phosphorus ratio (e.g., less than 0.5) may not be too much of a concern (Marcus, 1982). In fact, Heaney (1988) reported no deleterious effects of phosphorus in relation to calcium intake. Bell and colleagues (1977), however, found that by increasing the dietary phosphorus level in relation to calcium, such that the calcium-to-phosphorus ratio was less than 0.5, urinary calcium excretion was reduced, but parathyroid hormone secretion was increased, which leads to a loss of bone mineral. These researchers concluded that an average American diet which usually contains a lot of phosphate-containing additives may contribute to age-related bone loss. This was in comparison to a healthier diet which had a calcium-to-phosphorus ratio of approximately 0.7 to 0.8. In the present study, none of the three subject groups had a calcium-to-phosphorus ratio of 1:1, but the gymnasts had a significantly higher value (approximately 0.9) compared to the controls and runners (both approximately 0.7). None of these groups appear to be at risk of

diet-induced hyperparathyroidism, however, since their ratios are well above 0.5. Marcus (1982) suggested that this ratio of nutrients may not be critical to the skeleton in the adult, but may play a role during the growth years. The consumption of soft-drinks, particularly colas which contain approximately 20 mg/dL of phosphorus and no calcium (Lutwak, 1975), in place of milk in adolescents and young adults still growing, may result in a low calcium-to-phosphorus ratio, and increased parathyroid hormone-induced bone resorption.

Restricted eating is quite common among female athletes whose sport performance depends on body weight and/or aesthetics (Barr et al., 1994; Benardot et al., 1989; Nelson et al., 1986; Slavin, 1991). In the present study, all subject groups consumed less than the RDA for total kilocalories, with the gymnasts consuming the least (approximately 1700 kilocalories), although this intake was not significantly different compared to the runners (approximately 1800 kilocalories) and control subjects (approximately 1900 kilocalories). Since the athletes are also expending extremely high levels of energy training for their sport, up to twenty hours per week, it could be suggested that they require even more than the 2200 kilocalories per day. In this case, these runners and gymnasts appear to be in energy deficit, and the concept of "energy drain" (Malina, 1983; Warren, 1980) may play a role in menstrual cycle disturbances and loss of bone mineral related to insufficient caloric intake. Schweiger and colleagues (1988) found a strong positive correlation ($r=0.70$, $p<0.01$) between caloric intake and levels of progesterone in athletes, which led them to conclude that nutrition plays an important role in determining menstrual cycle status, even in apparently normal, asymptomatic eumenorrheic women. Those athletes who consumed fewer kilocalories also had lower progesterone levels and usually a shortened luteal phase of the menstrual cycle. This, in turn, could lead to potential bone loss (Drinkwater et al., 1984; Loucks, 1990; Prior et al. 1990). In the Schweiger et al. (1988) study, the correlation between total caloric intake and estrogen levels was also positive, but did not reach statistical significance ($r=0.36$, $p>0.05$). These researchers proposed that the luteal phase of the menstrual cycle is more susceptible to disruption from inadequate caloric

intake compared to estrogen production over the course of the menstrual cycle. In the present study, dietary analyses were not performed by menstrual cycle based on small subject numbers and large variations among nutrient intakes. It should be noted, however, that the accuracy of diet records for determining energy intake has been questioned, with under-reporting of food intake common, especially in female athletes. Recently, Edwards and colleagues (1993) found that highly trained female endurance runners had a significant imbalance between energy intake (based on food diaries) and energy expenditure (determined using doubly labeled water), but body weight did not change over the 7-day study period. In the present study, subjects actually increased body weight over the 8-month period, despite reporting a total kilocaloric intake which failed to meet the RDA. It is possible that these subjects failed to accurately (or honestly) record their dietary intake, despite the researcher's thorough instructions on how to keep accurate diet records and emphasis on its importance.

Another key nutrient related to bone health is calcium. The RDA for dietary calcium in this age group of women is 1200 mg per day. This is a relatively high intake and one which is usually not achieved in college-aged women (Benardot et al., 1989; Drinkwater et al., 1986; Nelson et al., 1986; Nieman et al., 1989; Perron and Endres, 1985). In the present study, all three groups of subjects were below the RDA for dietary calcium intake; yet, the gymnasts had the highest levels of intake (a mean of approximately 1100 mg/day), with the runners and the controls consuming approximately 800 mg/day and 900 mg/day, respectively. Although calcium intake also did not differ according to group and menstrual cycle status in the present study, the oligo/amenorrheic runners had the lowest levels at only 66% of the RDA. This level of calcium intake classifies their diet as poor in this nutrient, and may compound the already lower bone mass as a result of low estrogen levels. As Drinkwater and colleagues (1986) pointed out, low dietary intakes of calcium are of special concern for the bone health of amenorrheic athletes since there may be a compounding effect of lack of estrogen (i.e, increased rates of bone resorption) and

insufficient dietary calcium (i.e, less mineral available for deposition in the bone). In the present study, seven of the eighteen runners consumed a diet considered poor in the essential nutrient calcium (i.e., less than two-thirds of the RDA), with values for calcium intake ranging from approximately 41% to 59% of the RDA. If this pattern of calcium consumption was to continue over the long-term, these women would probably lose essential bone mineral since lack of dietary calcium would precipitate greater rates of bone resorption for other important physiological processes (Avioli, 1988; Heaney, 1988). This, in turn, could precipitate skeletal injury and future osteoporosis (Marcus, 1982). In support of this, Matkovic and colleagues (1979) studied bone mass in individuals residing in two different geographical regions of Yugoslavia, one where habitual calcium intake was high (approximately 1100 mg/day) and one where calcium intake was fairly low (approximately 500 mg/day). Their results showed that maximal bone density of the metacarpals was significantly higher in those individuals residing in the region where high dietary calcium was the norm. They also found a high incidence of femoral neck fracture in individuals from the low-calcium intake region. Matkovic and colleagues (1979) cautioned, however, that the major differences in bone mineral were established by the age of thirty, which suggests that once peak bone mass of the individual has been established, dietary calcium intake may not play as significant a role in maintaining bone mass. According to Recker and colleagues (1992), however, the women in the present study may still have been gaining bone mass, if their calcium intake was adequate (especially related to protein intake) and were engaged in physical activity. Two of the twelve gymnasts ate a poor diet in terms of calcium intake, with values of 59% and 64% of the RDA. Half (or seven) or the fourteen control subjects consumed insufficient levels of calcium, ranging from approximately 38% to 65% of the RDA.

Vitamin D is an important nutrient/hormone which affects calcium absorption. In the present study, dietary Vitamin D intake was poor among virtually all subjects; the runners consumed an average of 39% of the RDA, the gymnasts consumed an average of

44% of the RDA, and the control subjects consumed only 35% of the RDA on average. These results may not be as bleak as they seem, however; Vitamin D can be synthesized in the skin from ultraviolet radiation (DeLuca, 1988; Haddad, 1992), and since most of these young women (particularly the runners) spend quite a bit of time outdoors, they do not appear to be at risk for Vitamin D deficiency. DeLuca (1988) stated that the major determinant of calcium absorption from the intestinal tract was the circulating levels of the active form of Vitamin D, 1,25-dihydroxy Vitamin D. The production of the active form of Vitamin D is regulated by several factors, including the levels of dietary calcium and phosphorus and the presence of estrogen. Because of this complex interaction of nutrients and hormones, it becomes evident that estrogen withdrawal (either at menopause or in the amenorrheic athlete) could result in a deficiency of active Vitamin D, which in turn, could result in increased rates of bone resorption and urinary calcium loss, and a reduced rate of intestinal calcium absorption (Marcus, 1982).

Most researchers stress the importance of estrogen in the whole scheme of calcium balance and bone deposition/resorption processes (Dalsky, 1990; Gallagher and Nordin, 1975; Heaney, 1988; Nelson et al., 1986), and suggest that dietary calcium intake should be higher if estrogen is deficient. In the present study, nutrition did not differ significantly between groups according to menstrual cycle status, but that may have been due, in part, to the large variability in nutrient intake and small subject numbers involved. When all eumenorrheic athletes (n=20) were compared to all oligo/amenorrheic athletes (n=10) in terms of nutritional habits, some interesting trends were observed. These trends are confounded, of course, by the fact that runners are grouped with gymnasts, even though their respective bone mineral density values are significantly different; thus, diet and menstrual cycle status must be interacting differently in the gymnasts as compared to the runners. Both groups of eumenorrheic and oligo/amenorrheic athletes consumed only about 84% of the RDA (of a non-athletic college-aged woman) for total kilocalories. Approximately 16% of those kilocalories were consumed as protein (greater than 150% of

the RDA for protein). The eumenorrheic athletes consumed slightly more of their total caloric intake as fat (approximately 22%) and less as carbohydrate (approximately 64%) compared to the oligo/amenorrheic athletes (approximately 19% and 69%, respectively). The eumenorrheic athletes consumed a slightly higher percent of the RDA for calcium (approximately 78%) compared to the oligo/amenorrheic athletes (approximately 75%), although both groups had low dietary calcium intakes. Likewise, the eumenorrheic athletes consumed a slightly higher percent of the RDA for Vitamin D (but still only about 44%) compared to the oligo/amenorrheic athletes (approximately 35%). Vitamin D in its active form is a hormone which acts to increase intestinal absorption of calcium (DeLuca, 1988), and thus, insufficient levels will result in a negative calcium balance, with mineral being drawn out of the skeleton. As mentioned previously, though, these female athletes spend a lot of time outside exposed to ultraviolet radiation, which can be converted in the skin, liver and kidney to the active form of Vitamin D; so their low dietary levels are not as much of a concern as their insufficient intake of calcium.

Effect of Stress/Anxiety on Menstrual Cycle Status and BMD

Poor nutrition is only one piece of a complicated puzzle relating menstrual cycle status to bone health. High stress and anxiety levels are all too common in some female collegiate athletes when faced with the pressures of intense training, competing well, maintaining good grades in school, and possibly trying to have a social life. In the present study, inter-subject variation in stress/anxiety and mood scores was substantial and thus, no significant group differences or time effects were found, except for the Total Mood Disturbance (TMD) score (based on the Profile of Mood States) at baseline. The runners had significantly higher TMD scores at baseline compared to the controls, with the gymnasts exhibiting intermediate scores. This intuitively makes sense in that the runners were about to begin their fall competitive season and thus, the stress and worry of training

hard and competing well was manifesting itself, while the gymnasts had three months until competition began, and the controls did not have this worry. State anxiety scores were also significantly higher for the runners and the gymnasts compared to the controls, but only at the baseline time. It should be pointed out, however, that even though their scores were elevated compared to this group of college-aged control women, the runners' and gymnasts' scores were within the normal range of scores for TMD and State Anxiety reported for college-aged individuals (Edwards and Huston, 1984; McNair et al., 1971; Spielberger et al., 1970).

In a 1988 study, Schweiger and colleagues investigated the relationships between caloric intake, menstrual cycle status and stress in female endurance-trained athletes. These researchers found a significant positive correlation between caloric intake and luteal phase levels of progesterone ($r=0.70$, $p<0.01$), and a strong negative correlation between ratings of subjective stress from family, friends and partner and luteal phase levels of progesterone ($r=-0.80$, $p<0.01$). Stress from family, friends and partner was also significantly correlated with estrogen levels ($r=-0.55$, $p<0.05$), as was stress from work and studies ($r=-0.48$, $p<0.05$). Although non-significant, the correlations between luteal phase progesterone levels and stress from work and studies ($r=-0.39$) and stress from sport ($r=-0.41$) were noteworthy. These researchers concluded that the luteal phase of the menstrual cycle in athletes was specifically prone to disruption due to both caloric deficit and excessive stress from various sources. Cavanaugh and colleagues (1989) found very high anxiety levels in competitive female athletes, including gymnasts, and this was strongly related to menstrual cycle irregularities. In accordance with the present study, Edwards and Huston (1984) also could find no effect of time across a training season for Profile of Mood States scores in competitive gymnasts. Both early and late season scores were within the range of normal scores for college-aged individuals and athletes. This appears to be contradictory to the findings of Cockerill et al. (1992), who reported that high-intensity training negatively affected mood, as measured by the TMD score. They concluded that exercise which was so

intense as to cause amenorrhea was psychologically "self-defeating", in that their mood profile was not any better than a group of inactive women, even though less intense exercise has a beneficial effect on mood. In fact, Feicht and colleagues (1978) found that amenorrheic runners reported higher stress-related scores as a result of their intensive training compared to eumenorrheic runners. Schwartz and co-workers (1980) concluded that more amenorrheic athletes associated running with stress compared to regularly menstruating runners. The amenorrheic runners in this study were subjected to higher levels of stress (i.e., more intense exercise) compared to the eumenorrheic runners. It is possible that this stress could be the cause and the outcome of exercise-induced amenorrheic, or as Highet (1989) proposed, hypothalamic dysfunction is influenced by stress, which might disrupt the entire reproductive system.

The importance of being able to use these paper-and-pencil psychological questionnaires to evaluate stress and anxiety and mood profiles in an individual cannot be emphasized enough. For example, since excessive stress/anxiety has been associated with menstrual cycle irregularities and, in turn, possibly decreased bone mineral (Ding et al., 1988; Highet, 1989; Loucks and Horvath, 1984; Schwartz et al., 1980), it would be helpful if coaches and athletes could monitor stress/anxiety levels (without an invasive blood draw to measure cortisol levels) in order to control and manage this stress. In 1988, Ding and colleagues reported that physiological stress levels were related to exercise-associated amenorrhea. This, in turn, may affect bone metabolism and result in lower bone mass in highly stressed individuals. The mean cortisol level (glucocorticoids are the primary stress hormones) in the amenorrheic athletes was significantly higher compared to the eumenorrheic athletes and non-athletes, and even exceeded the upper limit of the normal range of values for cortisol. Bone mineral density of the lumbar spine was also significantly lower in the amenorrheic athletes compared to the eumenorrheic athletes. The amenorrheic athletes, as expected, had significantly lower circulating levels of estradiol compared to the other groups, but also had lower levels of progesterone compared to the

other groups. Both estrogen and progesterone have been reported as important to bone health (Marcus et al., 1985; Prior et al., 1990; Snow and Anderson, 1986). Ding and colleagues (1988) concluded that increased glucocorticoid levels may contribute to menstrual cycle disturbances and also decreased bone mineral by altering either or both of the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes. They suggested that increased circulating levels of cortisol may be due to physical and emotional stress resulting from their intensive training. Chronically-increased cortisol levels, as seen in the amenorrheic competitive athlete, may cause long-term suppression of the hypothalamic-pituitary-gonadal axis, and irreversible bone loss (Drinkwater et al., 1984; Prior et al., 1990). Even though Ding et al. (1988) could not find an independent effect of cortisol on bone density which was separate from the effects of hypo-estrogenemia, they proposed that bone health in these women was threatened by two sources: elevated cortisol levels and decreased estrogen levels. They suggested that the question of the combined effects of both hormones on bone mass over an extended period of time needs to be addressed more completely. Unfortunately, the large inter-subject variation in the POMS and STAI questionnaire measures in the present study made it impossible to conclude anything related to stress/anxiety and menstrual cycle status (since that did not change over the eight months) and bone mineral density changes in these women.

SUMMARY AND CONCLUSIONS

Physical activity has been shown to have a beneficial effect on bone, such that female athletes who train intensively in sports where high impact skeletal loading occurs (e.g., gymnastics) exhibit increased bone mineral density at specific sites over the course of the training season. Other female athletes, however, (e.g., runners) do not exhibit these increases in bone mass, despite their high level of training (i.e., up to 70 miles per week). This may be explained, in part, by the different skeletal loading patterns during running, where impact forces are two to five times body weight, compared to gymnastics, where impact forces can range from six up to fourteen times body weight. Activities which generate greater impacts with fewer repetitions appear to be more beneficial to bone compared to those activities which involve lesser impacts but with many cycles or repetitions.

Another major determinant of bone mineral density in these young women is menstrual cycle status. Intensive physical training may result in amenorrhea or oligomenorrhea, with subsequent lack of estrogen compared to eumenorrheic women. Physical training may also result in abnormal menstrual cycles, such as a shortened luteal phase and/or anovulation, which manifest in below normal levels of progesterone. Both estrogen and progesterone are important hormones for bone metabolism. In the present study, athletes were divided into those who were eumenorrheic (at least 10 menstrual cycles per year) or oligo/amenorrheic (0 to 9 menstrual cycles per year). All the control subjects were eumenorrheic. Eumenorrheic women with a shortened luteal phase and/or anovulatory cycle were also identified. Bone mineral density changes over the 8-month study were different depending on athletic group and menstrual cycle status.

Research Hypotheses

It was hypothesized at the beginning of this longitudinal study that lumbar spine bone mineral density would decline in those athletes with oligo- and amenorrhea compared to eumenorrheic athletes and non-athletic control subjects. At baseline, lumbar spine BMD was significantly lower in the runners compared to the gymnasts and controls, regardless of menstrual cycle status. Femoral neck BMD at baseline was significantly different among all three groups, and whole body BMD was significantly lower in the runners compared to the gymnasts. Due to these initial group differences for bone mineral density, menstrual cycle status was considered within each group, and subsequent analyses, including all percent change scores, were performed with the following five groups: eumenorrheic (ER) and oligo/amenorrheic (OAR) runners, eumenorrheic (EG) and oligo/amenorrheic (OAG) gymnasts, and eumenorrheic controls (EC).

Hypothesis #1: In the running group, the percent change in lumbar spine BMD was significantly different between ER (1.0±2.1%) and OAR (-0.9±1.5%). This supports the hypothesis that lumbar spine BMD would decline in the oligo/amenorrheic runners compared to the eumenorrheic runners. This was not true, however, for the oligo/amenorrheic gymnasts, who tended to increase lumbar spine BMD over the 8-month period (1.3±2.8%), but were not statistically different from the EG (3.6±2.0%). The OAR and OAG did not differ from the EC.

Hypothesis #2: This hypothesis stated that lumbar spine percent change would be greatest in the eumenorrheic gymnasts and runners compared to the eumenorrheic controls. This was supported in the gymnasts but not in the runners. The percent change in lumbar spine BMD was significantly greater in the EG compared to the eumenorrheic controls (0.7±1.3%). The percent change in lumbar spine BMD in ER and EC did not differ from one another.

Hypothesis #3: This hypothesis stated that the percent change in femoral neck BMD would be greater in the eumenorrheic gymnasts and runners compared to the controls. This was true only in the gymnasts, who had a tendency towards a positive percent change (2.7±2.8%) compared to the controls' tendency to lose BMD at the femoral neck (-0.9±2.2%).

Hypothesis #4: This hypothesis stated that the percent change in whole body BMD would be greater in the eumenorrheic gymnasts compared to the runners and controls. This was not supported, as none of the five groups differed significantly for the percent change in whole body BMD.

Hypothesis #5: This hypothesis stated that women with a shortened luteal phase of the menstrual cycle would exhibit a significant loss of BMD at the lumbar spine compared to eumenorrheic women with a normal luteal phase length. Due to small sample sizes, this hypothesis could not be adequately tested (i.e., no statistical significance was found), although there were some trends, particularly for the gymnasts and controls. Gymnasts with abnormal menstrual cycles (i.e., shortened luteal phase and/or anovulation) tended to have less of an increase in LS BMD over the 8-month period compared to the gymnasts with normal menstrual cycles. This pattern was apparent at all three sites of measurement (e.g., LS, FN, WB) in the control subjects: the percent change in BMD tended to be less (of an increase or even a decrease) in the women with abnormal cycles compared to those women with normal menstrual cycles.

Hypothesis #6: It was hypothesized that the incidence of menstrual cycle irregularities (defined as amenorrhea and oligomenorrhea) would be greater in the runners compared to the gymnasts. This was not true, since approximately one-third of both athletic groups exhibited oligo- or amenorrhea. Specifically, of the eighteen runners who completed the study, six had menstrual cycle irregularities (three were amenorrheic and three were

oligomenorrheic); and of the twelve gymnasts, four had irregular menstrual cycles (one had primary amenorrhea and three were oligomenorrheic).

Hypothesis #7: This hypothesis stated that training, inadequate nutrition and psychological stress/anxiety levels would be greater in the athletes compared to the controls. Control subjects were recruited specifically to be non-competitive but normally-active college-aged women, who were eumenorrheic. Thus, by definition, their training and menstrual cycle status were different compared to the athletes' , and this supports the hypothesis. The remaining variables in this hypothesis (i.e., nutritional state and psychological stress/anxiety levels) are only partially supported. In terms of dietary intake of key nutrients, the athletes consumed a significantly greater proportion of their total caloric intake in the form of carbohydrate and less as fat compared to the control subjects. The gymnasts (but not the runners) also had a higher calcium:phosphorus and calcium:protein ratio compared to the controls. Only at baseline were the psychological stress/anxiety levels different between the athletes and the controls. The runners and gymnasts were higher on the State Anxiety inventory and Total Mood Disturbance scores at baseline compared to the controls, although they were still within the normal range of scores for their age and gender.

Hypothesis #8: This hypothesis stated that athletes who were amenorrheic or oligomenorrheic would experience higher levels of training, higher stress/anxiety scores, and poorer nutritional habits compared to eumenorrheic athletes. This is supported only in terms of amount of training, in the runners, as the gymnasts' training was not quantifiable. When running mileage was averaged over the 8-month training period, the runners who had irregular menstrual cycles ran significantly more miles per week (54.0$\pm$11.7) compared to the women with regular menstrual cycles (38.1$\pm$15.0). The average number of intense training sessions per week for the runners did not differ according to menstrual cycle status. Dietary intakes of the nutrients measured did not differ among the groups according

to menstrual cycle status. Although the eumenorrheic athletes tended to consume more dietary calcium and Vitamin D compared to the oligo/amenorrheic athletes, and had a slightly higher percent of total kilocalories from fat, these were not significant differences. There were also no significant group by menstrual cycle status effects for any psychological stress/anxiety score. Thus, oligo/amenorrheic athletes (specifically runners) may train at a higher level (run more miles per week) compared to eumenorrheic athletes, but their nutritional and psychological stress/anxiety profiles were similar.

Directions for future research

The results of this study suggest that the type of impact loading during physical activity and the menstrual cycle status of the athlete interact to produce differential effects on bone mineral density over an 8-month period of time. Menstrual cycle irregularities (i.e., amenorrhea, oligomenorrhea, shortened luteal phase and/or anovulatory cycles) did not appear to significantly compromise bone mass in gymnasts at any skeletal sites. In runners, however, bone mineral density changes were minimal and not substantial enough to be of significant benefit. Some skeletal sites in runners showed a tendency to decrease after only 8 months. Some of the non-significant findings in the present study may be attributed to the low subject numbers in the subgroups, i.e., group by menstrual cycle status. Statistical power was lower for all variables when 5-group ANOVAs were performed compared to 3-group ANOVAs. Also, even small changes in BMD which are not statistically significant may have practical meaning, particularly in injury prevention. If subject numbers were increased, there would be more statistical power to detect differences among athletic groups and within each group according to menstrual cycle status.

Compliance to most of the many testing procedures in the present study was excellent; however, subjects were less willing to complete the two urine collections. These data (i.e., the effects of shortened luteal phase and/or anovulatory cycles on BMD changes over time) were not significant probably due to poor compliance by the subjects. Personal communication with some of the subjects (particularly the athletes) found that these urine collections, daily over two complete menstrual cycles were tedious and often impractical for athletes when travelling to competitions. Better planning for these collections with regard to ease of scheduling may help compliance in the future. In addition, a coincidental blood draw (possibly once a week for one menstrual cycle) with the urine collections would be insightful in terms of confirming the validity of these urinary reproductive hormone metabolites as markers for circulating hormonal levels.

Further research in this area needs to address several other concerns, i.e., tighter control of confounding factors know to affect bone, minimize variation within subject groups, and timing of testing. In the present study, intact athletic groups were recruited as subjects, and there was no intervention with respect to their training or diet. All subjects were free-living college students, some of whom happened to be competitive athletes; however, other aspects of their life could have confounded the experimental observations, even though an attempt was made to measure psychological stress/anxiety from all sources. Within each subject group, there was a certain amount of variation which may have affected the results in terms of significance of BMD changes. For example, the running group included women training for distances ranging from the 800 meters to the marathon (26.2 miles), with weekly mileage varying between approximately 21 and 75 miles/week. A more homogeneous group of runners may have shown different effects on BMD over time compared to the more varied group of runners in the present study. Likewise, not all the gymnasts trained the same, since some competed in the all-around (i.e., all four events) while others were event specialists (e.g., just one or two events). Those gymnasts who

trained for all four events underwent more intense training compared to the event specialists; this may have confounded the results of the present study. The timing of the post-test measures could have been planned more appropriately in the present study. For example, the post-test measures for the runners were performed immediately after completion of the track competitive season. However, post-testing in the gymnasts was delayed approximately four to five weeks after the completion of their competitive season, which may help explain why some variables showed no change or even a decrease (e.g., muscle strength). Since the gymnasts only took two weeks off training and then returned to the gym to practice new skills, it was believed that a significant amount of conditioning was not lost.

The critical factor for investigating changes in BMD was considered to be time. In the present study, an average of eight months elapsed between pre- and post-testing. This was a convenient time frame to use based on a collegiate athlete's training and school year, and a reasonable time period for investigator and subject involvement alike. Studies in the future should focus, however, on a longer period of time for observation and/or intervention (e.g., two to three years), since changes in BMD are quite small and are usually not evident in less than six months. In fact, a follow-up to the present study is currently underway with half of the runners and 9 of the 12 gymnasts continuing for a second year, as well as adding new team members to the study.

Two other considerations for future research related to bone mineral density changes in female athletes over time are: including athletes from a variety of different sports and measuring different skeletal sites. First, athletes in different sports train with varying methods, and since the "optimal" exercise regimen to improve bone mass is still unknown, studying a wider variety of athletes may help address this issue. Also, it may have been interesting to measure BMD at the radius in the present study, particularly in the gymnasts, since their training involves a lot of upper body work (e.g., bars). Training has been shown to have site-specific benefits on bone mass; thus, we might expect gymnasts to have higher BMD values for the radius and greater percent changes over time compared to runners, who typically do little upper-body training. Likewise, although swimmers do not experience the beneficial effects of gravitational forces on bone, strong muscular contractions and the consequent forces generated (particularly in the upper body) may augment BMD at the radial site.

The present study hypothesized that the magnitude of impact forces experienced by the gymnasts and runners accounts, at least in part, for the differential effects on bone mass over time. It would, therefore, be helpful if some biomechanical analyses were performed to investigate this question more objectively. Future studies should attempt to apply biomechanical principles to the study of changes in BMD over time, and actually measure the impact forces (e.g., force plate or force transducer data) being applied to the skeleton of these athletes. Quantifying the exercise regimens of the athletes, particularly in terms of skeletal loading patterns, is an important and necessary goal of any future research investigating the effects of physical activity on bone over time.

A next logical step in this research should address the issue of improving bone mass in those athletes whose exercise program is not resulting in the desired effect on bone. Intervention strategies to improve BMD among runners, or to at least minimize bone loss and reduce skeletal injuries, should be investigated. Some suggestions of possible intervention techniques which may benefit BMD include: estrogen replacement therapy in amenorrheic and oligomenorrheic athletes (who may be unwilling to decrease training and increase body weight in an attempt to become eumenorrheic); calcium supplementation, in both eumenorrheic and oligo/amenorrheic runners, since dietary calcium intake is typically below the RDA; and implementing different training regimens (e.g., decreasing weekly mileage but keeping intensity the same, and/or including some resistance-type training to enhance muscle strength). Athletes and coaches should be encouraged to think not only of training and competitive performance (short-term goals), but also to consider the long-term health effects of strenuous exercise training, particularly as it relates to the loss of bone mass and potential development of premature osteoporosis.

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APPENDICES

APPENDIX A

OREGON STATE UNIVERSITY INSTITUTION REVIEW BOARD APPROVAL AND INFORMED CONSENT

OREGON STATE UNIVERSITY

Committee for the Protection of Human Subjects

Chair's Summary of Review

Title: Bone mineral and menstrual cycle status in competitive female athletes: a longitudinal study (annual review)

Program Director: Christine Snow-Harter

Recommendation:

X Approval*

_____ Provisional Approval

_____ Disapproval

_____ No action

* The informed consent forms obtained from each subject need to be retained for the long term. Archives Division of the OSU Department of Budgets and Personnel Service is willing to receive and archive these on microfilm. At present at least, this can be done without charge to the research project. Please have the forms retained in archives as well as in your files.

Remarks: The IRB does not consider that there is additional risk to the subjects as a result of minor changes in protocol.

Date: 8/30/95

Redacted for privacy

Signature

If the recommendation of the committee is for provisional approval or disapproval, the program director should resubmit the application with the necessary corrections within one month.

Application for Approval of the OSU Human Subjects Board

BONE MINERAL AND MENSTRUAL CYCLE STATUS IN COMPETITIVE FEMALE ATHLETES: A LONGITUDINAL STUDY

Significance of the Project

Menstrual cycle abnormalities, such as loss of menstrual periods (amenorrhea) or infrequent menstrual periods (oligomenorrhea), are more common among competitive female athletes compared to non-athletes (Cann et al., 1984; Dalsky, 1990; Drinkwater et al., 1984; Lindberg et al., 1984; Loucks, 1990). The highest incidence is among long-distance runners, with up to 44% reporting menstrual cycle irregularities (Loucks, 1990), but they occur in other athletic groups as well (e.g., gymnasts, cyclists, swimmers). The hormonal changes which produce menstrual abnormalities, specifically low levels or lack of estrogen and/or progesterone, result in significantly lower bone mineral content (BMC). Selected factors theorized to play a role in BMC reduction as a result of menstrual dysfunction and estrogen and/or progesterone deficiency include: history of menstrual cycle irregularity; training intensity; composition of energy intake (e.g., low protein, calcium); body fat; and stress/anxiety levels (Schwartz et al., 1981). Several cross-sectional studies have shown that amenorrheic and oligomenorrheic athletes have a significantly lower lumbar spine BMC when compared to normally menstruating (eumenorrheic) athletes and non-athletes (Cann et al., 1984; Drinkwater et al., 1984; Lindberg et al., 1984, 1987; Marcus et al., 1985; Prior et al., 1990). The very limited cross-sectional data available on hip BMC among athletes with menstrual cycle irregularities indicates below normal values (Dugowson et al., 1991; Myburgh et al., 1990). The hip is of particular importance due to the incidence of osteoporotic fractures as a result of reduced bone mineral. The reduced bone mass among athletes with menstrual irregularities occurs despite the stimulatory effect of weight-bearing exercise and muscular contractions on BMC (Dalsky, 1990; Marcus et al., 1985; Snow-Harter et al., 1992).

A new concern among competitive athletes is that of changes in luteal phase length. In a recent longitudinal investigation by Prior et al. (1990), women with shortened luteal phases of the menstrual cycle had a significant reduction in lumbar spine BMC. Luteal phase changes occur in the absence of symptoms (i.e., there is no loss of menstrual periods), and are theorized to be more frequent in athletes.

The lower BMC observed among athletes with menstrual irregularities is of concern for two major reasons. First, it may lead to an increased susceptibility to debilitating skeletal injuries (e.g., stress fractures). Second, lower bone mineral content is associated with a greater risk of premature osteoporotic fractures and osteoporosis in

later years. Myburgh et al. (1990) showed that low BMC played a significant role in the etiology of stress fractures in athletes. These women were more likely to sustain a stress fracture if they had low BMC, accompanied by menstrual cycle irregularities and low dietary calcium intake. Other studies have also shown that amenorrheic athletes with low lumbar BMC are more susceptible to stress fractures (Barrow and Saha, 1988; Lindberg et al., 1984; Marcus et al., 1985). Dugowson et al. (1991) reported the first case of a hip stress fracture in an amenorrheic, competitive long-distance runner, as a result of non-traumatic stress on her musculoskeletal system.

To date, there has not been a longitudinal study examining BMC changes in female athletes who are strenuously training for competition. The purpose of this study is to evaluate factors proposed to reduce BMC in competitive collegiate female athletes over a 9-month training period. It is hypothesized that a significant reduction in BMC will occur over this time period in the athletes experiencing the greatest incidence of menstrual cycle irregularities. Results of this study will help identify factors which contribute to bone loss among these women. Two groups of competitive athletes, who train very different energy/muscular systems, but who both experience menstrual irregularities, will be investigated. Athletes will be compared to a non-competitive control group of women.

Methods

Subjects

Subjects volunteering for this study will be involved for a 9-month training period, from September (1992) through June (1993). The subjects recruited to participate in this study will be female collegiate runners and gymnasts. The Oregon State University gymnastics team will include 12 to 15 women, and the University of Oregon track team will include approximately 15 to 20 women. The coaches of both teams are very willing to have their athletes participate in this study, and look forward to gaining knowledge that they may use in the future when coaching their athletes. Subjects for the control group (n=15) will include college-aged females who do not participate in a regular training program (i.e., exercise less than 2 hours per week). They will be recruited from the Corvallis area, and will be matched for ethnicity with the athletes. Subjects will be 18 to 30 years of age, who are healthy, do not smoke or take any medication known to affect bone metabolism (e.g., thyroid or asthma medication), and who are nulliparous (have never given birth). The runners will be women who train seriously for middle-distance and distance events (i.e., events from 800 m to 10,000 m). They will be on a training regimen that includes fall and spring competitive seasons (e.g., cross-country and track racing, respectively), with a winter off-season. They will have been training seriously for at least the previous year, running a minimum of 4 to 5 days a week, at least 30 miles per week. The gymnasts will be women training for Pac-10 and NCAA competition. They will have been training intensively at least one year

prior to enrollment.

This study is restricted to the female gender because the risk of future osteoporosis is much more significant in women than men. Several of the risk factors, i.e., menstrual cycle irregularities and poor protein and calcium nutrition, are specific to women athletes.

Procedures

Prior to the study, all subjects will complete an exercise, health and nutrition questionnaire and provide informed consent. Bone mineral content of the lumbar spine (L2-L4), proximal femur and whole body will be measured at the beginning and end of the study, using the dual-energy X-ray absorptiometer (Hologic, Inc.). The whole body scan will enable determination of lean body mass and percent fat as well. Muscle strength will also be determined pre and post 9-month training period, using a Kin-Com isokinetic machine (Chattecx, Corp.). The muscle groups tested will include: trunk (back muscles), leg (quadriceps), hip (abductors and adductors), and arm (biceps). Once, at the beginning of the 9-month study period, subjects will perform a progressive exercise stress test to maximum on the treadmill, so that maximal oxygen consumption (VO_{2max}) can be determined. Subjects will continue to follow their regular training program throughout the 9-month study period, and will keep a daily activity log from which exercise intensity may be monitored. Control subjects will maintain their current level of physical activity (less than 2 hours per week or regular, structured exercise), current dietary habits and body weight. Copies of each subject's weekly workout schedule will be obtained from the coaching staff. Subjects will complete two psychological questionnaires - the Profile of Mood States (POMS) and the State-Trait Anxiety Inventory (STAI) - on four different occasions: September, December, March and June, which will be used to determine stress/anxiety levels. Each questionnaire takes approximately 5 to 10 minutes to complete. Subjects will monitor their menstrual cycle throughout the 9-month study period, noting any changes in cycle length or menstruation. Subjects will also monitor their body weight and note any fluctuations over time. Subjects will complete a 4-day diet record on the above-mentioned 4 occasions. For each 4-day period, subjects will record their dietary intake for two weekdays and two weekend days, consecutively. From these records, nutrient analyses will determine the total calories consumed, and the amount of carbohydrate, protein, fat, calcium and Vitamin D present in the diet. On two different occasions (November/December and February/March), eumenorrheic subjects will collect daily urine samples (1-2 ml), for one full menstrual cycle. An enzyme immunoassay technique developed by Munro et al. (1991) will be used to analyze the urine samples for levels of urinary progesterone metabolites and thus, provide information on luteal phase length. The investigator will call each subject once a week to discuss any problems and monitor progress.

Benefits and/or Risks to Subjects

The X-ray technique used to assess bone mineral content gives an accurate measure of bone density with a very low exposure to radiation. This radiation dose is considered safe to administer on several occasions to women in this age group (18 to 30 years) provided that the woman is not pregnant. If the subject is having normal menstrual periods, the bone scans will be conducted during the menstrual flow or within one week of onset. The external beam is the only ionizing radiation that the subject will be exposed to. No injections are given and there are no known hazards from radiation at such low levels. The calculated radiation exposure with this procedure per scan is approximately 2-5 millirads for a spine and hip scan and 1.5 millirads for a whole body scan. In comparison, a person can be expected to receive about 160 millirads per year from the environment, and about 40 millirads from a standard 2-position chest X-ray. Therefore, risk from participation in these bone scans is negligible, and there is no discomfort experienced from these procedures.

Muscle strength testing will be done on an isokinetic machine to measure strength in the back, legs, hips and arms. Isokinetic means that the speed at which force is applied is held constant throughout the exercise. Subjects will apply maximal force for 5 trials on each of 5 different exercises, with 30 seconds rest between trials, and approximately 5 minutes between exercises. Maximal effort trials will be preceded by a thorough warm-up for each specific exercise. There is minimal risk of injury from this muscle strength testing procedure, as it will be closely monitored by trained personnel and preceded by a proper warm-up. Testing in this laboratory with over 350 individuals, aged 18 to 80 years, has resulted in no problems with this protocol.

There is always the possibility of injury related to physical activity, especially an intensive 9-month training program which these competitive athletes are engaged in. This can be minimized, however, with good coaching, proper warm-up and cool-down, and careful monitoring of training with a daily training log.

The benefits of participation for the subjects include contributing to the scientific study of the etiology of athletic amenorrhea, and the effects of training and menstrual cycle status on changes on BMC. Knowledge of the relationships between menstrual cycle function, bone mineral content, muscle strength, stress/anxiety levels, calcium and protein nutrition, and exercise intensity over a 9-month training season will allow the athlete and coach to enhance the performance and health aspects of training. The subject will gain knowledge concerning her bone mineral density of the lumbar spine, proximal femur (hip) and whole body, her muscle strength of the back, hips, legs and arms, her menstrual status, her exercise intensity over various training seasons, her nutrition habits, and her psychological stress/anxiety levels over time. The whole body scan will also give her information about her body composition, including percent body fat and lean body mass. This evaluation will be offered at no charge to the subject for participating in the study, whereas normally, it would cost approximately $300.

Informed Consent

Refer to the attached copy of the subject's informed consent form. All subjects will receive an oral explanation of the significance of the study and the study procedures before signing the informed consent to participate.

Anonymity of Subjects

Subject anonymity will be maintained at all times. At no time will the subject's name appear on record forms or in computer files in reference to this study. A code number will be used to identify each subject's data and all records will be kept using this code number. Only the researchers will have knowledge of the subject's name. Confidentiality will be assured. When the results of this study are published in the scientific literature, only group data will be presented and no data will reveal any subject's identity. After completion of the study, the code numbers will be destroyed so that anonymity is assured.

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OREGON STATE UNIVERSITY BONE RESEARCH LABORATORY

INFORMED CONSENT

Bone Mineral and Menstrual Cycle Status in Competitive Female Athletes: A Longitudinal Study

It has been explained to me that the purpose of this study is to evaluate some of the factors proposed to alter bone mineral content (BMC) over a 9-month training period in competitive collegiate female athletes (runners and gymnasts) and non-competitive female controls. The conditions of "athletic amenorrhea" and oligomenorrhea describe the loss or infrequency of menstrual periods, respectively, among competitive female athletes. Proposed causes for this include: past history of menstrual irregularity, the stress of intensive training, low body fat, and inadequate nutrient intake. Female athletes may also experience changes in menstrual cycle phases without the loss of menstrual flow. These shortened luteal phases (luteal phase of cycle less than 10 days, from ovulation to onset of menses) or anovulatory cycles are proposed to reduce BMC due to the associated lowered hormone levels. Menstrual cycle irregularities may lead to significant losses of BMC which can increase susceptibility to stress fractures and result in risk of early osteoporotic fractures. Improved muscle strength in athletes, on the other hand, may counter the effects of menstrual cycle irregularity, enhancing BMC. The balance among these factors is important, and has not been studied across time. Results of this study will help to identify factors which make the most important contribution to BMC in female athletes over time.

I have been invited by Dr. Christine Snow-Harter (Principal Investigator) and Tracey L. Robinson (Student Investigator) to participate in this evaluation of factors affecting bone mineral content of my spine, hip and whole body. I have been selected because I am healthy, not pregnant and have no history of medical conditions that would affect my skeleton. It has been explained to me that this study will involve a 9-month period, from September (1992) through June (1993). I will be asked to participate in the following: (1) Bone mineral evaluation of the lumbar spine, L2-L4 (lower back), proximal femur (top of the thigh bone) and whole body will be performed at the beginning and end of the study. Information on my body composition (percent lean and fat tissue) will be derived from data collected during the whole body scan; (2) Muscle strength of the back, hips (abductors and adductors), legs (quadriceps) and arms (biceps) will be determined in September and June; (3) A progressive exercise stress test on the treadmill to determine my maximal aerobic capacity (VO_{2max}); (4) I will keep a daily exercise and activity log from which exercise intensity and volume will be assessed; (5) I will complete two psychological questionnaires - the Profile of Mood States (POMS) and the State-Trait Anxiety Inventory (STAI) - on four different occasions: September, December, March and June, which will be used to determine stress/anxiety levels. Each

questionnaire takes approximately 5 to 10 minutes to complete; (6) I will keep 4-day diet records on these same 4 occasions (two weekdays and two weekend days, consecutively), and nutrient analyses will determine the total caloric intake, amount of carbohydrate, protein, fat, calcium and Vitamin D present in the diet; (7) I will keep records of my menstrual cycle and weight throughout the 9-month study period, so changes can be noted; (8) If I am menstruating, I will be asked to collect a small urine sample (3-5 ml) upon rising in the morning, for each day of 2 complete menstrual cycles (once in November/December and once in February/March). I will keep these samples in the freezer until the investigator collects them, for analysis of progesterone metabolites. The investigator will call me once a week to discuss any problems and monitor progress. Prior to beginning the study, I will complete an exercise, health and nutrition questionnaire. This may take 30 to 45 minutes to complete.

I understand that I will be tested for bone mineral content on two different occasions, separated by a 9-month period. I will have the testing conducted during my menstrual flow or within 7 days of onset, unless I am amenorrheic or on birth control pills, in which case I can be tested anytime. I have been informed that if I am pregnant or plan to become pregnant during this 9-month period, I should not participate in this study. Further, if I become pregnant or begin taking birth control pills, I will be asked to inform the investigators immediately. I understand that if I sustain an injury (specifically, a stress fracture), I may be asked to undergo a scan on the injured area to determine its BMC.

I have been informed that the scan requires that I lie quietly on a table for 8 minutes for the spine and hip evaluations and 15 minutes for whole body mineral determination.

This technique used to assess bone mineral content gives an accurate measure of bone density with a very low exposure to radiation. It has been explained that this radiation dose is considered safe to administer on several occasions to women in my age group provided that the women are not pregnant. The external beam is the only ionizing radiation to which I will be exposed. No injections are given and there are no known hazards from radiation at such a low level. The calculated radiation exposure with this procedure per scan is approximately one-tenth of a standard chest X-ray or the equivalent of the background radiation one would receive flying across country. Therefore, risk from participation in this study is negligible. I further understand that I will experience no discomfort from the procedures.

I understand that I will keep record of my menstrual cycles and body weight throughout the 9-month study period. I will notify the investigators of any changes in my menstrual cycle (e.g., skipped cycle, shortened length of cycle, resumed menstruation). On two different occasions, November/December and February/March, I will collect urine samples (1-2 ml), upon rising in the morning, in containers supplied by the investigator, for a complete menstrual cycle. If I am amenorrheic or on estrogen

replacement therapy, I will not be asked to do this. I will freeze these samples immediately. The investigator will retrieve them for subsequent analysis of urinary progesterone metabolites. I understand that this is a simple procedure, which compares well to blood hormone assays, but with negligible risk or discomfort.

I understand that I will be muscle strength tested on two different occasions, separated by a 9-month period. An isokinetic machine will measure the strength in my back, hips, legs and arms. This testing will require that I push against a lever arm, designed to move at a constant speed throughout the exercise, with my legs, arms or back, with as much force as I can. The effort that I make to move the equipment will reflect the strength of the muscles that are being tested. I understand that I will receive a thorough warm-up before each muscle group is tested, including stretches recommended by the investigators. Each exercise will consist of 5 trials of maximal effort, with 30 seconds rest in between trials. Each exercise will be followed by a 5-minute rest period.

I understand that at the beginning of the study, I will perform a progressive exercise stress test on a motorized treadmill to determine my VO_{2max}. The test will begin at a slow (walking) speed and gradually the speed and treadmill elevation will increase until I become too fatigued to continue. The test will take approximately 15 to 20 minutes, with only the final few minutes being at a high intensity. During the test, I will breathe room air through a mouthpiece so that the amount of oxygen I am using can be determined. My heart rate will be checked continuously using a heart rate monitor.

I understand that the VO_{2max} test will be administered by trained laboratory personnel who are certified in CPR. Even so, there is a remote risk of death associated with the test of maximal aerobic capacity. In large, varied populations, this risk is one death per 20,000 tests. Since I am from a very low risk segment of the population (young and healthy) and will be screened to exclude individuals with known symptoms of heart disease, the risk is considerably less. Furthermore, the trained personnel administering the test will be continually monitoring for signs of exercise intolerance.

I understand that although the possibility of injury from the strength testing does exist, it is very slight since the testing will be closely monitored by trained personnel and preceded by a thorough warm-up, including stretching. Previous experience in testing over 300 individuals has resulted in no injury or soreness.

I understand that there is the possibility of injury related to my intensive 9-month training program, but this will be minimized with good coaching, proper warm-up and cool-down, and careful monitoring of training using a daily log.

I understand that if I am a control subject, I will maintain my current body weight, diet and activity level throughout the 9-month study period.

I understand that I will complete two psychological questionnaires, which will be used to assess my stress/anxiety levels, on 4 different occasions (i.e., in September, December, March and June). I will be completely honest in answering these questionnaires, as the investigators have explained to me the importance of this, and have assured me of complete confidentiality. It will take approximately 20 minutes to complete these questionnaires.

I understand that I will monitor my food intake on the same 4 occasions as above, using a 4-day diet record. I will do this by writing down everything I eat and drink for 4 consecutive days each time (i.e., 2 weekdays and 2 weekend days). I will be as honest and accurate as possible in keeping these records, after receiving instructions from the investigators and seeing food models. My dietary intake will be analyzed for total calories, carbohydrate, protein, fat, calcium and Vitamin D, using a computerized nutrient analysis program.

I understand that Oregon State University does not provide a research subject with compensation or medical treatment in the event a subject is injured as a result of participation in the research project. If I have any questions about the research or my rights, I understand that Dr. Christine Snow-Harter (Principal Investigator) at 737-6788 and Tracey L. Robinson (Student Investigator) at 737-6785 will be happy to answer them.

The benefits of my participation include contributing to the scientific study of some of the factors affecting bone mineral density: menstrual cycle status, muscle strength, nutrition, stress/anxiety levels and exercise intensity over a 9-month training season. I understand that I will gain knowledge concerning my bone mineral density, muscle strength, body composition, maximal aerobic capacity, menstrual cycle function, nutritional status, exercise intensity over various training seasons, and psychological stress/anxiety levels over time. I understand that I will receive a hard copy of my bone scan results for myself and my physician. Information on my bone mineral content will be valuable to me, to my doctor, and to my coach. Further, this evaluation is offered at no charge. The average cost of bone density assessment is $250-$300 and a body composition analysis is $20. I have been informed that this evaluation is not diagnostic and that any questions regarding my bone mineral density report should be directed to my physician.

I understand that the investigators may benefit from information contained in my records of previous physical exams as a member of Oregon State University's gymnastics team or University of Oregon's track team; e.g., menstrual history, injury history. I understand that this information will be accessed with complete confidentiality. Thus, I agree to release such medical records to the investigators upon their request.

I understand that my confidentiality will be maintained at all times. At no time will my name appear on record forms or in computer files in reference to the study. A

code number will be used to identify my data and all records shall be kept using the code number. Only the researchers will have knowledge of my name. I have been informed that the results of this study may be published in scientific literature and that any data that may be published is such a journal will not reveal my identity. The code number will be destroyed once the study is over and the investigators are finished with my data, so that my anonymity is assured.

I have been completely informed and understand the nature and purpose of this research. The researchers have offered to answer any further questions that I may have. I understand that my participation in this study is completely voluntary and I may withdraw from the study at any time without prejudice or loss of the benefits to which my participation entitles me. Questions about the research or any aspect of my participation should be directed to Dr. Snow-Harter at 737-6788 or to Tracey L. Robinson at 737-6785. I have read the foregoing and agree to participate.

Subject's Signature _____ Date _____

Address _____

_____

Investigator's Signature _____ Date _____

APPENDIX B

EXERCISE, HEALTH AND NUTRITION QUESTIONNAIRE

OREGON STATE UNIVERSITY BONE RESEARCH LABORATORY

Bone Mineral and Menstrual Cycle Status in Competitive Female Athletes: A Longitudinal Study

Exercise, Health & Nutrition Questionnaire

Code #: _____

Name: _____ Date: _____

Address: _____

_____

Telephone: (Home): _____ (Work): _____

Occupation and/or sports team: _____

Code #: _____

Date of Birth: _____ Age: _____

Height: _____

Weight: _____

Which best describes your ethnic identity?
____ Caucasian
____ African American
____ Asian American
____ Hispanic American
____ American Indian/Alaskan Native
____ other (please specify) _____

PAST HISTORY

Have you ever had?

Yes____No____ High cholesterol
Yes____No____ Heart murmur
Yes____No____ Heart trouble
Yes____No____ High blood pressure
Yes____No____ Lung disease
Yes____No____ Operations
Yes____No____ Back injury

If yes to any of the above, please explain _____
_____

FAMILY HISTORY

Is there a family history of any of the following conditions? If yes, please explain relationship.

Yes____No____ Diabetes
Yes____No____ Heart attacks
Yes____No____ High blood pressure
Yes____No____ High cholesterol
Yes____No____ Congenital heart disease
Yes____No____ Heart operations
Yes____No____ Osteoporosis
Yes____No____ Other _____

PRESENT SYMPTOMS REVIEW

Date of last medical/physical exam? _____

Physician? _____

Have you **recently** had any of the following? If so, please indicate the date(s).

Yes____No____ Chest pain Date(s)_____
Yes____No____ Shortness of breath Date(s)_____
Yes____No____ Heart palpitations Date(s)_____
Yes____No____ Cough on exertion Date(s)_____
Yes____No____ Coughing blood Date(s)_____
Yes____No____ Back pain Date(s)_____
Yes____No____ Painful, stiff or swollen joints Date(s)_____
Yes____No____ Other (please specify) _____

Please list your present medications (over-the-counter and prescription) and dosages here:
_____
_____
_____

Alcohol consumption

Do you drink alcohol regularly? Yes _____
No _____

If yes, how many drinks/week? _____

Smoking

Do you or did you ever smoke cigarettes? Yes _____
No _____

If yes, at what ages? from age ____ to age ____ number of cigarettes/day ____

Body Weight

What was your weight 1 month ago? _____
What was your weight 6 months ago? _____

OSTEOPOROSIS RISK FACTORS

Have you or your immediate family* had:

Yes____No____ rheumatoid arthritis
Yes____No____ osteoporosis
Yes____No____ an overactive thyroid gland
Yes____No____ an overactive parathyroid gland
Yes____No____ alcoholism
Yes____No____ chronic liver disease
Yes____No____ multiple myeloma
Yes____No____ the blood tumor, leukemia
Yes____No____ stomach ulcers
Yes____No____ lactase deficiency (inability to digest milk)
Yes____No____ anorexia nervosa or bulimia

*includes mother, father and/or siblings

For the following medications, please check whether you have taken them in the past, currently take them, or have never taken the drug:

past____current____never____ cortisone or similar drugs
past____current____never____ anabolic steroids
past____current____never____ thyroid hormone pills
past____current____never____ asthma medication
past____current____never____ Maalox or Mylanta antacids (**frequently**)
past____current____never____ lithium (**for over one year**)
past____current____never____ Tums/Rolaids

Please check yes or no for the following statements:

Yes____No____ I avoid milk and other dairy products
Yes____No____ I usually eat meat at least twice a day
Yes____No____ I drink more than 2 cups of coffee or tea daily (on average)
Yes____No____ I drink 2 or more cola soft drinks daily (on average)
Yes____No____ I have at least one alcoholic drink daily
Yes____No____ I follow a vegetarian diet

Please check yes or no for the following statements:

Yes____No____ I lost my period for a year or more before it came back
Yes____No____ I have had irregular (skipped) menstrual periods
Yes____No____ My menstrual period did not begin until after age 16
Yes____No____ I have a medical history of endometriosis
Yes____No____ I lost my period when exercising heavily

MENSTRUAL HISTORY

At what age did menarche occur? _____

If you lose you menstrual period when training, when does this occur? (e.g., all the time or only when competing?) _____
_____

Can you associate this change with any specific factor? (e.g., increased training intensity/duration?)_____
_____
_____

Do you currently take or have you ever taken birth control pills? Yes ____
No ____

If yes, please specify at what ages: from age _____ to age _____

Brand name and dosage: _____

Do you currently take or have you ever taken estrogens for hormone replacement therapy? Yes ____ No____

If yes, please specify when (ie., at what ages) and for how long?_____

Brand name and dosage: _____

Please state the number of menstrual periods you had each year at each of the following ages:

Age	Number of periods	Birth control pills?	Are you sure or estimating?
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			

27_____
28_____
29_____
30_____

Cola Beverages

How many cola beverages do drink daily?_____

How many years have you been drinking cola beverages on a regular basis?_____

Consumption of calcium-rich foods

How many 8 oz glasses of milk do you drink per day? _____ per week? _____
How many servings of cheese (1 oz) do you eat per day? _____ per week? _____
How many servings of yogurt (1 cup) do you eat per week? _____

Do you follow a vegetarian diet? Yes _____
No _____
If yes, how many years have you been a vegetarian? _____
Are you a strict vegetarian? _____ lacto-ovo? _____ other (explain)_____
_____

Nutritional Supplements

Do you take any vitamin and/or mineral supplements? Yes _____
No _____

Do you take any nutritional supplements (e.g., protein, carbohydrate)? Yes _____
No _____

If yes to either of the above 2 questions, please specify below:

Product name & brand	Dosage	# pills/day	years taken	at what ages?

History of Calcium Intake

For each life stage listed below, please indicate how often you included the following calcium-rich foods in your diet, using the following scale:

1 = at least 3 times/day
2 = at least once daily
3 = a few times a week
4 = less than once/week
5 = once or twice a month
6 = never or hardly ever

Your childhood (through age 12):

____ one glass (8 oz) of milk
____ one cup of yogurt/frozen yogurt
____ a serving (1 oz) of cheese
____ one cup of spinach
____ one cup of broccoli
____ a serving (3 oz) of salmon
____ one cup of cottage cheese
____ one medium orange
____ one cup of ice cream

Your teens (ages 13 to 19):

____ one glass (8 oz) of milk
____ one cup of yogurt/frozen yogurt
____ a serving (1 oz) of cheese
____ one cup of spinach
____ one cup of broccoli
____ a serving (3 oz) of salmon
____ one cup of cottage cheese
____ one medium orange
____ one cup of ice cream

Your early adulthood (ages 20 to 39):

____ one glass (8 oz) of milk
____ one cup of yogurt/frozen yogurt
____ a serving (1 oz) of cheese
____ one cup of spinach
____ one cup of broccoli
____ a serving (3 oz) of salmon
____ one cup of cottage cheese
____ one medium orange
____ one cup of ice cream

Past two years specifically:

____ one glass (8 oz) of milk
____ one cup of yogurt/frozen yogurt
____ a serving (1 oz) of cheese
____ one cup of spinach
____ one cup of broccoli
____ a serving (3 oz) of salmon
____ one cup of cottage cheese
____ one medium orange
____ one cup of ice cream

TRAINING PROGRAM

Runners

Predominant Event(s) (e.g., 800 m, 5 km): _____

What is your best racing time for these events? _____

Average weekly training mileage - summer? _____
fall? _____
winter? _____
spring? _____

Number of years of specific training? _____

Gymnasts

Predominant Events: _____

What are your best scores/performances in these events? _____

Average hours of training - summer? _____
fall? _____
winter? _____
spring? _____

Number of years of specific training? _____

Runners and Gymnasts

Supplemental training activities (e.g., weight training-free weights, machine; cycling):

Type: _____

Specific exercises for weight training (please list weight, repetitions, sets, frequency):
_____
_____
_____

Hours per week for each activity - summer? _____
fall? _____
winter? _____
spring? _____

Number of years for each supplemental activity? _____
_____
_____

Total average training time per week - summer? _____
fall? _____
winter? _____
spring? _____

PAST INJURIES

Circle any of the following musculoskeletal injuries you have had:

Fractures, Stress fractures, Sprains, Strains, Shin splints, Muscle pulls, Others (explain below)

For each of these injuries, indicate their location, frequency of occurrence, date, and length of time spent in rehabilitation:

APPENDIX C

ACTIVITY LOG

ACTIVITY LOG

Name _____
Week of _____
Phase/Training Season _____

* please include a description of your workout/run, the intensity of the workout (pace/mile), your average heart rate (if taken), your mileage, your body weight for that day, and any general comments about the workout

---

Sunday Month _____ Year _____

Monday Month _____ Year _____

Tuesday Month _____ Year _____

Wednesday Month _____ Year _____

Thursday Month _____ Year _____

Friday Month _____ Year _____

Saturday Month _____ Year _____

Total weekly mileage _____

APPENDIX D

SELF-EVALUATION (STAI) AND POMS QUESTIONNAIRES

SELF-EVALUATION QUESTIONNAIRE

Developed by Charles D. Spielberger
in collaboration with
R. L. Gorsuch, R. Lushene, P. R. Vagg, and G. A. Jacobs

STAI Form Y-1

Name _____ Date _____ S ___

Age _____ Sex: M ___ F ___ T ___

DIRECTIONS: A number of statements which people have used to describe themselves are given below. Read each statement and then blacken in the appropriate circle to the right of the statement to indicate how you feel *right* now, that is, *at this moment*. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	NOT AT ALL	SOMEWHAT	MODERATELY SO	VERY MUCH SO
1. I feel calm	①	②	③	④
2. I feel secure	①	②	③	④
3. I am tense	①	②	③	④
4. I feel strained	①	②	③	④
5. I feel at ease	①	②	③	④
6. I feel upset	①	②	③	④
7. I am presently worrying over possible misfortunes	①	②	③	④
8. I feel satisfied	①	②	③	④
9. I feel frightened	①	②	③	④
10. I feel comfortable	①	②	③	④
11. I feel self-confident	①	②	③	④
12. I feel nervous	①	②	③	④
13. I am jittery	①	②	③	④
14. I feel indecisive	①	②	③	④
15. I am relaxed	①	②	③	④
16. I feel content	①	②	③	④
17. I am worried	①	②	③	④
18. I feel confused	①	②	③	④
19. I feel steady	①	②	③	④
20. I feel pleasant	①	②	③	④

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SELF-EVALUATION QUESTIONNAIRE

STAI Form Y-2

Name _____ Date _____

DIRECTIONS: A number of statements which people have used to describe themselves are given below. Read each statement and then blacken in the appropriate circle to the right of the statement to indicate how you *generally* feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you generally feel.

	ALMOST NEVER	SOMETIMES	OFTEN	ALMOST ALWAYS
21. I feel pleasant	①	②	③	④
22. I feel nervous and restless	①	②	③	④
23. I feel satisfied with myself	①	②	③	④
24. I wish I could be as happy as others seem to be	①	②	③	④
25. I feel like a failure	①	②	③	④
26. I feel rested	①	②	③	④
27. I am "calm, cool, and collected"	①	②	③	④
28. I feel that difficulties are piling up so that I cannot overcome them	①	②	③	④
29. I worry too much over something that really doesn't matter	①	②	③	④
30. I am happy	①	②	③	④
31. I have disturbing thoughts	①	②	③	④
32. I lack self-confidence	①	②	③	④
33. I feel secure	①	②	③	④
34. I make decisions easily	①	②	③	④
35. I feel inadequate	①	②	③	④
36. I am content	①	②	③	④
37. Some unimportant thought runs through my mind and bothers me	①	②	③	④
38. I take disappointments so keenly that I can't put them out of my mind	①	②	③	④
39. I am a steady person	①	②	③	④
40. I get in a state of tension or turmoil as I think over my recent concerns and interests	①	②	③	④

Printed in U.S.A. Mark Reflex® by NCS ...3321

NAME _____ DATE _____

SEX: Male (M) Female (F)

IDENTIFICATION

Below is a list of words that describe feelings people have. Please read each one carefully. Then fill in ONE circle under the answer to the right which best describes HOW YOU HAVE BEEN FEELING DURING THE PAST WEEK INCLUDING TODAY.

The numbers refer to these phrases.

0 = Not at all
1 = A little
2 = Moderately
3 = Quite a bit
4 = Extremely

Col (C) O.P. (O)

	NOT AT ALL	A LITTLE	MODERATELY	QUITE A BIT	EXTREMELY
1. Friendly	0	1	2	3	4
2. Tense	0	1	2	3	4
3. Angry	0	1	2	3	4
4. Worn out	0	1	2	3	4
5. Unhappy	0	1	2	3	4
6. Clear-headed	0	1	2	3	4
7. Lively	0	1	2	3	4
8. Confused	0	1	2	3	4
9. Sorry for things done	0	1	2	3	4
10. Shaky	0	1	2	3	4
11. Listless	0	1	2	3	4
12. Peeved	0	1	2	3	4
13. Considerate	0	1	2	3	4
14. Sad	0	1	2	3	4
15. Active	0	1	2	3	4
16. On edge	0	1	2	3	4
17. Grouchy	0	1	2	3	4
18. Blue	0	1	2	3	4
19. Energetic	0	1	2	3	4
20. Panicky	0	1	2	3	4
21. Hopeless	0	1	2	3	4
22. Relaxed	0	1	2	3	4
23. Unworthy	0	1	2	3	4
24. Spiteful	0	1	2	3	4
25. Sympathetic	0	1	2	3	4
26. Uneasy	0	1	2	3	4
27. Restless	0	1	2	3	4
28. Unable to concentrate	0	1	2	3	4
29. Fatigued	0	1	2	3	4
30. Helpful	0	1	2	3	4
31. Annoyed	0	1	2	3	4
32. Discouraged	0	1	2	3	4
33. Resentful	0	1	2	3	4
34. Nervous	0	1	2	3	4
35. Lonely	0	1	2	3	4
36. Miserable	0	1	2	3	4
37. Muddled	0	1	2	3	4
38. Cheerful	0	1	2	3	4
39. Bitter	0	1	2	3	4
40. Exhausted	0	1	2	3	4
41. Anxious	0	1	2	3	4
42. Ready to fight	0	1	2	3	4
43. Good natured	0	1	2	3	4
44. Gloomy	0	1	2	3	4
45. Desperate	0	1	2	3	4
46. Sluggish	0	1	2	3	4
47. Rebellious	0	1	2	3	4
48. Helpless	0	1	2	3	4
49. Weary	0	1	2	3	4
50. Bewildered	0	1	2	3	4
51. Alert	0	1	2	3	4
52. Deceived	0	1	2	3	4
53. Furious	0	1	2	3	4
54. Efficient	0	1	2	3	4
55. Trusting	0	1	2	3	4
56. Full of pep	0	1	2	3	4
57. Bad-tempered	0	1	2	3	4
58. Worthless	0	1	2	3	4
59. Forgetful	0	1	2	3	4
60. Carefree	0	1	2	3	4
61. Terrified	0	1	2	3	4
62. Guilty	0	1	2	3	4
63. Vigorous	0	1	2	3	4
64. Uncertain about things	0	1	2	3	4
65. Bushed	0	1	2	3	4

MAKE SURE YOU HAVE ANSWERED EVERY ITEM.

POM 021

APPENDIX E

DIET RECORD SHEET

DIET RECORD SHEET

STUDY:

SUBJECT NO.

DAY OF WEEK: DATE / / NAME:

Please leave a blank space between each meal.
Continue diet record on back if necessary.
Use a separate sheet for each day.

NOTES:

Time	Location	Food Item	Brand	Preparation	Amount	Office Use Only	

Any questions call:
Nutrition & Food Mgmt.
737-0977
or
737-3561

Useful abbreviations:
cup.....C
ounce.....oz
teaspoon.....Tsp, Ts
tablespoon.....Tbsp, Tb

Useful Measurements:
1 cup=8 fl.oz.=16 Tbsp
1 lb.=454 gram=16 oz.
3 tsp.=1 Tbsp.=1/16 cup
1 tsp.=approx. 5gr solid

APPENDIX F

ILLUSTRATION OF HOLOGIC QDR-1000/W CALIBRATON WHEEL

Figure #10, Hologic QDR-1000™ Principle of Operation

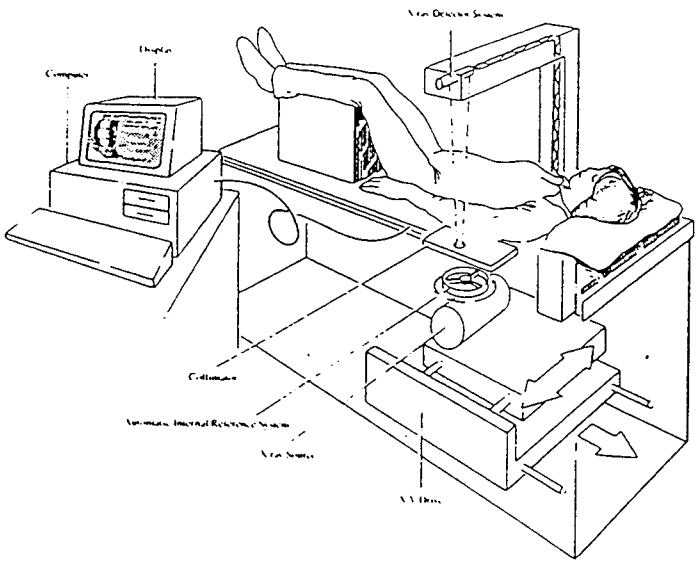
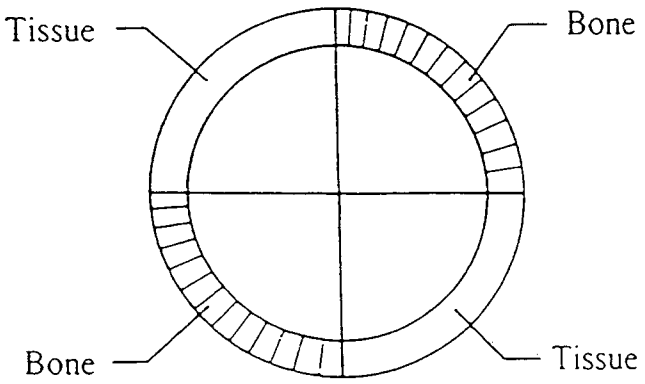


Figure #11, Calibration Wheel

The QDR-1000™ X-ray Bone Densitometer uses a proprietary Automatic Internal Reference System which employs a calibration wheel in order to achieve drift-free measurement.



Calibration Wheel

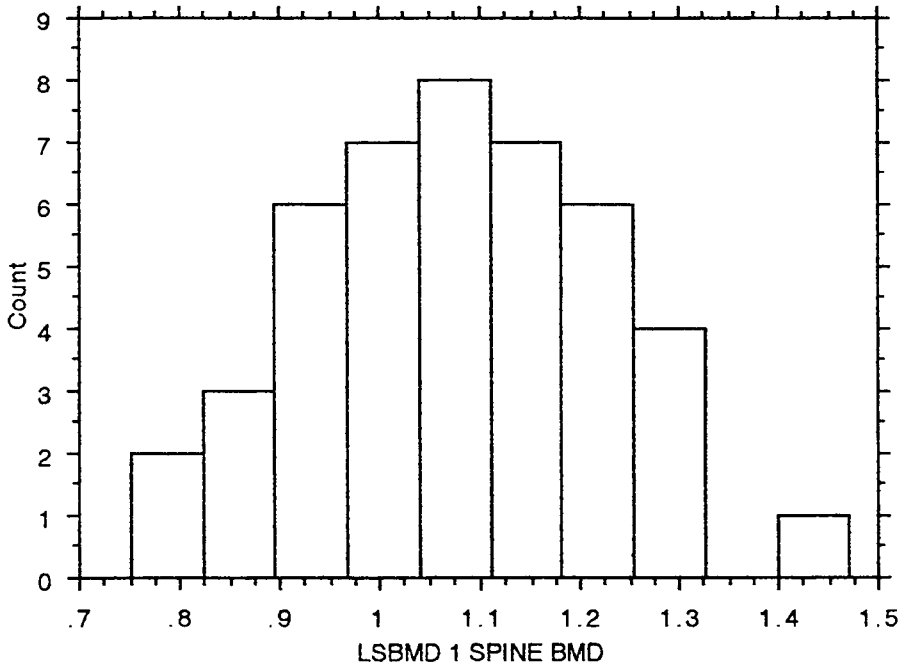
APPENDIX G

FREQUENCY DISTRIBUTIONS FOR ALL DEPENDENT VARIABLES

Frequency Distribution for LSBMD 1 SPINE BMD

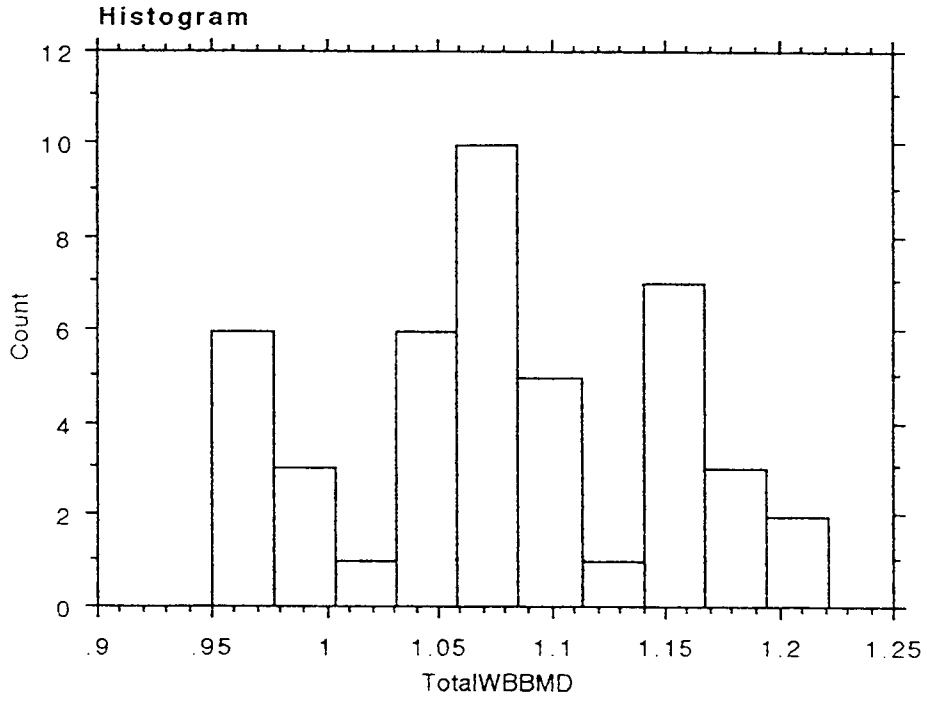
From (≥)	To (<)	Count
.751	.823	2
.823	.895	3
.895	.967	6
.967	1.039	7
1.039	1.111	8
1.111	1.183	7
1.183	1.255	6
1.255	1.327	4
1.327	1.399	0
1.399	1.471	1
	Total	44

Histogram



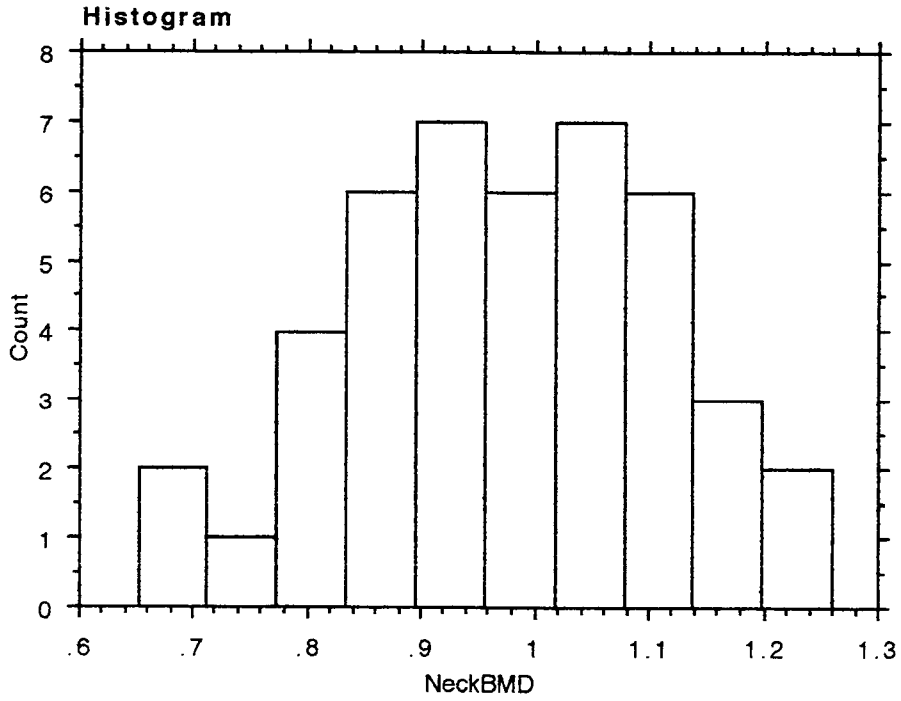
Frequency Distribution for TotalWBBMD

From (≥)	To (<)	Count
.949	.976	6
.976	1.004	3
1.004	1.031	1
1.031	1.058	6
1.058	1.086	10
1.086	1.113	5
1.113	1.140	1
1.140	1.167	7
1.167	1.195	3
1.195	1.222	2
	Total	44



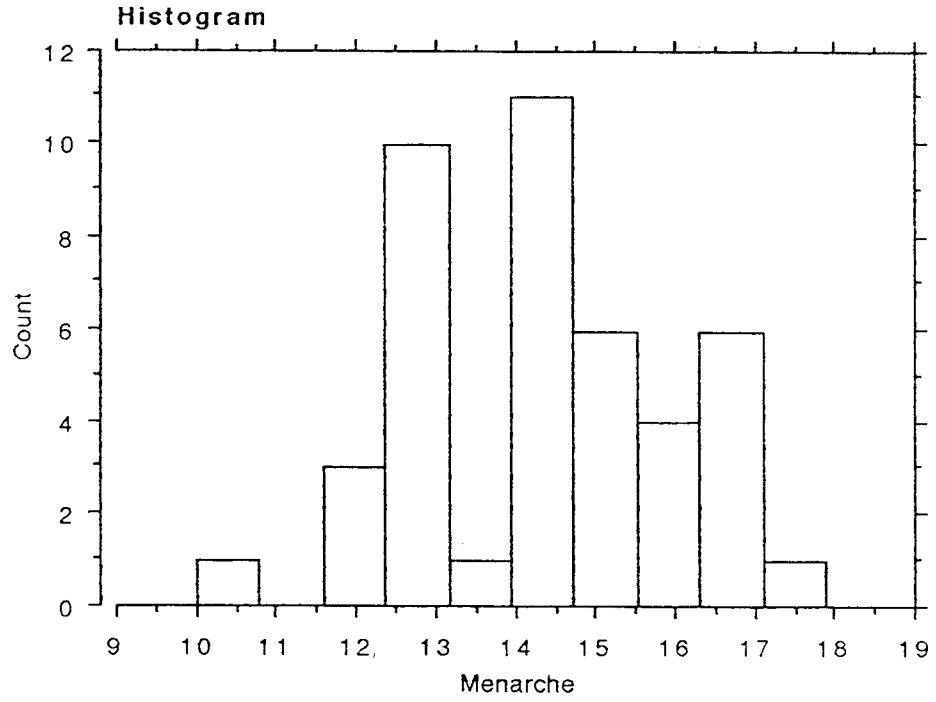
Frequency Distribution for NeckBMD

From (≥)	To (<)	Count
.651	.712	2
.712	.773	1
.773	.834	4
.834	.895	6
.895	.956	7
.956	1.016	6
1.016	1.077	7
1.077	1.138	6
1.138	1.199	3
1.199	1.260	2
	Total	44



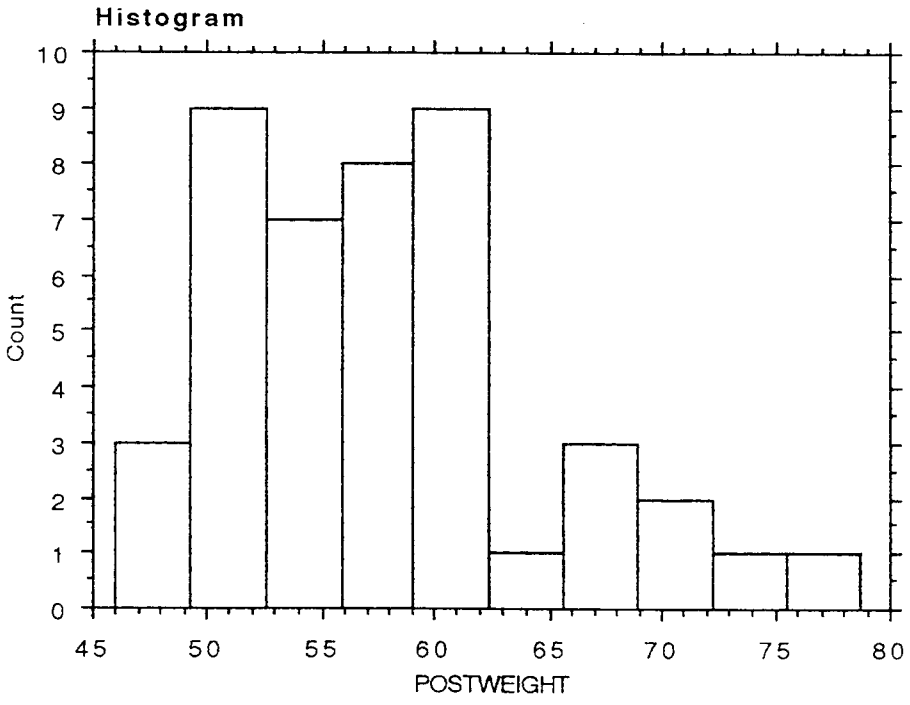
Frequency Distribution for Menarche

From (≥)	To (<)	Count
10.000	10.790	1
10.790	11.580	0
11.580	12.370	3
12.370	13.160	10
13.160	13.950	1
13.950	14.740	11
14.740	15.530	6
15.530	16.320	4
16.320	17.110	6
17.110	17.900	1
	Total	43



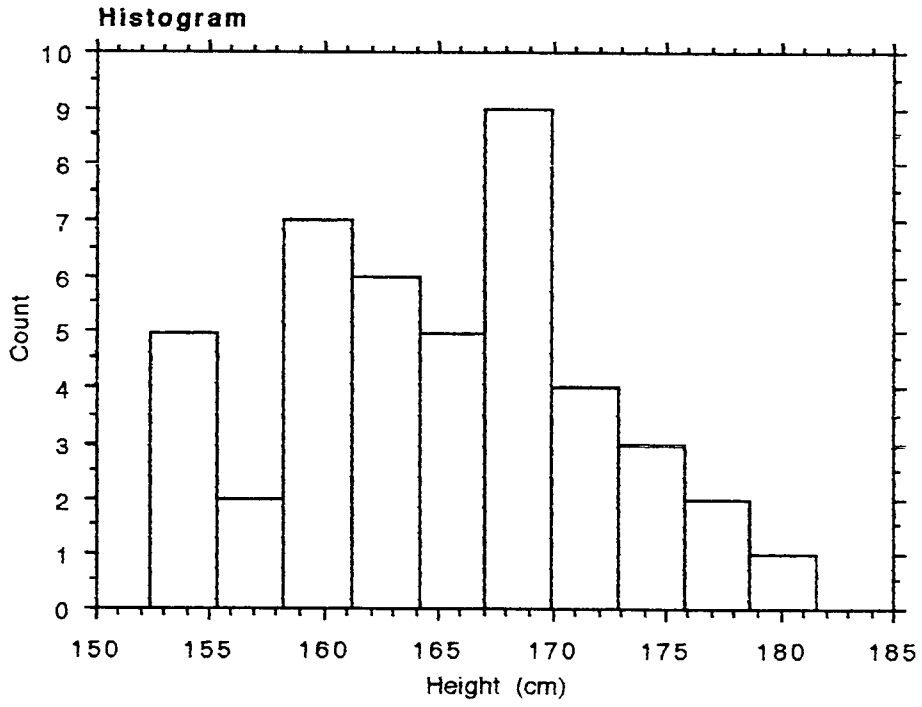
Frequency Distribution for POSTWEIGHT

From (≥)	To (<)	Count
45.954	49.234	3
49.234	52.515	9
52.515	55.795	7
55.795	59.076	8
59.076	62.356	9
62.356	65.636	1
65.636	68.917	3
68.917	72.197	2
72.197	75.478	1
75.478	78.758	1
	Total	44



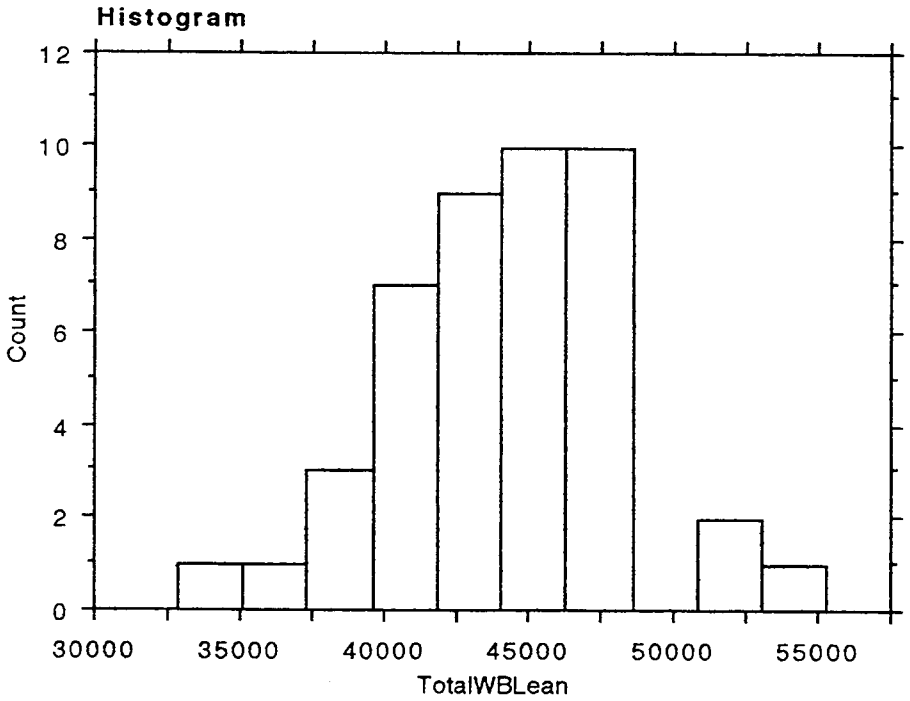
Frequency Distribution for Height (cm)

From (≥)	To (<)	Count
152.400	155.321	5
155.321	158.242	2
158.242	161.163	7
161.163	164.084	6
164.084	167.005	5
167.005	169.926	9
169.926	172.847	4
172.847	175.768	3
175.768	178.689	2
178.689	181.610	1
	Total	44



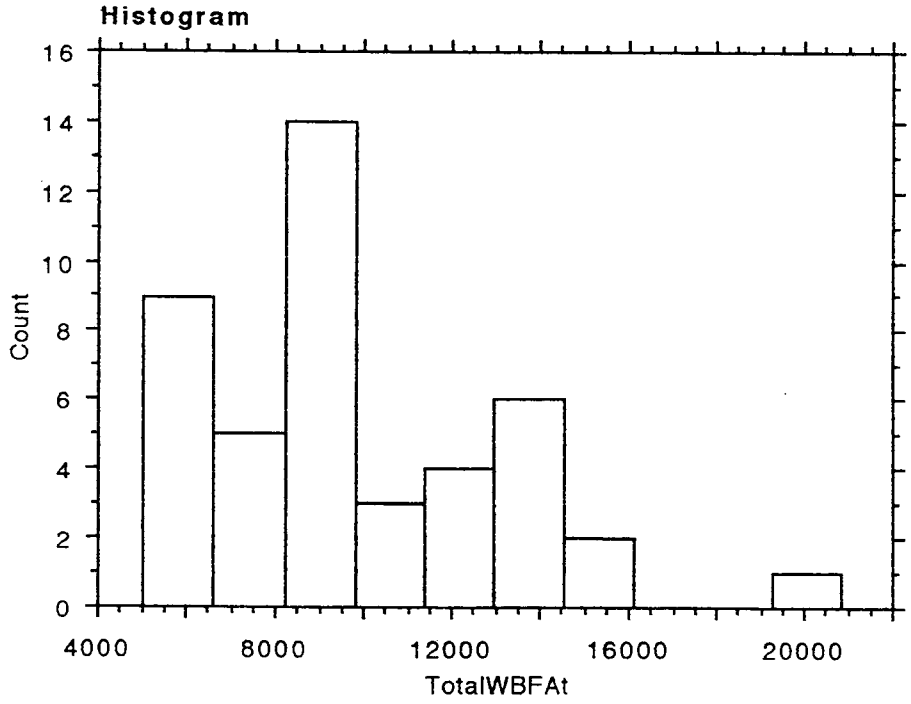
Frequency Distribution for TotalWBLean

From (≥)	To (<)	Count
32858.500	35103.930	1
35103.930	37349.360	1
37349.360	39594.790	3
39594.790	41840.220	7
41840.220	44085.650	9
44085.650	46331.080	10
46331.080	48576.510	10
48576.510	50821.940	0
50821.940	53067.370	2
53067.370	55312.800	1
	Total	44



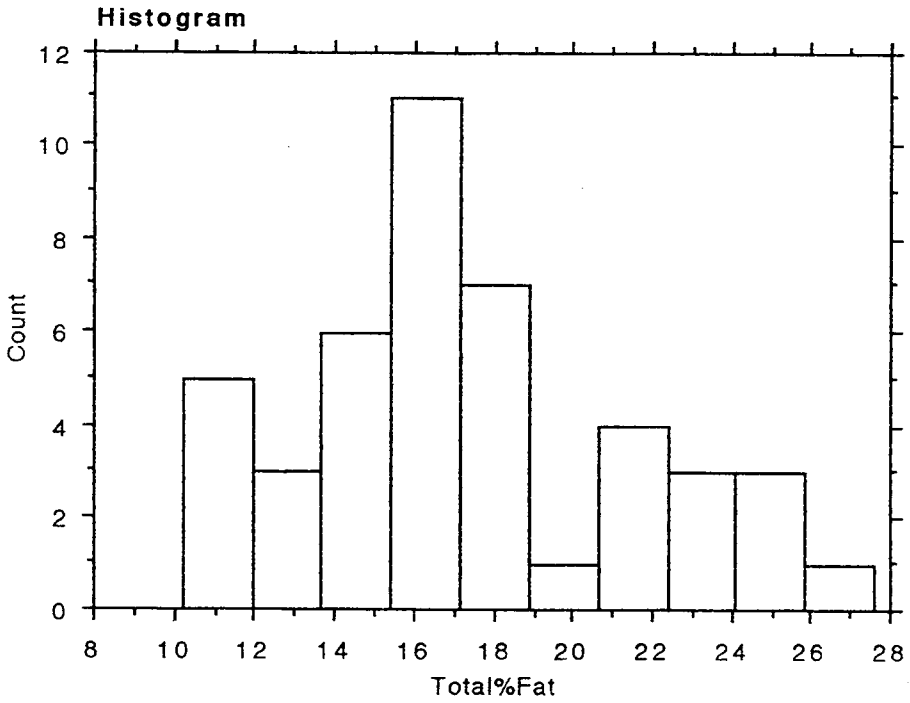
Frequency Distribution for TotalWBFAt

From (≥)	To (<)	Count
5055.900	6634.850	9
6634.850	8213.800	5
8213.800	9792.750	14
9792.750	11371.700	3
11371.700	12950.650	4
12950.650	14529.600	6
14529.600	16108.550	2
16108.550	17687.500	0
17687.500	19266.450	0
19266.450	20845.400	1
	Total	44



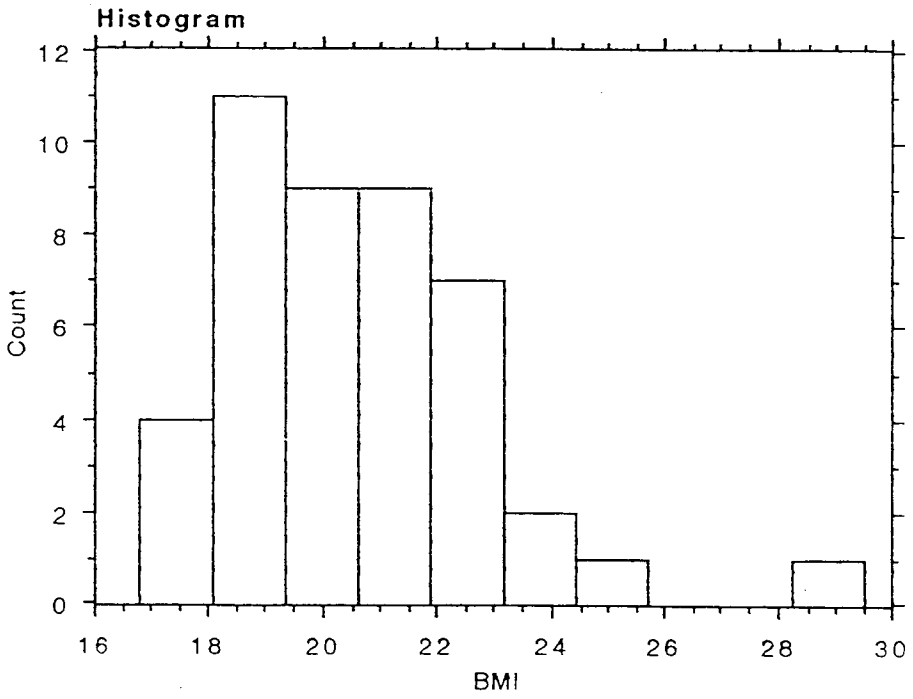
Frequency Distribution for Total%Fat

From (≥)	To (<)	Count
10.200	11.940	5
11.940	13.680	3
13.680	15.420	6
15.420	17.160	11
17.160	18.900	7
18.900	20.640	1
20.640	22.380	4
22.380	24.120	3
24.120	25.860	3
25.860	27.600	1
	Total	44



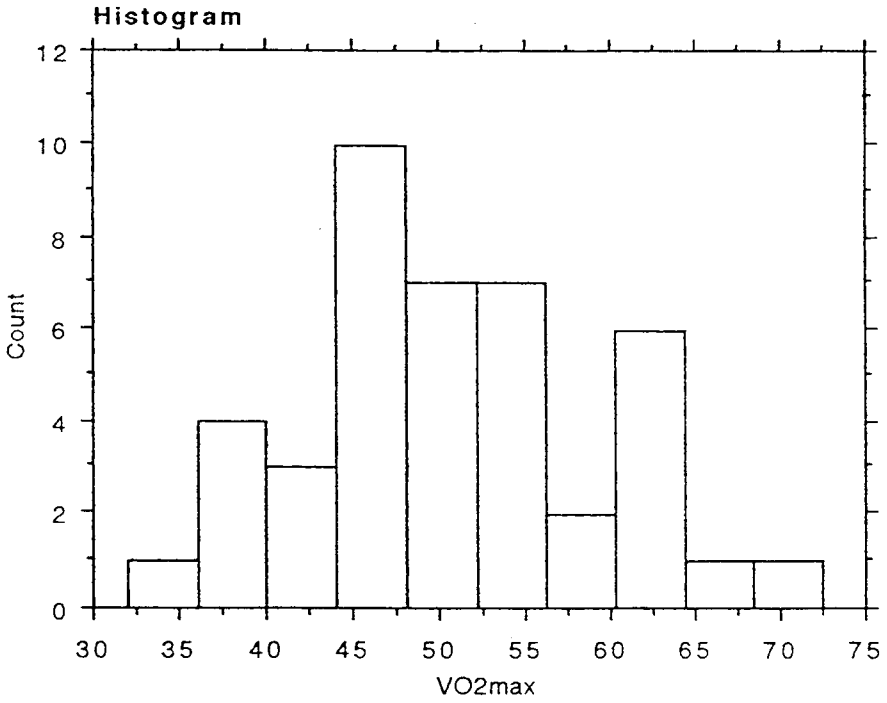
Frequency Distribution for BMI

From (≥)	To (<)	Count
16.800	18.071	4
18.071	19.342	11
19.342	20.613	9
20.613	21.884	9
21.884	23.155	7
23.155	24.426	2
24.426	25.697	1
25.697	26.968	0
26.968	28.239	0
28.239	29.510	1
	Total	44



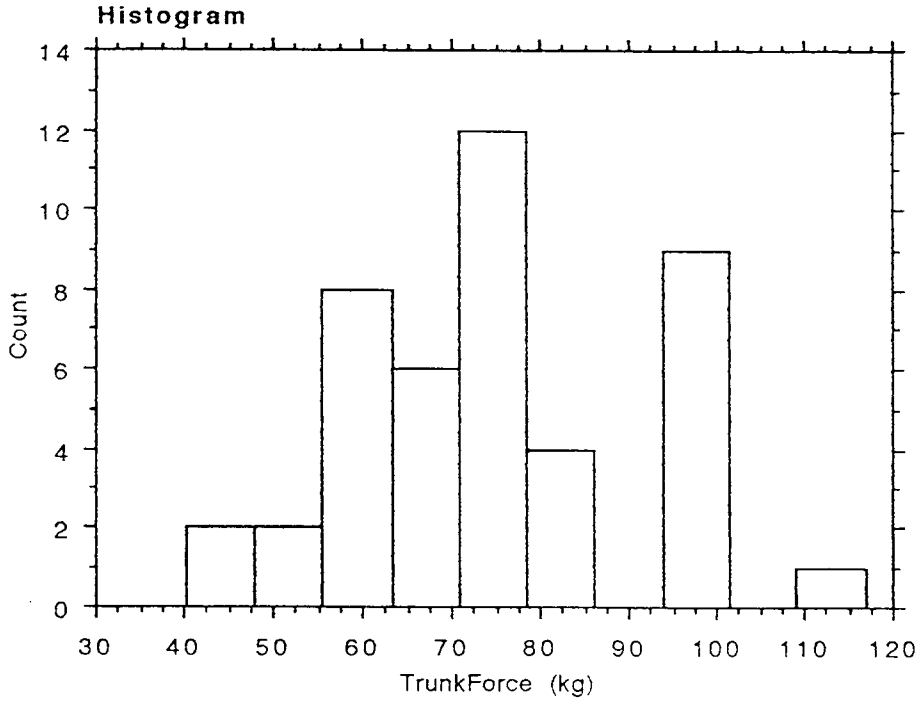
Frequency Distribution for VO2max

From (≥)	To (<)	Count
31.990	36.034	1
36.034	40.078	4
40.078	44.122	3
44.122	48.166	10
48.166	52.210	7
52.210	56.254	7
56.254	60.298	2
60.298	64.342	6
64.342	68.386	1
68.386	72.430	1
	Total	42



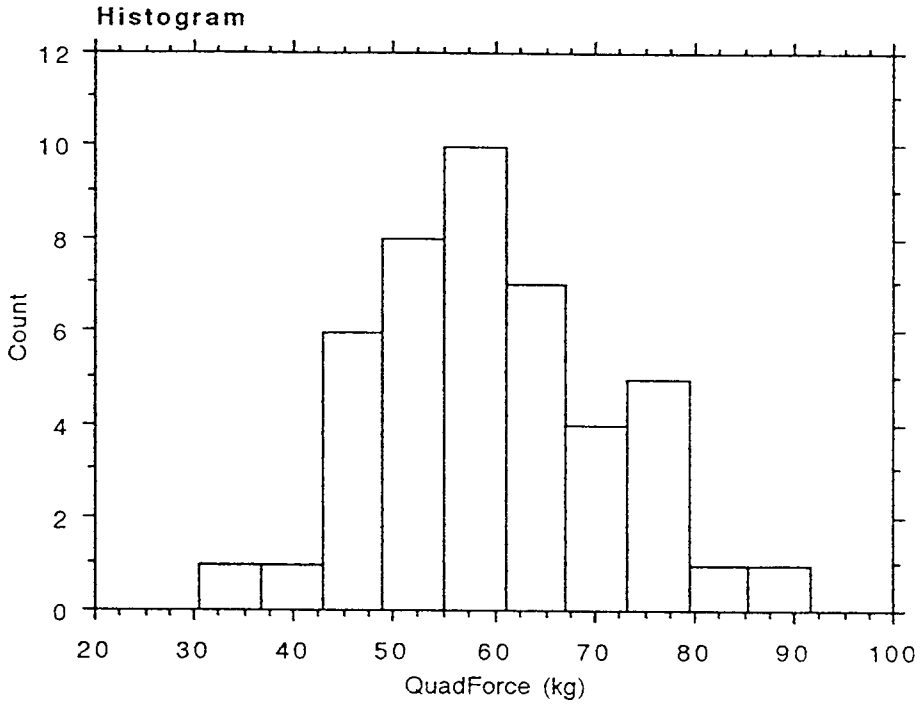
Frequency Distribution for TrunkForce (kg)

From (≥)	To (<)	Count
40.273	47.927	2
47.927	55.582	2
55.582	63.236	8
63.236	70.891	6
70.891	78.545	12
78.545	86.200	4
86.200	93.855	0
93.855	101.509	9
101.509	109.164	0
109.164	116.818	1
	Total	44



Frequency Distribution for QuadForce (kg)

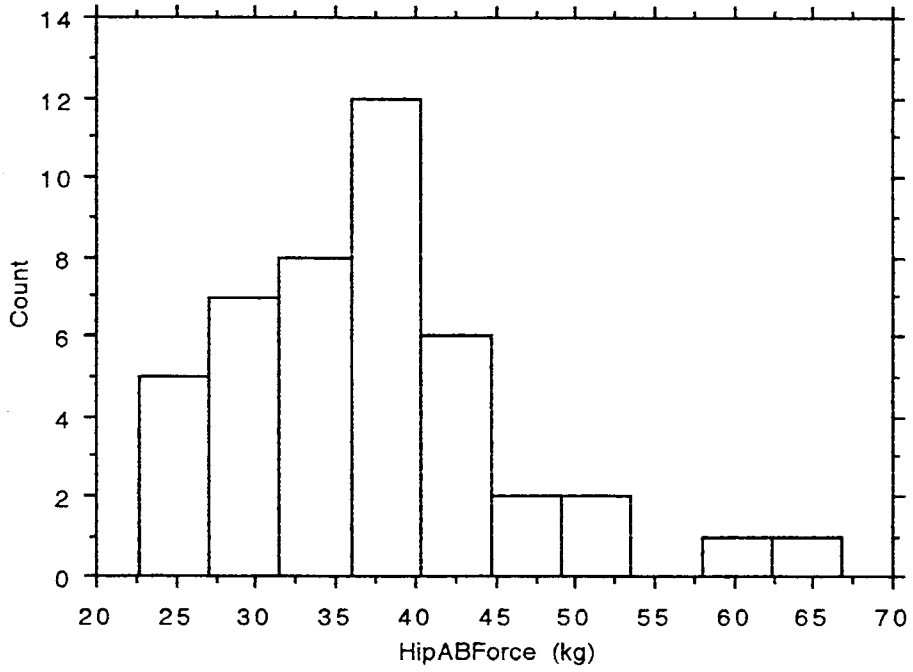
From (≥)	To (<)	Count
30.636	36.741	1
36.741	42.845	1
42.845	48.950	6
48.950	55.055	8
55.055	61.159	10
61.159	67.264	7
67.264	73.368	4
73.368	79.473	5
79.473	85.577	1
85.577	91.682	1
	Total	44



Frequency Distribution for HipABForce (kg)

From (≥)	To (<)	Count
22.682	27.095	5
27.095	31.509	7
31.509	35.923	8
35.923	40.336	12
40.336	44.750	6
44.750	49.164	2
49.164	53.577	2
53.577	57.991	0
57.991	62.405	1
62.405	66.818	1
	Total	44

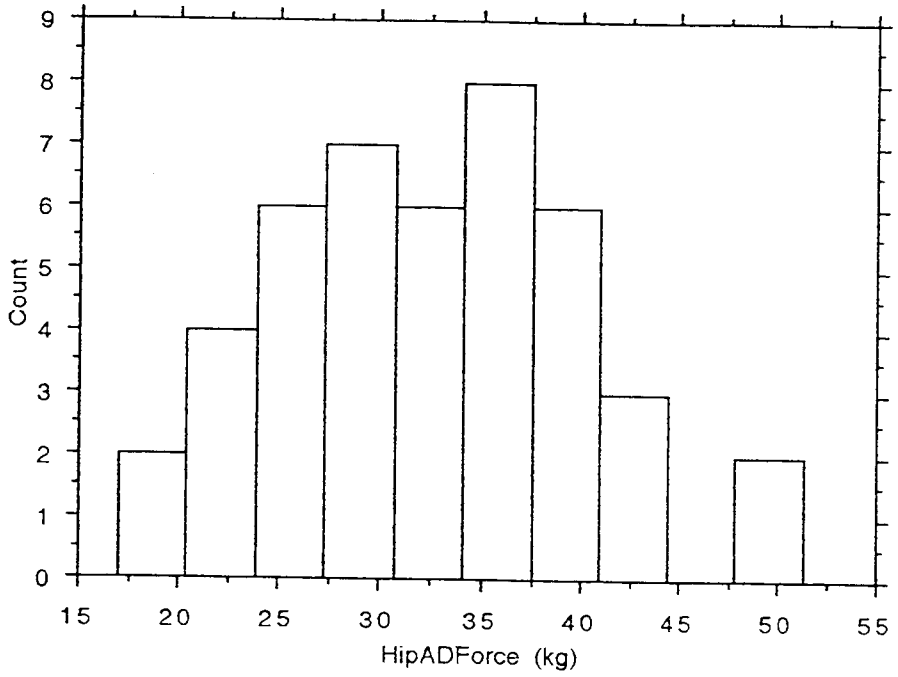
Histogram



Frequency Distribution for HipADForce (kg)

From (≥)	To (<)	Count
16.955	20.391	2
20.391	23.827	4
23.827	27.264	6
27.264	30.700	7
30.700	34.136	6
34.136	37.573	8
37.573	41.009	6
41.009	44.445	3
44.445	47.882	0
47.882	51.318	2
	Total	44

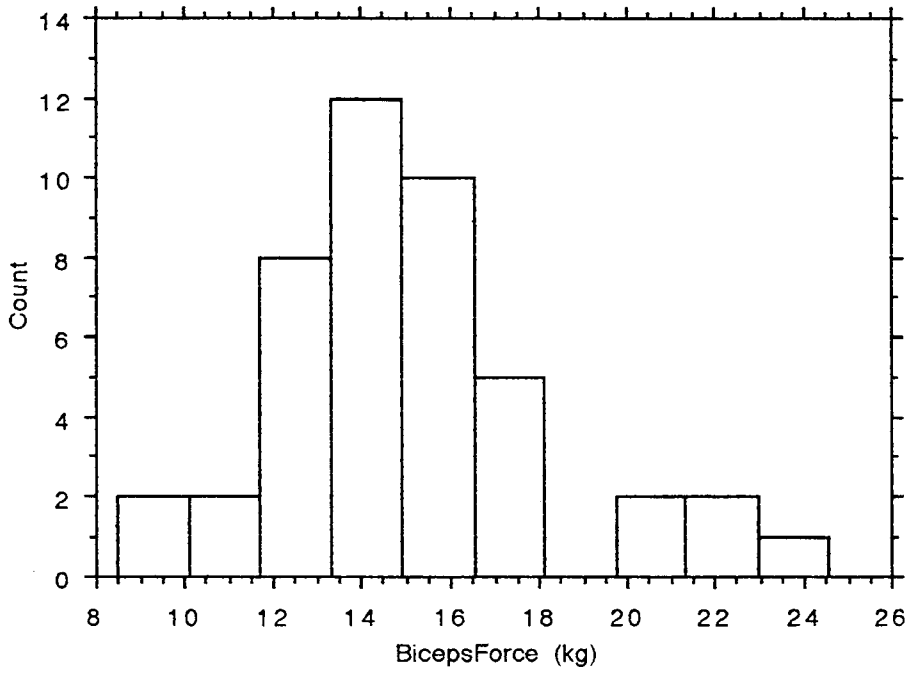
Histogram



Frequency Distribution for BicepsForce (kg)

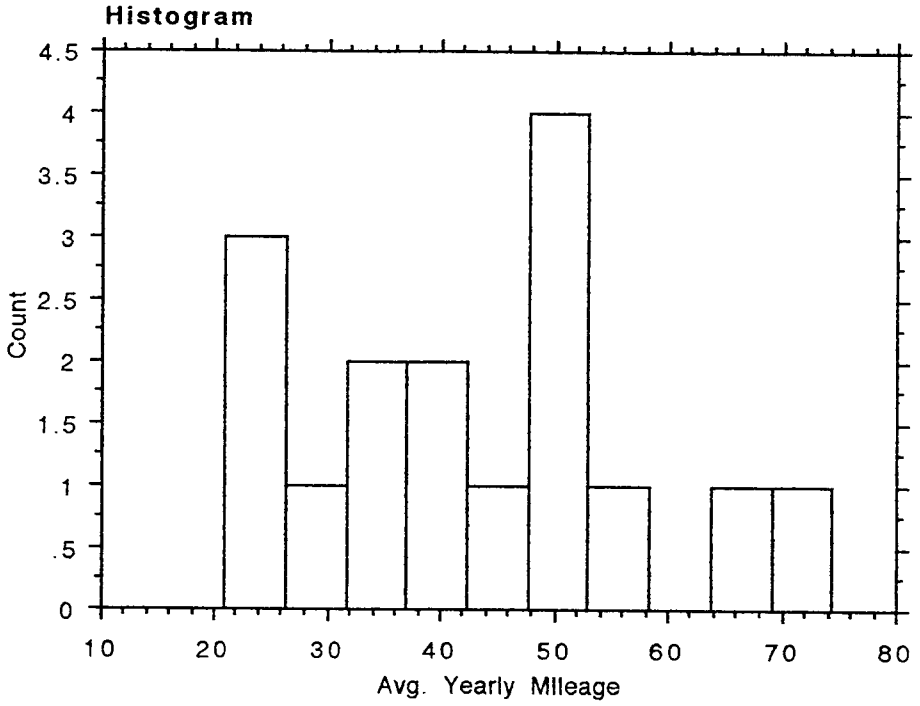
From (≥)	To (<)	Count
8.500	10.105	2
10.105	11.709	2
11.709	13.314	8
13.314	14.918	12
14.918	16.523	10
16.523	18.127	5
18.127	19.732	0
19.732	21.336	2
21.336	22.941	2
22.941	24.545	1
	Total	44

Histogram



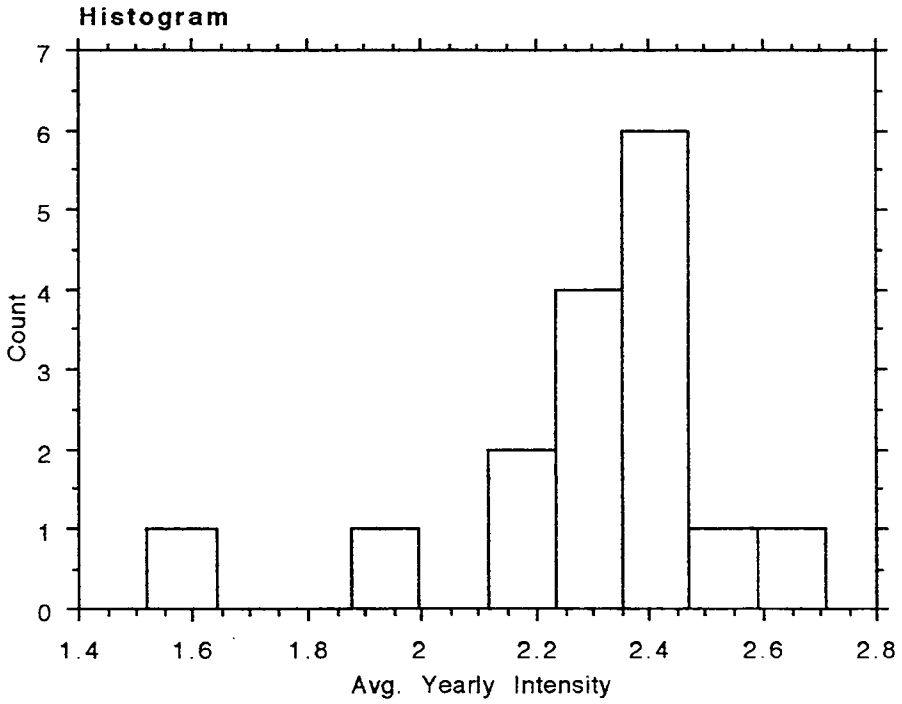
Frequency Distribution for Avg. Yearly Mileage

From (≥)	To (<)	Count
20.900	26.250	3
26.250	31.600	1
31.600	36.950	2
36.950	42.300	2
42.300	47.650	1
47.650	53.000	4
53.000	58.350	1
58.350	63.700	0
63.700	69.050	1
69.050	74.400	1
	Total	16



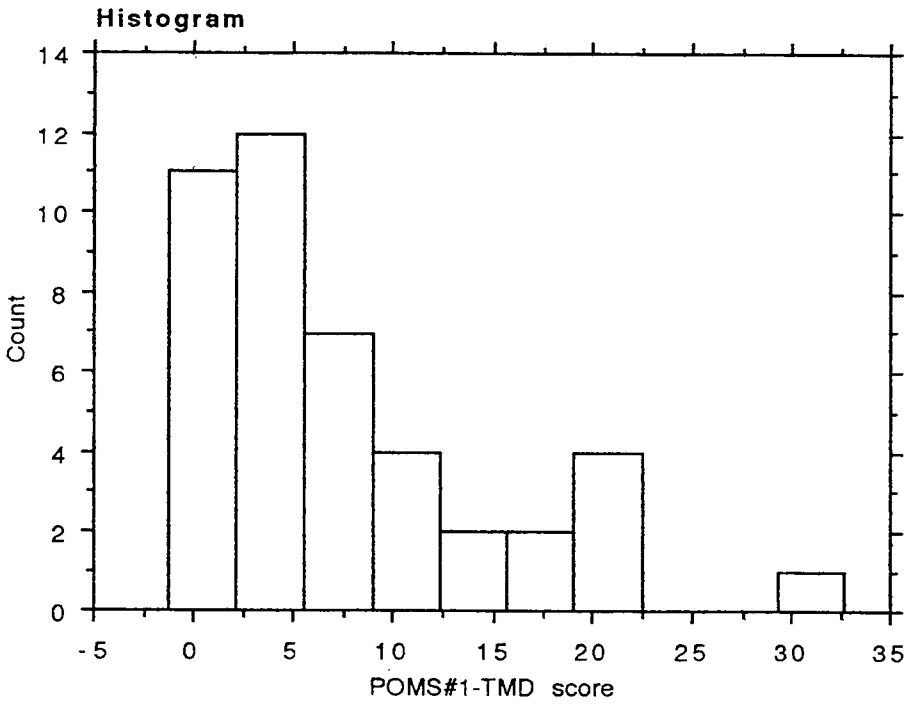
Frequency Distribution for Avg. Yearly Intensity

From (≥)	To (<)	Count
1.520	1.639	1
1.639	1.758	0
1.758	1.877	0
1.877	1.996	1
1.996	2.115	0
2.115	2.234	2
2.234	2.353	4
2.353	2.472	6
2.472	2.591	1
2.591	2.710	1
	Total	16



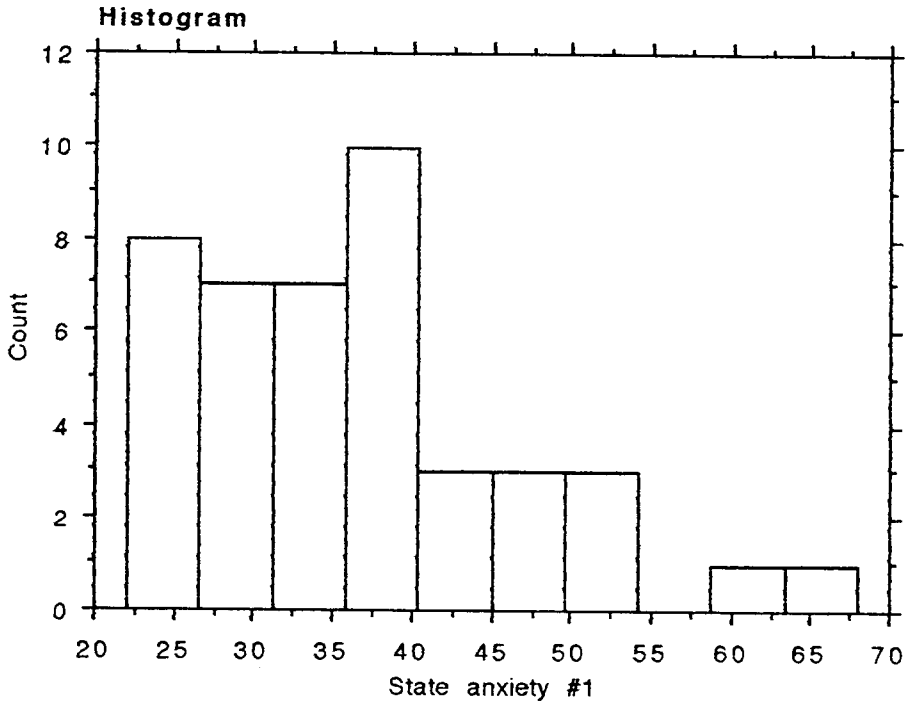
Frequency Distribution for POMS#1-TMD score

From (≥)	To (<)	Count
-1.250	2.150	11
2.150	5.550	12
5.550	8.950	7
8.950	12.350	4
12.350	15.750	2
15.750	19.150	2
19.150	22.550	4
22.550	25.950	0
25.950	29.350	0
29.350	32.750	1
	Total	43



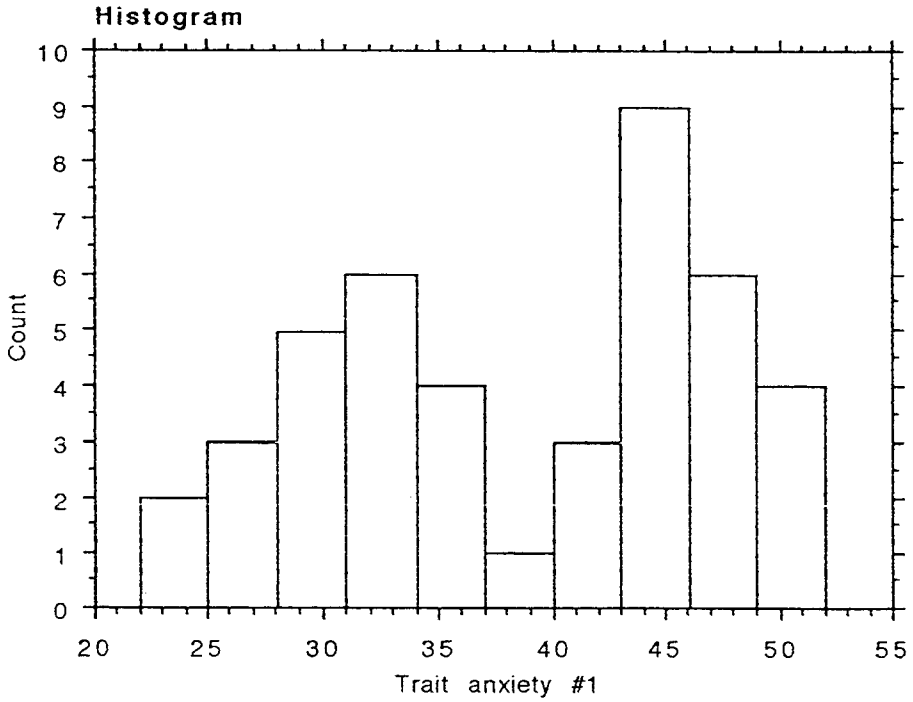
Frequency Distribution for State anxiety #1

From (≥)	To (<)	Count
22.000	26.600	6
26.600	31.200	7
31.200	35.800	7
35.800	40.400	10
40.400	45.000	3
45.000	49.600	3
49.600	54.200	3
54.200	58.800	0
58.800	63.400	1
63.400	68.000	1
	Total	43



Frequency Distribution for Trait anxiety #1

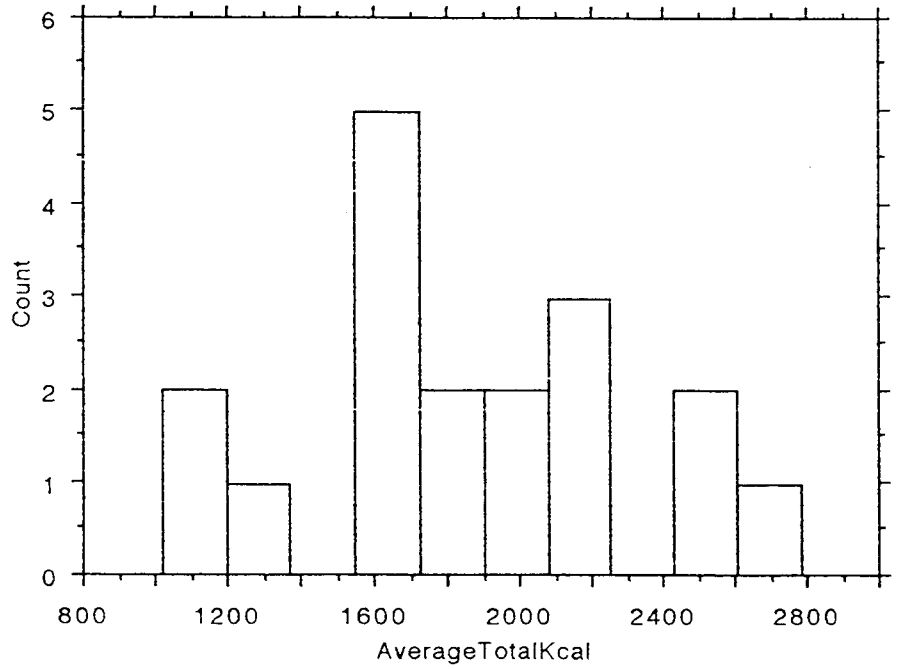
From (≥)	To (<)	Count
22.000	25.000	2
25.000	28.000	3
28.000	31.000	5
31.000	34.000	6
34.000	37.000	4
37.000	40.000	1
40.000	43.000	3
43.000	46.000	9
46.000	49.000	6
49.000	52.000	4
	Total	43



Frequency Distribution for AverageTotalKcal

From (≥)	To (<)	Count
1021.300	1197.595	2
1197.595	1373.890	1
1373.890	1550.185	0
1550.185	1726.480	5
1726.480	1902.775	2
1902.775	2079.070	2
2079.070	2255.365	3
2255.365	2431.660	0
2431.660	2607.955	2
2607.955	2784.250	1
	Total	18

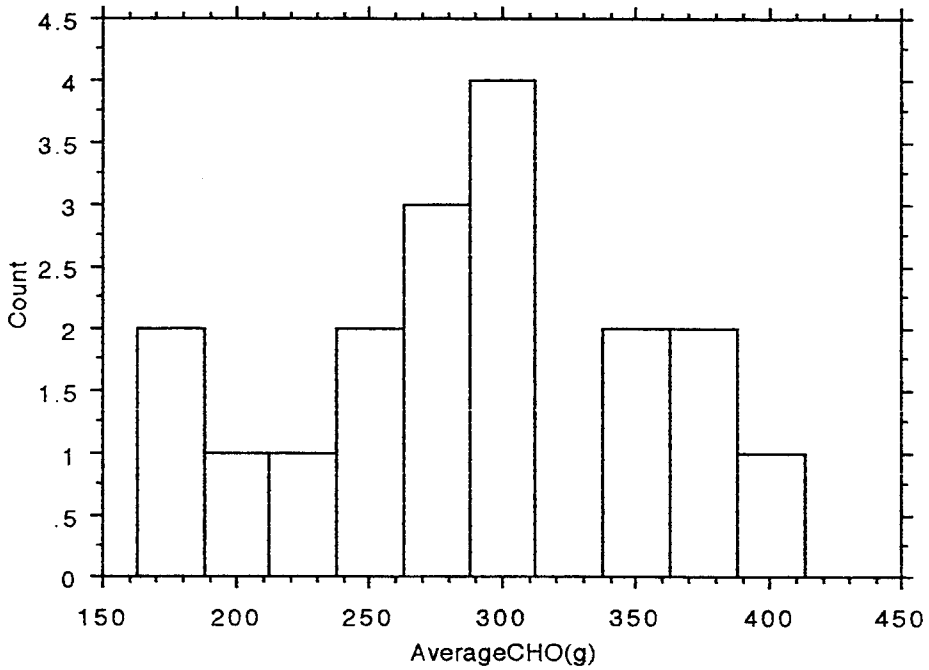
Histogram



Frequency Distribution for AverageCHO(g)

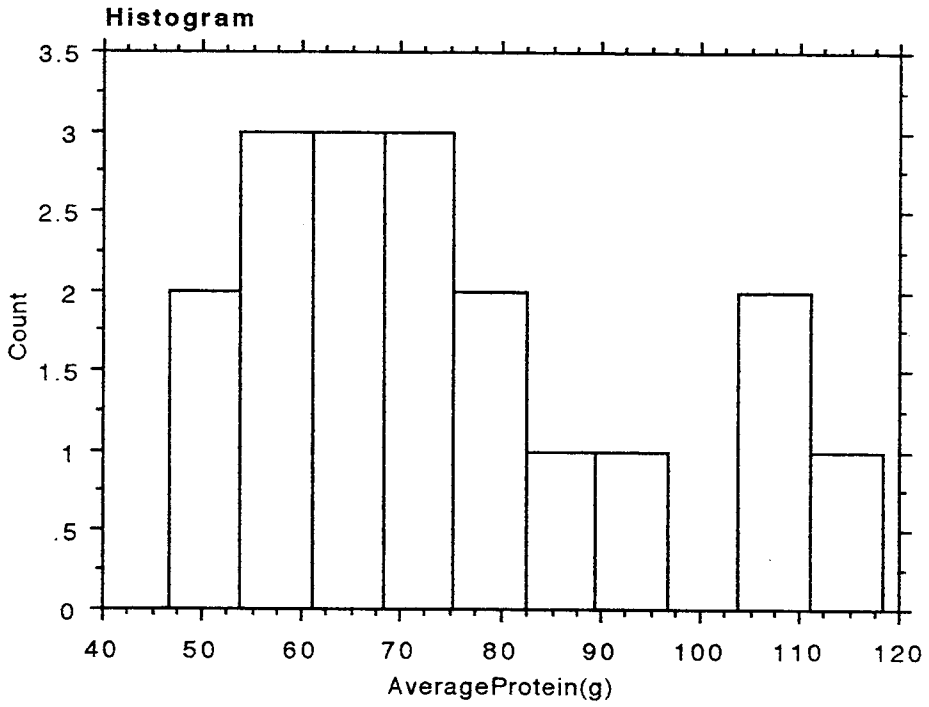
From (≥)	To (<)	Count
162.850	187.883	2
187.883	212.915	1
212.915	237.948	1
237.948	262.980	2
262.980	288.013	3
288.013	313.045	4
313.045	338.078	0
338.078	363.110	2
363.110	388.143	2
388.143	413.175	1
	Total	18

Histogram



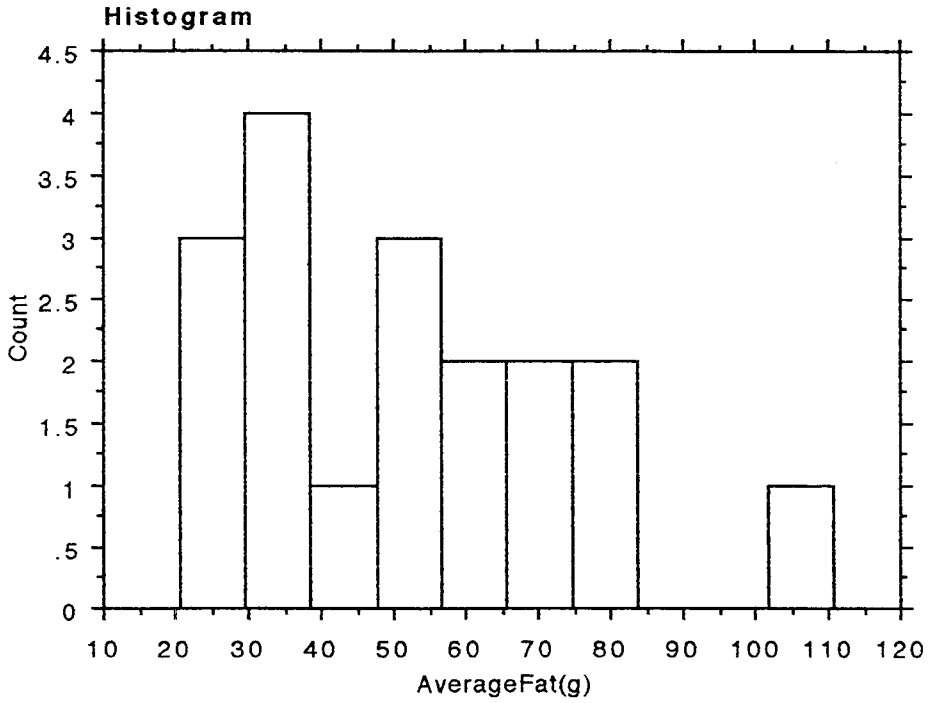
Frequency Distribution for AverageProtein(g)

From (≥)	To (<)	Count
46.700	53.855	2
53.855	61.010	3
61.010	68.165	3
68.165	75.320	3
75.320	82.475	2
82.475	89.630	1
89.630	96.785	1
96.785	103.940	0
103.940	111.095	2
111.095	118.250	1
	Total	18



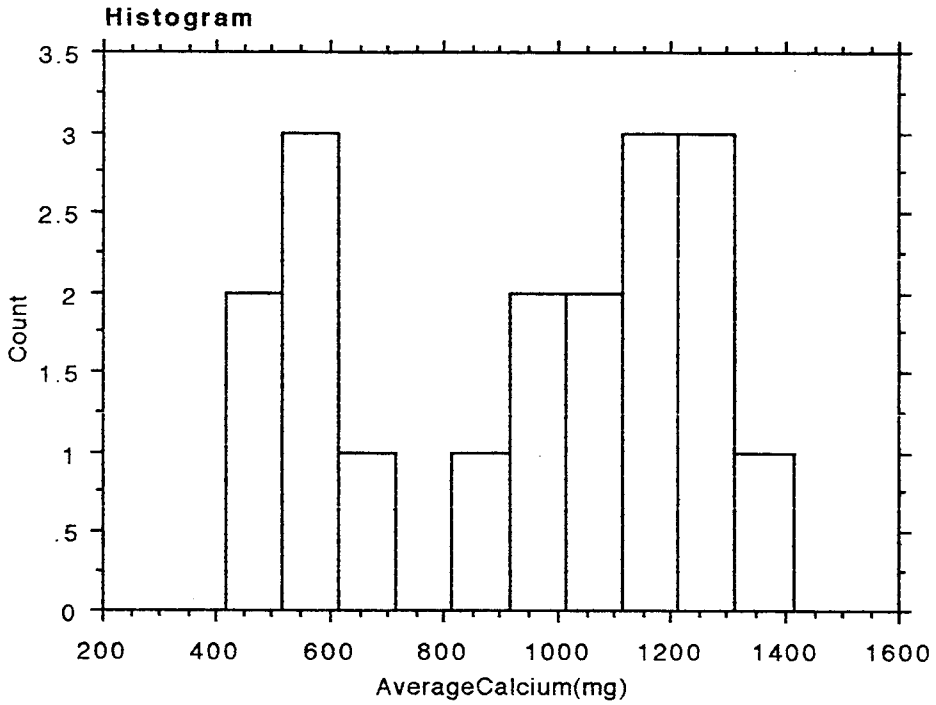
Frequency Distribution for AverageFat(g)

From (≥)	To (<)	Count
20.625	29.640	3
29.640	38.655	4
38.655	47.670	1
47.670	56.685	3
56.685	65.700	2
65.700	74.715	2
74.715	83.730	2
83.730	92.745	0
92.745	101.760	0
101.760	110.775	1
	Total	18



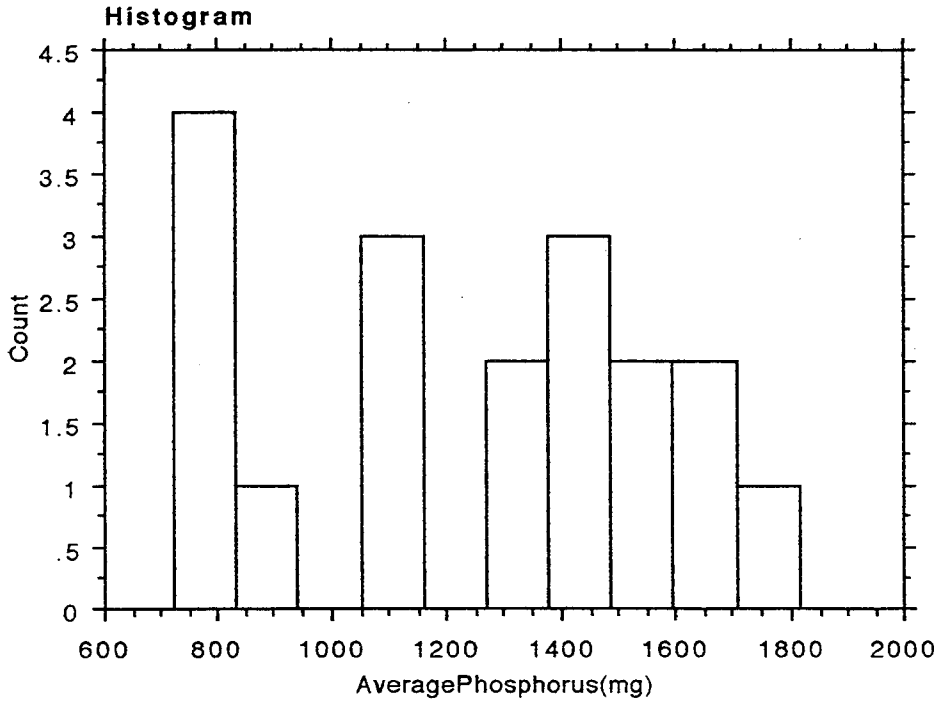
Frequency Distribution for AverageCalcium(mg)

From (≥)	To (<)	Count
414.725	514.707	2
514.707	614.690	3
614.690	714.672	1
714.672	814.655	0
814.655	914.637	1
914.637	1014.620	2
1014.620	1114.602	2
1114.602	1214.585	3
1214.585	1314.568	3
1314.568	1414.550	1
	Total	18



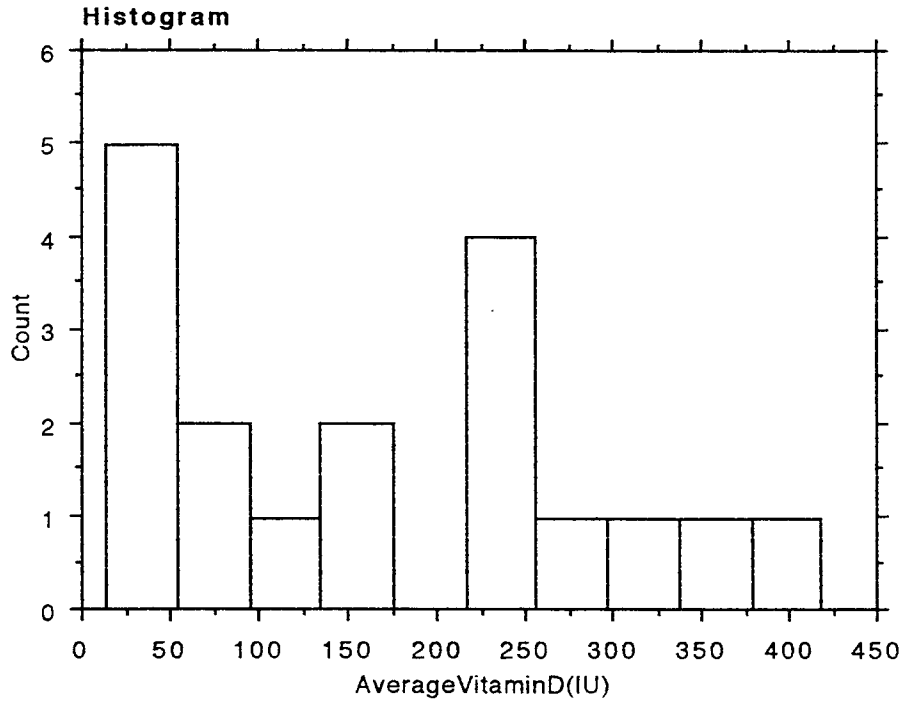
Frequency Distribution for AveragePhosphorus(mg)

From (≥)	To (<)	Count
723.325	832.500	4
832.500	941.675	1
941.675	1050.850	0
1050.850	1160.025	3
1160.025	1269.200	0
1269.200	1378.375	2
1378.375	1487.550	3
1487.550	1596.725	2
1596.725	1705.900	2
1705.900	1815.075	1
	Total	18



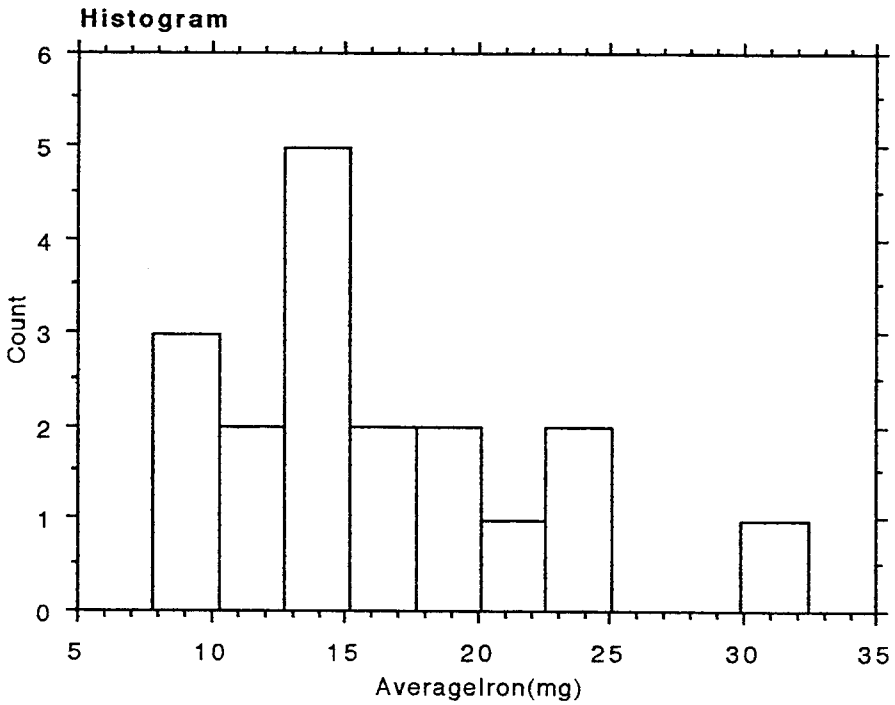
Frequency Distribution for AverageVitaminD(IU)

From (≥)	To (<)	Count
13.675	54.192	5
54.192	94.710	2
94.710	135.227	1
135.227	175.745	2
175.745	216.262	0
216.262	256.780	4
256.780	297.297	1
297.297	337.815	1
337.815	378.332	1
378.332	418.850	1
	Total	18



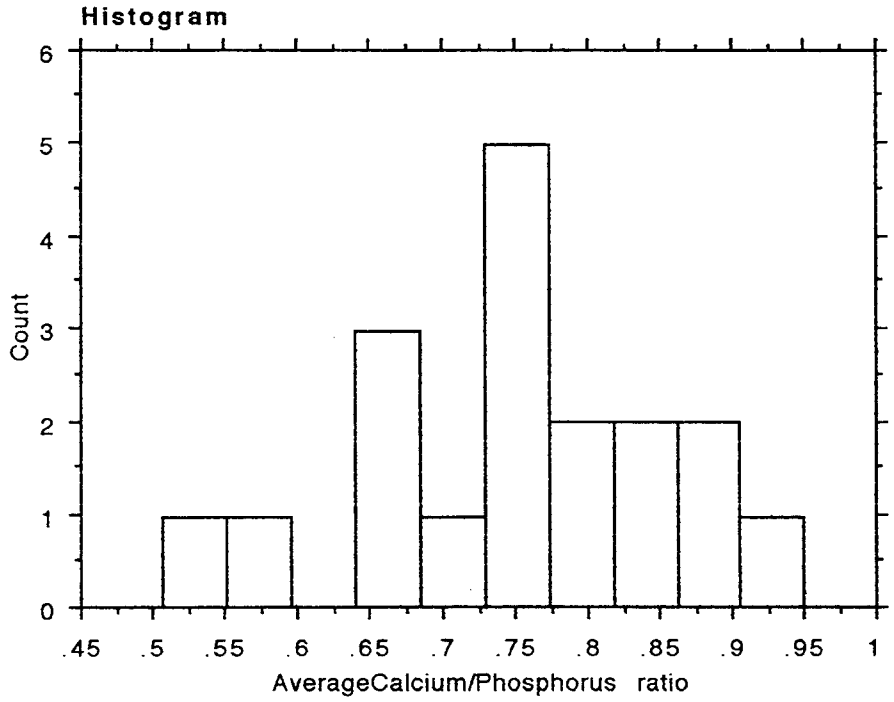
Frequency Distribution for AverageIron(mg)

From (≥)	To (<)	Count
7.875	10.332	3
10.332	12.790	2
12.790	15.247	5
15.247	17.705	2
17.705	20.163	2
20.163	22.620	1
22.620	25.078	2
25.078	27.535	0
27.535	29.993	0
29.993	32.450	1
	Total	18



Frequency Distribution for AverageCalcium/Phosphorus ratio

From (≥)	To (<)	Count
.508	.552	1
.552	.597	1
.597	.641	0
.641	.685	3
.685	.729	1
.729	.774	5
.774	.818	2
.818	.862	2
.862	.906	2
.906	.951	1
	Total	18



Frequency Distribution for AverageCalcium/Protein ratio

From (≥)	To (<)	Count
6.733E-3	7.910E-3	3
7.910E-3	9.087E-3	0
9.087E-3	.010	1
.010	.011	3
.011	.013	2
.013	.014	3
.014	.015	1
.015	.016	1
.016	.017	2
.017	.019	2
	Total	18