Changes in Bone Density
in Calcium Supplemented Adolescent Female Athletes
Experiencing Menstrual Dysfunction

by

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CHANGES IN BONE MINERAL DENSITY
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(ABSTRACT)

Thirteen adolescent runners experiencing menstrual dysfunction (mean no. menses = 5/yr) received dietary supplements of 1200 mg calcium carbonate and 400 IU vitamin D/d for 12 mos. Bone mineral content of the axial skeleton was measured by dual photon densiometry at the beginning and at the end of the 12 month supplementation period. Fasting plasma ionized calcium, intact PTH, 1,25(OH)2D3, estradiol, and thyroxin were also determined at the beginning and end of the study. Seven day dietary recalls were obtained for two separate weeks during the course of the study, one prior to the onset of supplementation and another six months later. Mean bone mineral density of the athletes increased (p<.05), but bone mineral density was observed to decrease in two of the subjects who had the lowest estradiol concurrent with the severest training regimen. Athletes' total plasma calcium and 1,25(OH)2D3 increased (p<.05). No measured variable correlated with the bone mineral density of the lumbar spine. There was a sig-
significant correlation between dietary calcium intake and total plasma calcium ($r = .50$), and dietary vitamin D intake and $1,25(OH)_2D_3$ ($r = .50$). Stepwise linear regression predicted a linear relationship between serum estradiol and bone mineral density, and no. menses/yr and bone mineral density. Multiple linear regression procedures indicated several predictive models incorporating variables that significantly affect BMD. The models include serum estradiol, no. menses/yr, body mass index, dietary calcium and dietary vitamin D intake. The increase in dietary calcium intake (192% RDA) and/or dietary vitamin D intake (190% RDA) due to supplementation did not appear to contribute to an increase in bone mineral density in estrogen compromised female athletic adolescents independent of growth.
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CHAPTER I
INTRODUCTION

Osteoporosis, reduced bone mass with normal mineralization is a major nutrition-related health problem. It has been estimated that 15 million Americans have osteoporosis (Bialack et al., 1986). Approximately 25% of white women in America have had one or more osteoporosis related fractures by the age of 65. Post menopausal osteoporosis occurs more frequently and causes more disability than any other form of osteoporosis. This high incidence has led many to seek new methods of prevention and treatment.

Sedentary lifestyle is considered to be a risk factor for osteoporosis, while weight bearing exercise is among the suggested methods of prevention (Smith, 1982). However, recent studies have reported that for females experiencing exercised induced amenorrhea, weight bearing exercise such as running does not promote an accretion in bone mass. Rather, these athletes have a reduced amount of bone mineral density which may predispose then to achieving a lower peak bone mass as well as to early onset of osteoporosis (Cann et al. 1984, Drinkwater et al. 1984).

While osteoporosis can be seen as an effect of athletic amenorrhea, poor calcium nutiture is considered to be among the causative risk factors. Calcium intake in the
United States has been studied extensively for the past 25 years. Notable are the Health and Nutrition Examination Surveys (Hanes I and II) and the Food Consumption Survey (FCS) of the USDA Department of Agriculture (Heaney, 1982). In general, these studies show similar patterns. The typical U.S. male consumes from 1.2 to 2.0 times as much calcium as the U.S. female with the difference being greatest between ages 15 and 50. During the time between 15 to 30 years of life when peak bone mass is developing, more than two thirds of all U.S. females ingest less than the RDA on any given day. Exacerbating the situation is evidence which suggests that women who lack estrogen have a requirement for calcium which is higher than the current RDA (Heaney, 1982). Adequate nutrition early in life is considered to be important in determining peak adult bone mass.

While it is not conclusively proven, it is apparent that calcium nutriture in the formative years affects the peak bone mass one is able to achieve in adulthood, and may have influence on fracture susceptibility as well (Johnson, 1981).

Statement of the Problem

It is now recognized that a substantial portion of female athletes will develop exercise induced menstrual
dysfunction along with other asymptomatic changes which may seriously affect their reproductive physiology and bone metabolism (Drinkwater et al. 1984, 1986). Compared with eumenorrheic athletes, amenorrheic athletes manifest reduced bone density, reduced circulating estradiol and progesterone, and increased musculoskeletal injury rates (Lloyd et al., 1987).

The purpose of the present study was to observe the effects of dietary calcium and vitamin D supplements on bone mineral density and hormonal and metabolic indices controlling calcium metabolism in adolescent athletes experiencing menstrual dysfunction.

Research Hypotheses

HO: Treatment with daily calcium and vitamin D supplementation for 12 months has no effect on subjects' bone mineral density as measured by dual photon densiometry.

HO: Treatment with daily calcium and vitamin D supplementation for 12 months has no effect on subjects' serum estradiol, intact parathyroid hormone, 1,25(OH)2D3, ionized calcium and total serum calcium measured via venipuncture pre and post supplementation.

HO: There is no relationship between training status, estrogen status, dietary intake of calcium and/or vitamin D, and initial bone mineral density.
HO: There is no relationship between the changes in bone mineral density and the changes in dietary calcium and/or vitamin D intake after supplementation.

Significance of the Study

Explanations for age-dependent reduction in skeletal mass cannot simply address events in the fifth decade of life, be they hormonal, nutritional, or otherwise. These factors must be considered over the entire life span.

Since important differences in bone mass are detectable by the second decade, it may prove essential to consider factors affecting bone mineral density during adolescence or earlier.

Presently there is a lack of information in the literature on training-induced hormonal and metabolic alteration in adolescent athletes experiencing menstrual dysfunction. These variables, along with dietary status, may decrease the extent to which these adolescents develop peak bone mass. This study will contribute information regarding the susceptibility of athletic adolescent females experiencing menstrual dysfunction, to bone loss and/or reduced bone accretion. Additionally, this study will present information regarding the effect of calcium and/or vitamin D supplementation on bone mineral density in these individuals.
Delimitations

The following delimitations were imposed by the investigator:

1. Athletic subjects (participating in regular endurance, 3d/wk, weight bearing exercise) were 13 female Caucasian volunteers (aged 14-18) within 85-110% of their ideal body weight who were experiencing menstrual dysfunction (secondary amenorrhea or oligomenorrhea).

2. Sedentary subjects (not presently participating in regular endurance, 3d/wk weight bearing exercise) were 7 female Caucasian volunteers (aged 12-18) not greater than 22% body fat, eumenorrheic.

3. All subjects were without history of smoking, recreational drug use, previous pregnancies, eating disorders, use of oral contraceptives within the last six months or cumulative oral contraceptive use of greater than six months.

4. Subject selection was dependent upon screening criteria.

5. The dietary supplement distributed to the athletes only, contained 1200 mg calcium carbonate and 400 IU vitamin D per two tablets.
Limitations

The following limitations affect the generalizability of the findings:

1. Due to a small sample size (n=13) results are limited to the experimental sample.

2. Adherence to dietary supplementation and dietary recall compliance was strictly voluntary so variation may have occurred.

3. Due to the bone density machine being moved after subjects' initial test, variation in recalibrating the machine may have occurred which may have affected subjects' post test measurements.

4. Interpretation of serum levels of ionized calcium is limited because the levels were adjusted to compensate for the change in pH that occurred due to the samples being frozen.

5. Interpretation of changes in blood and serum levels of hormones and metabolic factors is limited because such levels are the net result of synthesis, mobilization and catabolism and do not solely reflect the turnover rate of hormones or metabolic factors.

Definitions and Symbols

Terms and symbols regarding clarification for use in this study are as follows:
**Bone Mineral Density (BMD).** is the vertebral density of the lumbar spine (L1-L4) measured by dual photon densitometry.

**Eumenorrhea.** is defined as normal menstruation.

**Amenorrhea.** is defined as 0 menses per year.

**Oligomenorrhea.** is defined as 0-6 menses per year.

**Menstrual Dysfunction.** is defined as having 0-6 menses per year and/or a training induced interruption in normal menstruation.

**Basic Assumptions**

1. Subjects adhered to the dietary supplementation regimen.

**Summary**

Female athletes experience a higher incidence of menstrual irregularity and amenorrhea than the more sedentary female population. Current information is being presented which suggests that amenorrheic athletes may have reduced bone mass, lowering their peak bone mass development and, thus, making them more susceptible to osteoporotic fractures earlier in life. Inadequate calcium status is considered to be causative. Additionally, it is thought that the level of calcium needed to maintain calcium balance in amenorrheic athletes exceeds the current RDA. Documentation of the effect of calcium supplementation on bone den-
sity in amenorrheic athletes is not currently available.
CHAPTER II
REVIEW OF THE LITERATURE

To present the reported knowledge pertinent to the topic under investigation in this study, the review of the literature is divided into five sections: Exercise-Induced Amenorrhea and Skeletal Integrity, Calcium Requirements in Amenorrheic Athletes, Prevention of Bone Loss, Types of Calcium Supplements, Assessment of Bone Mineral Content.

Exercise-Induced Amenorrhea and Skeletal Integrity
A. Vertebral Bone Loss

Cann et al. (1980) were the first investigators to report a statistically significant decrease in bone content in amenorrheic athletic women. Twenty-five amenorrheic athletic patients aged 19-49 years served as subjects: of these, 7, who were not runners had hyperprolactinemia secondary to prolactinomas. Six additional women were runners with hypothalamic amenorrhea. The rest of the group, all non-runners had primary ovarian failure (premature menopause).

All subgroups of amenorrheic women had comparable bone loss as measured by quantitative computed tomography (CT). The bone mineral content in the amenorrheic women was 30% less at the spine and 13% less at the distal radius, the
latter measured by single photon absorptiometry. Of the 11 hypothalamic amenorrheic women 10 were long distance runners, 2 currently suffering from stress fractures. The amount of exercise was not controlled as this was not the original intent of the study. Radioimmunoassay confirmed a lower mean estradiol concentration in the amenorrheic women in four venous samples drawn at seven day intervals (amenorrheic group 38.58 pg/ml; eumenorrheic group 12.25 ng/ml).

Cann et al. concluded that the combination of decreased bone mass and increased skeletal stress of exercise may increase the rate of stress fractures in amenorrheic women during exercise or later in life. Cann et al. (1984) expanded the sample to include 11 hypothalamic amenorrheic (1 to 9 years) subjects (19-27 yrs), 10 of whom were regular participants in vigorous exercise programs; the amount of exercise was not assessed during the evaluation however. They had an average spinal mineral bone content of 128 mg/cm3 compared to 165.8 mg/cm3 in 50 eumenorrheic sedentary controls, a decrement of 24%. Differences in radial and metacarpal bone mineral content were not significant. No correlation of bone mass with age existed for the control group in this age range of 20-50 years, with non-significant changes of -0.2% per year in the spine and
+0.1% per year in the radius and metacarpals. Therefore, the differences in bone mass among the groups is not believed to reflect age related changes in bone. The mean age of the control group was 16 years older than that of the hypothalamic amenorrheic group. The subgroup of women with primary hypothalamic amenorrhea, however, was eight years younger than those women with secondary amenorrhea (19.7 v. 27.3 yrs). The rate of bone loss for the hypothalamic amenorrheic group was 4.5%. This was not statistically different from the other two groups; 4.8% hyperprolactinemic, 6.2% ovarian failure.

Estradiol levels were uniformly decreased in the amenorrheic women with concentrations in the group with hypothalamic amenorrhea in the low-normal, early follicular range (20-80 pg/ml). Serum concentrations of estradiol were not correlated with the body fat index within any of the groups, indicating a weak, if any, association of these parameters. Estradiol concentrations were not correlated with the decrease in bone mass relative to controls on an individual or a group basis. Estrone concentrations were not measured in these women. Cann et al. (1984) concluded that women with hypothalamic amenorrhea secondary to exercise also had a decrease in bone mass, and that this factor, combined with the skeletal stresses from vigorous
exercise, may increase the risk of stress fracture in these women during exercise or osteoporotic fractures later in life.

These findings of Cann et al. were surprising because it was assumed that the hypo-estrogenic amenorrheic athlete was protected from the adverse effects of estrogen deficiency on bone due to evidence of the positive effects of exercise on bone mass in athletes (Montoye 1985; Smith 1981; Yeater & Martin 1984). Furthermore, exercise has been shown to inhibit and even reverse bone loss in post-menopausal women not receiving estrogen therapy (Aloia et al. 1978; Krolner et al. 1983; Smith et al. 1981). However, the association between the hypoestrogenic state and diminished bone mass is well documented in women following a natural or surgical menopause (Cann et al. 1980; Lindsay et al. 1980) as well as in premenopausal women with amenorrhea associated with hyperprolactinaemia (Klibanski et al. 1980), and anorexia nervosa (Rigotti et al. 1984).

Several investigators subsequently attempted to replicate the work of Cann et al. (1984) using experimental designs more specifically directed toward evaluating bone mineral loss in female athletes. Drinkwater et al. (1984) compared the vertebral mineral density of 14 amenorrheic (0-3 menses/yr) athletes (11 runners, 3 crew members) with
that of 14 eumenorrheic athletes. The two groups of athletes were matched for age, height, weight, body composition, sport, and training regimens. A three-day dietary history showed no significant difference in nutritional intake. The athletic history of the two groups differed significantly; the mean weekly mileage of the eumenorrheic group being 24.9 miles and that of the amenorrheic group being 41.8 miles (p < 0.05).

Measurements of regional bone mass were made by single photon absorbtometry for the appendicular skeleton and by dual photon absorption of the axial skeleton. No differences were found between the amenorrheic and eumenorrheic groups with respect to the mineral content of the radius, but that of the vertebrae was significantly lower in the athletes belonging to the amenorrheic group (1.12 g/cm²) than the eumenorrheic group (1.30 g/cm²) (p < 0.05). When the values for vertebral mineral density were compared with those reported by Riggs et al. (1982) for 120 women representing a wide age span, the mean bone mineral density of the eumenorrheic women was close to that predicted (1.33 g/cm²) by an age-based regression equation. In contrast, the average bone mineral density of the amenorrheic athletes (25 years of age was equivalent to that of women 51.2 years of age (Drinkwater et al. 1984). Two of these ath-
letes had a vertebral mineral density below the fracture threshold as defined by Riggs et al. (1981) which is 0.965 g/cm². Radioimmunoassay also confirmed a significantly lower estradiol concentration in the amenorrheic athletes (38.58±7.03 pg/ml) versus the eumenorrheic athletes (106.00±9.80 pg/ml) (p<0.05). Estradiol levels were expressed as an average of four samples. A three-day dietary history showed no significant differences in nutritional intake, including calcium with and without supplements (amenorrheic: 1627 kcal/d, calcium 888 mg/d, with supplement 960 mg/d; eumenorrheic: 1965 kcal/d, calcium 912 mg/d, with supplement 1100 mg/d).

From this data the authors concurred with Cann et al. in that amenorrheic athletes may possess a reduced bone mass compared to eumenorrheic athletes and training may be a significant factor affecting estrogen levels which in turn may affect bone mass. However, Drinkwater et al. did note that amenorrheic athletes involved in sports other than running, should be evaluated as they observed that the amenorrheic crew members, for example, had an average vertebral mineral density of 1.22 g/cm² which is 0.13 g/cm² higher than that of amenorrheic runners.

Lindberg et al. (1984) studied the bone content of five groups of women. Group 1, 11 amenorrheic runners (no
menses for the previous six months); Group 2, 5 oligomenorrheic runners (menses occurred every six weeks to six months); Group 3, 15 eumenorrheic runners running a minimum of 20 miles per week: Group 4, 14 eumenorrheic normal controls not participating in regular exercise, and Group 5, 10 post-menopausal women. There were no significant differences in the physical characteristics in Groups 1 to 4. Mean age for all runners and non-runners was 30.4 years. Mean age for the post-menopausal group was 62 years. Heights were similar in all groups. Body weight was lower (p < 0.05) and ponderal index (height divided by the cubed root of the weight) higher in amenorrheic runners than in controls. There was no difference in age of menarche among Groups 1-4. Both amenorrheic and oligomenorrheic runners ran greater distances each week (data not reported) and had a longer duration of training than did normal runners (data not reported).

There was a significant (p < 0.05) reduction in both cortical (single photon absorbtometry) and trabecular radial density in the amenorrheic runners vs. the eumenorrheic runners and the eumenorrheic controls. In the 8 amenorrheic runners who had spinal measurements, the average lumbar spine mineral content (dual photon densiometry) was 1.078 g/cm², which is below the normal range of 1.2 to 1.6
g/cm².

All women had normal serum concentrations of calcium, phosphate, prolactin, and parathyroid hormone (mid-portion and intact molecule) with no differences between groups. Serum estradiol levels in amenorrheic and post-menopausal women tended to be lower and were similar to early follicular phase levels (data not given). Lindberg et al. concluded that the beneficial effects of exercise on maintaining bone mineral integrity can be overridden by other factors, and although estrogen may be the most significant factor affecting post-menopausal bone loss, the pathogenesis of the decrease in bone mineral content in amenorrheic runners is not clear.

To determine whether severe exercise training might reduce or even reverse the deleterious skeletal effects of amenorrhea, Marcus et al. (1985) evaluated bone mass in 17 elite distance runners, in whom training intensity, body composition, and endocrinological features were documented. Eleven of the women had experienced secondary amenorrhea (no menstrual bleeding whatsoever) for 1 to 7 years. Six women had maintained regular menses since menarche. Both groups were matched for aerobic capacity, body fat, exercise intensity, and age of menarche. The amenorrheic women were younger (18 to 22 years) verses cyclic women (19 to 29
years) (p< 0.05). Mineral density of the lumbar vertebrae (computed tomography) in the amenorrheic runners was lower (p< 0.02) than that in the cyclic women and age matched non athletic controls (151 mg/cm³, 182 mg/cm³, 166 mg/cm³) Mineral density of the radius was normal in both groups.

Age of menarche was similar for both groups (13 v. 13.5 years), but whereas the cyclic women began to train intensively 5.0 years after menarche, amenorrheic women had begun serious training very close to the onset of menses, 0.91 years (p< 0.02). Four amenorrheic women had initiated training before the first menstrual period. The subjects ran an average of 93 km each week (56-160 km); this average did not differ between groups.

Plasma estradiol was low in amenorrheic women (36.3±3.5 pg/ml) and higher in the cyclic women (92.5±27.4.0 pg/ml, p< 0.01). No woman had an elevated level of prolactin, luteinizing hormone or follicle stimulating hormone. Serum levels of calcium and phosphorus were normal in amenorrheic and cyclic women as were serum immunoreactive parathyroid hormone and alkaline phosphatase. Serum levels of calcifediol (25-OHD3) were within normal limits for all women and did not differ significantly between amenorrheic women and cyclic controls. Serum levels of calcitriol were also within normal limits but were significantly greater in
the cyclic women (48.0±6.0 pg/ml compared to 32.8±3.7 pg/ml, p< 0.05). No significant correlations were seen between the levels of vitamin D metabolites and immunoreactive parathyroid hormone, calcium excretion or serum estradiol levels. Free thyroxine was normal in all women, but was significantly lower in the amenorrheic women than in a group of 12 age-matched sedentary controls (1.15±0.06 compared to 1.45±0.06 ng/dl, p< 0.05). Serum triiodothyronine was lower in the amenorrheic women (88.2±3.9 ng/dl) than in the cyclic group (104±3.5 ng/dl, p< 0.02) or in the sedentary controls (124±6.3 ng/dl, p<0.001). Three day dietary intakes showed unexpectedly low calorie and calcium intake in both the cyclic (1715 kcal/d) and amenorrheic (1272 kcal/d) groups. Fifty percent of amenorrheic and 40% of cyclic women were not eating two-thirds of recommended allowances for calcium, and this figure was marginally altered by supplemental intake (cyclic 1129±300 mg/d; amenorrheic 783±98 mg/d).

When compared with the subjects of Cann et al. (1984), who were evaluated in the same laboratory, the amenorrheic runners had significantly lower spinal density than did age-matched eumenorrheic controls, but a significantly higher value than less active amenorrheic runners (121±6.9 mg/cm3, p< 0.05). These findings suggest that superior
athletes are able to exercise sufficiently to make up for the deleterious skeletal effects of amenorrhea, but it is unlikely that the average casual athlete can achieve performance levels seen in this group of women. Marcus et al. (1985) do not recommend that women who participate in endurance activity train to such a degree that menstrual function is compromised.

In a study similar to that of Drinkwater et al. (1984), Nelson et al. (1986) compared amenorrheic and normally menstruating athletes to investigate their bone mineral density, body composition, exercise training, and dietary habits. Eleven women were amenorrheic, having had no menstrual cycle in the last 12 months. Of these 11, two had started training before menarche and had irregular cycles before becoming amenorrheic. Seventeen trained women were eumenorrheic with normal cycles (<±5 day variation in cycle each month).

The physical characteristics of the two groups were similar except that the amenorrheic athlete were younger (25.5 yrs vs. 29.2 yrs, p<0.04) and had reached menarche at a later age 14.7 yrs vs. 12.8 yrs, p< 0.02). No significant differences between the two groups were found in body composition (amenorrheics 21% fat vs. eumenorrheics 19.7%), amount of training (amenorrheics 34.7±4.62 miles/wk vs.
eumenorrheics 39.8±2.83 miles/wk), or years of training (amenorrheics 6.9±0.80 yrs vs. eumenorrheics 6.2±0.65 yrs). The bone mineral density of the lumbar spine (dual photon densiometry) was significantly lower in the amenorrheic women (1.099±0.027 vs. 1.196±0.027 g/cm², p< 0.02) as was their mean estradiol level (8.5±1.88 pg/dl vs. 28.6±3.74 pg/dl, p< 0.01). Bone mineral density was positively correlated with estradiol levels (p< 0.008). There was a negative correlation between percent body fat and bone mineral density of the lumbar spine (p< 0.002). No other measured characteristic correlated with the bone mineral density of the lumbar spine. The bone mineral content of the radius shaft (single photon absorptiometry) did not differ between the two groups. A three-day dietary intake showed that the amenorrheic women reported a significantly lower daily energy intake (1730 kcal vs. 2250 kcal, p< 0.02). There was no difference between calcium intake for the amenorrheic women (886±312 mg/dl) and eumenorrheic women (1150±117 mg/dl). No correlation was found between dietary calcium intake and bone mineral content of the lumbar spine so that the low lumbar bone mineral content found in the amenorrheic group could not be associated with a low calcium content in the current diet. However Nelson et al. do contend that the associated endocrine and dietary dif-
ferences in the amenorrheic athletes could markedly affect calcium metabolism. Protein intake was less than the U.S. Recommended Dietary Allowance in 82% of amenorrheic women and 35% of eumenorrheic women.

The results of this study suggest that when weight-bearing exercise and a low energy intake are associated with amenorrhea, the accretion of a large bone mass in young trained women is not favored (Nelson et al. 1986). Furthermore, the significant positive linear correlation (r = 0.50) observed between spinal bone density and plasma estradiol levels does lend support to the role of estrogen deficiency in the bone loss exhibited by amenorrheic athletes. This relationship was not demonstrated by the previous reports of Cann et al. (1984), nor Drinkwater et al. (1984).

B. Radial Bone Loss

Studies restricting bone measures to the wrist have not been as consistent in revealing low bone mineral content in amenorrheic athletes. Berning et al. (1984) found no difference between 6 amenorrheic and 6 eumenorrheic runners; however, both groups had lower values than did 6 sedentary eumenorrheic controls.

Linnell et al (1984) reported finding no differences
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in radial bone mineral content in 10 amenorrheic runners (0-3 menses during the past year), 12 runners with regular menstrual cycles (10-12 menses during the past year), and 15 non-athletic eumenorrheic controls. There were no significant differences between the amenorrheic runners and the regularly menstruating runners regarding age (22.1±2.8 yrs vs. 22.5±4.6 yrs) height, weight, body density, relative fatness (the ratio of fat mass to total body weight as calculated from body density), and training distance (58.7±14.5 km/wk vs. 55±34.6 km/wk). The control subjects were similar in age and height to both groups of runners, but differed significantly (p< 0.05) in body weight (60.3±7.0 kg vs. amenorrheics 51.4±6.4 kg, eumenorrheics 54.5±6.30 kg), percent body fat (23.5±4.2% vs 12.2±6.2%, 13.8±6.8%) and body density (1.044g/cm3, vs 1.072g/cm3, 1.068g/cm3). The average daily intake of calcium (C) phosphorus (P) and C:P was not significantly different among the three subject groups. All groups were, however, above the RDA for calcium (controls 1038±877 mg/d, amenorrheics 870±428 mg/d, eumenorrheics 844±417 mg/d). The authors concluded that amenorrhea, independent of body composition was not related to reduced BMC/BW in their population of female runners. However, the combination of excessive...
Jones et al. (1985) studied the peripheral bone density (direct photon absorptiometry of the radius) of 39 women aged 18 to 43 with the diagnosis of secondary amenorrhea in an effort to define the population of amenorrheic women at risk for osteoporosis. Like Linnell et al. (1984), Jones et al. reported no differences in radial bone content in 8 amenorrheic athletes (aged 26.5±5.0 yrs; ran 30 miles/wk) and 25 eumenorrheic non-athletic controls (28.9±4.3 yrs). However, when comparing the radial bone content of 20 women (24.3±4.0 yrs) classified as having amenorrhea associated with weight loss, the average bone density of this group was significantly less than that of the controls (p< 0.05). The body composition of the two amenorrheic groups was not significantly different. The athletes had fewer months of amenorrhea than did the weight loss group. Linear regression analysis showed a significant correlation between months of amenorrhea and bone density (p< 0.001). The authors suggest that this result implies dietary restriction may be a factor in decreased bone density. Using the technique of peripheral bone densitometry Jones et al. (1985) have demonstrated that women with exercise induced amenorrhea have similar bone density to normal controls, whereas women with hypothalamic amenor-
rhea, on the basis of simple weight loss, are at risk for cortical bone loss.

With the exception of the data by Lindberg et al. (1984) none of the studies showed differences in radial bone density in amenorrheic athletes. This is not unexpected because estrogen deficiency results in a more rapid loss of trabecular bone (spine) than cortical bone (radius) (Cann et al. 1980). Snyder et al. (1984) were the only investigators who did not observe a decrement in spinal bone mineral content in 7 eumenorrheic, 5 oligomenorrheic and 4 amenorrheic elite, lightweight oarswomen, and 9 eumenorrheic non-athletic controls. According to Caldwell (1984), Snyder et al. suggest that normal levels of spinal bone mineral content in amenorrheic and oligomenorrheic oarswomen reflect the local effect of rowing in stressing the back and the arms. Smith et al. (1984) hypothesize that mineral is redistributed from areas of lesser to greater stress. Reduced spinal bone mineral content in runners may reflect a preferential redistribution of mineral from the spine to the lower extremities (Drinkwater et al. 1984).

Because the problem of reduced bone mineral density in athletic amenorrheic women has been recognized only recently, the roles of potentially related factors must
still be defined. This is further complicated because the mechanism responsible for athletic menstrual dysfunction itself is not, as yet, completely understood. Factors that must be considered include; age at onset of training and the duration of participation, intensity and volume of training, sport specificity, and adequacy of diet (Sinning & Little, 1987). Relative to diet, Drinkwater et al. (1984), Marcus et al. (1985) and Nelson et al. (1986) reported extremely low caloric intakes in the amenorrheic bone mineral-depleted athletes (1622 kcal/d, 1272 kcal/d, and 1730 kcal/d, respectively). With the exception of the sample studies by Nelson et al. (1986), body weight tended to be very low in the mineral depleted athletes. In the study by Cann et al. (1984) amenorrheic athletes were 10.7 kg. lighter than eumenorrheic controls (48.7±1.3 kg vs. 59.4±1.6 kg). The amenorrheic athletes evaluated by Drinkwater et al. (1984) were 3.5 kg. lighter than the eumenorrheic athletes, although the difference was not significant. Lindberg et al. (1984) reported that their amenorrheic athletes were significantly lighter than their eumenorrheic controls, but the specific values were not presented. The amenorrheic athletes studied by Marcus et al. (1985) were significantly lighter (4.1 kg) than their eumenorrheic athletes, even though the relative fat values were
similar. Since body-weight has been shown to be positively associated with bone mass (Jones et al. 1984; Linnell et al. 1984; Oyster et al. 1984), its role as a potentially confounding variable in studies of amenorrheic athletes cannot be ignored.

C. Irreversibility of Bone Loss

The data by Cann et al. (1984) indicate that the progression of bone loss exhibited by amenorrheic athletes is similar to that observed in post-menopausal women. This would suggest that, without early intervention, amenorrheic athletes will be predisposed to osteoporosis and fractures at an early age, and the bone loss may be irreversible.

To address the concern of irreversible bone loss in amenorrheic athletes Drinkwater (1986) re-evaluated the bone status of previously amenorrheic athletes (Drinkwater et al. 1984) who regained menses one to ten months following the initial tests.

Nine of the original group of fourteen amenorrheic athletes were re-tested. Seven of these women had regained menses one to ten months after the initial tests; two remained amenorrheic. Seven eumenorrheic athletes from the previous study (Drinkwater et al. 1984), who matched the former amenorrheic athletes on physical characteristics and
training regimen, also agreed to participate. The bone mineral density (BMD) was measured at two sites on the radius and at the lumbar vertebrae using single and dual photon densiometry, respectively. Changes in vertebral mineral BMD were significant ($p < 0.01$) for the formerly amenorrheic group (+6.3%) but not for the cyclic women (-0.3%). The cyclic women, however, continued to maintain a significantly higher vertebral BMD (1.369 g/cm²) than the women who resumed menses (1.198 g/cm²) ($p < 0.05$). A slight increase in radial density at S-1 and S-2 was not significant for either group. Two athletes who remained amenorrheic during this period continued to lose bone (-3.4%).

As in the original study, the number of miles run per week was significantly higher for the formerly amenorrheic athletes even though their mileage had decreased by 10% (38.7±6.3 miles/wk vs. 26.1±8.0 miles/wk; $p < 0.05$). The only significant differences between test 1 and test 2, other than age, were a 1.9 kg. increase in the body weight of the women who regained menses (55.0±3.9 kg vs 57.4±3.9 kg; $p < 0.01$) and their number of menses per year (0.6±0.3 vs. 7.4±1.3; $p < 0.01$). Six of the seven women from the original amenorrheic group reported an injury or illness that forced them to decrease their mileage and/or substitute other activities for running for several months. Five of
these women gained weight during this period. Five of the former amenorrheics reported an increase in consumption of dairy products and/or calcium supplementation during the previous year. Three of the cyclic women added dietary or supplemental calcium to their diets; three decreased their calcium intake. A retrospective estimate of daily calcium intake averaged 850 mg/d for cyclic women and 1300 mg/d for those resuming menses. The two who remained amenorrheic maintained their calcium intake between 500-900 mg/d. There were no significant correlations between changes in vertebral density and duration of amenorrhea, changes in body weight, age, number of menses during the intervening period, initial BMD, or any training factor.

The resumption of menses in these athletes followed a decrease in training and an increase in body weight. Thus the authors cannot discern one or the other as the causal factor; or an interaction of the two events. The peak value of estrogen for the four women who were cyclic when the second bone measurements were done is almost identical to that of cyclic athletes (205.4 pg/ml) in the previous study (Drinkwater et al. 1984). From this, the authors presume that estradiol levels were elevated to similar levels during the months in which the women experienced menstrual flow. Thus, the authors believe that it is
likely that an increase in circulating estradiol, coincident with resumption of menses, was the primary factor in reversing bone loss in six of seven formerly amenorrheic athletes. The continued decrease in vertebral BMD of the two women who remained amenorrheic lends support to that hypothesis.

D. Stress Fractures in Amenorrheic Athletes

Another concern regarding the skeletal integrity of amenorrheic athletes is the high incidence of stress fractures reported by different investigators. Lindberg et al. (1984) reported that, in the year before their study, 49% of the amenorrheic runners had stress fractures, whereas no stress fractures occurred in eumenorrheic athletes and controls. Marcus et al. (1985) found that 55% of their amenorrheic runners suffered stress fractures compared to only 17% of their eumenorrheic runners. It was suggested that the high incidence might be related to the osteopenic state of these women combined with the skeletal stress of vigorous exercise (Cann et al. 1984), although factors such as rapid changes in training intensity and abnormalities in running stride cannot be excluded (Marcus et al. 1985).

Warren et al. (1986) surveyed the incidence of scoliosis and fractures in 75 female professional ballet dancers. Scoliosis was present in 24% of the dancers.
Menarche was delayed in all of the dancers by an average by 2 years. There was a significant correlation \( r = 0.25, \ p < 0.03 \) between the frequency of scoliosis and the increase in age at menarche. Scoliosis and secondary amenorrhea were related, with those dancers having scoliosis having longer periods of amenorrhea \( (11.4 \pm 18.4 \text{ vs. } 4.1 \pm 1.4 \text{ months}) \). Fractures were reported in 61% of the dancers and of these, 69% were stress fractures, predominantly in the metatarsals. The relationship between stress fractures and menarchal age was not significant, however, in those subjects with stress fractures the incidence increased with menarchal age \( (p < 0.01) \). Secondary amenorrhea was twice as prevalent and amenorrhea was longer in those dancers with fractures. Marked hypo-estrogenism was found in 7 of 10 subjects whose endocrine profiles were determined. The authors concluded that a delay in menarche and prolonged intervals of amenorrhea that reflect hypo-estrogenism may predispose ballet dancers to scoliosis and stress fractures.

Lloyd et al. (1986) performed a retrospective, three phase study to evaluate the effect of menstrual status upon musculoskeletal injuries in women athletes. Initially, they collected the menstrual and running histories of women participants in a regional 10-km footrace. In this study 61%
of the respondents reported a continuous running program, and 39% reported an interruption of at least three months of their running program. The most common cause for interruption was injury. The women who stopped their running program because of injury were more likely to have irregular or absent menses while running \((p < 0.001)\) and less likely to have used exogenous estrogen in the form of oral contraceptives during their running program.

Secondly, Lloyd et al. (1986) obtained information on the relationship between bone injury and menstrual status by reviewing the sports medicine records of 207 collegiate women athletes. This data was subsequently combined with data from a larger population of more serious but still recreational runners participating in a national 10-km race. X-ray documented fractures occurred in 9% of women athletes with regular menses and in 24% of women athletes with irregular or absent menses \((p < 0.025)\). Logistic regression analysis of the data showed that the risk of injury was independent of age, height, weight, menarche, age at which running started, days/wk spent running, minute/mile, and mile/run, but is dependent on oral contraceptive use \((p < 0.001)\), menstrual history \((p < 0.0291)\) and years of running \((p < 0.0013)\). Each of these associations is independent of the others. The authors conclude that
premenopausal women who have absent or irregular menses, while engaged in vigorous exercise programs, are at increased risk for musculoskeletal injury.

E. Mechanism of Reduced Bone Mass in Amenorrheic Athletes

The mechanism for the decrease in trabecular bone mass in amenorrheic athletes is unclear. Causative factors suggested by Drinkwater et al. (1984) include; the hypoestrogenic status of the amenorrheic athlete, the interaction of low estrogen levels with some other variable(s) such as diet, training, physical characteristics, and menstrual history, or, a factor that has not yet been identified.

Although it is generally accepted that low estrogen levels after menopause or in premenopausal women with endocrine dysfunction are related to the osteopenia that is observed in these groups, the role of estrogen in bone dynamics is not fully understood. Since estrogen receptors have not been found in bone, it is generally assumed that the estrogen effect on bone is indirect. Heaney (1982) suggests that one such indirect route may be the effect of estrogen on calcium balance since there is evidence that a lack of estrogen increases the daily calcium requirement.

Drinkwater et al. (1984) also suggest that there is an
interaction between estrogen and exercise in their effect on specific skeletal areas since physical activity did not protect their amenorrheic athletes, as well as the amenorrheic athletes studied by Cann et al. (1984), from an apparent loss of vertebral bone.

Cann et al. (1984) and Linnell et al. (1984) suggest the predisposition of thin, amenorrheic athletes to bone loss may be due to low estradiol production by the ovary and decreased estrone production. The latter would be due to a reduction in the peripheral conversion of androstenedione to estrone which normally occurs in adipose tissue. Thin eumenorrhic athletes may also exhibit low estrone levels, but their ovarian estradiol levels appear to be sufficient to maintain bone mineralization (Linnell et al. 1984). Marcus et al (1985) suggest that decreased bone mass in amenorrheic athletes may reflect either bone loss or arrested skeletal maturation, the latter reflecting altered hypothalamic-pituitary function during the pubertal growth spirt, especially in athletes who begin training before or shortly after menarche.

In the most recent communication by Drinkwater et al. (1986) they reported that amenorrhea per se may not be indicative of a below-normal bone mineral density. This statement was based on the results of one athletic amenor-
rheic (5.5 yrs) who had a bone mineral density well above the mean of cyclic athletes despite the fact that her peak estrogen level was 32 pg/ml. After resuming menses, this subject maintained but did not increase vertebral bone mineral density. However, the authors do conclude that the beneficial effect of physical activity on bone mass is attenuated in most hypoestrogenic women.

Lloyd et al (1987) reported that increased dietary fiber intake is associated with menstrual dysfunction and subsequent reduction in bone mass in oligomenorrheic college athletes. Studied were three groups matched with respect to age, height, and weight; eumenorrheic athletes, oligomenorrheic athletes, and eumenorrheic sedentary controls. Both groups of athletes exhibited higher lean body mass and lower fat composition than controls. Menarche was delayed in the eumenorrheic (13.1 y) and oligomenorrheic (14.3 y) athletic groups compared with the sedentary subjects (12.2 y) (p< 0.05). Oligomenorrheic athletes exercised on more days per week than eumenorrheic athletes but the total time spent exercising was similar. Average bone density (quantitative tomography) tended (p= 0.10) to be lower in the oligomenorrheic athletes (158 mg/ml) compared with the eumenorrheic athletes (184 mg/ml) or sedentary control subjects (173 mg/ml). The athletes consumed sig-
nificantly \((p< 0.05)\) more kilocalories, carbohydrate, calcium and phosphorus than sedentary control subjects. Oligomenorrheic athletes consumed significantly \((p< 0.05)\) more fiber than did control subjects or eumenorrheic athletes \((5.72\,\text{g} \text{ v. } 2.97\,\text{g}, \text{ v. } 3.52\,\text{g})\) respectively.

In explaining the manner by which dietary fiber affects menstruation, the authors refer to studies reporting the fecal excretion of estrogen by vegetarians being greater than that by nonvegetarians, suggesting that dietary habits and food fiber levels alter circulating estrogen levels.

The authors note, however, that there are potentially confounding variables among the study groups such as the difference in body fat content between the two athletic groups, and the possibility of the oligomenorrheic athletes being calcium deprived due to their probable hypoestrogenic state, despite a calcium intake of over 800mg/d.

To understand causative factors contributing to bone loss in amenorrheic athletes, is to understand the mechanism(s) of amenorrhea in athletic women. To date, no mechanism has been demonstrated. However, several descriptive characteristics of amenorrheic athletes have led to a variety of proposed mechanisms involving body composition, training regimen, reproductive maturity, sport specificity,
diet and psychological stress. Basal hormone concentrations in eumenorrheic women after training and in amenorrheic athletes, as well as transitory endocrine responses to exercise are the basis for these proposals. Thus, it is not known whether amenorrhea is some direct effect of exercise itself or an indirect consequence of some of the above stated conditions coincidentally associated with exercise. Methodological errors in previous studies with regard to subject selection, definition of training, body composition measurements, and blood sampling contribute to variability in reported results. In reviewing the available data on athletic amenorrhea, Loucks & Horvath (1987), suggest that new methods for studying hypothalamic pathways in humans need to be developed for alterations in the control of the reproductive system in athletes to be understood. Once that is understood, only then can the mechanisms and clinical significance of the side effect of athletic amenorrhea; such as bone loss, begin to be understood.

**Calcium Requirements in Amenorrheic Athletes**

Bone density depends on the amount of bone made during growth and its subsequent rate of loss. Although the rate of bone loss has received more attention in the study of the pathogenesis of osteoporosis, it is becoming increas-
ingly clear that insufficient accumulation of skeletal mass by young adulthood predisposes a person to fracture later in life as age related bone loss ensues (Riggs & Melton, 1986).

There is an increasing amount of evidence that the consumption of calcium-rich foods during adolescence is a predictor of peak bone mass (Allen, 1986). Matkovic et al. (1979) demonstrated the effect of dietary calcium on peak bone mass and fracture rate. Two communities were matched for ethnic origin, physical activity, and living conditions. The only significant difference between the two communities was their calcium intake. One community consumed a low-calcium diet of 350-500 mg/d, while the other community consumed a higher calcium diet of 800-1100 mg/d. Both men and women in the high calcium group showed greater bone mass and bone density than those in the low calcium group by age 35. Importantly, bone mass did not increase in either group after age 35. The rate of age related bone loss was similar for both groups; but by age 70, the men in the high calcium group had retained a bone mass greater than the initial peak bone mass of men consuming the low calcium diet. Overall, there was a lower incidence of hip fractures among men in the high calcium group; women in the high calcium group had a lower fracture rate after age 59.
This study suggests that the higher incidence of fractures and the lower bone mass of the population receiving the 350-500 mg calcium diet were related to a reduced peak bone mass. Thus calcium intake early in life, by its influence on bone mass may be a critical factor in later susceptibility to osteoporosis.

Sandler et al. (1985) did a retrospective assessment of associations between postmenopausal bone density and milk consumption in the early stages of the life span. The study involved 255 white women aged 49-66 years. Bone density was assessed with a CT scanner in the dominant radius. Current calcium intake was estimated by the method of the 3-day diet record. Calcium intake in childhood, adolescence, and adulthood was assessed retrospectively from responses to questions concerning frequency of milk consumption at various stages of life.

No significant correlation was found between the current calcium intake and the baseline bone density, but women who reported drinking milk with every meal at any of the life stages had currently a significantly higher average calcium intake than women who drank milk less frequently (p < 0.001). Controlling for factors such as obesity, stature and age, known to affect bone density did not alter these results. Both higher peak skeletal mass as
well as early acquired life long favorable dietary habits are apparently implicated in this effect.

Although the data from this study indicated that women who reported drinking milk with every meal during childhood and adolescence had significantly higher bone densities than women who reported drinking milk less frequently, the number of women drinking milk with every meal steadily dropped in successive stages, and only 6% of the women in mid-adulthood drank milk with every meal. Thus, although the habit of consuming milk, developed early in life, tends to influence later consumption, apparently it does not overcome the popular notion that milk is needed by the growing young only. Data from national food consumption surveys and nutrition and health statistics of the U.S. population indicate that two-thirds of adults ingest less calcium than the RDA of 800mg (Heaney et al. 1982). Specifically, calcium intake of males ranges from 550-1300 mg/d with intakes highest during late adolescence (mean 1100 mg/d) and lowest by men 50 years and older (mean 700 mg/d); for females, the range is 400-1050 mg/d with intakes lowest after 35 years of age (mean 540 mg/d) (Chin, 1981). Findings from the most recent HANES survey reveal that on any given day 50% of U.S. females 15 years of age and above consume three-fourths or less of the RDA for calcium, and,
that after age 35, more than 75% of U.S. women have calcium intakes below the RDA (Abraham et al. 1982).

The calcium requirement for females 15-18 years of age is 1200 mg/d; for females over 19 years of age it is 800 mg/d. This recommended intake is intended to provide sufficient calcium to maintain calcium balance. However, evidence is accumulating from various types of studies to suggest that calcium intake in excess of 800 mg/d is necessary to achieve optimal skeletal health particularly in estrogen-deficient post-menopausal and amenorrheic women (Chin 1982, Heaney et al. 1982, Heaney et al. 1977).

Heaney et al. (1977) suggested that a lack of estrogen increases the daily requirement of calcium. They studied 130 women, 30-35 years, consuming self-selected diets and found that the women required 1.24g of calcium/d to maintain zero calcium balance. Heaney et al. (1978) reported that the premenopausal women required 0.99g of calcium/d, while postmenopausal women required 1.5g of calcium/d to maintain zero calcium balance.

Drinkwater et al. (1984) reported no difference in the calcium intake of amenorrheic and eumenorrheic subjects with or without supplementation. Both groups met the current RDA of 800mg/d. However, the decrease in calcium absorption, and the increase in calcium excretion in
estrogen deficient women has led Heaney (1982) to recommend a daily intake of 1.5g of elemental calcium to maintain calcium balance in low estrogen states. When applying these criteria to Drinkwater's subjects, the amenorrheic women are deficient in calcium, whereas the eumenorrheic women are meeting their daily requirements. Berning (1984) reported that amenorrheic distance runners have nutritionally inadequate diets, including low calcium intakes (mean 525mg/d). These amenorrheic runners had significantly lower bone density (single photon absorption) than sedentary controls consuming 800mg calcium/d (p< 0.05). Normally menstruating runners also had low calcium intakes (mean 617mg/d) and bone density than the controls, but not the amenorrheic runners (p< 0.05).

Diet is the first approach used by Cann (1982) for amenorrheic women who are concerned about osteoporosis. The suggested calcium intake is 1200-1500 mg/d for women 19 years and older. Currently there are no dietary recommendations for amenorrheic females under 19 years of age whose RDA is already 1200 mg/d, but recently Riggs (1986), suggested that dietary calcium requirements should be increased to 1500 mg/d for normal adolescents. Thus, a high calcium intake early in life, by increasing bone mass at skeletal maturity, may be advantageous in terms of
reducing risk of fracture in later years.

**Prevention and Management of Bone Loss**

The mainstays of prevention and management of bone loss are estrogen and calcium supplementation.

The first long term prospective, controlled study of estrogen treatment for the prevention of bone loss was initiated by Lindsay et al. (1984). They studied 120 patients for five years. Of these patients 24 were followed from oophorectomy, 64 from three years after oophorectomy, and 32 from six years after operation. None had received estrogen therapy before. Sixty-three women were treated with estrogen (24.8 ug menstranol/day) and 57 women were treated with a placebo preparation. Every six months during the first three years, and thereafter annually, photon absorptiometry measurements were made at the mid-point of the third metacarpal (single photon absorptiometry). In 46 of the 64 patients who started attending three years after oophorectomy, photon absorption estimations were carried out, at similar intervals, at the mid radius site in addition to the mid-metacarpel.

During the five year follow-up, the placebo-treated patients, followed from two months after operation, lost bone at a mean rate of 1.22mg/cm/yr, which represents a
fall in bone density of 2.7%/yr. The mean bone density of the menstranol-treated group fell by 0.02 mg/cm/yr or 0.4% per year; a result which was significantly different (p<0.001) from the loss of bone experienced by the control group.

The authors concluded that estrogen therapy has short term beneficial effects in retarding bone loss after a natural or artificial menopause, that continued well into the fifth year of treatment. The rate of bone loss was greatest immediately after loss of ovarian failure (mean loss 2.7%/yr), diminishing after three or more years to a steady mean loss of about 0.7% per annum. The response of patients whose therapy was delayed for three to six years after oophorectomy indicates an important increase in bone density which seemed to be maintained for the five year period of follow-up.

A number of other groups (Horsman et al. (1977) and Recker et al. (1977) subsequently confirmed the data reported by Lindsay et al. (1977).

Horsman et al. (1977), in a prospective trial in 72 postmenopausal women compared the effects on bone loss of no treatment, treatment with estrogen (enthinyloestradiol 25 or 50 ug/d for three weeks out of four), and treatment with calcium (two calcium gluconate tablets/d, providing
approximately 800 mg elemental calcium). Women were followed for at least two years and examined densiometrically (single photon absorption of the left distal ulna and radius). Women in the untreated group continued to lose bone during the two years whereas the estrogen group lost none (p<0.001). Loss in the calcium group was less than in the control, but not than in the estrogen treated group (p<0.05). The authors concluded that estrogen treatment prevents, and calcium treatment retards, postmenopausal bone loss.

Recker et al. (1977) studied the effects of estrogen therapy on bone mineral balance in postmenopausal women and compared it with the effect of calcium supplements. Sixty postmenopausal women between the ages of 55 and 65 were randomly divided into three groups. Group I (n=20) served as controls; group II (n=18) was given conjugated equine estrogen, 0.625 mg and methyltestosterone, 5 mg, 21 days of each month; group III (n=22) was given 2600 mg of calcium carbonate (1.04 g of elemental calcium) daily (mean intake of 1.543 g/d for group).

Bone mineral content of the metacarpals (radiogrammetry) and radius (single photon absorptiometry) was measured at the beginning of the study and at six month intervals thereafter for two years in each patient. Whole body
retention of calcium was obtained using a whole body rectilinear scanner. After one year, 12 members of the control group, 10 members of the estrogen/androgen treatment group, and 15 members of the calcium carbonate group were subjected to calcium balance and kinetic studies.

After two years, skeletal mass, as determined by radiogrammetry decreased by 1.18%/yr in the control group, 0.15% in the hormone group, 0.22%/yr in the calcium supplemented group; and 2.88%/yr in the control group, 0.73%/yr in the hormone group, and 1.83%/yr in the calcium supplemented group as determined by photon absorptiometry. The treatment groups differed significantly from those in the control group (p< 0.05) except for measures made with photon absorptiometry in the calcium supplemented group. Bone accretion and resorption decreased in the treatment groups (p< 0.05) as measured by calcium tracer kinetics, resorption more so than accretion. The authors concluded that postmenopausal sex-hormone replacement measurably decreases age-related bone loss by suppressing bone turnover, resorption more than accretion; calcium supplements produce the same effect but, at the dose used, were slightly less effective.

In a follow-up study, Lindsay et al. (1987) performed lateral radiographs of the lumbar spine in the women who
participated in their original study (Lindsay et al. 1977). The evaluations confirmed vertebral deformities in some of the placebo treated women after ten years but not in the estrogen replete women. From this data the authors suggest that estrogen would prevent at least 90% of the vertebral fractures among post-menopausal women. Epidemiological data (Weiss et al. 1980) suggest that estrogen treatment for postmenopausal women might reduce the incidence of hip fracture by as much as 50%, to about the incidence in men. The effect would appear to be greatest if estrogen is prescribed early in postmenopause and continued for at least five years.

Recently, Riis et al (1987) examined the effect of percutaneous estradiol and natural progesterone on postmenopausal bone loss. Fifty-seven women, who had experienced a natural menopause six months to three years previously, entered the study. The women were allocated to two treatment groups, receiving either percutaneous estradiol (estrogel cream, 0.6 mg of 17B-estradiol per gram, 28 day cycle) (n=29) or placebo (n=28) for one year. After the first year of treatment the code was broken; all women receiving 17B-estradiol continued with a supplement of 200 mg of micronized progesterone (utogestan capsules, 100 mg) from days 13 to 24, and the placebo group continued the
placebo cream. The study was double blind the first year and single blind the second year. The women were examined every three months during the two years of treatment. After 12 months, bone mineral content in the forearms (single photon absorptiometry) and the spine and total skeleton (dual photon absorptiometry) showed a significant decrease of 5-7% in the placebo group (p< 0.001) whereas it remained constant in all bone compartments in the estradiol group. The administration of progesterone did not influence the results.

Serum estrogens, the biochemical estimates of calcium metabolism, and the intestinal radiolabeled calcium absorption were also determined. In the hormone group there was a highly significant increase (p< 0.001) in serum estrone and estradiol after three months of treatment. Thereafter serum hormone concentrations were virtually unchanged. There was no significant change in serum calcium in either group, whereas both serum alkaline phosphatase and fasting urinary hydroxyproline were decreased in the hormone group, reaching significance after 12 months treatment (p< 0.01 to 0.001). Intestinal radiolabeled calcium absorption was unchanged in both groups. None of the variables appeared to be influenced by the addition of progesterone. The authors concluded that percutaneous estradiol is effective
as preventive therapy of postmenopausal bone loss; the addition of progesterone does not influence bone or calcium metabolism; the biochemical calcium metabolic variables changed during treatment in the same way as with oral estrogen treatment, that is, a decrease to the premenopausal level of bone turnover rate.

Additionally, the study demonstrated that four bone mass measurement systems (proximal bone mineral content, distal bone mineral content, total body bone mineral content and bone mineral density of the spine) were able to show the difference between groups. It was noted, however, that the standard error of the mean was lowest for the proximal bone mineral content (0.7% to 0.8% at 24 months) and highest for the bone mineral density of the spine (1.5% to 2.7% at 24 months). The authors believe this indicates that study of more patients is required to demonstrate the response to estrogen treatment if the spin scanner is used than if the forearm scanner is used.

Ettinger et al. (1987) designed a study to examine the rate of bone loss in untreated women undergoing spontaneous menopause and to test for possible protective effects of estrogen or calcium. Specifically, they tested the effectiveness of 0.3mg/d of conjugated estrogens in women having spontaneous menopause who were concurrently given calcium
supplements. They hypothesized that the effects of these two therapies might better prevent bone loss.

White, Asian and Hispanic women between the ages of 50 and 59, who had had spontaneous menopause 6 to 36 months before entry into the study served as subjects. Twenty-five women served as controls; they simply maintained their usual dietary calcium intake; 33 chose to supplement their dietary calcium intake up to 1500 mg/d by taking 1000 mg/d of oyster shell calcium (Os-Cal) in two divided doses; and 15 choose 0.3 mg/d of conjugated estrogens (Premerin) combined with the same calcium supplementation. Bone mineral content at the radial diaphyseal site was measured by single-photon absorptiometry. Quantitative computed tomography of the L1 and L2 vertebrae was used to measure spinal trabecular mineral content. These measurements were obtained at yearly intervals.

The treatment groups were not significantly different with regard to any baseline measurement. The range of total calcium intake from diet and from any supplemental calcium was 258 to 994 mg in control women, 1144 to 2950 mg in those taking calcium alone, and 1463 to 2498 mg in those taking calcium with estrogen. The mean daily intakes (± SD) were 662±208, 1863±369, and 1733±279 mg in the three groups.
After two years of follow-up, comparisons between the mean 2-year values and the mean baseline values showed a significant reduction of 9.0% (p= 0.002 compared with baseline) in untreated women and a mean of 10.5% (p= 0.001) in women given calcium supplements alone. The radial bone mineral content was also reduced by 2.1% (p< 0.05) for untreated women and 0.9% (p< 0.02) for calcium treated women.

Women taking both estrogen and calcium showed no significant changes in spinal trabecular mineral content (+2.3%), radial bone mineral content (+1.6%), and metacarpal combined cortical thickness (-0.2%). When compared with untreated women or with those taking calcium alone at 2 yrs, women treated with both estrogen and calcium had significantly greater preservation of spinal trabecular mineral content (p= 0.001 for both) and radial mineral content (p= 0.12, p= 0.09).

The following variables were not significantly related to loss of spinal mineral: age, months of menopause, race (descent), height, weight, parity, smoking habit, calcium intake, and level of activity. The basal levels of all three skeletal measurements or the changes in the radius or metacarpal after two years did not correlate significantly with changes in spinal mineral content. In the 56 women
not receiving estrogen, there was no relation between calcium intake and the spinal mineral loss after two years or between activity level and spinal mineral loss after two years. Estrogen use was the only independent variable showing a significant correlation in the stepwise multiple regression analysis. The authors conclude that calcium supplements alone do not protect against the accelerated bone loss that occurs at the menopause. However, by adding calcium to the diet, the amount of estrogen necessary to exhibit a protective effect on skeletal bone may be reduced, and thus, more acceptable to women.

A. Mechanism of Estrogen

Although the effectiveness of estrogen in prevention of bone loss is well known, the mechanism of action is far from evident.

Estrogen reduces urinary calcium and hydroxyproline levels, presumed to result from reduced skeletal turnover (Lindsay et al. 1987). Estrogen is also responsible for a serum phosphate reduction that is associated with an increased excretion of phosphate, which, in turn is characteristic of increased parathyroid hormone activity (Lindsay et al. 1976).

Data suggest that estrogen improves the intestinal
absorption of calcium (Heaney, 1982, 1986). Women with decreased estrogen absorb a smaller percentage of calcium (dietary and supplemented) than women with normal estrogen or women taking estrogen. Estrogen acts on the kidney to increase the enzyme 1a-hydroxylase (Bell, 1985). This enzyme is responsible for activating 25-OH-D3 to 1,25-OH-D3; the metabolite of vitamin D that functions to increase absorption of calcium, and to increase tubular reabsorption of calcium. It has been reported (Gallagher et al. 1979) that patients with post-menopausal osteoporosis have a significant decrease in 1,25-OH-D3.

Although there is data to support the hypothesis that administration of calcitriol increases calcium absorption (Gallagher et al. 1982; Riggs and Nelson, 1985), there is little evidence to support the direct stimulation of PTH or vitamin D by estrogens in humans. In search of an estrogen action that could explain the skeletal response, Bell et al. (1985) suggested that there is a direct increase in the secretion of calcitonin in response to estrogen. The only important biological action of calcitonin described so far is inhibition of osteoclast activity, and thus reduction in bone resorption. The observed biochemical effects of estrogen administration could follow this response. Further studies of the mechanism of estrogen action are
clearly needed in this important area of skeletal physiology.

B. Calcium Supplementation

To date, few studies evaluating the effect of calcium supplementation, independent of estrogen, on bone mass have been carried out. The two most widely cited studies are those by Horsman et al. (1977) and Recker et al. (1977).

These studies are generally considered to provide at least suggestive evidence of a preventive effect of calcium on bone loss in post-menopausal women. As presented earlier, Horsman et al. (1977) reported an intermediate bone loss in post-menopausal women supplemented with 800 mg elemental calcium/d for two years. That is, these women did not lose as much bone as did the controls, but lost more than the women treated with estrogen.

Recker et al. (1977) reported similar results when comparing the bone density of women treated with estrogen to those taking 1.04g calcium carbonate/d, to those serving as controls.

Nilas et al. (1984) studied 103 early postmenopausal women who received a daily supplement of 500 mg calcium carbonate for two years. All had a normal menopause six months to three years before. The participants were stratified into the following three groups according to their
calculated calcium intake. Group I had an intake below 55 mg/d (mean 430 mg/d); group II had an intake of 550 to 1150 mg/d (mean 880 mg/d); and group III had an intake above 1150 mg/d (mean 1640 mg/d). Overall the 103 women had a mean daily calculated total calcium intake of 910 mg/d (range 390-2350). Bone mineral content on the distal part of the forearm (single photon densiometry) and urine and blood biochemical values were determined at the start of the study and every three months for two years.

Results indicate that the initial and final values for bone mineral content in the three groups did not differ significantly. The changes in mean serum concentrations of calcium, phosphate, and N-terminal parathyroid hormone were small, insignificant and did not differ among the groups. There was continuous bone loss in all three groups, the yearly loss reaching 1.9%, 1.6%, and 2.0% respectively during the two years (p< 0.001). When the subjects were stratified according to initial calcium excretion rate no differences were seen in the rate of bone loss; nor when stratified according to the rate of bone loss, were there any differences in the initial or final calcium excretion values, and the estimated dietary calcium intakes were similar in the three groups. When comparing this data to data presented by Heaney et al. (1977) who reported that calcium
balance and intake are related and that in post-menopausal women (at least 6 months since her last menstrual period) a calcium intake above 1400 mg daily will lead to a positive calcium balance; the authors (Nilas et al. 1984) suggest that factors in addition to calcium intake and excretion determine bone mass. Further, the authors concluded that a calcium intake of 1000-2000 mg daily has little effect on loss of bone calcium in the early menopause.

Recently Riis et al. (1987) repeated the study by Nilas et al. (1984). Specifically, the purpose of their study was to examine the effect of 2000 mg of calcium daily, as compared with estrogen supplementation and placebo, on early post-menopausal bone loss. The authors choose 2000 mg of calcium daily based on the lack of effect demonstrated by a dosage of 500 mg daily in their last study (Nilas et al. 1984).

Forty-three women were treated with one of the following: 3 mg daily of percutaneous 17B-estradiol (28 day cycle) plus placebo calcium (n=15); placebo estrogen cream plus 2000 mg daily of calcium carbonate (n=15); or placebo estrogen cream plus placebo calcium (n=13). To study the
cyclic supplementation of 2000 mg of progesterone from days 13 through 24. The other two groups continued with the double blind design to examine the effect of calcium as compared with placebo. During the two year treatment all participants were examined every three months.

The bone mineral content of the forearms was measured by single-photon absorptiometry. The bone mineral density (ratio of bone mineral content to area) of the lumbar spine was measured by dual photon absorptiometry. The bone mineral content of the entire skeleton was likewise measured by dual photon absorptiometry on a whole body scanner. Intestinal absorption of calcium was measured by modification of the single isotope technique. The serum concentration of 25-hydroxyvitamin D was measured with a competitive protein binding assay, and 1, 25, dihydroxyvitamin D was measured with a receptor assay. Three measurements were used to assess the rate of bone formation. Serum alkaline phosphatase was measured enzymatically; plasma bone Gla-protein, a bone protein that reflects bone formation, was measured with a radioimmunoassay; whole-body retention of Tc-labeled diphosphonate was determined to assess an overall estimate of bone formation. The ratio of bone resorption was reflected by the fasting urinary excretion of hydroxyproline.
In the groups treated with calcium or placebo, the bone mineral content in the four bone compartments fell significantly \((p < 0.001)\), representing a bone-loss rate of 4 to 8 percent over two years. In the estrogen group however, the bone mass was unchanged throughout the study. The mean slopes (calculated from the bone measurement as a function of time) showed a significantly \((p < 0.05)\) slower rate of bone loss in the calcium group than in the placebo group when measured in the proximal forearm (mainly cortical bone); the same trend was seen for the entire skeleton (also mainly cortical bone) although it was not significant. There was no difference between the calcium and placebo groups in the bone mineral content in the distal forearm and the bone mineral density of the spine.

The estrogen group had a significant fall in all indices of bone accretion (serum level of alkaline phosphate, plasma level of bone-Gla-protein, and urinary excretion of Tc-phosphate) and in bone resorption (fasting urinary hydroxyproline) \((p < 0.01 \text{ to } 0.001)\) after 24 months of treatment. No significant changes were seen in either the calcium or the placebo group in any of these variables during the trial. In the calcium group, a significant decrease was observed in intestinal calcium absorption at 12 months of treatment \((p < 0.005)\) but at 24 months the dif-
ference had disappeared. There was no significant change in intestinal calcium absorption in the estrogen or placebo group.

The results indicate that calcium supplementation has a preventive effect on the loss of compact bone. Calcium however had no effect on bone mass in the spine or in a more distal part of the forearm with a higher content of trabecular bone. The authors suggest that an effect of calcium on trabecular bone in women early in the post-menopausal period is overridden by the high rate of bone turnover during this period of life, but it may become apparent later in life when the bone turnover rate has fallen.

From this study, the authors conclude that dietary calcium supplementation in the dosage used cannot be regarded as an effective alternative to estrogen or progesterone hormone replacement in the prevention of early post-menopausal bone loss. In contrast to estrogen treatment, which prevents bone loss throughout the skeleton, calcium treatment as compared with placebo seems to have an effect in retarding bone loss from cortical bone, but not from trabecular bone.

Thus, all studies reporting on calcium supplementation and bone mineral mass seem to be in agreement that calcium
may be effective in retarding cortical bone loss, but not trabecular bone loss, for which estrogen is required. Ettinger et al. (1987) basically concurred with these results in their report that taking estrogen, or combining estrogen therapy with calcium supplementation is more effective than calcium supplementation alone in preventing further bone loss in early post-menopausal women.

Polley et al. (1987) also studied the effect of calcium supplementation on forearm bone mineral loss in post-menopausal women, but came to different conclusions than the preceding authors (Ettinger et al. 1987; Riis et al. 1987). The purpose of this study was to establish whether calcium supplementation, with or without salt restriction, would reduce the rate of bone loss in the calcium-supplemented subjects as compared with their preceding rate of loss and as compared with the rate in untreated concurrent controls.

Two hundred and ten post-menopausal women with calcium intakes below 1000 mg participated in this study. The subjects were allocated to three treated and three untreated control groups. Groups 2 and 3 were selected at random for either supplementation with extra dairy products to yield a total calcium intake of at least 1250 mg (group 2) or supplementation with 1000 mg elemental calcium (glu-
conate, lactate, carbonate, and 370 mg (16 mmol) of sodium) to yield an intake of about 1700 mg calcium/d (group 3). Subjects in Group 1 (salt restriction) were selected at random from the same set for dietary product supplementation with an upper limit of 1950 mg (85 mmol) sodium per day. Simultaneous controls were chosen at random from each group to set a control to treated ratio of 1:3.

Forearm densitometry was determined at the beginning of the study, again 9 months later (period 1 when the intervention began and a third time at 18 months (period 2).

In the nine months before intervention, (period 1) there were highly significant (p< 0.001) falls in forearm mineral content (FMC) in all groups with no significant differences between them and no differences between the controls. After intervention, group 1 and group 3 demonstrated significant increase in FMC (p< 0.025). There was no significant difference in the controls FMC rate of change, but they appeared to lose FMC at a faster rate than did group 2. Thus, the authors report that they have shown that 136 normal post-menopausal women who were losing bone at a significant rate, when consuming a mean calcium intake of just over 700 mg daily, ceased to lose bone when their mean calcium intake was raised to about 1400 mg. When
treated groups were pooled and compared to controls, there was a significant difference in FMC ($p < 0.01$) between the two groups. When the data were confined to the subjects within 10 years of menopause (160) in all the effect of calcium became more significant. In this subset, the control group lost bone at a significant rate in period 2 whereas calcium-treated subjects did not ($p < 0.001$).

The authors conclude that their data are compatible with the concept that although calcium may be useful at any age, its effect may be statistically demonstrable only within 10 yrs of menopause when untreated controls are losing bone at a significant rate. When comparing their data to other workers who have failed to show an effect of calcium supplementation on vertebral bone loss (Ettinger et al. 1987; Riis et al. 1987) the authors suggest their effervescent mixture of calcium gluconate, lactate, and carbonate given in a single evening dose may be why bone rate was reduced. The authors believe it physiologically unlikely that calcium supplementation controls forearm bone loss but not vertebral bone loss in post-menopausal women who have experienced the onset of menopause within the past 10 years.

The authors (Polley et al. 1987) fail to acknowledge that Ettinger et al. (1987) and Riis et al. (1987) did
report a reduced loss of cortical bone, as measured by forearm densiometry, in post-menopausal women taking calcium supplements. Further, the authors did suggest that taking calcium supplements will promote a reduced rate in cortical bone loss but not in vertebral bone. Thus it may in fact be very likely that calcium supplementation affects cortical bone but not vertebral bone in post-menopausal women who have experienced the onset of menopause within the last ten years.

**Types of Calcium Supplements**

Postmenopausal and amenorrheic women have difficulty obtaining the higher levels of calcium recommended and may choose to take supplementary calcium (Solomons, 1986). Several forms of calcium supplements are available, but the amount of elemental calcium they contain varies widely. Calcium gluconate, lactate, and carbonate contain 9%, 13%, and 40% calcium respectively (108mg, 156 mg, and 480 mg elemental calcium respectively). Bone meal and dolomite are not acceptable sources of calcium because they may contain harmful amounts of lead, arsenic, mercury, and other potentially toxic metals (Slavin, 1985).

Many calcium supplements, especially those containing oyster shell calcium, also supply 125 or 25% of the USRDA for vitamin D. Without vitamin D the body cannot manufac-
nature the hormone 1,25, OH2 D3 needed for absorbing calcium. However, most people get enough vitamin D from sunlight, eggs, fatty fish, and fortified milk. For the most part, only the elderly may be exceptions, since they tend to spend less time outdoors and absorb less vitamin D from food than young people (Chapuy et al. 1987).

Calcium in a food or tablet is of no value if it is not absorbed; hence bioavailability is an important consideration, particularly with respect to supplements and fortified foods (Heaney, 1986). With the exception of spinach, the calcium of which is not very available, most natural foods seem to exhibit roughly similar availability. Smith et al. (1985) reported that calcium absorption from a typical mixed diet average 25% to 35% in women aged 20 to 60, and studies of isotopically labeled milk, chocolate milk, yogurt and cheese have shown that the calcium of these dairy products is absorbed at this same efficiency and that the sources are indistinguishable from one another (Recker et al. 1987; Smith et al. 1986).

Most of the supplements on the market today have not been adequately tested nor have most of the fortified foods. Thus, it is not known to what extent differences in bioavailability may influence the overall impact of a calcium supplement on calcium homeostasis or bone remodeling.
Also, little information is available concerning even the acute effects of supplemental calcium given as commonly prescribed, that is, in relatively large amounts taken with other nutrients.

Goddard et al. (1986) studied the short-term effects of calcium carbonate, lactate, and gluconate on the calcium parathyroid axis in normal elderly men and women. Subjects were 42 individuals (25 women, 17 men) aged 52-85 yr. The women were all post-menopausal; eight were currently taking estrogen replacement therapy. Fasting subjects were administered a standardized one gram calcium load on three occasions, using calcium carbonate, lactate, and gluconate in random sequence with 8 oz. milk or orange juice as carrier. Blood and urine were collected at baseline and followed for 6 hours following the load.

In response to oral calcium given with milk, serum urinary calcium rose significantly (p< 0.001) in all subjects. The increase in urine-calcium excretion continued over the duration of the test. With lactate and carbonate, the rise in calciuria was progressive through the last collection period; the response to gluconate declined after the third collection. Peak calciuria after carbonate, gluconate, and lactate did not differ significantly.

The rise in serum-calcium concentration progressed
through the third collection period following calcium lactate and carbonate. In response to calcium gluconate, the serum calcium concentration rose to a plateau level by 2 hours, and declined by the last collection period. The peak rise in serum calcium following lactate and carbonate did not differ significantly, but were both greater than the calcemic response to calcium gluconate (p< 0.01).

With orange juice as the carrier, significant rises (p< 0.001) in serum and urine calcium were also observed with each calcium salt. The calcemic responses were indistinguishable from those seen with the milk carrier, but the calciuric responses were approximately one-third lower with orange juice. The calciuric responses to the three salts did not differ significantly. The peak calcemic responses to carbonate and lactate exceeded that to gluconate, but this difference was marginally significant (Goddard et al. 1986)

In response to oral calcium (carbonate, lactate, and gluconate), serum iPTH decreased significantly (p< 0.001) and remained below basal levels throughout the test. None of these changes differed significantly, one from another. Phosphate excretion changed in a consistent manner following calcium ingestion. In each case phosphaturia rose significantly during the first post-calcium collection,
decreased during the next 2 hours, and rose during the final collection. Urinary excretion of cAMP did not change significantly at any time during the test.

The intent of this study was to examine the overall impact on calcium handling of clinically relevant doses of calcium salts, given as frequently prescribed. The results showed that acute responses to three commonly prescribed calcium salts were similar in a group of normal elderly volunteers. However, there was a subtle, yet significant difference observed in the time course following calcium administration. Unlike the response to the carbonate and lactate salts, the peak effects of calcium gluconate were reached by the end of the second collection period. In a previous balance study, Spencer et al. (1966) showed that calcium balance is more positive when calcium is provided as lactate rather than gluconate.

Heaney, (1987) reported that calcium carbonate is absorbed about as well as in food calcium, however it is recommended that this product be given with meals as absorption is poor in some women who take calcium carbonate on an empty stomach. Contrary to popular belief, gastric acidity is not necessary for absorption if calcium carbonate is coingested with meals (Recker, 1985). Solomons, (1986) reported that calcium carbonate may produce less
remodeling and strengthening of demineralized bone than dairy calcium. Recker and Heaney (1985) showed that 24 oz. of milk each day improved calcium balance and suggested that milk might be superior to calcium carbonate because it appeared to inhibit bone remodeling to a lesser degree. The authors stated that there was no apparent reason for the difference in remodeling rates between calcium carbonate and milk (Recker & Heaney, 1985). However, distaste for dairy products and lactose intolerance are both common, especially in the elderly and limits the widespread acceptability of milk (Goddard et al. 1987; Solomons, 1986).

Tablet formulation is an important additional factor in bioavailability of calcium from supplements. Some products are formulated precisely to break up in the stomach, and make calcium readily available for absorption. But other products, particularly certain generic calcium supplement tablets are so poorly formulated that they pass through the gastrointestinal tract essentially unaltered (Carr & Shangraw, 1987). Krzykowski et al. (1986) fed rats various calcium supplemented milk, finding minor differences in nonfat dry milk, calcium phosphate, oyster shell calcium, calcium carbonate, calcium lactate and chelated calcium as the calcium source. In some studies, calcium citrate has been reported to be better absorbed than food
calcium, and about the same as food calcium in other studies. (Heaney, 1987) No mention was made of whether these studies used different brands of calcium citrate. Calcium phosphate however, seems to be better tolerated, but somewhat less well absorbed than calcium carbonate or food calcium. Further testing of calcium phosphate is suggested (Heaney, 1987).

It is recommended that calcium dosages, as part of a multicomponent treatment program to help arrest further bone loss should be in the range of 1.5 to 3 gm/d, (Heaney, 1987); administered in multiple doses over the day (Goddard, 1986; Heaney, 1987). To be therapeutically useful, net absorption should be in excess of 300 mg/d.

The data reported by Goddard et al. (1987) provide little physiological basis for deciding between carbonate and lactate salts for normal elderly men and women. However, because calcium carbonate contains 40% calcium by weight, fewer pills are required to achieve a given total dose of calcium than with calcium lactate. That calcium gluconate has a shorter duration of effect supports the previous suggestion (Spencer et al. 1966) that this agent may be a less desirable agent.

Assessment of Bone Mineral Content

In detecting osteopenia, it should be noted that as
much as 30% of the bone can be lost from the vertebral body before that loss can be confirmed radiographically (Lane et al., 1984).

Quantifying bone mass and following its status during treatment can be accomplished using noninvasive diagnostic procedures. These techniques include tests such as cortical thickness measurements on appendicular bones, grading of structural changes in trabecular recognizable on x-ray films of the hip (Singh Index), and actual measurements of bone mineral by techniques such as neutron activation analysis (total body and regional measurements), compton scatter analysis (heel), quantitative computed tomography (QCT) (spine, radius, and hip), and photon absorptiometry (total body, spine, hip, radius, and heel (Wahner, 1986).

Clinically, the most promising result of this effort is the introduction of QCT and photon absorptiometry. Of these two the latter is more widely available and has been studied more.

In dual photon absorptiometry, the mass of bone mineral is measured in the lumbar spine (L2-L4) or the proximal femur. Total cortical and trabecular mineral is measured. In the spine, this is about 35% cortical and 65% trabecular bone. Bone mineral in the total skeleton can also be measured. The result is expressed as bone mass per unit
of area scanned (gm/cm²) and referred to as bone mineral
density (BMD) or area density. In single photon absorp-
tiometry, the bone mineral mass is measured in a cross-
section of the radius (or other long bone) one centimeter
in axial length. The unit is gm/cm, referred to as bone
mineral content (BMC). In QCT, trabecular bone is measured
from the center of several vertebral bodies from the lumbar
spine. The unit is gm/cm³, or density of anatomical tra-
becular bone tissue. Bone mineral in the entire vertebral
body can also be obtained but is of less value.

Several considerations should be addressed when deter-
mining which site to measure. Since fractures occur most
frequently at the spine and later in life at the hip, diag-
nostic efforts should be directed toward predicting frac-
tures at the spine and the hip. In selecting a procedure,
one may choose to measure bone mineral at these potential
fracture sites with either of the two methods or to measure
other generally appendicular skeletal sites as predictors
of spinal (and hip) bone mineral or fracture risk. There
is now undisputed evidence that trabecular bone in the
axial skeleton (hip, spine) has a higher turnover than cor-
tical bone, probably because of its greater surface area
(Riggs, 1986). Changes in bone mass that occur with disease
and therapy have an earlier onset and are most pronounced
at these trabecular bone sites. This is also true for osteoporosis and for age-related bone loss (Wahner, 1987).

In contrast, trabecular bone at appendicular bone sites where the bone marrow contains only fat is relatively inactive metabolically. Since 80% of the skeletal mass consists of cortical bone, total-body bone mineral measurements performed by dual-photon absorptiometry or neutron activation analysis do not appear sensitive enough for the early detection of bone loss in axial trabecular bone sites. The same is true for measurements on the midradius or distal one third of the radius where more than 90% of the bone mass is cortical bone (Wahner, 1985). Studies of other appendicular skeletal sites with more trabecular bone, for example the ultradistal radius with 60% and the calcaneus with 80%, have shown that although bone mineral at these sites is well correlated with bone mineral in the spine or hip, the correlation is not good enough for predicting bone mineral in the spine when managing a given patient; the error of such a prediction is between 12% and 15%.

In normal young adults, there is a high correlation of bone mineral in different areas of compact bone ($r > .9$), a moderate degree among areas of trabecular bone ($r > .7$ to $.5$), and even less correlation between areas of compact
and trabecular bone (Wahner, 1987). This correlation decreases when age and disease are included. For patient management, it is therefore best to use the spine as the reference site when osteoporosis is considered.

The accuracy in predicting the bone mineral content of the spine or hip from measurements of the appendicular skeleton is generally recognized, but it is not known if this is also true in predicting fracture risk at the spine or hips (Wasnich, 1985). This is an important issue because the use of single-photon absorptiometry may result in significant savings to the patient.

The use of QCT for bone mineral measurements is advantageous because CT instruments are generally available and measure trabecular bone at the sites of the fracture. Clinically useful measurements can be made with standard Ct instruments when special attention is given to strict quality and standardized calibration phantoms (Cann, 1980).

Compared with QCT for the spine, absorptiometry is more accurate and reproducible and the radiation dose and the cost of a test are lower (Wahner, 1987). For repeated studies and for studies of population groups, the dual-photon absorptiometry procedure is preferred.

**Summary**

It is now well documented that a substantial portion
of women athletes will develop exercise-induced menstrual dysfunction along with other asymptomatic changes which may seriously affect their reproductive physiology and bone metabolism. Compared with eumenorrheic control subjects and normally menstruating athletes, amenorrheic and oligomenorrheic athletes manifest reduced bone density, reduced circulating estradiol and progesterone, and increased musculoskeletal injury rates. The underlying physiologic mechanisms relating menstrual dysfunction to changes in bone metabolism have not been elucidated nor has the cause of exercise-induced amenorrhea been identified. Previous research has proposed mechanisms relating to: genetics, body composition, training regimen, reproductive maturity, sport specificity, diet, and physiological stress. Thus the treatment of choice regarding athletic amenorrhea and subsequent risk of reduced bone mineral density is currently being debated among the following; change of lifestyle (athletic regimen), hormone therapy and/or calcium supplementation.
CHAPTER III

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Changes in Bone Density
in Calcium Supplemented Adolescent Athletes
Experiencing Menstrual Dysfunction

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CHANGES IN BONE DENSITY
IN CALCIUM SUPPLEMENTED ADOLESCENT ATHLETES
EXPERIENCING MENSTRUAL DYSFUNCTION
by
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(ABSTRACT)

Thirteen adolescent runners experiencing menstrual dysfunction (mean no. menses 5/yr) received dietary supplements of 1200mg calcium carbonate and 400 IU vitamin D/d for 12 mos. Bone mineral content of the axial skeleton was measured by dual photon densiometry at the beginning and end of the 12 month period. Fasting plasma total and ionized calcium, intact PTH, 1,25(OH)2D3, estradiol, and thyroxin were determined at the beginning and end of the study. Seven day dietary recalls were obtained for two separate weeks during the course of the study, one prior to the onset of supplementation and another six months later. Mean bone mineral density of the athletes' increased (p< .05), but bone mineral density was observed to decrease in two of the subjects who had the lowest estradiol concurrent with the severest training regimen. Athlete's total plasma calcium and 1,25(OH)2D3 increased (p< .05). No measured variable
correlated with the bone mineral density of the lumbar spine. There was a significant correlation between dietary calcium intake and total plasma calcium \( (r = 0.50) \), and dietary vitamin D intake and \( 1,25(OH)2D3 \) \( (r = 0.50) \). Stepwise linear regression predicted a significant \( (p < 0.15) \) relationship between serum estradiol and bone mineral density, and no. menses/yr and bone mineral density. Multiple linear regression procedures indicated several predictive models incorporating variables that significantly affect bone mineral density. The models include plasma estradiol, no. menses/yr, body mass index, dietary calcium and dietary vitamin D intake. The increase in dietary calcium intake \( (192\% \text{RDA}) \) and/or dietary vitamin D intake \( (190\% \text{RDA}) \) due to supplementation did not appear to contribute to an increase in bone mineral density in estrogen compromised female athletic adolescents independent of growth.
Introduction

It is now recognized that a substantial portion of female athletes will develop exercise-induced menstrual dysfunction along with other asymptomatic changes which may seriously affect their reproductive physiology and bone metabolism (1). Compared with eumenorrheic control subjects, amenorrheic athletes manifest reduced bone density (2), reduced circulating estradiol and progesterone (3), and increased musculoskeletal injury rates (4). The underlying physiologic mechanism relating menstrual dysfunction to changes in bone metabolism have not been fully elucidated.

Previous studies have partially characterized nutritional and hormonal patterns in college students (5-8). However, these previous studies compared the nutritional and bone density patterns of eumenorrheic athletes with those of amenorrheic athletes. This study examines the effect of calcium and vitamin D supplements on bone density and hormonal indices controlling calcium metabolism in ado-
Methods

Experimental Subjects. Thirteen adolescent athletic females and ten sedentary adolescent females aged 14-18 were recruited from area high schools to participate as subjects. Because criteria for participation included that subjects be Caucasian and have less than 22% body fat, data for 3 of the sedentary subjects are not included. This decreased the mean age of the control group from 16 to 14.

All subjects received an oral and written explanation of the purpose of the study and procedures to be followed; all gave written consent prior to the beginning of the study. All procedures were approved by the University Institution Review Board for Research Involving Human Subjects. Each subject completed a detailed medical history questionnaire which included information regarding their exercise regimen and menstrual history. None of the athletes were using oral contraceptives.

Study Protocol. The thirteen athletic subjects participated in the study for 12 months; throughout the course of the study, beginning after initial testing, they were provided with calcium supplements containing 1200mg calcium carbonate and 400 IU vitamin D per two tablets. The subjects were instructed to take two tablets per day with
their morning meal for the duration of the study. No other dietary restrictions were imposed. Pretest and posttest protocols were identical (Figure 1.) The seven sedentary control subjects completed posttest bone measurement, dietary analysis, and body composition determination only (Figure 1.)

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Insert Figure 1 Here

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Experimental Procedures

**Bone Mineral Density.** Lumbar bone mineral density of the skeleton was determined by the dual photon densiometry technique originally described by Riggs et al. (9).

**Dietary Analysis.** Athletes' seven-day food intake records were collected prior to supplementation, and again at six months to determine if subjects' dietary intake varied over the course of the year. Subjects received oral and written instructions regarding documentation of dietary intake. A dietitian discussed the completed food records with each subject for further clarification regarding intake. Athletes' food records were analyzed for nutrient content using the Computerized Nutritional Analysis System, Department of Experimental Statistics, Louisiana State Uni-
versity. Control subjects completed 24 hour food recall intake records at the time of their bone mineral density test which were analyzed for nutrient composition using the Nutritionist III software package (N-Squared Computing, Inc., Silverton, OR).

**Body Composition.** As part of the determination of bone mineral density, all subjects were weighed on a physicians' scale and weight was recorded to the nearest 0.5kg, and height was recorded to the nearest cm. These data were used to determine subjects' body mass index (10).

Additional anthropometric measurements were determined on athletic subjects. Skinfold thickness, measured with Lange calipers (10mm$^2$ constant pressure), was determined at three sites; the triceps, supra-illiac, and thigh; the average of repeated (3) trial values was used as the representative score for a given site for estimation of body fat in adolescents (11).

**Sample Collection and Analysis.** Fasting blood samples (30mls) were taken from the anticubital vein by using a plastic 10 cc syringe and a 1 1/2 inch, 21 gauge needle. Blood was taken over a period of 3 to 4 minutes with minimum vacuum to reduce hemolysis. Blood was transferred to mineral free heparinized plastic tubes and centrifuged for
25 minutes at 2000 rpm. Serum was immediately separated from the red cells using an acid washed mohr pipet. Serum was stored, frozen and subsequently analyzed for mineral and hormone determination. All of the athletes had blood taken on the same day. Pre and post samples were taken approximately at the same time of the year so that subjects would be in similar training regimes.

Mineral Determination. Plasma was diluted (100 + 4.9mls 0.3% lanthanum=1:50) and analyzed for total calcium and total magnesium determination using a Perkin-Elmer Model 503 atomic absorption spectrophotometer (Perkin-Elmer Corporation, Norwalk, CT) equipped with a titanium-3-slot Boling burner head and with appropriate lamps for the two elements. Recovery samples consistently run at intervals in this laboratory indicate recoveries between 95 to 99 percent. All analyses were done in duplicate with duplication in the range of 2 to 5 percent.

Plasma ionized calcium was determined by a calcium specific electrode (Radiometer America Inc., Westlake, OH). Values were adjusted to correct for the change in pH that occurred due to the samples being frozen (Radiometer America Inc., Westlake, OH).

Hormone Analysis. Serum 1,25(OH)2D3 (Incstar, Stillwater, MN), estradiol and thyroxin (Diagnostic Products
Corp., Los Angeles, CA) intact PTH (Allegro Corp., Nicholas Institute, San Juan Capistrano, CA), were determined by radioassay. Coefficients of determination, evaluated by duplicate measurements of the same sample for 1,25, (OH)2D3, estradiol, thyroxin and intact PTH are 0.97, 0.99, 0.99, and 0.98 respectively. The within assay coefficients of variance of 1,25(OH)2D3, estradiol, and intact PTH were 9%, 6%, 8%, and 6% respectively. Intact PTH concentration less than the 14 pg/ml standard were assigned a minimal value of 14 pg/ml.

Data Analysis. Statistical Analysis Systems (SAS, Cary, N.C.) computer programs were used to conduct statistical analyses. Paired t-tests were performed to evaluate differences between athletes' pretraining and posttraining values for serum ionized calcium, total serum calcium, intact parathyroid hormone, estradiol, 1,25(OH)2D3, thyroxin, dietary intake of calories, calcium, vitamin D, dietary fiber, training history, menstrual history, weight, BMI, and BMD. Independent t-tests were performed to evaluate differences between the athletic and the control subjects' values for age, body weight, BMI, BMD, age of menarche, no. menses/yr, caloric intake, dietary calcium and vitamin D intake. Athletes were divided into two groups, those experiencing less than 6 menses per year
(group A) and those with greater than six menses per year (group B) Independent t-tests were performed to determine if differences existed between the groups with respect to BMD, BMI, caloric intake, dietary intake of calcium, vitamin D, fiber, training history, menstrual history, and endocrine and metabolic parameters. Multiple linear and stepwise regression statistical analyses were performed to assess the degree to which the dependent variable (BMD) could be explained by the independent variables: BMI, menstrual history (age of menarche, no. menses/yr), training history, plasma estradiol, 1,25(OH)2D3, ionized calcium, total calcium, intact PTH, and dietary intake of calcium, vitamin D, and fiber. Correlations were determined to identify relationships between variables. The level of significance was set at \( p < 0.05 \).

Results & Discussion

Subjects

The physical characteristics of the two groups studied are presented in Table 1. The average age of the athlete (16 ± 1.15 years) was significantly different \( (p < 0.01) \) from the average age of the control (14 ± 0.92). The mean BMI of the athletic group was significantly lower than the mean BMI of the control group (18.29 ± 0.15 \( \text{ht/m}^2 \) vs. 20.17 ± 0.88 \( \text{kg/m}^2 \)). The athletes' mean body fat was 16.2%.
The onset of menarche occurred at a significantly later age in the athletes as compared to the controls, and the athletes had fewer menses per year (mean no. = 5) compared to the controls (p < 0.05). The mean BMD of the athletic group significantly increased over the 12 month supplementation period (1.10 ± 0.01g/cm² to 1.12 ± 0.01g/cm²). However, their BMD at the completion of the 12 month period was not significantly lower than the controls' mean bone density (1.15 ± 0.02g/cm²), which was determined within two months after the athletes' post BMD measurement.

Dietary Intake

Dietary intake of the two groups is presented in Table 2. Although the athletes consumed fewer calories per day (1662 ± 117.2) than the controls (1853 ± 132.4), the difference was not significant. The distribution of energy between the two groups was similar.

Prior to supplementation, there was no difference in calcium intake between the two groups. They consumed almost 100% of their RDA (1200 mg) per day. Supplemented athletes consumed almost twice their RDA for calcium. There was no significant difference in vitamin D intake.
between the two groups. However, the athletes only consumed about half of their RDA (400 IU) for vitamin D per day prior to supplementation.

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Insert Table 2 Here

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Training Characteristics

Table 3 summarizes the training characteristics of the adolescent athletes. They ran an average of six days per week, covering approximately 30 ± 4.9 miles per week. Most of the athletes participated in both cross-country as well as track (indoor and outdoor), thus they trained approximately 9(+3) months per year. They had been running for an average of 3 years, indicating that they started running very close or prior to the onset of their menses. Ninety-two percent reported experiencing a change in their menstrual pattern during training. Specifically, if they ran longer distances, their menses was delayed and/or reduced in length and/or flow. Seventy-seven percent reported experiencing some type of training related injury during the years they had been training; stress fractures and sprains were the most common. Eighty-five percent participated in some other activity concurrent with their running program such as weight-lifting and swimming.
Endocrine/Metabolic Values

Table 4 presents the endocrine and metabolic response to calcium and vitamin D supplementation. Initial ionized plasma calcium was at the low end of the normal range (4.56 ± 0.01 mg/dl; reported normal range is 4.52 – 5.28 mg/dl) and did not change significantly (4.52 ± 0.02 mg/dl). Plasma total calcium was considered normal for values reported in this laboratory (10.91 ± 0.22 mg/dl) and significantly increased (11.7 ± 0.23 mg/dl) by the end of the supplementation period. The increase in intact parathyroid hormone (20.45 ± 2.90 pg/ml; to 23.38 ± 3.31 pg/ml; reported normal range 10–55 pg/ml) was not significant. The change in plasma estradiol (72.00 ± 14.49 pg/ml to 43.10 ± 14.47 pg/ml) was not significant. The increase in serum 1,25(OH)2D3 (12.86 ± 1.18 pg/ml to 17.98 ± 1.27 pg/ml) was significant (reported normal range is 12.7 – 32.2 pg/ml). Thyroxin was low at the beginning of the study, and decreased from 1.10 ± 0.04 ng/dl to 1.03 ± 0.05 ng/dl, a below normal (hypothyroid) level, (<1.10 ng/dl) over the 12 month period.

Athletic subjects had a mean of five menses per year.
They were divided into two groups based on whether they had more or less than six menses per year upon entry into the study (Table 5). Subjects with less than six menses per year (Group A) started to menstruate at a later age (p< .15) than females who had six or more menses per year (Group B). Group A ran more miles per week (p< .002) and had been training for more years than Group B. The dietary habits of the two groups differed in that Group A consumed significantly more protein (p< 0 .007), dietary calcium (p< 0.01), vitamin D (p< 0.05), and fiber than Group B. Subjects in Group A had significantly lower plasma estradiol (p< 0.004) values than subjects in Group B.

A significant correlation between dietary calcium intake and total plasma calcium(p< 0.03, r= .50), and dietary vitamin D intake and 1,25(OH)2D3 (p< 0.001, r= .50) was observed. No measured variable correlated with the bone mineral density of the lumbar spine. Models of combinations of independent variables that significantly predict a linear relationship to the dependent variable (BMD) are presented in Table 6. These models provide a means of assessing risk of bone mineral loss in populations who do
not as yet have a range established for normal levels of bone mineral density, such as adolescent athletes experiencing menstrual dysfunction. The significant difference in the athletes’ BMD was not related to the change in dietary calcium intake, dietary vitamin D intake, interaction of the two, or activity level.

No model was significant in predicting parathyroid hormone level.

Discussion

The athletes who participated in the present study possess physical, training and dietary characteristics similar to those of amenorrheic athletes who have been reported to have reduced bone mineral density; they are very lean; they train several days/week, have inadequate energy in the diet; and demonstrate hormonal aberrations in estradiol and thyroxin (2,12, 13).

Data regarding normal BMD for adolescents is not as yet reported in the literature. However, an age-based regression equation has been developed (Lunar Radiation Corp., Madison, WI; unpublished data) to interpret BMD scans from individuals not within the 20-50 year old age range.
When BMD is calculated by this equation, the mean BMD for the athletes is the same as the BMD value determined from their actual BMD scans, suggesting that they were within a normal range for their age prior to supplementation, as well as after supplementation, such that the additional dietary calcium and vitamin D did not significantly enhance the change in BMD that would have occurred due to growth alone.

Compared to the controls, the athletes had a lower BMD. The controls were significantly younger than the athletes, thus, one might expect that they would have a lower BMD comparatively. Another reason for this apparent discrepancy may, in part, be the effect of body weight on bone mass.

Body weight had been shown to be positively associated with bone mass (14-16). The body weight and BMI of the athletes is significantly lower than that of the controls. Thus, the difference in BMD is not surprising. The amenorrheic athletes (mean age 25 yrs) that were studied by Cann et al. (17) were significantly lighter than eumenorrheic controls, and had significantly less BMD than the controls. Lindburg et al. (18) and Marcus et al. (2) have reported similar findings when comparing body weight and bone density of amenorrheic and oligomenorrheic athletes to seden-
tary eumenorrheic controls.

Additionally, the reported BMD of the seven subjects who served as controls may not be representative of the BMD of that age group population. When calculated, the BMD for individuals that age is less than the BMD of the controls, as well as the older athletes. It is also suggested that the equation used to calculate BMD may not be correct.

Initiation of endurance training before complete maturation of hypothalamic-pituitary function may make females susceptible to amenorrhea (19). The age of menarche of the subjects studied is similar to the age of the amenorrheic athletes studied by Nelson (13), who had lower BMD than eumenorrheic athletes who reached menarche at an earlier age. Marcus (2) and Lindberg (18) did not find a difference in the age of menarche between the amenorrheic and eumenorrheic athletes they studied, but they did report that the amenorrheic athletes began training closer to the onset of menses than the eumenorrheic subjects. Thus, these subjects may have begun to train before maturation of the hypothalamic-pituitary function occurred.

Subjects in this study also began to train very close to the time (within the year) at which menarche occurred, or before the onset of menses as well. The literature supports support the theory that a later onset of menarche,
coupled with early initiation of endurance training, promotes menstrual dysfunction which has been shown to result in lower bone mineral density (2,18).

**Dietary Characteristics**

Prior to calcium supplementation, the athletes were ingesting almost 100% of their RDA for dietary calcium; with supplementation, that percentage increased to approximately 200%. However, that may not be representative of the total calcium that was available for absorption. Several factors have been associated with decreasing calcium absorption, namely protein and fiber (20,21).

The athletes who have less than six menses per year (group A) consumed a significantly greater amount of dietary protein relative to group B athletes, (> six menses per year). An increase in dietary protein, while enhancing calcium absorption, also increases urinary calcium excretion, thus decreasing overall calcium retention (20). However, athletes' intake of dietary protein (16% calories) is not considered an excessive amount and it is not thought that the difference in BMD between groups A and B is largely a factor of dietary protein intake.

Although none of the athletes in the study were vegetarians, and their overall dietary fiber intake was not above average, the subjects with lower bone mineral density
(group A) had a considerably higher intake of dietary fiber than subjects in group B.

In past studies of vegetarian women who consumed twice as much fiber as did nonvegetarian women, reduced frequency of breast cancer and reduced circulating estrone and estradiol levels were observed (21). Fecal excretion of estrogen by vegetarians was more than twice as great as that by non vegetarians (21). These studies suggest that dietary habits and food fiber levels alter circulating estrogen levels. Recently, Lloyd et al. (1) observed a significantly higher dietary fiber intake in oligomenorrheic collegiate athletes (5.72 ± 0.64 grams/day) compared to eumenorrheic collegiate athletes (3.52 ± 0.53 grams/day) or eumenorrheic sedentary collegiate controls (2.97 ± 0.31 grams/day). Bone density tended to be lower (p=.10) in the oligomenorrheic athletes compared with the eumenorrheic athletes or sedentary controls.

The authors did note some potentially confounding variables among their study groups. They suggest that since the oligomenorrheic athletes had the lowest fat percentage of the groups, that in turn may have affected their bone mineral measurement. Also, although the calcium intake of the oligomenorrheic athletes was above the RDA (800 mg/day), the authors recognize that this group may be
calcium deprived due to their hypoestrogenic state. They concluded that this association between increased fiber intake and menstrual irregularity may be related to alterations of endocrine status and mineral status (1).

The adolescent athletes in group A have a dietary fiber intake lower than that of the oligomenorrheic athletes studied by Lloyd et al. (1). The association between dietary fiber intake and menstrual irregularity may be related to alterations of endocrine and mineral status however, until further documentation of dietary fiber in foods is reported, this relationship cannot be seen as casual (12,22).

The mechanism responsible for increased fecal excretion of estrogens is not known although a variety of mechanisms, including direct estrogen adsorption by fiber and alteration of gut flora have been proposed (21). The theory that dietary fiber affects calcium absorption and calcium balance has also been proposed (23), but this is highly controversial. Some balance studies with human subjects have demonstrated increased calcium excretion consequent to increased dietary fiber intake (23). Conversely, other balance studies have shown no effect of increased fiber intake on calcium absorption (24). The difficulty of quantitatively measuring dietary fiber intake unless study pop-
ulations are provided with controlled diets is recognized. There are two reasons for this. First, the computer programs for nutrient analysis use limited data bases. Second, general agreement does not exist about dietary fiber content of the foods used in the data base. Recent advances in developing and implementing better methods to determine dietary fiber intake are, however, being reported (25).

Regardless of calcium intake, evidence shows that without estrogen, absorption of dietary calcium will be compromised (22). Thus, the dietary intake of the adolescent athletes, although supplemented, may still be inadequate if they do not have enough estrogen. The decrease in calcium absorption and the increase in calcium excretion in estrogen deficient women (, 30 mg/dl) has led Heaney (22) to recommend a daily intake of 1.5 grams of elemental calcium in low estrogen states to maintain calcium balance. This recommendation was made for women whose RDA for calcium is 800 mg/d. Riggs and Melton recommend that adolescents take in at least 1500 mg/d since adolescence is considered to be the prime time for accumulation of BMD (26). An intake of 1500 mg/d is above the RDA for female adolescents (1200 mg/d) who are not experiencing menstrual dysfunction. It would seem appropriate to assume that adoles-
cents who are then experiencing estrogen deprivation need even more calcium/day than the recommended 1500 mg. Basing the additional need on the same percentage increase recommended for older females (50%), it would seem that such adolescents would need to consume 2250 mg/d.

The supplemented athletes were consuming approximately 2280 mg/d of calcium for the duration of the experiment. Since plasma estradiol was low for several of the athletes, more dietary calcium may be required for these subjects. Also, accompanying parameters controlling calcium metabolism indicate that the amount of dietary calcium consumed by the estrogen-deficient athletes, even with supplementation may not be sufficient.

**Endocrine Features**

Calcium homeostasis is achieved through the coordinated actions of PTH, calcitonin, and 1,25(OH)2D3 via negative feedback (27).

When the ionized calcium concentration (the form of calcium that is most important physiologically and pathologically, and that regulates and is regulated by PTH) is low, PTH is secreted (27). Parathyroid hormone primarily acts on the bone; PTH activates formed osteoclasts, stimulates the formation of new osteoclasts, and delays the conversion of osteoclasts to osteoblasts. This leads to
increased bone resorption and increased plasma calcium and phosphate levels.

Parathyroid hormone also regulates the synthesis of 1,25(OH)2D3 in the kidney (27). This metabolite of vitamin D is important in affecting calcium and phosphate absorption from the intestine. In the absence of PTH, none of the 1,25(OH)2D3 is formed. Therefore, PTH exerts a potent effect in determining the functional effects of vitamin D in the body, specifically its effect on calcium absorption.

Parathyroid hormone also increases tubular reabsorption of calcium, with excessive renal phosphate excretion (27). A biochemical index of increased PTH activity is not only increased formation of 1,25(OH)2D3, but increased urinary phosphate excretion.

Conversely, when the calcium ion concentration is increased, the secretion of PTH is inhibited and calcitonin acts to reduce calcium adsorption from the bones, thus preventing an excessive rise in the calcium ion concentration. Without PTH, the conversion of 25OHD3 to 1,25(OH)2D3 is inhibited, decreasing the absorption of calcium from the intestinal tract, returning the calcium ion concentration back to its normal value.

Although it is generally accepted that low estrogen levels after menopause, or in premenopausal women with
endocrine dysfunction, are related to the osteopenia that is observed in these groups, the role of estrogen in bone dynamics is not fully understood. Since estrogen receptors have not been found in bone, it is generally assumed that the estrogen effect on bone is indirect (22). Heaney (22) suggests that one such route may be the effect of estrogen on calcium balance since there is evidence that a lack of estrogen increases the daily calcium requirement (22). Specifically, a decrease in estrogen will decrease calcium absorption and increase calcium excretion. Estrogen acts on the kidney to increase the enzyme 1a-hydroxylase (28). This enzyme is responsible for activating the conversion of 25OHD3 to 1,25(OH)2D3. It has been reported that patients with post menopausal osteoporosis have a significant decrease in 1,25(OH)2D3 (29).

Estrogen is also responsible for a serum phosphate reduction that is associated with an increased excretion of phosphate, which, in turn is characteristic of increased PTH activity (30).

Dietary information from the athletes in the present study indicated that they were consuming an adequate calcium intake prior to supplementation. This was also demonstrated by a normal total serum calcium level. The
increase in dietary calcium due to the supplement is directly related to the significant increase in total serum calcium. However, it is not the total serum calcium value that is associated with regulating calcium metabolism, but rather the ionized calcium molecule (31).

Mean ionized serum calcium was at the low end of the normal range. Within the literature, there is no distinction made regarding normal serum ionized calcium levels for individuals of different sexes and/or age groups. Both intact PTH and 1,25(OH)2D were low, but within the normal range.

Lindberg et al. (18) observed a reduction in both cortical and trabecular bone density in amenorrheic runners, but observed normal concentrations of serum total calcium, prolactin, and PTH (intact and mid-portion molecule) (data not reported). Serum estradiol levels, however, tended to be lower in the amenorrheic runners compared to eumenorrheic runners (data not reported).

In evaluating bone mass in a group of elite female distance runners in whom training intensity and endocrinological features were carefully documented, Marcus et al. (2) also reported normal serum levels of calcium (data not reported) and intact PTH in amenorrheic and eumenorrheic females (32.5 ± 2.4pg/ml vs. 37.4 ± 2.5 pg/ml). The ame-
norrheic females had significantly lower plasma estradiol levels compared to the eumenorrheic females (36.3 ± 3.5 pg/ml vs. 92.5 ± 27.4 pg/ml). Serum calcitriol was also normal, but significantly greater in cyclic women than in amenorrheic women (48.0 ± 6.0 pg/ml vs. 32.8 ± 3.7 pg/ml). The authors did not observe any significant correlation between the levels of vitamin D metabolites and intact PTH, calcium excretion or serum estrogen levels.

It is suggested that the adolescent athletes with low estrogen levels (< 30 mg/dl) were not able to absorb calcium sufficiently. There probably was a minimal amount of estrogen available to initiate the formation of 1,25(OH)2D3. However, this then resulted in only a minimal amount of 1,25(OH)2D3 being formed which enabled only a minimal amount of dietary calcium to be absorbed.

Despite the supplement, plasma ionized calcium concentration did not increase. This would suggest that the athletes were not consuming enough dietary calcium to compensate for their further reduction in estradiol, which in turn activated PTH and 1,25(OH)2D3 in an attempt to re-establish the plasma ionized calcium concentration. These changes are better demonstrated when the athletes are divided into groups based on the number of menses per year. Athletes with less than six menses per year have lower
estradiol and higher PTH values, despite a higher dietary calcium intake than athletes with more menses per year.

To date, the studies evaluating the effects of calcium supplementation and/or estrogen on bone mass provide suggestive evidence of a preventive effect of calcium on bone loss in post-menopausal women (32). However, the effect of calcium is not as protective as the effect of estrogen, or the combined effect of estrogen and calcium (33).

Horsman (34) and Recker (32) report an intermediate bone loss in post menopausal women supplemented with 800 mg of elemental calcium per day for two years. That is, these women did not lose as much bone as did the controls, but lost more than the women treated with estrogen (.625 conjugated estrogen/day). Similar findings were observed when treating women with 1.04 and 2.0 grams of calcium carbonate per day, respectively (32). Treatment with 2.0 grams however was observed to have a preventive effect on cortical bone but not trabecular bone accretion (35). Lindsay et al. (36) also observed less of a decrease in bone density (0.2%/year) in menstranol treated women after experiencing a natural or artificial menopause, compared to placebo treated patients who lost bone at a mean rate of 2.7%/year for the first three years of the study.

When comparing the effects of bone loss (ulna and


radius) of no treatment, treatment with estrogen (entiny-
lostranol 25) and treatment with calcium (800 mg/d),
Horsman et al. (34) observed that bone loss in the calcium
group was less than in the control but not than in the
estrogen treated group (p< .05). Recker et al. (32) did a
similar study but the calcium supplemented group was given
a larger dosage (2600 mg calcium carbonate per day) and the
estrogen group was given .625 mg conjugated equine
estrogen. After two years, skeletal mass (radiography)
decreased more in the control group (1.18%/year) and in the
hormonal group (0.22%/year) than the calcium group
(0.15%/year). However, skeletal mass, determined by photon
absorptiometry showed a 2.88%/year bone loss in the control
group, a 0.73%/year bone loss in the hormone group, and a
1.83%/year loss in the calcium group, indicating that sup-
plemental calcium may promote bone accretion in cortical
bone mass but not in trabecular skeletal bone mass.

Recently, Riis et al. (35) demonstrated that percuta-
neous estradiol (.6mg/28 day cycle) in post menopausal
women did not change either forearm mineral density or
total skeleton mineral density over a 12 month period com-
pared to a control group receiving a placebo who showed a
significant decrease in both forearm mineral content and
total skeletal content. Further, Riis et al. (35) reported
a significant increase in serum estrone and estradiol after only three months of treatment which then remained constant. There was no significant change in serum calcium in either group whereas both serum alkaline phosphatase and fasting urinary hydroxyproline were decreased in the hormone group, reaching significance after 12 months of treatment.

In the study by Ettinger et al. (33) they tested to see if the effects of estrogen plus calcium might better prevent bone loss in post menopausal women. A control group maintained their usual dietary calcium intake (258 to 994 mg/day), while one treatment group supplemented their dietary intake with 1000 mg of calcium per day (mean intake 1863 mg/day), and the other treatment group took 0.3 mg Premerin (estrogen) and 1000 mg calcium per day (mean intake 1733 mg/day).

Baseline measurements are not significantly different between groups. Follow-up measurements show a significant reduction in bone mass (quantitative tomography) of 9% in untreated women and 10.5% in women given calcium alone. Women taking both Premerin and calcium showed no significant change in spinal mineral measurements. The authors concluded that calcium supplementation alone does not protect against the accelerated bone loss that occurs at the
menopause. However, by adding calcium to the diet, the amount of estrogen necessary to exhibit a protective effect on skeletal bone mass may be reduced (33).

Basically, no study to date has shown a significant increase in vertebral skeletal bone mass in women supplemented with dietary calcium, independent of estrogen, regardless of the brand or dosage administered (32,34,37). Effects of calcium supplementation have shown a reduction in loss of cortical bone mass in post menopausal women (32,34,37). However, since it is the trabecular bone mass that is most affected by estrogen loss (22,26,30), and subjects women to increased fracture, the therapy that best prevents this type of bone loss is considered to be the desired therapy (22,26,30).

Although the data of this study indicate that bone mass increased during the supplementation period, bone mass did decrease in two of the subjects (Appendix H, Table 1, subjects 9 and 11). Subject 9 is a competitive cross country runner who was truly amenorrheic for the duration of the study. Although her average intake of dietary calcium was 2.3 grams per day for the year, her estradiol level did not change from 15 pg/ml (20 pg/ml is typical of post menopausal women) and her bone mineral density decreased from 1.163 g/cm² to 1.115 g/cm². Subject 11, had increased her
training throughout the year to prepare for a marathon competition that spring. During the year her dietary calcium intake was more than adequate (2.4 grams per day), but her serum estradiol level decreased from 60 pg/ml to 30 pg/ml with a concomitant decrease in bone mineral density (1.167 g/cm² to 1.130 g/cm²). These data are consonant with the previous studies that do not report preservation of skeletal bone mineral density in estrogen compromised females consuming calcium supplements. Also, both of these subjects demonstrated a low BMI, low calorie diet, and a rigorous training program. Such characteristics are typical of amenorrheic female athletes in whom a reduced bone mineral density has been observed (12,13).

In determining variables that predict bone mineral density previous authors have observed estrogen (estradiol) to be the only variable to correlate with bone mineral density (13,33,38). Nelson (13) reported a positive correlation (r= .50) between serum estradiol levels and bone mineral density of the lumbar spine and concluded that serum estradiol was the most significant factor associated with bone mineral density Ettinger et al. (33) reported that estrogen use was the only independent variable showing a significant correlation to loss of bone mass in the stepwise multiple regression analysis.
A significant correlation between level of plasma estradiol and bone mineral density, and number of menses per year and bone mineral density was observed in this study (p< .15, r= .50; p< .08, r= .50 respectively ). Recently Parker-Jones et al. (39) has reported a significant linear correlation between decreasing bone density with increasing months of amenorrhea (p< .001, r= .50).

In addition to stepwise linear regression, multiple linear regression analysis was done to identify models that would predict bone mineral density. Overall, the most significant models predicting bone mineral density include; BMI (a variable that has been previously established in affecting BMD); and what has now been shown to be an interdependent relationship between estrogen (either expressed by level of estradiol, number of menses per year, or age of menarche) and dietary calcium and or vitamin D intake.

No variable, specifically the change in dietary intake of calcium and /or vitamin D, was related to the change in BMD that occurred during the treatment period.

Based on the variables that have been statistically significant in predicting bone mineral density, the reports in the literature which indicate calcium supplementation does not affect vertebral bone mineral density independent
of estrogen, the decrease in bone mineral density in two of the calcium supplemented subjects participating in the study, the results of this study suggest that the increase that occurred in subjects' bone mineral density was not due to the additional calcium and/or vitamin D supplement.

Drinkwater et al. (40) have further demonstrated the importance of estrogen, not calcium, on bone accretion. After re-evaluating the bone status of previously amenorrheic athletes who regained menses one to ten months following initial testing (12), Drinkwater et al. (40) observed a significant increase in vertebral bone mineral density in those same subjects. The resumption of menses in the athletes followed a decrease in training and an increase in body weight. Thus, there is some confounding of the specific cause of resumption of menses in these subjects. Also, a retrospective estimate of daily calcium intake averaged 850 mg for cyclic athletes and 1300 mg for those women resuming menses. The authors believe that it is likely that an increase in circulating estradiol coincident with resumption of menses (peak value of estrogen for the women who were cyclic when the second bone measurements were done is almost identical to that of cyclic athletes, 205.4 pg/ml) was the primary factor in reversing bone loss in six of the seven formerly amenorrheic athletes. The one
subject that did not resume menses continued to lose bone mass and still maintained a low estradiol level. Shangold (41) substantiates the theory that estrogen is the critical factor affecting bone mass, and recommends treating amenorrheic females with estrogen replacement therapy.

Although the athletes in the present study demonstrate an overall increase in bone mineral density, this increase may not be representative of the normal growth rate experienced by sedentary or athletic adolescents who have sufficient estrogen to aid calcium deposition in bone. The athletes demonstrate a continuing decrease in serum estradiol levels, and report already having experienced several training related fractures. In light of the evidence that indicates that the consumption of calcium rich foods during adolescence is the best predictor of peak bone mass (26,42,43,44) and that estrogen is needed to sufficiently promote calcium retention (22), such effects in early adolescence could prohibit these individuals from developing their peak bone mass and result in their entering adulthood predisposed to more frequent and/or more severe bone injury.

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*measurements determined on sedentary control subjects.

Figure 1. Experimental design.
Table 1. Clinical parameters of athletic females and sedentary females.

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<td>49.07 ± 1.19</td>
<td>53.71 ± 2.17</td>
<td>0.05</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.20 ± 1.80</td>
<td>162.80 ± 5.11</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (ht/m²)</td>
<td>18.29 ± 0.15</td>
<td>20.17 ± 0.88</td>
<td>0.04</td>
</tr>
<tr>
<td>BMD (g/cm²) baseline</td>
<td>1.10 ± 0.01£</td>
<td>1.12 ± 0.01£</td>
<td></td>
</tr>
<tr>
<td>12 month</td>
<td>1.12 ± 0.01£</td>
<td>1.15 ± 0.02£</td>
<td>NS</td>
</tr>
<tr>
<td>Calculated BMD (g/cm²)*</td>
<td>1.12 ± 0.01£</td>
<td>1.10 ± 0.01£</td>
<td></td>
</tr>
<tr>
<td>Age of menarche</td>
<td>14 ± 0.44</td>
<td>11 ± 0.30</td>
<td>0.0008</td>
</tr>
<tr>
<td>No. of menses/year</td>
<td>5.16 ± 1.14</td>
<td>11.85 ± 0.14</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

£mean ± SEM
* based on the equation [0.3343 + (0.0459 x age)]
£ superscripts with different letters in the same column are significantly different (p< .05).
Table 2. Dietary characteristics of athletic females and sedentary females.

<table>
<thead>
<tr>
<th></th>
<th>Athletes</th>
<th>Controls</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=13</td>
<td>n=7</td>
<td></td>
</tr>
<tr>
<td>Energy (kcal/day)</td>
<td>1662 ± 117.2*</td>
<td>1853 ± 123.4</td>
<td>NS</td>
</tr>
<tr>
<td>Distribution of energy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>protein (%)</td>
<td>15 ± 0.69</td>
<td>15 ± 2.39</td>
<td>NS</td>
</tr>
<tr>
<td>fat (%)</td>
<td>36 ± 1.45</td>
<td>32 ± 3.39</td>
<td>NS</td>
</tr>
<tr>
<td>carbohydrate (%)</td>
<td>50 ± 1.39</td>
<td>52 ± 3.15</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>supplemented</td>
<td>1.12 ± 0.16</td>
<td>1.08 ± 0.15</td>
<td>NS</td>
</tr>
<tr>
<td>%RDA/d</td>
<td>97 ± 14.7</td>
<td>92 ± 15</td>
<td>NS</td>
</tr>
<tr>
<td>supplemented</td>
<td>190 ± 39**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IU/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>supplemented</td>
<td>257.81 ± 48</td>
<td>246.00 ± 55</td>
<td>NS</td>
</tr>
<tr>
<td>%RDA/d</td>
<td>57 ± 30</td>
<td>61 ± 53</td>
<td>NS</td>
</tr>
<tr>
<td>supplemented</td>
<td>190 ± 37**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* mean ± SEM
** significantly different from non-supplemented athlete or control (p< .05).
Table 3. Training characteristics of adolescent females.

<table>
<thead>
<tr>
<th>N</th>
<th>13*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miles/week</td>
<td>30 ± 4.9</td>
</tr>
<tr>
<td>Training pace (min)</td>
<td>7 ± 1.5</td>
</tr>
<tr>
<td>Days/week</td>
<td>6 ± 0.6</td>
</tr>
<tr>
<td>Months/year</td>
<td>9 ± 1.5</td>
</tr>
<tr>
<td>Years participating in sport</td>
<td>3 ± 2.8</td>
</tr>
<tr>
<td>Training related injury</td>
<td>77%</td>
</tr>
<tr>
<td>Participates in other sports</td>
<td>85%</td>
</tr>
<tr>
<td>Change in menses with training</td>
<td>92%</td>
</tr>
</tbody>
</table>

* mean ± SEM
Table 4. Mineral metabolism in 13 calcium and vitamin D supplemented female runners experiencing menstrual dysfunction

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>12 months</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionized serum calcium (mg/dl)</td>
<td>4.56 ± 0.01±</td>
<td>4.52 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Total serum calcium (mg/dl)</td>
<td>10.91 ± 0.22</td>
<td>11.68 ± 0.23</td>
<td>0.0091</td>
</tr>
<tr>
<td>Total serum magnesium (mg/dl)</td>
<td>2.1 ± 0.02</td>
<td>2.1 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Intact parathyroid hormone (pg/ml)</td>
<td>20.45 ± 2.90</td>
<td>23.38 ± 3.31</td>
<td></td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>72.00 ± 14.49</td>
<td>43.10 ± 14.47</td>
<td></td>
</tr>
<tr>
<td>1,25 (OH)2D (pg/ml)</td>
<td>12.86 ± 1.18</td>
<td>17.98 ± 1.27</td>
<td>0.0024</td>
</tr>
<tr>
<td>Thyroxin (ng/dl)</td>
<td>1.10 ± 0.04</td>
<td>1.03 ± 0.05</td>
<td></td>
</tr>
</tbody>
</table>

* mean ± SEM
Table 5. Comparison of indices measured in female athletes based on number of menses/year.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group A &lt; 6 menses/yr</th>
<th>Group B ≥ 6 menses/yr</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=7</td>
<td>n=6</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>16</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>49. ± 1.5*</td>
<td>49. ± 2.1</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>18. ± 0.7</td>
<td>19. ± 0.4</td>
<td></td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>1.12 ± 0.02</td>
<td>1.14 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Age of menarche</td>
<td>14. ± 0.5</td>
<td>13. ± 0.7</td>
<td>0.15</td>
</tr>
<tr>
<td>Training (mi/week)</td>
<td>39. ± 2.0</td>
<td>20. ± 3.9</td>
<td>0.01</td>
</tr>
<tr>
<td>Years of training</td>
<td>4. ± 0.4</td>
<td>3. ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Calories/day</td>
<td>1813. ± 225.0</td>
<td>1844. ± 308.0</td>
<td></td>
</tr>
<tr>
<td>protein (%)</td>
<td>16.4 ± 0.84</td>
<td>13. ± 0.51</td>
<td>0.01</td>
</tr>
<tr>
<td>fat (%)</td>
<td>34. ± 2.0</td>
<td>38. ± 1.8</td>
<td></td>
</tr>
<tr>
<td>carbohydrate (%)</td>
<td>51. ± 2.5</td>
<td>49. ± 1.8</td>
<td></td>
</tr>
<tr>
<td>Calcium (%RDA/day)</td>
<td>135.5 ± 18.0</td>
<td>69.1 ± 12.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Vitamin D (%RDA/day)</td>
<td>89.5 ± 7.1</td>
<td>68.7 ± 6.6</td>
<td>0.05</td>
</tr>
<tr>
<td>Dietary fiber (g/day)</td>
<td>3.2 ± 0.53</td>
<td>1.9 ± 0.31</td>
<td></td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>23.2 ± 4.4</td>
<td>17.2 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>39.8 ± 9.4</td>
<td>109.6 ± 17.8</td>
<td>0.01</td>
</tr>
<tr>
<td>1,25(OH)2D3 (pg/ml)</td>
<td>16.1 ± 1.5</td>
<td>16.1 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Ionized serum calcium (mg/dl)</td>
<td>4.6 ± 0.01</td>
<td>4.5 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Total serum calcium (mg/dl)</td>
<td>11.2 ± 0.24</td>
<td>10.6 ± 0.27</td>
<td></td>
</tr>
</tbody>
</table>

*mean ± SEM
Table 6. Regression models predicting BMD.

<table>
<thead>
<tr>
<th>Model 1</th>
<th>F &lt; 0.0117</th>
<th>Parameter Estimate</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.0480</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Menses per year</td>
<td>0.0462</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>Dietary calcium intake</td>
<td>0.0041</td>
<td>0.0009</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 2</th>
<th>F &lt; 0.0117</th>
<th>Parameter Estimate</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt. (kg)</td>
<td>0.0104</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.0560</td>
<td>0.011</td>
<td></td>
</tr>
<tr>
<td>Menses per year</td>
<td>0.0566</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.0007</td>
<td>0.0006</td>
<td></td>
</tr>
<tr>
<td>Dietary calcium intake</td>
<td>0.0046</td>
<td>0.0009</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 3</th>
<th>F &lt; 0.0136</th>
<th>Parameter Estimate</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of menses</td>
<td>0.0106</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>Menses per year</td>
<td>0.0391</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.0565</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>Dietary calcium intake</td>
<td>0.0037</td>
<td>0.0009</td>
<td></td>
</tr>
<tr>
<td>Dietary vitamin D intake</td>
<td>0.0001</td>
<td>0.0002</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER IV

SUMMARY

This study was conducted to assess the change in bone density, measured directly and reflected in anthropometric data, and calcium status via dietary data, blood calcium and other hormonal indices in response to a calcium and vitamin D supplement in athletic adolescent female athletes experiencing menstrual dysfunction.

Thirteen adolescent female runners participated in a 12 month study in which they consumed a dietary supplement containing 1200 mg calcium carbonate and 400 IU vitamin D per day.

Before and after the supplementation period, subjects' bone mineral density (dual photon densiometry) and body mass index was determined. Fasting blood samples (30mls) were also taken. Blood was transferred to mineral free, heparinized plastic tubes, and centrifuged. Serum was
separated from the red cells, stored, frozen and subsequently analyzed for total calcium and total magnesium (atomic absorption spectrophotometry), and serum ionized calcium (calcium specific electrode); 1,25(OH)2D3, estradiol and thyroxin were determined by radioassay. Dietary analysis was conducted on two separate seven day food intake records, one prior to calcium supplementation and one six months later. Intakes were analyzed for nutrient content using the Computerized Nutrient Analysis System (Louisiana State University).

Seven eumenorrheic sedentary adolescents participated as controls. Each subject had their bone mineral density (dual photon densiometry) and body mass index determined. The Nutritionist III software program (N-Squared Computing, Inc., Silverton, OR) was used to analyze nutrient content of 24 hour recalls. Statistical Analysis Systems (SAS, Cary, NC) computer programs were used to conduct statistical analysis. Paired t-tests were performed to evaluate differences between pretraining and posttraining variables. Multiple linear and stepwise regression were performed to assess the degree to which the dependent variable (BMD) could be explained by the independent variables. Correlations were determined to identify relationships between variables. The level of significance was set at p< .05.
The mean BMD of the athletes significantly increased over the 12 month supplementation period. This value was not significantly different from suggested BMD values for age-matched individuals, but it was significantly different from the younger control group. The mean BMI of the athletes was significantly lower than the mean BMI of the control group. The onset of menarche occurred at a significantly later age in the athletes, and the athletes had fewer menses per year compared to the controls.

The athletes consumed significantly fewer calories per day compared to the controls, but the distribution of energy between the two groups was not significantly different. Prior to supplementation there was no significant difference in calcium and vitamin D intake between the two groups.

Postsupplementation, the increase in the athletes' calcium and vitamin D intake was significant. Total serum calcium and 1,25(OH)2D3 significantly increased, but serum ionized calcium did not. Estradiol decreased, but the decrease was not significant. A significant correlation was observed between total serum calcium and dietary calcium intake (r=.50) and vitamin D intake and 1,25(OH)2D3 (r=.50). Stepwise linear regression indicated a significant correlation between estradiol and BMD (r=.50) and
number of menses per year and BMD ($r = .50$). Multiple linear regression indicated significant models predicting a linear relationship between several independent variables and BMD. The independent variables included; BMI, estradiol, dietary calcium and vitamin D intake.

The change in dietary calcium and vitamin D intake did not solely affect the change in BMD that was observed at the end of the study. The results of this study have implications regarding the role of calcium and estrogen in the treatment of athletic female adolescents experiencing menstrual dysfunction.

**Research Implications.** The results of this study contribute information regarding the physical, dietary, and hormonal alterations that occur in female athletes in response to calcium and vitamin D supplementation. The results also contribute information regarding risk assessment for reduced bone mineral density in female athletes experiencing menstrual dysfunction.

The adolescent athletes exhibit characteristics similar to amenorrheic athletes. They have a low BMI, experienced a delayed onset of menarche, began to train before, or very soon after menarche and experienced coincident menstrual dysfunction that was exacerbated with heavier bouts of training.
The dietary characteristics are also similar to those of amenorrheic athletes in that they consume a low energy diet, which is reflected in a low thyroxin level, despite their heavy activity schedules. Their dietary calcium intake (without supplementation), however, is higher than the usual dietary calcium intake reported by amenorrheic or oligomenorrheic athletes.

As expected, serum estradiol was low, and further decreased during the duration of the study. Despite the increased calcium intake, serum ionized calcium did not increase. Intact PTH and vitamin D increased, suggesting that the dietary calcium intake was not high enough to compensate for the loss of estrogen.

The regression models indicate that BMD is dependent on both estrogen and calcium. Body mass index is directly related to BMD but it may be a confounding variable when determining appropriate BMD levels. These regression models present an inexpensive method by which assessment/screening for females at risk for bone mineral loss can be conducted. By obtaining information regarding menstrual patterns and dietary intake, one can obtain important information regarding risk for osteoporosis without initially subjecting individuals to invasive measures.

This study supports the theory that athletes, exper-
iencing menstrual dysfunction, are at an increased risk for developing decreased vertebral bone mineral density and, without appropriate estrogen, additional calcium absorption will not occur regardless of intake.

Further, this study indicates that adolescent female athletes are in need of intervention/education regarding the effects of their training on their metabolism. By continuing to train at their present level they will experience further aberrations in endocrine function. Thyroxin will probably continue to decrease, which may predispose these athletes to substantial weight gain when they decrease the intensity or duration of their training regimen. Estrogen will also probably continue to decrease, and females will experience continued menstrual dysfunction and amenorrhea. This may prevent peak bone mass accumulation which may predispose them to additional fracture susceptibility and to early onset osteoporosis in later years.

**Recommendations for Further Studies.** It is recommended that further investigation and documentation of normal ranges of bone mineral density in adolescents be determined to establish possible fracture threshold ranges or risk ranges that can identify adolescents at risk for reduced bone mineral accumulation. This would involve screening of nonathletic amenorrheic females as well. If
adolescents are presently experiencing menstrual dysfunc-
tion it seems that their bone mineral density would not be
increasing at a normal rate. However, since these individ-
uals are still growing, growth and exercise at this age may
be compensating for mineral loss that might be occurring in
amenorrheic adolescent who are not engaging in some type of
weight bearing exercise. It has been reported that amenor-
rheic athletic females do not have as dense bones as do
eumenorrheic athletes, but they have denser bones than
sedentary amenorrheic females. The pattern of bone density
in similar type adolescents would be helpful in determin-
ing fracture risk and susceptibility to reduced bone mass
accumulation in the adolescent population.

Another recommendation is to establish normal ranges
of ionized calcium in adolescent females. The values
obtained in this study may be confounded due to the manner
in which they were analyzed and then corrected for pH vari-
ance. At present, normal ranges for ionized calcium for
different age groups and sexes are not available. Also
there is no distinction made between normal values obtained
from fresh or frozen blood samples.

Investigators presenting information on bone mineral
density and calcium supplementation have not reported ion-
ized calcium values. Since it has been established that
ionized calcium, not total calcium, is the ion that regulates calcium homeostasis, it would be important to know what appropriate ionized calcium levels are, and how these levels affect calcium homeostasis when individuals are supplemented with dietary calcium and/or vitamin D; especially in the growing adolescent or estrogen deficient amenorrheic or post menopausal female.

The differences in the bioavailability of different types of calcium supplements or calcium fortified foods, and the overall impact of a calcium supplement on calcium homeostasis or bone remodeling have not been adequately addressed. Calcium carbonate is considered to be absorbed equally as well as food calcium; however, differences in bone remodeling due to consumption of milk and calcium carbonate have been observed. Differences between the bioavailability of calcium carbonate, lactate, and gluconate have been reported as well. The type of supplement, dosage, and tablet formation, may affect its bioavailability. Additionally, it is also well established that ingestion of excess calcium can depress absorption of magnesium, iron, and zinc. Hence, the various doses of calcium supplements administered should not be evaluated independent of their effects on the bioavailability of other minerals. Before interpreting the effect of a supplement, this infor-
information needs to be documented.

Longitudinal studies assessing the degree to which menstrual dysfunction affects the bone growth process during adolescence, and its effect on the ability of such individuals to achieve peak bone mass will provide information on how to best prevent bone loss in adolescents susceptible to early onset osteoporosis. Estrogen therapy seems to be warranted in amenorrheic athletic females, and, over time, longitudinal studies would be able to suggest whether or not this type of therapy will allow amenorrheic athletes to achieve peak bone mass.
Methodology

Subjects and study design. Twenty adolescent females aged 15-18 were recruited from area high schools to participate as subjects. Criteria for selection of subjects required the individuals to be:

1. Caucasian.
2. Athletes; participating in regular endurance (>3d/wk) activities, within 85-110% of their ideal body weight, who were experiencing menstrual dysfunction (secondary amenorrhea or oligomenorrhea). Secondary amenorrhea is defined as cessation of menstruation once it has begun at puberty. Oligomenorrhea is defined as six or less menses per year.
3. Sedentary; not presently participating in regular (3d/wk) endurance activity, not greater than 22% body fat, eumenorrheic (normal menstruation).
4. Without any history of smoking, recreational drug use, previous pregnancies, eating disorders, use of oral contraceptives within the last six months, or cumulative oral contraceptive use of greater than six months.

All subjects received an oral and written explanation of the purpose of the study and procedures to be followed; all gave written consent prior to the beginning of the study (Appendix A). All procedures were approved by the
University Institution Review Board for Research Involving Human Subjects (Appendix B). Each subject completed a detailed medical history questionnaire which included information regarding their exercise regimen and menstrual history (Appendix C).

The thirteen athletic subjects participated in the study for 12 months. Pretest and post-test protocol was identical (Table 1). The seven sedentary control subjects completed post-test bone density measures, dietary analysis, and body composition determination.

Table 1

<table>
<thead>
<tr>
<th>Experimental Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Months</td>
</tr>
<tr>
<td>Procedures</td>
</tr>
<tr>
<td>bone density</td>
</tr>
<tr>
<td>body composition</td>
</tr>
<tr>
<td>dietary analysis</td>
</tr>
<tr>
<td>mineral analysis</td>
</tr>
<tr>
<td>hormone analysis</td>
</tr>
</tbody>
</table>

Throughout the course of the study, beginning after initial testing, athletes were provided with calcium supplements (Cal Sup 600) containing 1200 mg of calcium carbonate and 400 IU vitamin D per two tablets. These subjects were instructed to take two tablets per day with their
morning meal for the duration of the study. No other dietary restrictions were imposed on either the athletes or the controls during the study.

Procedures

Bone Mineral Density. The bone mineral density of the axial skeleton was determined by dual photon absorptiometry technique originally described by Riggs et al. (1982). See Appendix D.

Estimate of Body Composition. As part of the determination of bone mineral density, all subjects were weighed on a physicians' scale and weight was recorded to the nearest 0.5kg, and height was recorded to the nearest cm. These data were used to determine subject's Body Mass Index (BMI). See Appendix E.

Additional anthropometric measurements were determined on athletic subjects. Skinfold thickness measured with Lange calipers (10g mm constant pressure), was determined at three sites; the triceps, supra-illiac, and thigh; the average of repeated (3) trial values was used as the representative score for a given site (Behnke & Wilmore, 1974) for use in the equation of Jackson and Pollock (1980) for estimation of bone density in adolescents. See Appendix E.

Sample collection and analysis. Fasting blood samples
(30 ml) were taken from the anticubital vein by using a plastic 10 cc syringe and a 1 1/2 inch, 21 gauge needle. Blood was taken over a period of 3 to 4 minutes with minimum vacuum to reduce hemolysis. Blood was transferred to mineral-free, heparinized plastic tubes and centrifuged for 25 minutes at 2000 rpm. Serum was immediately separated from the red cells using an acid washed mohr pipet. Serum was stored, frozen and subsequently analyzed for mineral and hormone determination.

Mineral determination. Serum was diluted (100ul + 4.9mls 0.3% lanthanum=1:50) and analyzed for total calcium and total magnesium determination using a Perkin-Elmer Model 503 atomic absorption spectrophotometer (Perkin Elmer Corporation, Norwalk, CT) equipped with a titanium-3-slot Boling burner head and with appropriate lamps for the two elements. Recovery samples consistently run at intervals in this laboratory indicate recoveries between 95 to 99 percent. All analyses were done in duplicate with duplication in the range of 2 to 5 percent.

Serum ionized calcium was determined by a calcium specific electrode (Radiometer America Westlake, OH). Values were adjusted to correct the change in pH that occurred due to the samples being collected under anaerobic conditions and then frozen (Appendix F).
Hormone Analysis. Serum 1,25(OH)2D3 (Incstar, Stillwater, MN), estradiol and thyroxin (Diagnostic Products Corp. Los Angeles, CA), and IPTH (Nichols Institute, San Juan Capistrano, CA) were determined by radioassay (Appendix G). Coefficients of determination, evaluated by duplicate measurements of the same sample for 1,25(OH)2D3, estradiol, thyroxin, and IPTH are 0.97, 0.99, 0.99, and 0.98 respectively. The within assay coefficients of variance for 1,25(OH)2D3, estradiol, thyroxin and IPTH are 9%, 6%, 8%, and 6% respectively. IPTH concentration less than the 14 pg/ml standard were assigned a minimal value of 14 pg/ml.

Dietary Analysis. Athletes' seven-day food intake records were collected prior to supplementation, and again at 6 months to observe whether season variation affected their dietary intake. They were analyzed for nutrient content using the Computerized Nutritional Analysis System, Department of Experimental Statistics, Louisiana State University.

Control subjects completed twenty four-hour food intake records which were analyzed for nutrient content using the Nutritionist III software package (N-Squared Computing, Inc., Silverton, OR).

Data Analysis. Statistical Analysis System (SAS
Institute, Cary, N.C.) computer programs were used to conduct statistical analyses. Paired t-tests were performed to evaluate differences between pretraining and post-training values for serum ionized calcium, total calcium, estradiol, 1,25(OH)2D3, thyroxin, bone mineral density, physical characteristics, and dietary intake of calories, calcium, vitamin D, and fiber. Independent t-tests were performed to evaluate differences between the athletes' and the control subjects' values for age, body weight, BMI, BMD, menses/yr, calcium intake, vitamin D intake, and overall caloric intake. When the athletes were divided into two groups based on no. menses/yr, independent t-tests were also used to determine if differences existed between the groups in values for BMD, BMI, dietary intake of calories, calcium, vitamin D, fiber, training and menstrual history, endocrine and metabolic parameters. Multiple linear and stepwise regression were performed to assess the degree to which the dependent variable (BMD) could be explained by the independent variables such as: BMI, menstrual history (age of menarche, no. menses/yr), training history, serum estradiol, calcium, vitamin D, intact parathyroid hormone, and dietary intake of calories, calcium, vitamin D, and fiber. Correlations were determined to identify relationships between variables. The level of significance was set
at \( p < .05 \).

**Limitations.** The characteristics of the subjects; athletic female (14-18), experiencing menstrual dysfunction, allow the experimental findings from this study to be generalized only to a population possessing such characteristics.

Issues which may confound the claim that the observable results of the study can be attributed to the manipulation of the independent variable(s) rather than the uncontrolled variance include;

1. the machine used to measure athletic subjects' bone density was moved to a different location following initial testing.

2. serum ionized calcium values were adjusted due to the variance in pH that occurred as a result of the samples being collected under anaerobic conditions and then frozen.
APPENDIX A

INFORMED CONSENT
Consent of Participation

I have received an explanation of the study and understand the following:

As a subject I may be taking a dietary supplement containing 1200mg calcium carbonate and 400IU vitamin D, daily, during the period from August 10, 1986 to August 10, 1987. I understand that I am to maintain routine dietary and activity patterns throughout the study.

As a subject I will participate in a bone density test at the beginning and end of the study to evaluate bone mineral content. This procedure will be done by qualified personnel at the Osteoporosis Testing Center, Lynchburg, VA. The test will be approximately one hour. The dose of radiation is approximately 1% of the radiation obtained from an ordinary forearm x-ray. Subjects will be transported to and from Lynchburg by Tech personnel.

Several anthropometric measurements will be taken to assess body composition. Seven-day dietary recall sheets will be administered to determine mean dietary intake of each subject.

Two venous blood samples, approximately 30mls., will be taken pre and post study. Blood samples will be taken by a qualified medical technologist.
Individual bone density measures, blood analysis, dietary analysis and body composition measurements data will be available to each participant.

In lieu of a stipend, subjects will receive all of the above mentioned measurements, as well as dietary supplements free of charge.

If at any time a participant so desires or the investigators believe that the health of the subject may be impaired, the person may drop from the study.

Participants are invited to ask questions about procedures at any time.

No compensation or medical treatment, other than those normally available through student health services and emergency service by the rescue squad, is available if injury is suffered as a result of this research.

I understand the above and agree to participate in the study.

Signature __________________________________________

Guardian Signature __________________________________

Date _______________________________________________

L. Janette Taper, Investigator
APPENDIX B

HUMAN SUBJECTS REVIEW BOARD CONSENT
INVESTIGATION INVOLVING HUMAN SUBJECTS

Principal Investigator(s): Janette Taper

Project Title: Changes in bone density in amenorrheic athletes on calcium supplementation

Source of Support: [Departmental Research] [Sponsored Research] [Proposal No.]

1. The criteria for " expedited review" by the institutional Review Board for a project involving the use of human subjects and with minimal risk is one or more of the following. Please initial all applicable conditions and provide a substantiating statement of protocol.

2. a. Collection of:
   i) hair or nail clipping in a non-disfiguring manner;
   ii) deciduous teeth;
   iii) permanent teeth if patient care indicates need of extraction.

   b. Collection of secretions and external secretions: sweat, nasopharyngeal swab, placenta removed at delivery, amniotic fluid obtained at time of rupture of the membranes.

2. c. Recording of data from subjects 18 years or older, using non-invasive procedures routine employed in clinical practice. Exception does not include exposure to electromagnetic radiation outside the visible range.

2. d. Collection of blood samples by venipuncture (not exceeding 450 mL in a week period) and no more than twice a week from subjects 18 years or older, in good health and not pregnant.

2. e. Collection of supra- and subgingival dental plaque and calculus, provided the procedure is no more invasive than routine scaling of the teeth.

2. f. Voice readings.

2. g. Moderate exercise by healthy volunteers.

2. h. Study of existing data, documents, records, pathological specimens or diagnostic specimens.

2. i. Research on drugs or devices for which an investigational exemption is not required.

3. If the project involves human subjects who are exposed to "more than minimal risk" and are not covered by the criteria above 1 to 2) the IRB review must involve... the IRB board. Please check if the research involves more than minimal risk and provide a substantiating statement of protocol.

3. a. Human subjects would be involved in the proposed activity as either:
   i) Volunteers
   ii) Undergraduates
   iii) Pregnant women
   iv) Mentally Retarded
   v) Mentally Disabled

   Note that if children are involved in the research as human subjects, they may have to provide consent as well as their parents.

3. b. Whether or not the project may undergo "expedited review" or must be reviewed by the full institutional review board, it is necessary that the required informed consent forms also be reviewed. These should be submitted with the proposal. However, if there is insufficient time to meet the sponsor's deadline, verbal can be obtained up to twenty days after receipt of the proposal without jeopardizing the IRB certification to the prospective sponsor.

3. c. Minimal risk means that the risks of harm anticipated in the proposed research are not greater, considering the probability and magnitude, than those encountered in daily life or during performance of routine physical or psychological examinations or tests.

3. d. Subject at risk is an individual who may be exposed to the possibility of injury as a consequence of participation as a subject in any research, development or related activity which departs from the application of those established and accepted methods necessary to meet the needs, or which increases the ordinary risks of daily life, including the recognized risks inherent in a chosen occupation or field of science.

3. e. This is to certify that the project identified above will be carried out as approved by the Human Subject Review Board, and will neither be modified nor carried out beyond the period approved below without express review and approval by the Board.

Signature: Principal Investigator(s) Date

Signature: Departmental, Reviewer(s) Date

The Human Subjects Review Board has reviewed the protocol identified above, as it involves human subjects, and hereby approves the conduct of the project for... months, at which time the protocol must be resubmitted for approval to continue. Date

Signature: Board Chairman, Authorized Reviewer Date
APPENDIX C

MEDICAL INFORMATION
Medical Information

Name ________________________________

Age ____ Sex ____ Ht. ____ Wt. ____

Are you taking any medications on a regular basis?
no ____ yes ____

If yes, please list any and all medications you are taking (both prescription and non-prescription drugs).

Are you currently taking a vitamin and/or mineral supplement? _____ If yes, please list;

brand name of supplement(s) ______
dosage _______ frequency _______

Have you ever suffered a;
broken bone _____
bone fracture _____
sprain ___________

if yes, was the incident exercise related?

__________________________________________________________

Have you ever suffered a training related injury ?

__________________________________________________________

if yes, please elaborate.

Age of menarche ______ No. of menses per year ______
Does your menstrual pattern change during training?
if yes, please elaborate:

Are you currently: 

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<tr>
<th>Amenorrheic (0 menses/year)</th>
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<th>No</th>
<th>Since</th>
</tr>
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</tbody>
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<table>
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<th>Since</th>
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</thead>
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<table>
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<th>Since</th>
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</thead>
<tbody>
<tr>
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<td></td>
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</table>

Are you currently involved in a regular aerobic exercise program?

yes ___ no ___

if yes, no. of days/wk _______

average no. of miles/day ____

no. of months/year you run ____

no. of years you have been running ____

Are you involved in any additional type of athletic activities?

if yes, please elaborate (type of sport, no. of days/wk, length of workout):
APPENDIX D

BONE MINERAL DENSITY ASSESSMENT
Bone Density Measurement

Bone mineral density (BMD) of the axial skeleton was determined by dual photon absorptiometry, as described by Riggs et al. (1981). The dual photon absorption technique for measuring bone mineral content is based on measurements of radiogram transmission of two separate photon energies through a medium consisting of two different materials, bone and tissue. The dichromatic beam from a Gd\textsuperscript{153} source has photon electric peaks at 44 and 100 keV. The different energy dependence of the gamma-ray absorption coefficients in the two mediums permits the determination of the amount of bone present regardless of the amount of soft tissue.

The instrument consists of an Ohio Nuclear duall probe scanner frame with high-precision scanning mechanics, a Gd\textsuperscript{153} source, and a collimated NaI detector. The collimated photon beam traverses a 20-cm path across the spine in the area of interest and measurements are taken each second (the equivalent of one measurement every 12 mm). The distance between scanning paths is 4mm. The scanner is interfaced with a PDP-11V03 computer that also controls scanning pattern, speed, and data acquisition. The computer algorithm performs point-by-point determination of density of bone mineral, and an edge-detection program
recognized bone edges by evaluating the relative range in mineral density values between points.

Data are accumulated on disks and processed by a Data General NOVA XII/20 computer (NOVA Madison, WI). Intensity-modulated images of the spine are displayed on a 64x64 matrix. Lightpen interaction allows determination of the area of interest for immediate comparison of different parts of the skeleton. Bone mineral content (BMC) of the spine was assessed from scans of the lumbar (L1-L4) region. BMD expressed in grams per square centimeter, was derived by dividing BMC by the projected area of the spine. This value included vertebral bodies and disk interspaces. This technique has a coefficient of variation of 3%.
APPENDIX E

BODY COMPOSITION DETERMINATION
Body Composition Determination

Body Mass Index (BMI)

As part of the determination of bone mineral density, subjects were weighed on a physicians' scale and weight was recorded to the nearest 0.5 kg, and height was recorded to the nearest cm. These data were used to determine subject's BMI. $\text{BMI} = \frac{\text{wt/ht}^2}{\text{kg/m}^2}$

BMI highly correlates with other estimates of percent body fat, minimizes the effects of height on weight, and permits comparison within a population (Clark, 1988). A BMI of 21 corresponds to the 50th percentile of weight for a 19 year-old woman (Abraham, 1979). The BMI for a person with an anorectic physique is about 16 (Litt & Glader, 1986).

Body fat

Anthropometric measurements were also determined on athletic subjects. Skinfold thickness measured with Lange calipers (10g mm, constant pressure), were taken at three sites; the triceps, supra-illiac, and thigh; the average of repeated (3) trial values was used as the representative score for a given site (Behnke & Wilmore, 1974) for use in the equation of Jackson & Pollock (1980) for estimation of bone density.
BD = 1.0994921 - 0.0009929(X3) + 0.0000023(X3)^2 - 0.0001392(X4)

X3 = sum of triceps, thigh and suprailliac skinfolds, mm

X4 = age, years
APPENDIX F

IONIZED CALCIUM DETERMINATION
Serum Ionized Calcium Determination

Serum ionized calcium was determined by a calcium specific electrode (Radiometer America, Cleveland, OH). Values were adjusted by the following equation to correct for the change in pH that occurred due to the samples being frozen, and being collected under aerobic conditions.

\[ \text{Ca at pH 7.4} = [\text{Ca} \times 1 - 0.53 (7.4 - \text{pH})] \]

Normal serum ionized calcium values determined from venous blood are 1.14-1.29 (Tiepl, 1987). The panic range(s) are 0.5-0.7 mmol/l and 1.8-2.0 mmol/l respectively (Tiepl, 1978).
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<th>subject</th>
<th>Ca\textsuperscript{++}pH</th>
<th>corrected pre Ca\textsuperscript{++}pH</th>
<th>pH</th>
<th>corrected post</th>
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<td>1.12</td>
<td>0.91</td>
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<td>1.12</td>
<td>0.93</td>
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<td>1.10</td>
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<td>1.13</td>
<td>0.85</td>
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<td>1.16</td>
<td>0.87</td>
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<tr>
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<td>0.91</td>
<td>7.88</td>
<td>1.14</td>
<td>0.97</td>
</tr>
</tbody>
</table>
APPENDIX G

HORMONE ANALYSIS
Hormone Analysis

Serum 1,25 dihydroxyvitamin D (1,25(OH)2D) was determined by radioreceptor assay (Incstar, Stillwater, MN). The method is based on a thymus receptor that is specific for both 1,25(OH)2D2 and 1,25(OH)2D3. The assay involves a rapid extraction and preliminary purification of vitamin D metabolites from serum using a C19 cartridge with further purification of 1,25(OH)2D2 and 1,25(OH)2D3 from 25OHD2 and 25OHD3 on a silica cartridge. Quantitation is achieved using a nonequilibrium competitive protein binding assay. The addition of dextrin coated charcoal suspension, incubation, and centrifugation, separates the bound and free hormones. The supernatant, which contains the bound hormone, is decanted into ascertillation vial and counted. After correction for recovery, the final concentration of 1,25(OH)2D3 in the sample is expressed as picogram/ml (pg/ml).

Estradiol was determined by radioimmunoassay (Diagnostic Products Corp, Los Angeles, CA). The procedure is designed for the quantitative measurement of estradiol in serum. It is a no-extraction, solid phase 125I radioimmunoassay based on antibody coated tubes. 125I-labeled
estradiol competes with estradiol in the subject sample for antibody sites. After incubation, separation of bound hormone from free hormone is achieved by decanting. The sample tube containing the bound hormone is then counted in a gamma counter, the counts being inversely related to the amount of estradiol present in the subject sample. After correction for recovery, the final concentration of estradiol in the sample is expressed as picogram/ml (pg/ml).

The intact parathyroid hormone (IPTH) immunoassay (Allegro Corp. Nichols Institute, San Juan Capistrano, CA) system is a two-site immunoradiometric assay for the measurement of the biologically intact 84 amino-acid chain of PTH. Two different goat polyclonal antibodies to human PTH have been purified by affinity chromatography to be specific for well defined regions on the PTH molecule. One antibody is prepared to bind only the mid region and C-terminal PTH 39-84 and this antibody is immobilized onto plastic beads. The other antibody is prepared to bind only the N-terminal PTH 1-34 and this antibody is radiolabeled for detection.

The sample containing PTH is incubated simultaneously with an antibody coated bead and 125I-labeled antibody. Intact PTH present in the sample is bound by both the immobilized and labeled antibodies to form a "sandwich" com-
plex:

bead antiPTH(39-84) -- intact PTH(1-84) \(^{125}\)I
antiPTH(1-34)

Although mid region and C-terminal fragments are bound by the antibody coated bead, only the intact PTH 1-84 forms the sandwich complex necessary for detection. The capacity of the immobilized antibody has been adjusted to exhibit no interference by inactive fragments, even at very elevated levels.

At the end of the assay incubation, the bead is washed to remove unbound components and the radioactivity bound to the solid phase is measured in a gamma counter. Since the formation of a sandwich complex occurs only in the presence of an intact PTH molecule, the radioactivity of the bead bound complex is directly proportional to the amount of intact PTH in the sample.

A dose response curve of radioactivity vs. concentration is generated using results obtained from standards which are assayed concurrently with the unknowns. Concentrations of intact PTH present in the controls and patient samples are determined directly from this curve, and expressed in pg/ml.
APPENDIX H

DATA
Table 1. Physical characteristics of athletic females (n=13).

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>BMI (ht/m²)</th>
<th>BMD (g/cm²)</th>
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</thead>
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<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
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<td>166.0</td>
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<td>2</td>
<td>16</td>
<td>53.1</td>
<td>51.0</td>
<td>165.0</td>
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</tr>
<tr>
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<td>15</td>
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<td>4</td>
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<td>53.0</td>
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<td>49.0</td>
<td>52.0</td>
<td>160.0</td>
<td>163.0</td>
</tr>
</tbody>
</table>

\[ \overline{X} \quad 16 \quad 50.9 \quad 49.1 \quad 164.0 \quad 164.2 \quad 19.2 \quad 18.3 \quad 1.096 \quad 1.120* \]

SE 1.2 1.2 1.9 1.8 .45 .15 .20 .31

*p < .05

* bone mineral density decreased.
Table 2. Determination of subjects' bone mineral density based on an age-adjusted regression equation*.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Athletes (post)</th>
<th>Control</th>
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</thead>
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<tr>
<td></td>
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<td>BMD</td>
</tr>
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<td>1.1146</td>
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<tr>
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<td>.01</td>
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</table>

*\([0.3343 + (0.049 \times \text{age})]\)
Table 3. Menstrual and training history of female athletes (n=13).

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<tr>
<th>Subject</th>
<th>Age of Menses</th>
<th>Menses per yr</th>
<th>Training mi/wk</th>
<th>d/wk</th>
<th>mo/yr</th>
<th>yr change</th>
<th>Training w/ trading</th>
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### Table 4. Dietary information of female athletes (n=13).

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<th>Protein (%) (6 mos)</th>
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- **X** 1662 1802 16 15 35 36 50 50
- **SE** 117.2 187.5 .84 .69 1.5 1.5 1.4 1.4

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\[ \bar{X} = 1.12 \quad 2.18^* \quad 97.9 \quad 190.3^* \quad 257.8 \quad 628.5^* \quad 79.1 \quad 93.4^* \]

SE = .16 \quad .13 \quad 14.7 \quad 11.3 \quad 48.1 \quad 42.1 \quad 5.5 \quad 8.7

\( p < .05 \)

- data missing.
### Table 6. Serum ionized calcium and hormonal metabolism in calcium and vitamin D supplemented athletes (n=13)

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<thead>
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<th>Subject</th>
<th>Ionized calcium (mmol/L)</th>
<th>PTH (pg/ml)</th>
<th>Estradiol (pg/ml)</th>
<th>1,25(OH)2D3 (pg/ml)</th>
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(p < .05)

* data missing.
Table 7. Total serum calcium and magnesium in 13 calcium and vitamin D supplemented athletes.

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Table 8. Dietary fiber intake in female athletes (n=12).

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Table 9. Control subject data (n=7).

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<th>BMD (g/cm²)</th>
<th>Age of Menses</th>
<th>Menses per yr</th>
<th>Calcium (g/d) [%RDA/d]</th>
<th>Vitamin D (IU/d) [%RDA/d]</th>
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Table 10. Energy distribution of sedentary control subjects (n=7).

<table>
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<tr>
<th>Subject</th>
<th>Energy intake (calories/d)</th>
<th>Protein (g) (%)</th>
<th>Fat (g) (%)</th>
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eral density after resumption of menses in amenorrheic athletes. JAMA 1986;256:380-82.


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The vita has been removed from the scanned document
CHANGES IN BONE MINERAL DENSITY
IN CALCIUM SUPPLEMENTED ADOLESCENT ATHLETES
EXPERIENCING MENSTRUAL DYSFUNCTION

by
Janine Marie Baer

(ABSTRACT)
Thirteen adolescent runners experiencing menstrual dysfunction (mean no. menses = 5/yr) received dietary supplements of 1200 mg calcium carbonate and 400 IU vitamin D/d for 12 mos. Bone mineral content of the axial skeleton was measured by dual photon densiometry at the beginning and at the end of the 12 month supplementation period. Fasting plasma ionized calcium, intact PTH, 1,25(OH)2D3, estradiol, and thyroxin were also determined at the beginning and end of the study. Seven day dietary recalls were obtained for two separate weeks during the course of the study, one prior to the onset of supplementation and another six months later. Mean bone mineral density of the athletes increased (p<.05), but bone mineral density was observed to decrease in two of the subjects who had the lowest estradiol concurrent with the severest training regimen. Athletes’ total plasma calcium and 1,25(OH)2D3 increased (p<.05). No measured variable correlated with the bone mineral density of the lumbar spine. There was a significant correlation between dietary calcium intake and total plasma calcium (r=.50), and dietary vitamin D
intake and 1,25(OH)2D3 (r=.50). Stepwise linear regression predicted a linear relationship between serum estradiol and bone mineral density, and no. menses/yr and bone mineral density. Multiple linear regression procedures indicated several predictive models incorporating variables that significantly affect BMD. The models include serum estradiol, no. menses/yr, body mass index, dietary calcium and dietary vitamin D intake. The increase in dietary calcium intake (192% RDA) and/or dietary vitamin D intake (190% RDA) due to supplementation did not appear to contribute to an increase in bone mineral density in estrogen compromised female athletic adolescents independent of growth.