Oxytocin and Its Relationship to Body Composition, Bone Mineral Density, and Hip Geometry Across the Weight Spectrum

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Context: Oxytocin (OXT), an anorexigenic hypothalamic hormone anabolic to bone, may reflect energy availability. Basal serum OXT levels are lower in anorexia nervosa (AN, state of energy deficit) than healthy controls (HC) and negatively associated with spine bone mineral density (BMD). Reports are conflicting regarding OXT levels in overweight/obesity (OB, state of energy excess). Relationships between OXT and BMD in OB and hip geometry across the weight spectrum are unknown.

Objective: To determine whether overnight serum OXT levels are (1) elevated in OB and (2) associated with body composition, BMD, and hip geometry across the weight spectrum.

Design: Cross-sectional.

Setting: Clinical research center.

Participants: Fifty-nine women, ages 18 to 45 years: amenorrheic AN (N = 16), eumenorrheic HC (N = 24), eumenorrheic OB (N = 19).

Main Outcome Measures: Serum sampled every 20 minutes from 8 PM to 8 AM and pooled for integrated overnight OXT levels. Body composition, BMD, and hip structural analysis measured by dual x-ray absorptiometry.

Results: OXT levels were lowest in AN, higher in HC, and highest in OB ($P \leq 0.02$). There were positive associations between OXT and (1) body mass index ($P = 0.0004$); (2) total, visceral, and subcutaneous fat ($P \leq 0.0002$); (3) spine and hip BMD Z-scores ($P \leq 0.01$); and (4) favorable hip geometry, namely buckling ratio ($P \leq 0.05$). In a subset analysis of HC and OB, relationships between OXT and body composition, but not bone parameters, remained significant.

Conclusions: These data suggest OXT is a marker of energy availability and may be a mediator of bone density, structure, and strength. OXT pathways may provide targets for obesity and osteoporosis treatment. (***J Clin Endocrinol Metab*** **102: 2814–2824, 2017)**

Oxytocin (OXT) is a hypothalamic hormone synthesized in the supraoptic and paraventricular nuclei and secreted centrally, as well as peripherally via the posterior pituitary. Well-recognized for its effects on parturition and lactation, OXT has recently been implicated in energy regulation and bone homeostasis in

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Abbreviations: 25(OH)D, 25-hydroxyvitamin D; AN, anorexia nervosa; BMD, bone mineral density; BMI, body mass index; CV, coefficient of variation; DXA, dual x-ray absorptiometry; FS, femoral shaft; HC, healthy control; HSA, hip structural analysis; IBW, ideal body weight; IGF-1, insulinlike growth factor-1; IT, intertrochanteric region; NN, narrow neck; OB, overweight/obese; OXT, oxytocin; PA, posteroanterior; PTH, parathyroid hormone; SAT, subcutaneous fat; VAT, visceral fat.

preclinical studies; however, few studies have focused on this important area in the human.

OXT is a key regulator of energy balance in animal models through its effects on food intake, energy expenditure, and fat and muscle metabolism. OXT and OXT receptor null mice develop obesity, increased abdominal fat, and decreased muscle mass independent of food intake (1, 2). This may be because lack of OXT signaling indicates an energy deficit, and energy expenditure is decreased as a compensatory mechanism. Peripheral or central OXT administration (which raises serum OXT levels) decreases body weight (3) and fat mass (4) in diet-induced obese rodents while maintaining lean mass. These effects of OXT on body weight and fat mass may be mediated by decreased food intake (5, 6) and increased energy expenditure (7), as well as by enhanced lipolysis (8) through a direct action of OXT on adipocytes, which express the OXT receptor (9). In humans, OXT deficiency has been implicated in the pathogenesis of Prader-Willi, a genetic syndrome characterized by hyperphagia and severe early-onset obesity (10), and pilot studies in humans have shown that administration of intranasal OXT may decrease caloric intake (11, 12) and induce weight loss (13). We have previously demonstrated that in two states of energy deficit, anorexia nervosa (AN) and exercise-induced hypothalamic amenorrhea, overnight serum OXT levels are lower compared with healthy controls (HC) independent of estradiol levels, and associated with body mass index (BMI) (14, 15). However, reports are conflicting as to whether circulating OXT levels are increased (16), unchanged (17), or decreased (18) in overweight/obesity (OB) compared with HC.

In preclinical studies, OXT is anabolic to bone, promoting osteogenesis over adipogenesis and favoring osteoblastic over osteoclastic activity (19, 20). Both osteoblasts and osteoclasts have OXT receptors, and the effects of estrogen on bone mass in mice are mediated at least in part through OXT (21). OXT and OXT receptor null mice have severe osteoporosis with low bone turnover (22); subsequent OXT administration increases bone formation and improves bone microarchitecture (20). In wild-type rats, OXT also promotes net bone formation by increasing osteoblast proliferation and bone turnover (23). Similarly, in humans, lower serum OXT levels have been associated with a higher risk of osteoporosis in postmenopausal women (24). In addition, in premenopausal females, low overnight serum OXT levels are associated with reduced posteroanterior (PA) and lateral spine bone mineral density (BMD) in AN (14) and impaired bone microarchitecture and estimated strength (by finite element analysis) in amenorrheic athletes (25).

Whether OXT levels are high in OB, reflecting an energy surplus, and associated with body composition and bone parameters across the weight spectrum has not been studied. We hypothesized the following: 1) OXT is a marker of energy availability, and will therefore be (a) lowest in AN, intermediate in HC, and highest in OB; and (b) positively correlated with BMI, fat mass, and lean mass. 2) OXT is anabolic to bone, and will therefore be positively correlated with BMD Z-scores, positively correlated with the hip structural analysis (HSA) parameter section modulus, and negatively correlated with the HSA parameter buckling ratio, all of which have been associated with fracture risk (26, 27). We also hypothesized that the relationship between OXT and bone parameters would be independent of lean mass, which is a stronger predictor of BMD than fat mass (28) or BMI (29) and is associated with most HSA parameters across the weight spectrum (29).

Materials and Methods

Subjects were recruited from the community through advertisements and referrals from health care providers. We evaluated 59 women, 18 to 45 years of age: amenorrheic AN (N = 16), eumenorrheic HC (N = 24), eumenorrheic OB (N = 19). Subject clinical characteristics, dual x-ray absorptiometry (DXA) parameters, and hormone levels (but not OXT levels in OB) previously have been reported in subsets of AN, OB, and HC (14, 29–34). We are reporting on overnight serum OXT levels and their relationship to body composition, BMD, and HSA parameters in OB, as well as the relationship between OXT levels and visceral fat (VAT), subcutaneous fat (SAT), lean body mass, appendicular lean mass, femoral neck BMD, radial BMD, and HSA in AN, HC, and OB.

Subjects with AN met *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition, criteria, including fear of weight gain, body image disturbance, weight <85% of ideal body weight (IBW) as determined by 1983 Metropolitan Life tables (35), and amenorrhea for at least 3 consecutive months. HC subjects had a BMI of 18.5 to 24.9 kg/m^2. OB subjects had a BMI of ≥25 kg/m^2. Subjects were excluded if they had any disease (other than AN or obesity) or were taking any medication known to affect bone metabolism. Additional exclusion criteria included a prior self-reported diagnosis of diabetes mellitus, abnormal thyroid function, active drug or alcohol abuse, and pregnancy or breastfeeding within 6 months of the study. Additional exclusion criteria for the HC and OB groups included substantial medical problems, amenorrhea (current or past), disordered eating, or substantial anxiety or depression. No subject with AN, HC, or OB was taking hormonal contraception during the 3 months preceding study enrollment.

This study was approved by the Partners Human Research Committee, and written informed consent was obtained before any procedures. Subjects were admitted to the Clinical Research Center of Massachusetts General Hospital for an outpatient screening visit and an inpatient overnight visit for frequent blood sampling. At the screening visit, height, weight, and elbow breadth were measured, blood was drawn for screening

laboratory tests, and a comprehensive history and physical examination were performed. Frame size was determined by comparing elbow breadth to race-specific norms derived from the US Health and Nutritional Examination Survey-I (36). Percent IBW was calculated in subjects with AN as described previously. At the overnight visit, % IBW and BMI were reevaluated. Physical activity was quantified using the Paffenbarger scale (37). Whole body composition and BMD at the PA spine, lateral spine, total hip, femoral neck, total radius (comprising the distal 1/3, middistal, and ultradistal radius), distal 1/3 radius, and total body were assessed by DXA (Hologic 4500, Hologic, Inc., Waltham, MA). This technique has a precision of 0.01 g/cm^2 for BMD at the lumbar spine and 3% for fat mass (38). Sex- and race-specific BMD Z-scores were calculated from US Health and Nutritional Examination Survey-I reference data. Fat mass index (fat mass/height^2) and lean body mass corrected for height (lean body mass/height^2) were also calculated. An intravenous catheter was placed by 6 PM and subjects were allowed to acclimate to the environment. Subjects had blood samples drawn every 20 minutes from 8 PM to 8 AM for frequent sampling. Overnight serum samples were then pooled for integrated measures of OXT and cortisol. Fasting morning levels of serum leptin, insulinlike growth factor-1 (IGF-1), parathyroid hormone (PTH), and 25-hydroxyvitamin D [25(OH) D] were measured. OB and HC presented for the overnight visit during the follicular phase of the menstrual cycle.

Hip geometry and femoral strength were assessed via HSA of total hip DXA images. HSA parameters are known to correlate strongly with equivalent measurements assessed by three-dimensional quantitative computed tomography scan (39, 40), and section modulus (Z; index of bending and torsional strength, computed as the cross-sectional moment of inertia divided by the maximum distance from the center of mass to the medial or lateral bone edge) and buckling ratio (index of susceptibility to cortical buckling under compressive loads, computed as the maximum distance from the center of mass to the medial or lateral bone edge, divided by the estimated mean cortical thickness) in particular are associated with fracture risk (26, 27). Therefore, section modulus and buckling ratio were measured in testing hypothesis 2 (confirmatory analysis). In addition, we measured the following HSA parameters to explore their potential relationship with OXT (exploratory analysis): cortical thickness, cross-sectional area (index of resistance to axial forces), and cross-sectional moment of inertia (estimate of resistance to bending forces in a cross-section). Higher values correlate with more favorable hip geometry and femoral strength for all parameters except buckling ratio, in which higher values correlate with inferior femoral strength. HSA was performed at three sites: narrow neck (NN; narrowest part of the femoral neck), intertrochanteric region [IT; along the bisector of the angle of the axes of NN and femoral shaft (FS)], and FS (across the shaft 1.5 cm from the NN to the intersection of the neck and shaft axes) (39).

Biochemical analysis

Serum samples were stored at −80°C. Serum OXT was measured in unextracted serum by enzyme-linked immunosorbent assay (Assay Designs, Inc., Ann Arbor, MI). We previously reported a robust correlation between extracted and unextracted serum OXT levels (25). In-house quality controls had a mean of 152 and 338 pg/mL, respectively, and between-assay coefficient of variation (CV) of 15% and 18%, respectively. The sensitivity was 12.5 pg/mL. Serum cortisol was measured by chemiluminescent microparticle immunoassay (Architect System, Abbot Diagnostics, Abbott Park, IL; intra-assay CV ≤4.8%; total CV ≤7.7%; sensitivity 0.8 μg/dL). Serum IGF-1 was measured by chemiluminescent immunometric assay (Immulite 2000, Diagnostics Products Corp., Los Angeles, CA; intra-assay CV ≤3.9%; total CV ≤8.1%; sensitivity 20 ng/mL). Serum leptin was measured by radioimmunoassay (LINCO Research, a division of Millipore Inc., St. Charles, MO; intra-assay CV ≤8.3%; interassay CV ≤6.3%; sensitivity 0.5 ng/mL). Serum PTH was measured by chemiluminescent immunoassay in 39/59 subjects who had available stored serum (Beckman Coulter, Fullerton, CA; intra-assay CV ≤2.6%; interassay CV ≤5.8%; sensitivity 1 pg/mL). Serum 25(OH)D was measured by liquid chromatography-tandem mass spectroscopy in 39/59 subjects who had available stored serum (intra-assay CV <5%; interassay CV <8%; sensitivity 1 ng/mL). Serum estradiol was measured using a chemiluminescent immunoassay in 34/59 subjects who had available stored serum (Beckman Coulter, Fullerton, CA; total CV ≤12% to 21%; sensitivity 20 pg/mL).

Data analysis

We conducted analyses using JMP Statistical Discovery Software, version 12, Professional (SAS Institute, Inc., Cary, NC). Variables were assessed for normality using the Shapiro-Wilk test, and if nonnormal, nonparametric testing was performed or variables were log-transformed. We compared continuous variables across the three groups (*i.e.,* AN, OB, and HC) using Fisher's least significant difference test, which corrects for multiple comparisons when used for three-group comparisons. Linear regression analyses with Pearson correlation coefficients were used to investigate the associations between OXT levels and body composition, BMD, and HSA parameters in the three groups together, as well as in subgroups. *P* values were adjusted for multiple comparisons using Holm-Bonferroni correction. Multivariate least-square analyses were constructed to control for potential confounders. Stepwise regression analyses were performed to further investigate determinants of bone parameters. Statistical significance was defined as a two-tailed *P* value ≤0.05. Data are reported as mean ± standard deviation or as an R coefficient with an associated *P* value, unless otherwise noted.

Results

Clinical characteristics

Mean age was similar across the groups (Table 1). Mean height was also similar across the groups (AN 166.8 ± 5.7 cm, HC 164.1 ± 6.2 cm, OB 164.6 ± 7.1 cm). Per study design, mean BMI and %IBW were lower in AN ($P \leq 0.0003$) and higher in OB ($P < 0.0001$), compared with HC. Mean highest past BMI did not differ between AN and HC, and was greater in OB than HC ($P < 0.0001$). Mean lowest past BMI was lower in AN ($P < 0.0001$), and higher in OB ($P < 0.0001$), compared with HC. The mean duration of disease in AN was 5.9 ± 7.0 years, and the mean age at diagnosis was 20.3 ± 4.8 years. The race distribution across the groups was as follows: AN, 100% Caucasian; HC, 63% Caucasian, 12% African American, 21% Asian, 4% Hispanic; and OB, 53% Caucasian, 26% African American, 16% Asian, 5% Hispanic.

Per study design, HC and OB had regular menses with no history of amenorrhea, whereas AN had current amenorrhea with mean duration of 19.1 ± 5.4 months and total history of amenorrhea of 72.6 ± 17.4 months. Seventy percent (n = 38/54, five subjects had missing data) of subjects had previously taken some form of hormonal contraception, and the mean time since last oral contraceptive pill use was 5.7 ± 5.3 years. Mean hours of exercise per week was similar across the groups. Mean total fat, VAT, and SAT was lower in AN ($P \leq 0.003$), and higher in OB ($P < 0.0001$), compared with HC. Mean lean body mass and appendicular lean mass were similar in AN and HC and greater in OB than HC ($P < 0.0001$).

Mean PA spine and total radius BMD Z-scores were lower in AN ($P \leq 0.008$) and higher in OB ($P \leq 0.03$) compared with HC. Mean lateral spine, total hip, femoral neck, and total body Z-scores were lower in AN than HC ($P \leq 0.0005$) and comparable in OB and HC. Distal 1/3 radius BMD Z-scores did not differ across the groups ($P = 0.10$). Section modulus was similar between AN and HC and higher in OB compared with HC ($P \leq 0.02$) at most sites. Buckling ratio (a higher buckling ratio denotes lower strength) was higher in AN than HC ($P \leq 0.0002$) and comparable in OB and HC at all sites. Cortical thickness was lower in AN than HC ($P \leq 0.0004$) and comparable in OB and HC at all sites. Cross-sectional area was lower in AN ($P \leq 0.01$) and higher in OB ($P \leq 0.02$) compared with HC at most sites. Cross-sectional moment of inertia did not differ across the groups at most sites.

Hormone levels

Mean overnight serum OXT levels were lower in AN ($P = 0.02$) and higher in OB ($P = 0.01$) compared with HC (Table 1, Fig. 1). Fasting serum leptin levels were lower in AN ($P < 0.0001$) and higher in OB ($P < 0.0001$) compared with HC. Mean overnight serum cortisol levels were higher in AN ($P < 0.0001$) and similar in OB compared with HC. Mean serum IGF-1 levels were similar among the groups. Serum PTH levels were higher in OB compared with AN and HC ($P \leq 0.03$). Serum 25(OH)D levels were similar among the groups. Serum estradiol levels were lower in AN compared with HC and OB ($P \leq 0.05$) and comparable between HC and OB. Given that OXT may be a mediator of the effects of estradiol on bone, we assessed for a relationship between serum estradiol and serum OXT levels, but the relationship was not significant ($P = 0.14$). Given leptin's role as a marker of fat stores and energy reserves, we evaluated the relationship between OXT and leptin, and found a positive relationship ($R = 0.44$, $P = 0.001$).

Relationship between OXT and anthropometric measurements

Within the full cohort, there was a positive relationship between OXT and 1) BMI ($R = 0.44$, $P = 0.0004$), 2) total fat mass ($R = 0.47$, $P = 0.0001$), 3) VAT ($R = 0.47$, $P = 0.0002$), 4) SAT ($R = 0.47$, $P = 0.0002$), 5) lean body mass ($R = 0.30$, $P = 0.02$), and 6) appendicular lean mass ($R = 0.32$, $P = 0.01$) (Table 2, Fig. 2). All relationships remained significant after Holm-Bonferroni correction. There was also a positive relationship between OXT and 1) fat mass index (fat mass/height^2) ($R = 0.47$, $P = 0.0002$) and 2) lean body mass corrected for height (lean body mass/height^2) ($R = 0.33$, $P = 0.01$).

Relationship between OXT and BMD

We evaluated the relationship between OXT and BMD Z-scores, and found a positive relationship at the spine and hip: 1) PA spine ($R = 0.48$, $P = 0.0004$), 2) lateral spine ($R = 0.46$, $P = 0.001$), 3) total hip ($R = 0.33$, $P = 0.01$), and 4) femoral neck ($R = 0.36$, $P = 0.006$) (Table 2, Fig. 3). These relationships remained significant after Holm-Bonferroni correction. The relationship between OXT and absolute BMD (g/cm^2) was similar (PA spine BMD $R = 0.44$, $P = 0.0006$; lateral spine $R = 0.45$, $P = 0.001$; total hip $R = 0.33$, $P = 0.01$; femoral neck $R = 0.35$, $P = 0.006$). The relationship between OXT and BMD Z-scores or absolute BMD was not significant at the total radius, distal 1/3 radius, or total body. After controlling for BMI, the relationship between OXT and PA and lateral spine BMD Z-scores remained significant ($P \leq 0.05$). After controlling for lean mass, which exerts a greater effect on BMD than fat mass (29), the relationship between OXT and PA and lateral spine BMD Z-scores remained significant ($P \leq 0.05$). Given the effects of PTH and 25(OH)D on bone metabolism, we controlled for

Table 1. Subject Characteristics (Mean ± SD)

	AN (n = 16)	HC (n = 24)	OB (n = 19)	AN vs HC	AN vs OB	HC vs OB	Overall ANOVA
Clinical characteristics							
Age (y)	27.2 ± 6.7	26.5 ± 6.8	31.4 ± 9.1	–	–	–	0.12
BMI (kg/m^2)	18.3 ± 0.9	22.2 ± 1.4	32.3 ± 5.8	<0.0001	<0.0001	<0.0001	<0.0001
Percentage IBW (%)	80.2 ± 3.2	98.0 ± 6.5	135.6 ± 23.0	0.0003	<0.0001	<0.0001	<0.0001
Highest past BMI (kg/m^2)	23.5 ± 5.3	23.8 ± 1.9	34.4 ± 5.4	0.54	<0.0001	<0.0001	<0.0001
Lowest past BMI (kg/m^2)	16.4 ± 1.5	20.7 ± 1.5	24.9 ± 2.8	<0.0001	<0.0001	<0.0001	<0.0001
Hours vigorous activity/week	9.7 ± 14.3	6.2 ± 5.5	6.4 ± 11.9	–	–	–	0.60
Body composition							
Total fat (kg)	9.6 ± 2.2	16.5 ± 3.7	35.0 ± 11.5	0.003	<0.0001	<0.0001	<0.0001
VAT (g)	92.2 ± 48.3	208.8 ± 81.7	582.6 ± 282.7	<0.0001	<0.0001	<0.0001	<0.0001
SAT (g)	642.9 ± 151.5	1127.5 ± 292.8	2297.6 ± 689.7	0.001	<0.0001	<0.0001	<0.0001
Lean body mass (kg)	40.8 ± 3.9	43.1 ± 4.6	52.6 ± 7.8	0.23	<0.0001	<0.0001	<0.0001
Appendicular lean mass (kg)	17.5 ± 1.8	19.2 ± 2.5	23.6 ± 3.8	0.07	<0.0001	<0.0001	<0.0001
BMD							
PA spine BMD Z-score	−1.84 ± 1.13	−0.13 ± 0.81	0.63 ± 1.32	<0.0001	<0.0001	0.03	<0.0001
Lateral spine BMD Z-score	−1.99 ± 1.30	0.31 ± 0.94	0.88 ± 1.51	<0.0001	<0.0001	0.21	<0.0001
Total hip BMD Z-score	−1.20 ± 0.85	0.19 ± 1.02	0.57 ± 0.93	<0.0001	<0.0001	0.20	<0.0001
Femoral neck BMD Z-score	−1.08 ± 0.68	0.07 ± 1.07	0.54 ± 0.93	0.0005	<0.0001	0.11	<0.0001
Total radius BMD Z-score	−0.75 ± 0.67	0.06 ± 0.77	0.75 ± 1.13	0.008	<0.0001	0.01	<0.0001
Distal 1/3 radius BMD Z-score	−0.21 ± 0.53	0.15 ± 0.85	0.47 ± 1.14	–	–	–	0.10
Total body BMD Z-score	−1.00 ± 1.00	−0.04 ± 0.90	0.46 ± 1.07	0.005	<0.0001	0.10	0.0003
HSA							
NN cortical thickness (cm)	0.16 ± 0.02	0.21 ± 0.03	0.22 ± 0.03	0.0002	<0.0001	0.18	<0.0001
NN cross-sectional area (cm^2)	2.69 ± 0.36	3.07 ± 0.42	3.48 ± 0.55	0.01	<0.0001	0.005	<0.0001
NN cross-sectional moment of inertia (cm^4)	2.41 ± 0.87	2.41 ± 0.63	3.13 ± 1.13	0.78	0.01	0.01	0.02
NN section modulus (cm^3)	1.32 ± 0.32	1.48 ± 0.28	1.74 ± 0.45	0.17	0.001	0.02	0.003
NN buckling ratio	11.29 ± 3.11	8.21 ± 1.97	8.47 ± 2.20	0.0002	0.001	0.73	0.0004
IT cortical thickness (cm)	0.33 ± 0.06	0.41 ± 0.07	0.44 ± 0.07	0.0002	<0.0001	0.10	<0.0001
IT cross-sectional area (cm^2)	4.07 ± 0.69	4.91 ± 0.77	5.44 ± 0.79	0.001	<0.0001	0.02	<0.0001
IT cross-sectional moment of inertia (cm^4)	9.79 ± 2.48	11.24 ± 2.33	13.51 ± 5.23	–	–	–	0.17
IT section modulus (cm^3)	3.06 ± 0.53	3.88 ± 0.68	4.59 ± 1.05	0.0003	<0.0001	0.008	<0.0001
IT buckling ratio	10.11 ± 2.18	7.31 ± 1.53	6.95 ± 1.25	<0.0001	<0.0001	0.48	<0.0001
FS cortical thickness (cm)	0.46 ± 0.08	0.57 ± 0.09	0.61 ± 0.14	0.0004	<0.0001	0.24	<0.0001
FS cross-sectional area (cm^2)	3.64 ± 0.62	4.17 ± 0.52	4.50 ± 0.66	0.009	<0.0001	0.07	0.0004
FS cross-sectional moment of inertia (cm^4)	3.15 ± 0.83	3.32 ± 0.73	3.79 ± 1.14	–	–	–	0.09
FS section modulus (cm^3)	1.98 ± 0.38	2.17 ± 0.33	2.42 ± 0.50	0.10	0.003	0.09	0.01
FS buckling ratio	3.53 ± 0.76	2.74 ± 0.57	2.64 ± 0.55	0.0002	0.0001	0.62	0.0001
Laboratory							
Overnight OXT (pg/mL)	320.26 ± 165.32	446.73 ± 272.54	527.89 ± 197.20	0.02	0.0006	0.01	0.001
Leptin (ng/mL)	2.95 ± 1.94	8.78 ± 3.09	25.53 ± 13.52	<0.0001	<0.0001	<0.0001	<0.0001
Overnight cortisol (μg/dL)	11.01 ± 3.49	7.32 ± 1.89	6.19 ± 1.56	<0.0001	<0.0001	0.13	<0.0001
IGF-1 (ng/dL)	157.97 ± 89.45	193.71 ± 103.78	216.77 ± 106.51	–	–	–	0.10
PTH (pg/mL)	33.21 ± 9.50	35.29 ± 10.42	46.45 ± 16.43	0.68	0.02	0.03	0.03
25(OH)D (ng/mL)	15.31 ± 5.96	13.90 ± 4.31	11.05 ± 4.39	–	–	–	0.09
Estradiol (pg/mL)	24.06 ± 15.41	65.18 ± 62.47	41.70 ± 20.54	0.0002	0.05	0.23	0.001

SD, standard deviation.

PTH and 25(OH)D, and found that the relationship between OXT and PA spine, lateral spine, total hip, and femoral neck BMD Z-scores remained significant after controlling for PTH or 25(OH)D ($P \leq 0.05$).

A number of stepwise regression models were performed between OXT and BMD Z-scores. When OXT was entered into a stepwise regression model with other known endocrine determinants of BMD, namely IGF-1 and cortisol, OXT accounted for the following percentage variability in BMD Z-scores: 20% of the variability at the PA spine, 21% at the lateral spine, 11% at the total hip, and 13% at the femoral neck. In a stepwise

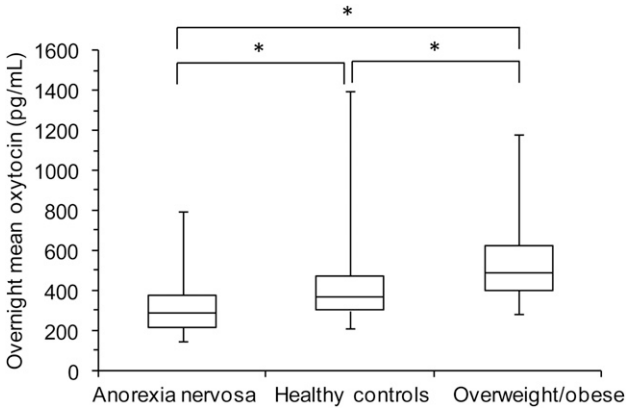

Figure 1. Box plot of overnight mean serum OXT levels in women with AN, HC, and OB. $*P < .05$.

regression model including OXT and BMI, OXT explained 4% of the variability in PA spine BMD Z-scores and 5% of the variability in lateral spine BMD Z-scores. In stepwise regression models including OXT and lean mass, or OXT and fat mass, OXT explained 8% of the variability in PA spine BMD Z-scores and 10% of the variability in lateral spine BMD Z-scores. When OXT, lean mass, and cortisol were added to a stepwise regression model, OXT explained 8% of the variability in PA spine BMD Z-scores and 6% of the variability in lateral spine BMD Z-scores. When OXT, lean mass, and IGF-1 were added to a stepwise regression model, OXT explained 8% of the variability in PA spine BMD Z-scores and 11% of the variability in lateral spine BMD Z-scores.

Mean OXT was significantly lower in subjects with a BMD Z-score <-1 at any site (379 ± 288 vs 476 ± 188 pg/mL, $P = 0.01$). Mean OXT was also significantly lower in subjects with a BMD Z-score <-2.5 at any site (256 ± 149 vs 459 ± 235 pg/mL, $P = 0.03$).

Relationship between OXT and HSA parameters

We then assessed the relationships between OXT and section modulus and buckling ratio, and found negative relationships between OXT and both NN and IT buckling ratio ($P \leq 0.01$) (Table 2, Fig. 4). These relationships remained significant after Holm-Bonferroni correction. After controlling for BMI, the relationship between OXT and NN buckling ratio remained significant ($P \leq 0.05$). After controlling for lean mass, the relationships between OXT and both NN and IT buckling ratio remained significant ($P \leq 0.05$). After controlling for PTH or 25(OH)D, the relationships between OXT and both NN and IT buckling ratio remained significant ($P \leq 0.05$). In an exploratory factor analysis, we found positive relationships between OXT and both NN and IT cortical thickness, as well as between OXT and both NN and IT cross-sectional area ($P \leq 0.05$). After controlling for BMI, lean mass, or PTH, the relationship between OXT and NN cortical thickness remained significant ($P \leq 0.05$). After controlling for 25(OH)D, the relationships between OXT and NN cortical thickness, IT cortical thickness, and IT cross-sectional area remained significant ($P \leq 0.05$).

A number of stepwise regression models were performed between OXT and HSA parameters. When OXT was entered into a stepwise regression model with other known endocrine determinants of BMD, namely IGF-1 and cortisol, OXT accounted for 13% of the variability in NN buckling ratio and 14% of the variability in NN cortical thickness. In a stepwise regression model including OXT and BMI, OXT explained 12% of the variability in NN buckling ratio and 5% of the variability in NN cortical thickness. In a stepwise regression model including OXT and lean mass, OXT explained 12% of the variability in NN buckling ratio, 10% of the variability in IT buckling ratio, and 8% of the variability in NN cortical thickness. In a stepwise regression model including OXT and fat mass, OXT accounted for 12% of the variability in NN buckling ratio. In a stepwise regression model including OXT, lean mass, and cortisol, OXT explained 12% of the variability in NN buckling ratio and 8% of the variability in NN cortical thickness. When OXT, lean mass, and IGF-1 were added to a stepwise regression model, OXT explained 13% of the variability in NN buckling ratio and 6% of the variability in NN cortical thickness.

Relationship among OXT and body composition, BMD, and HSA in subgroup analyses

We performed subgroup analyses of 1) HC and OB and 2) AN. In a subgroup analysis of HC and OB (n = 43), there was a positive relationship between OXT and 1) BMI ($R = 0.32$, $P = 0.04$), 2) total fat mass ($R = 0.38$, $P = 0.01$), 3) VAT ($R = 0.33$, $P = 0.03$), and 4) SAT ($R = 0.37$, $P = 0.02$), but none of the bone parameters was significant in this subgroup analysis. However, in a subgroup analysis of OB alone, there was a trend toward a significant relationship between OXT and femoral neck BMD Z-scores ($R = 0.40$, $P = 0.09$), NN buckling ratio ($R = 0.39$, $P = 0.09$), and NN cortical thickness (R = 0.43 $P = 0.06$). In a subgroup analysis of AN (n = 16), there was a trend toward a significant relationship between OXT and PA spine BMD Z-scores (R = 0.42, $P = 0.10$).

Discussion

We demonstrate that mean overnight serum OXT levels are higher in OB (a state of energy excess) and lower in AN (a state of energy deficiency) compared with HC and

Table 2. Association Between Overnight Mean OXT and Body Composition, Bone Parameters

	Overnight Mean OXT			
	R_1	R_2	R_3	P
Body composition				
BMI (kg/m^2)	0.44			0.0004[a]
Total fat (kg)	0.47			0.0001[a]
VAT (g)	0.47			0.0002[a]
SAT (g)	0.47			0.0002[a]
Lean body mass (kg)	0.30			0.02[a]
Appendicular lean mass (kg)	0.32			0.01[a]
BMD				
PA spine BMD Z-score	0.48	0.26[b]	0.35[c]	0.0004[a]
Lateral spine BMD Z-score	0.46	0.27[b]	0.36[c]	0.001[a]
Total hip BMD Z-score	0.33	0.15	0.23	0.01[a]
Femoral neck BMD Z-score	0.36	0.17	0.25	0.006[a]
Total radius BMD Z-score	0.21	−0.04	0.08	0.13
Distal 1/3 radius BMD Z-score	0.08	−0.03	−0.03	0.57
Total body BMD Z-score	0.25	0.04	0.11	0.06
HSA				
Confirmatory analysis				
NN section modulus (cm^3)	0.19	0.02	0.0007	0.14
NN buckling ratio	−0.34	−0.26[b]	−0.32[c]	0.007[a]
IT section modulus (cm^3)	0.23	−0.07	−0.0008	0.08
IT buckling ratio	−0.32	−0.15	−0.26[c]	0.01[a]
FS section modulus (cm^3)	0.10	−0.09	−0.17	0.45
FS buckling ratio	−0.23	−0.08	−0.18	0.08
Exploratory analysis				
NN cortical thickness (cm)	0.41	0.25[b]	0.31[c]	0.001
NN cross-sectional area (cm^2)	0.29	0.05	0.10	0.03
NN cross-sectional moment of inertia (cm^4)	0.08	−0.10	−0.17	0.56
IT cortical thickness (cm)	0.29	0.08	0.17	0.03
IT cross-sectional area (cm^2)	0.27	0.02	0.08	0.04
IT cross-sectional moment of inertia (cm^4)	0.07	−0.11	−0.13	0.59
FS cortical thickness (cm)	0.24	0.06	0.13	0.06
FS cross-sectional area (cm^2)	0.20	−0.02	−0.02	0.14
FS cross-sectional moment of inertia (cm^4)	0.06	−0.08	−0.21	0.63

R_1, β coefficient for overall model; R_2, β coefficient after controlling for BMI; R_3, β coefficient after controlling for lean mass; P, P value for overall model.

[a]Significant after Holm-Bonferroni correction.

[b]Significant after controlling for BMI.

[c]Significant after controlling for lean mass.

positively associated with BMI, fat mass, lean mass, and bone parameters in premenopausal women across the weight spectrum. Specifically, we show that nocturnal OXT levels are positively correlated with spine and hip BMD Z-scores, as well as negatively correlated with the DXA-derived hip geometry and femoral strength parameter, buckling ratio. In a subset analysis of HC and OB alone, relationships between OXT and body composition, but not bone variables, remained significant. This supports the concept that OXT is a marker of energy availability, and may be a mediator of bone density and structure.

Our data showing a positive association between OXT levels and BMI and fat mass are consistent with the concept that OXT is a marker of energy availability (*i.e.*, adiposity). Prior reports of OXT levels in OB were controversial with studies reporting higher (16), normal (17) or lower (18) fasting morning plasma or serum OXT levels compared with HC. These discrepancies were potentially due to differences in study populations, sampling procedures, and measurement techniques (16–18). For example, half of the OB subjects in Qian *et al.* had type 2 diabetes mellitus, which they reported was associated with lower fasting OXT levels (18). Whereas prior studies used a single blood sample to assess OXT levels, ours included samples taken every 20 minutes for 12 hours, yielding a more reliable, integrated measure of nocturnal OXT. In contrast to severe genetic variants of obesity, where OXT deficiency may be a causal factor, our finding of increased endogenous serum OXT in OB women, positively associated with BMI and body fat, suggests that OXT acts as an appropriate signal for energy availability. Studies of exogenous OXT administration in diet-induced obese rodents (3, 4, 8) and in

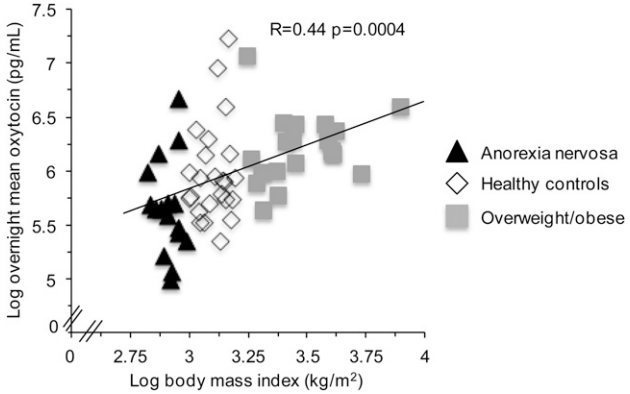

Figure 2. Positive linear relationship between log BMI and log overnight mean serum OXT.

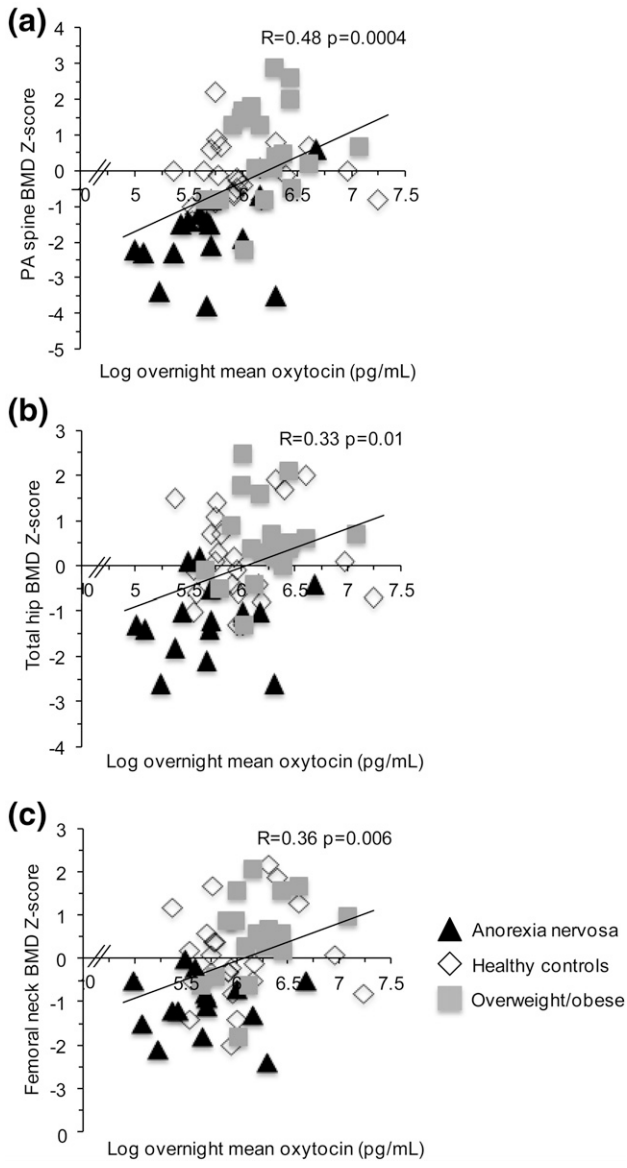

Figure 3. Positive linear relationship between log overnight mean serum OXT and (a) PA spine BMD Z-scores, (b) total hip BMD Z-scores, and (c) femoral neck BMD Z-scores. The relationship between OXT and PA spine BMD Z-scores remained significant after controlling for lean mass.

humans (11–13) suggest that OB is an OXT-sensitive state, and that further increasing OXT levels in OB may lead to a compensatory decrease in food intake, adiposity, and/or body weight. A recent study in humans demonstrated that the effects of exogenous OXT on decreasing food intake are stronger in obese compared with normal-weight men, indicating that obesity may be a particularly OXT-sensitive state (12). At the other end of the weight spectrum, we previously demonstrated that extracted OXT levels are lower in AN than HC (14). This report adds that unextracted serum OXT levels are also lower in AN than HC, which is not surprising as we have demonstrated that extracted and unextracted serum OXT levels using enzyme-linked immunosorbent assay are highly correlated (25). Whether low OXT levels in AN represent an adaptive response to energy deficiency is not clear. Although preclinical data suggest that estradiol may induce OXT, and OXT may be a mediator of the effects of estradiol on bone, we did not find a significant relationship between serum estradiol and OXT. This suggests that serum OXT may be modulated by factors other than estradiol, such as energy availability, or that the study lacked a large enough sample size to detect a relationship.

It is known that the OXT receptor is expressed in adipose tissue (9) and upregulated in some mouse models of obesity (41). We found a positive linear relationship between OXT and BMI, total fat, VAT, SAT, and serum OXT levels across the weight spectrum. In a secondary analysis excluding AN, the relationship between OXT and BMI as well as measures of body fat remained significant. These data are in agreement with previous studies showing that overnight serum OXT is positively correlated with BMI in amenorrheic athletes (25) and body fat and leptin levels in AN (14). Skeletal muscle also expresses the OXT receptor, and OXT deficient mice have decreased muscle mass characteristic of sarcopenia (2). We demonstrate a positive linear relationship between OXT levels and lean mass across the weight spectrum. This is consistent with preclinical data showing that OXT administration in old mice improves muscle regeneration, whereas attenuation of OXT signaling in young mice impairs muscle regeneration (2). Whether OXT is a key determinant in both skeletal muscle and adipose tissue development and maintenance in humans requires further investigation.

Preclinical data indicate that OXT is anabolic to bone. Both osteoblasts and osteoclasts have OXT receptors, and OXT promotes osteogenesis and osteoblastic over osteoclastic activity (19, 23). We now demonstrate that overnight serum OXT is positively associated with hip

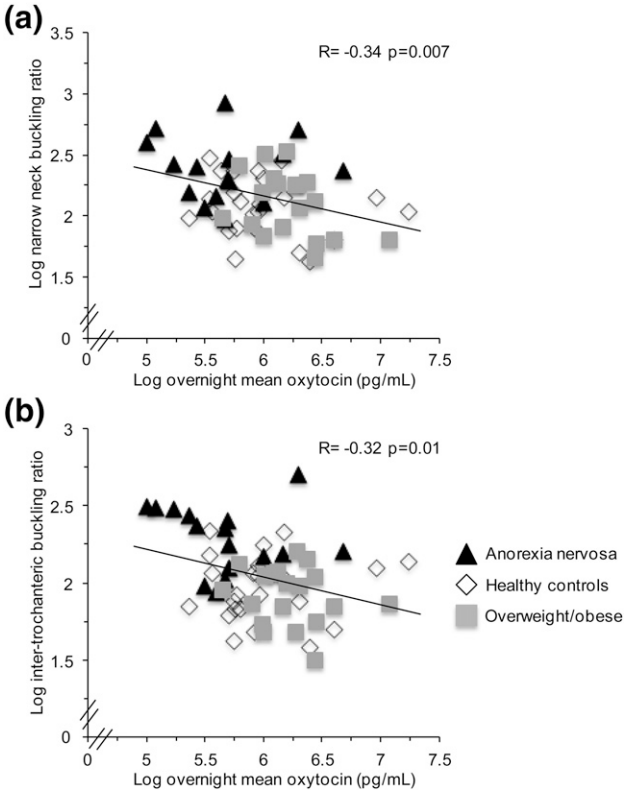

Figure 4. Negative linear relationship between log overnight mean serum OXT and (a) log NN buckling ratio and (b) log intertrochanteric buckling ratio. The relationship between OXT and log NN and log intertrochanteric buckling ratio remained significant after controlling for lean mass.

and spine BMD Z-scores across the weight spectrum in premenopausal women. The relationship between OXT and PA and lateral spine BMD Z-scores is independent of lean mass, which exerts a greater effect on BMD than fat mass (28) or BMI (29). We previously reported that low overnight serum OXT levels are associated with reduced PA and lateral spine BMD in premenopausal AN (14), and a prior cross-sectional study demonstrated an association between low morning serum OXT and osteopenia or osteoporosis in postmenopausal women (42). The substantial association between OXT and BMD at both trabecular (spine) and cortical (hip) sites suggests that OXT may impact both trabecular and cortical bone, which is consistent with preclinical data (22). Associations between OXT levels and BMD Z-scores were no longer significant in a subgroup analysis excluding AN, which may have been due to a smaller sample size (n = 43) and lack of range in BMD Z-scores when the AN group was excluded. Future studies in a larger number of women will be important to establish the role of OXT within subgroups (*i.e.*, underweight, normal weight, and OB) and to determine the effect of OXT administration on bone turnover markers and BMD.

We demonstrate that OXT levels are associated with DXA-derived hip geometry and femoral strength parameters, namely buckling ratio. Our findings are clinically relevant because buckling ratio is associated with an increased hip fracture risk independent of BMD (26, 27). Bachmann *et al.* previously reported that most HSA parameters are impaired in AN and superior in OB compared with HC (29). Our data also demonstrate that most HSA parameters are impaired in AN, but we found more similar results between OB and HC; this may be due to smaller sample sizes and type II error. We found that the association between OXT and buckling ratio was independent of lean mass, which has been associated with hip geometry and femoral strength (29). The significant association between OXT and buckling ratio after Holm-Bonferroni correction suggests that OXT may be a key mediator of hip geometry and femoral strength. The lack of significant relationships between OXT levels and HSA parameters in a subgroup analysis excluding AN may reflect a smaller sample size (n = 43) and lack of range in HSA parameters when the AN group was excluded; further study in larger samples will be important to determine the effect of OXT within these subgroups. In addition, whether low OXT levels increase fracture risk, and whether OXT administration can abate that risk, is unknown.

Limitations of our study include its cross-sectional nature, such that causality cannot be determined. We did not use rigorous exclusion criteria for diabetes mellitus, which was reported by Qian *et al.* to be associated with lower fasting serum OXT levels. Because patients with type 2 diabetes are often OB, exclusion of patients with undiagnosed diabetes mellitus might be expected to further strengthen the positive relationship that we report between OXT and BMI. Our ability to detect substantial associations between OXT and bone parameters when the AN group was excluded was likely limited by a smaller sample size and lack of range in BMD Z-scores; future studies in a larger number of women will be important to establish the role of OXT within subgroups (*i.e.*, underweight, normal weight, and OB). The study may have also lacked a large enough sample size to detect a relationship between estradiol and OXT; future studies in larger groups of women will be important to analyze the influence of estradiol on OXT and its association with body composition and bone parameters. Although an integrated measure of OXT, overnight serum OXT levels may not accurately represent 24-hour serum OXT levels. We previously found no evidence of diurnal variation in OXT levels (25), but this has not been clearly established. OXT may also have sex-specific effects, which could not be evaluated in this study. The racial distribution was different between the AN group and the HC and OB groups. BMD Z-scores are already race-specific, so these results

were likely not affected. In a subgroup analysis of Caucasian subjects, the differences and similarities among the groups remained the same as in the initial analysis. Both excess and deficit of body fat may affect the accuracy of some DXA-derived bone parameters (43). We addressed this by demonstrating that the relationships between OXT and PA and lateral spine BMD Z-scores, and between OXT and NN buckling ratio and cortical thickness, remained significant after controlling for BMI. DXA may also overestimate fat depot measurements compared with computed tomography scan, especially in AN (44). However, correcting DXA-derived VAT and SAT measurements for this underestimation using the regression equation from Bredella *et al.* did not change any of the significant associations between OXT and VAT or between OXT and SAT (43). DXA and HSA measurements are dependent on patient positioning, which was minimized by highly trained staff.

In conclusion, we have demonstrated that nocturnal OXT levels are higher in OB and lower in AN compared with HC and have shown that OXT levels are positively associated with BMI, fat and lean body mass, and bone parameters, specifically hip and spine BMD Z-scores and hip buckling ratio, across the weight spectrum. In a subset analysis of HC and OB alone, relationships between OXT and body composition, but not bone variables, remained significant. Our finding of increased endogenous serum OXT in OB women suggests that OXT acts as an appropriate signal for energy availability in this population; whether further increasing this OXT signal in OB will lead to compensatory weight loss is not yet known. These data also raise the question of whether OXT may be a mediator of bone density, structure and fracture risk in women. OXT pathways may provide targets for obesity and osteoporosis treatment.

Acknowledgments

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