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Hormone Predictors of Abnormal Bone Microarchitecture In Women with Anorexia Nervosa

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Abstract

Osteopenia is a complication of anorexia nervosa (AN) associated with a two- to three-fold increase in fractures. Nutritional deficits and hormonal abnormalities are thought to mediate AN-induced bone loss. Alterations in bone microarchitecture may explain fracture risk independent of bone mineral density (BMD). Advances in CT imaging now allow for noninvasive evaluation of trabecular microstructure at peripheral sites *in vivo*. Few data are available regarding bone microarchitecture in AN. We therefore performed a cross-sectional study of 23 women (12 with AN and 11 healthy controls) to determine hormonal predictors of trabecular bone microarchitecture. Outcome measures included bone microarchitectural parameters at the ultradistal radius by flat panel Volume CT (fpVCT); BMD at the PA and lateral spine, total hip, femoral neck and ultradistal radius by dual energy X-ray absorptiometry (DXA); and IGF-I, leptin, estradiol, testosterone and free testosterone levels. Bone microarchitectural measures, including apparent (app.) bone volume fraction, app. trabecular thickness, and app. trabecular number, were reduced ($p < 0.03$) and app. trabecular spacing was increased ($p = 0.02$) in AN vs. controls. Decreased structural integrity at the ultradistal radius was associated with decreased BMD at all sites ($p = 0.05$) except for total hip. IGF-I, leptin, testosterone and free testosterone levels predicted bone microarchitecture. All associations between both IGF-I and leptin levels and bone microarchitectural parameters, and most associations between androgen levels and microarchitecture remained significant after controlling for body mass index. We concluded that bone microarchitecture is abnormal in women with AN. Endogenous IGF-I, leptin and androgen levels predict bone microarchitecture independent of BMI.

Keywords

Anorexia nervosa; IGF-I; leptin; androgens; flat-panel volume CT

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Introduction

Anorexia nervosa (AN) is a psychiatric disease affecting 0.3% of the female population, and is characterized by self-induced chronic starvation and associated with severe bone loss and increased risk of fractures [1]. In a study of outpatient women with AN, mean age 24 years, 92% had osteopenia and 38% had osteoporosis by dual energy X-ray absorptiometry (DXA) [2]. A two- to threefold increase in fracture risk has been reported in this population [3,4]. The mechanism for AN-induced bone loss is multifactorial, and a low formation, high resorption state has been identified [5]. Low endogenous estrogen and testosterone secretion and decreased levels of nutritionally-dependent hormones, including IGF-I and leptin, have been implicated [6]. IGF-I, a nutritionally regulated anabolic hormone that is low in states of chronic starvation, is thought to be a particularly important mediator of AN-induced bone loss. Low levels of IGF-I in women with AN are associated with decreased levels of bone formation [5]. Administration of rhIGF-I increases markers of bone formation and, in estrogen-treated patients, is the only therapy that has been shown in a randomized, placebo-controlled study to improve bone mineral density (BMD) in women with AN [7]. In contrast, studies of estrogen-progestin therapy, effective in treating postmenopausal osteoporosis, have failed to show efficacy in preventing or improving AN-associated bone loss [7,8]. Serum androgen levels have been demonstrated to be low in women with AN [9] and short-term administration of low-dose testosterone increases procollagen type I C-terminal peptide (PICP), a marker of bone formation [10].

As in other populations at risk for osteoporosis, BMD has been the primary method used to evaluate skeletal integrity in women with AN in both clinical and research settings. However, it is increasingly clear that other measures of bone quality, particularly parameters of microarchitecture, are significant independent predictors of fracture risk in other populations [11–14] and therefore may be important in AN. Newer imaging technologies, including ultra-high resolution flat panel based volume CT (fpVCT), allow for noninvasive visualization of bone microstructure [15,16]. There is limited information about bone microarchitecture, and to our knowledge, there are no studies investigating the role of hormones in modulating microarchitectural parameters in AN. This study investigates the relationship between structural integrity of bone utilizing fpVCT and endocrine abnormalities in AN. Specifically, we hypothesized that IGF-I and other nutritionally-dependent hormones would predict parameters of bone microstructure.

Subjects and Methods

Protocol

23 women aged 18–45 years, including 12 women with AN and 11 HC of similar age, were studied. All women with AN were ambulatory and fulfilled all Diagnostic and Statistical Manual IV (DSM-IV) criteria for AN, including weight <85% of ideal body weight (7). Exclusion criteria for AN and HC included pregnancy, diabetes mellitus, thyroid, cardiac, liver or renal disease, or medications known to affect bone metabolism, including estrogens, progestins, androgens, bisphosphonates or recombinant parathyroid hormone. In addition, exclusion criteria for HC included history of disordered eating or amenorrhea. Subjects were referred to the study by eating disorder providers in the New England area or recruited through advertisements. The study was approved by the Partners Health Care Institutional Review Board, and written, informed consent was obtained from all subjects. Study visits took place at the Clinical Research Center at Massachusetts General Hospital. History, physical exam, and serum samples were obtained. Research dietitians measured metabolic weight and height and calculated body mass index (kg/m^2). Body composition and BMD at the AP spine (L1–L4), lateral spine (L2–L4), total hip, femoral neck and ultradistal radius were determined by DXA (Hologic QDR 4500, Waltham, MA).

Measurement of bone microarchitecture

Bone microarchitecture at the nondominant ultradistal radius, a site of predominantly trabecular bone previously shown to predict vertebral fractures [17], was assessed using ultra-high resolution flat panel based volume CT (fpVCT) (Siemens, Forchheim, Germany). The fpVCT prototype consists of a CT gantry with a bore diameter of 40 cm integrated with a modified X-ray tube and a 2-D digital flat-panel detector system. The imaging was conducted at 100kV, 30mA, with pulsed X-ray source in the 2x2 binning mode (voxel size $0.2 \times 0.2 \times 0.2 \text{ mm}^3$). A 50% X-ray duty cycle was used. Scan time was 20 seconds. The radiation dose of a distal radius fpVCT scan was 0.027 mSv. Trabecular structure parameters obtained by fpVCT were calculated using MicroView software (GE Healthcare, Waukesha, WI). For each distal radius, a 3-dimensional region of interest (ROI) was defined within the ultradistal radius to cover a maximum area of trabecular bone without including any cortical bone within the ROI. To improve reproducibility, all ROIs were placed by one observer. The observer was blinded to the patient status (AN vs. HC). Trabecular bone was then segmented from marrow with the individual threshold defined by the auto-threshold function of MicroView.

Apparent (app.) measures of trabecular structure were calculated, including app. trabecular bone volume (BV) fraction [app. BV/app. trabecular volume (TV), %], app. trabecular number (app. TbN, mm^{-3}) defined as the inverse of the mean spacing, app. trabecular thickness (app. TbTh, mm) and separation (app. TbSp, mm), derived from app. BV/TV and app. TbN using standard methods from histomorphometry (*i.e.* $\text{TbTh} = (\text{BV}/\text{TV})/\text{TbN}$ and $\text{TbSp} = (1 - \text{BV}/\text{TV})/\text{TbN}$). These parameters are defined as “apparent” because the spatial resolution is lower than that required for standard bone histomorphometry [18].

Biochemical Analysis

Serum IGF-I levels were measured using an Immulite 2000 automated immunoanalyzer (Diagnostic Products Corporation, Inc. [DPC], Los Angeles, CA), by a solid-phase enzyme-labeled chemiluminescent immunometric assay, with an intra-assay coefficient of variation (CV) of 2.3 – 3.9 and an analytical sensitivity of 20 ng/mL. Leptin levels were measured using a radioimmunoassay (RIA) kit from LINCO Research, a division of Millipore Inc. (St. Charles, MO). The intra-assay CV was 8.3 – 3.4%, and the sensitivity was 0.5 ng/mL. Serum testosterone was measured by RIA kit (DPC, Inc.) with a minimum detection limit of 2 ng/dL and an intra-assay CV of 4.1–10.5%. Sex hormone binding globulin (SHBG) was measured by immunoradiometric assay (DPC, Inc.), with a minimum detection limit of 0.5 nmol/L and an intra-assay CV of 2.8 – 5.3%. Free testosterone was calculated from total testosterone and SHBG by an equation based on the laws of mass action, which has been validated in comparison to free testosterone by equilibrium dialysis in women [19]. Estradiol levels were measured using a Chemiluminescent Microparticle Immunoassay kit from Architect (Abbott Laboratories, Abbott Park, IL), with a within-run CV of 1.5–6.4% for concentrations of 45–192 pg/mL and a functional sensitivity = 14 pg/mL.

Data Analysis

The means and standard error of the means (SEM) of clinical characteristics, hormone levels, BMD, and bone microstructure parameters were calculated for the AN and HC groups. The significance of differences between groups was calculated using the Student's *t* test at the 5% significance level. Linear regression analyses between trabecular structure parameters and BMD measurements at the different sites, and between trabecular structure, DXA and hormone levels were performed, and Pearson correlation coefficients are reported. Multivariate least-square analyses were constructed to control for BMI. Variables were log-transformed before being entered into the regression analyses. Statistical analysis was performed using JMP software (SAS Institute, Cary, NC).

Results

Patient Characteristics

Baseline clinical characteristics are described in Table 1. Women with AN and HC were of similar age. As expected, women with AN had lower weight, percent of ideal body weight (IBW), body mass index (BMI), percent fat mass, and lean body mass.

Bone Mineral Density and Trabecular Microarchitecture

Differences in BMD and trabecular microarchitecture between the groups are shown in Figures 1 and 2. Women with AN had lower mean BMD Z-scores than HC at all sites. Mean app. BV/TV, app. TbTh, and TbN were significantly lower and TbSp was higher in women with AN compared to HC. Table 2 summarizes the relationship between trabecular microarchitecture at the ultradistal radius as measured by fpVCT and bone mineral density by DXA. Decreased structural integrity at the radius was significantly associated with lower BMD at all sites except for total hip.

Relationship Between Bone Parameters and Hormones

Associations between bone parameters and hormones are shown in Table 3. IGF-I and leptin levels predicted greater structural integrity by all microarchitectural parameters measured. After controlling for BMI, the associations between both IGF-I and leptin and all of these measures were statistically significant. Testosterone levels were positively associated with bone volume fraction and trabecular thickness and inversely associated with trabecular spacing. The association with bone volume fraction remained significant after controlling for BMI. Free testosterone was positively associated with bone volume fraction and trabecular thickness. These associations remained significant after controlling for BMI. Estradiol levels were not significantly associated with microarchitectural parameters, though they were associated with BMD at several sites before, but not after, controlling for BMI.

Discussion

Hormonal abnormalities associated with severe undernutrition are important in the pathogenesis of bone loss in AN. However, they have not previously been examined in relation to trabecular microarchitecture, an important determinant of fracture risk. In contrast to postmenopausal osteoporosis where estrogen is the key factor, AN-mediated bone loss is driven by chronic starvation. We show that nutritionally-mediated hormones, including IGF-I and leptin, and androgen levels predict bone microstructure in women with AN.

Novel noninvasive imaging technology allowing for visualization of trabecular microarchitecture in humans introduces a new paradigm for evaluating bone quality. Emerging data indicate that bone microarchitecture offers important information about bone fragility [11–14]. Several studies have demonstrated increased fracture risk associated with abnormal bone microarchitecture and normal BMD, suggesting that microstructure may be a more sensitive measure than BMD in assessing such risk [20]. Parameters of bone microarchitecture are also abnormal in postmenopausal and corticosteroid-induced osteoporosis [21,22] and are associated with increased fracture risk independent of BMD [23]. A recent study of 82 postmenopausal women found that trabecular microstructure was a better predictor of recent spinal fracture than BMD [24]. Current treatments for osteoporosis, including bisphosphonates and parathyroid hormone [25–27], have been shown to preserve or improve bone microarchitecture, a mechanism that may contribute to the reduced fracture risk in patients prescribed these medications.

IGF-I is exquisitely sensitive to nutrient intake and has potent autocrine and paracrine effects on bone growth and remodeling. Experiments in cultured rat calvariae have shown that IGF-I stimulates DNA and collagen synthesis while reducing collagen degradation [28,29]. *In vitro* and *in vivo* research in animal models indicates that IGF-I is a critical factor in promoting normal longitudinal bone growth [30–32]. IGF-I is anabolic to bone, associated with bone formation and increased BMD, and is predictive of fracture risk [33–35]. AN is characterized by low levels of IGF-I and correspondingly reduced markers of bone formation [5,36,37]. Recombinant hIGF-I increases bone formation and is the only medical therapy to date shown in a randomized, placebo-controlled study to improve BMD in estrogen-treated women with AN [5,7]. A population-based study of 205 women and 269 men found that IGF-I levels were positively associated with trabecular thickness and negatively associated with trabecular number in young men, but not in older men or in women [38]. In contrast, in our study of women with AN, we demonstrate a clear relationship between IGF-I levels and favorable trabecular microarchitecture at the ultradistal radius, independent of BMI. This may be due to the importance of IGF-I deficiency in the pathogenesis of bone loss in our population. A prospective study comparing bone microarchitecture in women with AN receiving rhIGF-I to those on placebo will be important to establish causality.

Serum levels of leptin, an adipocyte-derived anorexigenic hormone, are low in women with AN, likely representing a physiologic adaptation to starvation as levels normalize with weight recovery [39,40]. Although leptin is known to modulate bone turnover through complex central and peripheral effects, the overall influence of leptin on bone is unclear and may differ based on skeletal site [41]. Increased BMD in obesity [42], low BMD in underweight populations [2,43], and an association between BMD and BMI [44] argue for a protective effect of leptin on bone in humans. However, mouse models of leptin deficiency demonstrate increased markers of bone formation and bone mass despite hypogonadism, and central administration of leptin reduces bone mass in leptin-deficient and wild type mice [45]. In contrast, *in vitro* and *in vivo* animal data suggest that peripheral leptin is anabolic to bone by promoting osteoblast proliferation and inhibiting osteoclastogenesis [46–48]. In our study, leptin remained a significant positive predictor of bone microarchitecture after controlling for BMI.

Gonadal steroids, specifically testosterone and estradiol are both important determinants of BMD. Testosterone and estradiol levels were associated with bone microarchitecture in healthy older men and women, but not in young men and women aged 20–39 years [38]. Hypogonadal men have been shown to have decreased trabecular networks and fraction of bone volume at the distal tibia compared to eugonadal men as assessed by μ MRI [49]. A study of ten hypogonadal men showed that with testosterone replacement, parameters of bone microarchitecture using this same technique improved [50]. A prospective observational study of early postmenopausal women reported that those receiving estrogen maintained or improved their bone microarchitecture at the distal tibia by μ MRI over 12 to 24 months, while those who were not on estrogen therapy demonstrated deterioration in microarchitecture [51]. In AN, both total and free testosterone [9] are low, and low-dose transdermal testosterone administration increases surrogate markers of bone metabolism in this population [10], although effects on bone mass or microarchitecture are not known. Two randomized, placebo-controlled studies have shown that estrogen administration is not effective at increasing BMD in women with AN [7,52]. In the current study, we report that testosterone predicts bone volume fraction and free testosterone predicts bone volume fraction and trabecular thickness, independent of BMI. In contrast, we did not find significant associations between estradiol levels and parameters of bone microstructure.

There is limited research on trabecular microarchitecture in women with AN-induced bone loss, with only two published studies, neither of which examined endocrine predictors. Milos *et al.* found decreased BV/TV, decreased TbN, and increased TbSp but no difference in TbTh

at the distal radius by three-dimensional peripheral quantitative computer tomography in a group of 36 women with AN compared to 30 healthy women [53]. Galusca et al. reported decreased TbN and increased TbSp but no difference in BV/TV or TbTh at the distal radius and tibia in women with a greater than two year history of AN; there were no differences in trabecular microarchitecture between those with a more recent diagnosis of AN and healthy controls; these parameters were associated with disease duration [43]. In children with AN who should be accruing bone mass, we recently reported altered trabecular microarchitecture despite normal BMD, indicating that changes in microarchitecture may precede BMD abnormalities in this population [54].

Importantly, we found that trabecular microstructure at the ultradistal radius predicts BMD at multiple skeletal sites, including the spine, the region most severely affected in AN. Using this peripheral site rich in trabecular bone and previously shown to predict vertebral fractures [17] as the focus of microarchitecture assessment and measure of response to therapy is an important area of future investigation.

In conclusion, we demonstrate that nutritionally-regulated IGF-I, leptin and androgens, anabolic hormones deficient in women with AN, are correlates of bone microarchitecture in this population. In addition, we report abnormal trabecular microstructure in women with AN. Microarchitecture at the ultradistal radius assessed using noninvasive high-resolution CT technology correlates with BMD at multiple sites including the spine, the most severely affected skeletal site in women with AN. This technique may provide additional information about bone fragility and risk of fracture. Because this is a cross-sectional study, causality cannot be established. Prospective randomized controlled trials investigating the effect of hormone administration on trabecular bone microarchitecture will be important.

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Figure 1.

Bone mineral density. Women with AN (black) had significantly lower Z-scores at the PA spine (-1.68 ± 0.28 vs. -0.08 ± 0.33), lateral spine (-1.64 ± 0.26 vs. 0.50 ± 0.38), ultradistal radius (-0.72 ± 0.18 vs. 0.77 ± 0.33), total hip (-1.14 ± 0.29 vs. 0.32 ± 0.36), and femoral neck (-1.18 ± 0.28 vs. 0.28 ± 0.39), compared to controls (grey) (* $p=0.006$).

Figure 2.

Trabecular structure. Women with AN (black) had lower A) BV/TV (0.34 ± 0.02 vs. $0.43 \pm 0.01\%$), B) TbTh (0.28 ± 0.01 vs. 0.31 ± 0.01 mm), and C) TbN (1.19 ± 0.05 vs. 1.38 ± 0.05 mm⁻³), and higher D) TbSp (0.58 ± 0.05 vs. 0.43 ± 0.02 mm), compared to controls (grey) ($p=0.02$). BV/TV, apparent bone volume fraction; TbTh, apparent trabecular thickness; TbN, apparent trabecular number; TbSp, apparent trabecular spacing.

TABLE 1

Clinical and biochemical characteristics

	HC	AN	<i>p</i> -value
Age (yrs)	27.0±1.8	28.8±1.7	NS
Weight (kg)	65.3±2.6	49.6±1.6	<0.001
Percent of IBW (%)	103.6±2.5	77.6±1.8	<0.001
Body mass index (kg/m ²)	23.8±0.7	17.6±0.4	<0.001
Percent fat mass (%)	32.3±1.4	17.7±1.2	<0.001
Lean body mass (kg)	42.8±1.7	37.9±1.0	0.02
Age at menarche (yrs)	12.7±0.6	13.7±0.5	NS
Age of AN onset (yrs)	-	23.0±1.9	-
Illness duration (months)	-	39.1±7.2	-

Data presented as mean±SEM. *p*-values indicated significance of difference between HC and AN.

TABLE 2

Bivariate correlations between trabecular microarchitecture at the ultradistal radius and BMD at all sites

	app. BV/TV (%)	app. TbTh (mm)	app. TbN (mm ⁻³)	TbSp (mm)
AP spine BMD (g/cm ²)	0.59**	0.47*	0.55**	-0.45**
Lateral spine BMD (g/cm ²)	0.57**	0.46*	0.53**	-0.53**
Ultradistal radius BMD (g/cm ²)	0.54**	0.45*	0.50*	-0.50*
Total hip BMD (g/cm ²)	0.36 [†]	0.33	0.29	-0.31
Femoral neck BMD (g/cm ²)	0.61**	0.44*	0.58**	-0.57**

[†]
 $p < 0.10$,*
 $p = 0.05$,**
 $p < 0.01$

TABLE 3

Relationship between bone parameters and hormones

	IGF-1	Leptin	Estradiol	Testosterone	Free Testosterone
Bone volume fraction (app. BV/TV, %)	0.55 ^{**a}	0.72 ^{**a}	0.25	0.55 ^{*a}	0.43 ^{*a}
Trabecular thickness (app. TbTh, mm)	0.42 ^{*a}	0.56 ^{**a}	0.19	0.67 ^{**}	0.52 ^{*a}
Trabecular number (app. TbN, mm ⁻³)	0.50 ^{*a}	0.61 ^{**a}	0.26	0.32	0.23
Trabecular spacing (TbSp, mm)	-0.56 ^{**a}	-0.65 ^{**a}	-0.21	-0.44 [*]	-0.37
AP spine BMD (g/cm ²)	0.23	0.69 ^{**}	0.57 [*]	0.27	0.13
Lateral spine BMD (g/cm ²)	0.02	0.72 ^{**a}	0.66 ^{**}	0.29	0.11
Ultradistal radius BMD (g/cm ²)	0.17	0.65 ^{**}	0.48 [*]	0.40 [†]	0.07
Total hip BMD (g/cm ²)	0.1	0.36	0.43 [†]	0.33	0.15
Femoral neck BMD (g/cm ²)	0.36 ^a	0.70 ^{**a}	0.49 [*]	0.44 [*]	0.13

[†] $p < 0.10$,^{*} $p = 0.05$,^{**} $p < 0.01$,^a $p < 0.05$ after controlling for BMI