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BONE MINERAL CONTENT OF AMENORRHEIC AND EUMENORRHEIC ATHLETES

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Abstract This study was designed to determine whether the hypoestrogenic status of 14 amenorrheic athletes was associated with a decrease in regional bone mass relative to that of 14 of their eumenorrheic peers. The two groups of athletes were matched for age, height, weight, sport, and training regimens. Bone mass was measured by dual-photon and single-photon absorptiometry at the lumbar vertebrae (L1 to L4) and at two sites on the radius. Vertebral mineral density was significantly lower in the amenorrheic group (mean, 1.12 g per square centimeter) than in the eumenorrheic group (mean, 1.30 g per square centimeter). There was no significant difference at either radial site. Radioimmunoassay confirmed a lower mean estradiol concentration (amenorrheic group, 38.58 pg per milliliter; eumenorrheic group, 106.99 pg per millili-

THE prevalence of secondary amenorrhea among female athletes has been reported to range from 3.4 to 43 per cent, depending on the investigator's definition of amenorrhea and selection of subjects.^{1,2} Among highly trained endurance athletes, for example, 25 to 40 per cent of the women report fewer than three menses per year.^{2,3} Concern about the clinical implications of this phenomenon centered originally on possible detrimental effects on reproductive function. More recently, attention has been drawn to the potential adverse consequences of prolonged low levels of estrogen on bone mass.

Although the precise mechanism by which estrogen affects bone mineralization is unknown, numerous studies have shown that low estrogen states, such as those observed in hyperprolactinemia or after surgical or natural menopause, are associated with low levels of skeletal bone mass.⁴⁻⁶ The hypothesis that hypoestrogenic amenorrheic athletes may also have decreased bone mass would seem plausible were it not for the fact that physical activity has been shown to inhibit and even reverse bone loss in postmenopausal

ter) and progesterone peak (amenorrheic group, 1.25 ng per milliliter; eumenorrheic group, 12.75 ng per milliliter) in the amenorrheic women, in four venous samples drawn at seven-day intervals. A three-day dietary history showed no significant differences in nutritional intake, including calcium with and without supplements. The two groups were similar in percentage of body fat, age at menarche, years of athletic participation, and frequency and duration of training but differed in number of miles run per week (amenorrheic group, 41.8 miles [67.3 km]; eumenorrheic group, 24.9 miles [40.1 km]). We conclude that the amenorrhea that is observed in female athletes may be accompanied by a decrease in mineral density of the lumbar vertebrae. (N Engl J Med 1984; 311: 277-81.)

women.^{7,8} Since the frequency, duration, and intensity of training regimens for endurance athletes far exceed those prescribed for postmenopausal women, one might expect the activity to exert a protective effect against bone loss in the amenorrheic athlete. This study was designed to test that assumption.

METHODS

Subjects

Twenty-eight women athletes participated in this study after giving their informed consent in accordance with procedures established by the University of Washington Human Subjects Review Committee. Fourteen of the women were amenorrheic, having had no more than one menstruation in the preceding 12 months. From a larger pool of potential subjects, 14 eumenorrheic women were selected to match the amenorrheic athletes for the following variables and in order of priority: sport, age, weight, height, and the frequency and duration of daily training sessions. Eleven of the subjects in each group were runners; the remaining three were crew members. Prestudy interviews with one of us (K.N.) eliminated amenorrheic women who had a history of eating disorders, who were undergoing hormone-replacement therapy, or whose amenorrhea or other cyclic irregularities predated the beginning of training. All the women were nonsmokers, in good health, and had not used oral contraceptives during the preceding six months. All had experienced menarche, and the majority of the amenorrheic athletes, 12 of 14, had normal cycles before they began training. The other two subjects began training before the menarche.

Protocol

The subjects reported to the laboratory at 8:00 a.m. after an overnight fast on four separate occasions at seven-day intervals. A 30-ml sample of venous blood was drawn after a 20-minute sitting

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rest. The samples were allowed to clot and then were centrifuged, and the serum was stored at -20°C until it was assayed for estradiol, progesterone, testosterone, and prolactin. During the same month the women were scheduled for determination of regional bone mass by photon absorptiometry and for estimation of body density by hydrostatic weighing. During the first visit each subject completed a brief questionnaire concerning her menstrual history and athletic activities.

A registered nutritionist met with the women during one of the four laboratory sessions to explain procedures for maintaining a three-day dietary diary. Diaries were returned directly to the nutritionist, who then conferred individually with each subject to verify the information before coding it for computer analysis.

Regional Bone-Mass Measurements

Single-photon and dual-photon absorptiometry were used to measure regional bone mass at the distal radius and lumbar vertebrae, respectively. Single-photon measurements were made on the nondominant arm with a Norland-Cameron Bone Mineral Analyzer, Model 178, at two sites: S_1 and S_2 — one tenth and one fifth, respectively, of the forearm length proximal to the styloid process. The S_1 site contains both cortical and trabecular (12 to 20 per cent) bone, whereas the S_2 site consists primarily of cortical bone.⁹ Bone mineral content was measured in grams per centimeter, and bone mineral density in grams per square centimeter, the latter derived by dividing bone mineral content by the radial bone width. In our laboratory this technique has a coefficient of variation of 2 per cent at the S_1 site and 3 per cent at the S_2 site.

The bone mineral density of the axial skeleton was determined by the dual-photon absorptiometry techniques originally described by Mazess et al.¹⁰ and Riggs et al.¹¹ This technique measures the transmission of a collimated beam of photons (derived from gadolinium-153) of two distinct energies (42 and 100 keV) through bone and soft tissue. A transmission scan is obtained by counting the 42-keV and 100-keV gamma rays of gadolinium-153 after they have been attenuated by bone and soft tissue; 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.

For spine measurements, the mineral content of the individual vertebrae L1 through L4 was determined. The vertebrae at this site are estimated to contain 50 to 66 per cent trabecular bone.¹² Bone mineral density, expressed in grams per square centimeter, was derived by dividing the mineral content by the projected area of the spine (L1 through L4). This value includes vertebral bodies and disk interspaces. In our laboratory this technique has a coefficient of variation of 3 per cent.

Hormone Assays

Estradiol and testosterone were measured by previously described radioimmunoassays.^{13,14} Progesterone was measured by radioimmunoassays with use of reagents purchased from Diagnostic Products. The prolactin radioimmunoassay used a reference preparation (RP-1) and first antibody (AFP-C 11590) obtained from the National Hormone and Pituitary Program. Radioiodinated prolactin was purchased from Diagnostic Products.

Hydrostatic Weighing

Underwater weights were measured in an immersion tank in water maintained at 36 to 39°C . The calculated underwater weight was corrected for air remaining in the lungs after forced exhalation by measuring residual volume before immersion, with use of the nitrogen-dilution method described by Wilmore.¹⁵ Percentage of body fat was estimated with use of the formula of Brožek et al.: percentage of body fat = $100 (4.570/D - 4.142)$.¹⁶

Data Analysis

Comparisons between the amenorrheic and eumenorrheic groups were made with Student's two-tailed t-test for uncorrelated data. Pearson's product moment correlation was used to describe the relations among selected variables.

Table 1. Physical Characteristics and Training Regimens of 14 Amenorrheic and 14 Eumenorrheic Athletes.*

	AMENORRHEIC	EUMENORRHEIC	P VALUE
Age (yr)	24.9±1.3	25.5±1.4	NS
Height (cm)	166.1±2.5	165.7±2.2	NS
Weight (kg)	54.4±2.3	57.9±2.2	NS
Body fat (%)	15.8±1.4	16.9±0.8	NS
Lean body mass	45.6±1.6	48.0±1.6	NS
Age at menarche (yr)	12.5±0.5	12.8±0.4	NS
Duration of amenorrhea (mo)	41.7±7.4		
Length of participation in sport (yr)	7.0±1.6	6.6±1.1	NS
Training			
Days/week	6.2±0.2	5.6±0.3	NS
Hours/day	1.6±0.2	1.8±0.3	NS
Time for 10-km race (min:sec) †	39:06±1:15	45:59±1:49	NS
Miles run/week ‡	41.8±5.2	24.9±3.0	<0.01
Minutes/mile ‡§	7:32±0:20	7:47±0:18	NS

*Plus-minus values are means ±S.E.M. NS denotes not significant.

†N = eight eumenorrheic and seven amenorrheic athletes.

‡N = 12 eumenorrheic and 10 amenorrheic athletes.

§Pace of long, slow distance runs.

RESULTS

The physical characteristics and training regimens of the two groups of athletes were similar (Table 1). Although body composition had not been considered in matching the subjects, amenorrheic and eumenorrheic women had a similar percentage of body fat. The only marked difference between the amenorrheic and eumenorrheic groups in athletic history was the number of miles run per week.

Although neither bone mineral content nor bone mineral density at the two sites along the radius differed between groups (Table 2), the mineral density of the lumbar vertebrae was significantly lower in the amenorrheic group of athletes. Although the mineral content and mineral density at S_1 and S_2 were significantly related ($r = 0.72$), there was no significant relation between the mineral density of the vertebrae and that at either radial site (S_1 , $r = 0.31$; S_2 , $r = 0.34$).

Estradiol levels, expressed either as an average of four samples or as the peak value, were significantly lower in the amenorrheic group (Table 3). Eight of these women had estradiol values under 45 pg per milliliter in each of their four samples. Three of the

Table 2. Bone Mineral Content and Density in 14 Amenorrheic and 14 Eumenorrheic Athletes at Two Forearm Sites and at the L1 through L4 Lumbar Vertebrae.*

SITE	AMENORRHEIC	EUMENORRHEIC	P VALUE
Radius			
S_1			
Mineral content (g/cm)	0.89±0.03	0.85±0.03	NS
Mineral density (g/cm ²)	0.53±0.02	0.54±0.01	NS
S_2			
Mineral content (g/cm)	0.91±0.02	0.88±0.03	NS
Mineral density (g/cm ²)	0.67±0.02	0.67±0.02	NS
Vertebrae (g/cm ²)	1.12±0.04	1.30±0.03	<0.01

*Plus-minus values are means ±S.E.M. NS denotes not significant.

Table 3. Hormone Levels in 14 Amenorrheic and 13 Eumenorrheic Athletes.*

HORMONE	AMENORRHEIC	EUMENORRHEIC	P VALUE
Estradiol (pg/ml)			
Mean	38.58±7.03	106.99±9.80	<0.01
Peak	67.75±13.77	205.39±20.6	<0.01
Progesterone (ng/ml)			
Peak	1.25±1.00	12.75±2.4	<0.01
Testosterone (ng/ml)	0.47±0.04	0.42±0.04 †	NS
Prolactin (ng/ml)			
Peak value	23.73±3.74	44.74±7.34	<0.02
Mean	17.04±2.28	33.96±6.80	<0.02

*Plus-minus values are means ±S.E.M. NS denotes not significant.

†N = 14.

other women had at least one sample in which the value was between 45 and 100 pg per milliliter, and the remaining three had one or more samples in which the value exceeded 100 pg per milliliter. Twelve of the eumenorrheic women had an estradiol profile that was characteristic of women with regular cycles, and at least one estradiol value was above 130 pg per milliliter. The other two women reported menstruating within 12 days of entering the study and having regular periods during the previous 12 months.

All four progesterone values for 13 of the amenorrheic women were less than 0.65 ng per milliliter, suggesting that ovulation had not occurred. One woman had a peak concentration of 13.1 ng per milliliter that was coincident with an estradiol level of 93.6 pg per milliliter, but she did not experience menstrual flow. Progesterone concentrations for the eumenorrheic group indicated the presence of an ovulatory cycle in 11 of the subjects. The peak progesterone level in two of the other women was under 2.0 ng per milliliter, suggesting the presence of an anovulatory cycle. Both women had peak estradiol levels in excess of 250 pg per milliliter. No conclusions can be drawn about the remaining member of this group, since two of her samples were lost through technical error.

Prolactin levels also differed between groups (Table 3). Two women had at least one prolactin value above 70 ng per milliliter and a four-sample average that was above 60 ng per milliliter. Both were in the eumenorrheic group and reported the occurrence of regular

Table 4. Three-Day Dietary History in 14 Amenorrheic and 14 Eumenorrheic Athletes.*

	AMENORRHEIC	EUMENORRHEIC	P VALUE
Total caloric intake/day (kcal)	1622.7±145.1	1965.1±98.4	NS
Carbohydrate/day (g)	222.2±19.6	255.4±14.8	NS
Fat/day (g)	56.7±8.0	79.3±8.2	NS
Protein/day (g)	66.43±6.72	66.46±6.87	NS
Calcium (mg)			
Without supplements	888±105	912±130	NS
With supplements †	960±98	1100±153	NS
Phosphorus (mg)	1167±109	1241±128	NS
Calcium/phosphorus	0.92±0.17	0.88±0.09	NS

*Plus-minus values are means ±S.E.M. NS denotes not significant.

†Seven subjects in each group included calcium supplements in their daily intake.

cycles during the previous 12 months. The relation between prolactin and estradiol was significant for both mean values ($r = 0.54$, $P < 0.01$) and peak concentrations ($r = 0.44$, $P < 0.02$).

Testosterone levels did not differ between groups and were within the normal range for women (Table 3).

The dietary intake of the two groups was similar (Table 4). However, the lower total intake of calories ($P < 0.06$) and of fat ($P < 0.06$) of the amenorrheic group closely approached the level of significance ($\alpha = 0.05$) established for this study. There was no difference between the amenorrheic and eumenorrheic women in calcium intake, either through diet alone or through diet plus supplements. For both groups, intake exceeded the current recommended dietary allowance of 800 mg per day.

Although both mean and peak estradiol values were significantly different between groups, neither value was significantly correlated with bone mineral density at any site. With the exception of a significant relation between age and mineral density of the radius (S_1 , $r = 0.52$; S_2 , $r = 0.48$), none of the physical characteristics, training factors, hormone levels, or dietary variables were related to the mineral density of the radius or the lumbar vertebrae.

DISCUSSION

Neither physical activity nor menstrual state resulted in any marked deviations from the norm in the mineralization of the predominantly cortical bone of the radius. The observed values for bone mineral content in both groups of athletes fell within ± 0.02 g per centimeter (S_1) and ± 0.04 g per centimeter (S_2) of the norms for nonathletes with regular cycles who were tested in the same laboratory. When the values for vertebral mineral density were compared with those reported by Riggs et al.¹⁷ 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 per square centimeter) by an age-based regression equation. In contrast, the average bone mineral density of the amenorrheic athletes was equivalent to that of women 51.2 years of age. Two of these athletes had a vertebral mineral density below the fracture threshold as defined by Riggs et al.,¹¹ which is 0.965 g per square centimeter.

Cann et al.¹⁸ recently reported a similar finding in a descriptive study of amenorrheic women, including a subgroup of 11 women who were classified as having hypothalamic amenorrhea. The regular participation of 10 of this group in physical activity suggested a possible association between exercise-induced amenorrhea and a decrease in vertebral mineral content. Since the study was not originally designed to examine the relation between "athletic amenorrhea" and bone mineral content, there was some question about whether the amenorrhea was indeed related to exercise and whether other uncontrolled factors might explain the decrease in vertebral mineral content. For

example, the extremely low percentage of torso body fat of the hypothalamic amenorrheic group, which was 11 per cent of the control value, raised the possibility that dietary factors may have contributed to both the amenorrhea and the decrease in bone mineral content. The results of the present study, which was designed to maximize the probability that the amenorrhea was associated with exercise and to examine factors related to diet and the training program, should alert physicians to the possibility that some amenorrheic athletes may indeed have a decrease in bone density. This may be due to their hypoestrogenic status, to the interaction of low estrogen levels with some other variable, or to 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.¹⁹ One such indirect route may be the effect of estrogen on calcium balance, since there is ample evidence that a lack of estrogen increases the daily calcium requirement.²⁰ There was no difference in the calcium intake of our amenorrheic and eumenorrheic subjects, either with or without supplementation. Both groups met the current recommended dietary allowance of 800 mg per day. However, the decrease in calcium absorption and the increase in calcium excretion in estrogen-deficient women has led Heaney²⁰ to recommend a daily intake of 1.5 g of elemental calcium to maintain calcium balance in low-estrogen states. When these criteria are applied, our amenorrheic women are deficient in calcium, whereas the eumenorrheic women are meeting their daily requirement. When the figures derived by Heaney²⁰ from treated and untreated postmenopausal women are used, the difference in calcium balance between groups is as much as 30 mg per day.

The amount of physical activity reported by our amenorrheic athletes did not protect them from an apparent loss of vertebral bone. This should not be interpreted as negating the value of exercise in maintaining skeletal integrity, but it does suggest an interaction between estrogen and exercise in their effect on specific skeletal areas. In both our study and that of Cann et al.,¹⁸ the radius, which is predominantly cortical bone, was unaffected, whereas the mineral content of the vertebrae, which have more trabecular bone, was lower in the amenorrheic women. This is contrary to what has been found in male runners. Dalen and Olsson,²¹ using x-ray spectrophotometry, reported a mean bone mineral content approximately 20 per cent higher in the appendicular skeleton of cross-country runners than in that of controls, but no significant differences between groups at the third lumbar vertebra. Male marathon runners also had higher indexes of skeletal mass than did sedentary

controls, but both measures of bone mineral content — total body neutron activation and photon absorptiometry of the radius — reflect predominantly cortical bone.²² At present there are not enough data to draw any firm conclusions concerning a differential effect of physical activity and estrogen on cortical and trabecular bone. However, the data do provide a basis for recommending that sites having a high proportion of trabecular bone be included in an evaluation of the effect of diminished estrogen stimulation on the bone mineral content of amenorrheic athletes.

Our results should be viewed as an impetus for further research rather than as a signal to amenorrheic athletes to cease their strenuous conditioning programs. Additional verification of our observations is needed to ensure that our findings can be generalized to a wider population. Amenorrheic athletes from sports other than running should be evaluated, particularly athletes from sports that place less emphasis on low percentage of body fat and involve greater mechanical strain on the spine. Our amenorrheic crew members, for example, had an average vertebral mineral density of 1.22 g per square centimeter, which is 0.13 g per square centimeter higher than that of amenorrheic runners. Additional skeletal areas should also be examined, particularly those that are subjected to repeated mechanical stresses during running, such as the femur and tibia. Numerous questions remain to be answered regarding the long-term effect of amenorrhea on the skeletal integrity of female athletes.

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