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Serum Osteoprotegerin in Adolescent Girls with Anorexia Nervosa

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Low bone mineral density (BMD) in adolescents with anorexia nervosa (AN) is associated with a low bone turnover state. Osteoprotegerin (OPG), a cytokine that acts as a decoy receptor for receptor activator of nuclear factor-κB ligand , decreases bone resorption by inhibiting differentiation of osteoclast precursors and activation of mature osteoclasts, and by stimulating osteoclast apoptosis. We compared OPG levels in 43 adolescent girls with AN with 38 controls and examined bone density, bone turnover, and hormonal parameters. Girls with AN had lower fat mass, lean body mass, lumbar BMD z-scores, and lumbar bone mineral apparent density than controls. OPG levels were higher in girls with AN than in controls (44.5 ± 22.5 pg/ml *vs.* 34.5 ± 12.7 pg/ml, *P* = 0.02). Osteocalcin, deoxypyridinoline, estradiol, free testosterone, IGF-I, and leptin were lower in AN than in healthy adolescents. OPG values correlated negatively with body mass index (r = −0.27, *P* = 0.02), percent fat mass (r = −0.35, *P* = 0.0002), leptin (r = −0.28, *P* = 0.02), lumbar BMD z-scores (r = −0.25, *P* = 0.03), and lumbar bone mineral apparent density (r = −0.26, *P* = 0.03). In conclusion, adolescent girls with AN have higher serum OPG values than controls. OPG values correlate negatively with markers of nutritional status and lumbar bone density z-scores and may be a compensatory response to the bone loss seen in this population. (*J Clin Endocrinol Metab* 88: 3816–3822, 2003)

OSTEOPROTEGERIN (OPG) AND RANKL [receptor activator of nuclear factor-κB (RANK) ligand] have been identified and recognized as important regulators of bone resorption (reviewed in Refs. 1 and 2). RANK (the receptor for RANKL) is a type I transmembrane protein of the TNF receptor superfamily and is expressed on osteoclast precursors, whereas RANKL, a cell-bound polypeptide, is expressed on the cell surface of osteoblasts. When RANK recognizes and binds to RANKL through cell-cell interactions, osteoclast precursors differentiate into mature osteoclasts, resulting in increased bone resorption. RANKL also activates mature osteoclasts and inhibits osteoclast apoptosis. OPG is also a member of the TNF receptor superfamily, but unlike other members of this superfamily, it lacks a transmembrane domain. Acting as a soluble decoy receptor preventing the binding of RANKL to RANK, OPG inhibits osteoclast differentiation and activation, and, stimulates osteoclast apoptosis. Studies have demonstrated OPG and RANKL to be regulated by a number of hormones. Estrogen increases OPG levels (3), whereas PTH, testosterone, IGF-I, and cortisol (3–5) decrease levels of OPG.

Anorexia nervosa (AN) is associated with extensive and prevalent bone loss such that osteopenia has been reported in 92%, and osteoporosis in as many as 38% of adult women with this disorder (6, 7). In adult women with AN, an uncoupling of bone turnover with decreased bone formation and increased bone resorption is associated with low bone density (8). Decreased bone mineral density (BMD) has been identified in adolescent girls with AN at a time of maximal bone accrual in normal girls (9–12). In girls with AN, we have shown a decrease in markers of both bone formation and bone resorption, suggesting a state of decreased bone turnover (11, 12). Therefore, underlying defects in bone metabolism may differ in AN depending on the age of the patients. No studies to our knowledge have investigated serum OPG levels in AN or determined the role of OPG in the bone loss seen in adolescents with this disorder.

Critical hormonal factors that regulate OPG are disrupted in AN due to profound undernutrition and are either undersecreted (IGF-I, estrogen, and testosterone) or oversecreted (cortisol). Low bone density has been demonstrated to correlate with markers of nutritional status including body mass index (BMI) (9, 13), lean body mass (10–12, 14), and fat mass (10). Very low levels of IGF-I occur in AN as a consequence of undernutrition, and markers of bone formation correlate with levels of IGF-I (11, 15). In addition, hypothalamic hypogonadism results in low levels of estrogen and free testosterone (11, 12, 16). Hypercortisolemia has been reported in adult women with AN (17–19), although this has not been consistently observed in adolescents with this disorder (11, 12, 15). These hormonal alterations likely contribute to the low bone mass that is characteristic of AN. How-

Abbreviations: AN, Anorexia nervosa; BA, bone age; BCE, bone collagen equivalent; BMD, bone mineral density; BMI, body mass index; BSAP, bone-specific alkaline phosphatase; CA, chronological age; CV, coefficient of variation; DHEAS, dehydroepiandrosterone sulfate; DPD, deoxypyridinoline; IGFBP, IGF binding protein; LBMAD, lumbar bone mineral apparent density; LBMD, lumbar BMD; NTX, *N*-telopeptide; OC, osteocalcin; OPG, osteoprotegerin; RANK, receptor activator of nuclear factor-κB; RANKL, RANK ligand.

ever, the effects of these hormonal changes on serum OPG levels in AN are yet to be determined.

To better understand underlying mechanisms of low bone mass in adolescents with AN, we 1) determined levels of OPG in adolescent girls with AN with and without osteopenia and in healthy controls of similar age and pubertal stage; and 2) investigated the relationship of OPG levels to bone density and markers of bone turnover.

Subjects and Methods

Subjects

We studied 43 Caucasian girls with AN and 38 healthy Caucasian girls. Bone density, hormonal data, and biochemical data, with the exception of OPG values, from 18 AN and 19 controls have been reported previously (12). Girls with AN met the Diagnostic and Statistical Manual-IV (revised) criteria for AN and ranged in age from 12.8–18.7 yr. Eight girls were premenarchal, and 35 girls had secondary amenorrhea. Girls with a history of intake of estrogen or other medications known to affect bone metabolism within 3 months of study initiation were excluded from the study. No girl had any condition other than AN known to affect bone metabolism. Girls with AN were recruited through mass mailings to physicians, nutritionists, and therapists in the Boston area, and through referrals from Eating Disorder centers in New England. The control population ranged in age from 12.1–18.0 yr. Six were premenarchal, and 32 were postmenarchal. None of the controls were receiving medications or had medical conditions known to affect bone metabolism. Controls were recruited through postings in offices of primary care providers and advertisements in Massachusetts General Hospital and affiliated clinics. The study was approved by the institutional review board of our hospital, and informed consent was obtained from all subjects and their parents.

Experimental protocol

Subjects were screened at an initial visit to the General Clinical Research Center of Massachusetts General Hospital, and eligibility was determined based on history, physical examination, and laboratory reports, which included potassium, glucose, LH, FSH, TSH, prolactin, and a hematocrit. Eligible patients underwent a repeat medical and menstrual history and a physical examination at the time of the study visit. Healthy postmenarchal girls were studied in the early follicular phase (d 1–7) of their menstrual cycles to control for effects of changing levels of gonadal steroids across a menstrual cycle. Tanner staging for pubic hair was performed for all subjects. In addition, blood was drawn in the fasting state, and a second morning 2-h urine sample (for assessing markers of bone resorption) was collected. Subjects also brought in a 24-h urine collection for estimation of urinary free cortisol, and a completed 4-d food diary, as per instructions provided at the time of the screen. Each subject had a bone density test, and bone age (BA) determination was made.

Methods

Anthropometric measurements and pubertal staging. Subjects were weighed in a hospital gown on an electronic scale after an overnight fast. Heights were obtained in triplicate on a single stadiometer and averaged. BMI percentiles were obtained from Centers for Disease Control and Prevention 2000 charts (20). Subjects with AN and controls were matched for pubertal stage and for BA to control for changes in bone density and in bone markers that occur through puberty. BA was determined using the methods of Greulich and Pyle (21). BA is highly correlated with pubertal maturation and is delayed in conditions of undernutrition and hypogonadism. Girls with a BA less than 15 yr were determined to be immature for pubertal stage, whereas those with a BA 15 yr or older were determined to be mature for pubertal stage.

Tanner breast stage is often difficult to determine in this population because of associated breast atrophy. Many adolescents with AN go through puberty at a normal age and therefore have had full pubertal maturation before onset of AN. In such cases, although breast tissue appears immature, BA may be mature due to the past exposure to sex steroids and normal nutrition. This is a different physiological state than an adolescent who develops AN early in development and has both immature breast tissue and delayed BA. We therefore used pubic hair rather than breast development to determine pubertal (Tanner) stage. Girls in Tanner stages I, II, and III were classified as being in early puberty, whereas those in Tanner stages IV and V were classified as being in late puberty.

Laboratory analyses. OPG levels were measured by Amgen, Inc. (Thousand Oaks, CA), using their in-house human endogenous OPG ELISA. This assay uses mouse monoclonal antibody for capture and a rabbit polyclonal antibody for detection. Reported OPG values include monomeric and dimeric forms of OPG, and OPG bound to RANKL. The assay has a minimum detection limit of 2.34 pg/ml. Between test coefficients of variation (CV) using control sera are 0.91% (60.00 pg/ml), 1.57% (40.00 pg/ml), 0.69% (15.00 pg/ml), 1.82% (7.50 pg/ml), 2.7% (3.75 pg/ml), and 2.64% (2.34 pg/ml).

Serum osteocalcin (OC) was measured by an immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA). This assay had a minimum detection limit of 0.5 μg/liter and an intraassay CV of 3.5–5.2%. ELISAs were used to determine levels of bone-specific alkaline phosphatase (BSAP) (Quidel, Inc., Mountain View, CA, sensitivity 0.7 U/liter, CV 3.9–5.8%), *N*-telopeptide (NTX) (Ostex International, Inc., Seattle, WA; limit of detection 20 nmol bone collagen equivalent (BCE), CV 5–19%) and deoxypyridinoline (DPD) (Quidel, Inc.; limit of detection 1.1 nmol/liter, CV 4.3–8.4%)

Estradiol levels were measured by ultrasensitive RIA (Diagnostic Systems Laboratories, Inc., Webster, TX). The detection limit of this assay is 2.2 pg/ml with a CV of 6.5–8.9%. We also used RIA to measure free testosterone (DiaSorin, Inc., Stillwater, MN; detection limit 0.18 pg/ml, intraassay CV 3.7–6.2%), serum dehydroepiandrosterone sulfate (DHEAS) (Coated Tube RIA, DiaSorin, Inc.; detection limit 1.1 μg/dl and intraassay CV 3.8–5.3%) and serum leptin (Linco Diagnostics, St. Louis, MO; sensitivity 0.5 ng/ml and intraassay CV 3.4–8.3%). Serum IGF-I was measured using an acid-alcohol extraction and RIA kit (Nichols Institute Diagnostics; detection limit 0.06 ng/ml, intraassay CV 2.4–3.0%). We measured serum IGF binding protein-3 (IGFBP-3) using an immunoradiometric assay (Coated Tube IRMA, Diasorin, Inc.) with a detection limit of 0.5 ng/ml and an intraassay CV of 1.8–3.9%. Free testosterone, IGFBP-3, and DHEAS values were available in 21 AN and 22 controls.

Serum was frozen and stored at −80 C, and all assays were run in duplicate.

Bone density measurements

Lumbar BMDs (LBMDs) were measured using a QDR-4500 dual energy x-ray absorptiometer (Hologic, Inc., Waltham, MA). The SD for lumbar spine BMD is 0.01 g/cm^2 and does not vary with bone density. z-Scores were calculated from the applet of Bachrach, Hastie, and Narasimhan (http://www-stat-class.stanford.edu/pediatric-bones/) for chronological age (CA) and BA. Although this applet is based on measurements using a Hologic QDR 1000 bone densitometer, studies have demonstrated very minimal differences in bone density measurements at the lumbar spine using a Hologic QDR 4500 machine *vs.* a Hologic QDR 1000 machine (r^2 = 0.985 and 0.990, with mean BMD differences of 0.68% and 0.003 g/cm^2) (22, 23). Bone mineral apparent density (BMAD), an estimate of volumetric bone density, was calculated using the formula described by Katzman *et al.* (24).

Statistical analysis

All data are expressed as mean ± SD. Student's *t* test was used to calculate differences between means in girls with AN and controls. ANOVA was first used to perform a three group analysis comparing girls with AN and low bone density (z-score < -1) with girls with AN whose BMD z-score was at least −1 and controls. When ANOVA was significant, we performed the Tukey-Kramer's test for comparisons within all groups. Correlational and multiple regression analyses were used to determine predictors of OPG levels and also to determine if OPG predicted bone density and levels of bone turnover markers.

Results

Demographic, body composition, and bone density data

Adolescent girls with AN and healthy controls had similar CA (15.9 ± 1.6 *vs.* 15.2 ± 1.8 yr, *P* value not significant) and BA (15.5 ± 1.6 *vs.* 15.7 ± 1.8 yr, *P* value not significant). Twenty-three girls (13 AN and 10 controls) were pubertally immature (based on BA), whereas 58 (30 AN and 28 controls) had a mature BA. Twenty girls (12 AN and 8 controls) were early pubertal (Tanner stages I–III), whereas 61 (31 AN and 30 controls) were late pubertal (Tanner stages IV and V). As expected, weight, BMI, lean body mass, and percent fat mass were all significantly lower in girls with AN than in controls (weight: 45.2 ± 5.2 *vs.* 59.0 ± 10.3 kg, $P < 0.0001$; BMI: 16.6 ± 1.1 *vs.* 22.5 ± 3.5 kg/m^2, $P < 0.0001$; lean body mass: 35.5 ± 4.0 *vs.* 38.7 ± 5.8 kg, $P = 0.005$, % fat mass 18.2 ± 5.1 *vs.* 31.1 ± 5.3%, $P < 0.0001$). Compared with controls, girls with AN had significantly lower LBMD z-scores for CA (−0.94 ± 0.98 *vs.* −0.23 ± 0.49 g/cm^2, $P = 0.0001$) and BA (−0.95 ± 0.85 *vs.* −0.33 ± 0.47 g/cm^2, $P = 0.0002$), and lumbar BMAD (LBMAD) was also significantly lower in AN than in controls (0.12 ± 0.01 *vs.* 0.13 ± 0.01 g/cm^3, $P = 0.001$). The mean duration of amenorrhea in postmenarchal girls with AN was 11.7 ± 10.2 months, whereas the mean duration since diagnosis of AN was 9.9 ± 10.8 months.

Hormonal and bone turnover data

Significantly lower levels of IGF-I and leptin were seen in the AN group compared with controls (IGF-I: 260 ± 135 *vs.* 463 ± 183 μg/liter, $P < 0.0001$; leptin: 3.2 ± 2.3 *vs.* 13.7 ± 6.0 μg/liter, $P < 0.0001$). Girls with AN had lower levels of estradiol than healthy adolescents (54.7 ± 21.3 *vs.* 70.9 ± 23.0 pmol/liter, $P = 0.002$). Value of free testosterone, DHEAS, and urinary free cortisol did not differ in the two groups. Levels of OC and BSAP, markers of bone formation, and of DPD and NTX, markers of bone resorption, were lower in girls with AN than in controls (OC: 39.6 ± 23.9 *vs.* 55.9 ± 32.5 μg/liter, $P = 0.01$; BSAP: 30.8 ± 19.3 *vs.* 42.2 ± 30.2 U/liter, $P = 0.05$; DPD: 9.5 ± 4.0 *vs.* 14.2 ± 10.9 nmol/mmol creatinine, $P = 0.01$; NTX: 113 ± 67 *vs.* 172 ± 148 nmol BCE/mmol creatinine, $P = 0.02$).

In the whole group, leptin levels correlated positively with lumbar spine BMD z-scores ($r = 0.42$, $P = 0.0002$) and LBMAD ($r = 0.40$, $P = 0.0003$). Levels of IGF-I also correlated positively with lumbar spine BMD z-scores ($r = 0.32$, $P = 0.005$) and LBMAD ($r = 0.23$, $P = 0.04$). When multiple regression analyses were performed with leptin, fat mass, and lean body mass, significant predictors of lumbar spine BMD z-scores were fat mass (accounting for 23% of the variability) and lean body mass (accounting for 7% of the variability), whereas fat mass was the only significant predictor of lumbar spine BMAD (accounting for 19% of the variability). A positive correlation was observed between levels of OC and lumbar spine BMD z-scores ($r = 0.24$, $P = 0.03$). No correlations were noted between bone density z-scores or BMAD and other markers of bone turnover, IGFBP-3, free testosterone, DHEAS, or estradiol.

Serum OPG levels

Serum OPG levels were significantly higher in adolescents with AN than in healthy adolescents (44.5 ± 22.5 pg/ml *vs.* 34.5 ± 12.7 pg/ml, $P = 0.02$) (Fig. 1).

In a three-group analysis comparing OPG values in adolescent girls with AN and low bone density z-scores ($z < -1$), girls with AN and normal bone density z-scores ($z \geq -1$) and controls (Table 1), OPG values were the highest in girls with AN whose bone density z-scores were less than −1, with the values being significantly higher than in controls. Bone density measures, as expected, were significantly lower in this group compared with the other two groups. This group also had lower weight, BMI, lean body mass, and fat mass than the control group. In addition, the ratio of BA/CA and levels of OC, IGF-I, leptin, and estradiol were significantly lower in this group than in controls. Girls with AN whose BMD z-scores were at least −1 had OPG values intermediate between those in girls with AN and low bone density z-scores and controls, but the value of OPG was not significantly different from the other two groups. This group of girls with AN also had lower mean weight, BMI, fat mass, ratio of BA/CA, and levels of IGF-I, leptin, and estradiol than the control group. Bone density measures in this group were not different from those in healthy adolescents. The only difference in nutritional parameters that could account for differences in bone density measures in the two groups of adolescent girls with AN was the lower lean body mass in the group with the lower bone density z-scores. Free testosterone, DHEAS, and urinary free cortisol were not different in the three groups.

In the whole group, a significant correlation was observed between OPG and BMI ($r = -0.27$, $P = 0.02$), and OPG and percent fat mass ($r = -0.35$, $P = 0.002$), such that girls with the lowest BMI and lowest fat mass had the highest levels of OPG. No correlation existed between levels of OPG and lean body mass. An inverse correlation was also observed between leptin levels and serum OPG ($r = -0.28$, $P = 0.02$). In a stepwise regression model including BMI, percent fat mass, and leptin, percent fat mass was the only significant predictor of serum OPG values, contributing to 13% of the variation in serum OPG values. No correlation was observed between OPG and other hormonal parameters (IGF-I, IGFBP-3, estradiol, free testosterone, or urinary free cortisol).

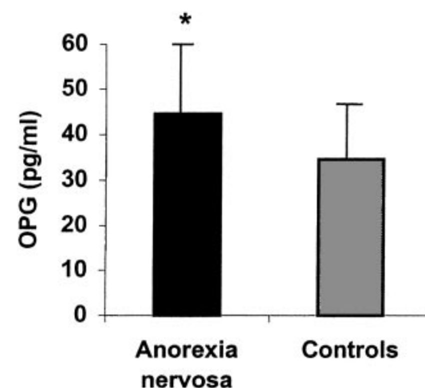

FIG. 1. OPG values in girls with AN and BA-matched controls. Girls with AN had significantly higher OPG values than controls (44.5 ± 22.5 *vs.* 34.5 ± 12.7, $P = 0.02$).

TABLE 1. Comparison of demographic, bone density, bone turnover, and hormonal characteristics in girls with AN and lumbar BMD z-scores < −1 and ≥ −1 and controls

	AN with lumbar BMD z ≥ −1 (n = 20)	AN with lumbar BMD z < −1 (n = 23)	Healthy controls (n = 38)	ANOVA *P*
Age (yr)	15.5 ± 1.6	16.2 ± 1.6	15.2 ± 1.8	ns
BA (yr)	15.2 ± 1.6	15.7 ± 1.6	15.7 ± 1.8	ns
BA/CA	0.98 ± 0.05[a]	0.97 ± 0.04[a]	1.03 ± 0.05	<0.0001
Weight (kg)	47.7 ± 6.0[a]	43.0 ± 3.3[a,b]	59.0 ± 10.3	<0.0001
BMI (kg/m^2)	17.0 ± 1.1[a]	16.2 ± 0.9[a]	22.5 ± 3.5	<0.0001
Lean body mass (kg)	37.0 ± 4.5	34.1 ± 3.0[a]	38.7 ± 5.8	0.003
% Fat mass	18.6 ± 5.5[a]	17.8 ± 4.7[a]	31.1 ± 5.3	<0.0001
Lumbar (AP) BMD (g/cm^2)	0.97 ± 0.08	0.83 ± 0.07[a,b]	0.96 ± 011	<0.0001
Lumbar (AP) BMD z-score (CA)	−0.22 ± 0.47	−1.63 ± 0.61[a,b]	−0.23 ± 0.49	<0.0001
Lumbar (AP) BMD z-score (BA)	−0.15 ± 0.52	−1.46 ± 0.67[a,b]	−0.33 ± 0.47	<0.0001
Lumbar (AP) BMAD	0.13 ± 0.01	0.11 ± 0.01[a,b]	0.13 ± 0.01	<0.0001
OPG (pg/ml)	41.6 ± 21.8	46.9 ± 23.2[a]	34.5 ± 12.7	0.04
Osteocalcin (μg/liter)	42.2 ± 26.6	37.5 ± 21.9[a]	55.9 ± 32.5	0.04
Bone-specific AP (U/liter)	31.4 ± 17.7	30.3 ± 20.8	42.2 ± 30.2	ns
N-telopeptide (nmol BCE/mmol cr)	114 ± 53	112 ± 78	172 ± 148	0.08
Deoxypyridinoline (nmol/mmol cr)	9.3 ± 4.2	9.7 ± 4.0	14.2 ± 10.9	0.05
IGF-I (μg/liter)	277 ± 160[a]	247 ± 114[a]	463 ± 183	<0.0001
Leptin (μg/liter)	3.3 ± 2.1[a]	3.1 ± 2.4[a]	13.7 ± 6.0	<0.0001
Estradiol (pmol/liter)	53.0 ± 20.3[a]	56.4 ± 22.0[a]	70.9 ± 23.0	0.007
Free testosterone (pmol/liter)[c]	5.5 ± 1.0	6.9 ± 2.7	7.9 ± 3.4	ns
DHEAS (mmol/liter)[c]	3.9 ± 2.3	4.3 ± 1.2	4.9 ± 2.9	ns
Urinary free cortisol (μg/d)	43.1 ± 29.4	36.5 ± 20.2	41.4 ± 22.1	ns

Mean ± SD.
ns, Not significant; AP, alkaline phosphatase; cr, creatinine.
[a] Significantly different from controls; [b] significantly different from AN with lumbar BMD z ≥ −1.
[c] Data available for 21 AN and 22 controls.

A negative correlation was noted between LBMD and OPG levels (r = −0.22, *P* = 0.05), LBMD z-scores (for CA) and OPG (r = −0.25, *P* = 0.03), and LBMAD and OPG (r = −0.26, *P* = 0.03) (Fig. 2, A and B). When the two outliers were excluded, the correlation coefficient was −0.33, *P* = 0.004 for LBMD, and −0.29, with a *P* value of 0.01 for LBMD z-scores and BMAD. Conversely, markers of bone turnover did not correlate with serum OPG values.

No correlation was observed between OPG and CA, and no difference was noted between OPG levels in girls with immature BA (BA < 15 yr, n = 23) *vs.* girls of mature BA (BA ≥ 15 yr, n = 58). However, a negative correlation was observed between OPG and the ratio of BA to CA (BA/CA) (r = −0.24, *P* = 0.03) (Fig. 3). With exclusion of the same two outliers described above, the correlation coefficient was −0.31, and the *P* value 0.008. BA/CA correlated positively with LBMD z-scores (r = 0.38, *P* = 0.0005). Because delayed puberty and lower BA/CA would be expected in girls with lower bone density z-scores, we performed multiple regression analysis including lumbar bone density z-scores and the ratio of BA/CA, and the latter was no longer a significant predictor of OPG values. No differences were observed between OPG values in girls in early *vs.* late puberty (by pubic hair staging).

In healthy controls taken alone, OPG correlated negatively with percent body fat (r = −0.34, *P* = 0.04) and there was a trend toward a negative correlation with LBMAD (r = −0.30, *P* = 0.06). A positive trend was observed between values of IGFBP-3 and OPG (r = 0.43, *P* = 0.07) and a stronger correlation between free testosterone values and OPG (r = 0.50, *P* = 0.03). On stepwise regression, free testosterone values contributed to 25% of the variability in OPG values, whereas

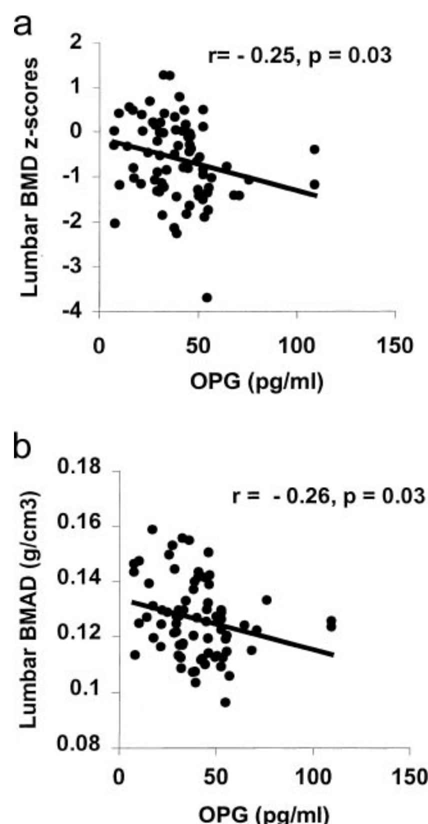

FIG. 2. Correlation between OPG values and LBMD z-scores and LBMAD. A negative correlation was noted between OPG values and LBMD z-scores (r = −0.25, *P* = 0.03) and with LBMAD (r = −0.26, *P* = 0.03).

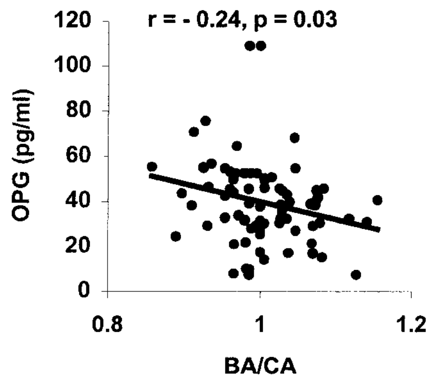

FIG. 3. Correlation between OPG values and the ratio of BA to CA (BA/CA). A negative correlation was observed between OPG values and BA/CA (r = −0.24, P = 0.03).

IGFBP-3 contributed to 5% of this variability. These correlations were not observed in girls with AN.

Discussion

Our data indicate higher levels of serum OPG in adolescent girls with AN than in BA-matched controls, and a negative correlation between OPG and markers of nutritional status, including BMI, fat mass, and leptin. In particular, girls with AN and low bone density had higher OPG values than did controls, whereas OPG values in girls with AN and normal bone density z-scores did not differ from that in controls. OPG levels correlated inversely with lumbar spine bone density z-scores. Estrogen and urinary free cortisol did not predict OPG levels, whereas a positive correlation was observed between free testosterone and OPG in healthy adolescents. We found that, in normal adolescents, OPG levels were comparable in early and late puberty.

OPG levels have been demonstrated to be higher in osteoporotic postmenopausal women than in nonosteoporotic postmenopausal women (25), though not consistently (26). Levels have been shown to be higher in menopausal women than in younger women despite lower estrogen levels in postmenopausal women. It has been suggested that this may be a compensatory response to bone loss, with the higher OPG levels subsequently reducing osteoclast differentiation and activation and increasing osteoclast apoptosis. Consistent with this is the finding that administration of a single 3-mg/kg sc dose of OPG decreased levels of NTX by 80% in postmenopauasal women (27). Adolescent girls with AN have been demonstrated to have decreased bone turnover with a decrease in markers of both bone formation and bone resorption (12). The decrease in markers of bone resorption may reflect the suppressive effects of higher OPG levels on osteoclastic activity in this population. If this were so, however, one would expect low levels of bone resorption markers in postmenopausal women with osteoporosis, and this has not been reported. In our study, a negative correlation was noted between lumbar spine BMD z-scores and OPG, and LBMAD and OPG, suggesting that higher OPG values in girls with lower BMD may indeed be a compensatory phenomenon. However, like Ueland *et al.* (28), we did not find a significant association between OPG and markers of bone resorption.

We noted higher OPG levels in girls with lower BMI, fat mass, and leptin levels. In particular, fat mass was an important predictor of serum OPG values. This may again reflect a compensatory response because girls with AN have significant decreases in body fat (10, 11), and the greatest decreases in fat mass are seen in girls with the lowest weights and BMIs. Girls with the lowest BMIs are also more likely to suffer bone loss (9, 10). However, a decrease in lean body mass is a more important predictor of bone loss than is a decrease in BMI or fat mass (10, 11), and no correlations were observed between lean body mass and serum OPG values in this study. This finding was unexpected in that girls with AN and low bone density z-scores differed from girls with AN and normal bone density z-scores only in their mean lean body mass, which was significantly lower than that in controls, whereas lean body mass in girls with AN and normal bone density z-scores was comparable to that in controls. These data suggest a role for fat mass possibly independent of bone loss in regulating levels of serum OPG, or that a larger number of patients may be necessary to detect significances.

The inverse relationship noted between levels of OPG and leptin is also of interest in the light of recent data suggesting an independent role for leptin in predicting BMD in postmenopausal women (29). These authors demonstrated a positive correlation between leptin and femoral neck BMD, and a negative correlation between leptin and bone resorption markers, suggesting that leptin levels may inhibit the increased bone resorption that is characteristic of postmenopausal osteoporosis. In this study, we likewise observed a positive correlation between leptin and bone density z-scores and BMAD, though these significances were lost on multiple regression analyses including fat mass and lean body mass. Leptin has also been shown to stimulate human osteoblastic cell proliferation and mineralization (30) and to inhibit osteoclast generation from human peripheral blood mononuclear cells (31). However, these studies demonstrated a local increase in OPG levels following administration of leptin to cell cultures (30, 31). We did not observe any correlations between leptin levels and markers of bone turnover, and we noted a negative correlation between leptin and OPG levels in our study.

Serum OPG is regulated by many hormones including estrogen, testosterone, PTH, cortisol, GH, and IGF-I. In this study, lower levels of estrogen and IGF-I and a trend toward lower levels of free testosterone were observed in girls with AN than in matched controls, whereas urinary free cortisol values were not different in the two groups. Estradiol values were lower in both groups of girls with AN compared with controls, irrespective of bone density status. Because estradiol increases OPG production by osteoblasts, decreased estradiol secretion would be expected to result in decreased production of OPG. Conversely, in this study, girls with AN had low estradiol and high OPG levels, and we found no correlation between OPG and estradiol values, similar to a report by Khosla *et al.* (32). However, unlike Khosla *et al.* (32), we did find a positive correlation between free testosterone values and OPG in our healthy controls. One possible reason for the lack of an association between estradiol and OPG values is that we measured circulating rather than local OPG.

In addition, our healthy subjects were studied in the early follicular phase of their menstrual cycles when estradiol values are at a physiological nadir. These nadir values do not reflect estradiol values at other stages of the menstrual cycle, and it cannot be determined from this study if OPG values are affected by net estradiol effects over a menstrual cycle as opposed to nadir values measured in this study.

High cortisol values have been reported in adult women with AN (17–19), and glucocorticoid excess has been demonstrated to result in increased serum OPG (28). In our study, as in other studies examining adolescent girls with AN (12, 15), urinary free cortisol values were not higher in AN girls compared with controls, and we did not find an association between cortisol values and OPG. Therefore, hypercortisolemia is unlikely to be an important mechanism of bone loss in adolescents with AN. We noted a weak association between IGFBP-3 levels and OPG values in controls, but not in anorectic subjects. No relationship was observed between serum IGF-I and OPG values, similar to findings of Ueland *et al.* (28). Data on the effects of administration of GH and IGF-I on OPG production are conflicting, with reports of both an increase in local OPG production following recombinant human GH administration (33), and a decrease in OPG values following administration of recombinant human IGF-I (4).

In our subjects, a negative correlation was noted between the ratio of BA to CA and OPG values, suggesting a role for pubertal maturation on OPG levels. More mature adolescents, however, have higher levels of estrogen and would therefore be expected to have higher levels of OPG. Alternatively, bone turnover is greater in the early adolescent years and decreases in late adolescence (34), and the higher OPG values at a younger BA may reflect a compensatory response to the extensive bone remodeling occurring in younger adolescents. The lack of this extensive remodeling in both mature and immature adolescents with AN (characterized by a low bone turnover state), may explain why this negative correlation with the ratio of BA to CA was not observed in subjects with AN taken alone. Changes in body fat distribution with pubertal maturation may also contribute to lower serum OPG values with increasing pubertal maturity. However, in this study, leptin levels and percent fat mass correlated positively with the ratio of BA to CA. In addition, BA/CA was not a significant predictor of OPG values on multiple regression analysis including LBMD z-scores, suggesting that the association of pubertal maturation and OPG values might merely reflect the delayed skeletal maturation expected in girls with AN with lower bone density z-scores.

A confounder to this study is that we measured circulating OPG rather than OPG produced locally in bone, and serum levels may not accurately reflect local production of OPG in bone. It would also be useful to measure levels of RANKL and the ratio of OPG to RANKL. Currently available serum assays for RANKL, however, have not been verified to represent bone turnover.

We thus demonstrate higher OPG values in adolescent girls with AN and low bone density z-scores than in controls, possibly subsequent to a compensatory response to the lower bone density seen in this population. We also show a negative correlation of OPG with markers of nutritional status, especially with fat mass, and with maturity, as determined by the ratio of BA to CA. In healthy adolescents, OPG correlates positively with testosterone values. Studies with larger numbers of adolescents are necessary to confirm a decrease in OPG levels with increasing pubertal maturity.

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