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Abnormal Bone Mineral Accrual in Adolescent Girls with Anorexia Nervosa

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Anorexia nervosa (AN) is increasingly common in adolescent girls and occurs at a time of peak bone mass formation. Osteopenia is common in adolescent girls with AN, and in a cross-sectional study, we have reported low bone formation markers in such girls. To determine the impact of chronic undernutrition on bone mineral accrual in contrast to healthy controls, we prospectively measured bone mineral density (BMD) and body composition by dual energy x-ray absorptiometry, bone metabolism markers, and nutritional and hormonal status at baseline, 6 months, and 12 months in 19 adolescent girls with AN (mean \pm SEM, 15.4 \pm 0.4 yr) and 19 controls of comparable chronological and skeletal age. Overall, nutritional status in subjects with AN improved (mean percentage increase in body mass index from baseline, 9.2 \pm 1.9% and 15.2 \pm 2.6% at 6 and 12 months, respectively), with 11 subjects having recovered weight at 12 months. However, lumbar BMD at 12 months (AN, 0.88 \pm 0.02 g/cm², vs. control, 0.98 \pm 0.03 g/cm²; P = 0.008) remained significantly reduced in AN compared with controls, even in recovered subjects. This was due to significant increases in lumbar BMD in controls vs. no change in AN subjects over the year (0.003 \pm 0.001 g/cm²-month vs. 0.000 \pm 0.001 g/cm²-month, respectively; P = 0.04). The most significant determinant of change in lumbar BMD at 12 months was change in lean body mass in both AN (r = 0.62; P = 0.008) and control (r = 0.80; P = 0.0006) groups. There were significant increases in surrogate markers of bone turnover in

subjects with AN compared with controls as assessed by osteocalcin (AN, 0.9 \pm 0.4 μ g/liter-month, vs. control, -1.1 \pm 0.4 μ g/liter-month; P = 0.0007), bone-specific alkaline phosphatase (AN, 0.6 \pm 0.5 U/liter-month, vs. control, -1.5 \pm 0.4 U/liter-month; P = 0.002), deoxypyridinoline [AN, 0.1 \pm 0.1 nmol/mmol creatinine (cr)-month, vs. control, -0.4 \pm 0.1 nmol/mmol cr-month; P = 0.005], and N-telopeptide (AN, 4 \pm 4 nmol BCE/mmol cr/month, vs. control, -9 \pm 4 nmol BCE/mmol cr/month; P = 0.01). Changes in IGF-I levels over the year were highly correlated with changes in bone turnover over the same period in AN (osteocalcin, r = 0.77; P = 0.001; deoxypyridinoline, r = 0.66; P = 0.01). A rise in N-telopeptide over the year was correlated with an increase in all bone mineral measures, including lumbar bone mineral content (r = 0.58; P = 0.03) and BMD (r = 0.53; P = 0.05) and total bone mineral content (r = 0.69; P = 0.006) and BMD (r = 0.69; P = 0.006) in the AN group.

Therefore, despite recovery over 1 yr, poor bone mineral accrual persists in adolescent girls with AN in contrast to rapid bone accrual in healthy girls. Normalization of bone turnover markers occurs in association with nutritional recovery and an increase in the nutritionally dependent bone trophic factor IGF-I. A rise in bone turnover markers may be an early indicator of increase in BMD in recovering girls with AN. (*J Clin Endocrinol Metab* 87: 4177–4185, 2002)

OSTEOPENIA OCCURS IN approximately 50% of adolescent girls with anorexia nervosa (AN), and the onset of disease occurs at a time of peak bone mass development (1–4). Reduced bone mass is seen after a brief duration of illness and may persist despite recovery (2, 4–6). Adult women with AN have widespread osteopenia and osteoporosis, presumably attributed to bone loss (7). However, adult women with AN with disease onset in adolescence have lower bone mass than those with adult-onset AN (8). Because the majority of bone mineral is accrued during adolescence, any process that interferes with normal bone mineral accretion may lead to permanent deficits. It is unclear whether catch up can occur in bone mineral density (BMD) in adolescent girls with AN with recovery of weight. Previous

reports (2, 4–6) have not included prospective data regarding change in BMD with recovery compared with controls of the same chronological age (CA) and skeletal maturation.

We have previously reported low spinal BMD in teenage girls with AN compared with CA- and bone age (BA)-matched controls (3). In that study, a decrease in bone formation markers [osteocalcin (OC) and bone-specific alkaline phosphatase (BSAP)] was observed, indicating that low bone formation occurs in association with low BMD in girls with AN. Moreover, the low bone formation markers in such girls were significantly associated with serum levels of IGF-I, a bone trophic hormone that is highly dependent on nutritional status.

In this study, we hypothesized that reduced bone mineral accrual occurs in girls with AN during puberty and persists in association with chronic undernutrition. Therefore, we prospectively examined 38 adolescent girls (19 AN and 19 control) to determine whether 1) low bone formation markers in adolescents with AN are associated with abnormal bone mass accretion, 2) improvement in BMD and markers of bone metabolism can be predicted by nutritional status,

Abbreviations: AN, Anorexia nervosa; BA, bone age; BMC, bone mineral content; BMD, bone mineral density; BMI, body mass index; CA, chronological age; cr, creatinine; BSAP, bone-specific alkaline phosphatase; DHEAS, dehydroepiandrosterone sulfate; DPD, deoxypyridinoline; E₂, estradiol; IGFBP-3, IGF binding protein-3; OC, osteocalcin; NTX, N-telopeptide; RDA, recommended daily allowance; T, testosterone; TBBMC, total body BMC; TBBMD, total body BMD.

including body composition and levels of IGF-I, and 3) reductions in BMD and bone formation markers persist despite recovery of weight and menstrual function.

Subjects and Methods

Subjects

We studied 38 Caucasian adolescent girls, 19 with AN and 19 healthy controls. Fourteen of the AN and 12 of the controls participated in a previously reported cross-sectional bone density study (3). The AN patients were 12.9–17.8 yr of age at baseline. All met Diagnostic and Statistical Manual IV criteria for AN with a mean duration since diagnosis of 14 ± 3 months (range, 1–48 months). One patient had concomitant bulimia. Patients had either primary (failure of menarche by age 16 yr; $n = 1$) or secondary ($n = 12$; mean duration, 21 ± 4 months; range, 6–36 months) amenorrhea or were premenarchal ($n = 6$). Two patients with secondary amenorrhea had taken estrogen previously (for 6–18 months), but not within 3 months of the study. Patients receiving hormonal or other medications known to affect bone metabolism were excluded. A history of traumatic bone fracture was reported during childhood or adolescence in 26% of AN patients, but none occurred within 6 months of the study and none were nontraumatic. Subjects were recruited through mass mailings to primary health care providers and through collaboration with eating disorder programs in the greater Boston area.

A control group of 19 healthy Caucasian female adolescents, ages 12.1–17.8 yr, was recruited from primary care providers and by advertisement in community newspapers. All had a body mass index (BMI; kg/m^2) between the 25th and 95th percentiles for age, based on published standard charts (9). Three control subjects, ages 12.1–13.1 yr, were premenarchal. All postmenarchal adolescents menstruated regularly. None of the controls had a history of an eating disorder. Potential control subjects with a medical condition, or receiving hormonal or other medications known to affect bone metabolism, were excluded. A history of traumatic bone fracture during childhood or adolescence was reported in 23% of control subjects, but none occurred within 6 months of the study.

The study protocol was approved by the Massachusetts General Hospital Subcommittee on Human Subjects, and informed consent was obtained from all subjects and their parents.

Experimental protocol

All subjects were evaluated at a screening outpatient visit at the General Clinical Research Center at Massachusetts General Hospital, at which time eligibility for study participation was determined and instruction in collection of urine samples and completion of 4-d food records given. This was followed by an outpatient baseline visit during which study measurements were performed. After the baseline study visit, all subjects were asked to return at 6 and 12 months for repeat measurements. All menstruating subjects were studied during the follicular phase of the menstrual cycle to control for the effects of cycle stage on biochemical and hormonal measurements. A medical and menstrual history and physical examination were performed at all visits. A 2-h, second morning urine sample was collected before all visits. All blood samples were drawn in the fasting state. Urine and serum samples were obtained for biochemical and hormonal measurements as outlined below. Bone density and body composition were assessed in all subjects at each visit.

Methods

Anthropometric measurements. Subjects' heights were measured using a single stadiometer (average of triplicate measurements), and weights were measured in a hospital gown on an electronic scale. BMI was calculated, and BMI percentile was determined from published charts (9).

Classification of subjects by BA. All AN and control subjects had their BA determined by x-ray of the left hand and wrist using the methods of Greulich and Pyle (10). For the analysis of biochemical parameters that are dependent on pubertal stage, such as gonadal steroids and markers of bone formation and resorption, we analyzed groups according to

maturity. Because Tanner breast staging is difficult in girls with AN who are hypoestrogenemic, we classified the maturational stage of subjects by BA, subdividing the subjects into immature ($BA < 15$ yr) and mature ($BA \geq 15$ yr) groups. We used the cut-off BA of 15 yr as mature in that all epiphyses are near fusion and 99% of final adult height has been attained (10).

Biochemical assessment. Serum-ionized calcium, phosphorus, TSH, and urine creatinine were measured in the hospital laboratory using published methods (11).

Serum estradiol (E_2) was measured by ultra-sensitive RIA (Diagnostic Systems Laboratories, Inc., Webster, TX). The detection limit of the E_2 assay is 8.1 pmol/liter, and the coefficient of variation is 6.5–8.9%. Free testosterone (T) was measured by RIA (DiaSorin, Inc., Stillwater, MN), with a detection limit of 0.6 pmol/liter and an intra-assay coefficient of variation of 3.7–6.2%. Serum dehydroepiandrosterone sulfate (DHEAS) was measured by RIA (Coated Tube RIA, DiaSorin, Inc.) with a detection limit of 0.03 $\mu\text{mol}/\text{liter}$ and an intra-assay coefficient of variation of 3.8–5.3%. Serum IGF-I was measured by an acid-alcohol extraction and RIA kit (Nichols Institute Diagnostics, San Juan Capistrano, CA), with a detection limit of 0.06 $\mu\text{g}/\text{liter}$ and an intra-assay coefficient of variation of 2.4–3.0%. Serum IGF binding protein-3 (IGFBP-3) was measured using an immunoradiometric assay (Coated Tube IRMA, DiaSorin, Inc.) with a detection limit of 0.5 $\mu\text{g}/\text{liter}$ and an intra-assay coefficient of variation of 1.8–3.9%. Serum leptin was measured by RIA (Linco Research, Inc., St. Charles, MO) with a sensitivity of 0.5 $\mu\text{g}/\text{liter}$ and an intra-assay coefficient of variation of 3.4–8.3%. Serum OC was measured using an immunoradiometric assay (Nichols Institute Diagnostics) with a sensitivity of 0.5 $\mu\text{g}/\text{liter}$ and an intra-assay coefficient of variation of 3.2–5.2%. BSAP was measured by enzyme-linked immunoassay (Quidel, Inc., Mountain View, CA) with a sensitivity of 0.7 U/liter and a coefficient of variation of 3.9–5.8%. Urine deoxypyridinoline (DPD) and N-telopeptide (NTX) were measured in a 2-h, second morning urine sample normalized for creatinine excretion. DPD was measured by enzyme-linked immunoassay (Quidel, Inc.) with a minimum detection limit of 1.1 nmol/liter and an intra-assay coefficient of variation of 4.3–8.4%. NTX was measured by enzyme-linked immunoassay (Ostex International, Inc., Seattle, WA) with a detection limit of 20 nmol BCE and an intra-assay coefficient of variation of 5–19%. Serum was frozen so that hormone determinations for each subject could be performed in the same assay. All samples were run in duplicate.

Bone density and body composition. Total body and lumbar spine (L1-L4) bone density was measured in the anteroposterior and lateral projections by dual energy x-ray absorptiometry (QDR-4500, Hologic, Inc., Waltham, MA). The SD value for lumbar bone density measurement is 0.01 g/cm^2 and does not vary with bone density. Whole body dual energy x-ray absorptiometry was performed using a Hologic QDR-4500 densitometer to determine fat and lean body mass, which has been validated in children and young adults (12, 13).

Nutrition and activity assessment. Calcium and vitamin D intake data were obtained through computer analysis (Nutrition Data Systems, version 2, Minneapolis, MN) of 4-d food records and history of dietary intake over the month before the study visit. Typical exercise and activity of subjects over the past year was determined using a validated questionnaire (14).

Statistical analysis

Longitudinal markers were measured at baseline, 6 months, and 12 months. A random slopes model (PROC MIXED in SAS) was used to analyze these data. The model specifies that each patient has a random intercept and slope, and the test for a group effect determines whether the mean slope varies between groups. This method is similar to comparing the change from baseline in each group, however it can appropriately incorporate patients who were missing their 12-month follow-up. *T* tests were used to compare baseline measures and percentage change at 6 and 12 months.

Recovery from AN was defined as a 10% or greater increase in BMI from baseline, regardless of resumption of menses.

Results

We studied 19 adolescent girls with AN and 19 healthy controls. Baseline data on 14 of the AN and 12 of the control

subjects has been previously published (3). All subjects were evaluated at baseline and asked to return at 6 and 12 months. All available data were collected and analyzed. Reasons for not attending follow-up visits included hospitalization (AN, $n = 4$ visits) or loss of interest (AN, $n = 3$; control, $n = 3$ visits). Three control subjects were disqualified between the 6- and 12-month visits because they started taking oral contraceptive pills for birth control. Data were available for 14 AN and 18 control subjects at 6 months and for 17 AN and 14 controls at 12 months. Incomplete capture of subjects for follow-up visits did not result in any differences in BA or height between the groups at 6 and 12 months. Six AN subjects at 6 months and 11 at 12 months were weight recovered.

Clinical and anthropometric data

Clinical and anthropometric data for patients and controls at baseline are summarized in Table 1. There were no significant differences between patients and controls in BA, CA, or height. BA/CA and weight were significantly lower in AN patients compared with controls. Mean duration of AN was 14 ± 3 months, and duration of amenorrhea in postmenarchal patients ($n = 12$) was 21 ± 4 months. All subjects were Caucasian. No subjects regularly smoked cigarettes or consumed alcohol.

Body composition data at each time point and rates of change are summarized in Table 2. BMI, lean body mass, and percentage body fat were significantly lower in AN at baseline. In the AN group, nutritional status improved (percentage increase in BMI from baseline, $9.2 \pm 1.9\%$ at 6 months; $15.2 \pm 2.6\%$ at 12 months). Six subjects at 6 months and 11 subjects at 12 months had recovered weight (10% or greater increase in BMI), and four subjects at 6 months and eight subjects at 12 months had recovered weight and menstrual function. Analysis of rate of change over time (Table 2) demonstrated a significantly greater increase in lean body mass in AN compared with control such that at 6 and 12 months lean body mass in AN was no longer different from control. The AN group had a significant increase in percentage body fat compared with control. However, percentage body fat remained lower than control at 6 and 12 months.

There was no difference in linear growth velocity between AN and control, nor between recovered and nonrecovered AN over the year (data not shown).

TABLE 1. Baseline clinical and anthropometric data

	AN (n = 19)	Control (n = 19)	P value
CA (yr)	15.4 ± 0.4 [12.9–17.8]	14.6 ± 0.4 [12.1–17.8]	NS
BA (yr)	15.0 ± 0.4 [12–18]	15.1 ± 0.4 [13–18]	NS
BA/CA	0.98 ± 0.01	1.04 ± 0.01	0.003
Height (cm)	162.3 ± 1.4	162.7 ± 1.4	NS
Weight (kg)	43.5 ± 1.7	58.0 ± 1.7	<0.0001
Duration of illness (months)	14 ± 3 [1–48]		
Amenorrhea			
No. premenarchal	6	3	
No. primary	1	0	
No. secondary	12	0	
Duration (months)	21 ± 4 [6–36]		
Activity (h/wk)	17.8 ± 2.2	11.9 ± 2.6	0.07

All values are expressed as mean \pm SEM. Ranges are in brackets. The P value is for comparison between AN and control subjects by *t* test. NS, Not significant.

Nutritional intake and activity data

Analysis of calcium and vitamin D intake by either 4-d food records or diet history was comparable. Therefore, only intake by food records is reported. Forty-two percent of AN patients and 50% of controls had a calcium intake less than the recommended daily allowance (RDA) of calcium (1300 mg/d) at baseline. Both groups also had a high proportion of subjects with dietary calcium deficiency at follow-up (AN, 38% at 6 months, 33% at 12 months; control, 87% at 6 months, 50% at 12 months). This high degree of dietary calcium deficiency occurred despite a range of supplementation in AN (28% at baseline, 38% at 6 months, 54% at 12 months) and control subjects (32% at baseline, 13% at 6 months, 17% at 12 months). In 42% of AN patients and 50% of controls, vitamin D intake was less than the RDA at baseline, with a high proportion of subjects (AN, 69% at 6 months, 53% at 12 months; control, 100% at 6 months, 64% at 12 months) continuing to have dietary deficiency at follow-up. A high rate of dietary deficiency occurred despite many subjects taking vitamin D supplements (AN, 42% at baseline, 92% at 6 months, 73% at 12 months; control, 44% at baseline, 33% at 6 months, 50% at 12 months).

Historical assessment of activity level demonstrated that AN subjects tended to be more physically active than controls at baseline (Table 1), whereas at follow-up there was no difference between the groups.

Bone density data

Bone density data at each time point and rate of change in bone density in each group are provided in Table 3. At baseline, lumbar spine (L1-L4) BMD was significantly lower in AN subjects and remained significantly reduced at 6- and 12-month follow-up visits. Similar differences in lumbar bone mineral apparent density, a calculated volumetric density, were demonstrated using the formula of Katzman *et al.* (15). Lumbar BMD was more than 1 SD below the mean of the controls in 37% of AN subjects at baseline, 57% at 6 months, and 71% at 12 months. Twenty-four percent of AN subjects had a lumbar BMD more than 2 SD below the normal mean at 12 months. The increasing frequency of osteopenia in AN was due to a significantly greater rate of change in BMD in controls than in AN. Similarly, the increase in lumbar bone mineral content (BMC) was significantly greater in control

TABLE 2. Body composition data in AN and control at each time point and change over time (slope)

	Baseline	6 months	12 months	Slope change/month	P value for slope
BMI (kg/m ²)					
AN	16.4 ± 0.5 ^a	18.0 ± 0.7	18.9 ± 0.6	0.20 ± 0.04	0.005
Control	22.0 ± 0.5	22.4 ± 0.6	22.2 ± 0.7	0.04 ± 0.04	
Lean body mass (kg)					
AN	33.6 ± 1.0 ^a	35.7 ± 1.3	37.8 ± 1.1	0.33 ± 0.06	0.04
Control	37.4 ± 1.0	38.5 ± 1.2	38.1 ± 1.2	0.14 ± 0.06	
Percentage body fat					
AN	17.7 ± 1.4 ^a	20.2 ± 1.9	22.8 ± 1.7	0.41 ± 0.11	0.009
Control	31.7 ± 1.4	30.8 ± 1.7	32.2 ± 1.9	-0.03 ± 0.11	

Baseline, 6-, and 12-month values are expressed as mean ± SEM. Slope refers to the rate of change per month. The *P* value for the slope refers to the difference in slopes between AN and control subjects. For AN subjects, *n* = 19 at baseline, 14 at 6 months, and 17 at 12 months. For control subjects, *n* = 19 at baseline, 18 at 6 months, and 14 at 12 months.

^a *P* value < 0.05 for comparison between AN and control subjects at baseline by *t* test.

TABLE 3. Bone density data in AN and control at each time point and rate of change (slope)

	Baseline	6 months	12 months	Slope change/month	P value for slope
Lumbar (L1–4) BMD (g/cm ²)					
AN	0.89 ± 0.02 ^a	0.88 ± 0.03	0.88 ± 0.02	0.000 ± 0.001	0.04
Control	0.96 ± 0.02	0.98 ± 0.02	0.98 ± 0.03	0.003 ± 0.001	
Lumbar (L1–4) BMC (g)					
AN	46 ± 2	44 ± 2	47 ± 2	0.05 ± 0.08	0.009
Control	50 ± 2	51 ± 2	52 ± 2	0.37 ± 0.08	
Lateral spine BMD (g/cm ²)					
AN	0.70 ± 0.02 ^a	0.69 ± 0.02	0.70 ± 0.02	0.000 ± 0.001	NS
Control	0.80 ± 0.02	0.81 ± 0.02	0.80 ± 0.02	0.000 ± 0.001	
Volumetric lumbar BMD (g/cm ³)					
AN	0.12 ± 0.00 ^a	0.12 ± 0.00	0.12 ± 0.00	0.0003 ± 0.0001	NS
Control	0.13 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.0000 ± 0.0001	
Total BMD (g/cm ²)					
AN	1.00 ± 0.02	0.98 ± 0.02	0.98 ± 0.01	0.0015 ± 0.0008	0.09
Control	1.05 ± 0.02	1.05 ± 0.02	1.05 ± 0.02	0.0006 ± 0.0008	
Total BMC (g)					
AN	1705 ± 55	1662 ± 57	1720 ± 51	1.6 ± 2.5	0.07
Control	1838 ± 55	1891 ± 50	1903 ± 56	7.2 ± 2.6	

Baseline, 6-, and 12-month values are expressed as mean ± SEM. Slope refers to the rate of change per month. The *P* value for the slope refers to the difference in slopes between AN and control subjects. NS, Not significant. For AN subjects, *n* = 19 at baseline, 14 at 6 months, and 17 at 12 months. For control subjects, *n* = 19 at baseline, 18 at 6 months, and 14 at 12 months.

^a *P* value < 0.05 for comparison between AN and control subjects at baseline by *t* test.

subjects. The control group had a significantly greater monthly increase in lumbar BMD of 0.003 ± 0.001 g/cm²-month compared with 0.000 ± 0.001 g/cm²-month in the AN group (*P* = 0.04). Change in lumbar BMD was not different in AN subjects who recovered (*n* = 11) compared with those who did not recover (*n* = 6) at 12 months, such that lumbar BMD remained reduced even in subjects who were weight recovered at 12 months (AN, 0.88 ± 0.03 g/cm², vs. control, 0.98 ± 0.03 g/cm²; *P* = 0.02). There were no recovered subjects whose lumbar BMD z-score increased from below -1 to above -1. As illustrated in Fig. 1, those in the AN group who recovered weight had variable changes in lumbar BMD over the year, with the rate of change not significantly different from those without weight recovery. The percentage change in lumbar BMD in patients who recovered weight (-2.3% to 9.2%) was similar to those who recovered menses as well as weight (-3.1% to 5.5%). Two AN subjects who had a decrease in BMI at 12 months had a decrease of 3.8% and 5.3% in lumbar BMD over the year. All but one control subject had a positive change of variable degree (0.1–18.8%) in lumbar BMD over the year. There was a trend toward greater increase in lumbar BMD over the year in subjects who had immature skeletal maturation at baseline

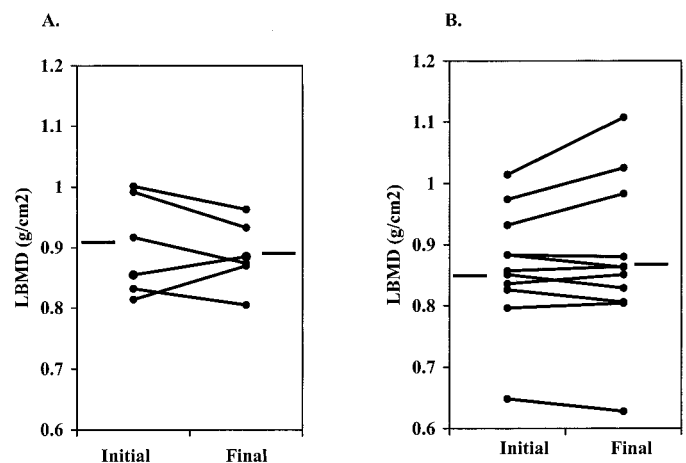


FIG. 1. Comparison of initial and final lumbar BMD (LBMD) in non-recovered AN (A; *n* = 6) and recovered AN (B; *n* = 11). No significant difference was seen in the mean final BMDs in the two groups.

(AN, *n* = 8; control, *n* = 7), in both AN (immature, *n* = 8, $2.5 \pm 1.6\%$, vs. mature, *n* = 11, $-1.2 \pm 1.3\%$; *P* = 0.09) and control (immature, *n* = 7, $7.5\% \pm 2.1\%$, vs. mature, *n* = 12, $2.0 \pm 1.8\%$; *P* = 0.07) groups.

There was a trend toward lower total body BMD (TBBMD) and BMC (TBBMC) in AN at baseline as well as a trend toward greater increase in TBBMD and TBBMC in controls compared with AN (Table 3).

In the AN group, change in lean body mass from baseline to 12 months had the strongest correlation with change in bone density over 12 months. This relationship was true for lumbar BMD ($r = 0.62$; $P = 0.008$; Fig. 2), lumbar BMC ($r = 0.51$; $P = 0.04$), TBBMD ($r = 0.70$; $P = 0.002$), and TBBMC ($r = 0.81$; $P = 0.0003$). In addition, there was a tendency for the change in lean body mass over the first 6 months to be correlated with the change in bone density in the following 6 months at the lumbar anteroposterior ($r = 0.56$; $P = 0.06$) and lateral ($r = 0.56$; $P = 0.07$) spine. Similarly, in the control group, change in lean body mass over the 12-month study period had the strongest correlation with change in BMD and BMC over the same period, including lumbar BMD ($r = 0.80$; $P = 0.0006$; Fig. 2), lumbar BMC ($r = 0.71$; $P = 0.004$), and total BMD ($r = 0.51$; $P = 0.06$). Change in lean body mass in the first 6 months was strongly correlated with change in the following 6 months in lumbar BMD ($r = 0.75$; $P = 0.003$) and lumbar BMC ($r = 0.75$; $P = 0.002$).

Biochemical data

TSH, serum-ionized calcium, and phosphorus were normal in all subjects at all time points studied. Hormonal data at baseline are analyzed on the basis of maturational stage (immature, BA < 15 yr; mature, BA \geq 15 yr) and summarized in Table 4. Baseline IGF-I levels were significantly reduced in both immature and mature AN compared with control, with the most marked reduction in the mature group to less than 50% of control levels. Analysis of rate of change over time demonstrated that the AN group had a significantly greater increase in IGF-I compared with control (AN, 8 ± 2 $\mu\text{g/liter}\cdot\text{month}$, vs. control, -4 ± 2 $\mu\text{g/liter}\cdot\text{month}$; $P = 0.0008$), such that at 6 and 12 months, IGF-I levels were not different from control in the immature group (data not shown). However, in the mature group, despite significant increases in IGF-I, levels remained reduced at 6 and 12 months (AN, 300 ± 47 $\mu\text{g/liter}$, vs. control, 499 ± 36 $\mu\text{g/liter}$; and AN, 356 ± 42 $\mu\text{g/liter}$, vs. control, 522 ± 45 $\mu\text{g/liter}$, respectively). In recovered subjects at 12 months, IGF-I levels did not differ

from those in nonrecovered subjects. In both AN and control groups, change in IGF-I over 12 months was strongly correlated with change in nutritional markers over 12 months, including BMI (AN, $r = 0.76$; $P = 0.001$; and control, $r = 0.71$; $P = 0.004$) and leptin (AN, $r = 0.66$; $P = 0.009$; and control, $r = 0.55$; $P = 0.03$). There were no differences in IGFBP-3 levels between the groups at any time point studied.

Serum E_2 levels at baseline were significantly reduced in the immature AN group compared with follicular phase control levels, but not different in the mature group (Table 4). At follow-up, there were no differences in serum E_2 levels in either maturational group. There was a trend toward a greater increase in serum E_2 levels over time in the AN compared with the control group (AN, 2.8 ± 1.1 pmol/liter \cdot month, vs. control, 0.1 ± 0.9 pmol/liter \cdot month; $P = 0.08$). Change in E_2 over the first 6 months of observation in the AN group was correlated with change in BMI ($r = 0.76$; $P = 0.004$) and percentage body fat ($r = 0.65$; $P = 0.02$). These correlations were not present in the control group. There was no difference in the rate of change in E_2 levels in recovered vs. nonrecovered AN.

Free T levels at baseline were significantly reduced in the mature AN group, but not in the immature group (Table 4). Analysis of rate of change over time demonstrated a significantly greater increase in free T in the AN compared with the control group (AN, 0.07 ± 0.03 pmol/liter \cdot month, vs. control, -0.1 ± 0.7 pmol/liter \cdot month; $P = 0.02$). DHEAS levels were not different between AN and control in either maturational group at any time point studied.

Bone turnover markers at baseline are summarized in Table 4. In both immature and mature AN groups, baseline levels of OC, BSAP, DPD, and NTX were significantly reduced compared with control levels. At follow-up, bone turnover markers in the AN group increased, whereas they decreased in controls, resulting in a markedly different change in marker levels between the groups over the 12-month period (OC: AN, 0.9 ± 0.4 $\mu\text{g/liter}\cdot\text{month}$, vs. control, -1.1 ± 0.4 $\mu\text{g/liter}\cdot\text{month}$, $P = 0.0007$; BSAP: AN, 0.6 ± 0.5 U/liter \cdot month, vs. control, -1.5 ± 0.4 U/liter \cdot month, $P = 0.002$; DPD: AN, 0.1 ± 0.1 nmol/mmol cr \cdot month, vs. control, -0.4 ± 0.1 nmol/mmol cr \cdot month, $P = 0.005$; and NTX: AN, 4 ± 4 nmol BCE/mmol cr \cdot month, vs. control, -9 ± 4 nmol

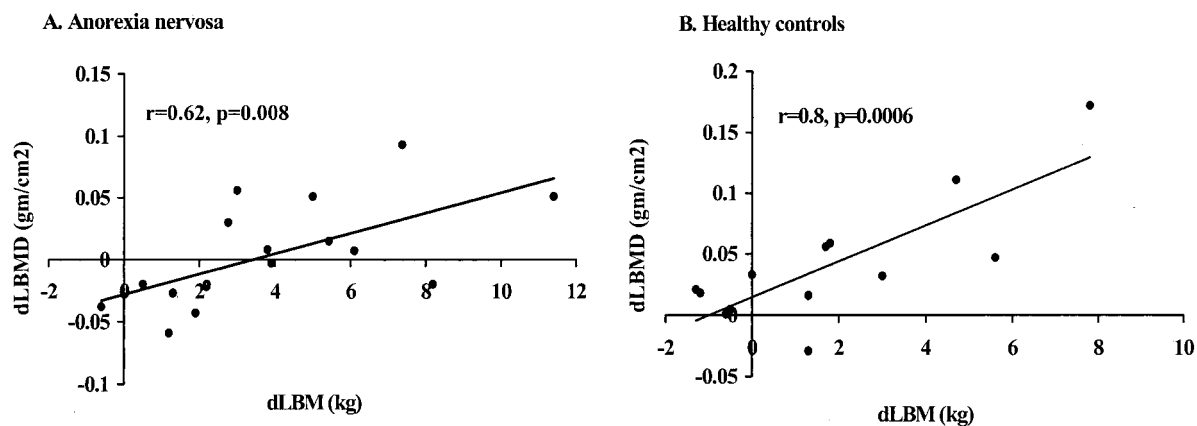


FIG. 2. Correlation between change in lean body mass (dLBM) and change in lumbar BMD (dLBMD) over 1 yr in adolescents with AN (A) and healthy controls (B).

FIG. 3. Percentage change in markers of bone formation (left) and bone resorption (right) over 1 yr. Percentage changes in levels of OC and BSAP were significantly higher in recovered AN than in nonrecovered AN ($P = 0.03$ for OC; $P = 0.0007$ for BSAP) and controls ($P = 0.0003$ for OC; $P < 0.0001$ for BSAP). Percentage changes in levels of markers of bone resorption (DPD and NTX) were significantly higher in recovered AN than in controls ($P = 0.02$ for DPD; $P = 0.002$ for NTX). *, Significantly different from healthy controls; **, significantly different from recovered AN.

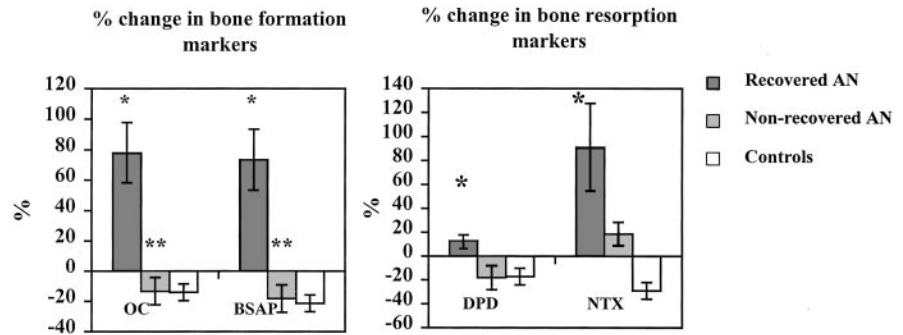


TABLE 4. Baseline hormone and bone turnover marker concentrations according to maturity

	Immature		Mature	
	AN (n = 8)	Control (n = 7)	AN (n = 11)	Control (n = 12)
IGF-I ($\mu\text{g/liter}$)	390 \pm 50 ^a	587 \pm 54	251 \pm 37 ^a	566 \pm 35
IGFBP-3 ($\mu\text{g/liter}$)	4564 \pm 258	4920 \pm 276	4040 \pm 229	4533 \pm 219
E ₂ (pmol/liter)	53.6 \pm 5.5 ^a	77.5 \pm 6.6	63.9 \pm 7.7	73.8 \pm 7.0
Free T (pmol/liter)	6.8 \pm 0.8	6.7 \pm 0.8	5.7 \pm 0.9 ^a	8.6 \pm 0.9
DHEAS ($\mu\text{mol/liter}$)	3.5 \pm 0.5	3.4 \pm 0.5	4.9 \pm 0.8	6.0 \pm 0.8
Leptin ($\mu\text{g/liter}$)	3.7 \pm 1.4 ^a	10.3 \pm 1.5	2.4 \pm 1.4 ^a	13.9 \pm 1.4
OC ($\mu\text{g/liter}$)	59.6 \pm 9.6 ^a	109.3 \pm 10.3	33.9 \pm 6.1 ^a	55.8 \pm 5.9
BSAP (U/liter)	48.1 \pm 13.6 ^a	108.7 \pm 14.5	21.0 \pm 3.5 ^a	32.9 \pm 3.4
DPD (nmol/mmol cr)	12.8 \pm 2.4 ^a	23.2 \pm 2.5	8.2 \pm 1.4 ^a	13.4 \pm 1.4
NTX (nmol BCE/mmol cr)	175 \pm 112	517 \pm 120	86 \pm 17 ^a	142 \pm 16.4

Baseline hormone and bone turnover marker data in immature (BA < 15 yr) and mature (BA \geq 15 yr) subjects. Values are expressed as mean \pm SEM.

^a P value < 0.05 for comparison between AN and control in each group by t test.

BCE/mmol cr-month, $P = 0.01$). In the immature AN group, there were persistent reductions in OC (AN, 60.3 \pm 17.0 $\mu\text{g/liter}$, vs. control, 112.0 \pm 15.0 $\mu\text{g/liter}$), DPD (AN, 12.9 \pm 2.7 nmol/mmol cr, vs. control, 21.3 \pm 3.0 nmol/mmol cr) and NTX (AN, 154 \pm 56 nmol BCE/mmol cr, vs. control, 494 \pm 61 nmol BCE/mmol cr) compared with control at 6 months. There were no differences between the immature AN and control groups at 12 months. In the mature group, OC at 6 months (AN, 31.8 \pm 5.5 $\mu\text{g/liter}$, vs. control, 50.4 \pm 4.2 $\mu\text{g/liter}$) was the only reduced marker of bone turnover in AN at follow-up.

There were significantly greater changes in bone formation markers in AN subjects who recovered compared with those who did not, as determined by BSAP and OC (Fig. 3).

In AN subjects, change in lean body mass over the year was strongly correlated with change in bone turnover over the year, as assessed by BSAP ($r = 0.61$; $P = 0.02$), NTX ($r = 0.62$; $P = 0.02$), DPD ($r = 0.55$; $P = 0.04$), and OC ($r = 0.48$; $P = 0.08$). In addition, change in IGF-I levels over the year was highly correlated with change in bone turnover over the same period, as measured by OC ($r = 0.77$; $P = 0.001$) and DPD ($r = 0.66$; $P = 0.01$). In addition, there was a trend toward correlation with BSAP ($r = 0.47$; $P = 0.09$). There was also a strong correlation between change in free T and change in bone formation markers OC ($r = 0.58$; $P = 0.03$) and BSAP ($r = 0.53$; $P < 0.05$) over the year in the AN group. There were no such correlations in the control group between bone turnover and lean body mass, IGF-I, or free T levels. There was no correlation between change in E₂ levels and bone turnover markers in either group.

In the AN group, an increase in specific bone turnover markers was predictive of an increase in bone mineral. Over the year, increased NTX was correlated with an improvement in all bone mineral measures over the same period, including lumbar BMC ($r = 0.58$; $P = 0.03$), and BMD ($r = 0.53$; $P = 0.05$), lateral spine BMD ($r = 0.58$; $P = 0.04$), and total BMC ($r = 0.69$; $P = 0.006$) and BMD ($r = 0.69$; $P = 0.006$). An increase in NTX for the first 6 months tended to predict a rise in lumbar BMC ($r = 0.55$; $P = 0.06$) and BMD ($r = 0.51$; $P = 0.09$) for the subsequent 6 months. In addition, an increase in BSAP in the first 6 months was correlated with a rise in lumbar BMC during the following 6 months ($r = 0.70$; $P = 0.02$).

Discussion

We prospectively investigated changes in bone density and bone metabolism markers over 1 yr in adolescent girls with AN compared with healthy adolescents of comparable skeletal maturation. Our data indicates that despite improvement in nutritional status and recovery of menstrual function, girls with AN had reduced bone mineral accrual and progressive spinal osteopenia over the year compared with their healthy counterparts. Change in lean body mass was the most significant determinant of change in lumbar BMD in both AN and healthy girls. At the end of the 12-month study period, none of the AN subjects had an improvement in lumbar BMD z-score from below -1 to above -1 , and 24% of AN subjects had a lumbar BMD more than 2 sd below the mean of the healthy girls. However, the low levels of bone

turnover markers present in girls with AN at baseline significantly increased in association with improvement in nutritional status, specifically an increase in lean body mass and the nutritionally dependent bone growth factor IGF-I. In addition, we found that the rise in the surrogate marker of bone resorption NTX over the year was correlated with improvement in lumbar and total BMC and BMD in the AN group. Moreover, our data suggests that an increase in the bone turnover markers NTX and BSAP in AN over time may be predictive of an increase in lumbar BMC and BMD.

The onset of AN frequently occurs during adolescence when maximal bone mineral accrual takes place, thereby making adolescent girls at high risk for reduced peak bone mass. Cross-sectional studies have shown that approximately half of adolescent girls with AN have low BMD involving trabecular and cortical bone (1–4). However, there is little prospective data available regarding the progression of osteopenia. In a prospective study of adolescent girls with AN by Bachrach *et al.* (2), spinal BMD was assessed by dual-photon absorptiometry in 15 girls over 12–16 months. In these girls, mean lumbar BMD was unchanged, but the change among patients varied considerably with weight loss or gain (–13.9% to 30.3%, respectively). In addition, one third of females examined retrospectively who had recovered from AN during adolescence had persistent osteopenia (2). Biller *et al.* (8) found that women with AN who had disease onset during adolescence had lower spinal bone density compared with those with disease onset in adulthood. The majority of published data in adolescents with AN is not prospective, and therefore little is known about bone mineral accrual during this critical period. Specifically, because height and bone maturation significantly impact BMD measurements and there is little available regarding normal prospective data on changes in BMD in adolescents, comparison of AN with healthy adolescents of similar stature and bone maturation is critical. To date, no such studies have been performed. Our data are in agreement with Bachrach *et al.* (2) in that our two subjects who lost weight did have evidence of bone loss, with a decrease in lumbar BMD of –3.8 and –5.9% after 1 yr. However, we found that healthy girls had greater gains in bone mineral than girls with AN, even among weight recovered subjects, suggesting that poor bone mineral accrual contributes to osteopenia in girls with AN. Moreover, no recovered subjects had an improvement in spinal BMD z-score from below –1 to greater than –1, indicating that there was no catch-up in bone mineral accretion with recovery over the study period.

In healthy females, bone mass increases throughout childhood with maximal bone mass accrual occurring in early to mid puberty, and slowing in late puberty (16–19). Longitudinal data from healthy girls demonstrate that gain in bone mass is most pronounced between 11 and 14 yr of age, and falls significantly after 16 yr of age and/or 2 yr after menarche (19). Therefore, it would be expected that bone mineral accrual in girls with AN would differ depending on the maturational stage. Because Tanner breast staging is difficult in girls with AN who are hypoestrogenemic, we classified girls as immature or mature based on skeletal maturation, which is a more objective measure. As expected, bone mineral accrual differed in each group by maturational stage,

with the immature girls tending to have greater gains in bone mineral over the year than the mature girls. In addition, mean changes in BMD in immature AN indicate low bone mineral accrual, whereas changes in the mature group indicate bone loss. These data indicate that the pathogenesis of osteopenia in AN may differ depending on maturational stage.

The determinants of change in BMD in adolescents with AN have not been extensively examined. We and others have shown that body mass, particularly lean tissue, is a major determinant of BMD in healthy children and adolescents (3, 20). Estrogen has been proposed as a contributing factor to the low bone density in AN because primary or secondary amenorrhea is a nearly universal feature. In adolescents, lower lumbar bone density is seen in amenorrheic teens compared with those with normal menses but is not significant when patients are controlled for body weight (21–22). Bachrach *et al.* (2) found a significant correlation between change in BMI and change in lumbar BMD in adolescent girls with AN with weight recovery, even before resumption of menses. We did not find a correlation between bone density in AN and menstrual status, E₂ levels, or duration of amenorrhea. Our data indicate that lean body mass specifically was a major determinant of change in total and lumbar BMD in girls with AN, as well as healthy controls. Moreover, change in lean body mass over the first 6 months of study was predictive of change in lumbar BMD over the subsequent 6 months, indicating that an improvement in lean body mass may predict an increase in spinal bone density in AN. Girls with a significant change in estrogen status, as demonstrated by resumption of menses, did not have greater gains in BMD compared with girls who gained weight only. In addition, change in E₂ levels over the study period did not correlate with change in BMD or bone turnover markers. These data indicate that change in nutritional status is an important predictor of change in BMD, independent of estrogen status. It is likely that not all girls who resumed menses were having ovulatory cycles, although this was not tested. Whether or not prolonged suppression of bone mineral accrual was related to lack of resumption of ovulatory menstrual cycles is not known.

Change in BMD is a slow process, and the impact of recovery on BMD may not be apparent over a relatively short time frame. Examination of indirect measures of bone turnover provides a more immediate view of bone mineral status to study the impact of weight and gonadal recovery on bone. It is important to note that in normal adolescents, both bone formation and resorption markers are at very high levels (23–25) due to rapid bone growth, mineralization, and remodeling, in contrast to relatively low levels of turnover markers in adulthood during which time only remodeling occurs. Bone turnover decreases toward adult levels during late puberty (23–25). The abnormalities in bone metabolism in adolescents with AN and their long-term impact are not completely understood. We were the first to describe low bone formation in mature adolescent girls with AN compared with CA- and BA-matched controls (3). In this report, we demonstrate that low bone turnover also occurs in immature girls with AN. To date, there are no published prospective data on bone turnover in adolescents with AN during recovery compared with healthy girls of similar

maturational stage. In contrast to no change in mean spinal BMD in AN subjects at 12 months, there were major changes in bone turnover in association with nutritional recovery. AN subjects, who had a mean 15.2% increase in BMI over the year, had significantly greater increase in OC, BSAP, DPD, and NTX compared with controls, such that by 12 months bone turnover was no longer different between the groups. These data suggest that bone turnover in subjects with AN undergoing nutritional recovery increases from abnormally suppressed levels to levels equivalent to that of healthy control girls. In contrast, levels of bone turnover markers in the healthy girls decreased as expected as they continued through puberty.

When assessing bone metabolism in growing children, levels of bone turnover markers may reflect changes in mineralization, changes in growth of bones, or both. Examination of the correlation between bone turnover markers and measurements of bone size and density by computed tomography in children found that bone resorption markers correlated negatively with bone size and volume, whereas bone formation markers correlated negatively with true bone density; however, these data are not prospective (26). These observations are consistent with the known decrease in bone turnover markers that occurs in late puberty with consolidation of bone density and completion of bone growth. Our interpretation of the prospective changes in bone turnover markers in our subjects is that they most likely reflect changes in bone mineralization rather than growth, because there were no significant differences in lumbar bone area, linear growth, or height between the groups.

Increases in bone turnover markers occurred even without recovery from AN, but increases in bone formation were greater in those AN subjects who increased BMI by 10% or more compared with those who did not. Changes in lean body mass and IGF-I levels were the strongest correlates of change in bone turnover in AN over the year of study. We have previously reported a strong correlation between IGF-I, an important bone trophic factor, and markers of bone formation, OC and BSAP, in adolescent girls with AN (3). As noted above, in this study we show that change in IGF-I is also strongly correlated with longitudinal changes in bone formation and resorption. Both markers of bone formation and resorption increased in association with a rise in IGF-I, similar to that reported in studies of adult patient populations under IGF-I administration (27–29), including adult women with AN (30). The increase in markers of bone resorption may relate to IGF-I indirectly activating osteoclast activity through stimulation of osteoblasts, as suggested by *in vitro* study (31). The normalization of suppressed bone turnover seen in our AN subjects is similar to that demonstrated in GH-deficient children under recombinant human GH therapy (32–33).

Reduced levels of androgens may potentially contribute to the low bone density seen in AN. Serum free T levels were significantly reduced at baseline in the mature AN group only, whereas serum DHEAS did not differ between the groups at either maturational stage, suggesting a relatively greater deficiency in gonadal *vs.* adrenal androgens in mature AN. These data may reflect increased androgen secretion from the ovaries in more mature healthy girls. Changes

in levels of free T in AN were correlated with change in bone formation markers over the year, suggesting that free T has an anabolic role in bone metabolism in adolescents with AN, as has been reported in normal adult women (34).

Of particular significance is that an increase in specific bone turnover markers was predictive of an increase in BMD in the AN group. An increase in BSAP in the first 6 months was correlated with a rise in lumbar BMC during the following 6 months. In addition, there was a trend toward an increase in NTX for the first 6 months to predict a rise in lumbar BMC and BMD for the subsequent 6 months in AN subjects. Over the year, an increase in NTX correlated with an improvement in all bone density measures over the same period, including lumbar BMC and BMD, lateral spine BMD, TBBMC, and TBBMD in the AN group. These data indicate that bone metabolism in AN normalizes over the course of 1 yr with nutritional recovery, and a rise in specific bone turnover markers from a previously suppressed state may be an indicator of recovery of bone mineral accrual.

Potentially important environmental factors for preservation of bone health include exercise and calcium intake, neither of which correlated with bone density or bone turnover in our subjects. However, a large proportion of both AN and healthy subjects were found to have dietary calcium and vitamin D deficiency, during a time when skeletal needs for calcium are greatest (35), and despite supplementation in many subjects. Although the study participants were not instructed to take supplements, they were educated about the importance of calcium for bone health as well as the RDA for calcium and vitamin D for adolescents at each visit. There was no improvement in intake of these nutrients during the study in either group. Our data support the findings of other studies in which a majority of adolescent girls are found to be deficient in dietary calcium intake (36, 37) and suggest that many girls may require concentrated efforts at education and dietary supplementation to meet skeletal needs during adolescence.

Our data demonstrate that osteopenia may persist in adolescent girls with AN despite recovery over 1 yr and that, in contrast to bone loss in adults with AN, poor bone mineral accrual is a critical component of the osteopenia seen in this population. These are the first data to demonstrate normalization of bone turnover markers in adolescents recovering from AN, in association with an increase in lean body mass and the nutritionally dependent bone trophic hormone IGF-I. The rise in bone turnover markers may be an early indicator of an increase in BMD in girls with AN undergoing nutritional recovery. Further investigation is required to determine whether ongoing recovery will lead to an improvement in BMD in this population.

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