Bone Parameters in Anorexia Nervosa and Athletic Amenorrhea: Comparison of Two Hypothalamic Amenorrhea States

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Objective: We have reported low bone mineral density (BMD), impaired bone structure, and increased fracture risk in participants with anorexia nervosa (AN) and normal-weight oligoamenorrheic athletes (OAs). However, data directly comparing compartment-specific bone parameters in participants with AN, OAs, and controls are lacking.

Design: A total of 468 female participants 14 to 21.9 years old were included: 269 with AN, 104 OAs, and 95 normal-weight eumenorrheic controls. Dual-energy x-ray absorptiometry was used to assess areal BMD (aBMD) of the whole body less head (WBLH), spine, and hip. High-resolution peripheral quantitative computed tomography was used to assess volumetric BMD (vBMD), bone geometry, and structure at the non–weight-bearing distal radius and weight-bearing distal tibia.

Results: Participants with AN had lower WBLH and hip aBMD *z* scores than OAs and controls ($P < 0.0001$). Participants with AN and OAs had lower spine aBMD *z* scores than controls ($P < 0.01$). At the radius, total and cortical vBMD, percentage cortical area, and thickness were lower in the AN and OA groups than in controls ($P \leq 0.04$); trabecular vBMD was lower in participants with AN than controls. At the tibia, participants with AN had lower measures for most parameters compared with OAs and controls ($P < 0.05$); OAs had lower cortical vBMD than controls ($P = 0.002$). Participants with AN and OAs had higher fracture rates than controls. Stress fracture prevalence was highest in OAs ($P < 0.0001$); nonstress fracture prevalence was highest in participants with AN ($P < 0.05$).

Conclusion: AN is deleterious to bone at all sites and both bone compartments. A high stress fracture rate in OAs, who have comparable WBLH and hip aBMD measures to controls, indicates that BMD in these women may need to be even higher to avoid fractures. ***(J Clin Endocrinol Metab* 103: 2392–2402, 2018)**

We and others have reported lower areal bone mineral density (aBMD) and higher fracture risk in adolescent girls and young women with anorexia nervosa (AN) compared with controls (1–9). Similarly, using high-resolution peripheral quantitative computed tomography (HRpQCT), we have shown that both

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Abbreviations: 25(OH)D, 25-hydroxy-vitamin D; aBMD, areal bone mineral density; AN, anorexia nervosa; BMD, bone mineral density; BMI, body mass index; CI, confidence interval; DXA, dual-energy x-ray absorptiometry; EA, eumenorrheic athlete; EBW, expected body weight; FHA, functional hypothalamic amenorrhea; HRpQCT, high-resolution peripheral quantitative computed tomography; mBMI, median body mass index; OA, oligoamenorrheic athlete; OR, odds ratio; vBMD, volumetric bone mineral density; WBLH, whole body less head.

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cortical and trabecular microarchitecture are altered and volumetric bone mineral density (vBMD) and bone strength reduced in participants with AN compared with controls (10–12). We have also demonstrated that oligoamenorrheic athletes (OAs) have lower spine and hip aBMD than eumenorrheic athletes (EAs) and lower spine aBMD than nonathletes, associated with a higher risk of stress fracture (13, 14). Furthermore, at the distal radius, OAs have lower bone strength than nonathletes. In contrast, at the distal tibia, OAs lose the advantage of weight-bearing exercise seen in EAs (15–17). Both AN and oligoamenorrhea are conditions of functional hypothalamic amenorrhea (FHA), a common cause of secondary amenorrhea associated with weight loss, excessive exercise, or stress (18, 19). FHA results from impaired pulsatile secretion of gonadotropin-releasing hormone, which in turn leads to impaired gonadotropin secretion. The final consequence is varying degrees of hypoestrogenism, with negative effects on the skeletal system (20–22).

Low aBMD and vBMD in AN are related to low body weight and multiple associated hormone deficiencies, including estrogen deficiency (which manifests as oligoamenorrhea) (23–27). Similarly, low bone mineral density (BMD) in normal-weight oligoamenorrhea results from hormonal changes associated with relative energy deficiency (even when body weight is normal), including estrogen deficiency (15–17, 28). However, exercise activity in oligoamenorrhea may have some protective effects on bone, given the osteogenic effect of mechanical loading. Studies directly comparing the skeletal effects of AN and exercise-induced amenorrhea in adolescents and young adults are currently lacking, and understanding differences in bone parameters in these two conditions associated with amenorrhea and low energy availability may help delineate mechanisms that lead to bone loss. Furthermore, AN typically triggers early referrals to adolescent medicine physicians and endocrinologists. However, exercise-induced amenorrhea in the normal-weight athlete often comes to medical attention much later unless there are clinical fractures.

The aim of this study was to evaluate and compare the effects of AN and exercise-induced amenorrhea on bone parameters and to identify groups at high risk for fracture. We hypothesized that oligoamenorrheic adolescent girls and young adults with AN would have greater impairment of bone parameters compared with OAs and eumenorrheic controls, particularly at weight-bearing sites, where effects of weight-bearing activity would be protective regardless of gonadal status. We also hypothesized that participants with AN would have the highest prevalence of fracture (by self-report), followed by OAs, followed by controls.

Participants and Methods

A total of 468 adolescent and young adult women between the ages of 14 and 21.9 years were included in the study (269 with AN, 104 OAs, and 95 normal-weight eumenorrheic controls). The group with AN was drawn from participants who had been screened for past or current AN studies being conducted at the Neuroendocrine and Pediatric Endocrine Units of Massachusetts General Hospital between 2002 and 2016 (8, 9, 29, 30). The study psychologist or psychiatrist confirmed the diagnosis of AN according to *Diagnostic and Statistical Manual of Mental Disorders,* 4th Edition (Text Revision) (31) or 5th Edition (32) criteria (depending on whether assessments were done before or after the 2013 publication of the 5th Edition) by reviewing clinical data and conducting a clinical interview including items from the Structured Clinical Interview for the 4th Edition. To establish the criterion of low weight, participants with AN were required to meet at least two of three criteria [percentage median body mass index (% mBMI) for age, percentage expected body weight (% EBW) for height, or % EBW for age <90%]. Participants with AN were enrolled in treatment programs with instructions to refrain from purposeful physical activity.

Enrolled athletes were engaged in weight-bearing exercise for ≥4 hr/wk or ≥20 miles of running per week for ≥6 months in the preceding year (14–17). Oligoamenorrhea was defined as lack of menstrual periods for ≥3 months within the ≥6 preceding months of oligoamenorrhea (cycle length >6 weeks) or absence of menses at >15 years (14–17). Controls were required to have a body mass index (BMI) between the 10th and 90th percentiles for age and ≥9 menstrual cycles in the preceding year, with menstrual cycle length between 21 and 35 days. None of the OAs met criteria for AN at the time of study entry; 27 had a current or past history of milder forms of disordered eating. Participants were assessed for hours per week of weight-bearing activity for the preceding year as a known determinant of bone density and structure; these data were available for a subset of 51 participants with AN, 90 OAs, and 38 controls. Furthermore, data for vitamin D and calcium intake (from diet and supplements) were available through 4-2day food records for 72 participants with AN, 48 OAs, and 54 controls (Nutrient Data System for Research software version 2008; University of Minnesota Nutrition Coordinating Center, Minneapolis, MN). Levels of 25-hydroxy-vitamin D [25(OH)D] were available for 67 participants with AN, 81 OAs, and 45 controls. An immunochemiluminometric assay was used to measure 25(OH)D (Labcorp, Burlington, NC; sensitivity 4.0 ng/mL; intra-assay coefficient of variation 4.8% to 7.7%)

The Partners HealthCare Institutional Review Board approved this study. Informed consent was obtained from all participants ≥18 years old and from parents of participants <18 years old. Assent was obtained from all participants <18 years old. Participants were recruited through advertisements and referrals from eating disorder centers and regional practitioners. Participants were weighed on a calibrated scale while wearing a hospital gown, and height was measured in triplicate on a stadiometer. Menarchal age and bone age (from x-rays of the left hand and wrist) were evaluated by the study endocrinologist. We used Centers for Disease Control and Prevention tables to calculate the height z score, BMI z score, % mBMI [(BMI of participant/50th percentile BMI for age and sex) * 100], % EBW for height [(weight of

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participant/weight corresponding to the height percentile of the participant) * 100], and % EBW for age [(weight of participant/50th percentile weight for age and sex) * 100] (33).

Participants with AN were further dichotomized based on duration of amenorrhea: no amenorrhea or <1 year of amenorrhea (excluding premenarchal participants) vs ≥1 year of amenorrhea (referring to consecutive months of missed menses). The latter group also included those postmenarchal at ≥16 years of age (>1 year past the upper limit of normal for menarche).

Other causes of oligoamenorrhea (premature ovarian failure, hyperprolactinemia, thyroid dysfunction, and hyperandrogenism) were ruled out. Participants on medications that affect bone metabolism (e.g., estrogen/progesterone combination pills, injectable hormonal contraceptives, glucocorticoids, and anticonvulsants) were excluded. We did not exclude participants on calcium or vitamin D supplements. Lifetime fracture history (stress and nonstress) was obtained from all participants by self-report, and directed questions were asked about the site of fracture, mechanism of injury, how the diagnosis was confirmed, and the management (8, 14).

aBMD assessment

aBMD of the whole body less head (WBLH), lumbar spine, and total hip was determined with dual-energy x-ray absorptiometry (DXA) (Hologic 4500 A; Hologic Inc., Waltham, MA). aBMD *z* scores were calculated for sex, age, and race according to reference curves from the BMD in Childhood Study and adjusted for height *z* scores (34). All consecutive participants who met criteria for AN and oligoamenorrhea and had a DXA scan performed were included in the analyses. Similarly, all consecutive participants who met criteria for normal-weight eumenorrheic controls and underwent a DXA scan were included as controls. Comparisons of aBMD in a subset of AN vs control participants and of OAs vs EAs and nonathletes have been previously reported (8, 9, 14–16, 29, 30). However, no reports thus far have directly compared participants with AN vs OAs vs eumenorrheic controls.

Bone microarchitecture assessment

vBMD, bone geometry, and structure were assessed at the distal radius and tibia with HRpQCT (XtremeCT; Scanco Medical AG, Bassersdorf, Switzerland). Measurements were performed at the nondominant wrist and leg unless there was an acute fracture at those sites, in which case the nonfractured side was assessed. Outcome variables computed by automated analysis included area (mm^2) and vBMD (mg hydroxyapatite/cm^3) for total, trabecular, and cortical regions, cortical thickness (mm) and perimeter (mm), and trabecular thickness (mm), number (1/mm), and separation (mm). Comparisons of microarchitecture data in a subset of participants with AN vs eumenorrheic control participants and OAs vs eumenorrheic controls have been previously reported (14–16). However, we are now reporting data for additional 49 participants with AN, 56 OAs, and 24 controls.

Statistical analysis

We conducted analyses with JMP Statistical Discovery Software (SAS Institute Inc., Cary, NC). We used analysis of variance or the Kruskal-Wallis test to compare differences across groups (depending on data distribution), followed by the Tukey-Kramer or Steel-Dwass test, respectively, to adjust for multiple comparisons. The Fisher's exact test was used to compare proportions across groups. Multivariate analysis was used to define the determinants of bone density and structure for the entire group and of stress and nonstress fractures in participants with AN and OAs. The odds ratio (OR) was calculated for stress fractures in the OA group and nonstress fractures in the AN group after we controlled for BMI *z* scores, duration of amenorrhea, and exercise activity.

Results

Subject characteristics

Baseline clinical characteristics are described in Table 1. Groups did not differ for mean height and height *z* scores. Per study design, participants with AN had lower mean weight, BMI, BMI *z* scores, % mBMI, and bone age than OAs and controls, and OAs had lower mean BMI, BMI *z* scores, and % mBMI than controls. Mean age at menarche was greater in OAs and participants with AN than controls and in OAs compared with participants with AN. All groups included a small proportion of premenarchal participants. In the AN group, 31.6% of participants were not amenorrheic. Mean duration of amenorrhea preceding the study visit did not differ in OAs vs participants with AN. A total of 84% of participants were white. Calcium and vitamin D intake from food and supplements and vitamin D levels were higher in participants with AN and OAs than in controls in a subset of participants.

BMD

Participants with AN had lower WBLH and hip aBMD *z* scores than OAs and controls ($P < 0.0001$ for all comparisons), whereas OAs and controls did not differ for these measures. Spine aBMD *z* scores were lower in participants with AN than OAs and controls ($P < 0.05$ and $P < 0.0001$, respectively) and lower in OAs vs controls ($P < 0.001$) [Fig. 1(a)]. These results remained significant after we excluded premenarchal participants (data not shown). Differences between groups for aBMD *z* scores persisted after we controlled for age, race, and height *z* scores, except for an attenuation in the difference between OAs and controls for spine aBMD *z* scores.

Overall, BMI *z* scores correlated positively with aBMD *z* scores at one or more sites in each of the groups, whereas duration of amenorrhea correlated negatively with aBMD *z* scores at the spine and hip in OAs ($r \leq -0.25$, $P \leq 0.02$) and for the whole body and spine in participants with AN ($r \leq -0.16$ for both, $P \leq 0.02$). Of note, regardless of the duration of amenorrhea, participants with AN had lower WBLH and hip aBMD *z* scores than OAs and controls ($P \leq 0.0001$) and lower spine aBMD *z* scores than controls ($P < 0.0001$ for both)

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Table 1. Clinical Characteristics of Participants With AN, OAs, and Controls

	Participants With AN (n = 269)	OAs (n = 104)	Controls (n = 95)	*P* (ANOVA)	*P*, AN vs OA	*P*, OA vs Controls	*P*, AN vs Controls
Age, y	18.2 ± 0.1	18.7 ± 0.2	18.1 ± 0.2	0.03	0.04	0.07	—
Height, cm	164.8 ± 0.4	165.2 ± 0.6	163.4 ± 0.7	NS	—	—	—
Height *z* score	0.27 ± 0.06	0.34 ± 0.09	0.07 ± 0.11	NS	—	—	—
Weight, kg	47.5 ± 0.3	56.1 ± 0.8	58.2 ± 0.7	<0.0001	<0.0001	0.04	<0.0001
Bone age, y	16.8 ± 0.1	17.3 ± 0.1	17.3 ± 0.1	0.0007	0.002	—	0.01
BMI, kg/m^2	17.4 ± 0.1	20.5 ± 0.2	21.8 ± 0.2	<0.0001	<0.0001	<0.0001	<0.0001
BMI *z* score	−1.71 ± 0.06	−0.38 ± 0.07	0.11 ± 0.06	<0.0001	<0.0001	0.0003	<0.0001
% mBMI	82.4 ± 0.4	96.5 ± 1.0	103.0 ± 1.0	<0.0001	<0.0001	<0.0001	<0.0001
Age at menarche, y	12.9 ± 0.1	13.7 ± 0.2	12.4 ± 0.1	<0.0001	<0.0001	<0.0001	0.008
Duration of amenorrhea, mo	6.6 (1.7–11.8)	3.9 (0.8–10.1)	—	NS	—	—	—
Premenarchal participants	8.1%	4.8%	2.1%	0.10	—	—	—
Weight-bearing activity, h/wk	4.3 (2.0–7.0)	8.5 (5.9–12.0)	1.0 (0–3.9)	<0.0001	<0.0001	<0.0001	<0.0001
Vitamin D intake from food and supplements, IU/d	495.6 ± 48.1	652.0 ± 93.8	289.2 ± 38.0	0.0004	—	0.0003	0.03
Calcium intake from food and supplements, mg/d	1787 ± 104	1584 ± 112	1021 ± 69	<0.0001	—	0.0007	<0.0001
25(OH)D, ng/mL	34.9 ± 1.1	38.6 ± 1.5	24.5 ± 1.4	<0.0001	—	<0.0001	<0.0001
25(OH)D <20 ng/mL, %	4.5%	0.0%	35.6%	<0.0001	0.09	<0.0001	<0.0001
25(OH)D <30 ng/mL, %	34.3%	25.9%	75.6%	<0.0001	—	<0.0001	<0.0001

Means ± standard error of the mean or median (1st quartile–3rd quartile) depending on data distribution.

Abbreviations: ANOVA, analysis of variance; NS, not significant.

[Fig. 1(b)]. Spine aBMD *z* scores were also lower in participants with AN with amenorrhea for ≥1 year than in OAs (P = 0.02) and lower in OAs than in controls (0.002). Surprisingly, hours per week of weight-bearing activity over the preceding year did not correlate with aBMD *z* scores at any site in any group. Furthermore, vitamin D intake and 25(OH)D levels were not associated positively with bone measures except for weak positive associations with total and trabecular bone area at the distal radius and tibia.

After we added BMI *z* scores to the multivariate model that included age, race, and height *z* scores, WBLH and total hip aBMD *z* scores remained lower in participants with AN than in OAs (P = 0.02 for both), whereas for the spine, differences between groups were lost. WBLH and total hip aBMD *z* scores remained lower in participants with AN than in OAs after we added duration of amenorrhea (P = 0.009, P = 0.02, respectively) and exercise levels (P = 0.006 and P = 0.03) incrementally to this model.

aBMD *z* scores ≤ −2 are considered low for age. About one-fifth of all participants with AN and OAs had spine aBMD *z* scores ≤ −2 (21.8% and 25.3%, respectively), compared with 1.2% of controls ($P < 0.0001$ for AN vs controls and OAs vs controls). A significantly higher proportion of participants with AN had aBMD *z* scores ≤ −2 at the hip compared with OAs and controls (15.7% vs 1.1% and 0%, respectively, $P < 0.0001$ for both participants with AN vs OAs and participants with AN vs controls). For WBLH measurements, the AN group was about four times more likely to have aBMD *z* scores ≤ −2 than OAs and controls (20.5% vs 5.4% and 4.7%, P = 0.0004, P = 0.0005, respectively). We also examined the proportion of participants with AN, OAs, and controls with aBMD *z* scores < −1 at the different sites. *Z* scores of < −1 were noted in 57.6%, 40.7%, and 23.3% of participants with AN, OAs, and controls, respectively, at the spine ($P < 0.0001$); in 45.5%,

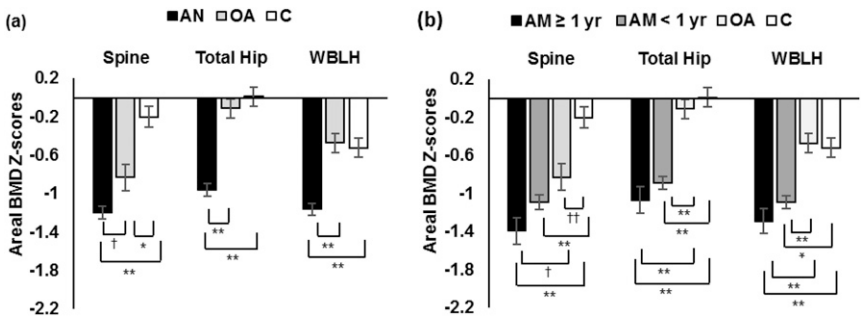

Figure 1. Comparison of aBMD *z* scores at the lumbar spine (spine), total hip, and WBLH. (a) Participants with AN, OAs, and controls. Participants with AN had lower aBMD *z* scores at all sites compared with the other two groups. Furthermore, OAs had lower aBMD *z* scores at the lumbar spine than controls. (b) Participants with AN and amenorrhea ≥1 year, participants with AN and amenorrhea <1 year, OAs, and controls. Regardless of the duration of amenorrhea, aBMD *z* scores were lower in both groups of participants with AN compared with OAs and controls at the total hip and WBLH. At the spine, BMD *z* scores were lower in both groups of participants with AN compared with controls, in participants with AN with amenorrhea ≥1 year compared with OAs, and OAs compared with controls. †$P < 0.05$; ††$P < 0.01$; *$P < 0.001$; **$P < 0.0001$. AM, amenorrhea; C, controls.

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23.4%, and 17.1% of participants with AN, OAs, and controls, respectively, at the hip ($P < 0.0001$); and in 55.2%, 32.3%, and 27.1% of participants with AN, OAs, and controls, respectively, for the WBLH ($P < 0.0001$). The proportion of participants with AN with low BMD z scores based on duration of amenorrhea is reported in Table 2.

Bone microarchitecture

In a subset of participants with AN, OAs, and controls, we assessed microarchitecture parameters at the distal radius (66 participants with AN, 88 OAs, 40 controls) and distal tibia (51 participants with AN, 86 OAs, 39 controls) by using HRpQCT. Data for 17 participants with AN for the distal radius and for 32 OAs and 16 controls for both distal radius and tibia measurements have been previously published (11, 15, 16, 35). The subsets were comparable to the larger group for clinical characteristics (data not shown).

Distal radius (non–weight-bearing bone)

HRpQCT of the non–weight-bearing radius showed lower total and cortical vBMD, cortical area, percentage cortical area, and cortical thickness in participants with AN and OAs compared with controls ($P \leq 0.02$ for all; Fig. 2 and Table 3). Participants with AN had lower trabecular (total, meta, and inner) vBMD than controls ($P < 0.05$ for all), whereas OAs had borderline lower total and inner trabecular vBMD than controls ($P \leq 0.05$) (Fig. 2). Both participants with AN and OAs had lower calculated strength estimates than controls ($P = 0.0009$ and $P = 0.04$) (Table 3). After we controlled for age, race, and height z scores, differences between participants with AN and controls persisted for total and trabecular vBMD, cortical area and thickness, and estimated strength ($P < 0.05$). After we controlled for BMI z scores (with and without duration of amenorrhea), these differences between groups were lost. Adding hours per week of exercise activity to the model did not further change the results.

Distal tibia (weight-bearing bone)

HRpQCT of the weight-bearing tibia showed that participants with AN had lower measures of total and trabecular (meta) vBMD, percentage cortical area and thickness, and percentage trabecular area and number compared with OAs and controls and lower cortical vBMD than controls ($P \leq 0.05$ for all, Fig. 2 and Table 3). OAs had lower cortical vBMD than controls ($P = 0.002$) (Fig. 2) despite greater cortical perimeter ($P = 0.046$), but these groups did not differ for other cortical or trabecular parameters (Table 3). After we controlled for age, race, and height z scores, compared with OAs participants with AN had lower total and cortical area, cortical thickness, total vBMD, trabecular number, and calculated strength, and compared with controls they had lower cortical area and thickness, total and trabecular vBMD, trabecular number, and calculated strength ($P < 0.05$). Differences between groups were no longer evident after we also controlled for BMI z scores (with or without duration of amenorrhea). Adding hours per week of exercise activity to the model did not further change the results.

History of fractures

Overall fracture prevalence (stress and nonstress) was comparable in participants with AN (38.2%) and OAs (42.6%) and significantly higher than in controls (22.4%) ($P = 0.008$ and $P = 0.005$ for comparisons of participants with AN vs controls and OAs vs controls, respectively, Fig. 3). However, a higher proportion of OAs than AN and controls had stress fractures (25% vs 5.0% and 1.2%, respectively, $P < 0.0001$ for both). In

Table 2. Comparison of aBMD Measurements at WBLH, Lumbar Spine, and Total Hip in Participants With AN (Postmenarchal or ≥16 y Old), OAs, and Controls, Factoring In the Duration of Amenorrhea

BMD z- Scores	Participants With AN: ≥1 y Amenorrhea	Participants With AN: <1 y Amenorrhea	OAs	Controls	ANOVA P	Participants With AN: AM ≥1 y vs OAs	Participants With AN: AM <1 y vs OAs	Participants With AN: AM ≥1 y vs Controls	Participants With AN: AM <1 y vs Controls	Participants With AN: AM <1 y vs ≥ 1 y	OAs vs Controls
Lumbar spine	N = 66	N = 175	N = 91	N = 86							
$z < -1$	60.9%	54.3%	40.7%	23.3%	<0.0001	0.01	0.04	<0.0001	<0.0001	—	0.02
$z < -2$	25.0%	19.4%	25.3%	1.2%	<0.0001	—	—	<0.0001	<0.0001	—	<0.0001
Hip	N = 60	N = 171	N = 94	N = 76							
$z < -1$	53.3%	41.5%	23.4%	17.1%	<0.0001	0.0002	0.002	<0.0001	<0.0001	0.08	—
$z < -2$	21.7%	12.9%	1.1%	0%	<0.0001	<0.0001	0.001	<0.0001	0.0005	0.08	—
WBLH	N = 62	N = 165	N = 93	N = 85							
$z < -1$	61.3%	52.1%	32.3%	27.1%	<0.0001	0.0005	0.002	<0.0001	0.0002	—	—
$z < -2$	24.2%	18.2%	5.4%	4.7%	<0.0001	0.001	0.006	0.0008	0.005	—	—

Data shown as proportions; *P* values for proportions are not corrected for multiple comparisons.

AN: AM ≥1 y: AN with AM for ≥1 y (includes those with primary AM at ≥16 y).

AN: AM <1 y: AN with AM for <1 y or no AM (does not include premenarchal girls <15 y old).

Abbreviations: AM, amenorrhea; ANOVA, analysis of variance.

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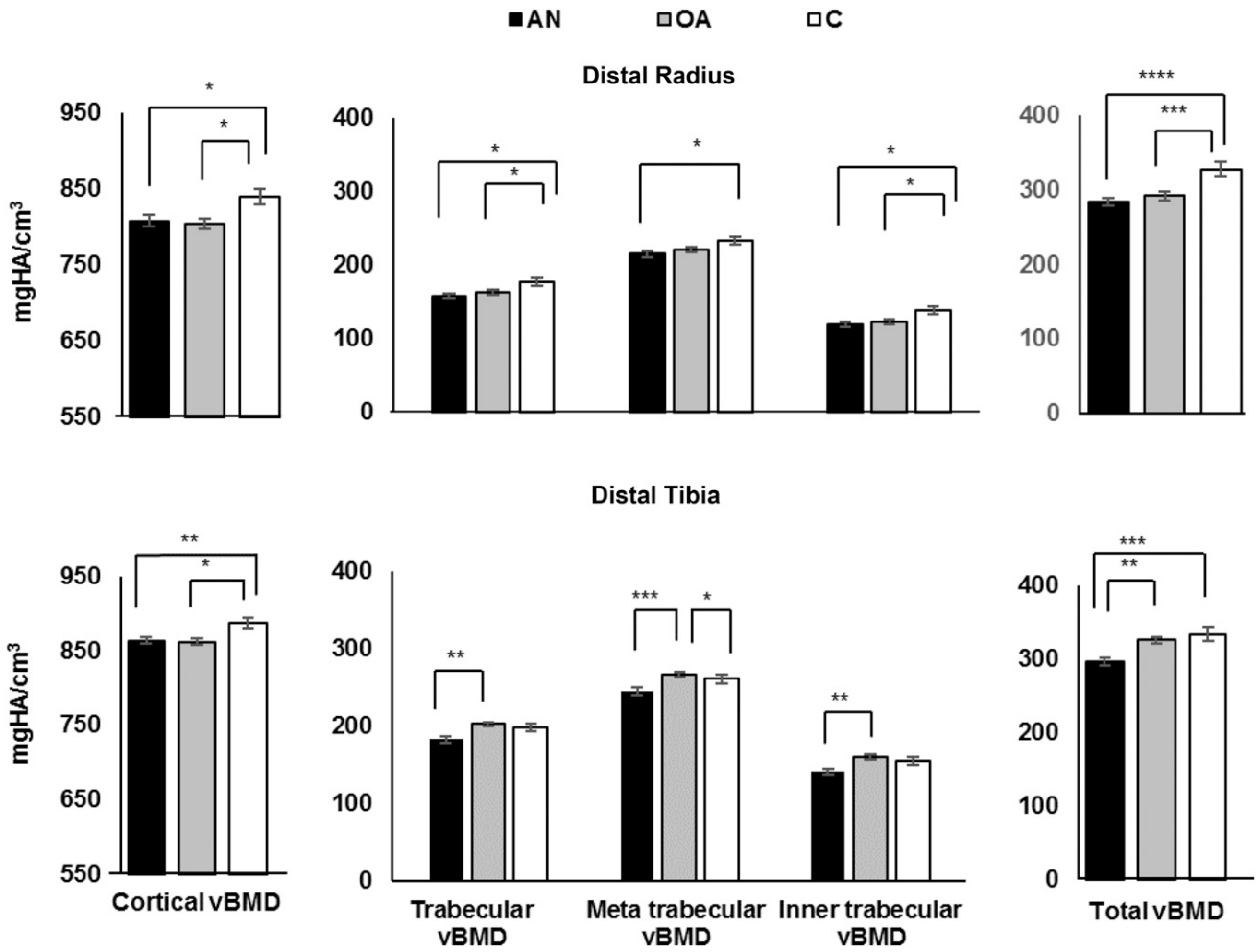

Figure 2. Comparison of vBMD at the distal radius and distal tibia in participants with AN, OAs and controls. Participants with AN had lower cortical, trabecular, and total vBMD at the distal radius and lower cortical and total vBMD at the distal tibia compared with controls. They also had lower trabecular and total vBMD of the distal tibia than OAs. OAs had lower cortical, trabecular, and total vBMD at the distal radius and lower cortical vBMD at the distal tibia than controls. $*P \leq 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$. C, controls; mgHA, milligrams hydroxyapatite.

contrast, a higher proportion of participants with AN than OAs and controls had nonstress fractures (35.7% vs 20.0% and 21.2%, respectively, $P = 0.005$ for participants with AN vs OAs and $P = 0.01$ for participants with AN vs controls, Fig. 3). Participants with AN with amenorrhea for ≥1 year did not significantly differ from participants with AN with amenorrhea for <1 year for overall fracture rate (40.0% vs 33.8%), nonstress fracture rate (38.9% vs 32.4%), or stress fracture rate (6.7% vs 1.4%).

After we controlled for BMI z scores, duration of amenorrhea, and exercise activity, the OR for stress fracture was 7.5 for OAs vs participants with AN [95% confidence interval (CI), 2.1 to 33.7], whereas OR for nonstress fracture was 4.1 for participants with AN vs OAs (95% CI, 1.5 to 11.8). After we controlled for these covariates, compared with the control group, the OR for having all fractures was 6.8 in participants with AN (95% CI, 1.9 to 27.5) and 5.2 in OAs (95% CI, 1.7 to 18.1); the OR for having stress fractures was 14.7 in OAs (95% CI, 2.4 to 289.1) and for having nonstress fractures was 5.8 in participants with AN (95% CI, 1.5 to 24.5).

In a multivariate model that included aBMD z scores (spine, total hip, or whole body), BMI z scores, duration of amenorrhea, and exercise activity, we found that in the OA (but not AN) group, spine and WBLH (but not total hip) aBMD z scores were associated with risk for stress fractures ($P = 0.03$ and $P = 0.04$, respectively) and all fractures ($P = 0.04$ and $P = 0.01$, respectively). In this model, BMI z scores were protective against nonstress fractures in OAs. Duration of amenorrhea did not independently predict risk of fractures in this model.

Discussion

In adults, the severity of bone loss has been demonstrated to be greater in participants with AN than in normal-weight patients with hypothalamic amenorrhea,

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Table 3. HRpQCT Data for the Distal Radius and Distal Tibia in Participants With AN, OAs, and Controls

Distal Radius	Participants With AN (n = 66)	OAs (n = 88)	Controls (n = 40)	*P* ANOVA	Participants With AN vs OAs	OAs vs Controls	Participants With AN vs Controls
Cortical parameters							
Cortical area, mm^2	43.8 ± 1.2	46.3 ± 1.4	52.3 ± 2.0	0.002	—	0.02	0.001
% Cortical area	17.3 ± 0.6	17.8 ± 0.6	20.8 ± 0.9	0.005	—	0.02	0.006
Cortical thickness, mm	0.66 ± 0.02	0.68 ± 0.02	0.78 ± 0.03	0.002	—	0.01	0.002
Cortical perimeter, mm	67.1 ± 0.6	67.9 ± 0.6	66.3 ± 0.8	NS	—	—	—
Trabecular parameters							
Trabecular area, mm^2	215.1 ± 5.0	216.9 ± 4.9	202.3 ± 6.1	NS	—	—	—
% Trabecular area	82.9 ± 1.5	80.7 ± 0.6	78.1 ± 0.9	0.02	—	—	0.01
Trabecular number, 1/mm	1.93 ± 0.04	1.95 ± 0.03	2.03 ± 0.04	NS	—	—	—
Trabecular thickness, mm	0.068 ± 0.001	0.070 ± 0.001	0.073 ± 0.002	0.09	—	—	0.07
Trabecular separation, mm	0.46 ± 0.01	0.45 ± 0.01	0.43 ± 0.01	NS	—	—	—
Estimated strength, N	2686 ± 49	2798 ± 47	2993 ± 64	0.002	—	0.04	0.0009
Distal Tibia	**Participants With AN (n = 51)**	**OAs (n = 86)**	**Controls (n = 39)**	***P* ANOVA**	**Participants With AN vs OAs**	**OAs vs Controls**	**Participants With AN vs Controls**
Cortical parameters							
Cortical area, mm^2	101.7 ± 2.3	121.2 ± 2.7	120.4 ± 3.9	<0.0001	<0.0001	—	0.0004
% Cortical area	16.3 ± 0.4	18.5 ± 0.5	19.5 ± 0.8	0.002	0.01	—	0.002
Cortical thickness, mm	1.05 ± 0.02	1.21 ± 0.03	1.24 ± 0.04	0.0001	0.0005	—	0.0006
Cortical perimeter, mm	97.4 ± 0.8	100.9 ± 0.8	97.6 ± 1.2	0.008	0.02	0.046	—
Trabecular parameters							
Trabecular area, mm^2	529.0 ± 10.4	550.6 ± 12.0	510.0 ± 15.6	0.09	—	0.09	—
% Trabecular area	83.4 ± 0.4	81.5 ± 0.5	80.3 ± 0.8	0.002	0.03	—	0.002
Trabecular number, 1/mm	1.78 ± 0.04	1.92 ± 0.03	1.92 ± 0.04	0.008	0.008	—	0.04
Trabecular thickness, mm	0.086 ± 0.002	0.088 ± 0.001	0.086 ± 0.002	NS	—	—	—
Trabecular separation, mm	0.49 ± 0.01	0.44 ± 0.01	0.44 ± 0.01	0.002	0.003	—	0.02
Estimated strength, N	5367 ± 88	5952 ± 83	5891 ± 122	<0.0001	<0.0001	—	0.003

Data shown as mean ± standard error of the mean.

Abbreviations: ANOVA, analysis of variance; NS, not significant.

indicating the critical effect of nutritional status on bone in addition to that of estrogen deficiency, also known to be deleterious to bone (23). However, no such comparisons are available in adolescents. In this study, we compared bone density, bone structure parameters, and reported fracture rate in adolescents and young adults with two common causes of hypothalamic amenorrhea (AN and exercise-induced amenorrhea) and hypothesized that participants with AN would have the greatest skeletal impact and the highest risk for fracture, followed by OAs, followed by controls. This study evaluated the impact of low weight and amenorrhea (both known to be deleterious to bone) and weight-bearing activity (known to be beneficial to bone) on bone parameters at different sites.

We found that adolescents and young adults with AN had lower aBMD at all sites compared with OAs and controls. However, the normal-weight OAs also had lower spine aBMD than controls (36–39). A key difference between groups was nutritional status. As expected, participants with AN had lower weight parameters than OAs and controls; furthermore, OAs (though normal weight) had lower BMI *z* scores and percentage mBMI than controls, indicative of a relative energy deficit state in OAs. Although weight-bearing activities during adolescence and young adulthood, a critical period for bone accrual, have positive effects on bone, our data indicate that hypoestrogenism negates this health benefit at the spine, as indicated by lower spine aBMD in OAs than controls. Furthermore, in participants with AN, the additional deleterious effect of low weight may contribute to even lower spine aBMD than seen in OAs. Consistent with this finding, BMI *z* scores correlated positively and duration of amenorrhea correlated negatively with aBMD *z* scores at specific sites in participants with AN and OAs. However, higher total

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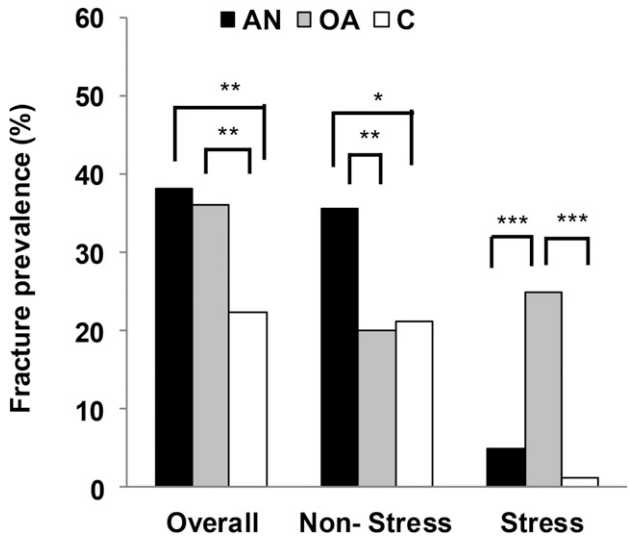

Figure 3. Comparison of fracture prevalence in participants with AN, OAs, and controls (C). Whereas OAs had a higher prevalence of stress fractures than participants with AN and controls, participants with AN had a higher prevalence of nonstress fractures than OAs and controls. Total fracture prevalence was higher in both OAs and participants with AN compared with controls. $*P < 0.05$; $**P < 0.01$; $***P < 0.0001$. C, controls.

hip and WBLH aBMD *z* scores in OAs vs participants with AN, even after we adjusted for BMI *z* scores and duration of amenorrhea, support the beneficial effect of exercise at these sites. Of note, these measures remained significantly higher in OAs than in participants with AN, even after we added hours per week of weight-bearing physical activity over the past year to the multivariate model. This finding may indicate that this measure of exercise activity does not capture the cumulative effect of exercise activity over many years, which may otherwise have accounted for the persistent difference in aBMD *z* scores in OAs and participants with AN. Vitamin D intake and 25(OH)D levels were higher in participants with AN and OAs than controls and were only weakly and positively associated with total and trabecular bone area.

Although aBMD is a useful determinant of fracture risk, it can be artifactually high in tall subjects and low in short subjects (40, 41). The amount of fat surrounding bone is another factor that can affect aBMD (42). Also, it does not provide measures of bone geometry and structure or differentiate between trabecular and cortical bone compartments. HRpQCT allows us to evaluate vBMD (not affected by stature), bone geometry, and microarchitecture (43). Previous studies have shown that vBMD and both cortical and trabecular geometry and microarchitecture are altered in girls with AN at the distal radius compared with controls (11, 12). Cortical area and thickness are lower, and cortical porosity is higher; trabecular vBMD, number, and thickness are lower, and trabecular separation is higher (13). Our group has also examined HRpQCT findings in OAs compared with EAs and nonathletes and reported that athletic activity is associated with greater total and trabecular area and greater cortical perimeter at the weight-bearing tibia, whereas amenorrhea is associated with lower trabecular vBMD at the radius and with lower total vBMD and trabecular number and greater trabecular separation at the tibia (despite weight-bearing activity) (15, 16).

Consistent with these reports, we found that participants with AN and OAs had lower vBMD and impaired cortical parameters compared with controls at the distal radius. In addition, participants with AN had significant impairment in trabecular parameters at this non–weight-bearing site compared with controls. There were no differences in microarchitecture between OAs and participants with AN at this site. However, the main difference between participants with AN and OAs was at a weight-bearing site, the distal tibia, for both cortical and trabecular parameters. Participants with AN had lower total and trabecular vBMD than OAs, in addition to greater impairment in certain cortical and trabecular parameters such as cortical area, thickness, and trabecular number. This finding might reflect the protective effect of weight-bearing activity in OAs for these microarchitectural parameters. OAs did not differ from controls for most structural parameters at this site, except that they had lower cortical vBMD than controls. Of note, differences between groups were lost after we controlled for BMI *z* scores (with or without duration of amenorrhea and with or without hours per week of exercise activity), emphasizing the critical impact of nutritional status on bone geometry and structure in these conditions.

We have previously reported a higher fracture prevalence in adolescents with AN than controls (8). Furthermore, although athletic activity should have favorable skeletal effects, OAs (even when of normal weight) lack many bone health benefits of weight-bearing exercise and are more susceptible to stress fractures than normal-weight nonathletes (15). Thus far, there are no data comparing fracture prevalence in adolescents and young adults with AN vs OAs and controls. Controls typically should not get stress fractures because they do not engage in repetitive weight-bearing activities.

When we compared the fracture rate in participants with AN vs OAs, the overall fracture rate was similar in both groups and higher than in controls. However, when we examined stress and nonstress fracture rates in these groups, participants with AN had a higher nonstress fracture rate than controls and OAs (OR = 5.8 and 4.1, respectively, after we controlled for risk factors such as BMI *z* scores, duration of amenorrhea, and weight-bearing exercise activity). In contrast, the stress fracture rate was 15 times higher in OAs than in controls and

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7.5 times higher in OAs than in participants with AN after we controlled for these covariates. After we controlled for the same risk factors, important DXA determinants of stress fracture in OAs were spine and WBLH (but not total hip) aBMD z scores. We have previously reported that among OAs, spine and whole body (but not total hip or femoral neck) aBMD z scores were lower in those with a history of fracture compared with those without a history of fracture (11). The lack of association of fracture rates with hip aBMD z scores in both studies may relate to the beneficial effect of weight-bearing exercise on bone at this site. Furthermore, the AN group was four times more likely to have nonstress fractures than OAs. We have previously shown that fracture prevalence is higher in participants with AN compared with normal-weight controls even without a significant reduction in aBMD (8). Similarly, aBMD z scores of the spine, total hip, and WBLH were not associated with fracture risk in participants with AN.

Nevertheless, given that low BMI and amenorrhea are key determinants of impaired bone density, microarchitecture, and strength estimates in FHA, a presumed strategy to reduce fracture risk is optimizing energy availability and menstrual recovery, which typically requires a treatment team (dietician, psychologist or psychiatrist, and eating disorder specialist or sports medicine physician at the very least) and avoidance of repetitive or high-impact activities, particularly when aBMD is low (44). Calcium and vitamin D intake should be optimized through diet and supplements, with a target 25(OH)D level between 32 and 50 ng/mL. However, the effect of these interventions on fracture risk remains to be determined in large prospective studies. Of note, combined oral hormonal contraceptives do not improve aBMD in FHA (or cause only minimal increases that are not clinically significant) (45). In contrast, physiologic estrogen replacement with transdermal 17-β estradiol does improve aBMD z scores in adolescents with AN, presumably because transdermal estradiol administration avoids hepatic first-pass metabolism and does not suppress insulin like growth factor-1, a key bone trophic factor during adolescence (29). Other studies have reported maintenance of aBMD z scores by using a combined oral hormonal contraceptive with oral dehydroepiandrosterone in young women with AN (46) and improved aBMD with risedronate in adults with AN (47) and teriparatide in older women with AN (48). None of these studies assessed the effect of these interventions in reducing fracture risk.

A limitation of our study is that it is cross-sectional, with the inherent limitations of such studies. Furthermore, fracture counts were based on self-report. However, we obtained a detailed fracture history from our participants (as described in the Methods section) to rule out sprains and other injuries. Also, in participants with AN we did not have a reliable method to calculate the total duration of illness (because the exact time of onset of illness is difficult to determine) and instead used duration since diagnosis as a predictor of bone status, which is typically shorter than the duration of illness. Finally, our participants were only female and mostly white. Additional studies are therefore needed to address this question in men, boys, and different racial and ethnic groups.

A strength of our study is that it compares the impact of AN and oligoamenorrhea on bone parameters and fracture risk in adolescents, with larger numbers of participants than most reported cohorts in adults (3, 4, 6, 7). It also compares HRpQCT data in these groups. Our data demonstrate that estrogen status alone does not account for the many differences in bone parameters between groups, and they point to the independent and critical effects of nutrition on these parameters. Furthermore, amenorrhea in OAs (even when participants are normal weight) increases the risk of stress fracture compared with controls, suggesting that traditional measures of bone mass are not useful in determining stress fracture risk in this group.

Conclusion

Low weight and amenorrhea (in AN) are deleterious to bone at all sites and affect both bone compartments. Normal-weight OAs have lower spine aBMD and lower cortical vBMD, area and thickness at non–weight-bearing sites, and lower cortical vBMD at weight-bearing sites than controls (indicative of effects of oligoamenorrhea), whereas most other bone parameters do not differ (indicative of the compensatory effect of normal weight or mechanical loading). A high stress fracture rate in OAs, who have comparable aBMD measures at multiple sites (WBLH, hip, and distal tibia) as controls, indicates that BMD in these young women may need to be higher than in controls to avoid fractures from weight-bearing activity (49).

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