Physiologic and behavioral indicators of energy deficiency in female adolescent runners with elevated bone turnover[1–3]

Michelle T Barrack, Marta D Van Loan, Mitchell J Rauh, and Jeanne F Nichols

ABSTRACT

Background: Female adolescent runners have an elevated prevalence of low bone mass for age—an outcome that may be partially due to inadequate energy intake.

Objective: The objective was to evaluate diet, menstrual history, serum hormone concentrations, and bone mass in female adolescent runners with normal or abnormal bone turnover.

Design: Thirty-nine cross-country runners (age: 15.7 ± 0.2 y) participated in the study, which included a 7-d dietary assessment with the use of a food record and daily 24-h dietary recalls; serum measures of insulin-like growth factor I, estradiol, leptin, parathyroid hormone, progesterone, triiodothyronine, 25-hydroxycholecalciferol, bone-specific alkaline phosphatase (BAP), and cross-linked C-telopeptides of type I collagen (CTX); an evaluation of height, weight, bone mass, and body composition with the use of dual-energy X-ray absorptiometry; and a questionnaire to assess menses and sports participation. Age- and sex-specific BAP and CTX concentrations of at least the 97th percentile and no greater than the third percentile, respectively, were considered abnormal.

Results: All abnormal BAP and CTX concentrations fell within the elevated (≥97%) range. Runners with an elevated bone turnover (EBT) (n = 13) had a lower body mass, fewer menstrual cycles in the past year, lower estradiol and 25-hydroxycholecalciferol concentrations, and a higher prevalence of body mass index <10% for age, vitamin D insufficiency, amenorrhea, and low bone mass. Girls with EBT consumed less than the recommended amounts of energy and had a higher prevalence of consuming <1300 mg Ca than did those with normal bone turnover.

Conclusions: Runners with EBT had a profile consistent with energy deficiency. Nutritional support to increase energy, calcium intake, and 25-hydroxycholecalciferol concentrations may improve bone mineral accrual in young runners with EBT. This trial was registered at clinicaltrials.gov as NCT01059968. *Am J Clin Nutr* 2010;92:652–9.

INTRODUCTION

The adolescent years represent a critical period for bone mineral accrual. In healthy individuals, the rate of bone mineral accumulation reaches its peak between Tanner Stages 2 and 4, with rapid gains occurring until age 16 y (1–4). It appears necessary to maximize bone mineral accrual during this time because equivalent increases may not be attainable in the latter adolescent years or young adulthood.

Previous laboratory studies have shown the importance of maintaining sufficient energy status for normal functioning of hormones that regulate bone formation and bone resorption (5–8). In one study, biomarkers of bone formation decreased after only 5 d of an energy intake and exercise regimen that yielded low energy availability (<30 kcal/kg fat-free mass daily) in young adult sedentary women, which emphasizes the close relation between systemic energy status and bone turnover in this population (5). This finding and observations from other studies suggest that energy may play an equal, if not more important role, in bone mineral accrual than other bone-building nutrients (eg, calcium and vitamin D) because the hormone profile resulting from energy restriction reduces calcium absorption and enhances the mobilization of calcium from bone (4–10).

Some populations of adolescents may be at risk of consuming inadequate energy and, as a result, may accrue inadequate bone mass during the critical bone-building years. Adolescents with a diagnosis of anorexia nervosa are one such population because they practice extreme dietary restriction and have suppressed bone mineral gains (11–13). Recently, an elevated prevalence of low bone mass for age was observed in a sample of adolescent endurance runners (14, 15)—a finding consistent with that of studies in collegiate runners (16–18). Variables associated with lower bone mass in the adolescent runners included higher levels of training, dietary restraint, and menstrual irregularity (14, 19). It was hypothesized that each of these factors either reduced the girls' energy status (ie, higher training and dietary restraint) or were indicative of low energy availability (ie, menstrual irregularity); however, energy intake was not directly measured [14, 15].

The recent development of normal pediatric reference curves for serum markers of bone formation and resorption (20) facilitates the short-term evaluation of the relation between an individual's energy status and their bone turnover (21). This is important because the effect of negative behaviors on bone mass

[1] From the Graduate Group in Nutritional Biology, University of California Davis, Davis, CA (MTB); the US Department of Agriculture, Agricultural Research Service, Western Human Nutrition Research Center, University of California Davis, Davis, CA (MDVL); the Graduate Program in Orthopaedic and Sports Physical Therapy, Rocky Mountain University of Health Professions, Provo, UT (MJR); and the School of Exercise and Nutritional Sciences, San Diego State University, San Diego, CA (MJR and JFN).

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[3] Address correspondence to MT Barrack, 817 South Westgate Avenue, no. 101, Los Angeles, CA, 90049. E-mail: michellebarrack@gmail.com.

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can take up to 6–12 mo to be recognized (3). Therefore, short-term evaluation of bone mass with the use of biomarkers of bone turnover may play a critical role in identifying adolescents at risk of suppressed bone mineral accrual.

Whereas mounting evidence from previous studies suggests that a relative energy deficit may lead to low bone mass in adolescent runners (14, 15), this has not been directly evaluated. Therefore, our current study aimed to evaluate the relation between adolescent runners' nutritional intake and biomarkers of bone turnover and to assess runners' body mass and body composition, menstrual status, sports participation history, serum hormone concentrations, and bone mass.

SUBJECTS AND METHODS

Subjects

Participant recruitment occurred during the first 2 wk of the fall 2008 cross-country season. Female high school cross-country runners aged 14–17 y competing in the fall 2008 season, who previously competed in at least one season of an endurance running sport (cross-country or track and field in which their primary event was ≥1 mile), were currently running ≥25 miles/wk, and were not taking medication known to affect bone mass were invited to participate. Informed participant assent and parent consent were obtained before data collection. This study was approved by the San Diego State University and University of California, Davis, Institutional Review Boards.

Data collection

Two to 5 d before the 7-d data collection period, trained graduate student research assistants provided a 60-min instructional training in-service to review the protocol for the upcoming week of data collection and to instruct participants on proper techniques for recording their dietary intake. On days 1–7 of the data collection period, the girls recorded their dietary intake and underwent daily dietary recalls. On the morning of the day 8, the girls arrived in a fasting state at our research center and provided a small sample of blood. After the blood draw, the girls completed several questionnaires that inquired about their menstrual function, training volume, sports participation, dietary supplement use in the past year, and injury history. In addition, the girls underwent a dual-energy X-ray absorptiometry (DXA) scan to measure their bone mass and body composition. Before the DXA scan, height and weight were measured to the nearest 0.1 kg and 0.1 cm, respectively, while the subjects were shoeless.

Blood collection

The girls provided a fasted, morning blood sample to minimize variability due to circadian rhythm effects on concentrations of some hormones. A certified phlebotomist drew all of the blood samples using standard sterile techniques into an 8.5-mL evacuated tube (SST Vacutainer; Becton Dickinson, Franklin Lakes, NJ). After clotting, the blood samples were centrifuged at 4°C, 1500 × *g*, for 15 min, and the remaining serum was portioned into aliquots and frozen at –80°C until analyzed.

Serum biomarkers of bone turnover and hormones

All serum assays were conducted at the US Department of Agriculture, Agricultural Research Service, Western Human Nutrition Research Center on the campus of the University of California, Davis. The Serum Crosslaps ELISA (Immunodiagnostic Systems Inc, Fountain Hills, AZ) was used to analyze serum cross-linked C-telopeptides of type I collagen (CTX). The intra- and interassay precision CVs were <6% and <10%, respectively. The Metra bone-specific alkaline phosphatase (BAP) immunoassay (Quidel Corporation, Santa Clara, CA) was used to analyze BAP. This kit had intra- and interassay precision CVs of 4–6% and 5–8%, respectively.

Estradiol, IGF-I, intact PTH, progesterone, and free T_3 were evaluated by Immulite Immunoassay (Siemens's Health Care Diagnostics, Deerfield, IL). The intraassay precision CVs for each were 10.3%, 2.5%, 3.6%, 5.0%, and 5.7%, respectively. Twenty-five hydroxycholecalciferol was measured by radioimmunoassay (DiaSorin Inc, Stillwater, MN). The mean intraassay precision CV was 8.81%.

Bone turnover classification

Pediatric reference ranges for serum concentrations of BAP and CTX were used to classify runners with normal or abnormal bone turnover (20). Age- and sex-specific BAP and CTX concentrations of at least the 97th percentile and no greater than the 3rd percentile, respectively, were considered abnormal.

Dietary intake

To optimize the accuracy of the diet assessment, a 7-d diet record and daily 24-h recalls measured the runners' intakes. The girls recorded the description and the amount of all food, drink, and supplement items consumed during each day of the 7-d assessment period. In addition, trained nutrition graduate students interviewed the girls each evening to assess their intake during the previous 24 h. Runners were encouraged to continue their normal eating patterns and to note whether their diet was typical and, if not, why (eg, if they were sick, ate out more often, or were at a friends' house). Nutrition interviewers used the multiple pass method of interview from the Nutrition Data System for Research (NDSR, University of Minnesota, Minneapolis, MN) software for the 24-h recall. Data collected from the 7-d diet log and the 24-h recall were analyzed by using the NDSR analysis software.

NDSR uses the University of Minnesota Nutrition Coordinating Center (NCC) Food and Nutrient Database, which includes 18,000 foods and 7000 brand products, derives its nutrient data from the US Department of Agriculture, Nutrient Data Laboratory, food manufacturers, and data available from the scientific literature (22). The NDSR diet reports provide an assessment of 160 nutrients and 9 food groups. This software is considered the gold standard tool for analyzing an individual's diets (22). Food or supplement items not included in the NCC database were added by using food labels, nutrition information provided by the manufacturers or restaurant food chains, or recipes provided by runners that were used when cooking at home.

The girls were instructed on how to record their dietary intake for the 7-d dietary assessment period. During the training period, the girls participated in several instructional exercises in which they were supervised and monitored while measuring various

amounts of different solid and liquid food items. After being measured, the food in each serving cup or spoon was poured in different-sized plates, bowls, or cups or placed in 1 or 2 of their own hands to allow them to visualize each serving amount. Research assistants then took pictures of the girls with 8, 4, 2, and 1 fluid ounce(s) of snack mix in their hands to provide a visual image for them to use when estimating amounts of snack food items consumed when away from home. Visual images of various serving size amounts were also included in their training binder. In addition, girls measured and recorded the distance, in inches, from the tip of their thumb to the tip of their index finger, allowing them to have a reference measure always "on hand" to measure the portion sizes of pizza, casserole dishes, breads, etc, when they were away from home.

Menstrual irregularity

Girls were classified with primary amenorrhea if they were age ≥15 y and had not begun menstruating. Secondary amenorrhea was defined as the absence of ≥3 consecutive menstrual cycles in the past year (23), and oligomenorrhea was defined as menstrual cycle lengths >35 and <90 d during the past year (24).

Bone mass

Bone mineral content (BMC; in g) and areal bone mineral density (BMD; in g/cm^2) at the spine (L1-L4), proximal femur, and total body and body composition were assessed by DXA with a Lunar DPX-NT densitometer (GE Medical Systems Lunar, Madison, WI). The runners were categorized with low bone mass for their age if their BMD Z-scores at the spine or total body were ≥1 SD or ≥2 SD below the age- and ethnicity-matched, sex-specific reference data from the GE/Lunar pediatric database (Z-score of ≤ −1 or ≤ −2) (24). The GE Lunar Femur Strength Analysis Software (GE Medical Systems Lunar) was used to measure hip axis length and provided estimates of the hip structural properties [ie, cross-sectional area (CSA) and cross-sectional moment of inertia (CSMI)]. Quality assurance tests were performed on each morning of testing. The CV in BMD in our laboratory is 0.6% for the total hip, 1.2% for the spine (L1-L4), and 1.0% for the total body.

Sports participation

In the sports history section of the questionnaire, girls reported the number of years and months per year of competition in and/or training for all organized sports in which they participated from age 10 y to the current year. Months of participation in an endurance running or non–lean build, impact loading, or aesthetic sport were recorded. Endurance running included cross-country or track and field, where the runner's main event was ≥1 mile (1609 m). Non–lean build, impact loading sports were basketball, field hockey, lacrosse, martial arts, rugby, soccer, softball, tennis, track and field (sprinting and field events), and volleyball. Aesthetic sports included all forms of dance, gymnastics, and ice skating.

Statistics

Values are expressed as means ± SEMs. Analysis of variance and analysis of covariance were used to determine whether there were significant differences between mean values for the normal and abnormal bone turnover groups. Chi-square tests assessed prevalence estimates between groups. Odds ratios and 95% CIs were calculated to determine risk factors for abnormal bone turnover. All analyses were performed by using the Statistical Package for Social Sciences version 16.0.

RESULTS

Descriptive characteristics

Because, under normal conditions, biomarkers of bone turnover and some of the analyzed serum hormones (eg, leptin, IGF-I, and estradiol) vary throughout adolescence, mean differences were assessed for the sample as a whole and stratified by age. Younger (age 14–15 y) runners had higher BAP and CTX values than did older (age 16–17 y) runners (BAP: 70.0 ± 5.2 compared with 34.4 ± 5.2, $P < 0.001$; CTX: 1.9 ± 0.1 compared with 1.3 ± 0.1, $P < 0.005$). Thirteen (33%) runners met the criteria for abnormal bone turnover (8 aged 14–15 y and 5 aged 16–17 y). Of these 13 runners all BAP and CTX values fell within the elevated (≥97th percentile) range, which indicated elevated bone turnover (EBT). For both age groups, runners with EBT had significantly higher serum BAP and CTX concentrations than did those with normal turnover (**Figure 1**). Within the sample, BAP and CTX were strongly correlated ($r = 0.82$, $P < 0.001$).

Compared with those with normal turnover, runners with EBT were younger and began running competitively at a younger age (**Table 1**). They had a significantly lower mean body weight, fat mass, lean tissue mass, and body mass index (BMI) and reported fewer menstrual cycles in the past year (Table 1). The number of months or seasons that girls competed in or trained for an endurance running sport did not differ significantly between groups (Table 1). Runners with EBT reported a 23.1% ($n = 3$), 38.5% ($n = 5$), and 0% ($n = 0$) prevalence of primary amenorrhea, secondary amenorrhea, and oligomenorrhea, respectively. Two additional runners with EBT had not yet begun menstruating, but were aged <15 y and therefore were not classified with primary amenorrhea. Runners with normal bone turnover reported a 0% ($n = 0$), 3.8% ($n = 1$), and 34.6% ($n = 9$) prevalence of primary amenorrhea, secondary amenorrhea, and oligomenorrhea, respectively. Prevalence estimates of primary amenorrhea ($\chi^2 = 7.7$, $P < 0.01$), secondary amenorrhea ($\chi^2 = 9.9$, $P < 0.005$), and total clinical menstrual irregularities ($\chi^2 = 3.6$, $P = 0.06$) were higher in those with EBT. Furthermore, runners with EBT had a higher prevalence of BMI <10% for age, late age at menarche, and low bone mass (**Figure 2**).

Energy and macronutrient intakes

Runners with EBT had a significantly higher prevalence of consuming <2000 kcal/d (53.8% compared with 19.2%; $\chi^2 = 4.88$, $P < 0.05$). Further, runners with EBT trended toward lower mean daily intakes of energy ($P = 0.07$), carbohydrate ($P = 0.09$), and dietary fat ($P = 0.09$) than did those with normal turnover (**Table 2**). Total calorie and carbohydrate ($g \cdot kg^{-1} \cdot d^{-1}$) intakes fell below or in the lower portion of the recommended range for all runners (Table 2) (25–27). All runners additionally consumed less than the recommended amount of fiber (Table 2) (25).

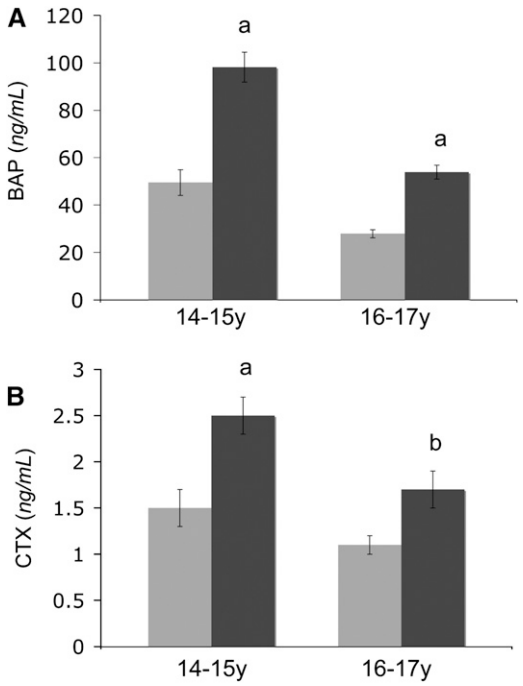

FIGURE 1. Mean (±SEM) concentrations of (A) bone alkaline phosphatase (BAP) and (B) cross-linked C-telopeptides of type I collagen (CTX) in runners with normal (■; age 14–15 y, $n = 11$; age 16–17 y, $n = 15$) or elevated (■; age 14–15 y, $n = 8$; age 16–17 y, $n = 5$) bone turnover. [a,b]Significantly different from control (ANOVA): [a]$P < 0.005$, [b]$P < 0.05$.

TABLE 1
Descriptive characteristics of runners with normal or elevated bone turnover[1]

	Normal bone turnover ($n = 26$)	Elevated bone turnover ($n = 13$)
Chronologic age (y)	16.3 ± 0.2	15.5 ± 0.3[2]
Age at which running began (y)	13.6 ± 0.2	12.8 ± 0.3[2]
Age at menarche (y)	13.4 ± 0.3	14.4 ± 0.5
Gynecologic age (y)[3]	2.9 ± 0.3	1.9 ± 0.5
Height (cm)	165.9 ± 1.0	163.2 ± 1.5
Weight (kg)	56.9 ± 1.4	48.6 ± 1.9[4]
Body fat (%)	23.5 ± 1.2	19.1 ± 1.7[2]
Fat mass (kg)	12.9 ± 0.9	9.2 ± 1.2[4]
BMI (kg/m^2)	20.7 ± 0.4	18.2 ± 0.6[4]
Lean tissue mass (kg)	41.1 ± 0.7	36.8 ± 1.0[4]
Number of menses[5]	9.5 ± 0.7	3.7 ± 1.1[4]
Lifetime endurance running seasons[6]	4.4 ± 0.4	3.8 ± 0.6
Running months[7]	19.7 ± 2.1	18.8 ± 2.9
Non–lean build months[7]	33.2 ± 5.1	21.8 ± 7.1
Aesthetic months[7]	10.6 ± 4.3	18.0 ± 6.0

[1] All values are means ± SEMs.
[2,4] Significantly different from normal bone turnover (ANOVA): [2]$P < 0.05$, [4]$P < 0.005$.
[3] Defined as age at menarche.
[5] Number of menstrual cycles in the past year.
[6] Lifetime number of seasons in an endurance running sport.
[7] Total lifetime months of participation in an endurance running; non–lean build, impact loading; or aesthetic sport.

Micronutrient intakes

Runners with EBT had lower mean intakes of each micronutrient assessed than did those with normal bone turnover; however, none of the intakes were significant at an α level of 0.05 (**Table 3**). Runners with EBT trended toward consuming a lower amount of calcium daily ($P = 0.09$). In a comparison of the mean daily intakes with the recommended age- and sex-specific Recommended Dietary Allowance or Adequate Intake (AI) (28–30), runners with normal turnover and EBT met the recommended intakes of ≥14 of the 18 nutrients assessed (Table 3). Both the EBT and normal turnover groups consumed below the AI of 1300 mg Ca/d and below the RDA of 360 mg Mg/d (28). A significantly higher percentage of runners with EBT consumed below the AI for calcium (Table 3) (28).

Serum hormone concentrations

The runners' mean leptin, 25-hydroxycholecalciferol, PTH, IGF-I, estradiol, free T_3, and progesterone concentrations are shown in **Table 4**. Compared with older runners (age: 16–17 y, $n = 20$), younger runners (age: 14–15 y, $n = 19$) had lower leptin (4.7 ± 0.8 compared with 6.9 ± 0.7 ng/mL; $P < 0.05$) and higher IGF-I (336.9 ± 12.4 compared with 291.7 ± 12.1 ng/mL, $P < 0.05$) concentrations. A comparison of runners by bone turnover status showed that runners with EBT had lower serum estradiol (37.8 ± 11.6 compared with 71.4 ± 8.2 pg/mL, $P < 0.05$) and 25-dyhydroxycholecalcifeol (32.3 ± 1.5 compared with 36.5 ± 1.0 ng/mL, $P < 0.05$) concentrations. Furthermore, a significantly higher prevalence of runners with EBT had vitamin D insufficiency [25(OH)D <30 ng/mL] (27.3% compared with 3.8%, $P < 0.05$) (31). When stratified by age, younger runners with EBT had significantly lower leptin and 25-hydroxyvitamin D concentrations (Table 4).

Bone mass and indicators of bone strength

Bone mineral content at the total body, femoral neck, total hip, and lumbar spine was significantly lower in runners with EBT (**Table 5**). The age- and sex-specific lumbar spine BMD Z-scores were lower in those with EBT than in those with normal bone turnover (Table 5). However, when adjusted for age and/or height and weight, only the femoral neck BMC remained significantly lower (Table 5). Runners with EBT also had a lower mean hip CSA and CSMI (Table 5).

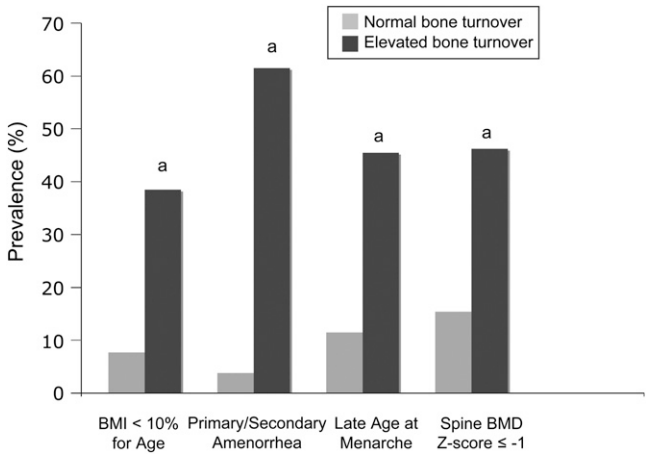

FIGURE 2. Prevalence estimates in runners with normal ($n = 26$) or elevated ($n = 13$) bone turnover. BMD, bone mineral density. [a]$P < 0.05$ (chi-square test).

TABLE 2
Energy and macronutrient intakes

Intake	Normal bone turnover (n = 26)	Elevated bone turnover (n = 13)	Recommended daily intake[1]
Energy (kcal/d)	2346 ± 83[2]	2084 ± 116[3]	2300–2800
Carbohydrate (g/d)	335 ± 13	296 ± 19[3]	≥130
Carbohydrate (g · kg^{-1} · d^{-1})	5.9 ± 0.3	6.3 ± 0.4	6–10
Protein (g/d)	77 ± 3	74 ± 5	≥46
Protein (g · kg^{-1} · d^{-1})	1.4 ± 0.1	1.6 ± 0.1	1.2–1.4
Fat (g/d)	84 ± 4	72 ± 5[3]	—
Fiber (g/d)	21 ± 1	22 ± 2	26
Carbohydrate (%)	57 ± 1	57 ± 1	45–65
Protein (%)	13 ± 1	14 ± 1	10–30
Fat (%)	32 ± 1	31 ± 1	20–35
Water (mL/d)	2764 ± 139	2599 ± 197	—

[1] Recommendations from the Food and Nutrition Board, Institute of Medicine, Dietary Reference Intakes for girls aged 14–18 y, and from the American College of Sports Medicine, American Dietetic Association, and Dietitians of Canada Position Stands.
[2] Mean ± SEM (all such values).
[3] Values trended lower than normal bone turnover, $P < 0.10$ (ANOVA).

Predictors of elevated bone turnover

Secondary amenorrhea, a BMI <10% for age, and consumption of <1300 mg Ca/d were significant predictors of EBT, with odds ratios (ORs) ranging from 5.5 to 20.8 (**Table 6**). Consumption of <2000 kcal/d trended ($P = 0.06$) toward being a significant risk factor (Table 6). Protective factors of EBT included beginning competitive running at an older age as well as having a higher gynecologic age, more menses in the past year, and a higher lean tissue mass (Table 6).

DISCUSSION

To our knowledge, this is the first study to report the relation between energy intake and biomarkers of bone turnover in female

TABLE 3
Micronutrient intakes and percentages of runners who consumed less than the recommended intakes[1]

	Normal bone turnover (n = 26)			Elevated bone turnover (n = 13)			Recommended daily intake[2]
	Intake	Consumed	<RDA/AI	Intake	Consumed	<RDA/AI	
		n	%		n	%	
Minerals							
Calcium (mg/d)	1262 ± 66[3]	13	50	1055 ± 97[4]	11	85[5]	1300
Phosphorus (mg/d)	1349 ± 56	11	42	1246 ± 77	7	54	1250
Iron (mg/d)	25.7 ± 2.5	4	15	18.5 ± 3.6	5	39	15
Magnesium (mg/d)	341 ± 17	18	69	316 ± 25	10	77	360
Zinc (mg/d)	13.8 ± 0.9	4	15	12.5 ± 1.2	2	15	9
Copper (μg/d)	1.9 ± 0.1	0	0	1.7 ± 0.2	1	8	0.890
Vitamins							
Vitamin A (IU)	8741 ± 1046	2	8	6013 ± 1491	1	8	2300
Vitamin D (IU)	296 ± 36	10	39	208 ± 52	6	46	5
Vitamin E (IU)	40 ± 14	17	65	22 ± 19	8	62	22
Vitamin K (μg/d)	104.5 ± 15.8	10	39	96.1 ± 22.5	6	46	75
Vitamin C (mg/d)	221 ± 50	8	31	115 ± 71	3	23	65
Pantothenic acid (mg/d)	9 ± 2	8	31	7 ± 3	4	31	5
Folate (μg/d)	647 ± 49	5	19	560 ± 70	3	23	400
Thiamine (mg/d)	5.1 ± 2.2	0	0	2.9 ± 3.2	0	0	1
Riboflavin (mg/d)	5.5 ± 2.2	0	0	3.0 ± 3.1	0	0	1
Niacin (mg/d)	32.3 ± 2.8	0	0	26.8 ± 4.0	1	8	14
Vitamin B-6 (mg/d)	4.0 ± 1.1	0	0	3.0 ± 1.5	1	8	1.2
Vitamin B-12 (μg/d)	19.7 ± 9.8	0	0	6.3 ± 14.0	0	0	2.4

[1] RDA, Recommended Dietary Allowance; AI, Adequate Intake.
[2] Recommendations from the Food and Nutrition Board, Institute of Medicine, Dietary Reference Intakes for girls aged 14–18 y, and from the American College of Sports Medicine, American Dietetic Association, and Dietitians of Canada Position Stands.
[3] Mean ± SEM (all such values).
[4] Significantly different from normal bone turnover, $P < 0.05$ (ANOVA).
[5] Values trended lower than normal bone turnover, $P < 0.10$.

TABLE 4
Serum concentrations of hormones relevant to bone turnover[1]

	Normal bone turnover[2] (n = 26)	Elevated bone turnover[3] (n = 13)
Leptin (ng/mL)		
14–15 y	6.1 ± 0.7	2.8 ± 0.9[4]
16–17 y	6.7 ± 1.0	7.4 ± 1.7
25(OH)D_3 (ng/mL)		
14–15 y	36.1 ± 1.3	31.3 ± 1.7[4]
16–17 y	36.8 ± 1.4	34.1 ± 2.7
PTH (pg/mL)		
14–15 y	35.7 ± 4.0	46.2 ± 4.7
16–17 y	38.4 ± 4.0	39.5 ± 7.0
IGF-I (ng/mL)		
14–15 y	331.2 ± 13.7	344.8 ± 16.1
16–17 y	280.7 ± 15.5	324.6 ± 26.8
Estradiol (pg/mL)		
14–15 y	72.9 ± 13.3	36.4 ± 15.6[5]
16–17 y	70.2 ± 10.8	39.9 ± 18.8
Free T_3 (pg/mL)		
14–15 y	3.2 ± 0.2	3.4 ± 0.1
16–17 y	3.2 ± 0.1	3.0 ± 0.2
Progesterone (ng/mL)		
14–15 y	3.5 ± 1.0	1.5 ± 1.2
16–17 y	3.7 ± 1.0	1.5 ± 1.8

[1] All values are means ± SEMs. 25(OH)D_3, 25-hydroxyvitamin D_3; PTH, parathyroid hormone; IGF-I, insulin-like growth factor I; T_3, triiodothyronine.
[2] Normal bone turnover: age 14–15 y (n = 11), age 16–17 y (n = 15).
[3] Elevated bone turnover: age 14–15 y (n = 8), age 16–17 y (n = 5).
[4] Significantly different from normal bone turnover, $P < 0.05$ (ANOVA).
[5] Values trended lower than normal bone turnover, $P < 0.10$ (ANOVA).

TABLE 5
Indicators of bone strength in runners with normal or elevated bone turnover[1]

	Normal bone turnover (n = 26)	Elevated bone turnover (n = 13)
Total-body BMC (g)	1980.7 ± 54.1	1701.9 ± 76.5[2]
Adjusted	1900.0 ± 32.2	1864.0 ± 47.9
Femoral neck BMC (g)	5.0 ± 0.1	4.3 ± 0.2[3]
Adjusted	4.9 ± 0.1	4.5 ± 0.2[2]
Total hip BMC (g)	33.0 ± 0.7	29.0 ± 1.0[3]
Adjusted	32.3 ± 0.6	30.5 ± 0.9
Lumbar spine BMC (g)	57.7 ± 1.8	49.7 ± 2.6[2]
Adjusted	55.9 ± 1.5	53.4 ± 2.3
Total-body BMD Z-score	0.39 ± 0.15	−0.12 ± 0.22
Adjusted	0.25 ± 0.15	0.16 ± 0.22
Lumbar spine BMD Z-score	−0.25 ± 0.14	−0.74 ± 0.20[2]
Adjusted	−0.33 ± 0.14	−0.58 ± 0.21
Hip CSA	158.3 ± 3.9	137.5 ± 5.5[3]
Hip CSMI	9588.9 ± 427.6	7238.2 ± 604.7[3]

[1] All values are means ± SEMs. BMC, bone mineral content; BMD, bone mineral density; CSA, cross-sectional area; CSMI, cross-sectional moment of inertia. BMC was adjusted for age, height, and weight; BMD Z-score was adjusted for height and weight.
[2,3] Significantly different from normal bone turnover (ANOVA and ANCOVA): [2]$P < 0.05$, [3]$P < 0.005$.

adolescent endurance runners. In our sample, all girls with abnormal bone turnover had elevated values, because no girl had BAP or CTX values in the lowest 3% for their age. Furthermore, BAP and CTX were strongly correlated. Therefore, in our sample, EBT values were associated with increased bone resorption. Elevated resorption during the early to midpubertal years, especially if chronic, may lead to suppressed bone mineral accrual during the period when the rate of bone formation should be at its peak.

Previous laboratory studies in young adults, regularly menstruating women who, under controlled conditions, were restricted to a diet and exercise regimen that induced low energy availability (<30 kcal · kg FFM^{-1} · d^{-1}) had hormonal changes preceding subclinical and clinical menstrual disturbances (ie, suppressed luteinizing hormone and follicle stimulating hormone pulsatility and estradiol levels) (5, 6). Furthermore, patients with anorexia nervosa who follow a severely low-calorie diet, resulting in a markedly low body mass, develop irregular menses or a loss of menstrual function (32–34). In our sample, runners with EBT had an elevated, significantly higher prevalence of BMI <10% for age (36%) and primary or secondary amenorrhea (57%) compared with runners with normal turnover. Therefore, traits that characterized our sample of runners with EBT appeared consistent with traits in populations with known chronic energy deficiencies.

Findings from our assessment of 7-d dietary intakes of runners further support the theory that runners with EBT were consuming inadequate amounts of energy. At ≈2080kcal/d, the mean energy intake in runners with EBT trended lower than that in those with normal bone turnover. Furthermore, more than half of the runners with EBT consumed <2000 kcal/d—an energy intake ≥300 kcal/d less than that recommended for their age and level of activity. Although 2000 kcal may be adequate for sedentary or moderately active teens (25), it may not be sufficient for adolescent girls training or competing daily in an energy-demanding competitive endurance sport. On the basis of the 2005 Institute of Medicine Dietary Reference Intake Report (25), active to very active adolescent girls require ≥2300–2800 kcal/d to maintain a healthy body weight, to allow for normal growth and development as well as to provide sufficient energy for their exercise activities. Thus, our findings suggest that the runners with EBT were consuming inadequate calories, which, paired with their high level of activity, may have led to an energy deficiency.

In addition, the hormone profile of runners with EBT appeared consistent with a low energy state. Adolescents with a diagnosis of anorexia nervosa, who, by definition, consume markedly low

TABLE 6
Variables that contributed significantly to the prediction of elevated bone turnover

	Odds ratio (95% CI)
Risk factors	
Secondary amenorrhea	20.83 (2.04, 212.97)
BMI (kg/m^2) <10% for age	7.50 (1.21, 46.50)
Calcium <1300 mg/d	5.5 (1.01, 29.85)
Energy intake <2000 kcal/d	4.2 (0.94, 18.71)
Protective factors	
Began running at older age (y)	0.49 (0.25, 0.99)
Older chronologic age (y)	0.52 (0.27, 0.99)
Higher number of menses in the past year	0.71 (0.57, 0.88)
Higher lean tissue mass (kg)	0.71 (0.56, 0.91)

amounts of calories daily have low serum concentrations of estradiol, leptin, and IGF-1 (35–37). The runners in the current sample, such as those with anorexia nervosa, had hypoestrogenism. Leptin concentrations were lower in the younger (age: 14–15 y) runners with EBT. Two older adolescent runners with EBT had higher leptin concentrations than did the remaining older runners with EBT (12.3 ± 1.2 compared with 4.2 ± 0.9 ng/mL, $P < 0.05$). When these runners' elevated concentrations were excluded, leptin was significantly lower in the entire sample of runners with EBT than in the runners with normal bone turnover (3.2 ± 0.9 compared with 6.4 ± 0.6 ng/mL, $P < 0.005$). Therefore, most of the runners with EBT also had lower leptin concentrations.

In healthy adolescent girls, mean IGF-I concentrations have been reported to peak at 450–600 ng/mL between Tanner Stages 3 and 4 (at approximately age 14.5 y) (38–40) and then gradually decline throughout the remaining adolescent years (38, 39). As expected, younger (age 14–15 y) runners had higher IGF-I concentrations than did the older (16–17 y) runners. However, mean IGF-I concentrations for runners with both normal and EBT were lower than concentrations previously reported in healthy, adolescent girls. As a result, further research is necessary to evaluate the relation between endurance running and the endogenous production of IGF-I during puberty. It is speculated that running may have led to a mild energy deficit in all the endurance runners, thus contributing to their low serum IGF-I concentrations.

Prior assessments of young adult female endurance runners have reported associations between an energy deficit, menstrual disturbances, and/or disrupted bone turnover (41–44). Furthermore, Christo et al (8) observed reduced markers of bone formation and bone resorption in amenorrheic than in eumenorrheic athletes—a finding consistent with suppressed bone mineral accrual. However, their observation of lower bone resorption in those with amenorrhea is dissimilar to the correlation observed in the current study between amenorrhea and elevated CTX. Other reports, though, have documented elevated BAP and/or bone resorption in athletes, patients with anorexia nervosa, or postmenopausal women with an energy and/or estrogen deficiency (7, 45, 46). These findings are similar to our observations. However, the inconsistency in the relation between markers of bone resorption and menstrual status in Christo et al's study and the current study warrant further evaluation of biomarkers of bone turnover in adolescent endurance athletes.

In addition to lower energy intakes, runners with EBT had a significantly higher prevalence of consuming less than the age-specific AI of 1300 mg Ca/d and had significantly lower serum 25-hydroxycholecalciferol concentrations. Furthermore, runners with EBT had a pattern of lower intakes of each micronutrient assessed; however, these group differences were not significant. These observations suggest that runners with EBT may also be consuming inadequate amounts of various micronutrients, particularly those that play a role in bone formation.

Limitations

Despite our best attempts to have the runners provide accurate dietary records, some runners may have under- or overreported their intakes. On administering the menstrual and sports history questionnaire, we attempted to provide an environment that promoted privacy and we ensured runners of the confidentiality of their responses. However, runners may have provided inaccurate responses. Furthermore, runners were not standardized for the phase of their menstrual cycle. Therefore, estradiol concentrations may have been subject to additional variance. In the current study, only endurance runners were assessed; however, additionally evaluating nonathlete controls and nonendurance runner athletes may have provided a more comprehensive understanding of the relation between diet, exercise, and bone formation during the adolescent years.

Conclusions

Our sample of female adolescent endurance runners with EBT and elevated bone resorption had physiologic and behavioral traits consistent with an energy deficiency. Furthermore, runners with EBT had a significantly higher prevalence of consuming less than the age-specific AI for calcium and a lower serum 25-dihydroxyvitamin D concentration, which may have further contributed to their high bone resorption. Findings from this study provide support for monitoring bone turnover in adolescent runners, because it may aid in identifying those at risk of an energy deficiency and, therefore, suppressed bone mineral accrual during the critical bone-building years. Furthermore, because adolescent endurance runners may be at risk of low energy and calcium intakes and vitamin D insufficiency, they may be candidates for nutritional monitoring and education.

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