Bone Metabolism in Adolescent Boys with Anorexia Nervosa

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Background: Anorexia nervosa (AN) is a condition of severe undernutrition associated with low bone mineral density (BMD) in adolescent females with this disorder. Although primarily a disease in females, AN is increasingly being recognized in males. However, there are few or no data regarding BMD, bone turnover markers or their predictors in adolescent AN boys.

Hypotheses: We hypothesized that BMD would be low in adolescent boys with AN compared with controls associated with a decrease in bone turnover markers, and that the gonadal steroids, testosterone and estradiol, and levels of IGF-I and the appetite regulatory hormones leptin, ghrelin, and peptide YY would predict BMD and bone turnover markers.

Methods: We assessed BMD using dual-energy x-ray absorptiometry and measured fasting testosterone, estradiol, IGF-I, leptin, ghrelin, and peptide YY and a bone formation (aminoterminal propeptide of type 1 procollagen) and bone resorption (N-telopeptide of type 1 collagen) marker in 17 AN boys and 17 controls 12–19 yr old.

Results: Boys with AN had lower BMD and corresponding Z-scores at the spine, hip, femoral neck, trochanter, intertrochanteric region, and whole body, compared with controls. Height-adjusted measures (lumbar bone mineral apparent density and whole body bone mineral content/height) were also lower. Bone formation and resorption markers were reduced in AN, indicating decreased bone turnover. Testosterone and lean mass predicted BMD. IGF-I was an important predictor of bone turnover markers.

Conclusion: AN boys have low BMD at multiple sites associated with decreased bone turnover markers at a time when bone mass accrual is critical for attainment of peak bone mass. **(*J Clin Endocrinol Metab* 93: 3029–3036, 2008)**

Anorexia nervosa (AN), a condition of severe undernutrition, is associated with very low bone mineral density (BMD) in adolescent girls and adult women (1–5). Low BMD in AN girls (1, 2, 4–6) is associated with decreased levels of bone turnover markers (4, 5), in contrast to adults with AN, who have an uncoupling of these markers (3). Although predominantly a disease of females, AN is increasingly recognized in males (7, 8), and yet there are no controlled studies assessing BMD and bone turnover markers in AN boys. In the one uncontrolled study published (9), which did not examine bone turnover markers, boys with the lowest BMD had the longest duration of illness, and lowest physical activity and calcium intake.

BMD assessment by dual-energy x-ray absorptiometry (DXA) has inherent flaws in that it measures areal rather than true or volumetric BMD, and underestimates BMD in shorter children and overestimates BMD in taller children (10). Given the high prevalence of short stature in AN boys (11), it is important to examine height adjusted measures such as lumbar

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Abbreviations: AN, Anorexia nervosa; BMAD, bone mineral apparent density; BMD, bone mineral density; BMI, body mass index; CV, coefficient of variation; DXA, dual-energy x-ray absorptiometry; FAI, free androgen index; NTX, N-telopeptide; PINP, aminoterminal propeptide of type 1 collagen; PYY, peptide YY; SDS, SD scores; WB BMC, whole-body bone mineral content.

spine bone mineral apparent density (BMAD) (12), reported to be the most consistent predictor of upper limb fracture risk in children (13), and whole-body bone mineral content (WB BMC) adjusted for height, which compares well with peripheral quantitative computed tomography data for bone strength (14). Of note, surrogate markers of bone formation and bone resorption provide information regarding the state of bone turnover but have not been examined in males with AN.

During adolescence, a marked increase in bone accrual occurs as a consequence of rising GH, IGF-I (15), and gonadal steroid levels. Increases in GH and IGF-I secretion induce increases in periosteal bone apposition in early to mid puberty, and rising levels of gonadal steroids cause decreases in endosteal bone resorption in later puberty. Whereas estradiol is primarily antiresorptive, testosterone has both direct bone anabolic effects and antiresorptive effects from aromatization to estradiol (16). Conditions of severe undernutrition, including AN, are associated with low IGF-I and hypothalamic amenorrhea in females (5). Although hypogonadotropic hypogonadism has not been as well characterized in males with AN, this is anticipated, given the negative energy balance state, an important predictor of impaired gonadotropin pulsatility (17, 18). Only one study has examined associations of IGF-I and testosterone with BMD in AN boys and reported no associations between these variables (9). In addition, recent data demonstrate that orexigenic and anorexigenic hormones such as ghrelin, leptin, and peptide YY (PYY) affect gonadotropin secretion (19–22) and impact bone metabolism (23–27). Ghrelin, a stomach-derived orexigenic hormone, is elevated in females with AN and has osteoblast proliferative effects (25, 26). PYY is also high in AN girls and inversely predicts bone turnover markers (28). Leptin, an adipokine that is down-regulated in females with AN, has been variably reported to be a positive (24, 29) or negative (27) regulator of bone metabolism. Low leptin in particular is seen in states of low fat mass but has not been assessed in boys with AN.

In addition to hormonal changes, adolescence is characterized by changes in body composition, and boys in particular have marked increases in lean mass, a consistent predictor of BMD (4, 5, 30), likely from biomechanical forces exerted by the pull of muscle on growing bone. Body composition in AN boys and associations with BMD have not been reported.

In this study, we examined absolute and height-adjusted measures of BMD and levels of bone turnover markers in adolescent boys with AN and controls of similar maturity as well as predictors of these measures. We hypothesized that adolescent AN boys would have lower BMD and bone turnover markers than controls, even after adjusting for stature, and that BMD would be predicted by body composition, gonadal steroids, IGF-I, and appetite-regulating hormones.

Subjects and Methods

Subject selection

Seventeen adolescent boys with AN (diagnosed by *Diagnostic and Statistical Manual of Mental Disorders*-IV criteria) (chronological age 16.0 ± 1.8 yr, bone age 16.2 ± 1.5 yr), and 17 healthy boys of comparable chronological (15.6 ± 1.9 yr) and bone age (16.0 ± 2.3 yr) were examined. All subjects were between 12 and 19 yr old. Mean duration since diagnosis was 11.9 ± 16.3 months and age at diagnosis, 15.0 ± 1.9 yr in AN. None of the controls had a present or past history of eating disorders, and all were greater than 90% ideal body weight based on the 50th percentile of body mass index (BMI) for age. Six AN boys were on selective serotonin reuptake inhibitors, three on a serotonin norepinephrine reuptake inhibitor, two on risperidone, one on olanzapine, one on valproate, and 11 on multivitamin/calcium supplements. In contrast, only two controls were on multivitamin/calcium supplements. Subjects were mostly white but did include one black and one Asian boy (both controls). For boys with AN, methods of recruitment included referrals from Eating Disorders Units in New England and the Hospital for Sick Children (Toronto, Canada). Controls were recruited through mass mailings to pediatricians, nutritionists, and psychiatrists. The study was approved by the Institutional Review Board of Partners Health Care (Boston, MA) and the Research Ethics Board of the Hospital for Sick Children. Informed assent and consent obtained from all subjects and their parents.

Experimental protocol

Subjects were admitted to the General Clinical Research Center of Massachusetts General Hospital, Boston, or the Clinical Investigation Unit of the Hospital for Sick Children. Conditions other than AN that may affect bone metabolism excluded subjects from study participation. All subjects had hematocrits greater than 30%, potassium greater than 3 mmol/liter, FSH less than 10 U/liter, and glucose greater than 50 mg/dl. At the study visit, height was measured in triplicate and averaged. Weight was measured on an electronic scale in the fasting state. BMI was calculated as the ratio of weight (in kilograms)/height (in meters)^2. Bone age was read by a single pediatric endocrinologist (31).

Fasting blood was obtained for IGF-I, testosterone, SHBG, estradiol, active ghrelin, PYY, and leptin. BMD and body composition were measured by DXA (Hologic 4500, Hologic, Waltham, MA). Z-scores were calculated using Hologic reference databases (32). To correct for body size, BMAD was calculated for the spine (10) and measures of WB BMC/height examined. The coefficient of variation (CV) for spine and WB BMD was 1.1 and 0.8% and fat and lean mass 2.1 and 1.0%, respectively. Cross-calibration of the Hologic 4500 instruments at the two sites was performed using phantoms provided by Synarc (San Francisco, CA). We measured fasting levels of one serum marker of bone formation, namely aminoterminal propeptide of type 1 collagen (PINP), and bone resorption, namely N-telopeptide (NTX).

Activity scores were derived from an exercise questionnaire validated for use in adolescents (33) and calcium and vitamin D intake assessed using a food frequency questionnaire administered by dietitians at the respective sites. Data regarding calcium and vitamin D intake were available in 16 AN boys and 13 controls.

Biochemical assessment

RIA was used to measure serum leptin (Linco Diagnostics, Inc., St. Louis, MO; sensitivity 0.5 ng/ml, CV 3.4–8.3%) and active ghrelin (Linco, St. Louis, MO; sensitivity 7.8 pg/ml, CV 7.4%), and immunoradiometric assay to measure IGF-I (detection limit 2.06 ng/ml, CV 3.9%). PYY was measured using ELISA (Millipore/Linco Research Inc., St. Charles, MO; sensitivity 1.4 pg/ml; CV 1.5%, −2.7%). RIA was used to measure testosterone (Diagnostic Products Corp., Los Angeles, CA; CV 5.1–9.8%, sensitivity 4 ng/dl) and estradiol (Diagnostic Systems Laboratories, Webster, TX; CV 6.5–8.9%, sensitivity 2.2 pg/ml) and immunoradiometric assay to measure SHBG (Diagnostic Products; CV 2.8–5.3%; sensitivity 0.04 nmol/liter). Free androgen index (FAI) was calculated as total testosterone3.47/SHBG. PINP was measured by a RIA (Orion Diagnostica Oy, Espoo, Finland; detection limit 2 ng/ml; CV 6.5–10.2%) and NTX by an enzyme immunoassay [Osteomark-Wampole Laboratories, Inverness Medical Professional Diagnostics, Princeton, NJ; detection limit 2.5 nM bone collagen equivalent, CV 4.6%]. Samples were stored at −80 C until analysis and run in duplicate.

TABLE 1. Clinical characteristics of adolescent boys with AN and healthy adolescent boys

	Controls (n = 17)	AN (n = 17)	*P*
Chronological age (yr)	15.6 ± 1.9	16.0 ± 1.8	NS
Bone age (yr)	16.0 ± 2.4	16.2 ± 1.5	NS
Testicular volume (ml)	17.8 ± 6.6	11.5 ± 6.2	0.01
Height (cm)	169.3 ± 8.8	170.7 ± 10.3	NS
Height SDS	0.05 ± 1.27	−0.12 ± 1.12	NS
Weight (kg)	61.2 ± 9.6	50.7 ± 9.7	0.003
Weight SDS	0.10 ± 0.76	−1.04 ± 0.72	<0.0001
BMI (kg/m^2)	21.3 ± 2.6	17.2 ± 1.6	<0.0001
BMI SDS	0.12 ± 0.75	−1.19 ± 0.42	<0.0001
Fat mass (kg)	10.2 ± 3.5	6.9 ± 2.2	0.002
Lean mass (kg)	50.4 ± 8.5	43.7 ± 8.4	0.03
Body fat (%)	16.3 ± 4.9	13.1 ± 3.4	0.04
Composite activity scores (h/wk)	18.1 ± 17.8	12.1 ± 9.8	NS

Mean ± SD. NS, Not significant.

Statistical analysis

Data were analyzed using the JMP program (version 4; SAS Institute Inc., Cary, NC) and are presented as mean ± SD. The Student *t* test was used to calculate differences between means. Where data were not normally distributed, logarithmic conversions were performed to approximate a normal distribution. This was required for active ghrelin. $P < 0.05$ was considered significant. Univariate and mixed model stepwise regression analyses ($P = 0.15$ for entry and $P = 0.10$ to leave the model) were used to determine predictors of hormonal and BMD measures. All correlations relate to the group as a whole, unless otherwise specified. Variables entered into the regression model were based on data from correlational analysis and known predictors of various parameters.

Results

Clinical characteristics (Table 1)

As per study design, AN boys did not differ from controls for chronological or bone age. However, in AN, duration since diagnosis correlated inversely with the difference between bone age and chronological age (r = −0.67, $P = 0.004$), such that boys with longest duration since diagnosis had the lowest bone age in relation to chronological age.

As expected, AN boys had lower weight, BMI, and fat and lean mass than controls. However, height and height SD scores (SDS) did not differ. Although AN boys were not shorter than controls, within AN boys, height SDS was associated strongly and positively with BMI SDS (r = 0.71, $P = 0.001$) and inversely with duration since diagnosis (r = −0.59, $P = 0.01$). Midparental height (genetic potential) and predicted adult height (based on current height and bone age) did not differ between groups, and neither did corresponding SDS. The difference between predicted adult height and midparental height was lower in AN than controls but did not reach significance (−0.91 ± 5.0 *vs.* 2.8 ± 9.3 cm). Similar results were observed for differences in corresponding SDS. Duration since diagnosis correlated inversely with the difference between height SDS and midparental height SDS (r = −0.62, $P = 0.008$), such that boys with longer duration of illness had lower height SDS, compared with genetic potential.

TABLE 2. Hormonal alterations in adolescent boys with AN and controls

	Controls (n = 17)	AN (n = 17)	*P*
IGF-I (ng/ml)	580 ± 238	483 ± 275	NS
Testosterone (ng/dl)	413.8 ± 223.2	186.8 ± 147.7	0.004
SHBG (nmol/liter)	43.1 ± 28.0	42.9 ± 20.8	NS
FAI	45.8 ± 31.3	21.0 ± 21.7	0.02
Estradiol (pg/ml)	18.0 ± 7.2	12.0 ± 4.6	0.01
Active ghrelin (pg/ml)	48.1 ± 29.6	58.2 ± 22.1	NS
Leptin (ng/ml)	3.5 ± 3.2	2.0 ± 0.9	NS
PYY (pg/ml)	64.3 ± 28.7	119.2 ± 57.5	0.003
Glucose (mg/dl)	85.6 ± 4.5	75.2 ± 12.1	0.009

Mean ± SD. NS, Not significant.

Compared with controls, AN boys had higher calcium (1706 ± 544 *vs.* 1172 ± 396 mg/d, $P = 0.006$) and vitamin D intake (536 ± 248 *vs.* 285 ± 202 IU/d, $P = 0.007$) from food and supplements. Overall, more AN than controls met the dietary reference index for calcium (87 *vs.* 33%, $P = 0.005$) and vitamin D intake (69 *vs.* 15%, $P = 0.005$).

Hormonal parameters and their predictors

The groups did not differ for Tanner stage (4.4 ± 1.0 in controls *vs.* 4.1 ± 1.4 in AN). However, AN boys had lower testicular volume than controls (Table 1) and lower testosterone, FAI, and estradiol (Table 2). PYY was higher in AN, whereas ghrelin and leptin did not differ between groups. Predictors of hormonal parameters are reported in Table 3. Associations of testosterone with various parameters were similar to those of FAI, and only associations with testosterone are reported. BMI and fat and lean mass correlated positively with gonadal steroids and inversely with PYY. We observed positive associations of BMI and fat mass with leptin and inverse associations of lean mass with ghrelin. We found no associations of BMI, fat mass, or leptin with IGF-I, although PYY correlated inversely with IGF-I. Active ghrelin and PYY correlated inversely with testicular volume and gonadal steroids. Within AN, duration since diagnosis predicted ghrelin (r = 0.62, $P = 0.01$) but not leptin or PYY. Strong positive associations were observed between gonadal steroids and IGF-I. On regression modeling (BMI, ghrelin, and leptin added to the model), BMI and leptin predicted testosterone (56% of variability). When PYY was added to the model, predictors of testosterone were BMI and PYY (69% of variability explained).

Bone density and markers of bone turnover and their predictors

Boys with AN had lower BMD and BMD Z-scores at the spine, hip, and whole body than controls (Table 4 and Fig. 1). In addition to the total hip, measures of femoral neck, trochanteric and intertrochanteric BMD, and corresponding Z-scores were lower in AN. Height-adjusted measures of bone density including lumbar BMAD and WB BMC/height Z-scores were also lower. Lumbar and WB BMD in AN was 90 and 95% of that in controls, whereas hip, femoral neck, and trochanteric and intertrochanteric BMD were 86, 88, 85, and 85% of control values,

TABLE 3. Predictors of hormonal parameters for all subjects

	BMI	Fat mass	Lean mass	Testicular volume	Testo-sterone	Estradiol	IGF-I	Ghrelin[a]	Leptin
Testosterone	0.66[b]	0.45[c]	0.74[d]	0.75[d]		0.63[b]	0.41[c]	−0.37[c]	0.05
Estradiol	0.49[e]	0.23	0.59[e]	0.56[e]	0.63[b]		0.32	−0.52[e]	−0.11
IGF-I	0.09	0.16	0.23	0.45[c]	0.41[c]	0.32		−0.14	0.01
Ghrelin*	−0.22	0.03	−0.47[c]	−0.46[c]	−0.37[c]	−0.52[e]	−0.14		0.28
Leptin	0.48[e]	0.70[d]	−0.06	−0.15	0.05	−0.11	0.01	0.28	
PYY	−0.45[c]	−0.37[c]	−0.50[e]	−0.60[e]	−0.62[e]	−0.42[c]	−0.50[c]	0.28	−0.01

[a] Log conversions performed to approximate a normal distribution.

[b] $P < 0.001$.

[c] $P < 0.05$.

[d] $P < 0.0001$.

[e] $P < 0.01$.

respectively. More AN boys than controls had BMD Z-scores less than −1 at the hip and its subregions (Table 4). The difference between groups was even more marked when Z-scores were calculated based on bone age (data not reported). Levels of PINP and NTX were significantly lower in AN than controls (Fig. 2).

BMD measures were positively associated with BMI, lean mass, and gonadal status but not fat mass (Table 5). PYY was an inverse predictor of BMD. In AN boys, duration since diagnosis, activity scores, and calcium or vitamin D intake did not predict BMD. On regression modeling to account for confounding variables, with bone age, BMI, lean mass, total testosterone, estradiol, and PYY entered into the model (Table 6), we observed that testosterone independently predicted lumbar BMD, whereas BMI was the single most significant predictor of hip and femoral neck BMD. Lean mass and bone age predicted WB BMC/height measures, and lean mass was also an independent predictor of lumbar and WB BMD. BMD Z-scores at the corresponding sites were predicted by similar variables and are not reported. Figure 3 shows associations of BMD with independent predictors assessed using regression modeling.

IGF-I was a strong predictor of PINP (r = 0.53, $P = 0.007$) and a weaker predictor of NTX (r = 0.39, $P = 0.06$). On regression modeling, IGF-I was the sole independent predictor of NTX (accounting for 15% of the variability), whereas IGF-I and lean mass accounted for 44% of the variability in PINP. Other hormonal and body composition parameters did not predict bone turnover markers. PINP correlated positively with NTX (r = 0.63, $P = 0.001$).

Discussion

We report for the first time lower BMD and height adjusted measures of BMD, including corresponding Z-scores, at multiple sites in adolescent boys with AN, compared with controls. In addition, we report for the first time that lower BMD in AN boys is associated with a reduced bone turnover state as indicated by surrogate markers of bone formation and resorption. In addition to low BMI and lean mass, low testosterone is an important predictor of low BMD.

TABLE 4. Bone density and bone turnover markers in adolescent boys with AN and healthy adolescent boys

	Controls (n = 17)	AN (n = 17)	*P*
Lumbar spine BMD Z-score	−0.14 ± 0.81	−1.09 ± 0.98	0.004
Lumbar BMD Z-score less than −1	23.5%	50.0%	NS
Lumbar spine BMAD Z-score	−0.07 ± 0.16	−0.32 ± 0.33	0.008
Lumbar spine BMAD Z-score less than −1	0%	5.9%	NS
Total hip BMD Z-score	−0.06 ± 0.81	−1.23 ± 0.99	0.0008
Total hip BMD Z-score less than −1	23.5%	58.8%	0.04
Femoral neck BMD Z-score	−0.23 ± 0.94	−1.17 ± 0.82	0.004
Femoral neck BMD Z-score less than −1	17.7%	64.7%	0.007
Trochanteric BMD Z-score	−0.08 ± 0.64	−1.22 ± 0.80	<0.0001
Trochanteric BMD Z-score less than −1	5.9%	70.6%	0.0001
Intertrochanteric BMD Z-score	0.07 ± 0.88	−1.14 ± 1.18	0.002
Intertrochanteric BMD Z-score less than −1	17.7%	58.8%	0.02
Whole-body BMD Z-score	−0.27 ± 0.76	−1.09 ± 1.14	0.02
Whole-body BMD Z-score less than −1	23.5%	43.8%	NS
WB BMC/height Z-score	0.13 ± 0.75	−0.80 ± 0.77	0.001
WB BMC/height Z-score less than −1	0%	29.4%	0.02
PINP (ng/ml)	259.3 ± 112.8	166.3 ± 99.7	0.04
NTX/cr (nmol BCE)	320.8 ± 205.5	166.6 ± 134.7	0.04

Mean ± SD. cr, Creatinine; BCE, bone collagen equivalent; NS, not significant.

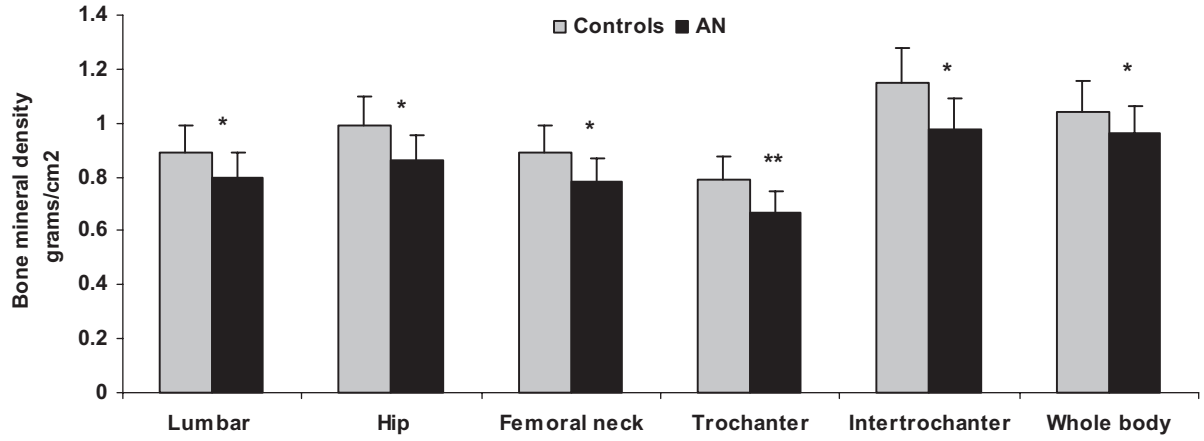

FIG. 1. Bone density in adolescent boys with anorexia nervosa and controls. Bone density at the lumbar spine, total hip, and its subregions (femoral neck, trochanter, intertrochanteric region) and the whole body was significantly lower in boys with AN than in controls. *, $P < 0.05$; **, $P < 0.01$.

There are no controlled studies thus far that have reported on bone metabolism in boys with AN, and the only uncontrolled study showed that a longer duration of illness, lower levels of physical activity, and lower calcium intake predicted lower spine BMD (9). Unlike our study, this study did not compare AN boys against controls of comparable maturity or control for height.

We observed markedly lower BMD at the spine, hip, femoral neck, trochanter, intertrochanteric region, and whole body in boys with AN, compared with controls of similar maturity, and these differences persisted after adjustments for bone age and for stature, such that lumbar BMAD and WB BMC/height were lower in AN than controls. These data are concerning for low BMD and possibly a decreased rate of bone mass accrual during adolescence, a period characterized by marked increases in bone accrual (34), toward achievement of peak bone mass (35). This also raises concerns regarding bone health and fracture risk in later life. We previously demonstrated that girls with AN have low BMD, and spine BMD is affected preferentially to the hip in females (4). In contrast, in AN boys, the hip and its subregions are affected at least as severely as the spine, based on the proportion of boys whose Z-scores at these sites are less than −1.

Body composition (4, 6, 36), the gonadal steroids, and IGF-I (37–39) are key determinants of BMD, and in AN girls, the appetite-regulating peptides are also important predictors (40). We therefore examined these parameters in our subjects. As expected, AN boys had lower fat mass, lean mass, testosterone, and estradiol and higher PYY. Unlike in AN girls, ghrelin was not significantly higher in AN boys (41), and IGF-I did not differ between groups. IGF-I levels change rapidly with weight changes, and our subjects were in active nutritional rehabilitation programs. Although BMI was very low in AN boys, an increasing trend from nutritional intervention may explain why IGF-I did not differ between groups. Leptin levels are markedly reduced in AN girls (to 27% of values in controls) (42) and were reduced to a lesser extent in AN boys in our study (to 57% of control values). Because leptin is primarily secreted by adipocytes, relatively lesser decreases in leptin in boys, compared with girls, likely reflect lesser decreases in fat mass in AN boys (32% reduction, compared with controls) *vs.* AN girls (49% reduction) (43) and differences in body composition in pubertal boys *vs.* girls.

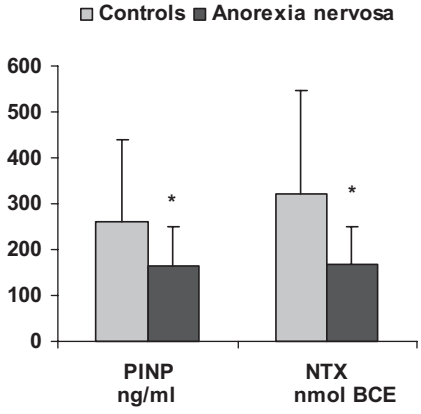

FIG. 2. Bone turnover markers in adolescent boys with AN and controls. Levels of PINP (a bone formation marker) and NTX (a bone resorption marker) were significantly lower in boys with AN than controls, suggestive of a reduced state of bone turnover. *, $P < 0.05$. BCE, Bone collagen equivalent.

Important predictors of BMD were testosterone, lean mass, BMI, and bone age. The gonadal steroids have positive effects on trabecular bone (39), and consistent with this, testosterone was an important and independent predictor of spine BMD. However, testosterone also has important effects on cortical bone, and significant involvement of sites of cortical bone in our study may also be a consequence of lower testosterone levels. In addition, we have demonstrated in AN girls that lean mass and BMI are important predictors of BMD at sites of primarily cortical bone, such as the hip and whole body (4), and our findings in AN boys are consistent with these reports. In contrast to our studies in AN girls, however, the appetite-regulating peptides were not significant predictors of BMD. We did observe inverse associations of PYY with BMD, but PYY did not remain an independent predictor of BMD on regression modeling.

Consistent with the other study in AN boys (9), we found no associations of IGF-I with bone mass. However, in contrast to findings in this study, we also found no associations of BMD with duration since diagnosis, calcium intake, or activity scores. In fact, in our study, the majority of AN boys were on supplements, and more AN than controls met the DRI for calcium and vitamin D intake. This likely reflects the growing awareness on the part

TABLE 5. Body composition and hormonal predictors of bone density measures for all subjects

	BMI	Fat mass	Lean mass	Testicular volume	Testosterone	FAI	Estradiol	PYY
Lumbar BMD	0.59[a]	0.22	0.79[b]	0.67[a]	0.72[b]	0.69[b]	0.44[c]	−0.46[c]
Lumbar BMAD	0.54[d]	0.20	0.52[d]	0.65[a]	0.62[a]	0.64[a]	0.29	−0.34
Hip BMD	0.64[b]	0.24	0.68[b]	0.52[d]	0.58[d]	0.53[d]	0.40[c]	−0.31
Femoral neck BMD	0.57[a]	0.15	0.58[a]	0.52[d]	0.54[d]	0.50[d]	0.41[c]	−0.36[c]
Whole-body BMD	0.45[d]	0.16	0.68[b]	0.66[a]	0.47[c]	0.48[d]	0.32	−0.29
WB BMC/height	0.62[a]	0.27	0.81[b]	0.53[d]	0.60[a]	0.56[d]	0.45[c]	−0.37

[a] $P < 0.001$.

[b] $P < 0.0001$.

[c] $P < 0.05$.

[d] $P < 0.01$.

of providers of risk for low BMD with low weight and recommendations to take daily supplements. In addition, in adults, increased physical activity and a negative energy balance state have been related to low BMD and increased fracture risk (44, 45), prompting efforts to curtail exercise in AN. Interestingly, studies suggest that excessive physical activity is more likely in males than females with AN (46, 47). The lack of difference in activity scores between subjects and controls may reflect concerns on the part of eating disorder providers regarding associations of very excessive activity with low BMD and recommendations to reduce activity. Of note, the issue of physical activity in AN remains controversial, and one study in AN girls reported that exercise was a positive predictor of BMD (48).

For the first time, we demonstrate that bone turnover markers (both bone formation and bone resorption markers) are reduced in AN boys, compared with controls, who are in a state of increased bone turnover (49). These data are consistent with our reported data in girls with AN (50). The sole significant predictor of both bone turnover markers was IGF-I, consistent with studies in AN girls (36) and the known bone trophic effects of IGF-I (51).

Adjustments for height when assessing BMD are especially important in conditions associated with short stature (10, 12), and AN, a condition of chronic undernutrition, has been associated with statural deficits. Although we did adjust for height in this study, we did not find any significant differences in stature in AN boys, compared with controls. This is contrast to older studies, which did report short stature in AN (11, 52, 53). It is possible that AN is being diagnosed earlier now, and statural deficits are being prevented by prompt implementation of therapy. Of note, AN boys did demonstrate a positive association of height SDS with BMI SDS, and an inverse association with duration since diagnosis, such that boys with the lowest BMI SDS and longest duration since diagnosis had the lowest height SDS. These data indicate that greater severity and length of illness are associated with shorter stature, consistent with older studies (11, 52, 53).

Limitations of this study relate to use of DXA in assessing BMD in general and more specifically in populations with changing body composition. We have discussed the impact of differences in height on DXA-reported measures of BMD, and corrections to adjust for extremes of height. However, these corrections increase the accuracy of projection techniques to only a limited extent (54). In addition, changes in body composition and fat distribution can cause an over- or underestimation of BMD by DXA (55, 56), and significant alterations in body composition are characteristic of AN. We previously demonstrated, using dual-energy quantitative computed tomography, that marrow fat is increased (57), and sc fat decreased in AN (58).

TABLE 6. Predictors of bone density (mixed model stepwise regression modeling: bone age, BMI, lean mass, total testosterone, estradiol)

	Parameter estimate	F ratio	*P*	Cumulative variability explained by model
Lumbar spine BMD (g/cm^2)				
Lean mass	4.5×10^{-3}	3.7	<0.0001	0.64
Testosterone	1.9×10^{-4}	4.7	0.06	0.69
Bone age	2.0×10^{-2}	3.7	0.07	0.74
Lumbar spine BMAD (g/cm^3)				
Testosterone	5.1×10^{-5}	19.2	0.0002	0.46
Hip BMD (g/cm^2)				
BMI	0.037	30.2	<0.0001	0.57
Femoral neck BMD (g/cm^2)				
BMI	0.028	16.7	0.0005	0.42
Whole-body BMD (g/cm^2)				
Lean mass	6.4×10^{-3}	14.1	0.001	0.38
WB BMC/height (g/cm)				
Lean mass	0.14	13.7	<0.0001	0.64
Bone age	0.45	4.6	0.04	0.70

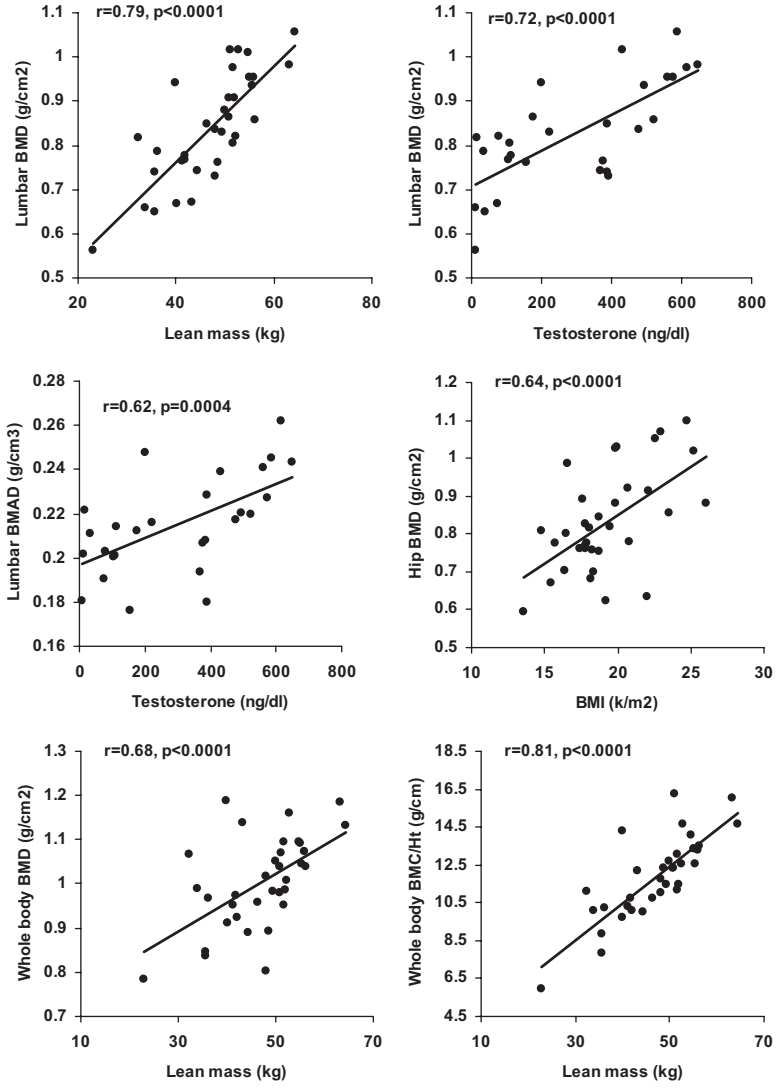

FIG. 3. Predictors of bone density in adolescent boys with AN and in controls. Lean body mass and testosterone levels were independent predictors of lumbar bone density measures, whereas BMI was the most significant and independent predictor of bone density at the hip. Lean mass predicted whole-body BMD and bone mineral content adjusted for height.

These alterations could lead to an underreporting of BMD by DXA. However, we have also shown that BMD measured by dual-energy computed tomography is lower in AN women than controls (59), indicating that low BMD reported by DXA in AN is real. Recent studies also indicated changes in bone microarchitecture in women with AN, including a decrease in trabecular number and cortical thickness (60), suggesting that DXA measurements of low BMD are likely valid. Other modalities for assessing BMD are under investigation in AN, but until these are validated, DXA remains the tool available to assess BMD in this condition.

We thus demonstrate lower BMD in adolescent AN boys than in controls, even after adjusting for height, raising concerns with regard to achievement of optimal peak bone mass. Boys with AN are hypogonadal, and lower BMD is predicted by lower testosterone, BMI, and lean mass. Because BMI is an important predictor of low BMD, emphasizing weight recovery is critical.

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