Oxytocin, a New Determinant of Bone Mineral Density in Post-Menopausal Women: Analysis of the OPUS Cohort

Véronique Breuil, Patricia Panaia-Ferrari, Eric Fontas, Christian Roux, Sami Kolta, Richard Eastell, Hedi Ben Yahia, Sylvie Faure, Fatma Gossiel, Claude-Laurent Benhamou, Liana Euller-Ziegler, and Ez-Zoubir Amri

Department of Rheumatology (V.B., H.B.Y., L.E.-Z.), Nice University Hospital, Archet Hospital, Faculté de Médecine (V.B., L.E.-Z., E.-Z.A.), University Nice Sophia Antipolis, Department of Hormonolgy (P.P.-F., H.B.Y., S.F.), Nice University Hospital, St Roch Hospital, and Department of Clinical Research (E.F.), Nice University Hospital, Cimiez Hospital, Nice F-06003, France; Department of Rheumatology (C.R., S.K.), INSERM, Unité 1153, Paris Descartes University, Cochin Hospital, F-75014 Paris, France; Academic Unit of Bone Metabolism (R.E., F.G.), University of Sheffield, Sheffield S10 2JF, United Kingdom; Centre National de la Recherche Scientifique (H.B.Y., E.-Z.A.), Institut de Biologie Valrose Unité Mixte de Recherche 7277, and INSERM (H.B.Y., E.-Z.A.), iBV, Unité 1091, F-06100 Nice, France; and INSERM (C.-L.B.), Unité 658, Centre Hospitalier Régional d'Orléans, F-45032 Orléans Cedex 1, France

Introduction: Oxytocin (OT), a neurohypophysial hormone regulated by estrogen and leptin, may play a role in bone metabolism in humans as suggested by animal studies. This study assessed the relationship between OT and bone status in a large population of postmenopausal women.

Subjects and Methods: Subjects were included in the Osteoporosis and Ultrasound study, a 6-year prospective study in a population-based cohort. Final visit data were used for this cross-sectional study. OT, leptin, and estradiol serum levels were measured in 1097 postmenopausal women and compared with bone mineral density (BMD), fractures, and the bone turnover markers (BTMs) procollagen type 1 N-terminal propeptide, bone alkaline phosphatase, and C-telopeptide of type 1 collagen.

Results: The median age was 70.8 years, 16% were osteoporotic, 48% were osteopenic, and 29% had at least one fracture. The OT serum level was related to spine ($r = +0.12$, $P = .0002$) and total hip BMD ($r = +0.21$, $P < .0001$) and with BTM (procollagen type 1 N-terminal propeptide: $r = -0.13$, $P < .0001$, bone alkaline phosphatase: $r = -0.07$, $P = .02$, C-telopeptide of type 1 collagen: $r = -0.18$, $P < .0001$). The relationship of OT with BMD was independent of BTM. After adjustment for confounding factors, the correlation between OT serum level and BMD remains significant at the hip in women with unmeasurable estradiol or leptin above the median value. There was no significant relationship between OT serum levels and fractures.

Conclusion: High OT levels are associated with high BMD, especially at the hip in women with low estradiol or high leptin serum levels. The mechanism may be explained by the effect of OT on bone turnover. (***J Clin Endocrinol Metab*** **99: E634–E641, 2014)**

Many factors are involved in the control of bone remodeling. Recently the role of a hypothalamic nonapeptide, the oxytocin (OT), has been suggested in the control of bone remodeling.

OT is a neuropeptide predominantly synthesized within the magnocellular neurones of the hypothalamus and recognized as the most prevalent hypothalamic-specific mRNAs (1). It is also synthesized in some peripheral

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received November 16, 2013. Accepted January 13, 2014.
First Published Online January 21, 2014

Abbreviations: ALP, alkaline phosphatase; BMD, bone mineral density; BMI, body mass index; CTX, type I collagen C-terminal telopeptide; DXA, dual-energy X-ray absorptiometry; IQR, interquartile range; OP, osteoporosis; OPUS, Osteoporosis and Ultrasound Study; OT, oxytocin; PINP, procollagen type I N-terminal propeptide; sBMD, standardized BMD; WHO, World Health Organization.

tissues, including the reproductive system, heart, and bone (1). OT and its receptors are positively regulated by estrogens, through estrogen receptor-β because oral administration of estrogen induces an increase in OT serum levels within 12 hours in humans (2–4). The relationship between OT and leptin is complicated and not well understood: in the central nervous system, Honda et al (5)suggested that leptin inhibits OT secretion, but Yamamoto et al (6) found that leptin had no effects on plasma OT secretion and OT gene expression in the supraoptic nucleus. At the peripheral level, during lactation in a rat model, plasma leptin and OT are highly correlated (7, 8). Interestingly, leptin stimulates OT secretion in vitro by ovarian cells (7, 8). OT is involved in several central and peripheral functions including parturition, milk letdown reflex, and social behavior (1).

Human osteoblasts and osteoclasts express the OT receptor (9, 10). Intramuscular injection of OT in rats causes a decrease in serum calcium and soluble receptor activator of nuclear factor-κB ligand, an increase in osteoprotegerin, and a weak effect on bone remodeling in favor of bone formation (11). Subcutaneous OT injection reverses bone loss in ovariectomized mice and enhances bone microarchitecture and biomechanical strength (12). OT and OT receptor knockout mouse develop osteoporosis (OP), through a decrease in bone formation related to a decrease in osteoblast differentiation; this bone deleterious effect could be rescued by ip injection of OT (13). In a preliminary study of 20 postmenopausal osteoporotic women with at least one bone fracture compared with 16 healthy controls, low OT serum levels was significantly associated with severe OP, independently of other factors associated with OP or known to regulate OT serum levels, such as age, estradiol, or leptin (12, 14). In amenorrheic athletes, nocturnal OT secretion is decreased and is associated with site-dependent microarchitectural parameters (15).

Altogether these observations suggest the involvement of OT in the pathophysiology of postmenopausal OP. Thus, the aim of this study was to further evaluate the relationships between OT serum level and bone status in the frame of a large European cohort of postmenopausal women, from the Osteoporosis and Ultrasound Study (OPUS). We analyzed this relationship according to two determinants of OT secretion and bone metabolism, ie, estradiol and leptin serum levels (2, 15, 16).

Subjects and Methods

Subjects

The study subjects were postmenopausal women participating in the OPUS cohort, a study that included five European centers (Aberdeen, United Kingdom; Berlin, Germany; Kiel, Germany; Paris, France; and Sheffield, United Kingdom) and was coordinated by the Medical Physics Research Group in Kiel, Germany. Participants in the OPUS study were recruited from random population samples between April 1999 and April 2001, as previously described (16). At baseline, 2419 women in the age group of 55–79 years were included and 1566 had further evaluation approximately 6 years later (median 5.99 y, range 4.5–7.5 y). The present study has been conducted on the 6 years of follow-up visit data because of technical concerns (requirement of at least 1 mL of serum for biological measurements that were no more available at the inclusion visit). All subjects provided informed consent before OPUS cohort inclusion, and the study was approved by an appropriate ethics committee. The present study has been conducted in accordance with the French national regulations regarding patient consent and ethical review.

Methods

Bone mineral density (BMD) assessment

BMD was measured by dual-energy X-ray absorptiometry (DXA) of the spine and the proximal femur in posteroanterior projection (Hologic QDR-4500; Hologic, at Kiel, Paris, and Sheffield) or in anteroposterior projection (Lunar Expert devices; GE Lunar, at Aberdeen and Berlin). Lumbar spine, total hip, and femoral neck measurements were standardized, and because we used DXA devices of different brands, cross-calibration of BMD was done using published formulae and was expressed in milligrams per square centimeter (sBMD) (17, 18). Both the Aberdeen and Berlin centers replaced their densitometers by a different Lunar model (Prodigy) during the study. For consistency, a single European spine phantom was circulated to all the centers. At each center, the phantom was measured on 10 occasions during each of the two visits, and follow-up measurements were standardized against the baseline mean. In the present study, we analyzed sBMD results at the 6-year follow-up visit. BMD status was defined according to World Health Organization (WHO) definition (WHO 1994), taking into account the lumbar spine T-score and the total hip T-score: normal (T-score > -1 SD), osteopenia (-2.5 SD $<$ T-score ≤ -1SD), osteoporosis (T-score ≤ -2.5 SD); the global T-score was defined by the lower T-score of both sites.

Questionnaires

Each participant completed the OPUS Risk Factor Questionnaire, a modified version of the Risk Factor Questionnaire used in the European Vertebral Osteoporosis Study (19), which was administered by interview. It included a family history of osteoporosis, a self-reported medical history, previous fractures, and medications known to affect skeletal metabolism. Height and weight were also measured, with the participant wearing lightweight clothing and no shoes. At the 6-year follow-up visit, a second questionnaire was completed, focusing mainly on any changes since the first visit.

Fractures

Lateral lumbar and thoracic spine radiographs were performed at both visits, according to standardized procedures. All X-rays were analyzed at a single site (Berlin) by one radiologist. Nonvertebral fractures were captured through self-report, and incident fractures were then verified by radiographs or medical records. All nonvertebral fractures at any skeletal site, whether

caused by high or low trauma, were included. Details of the estimated degree of trauma and the site of fracture (hip, wrist, proximal humerus, or other) were recorded.

Biochemical measurements

Venous blood was taken between 12:00 and 15:00 PM, and samples were left to clot at room temperature for 30 minutes and then centrifuged. Aliquots of the serum supernatant were stored at −80°C until assay.

Oxytocin RIA (Phoenix Pharmaceuticals) was performed according to manufacturers' instructions. The detection limit was 2.5 pg/mL (corresponding to 0.3 pg/mL because samples were concentrated eight times), and intraassay variability was 15% as reported by the manufacturers.

Serum samples were extracted to eliminate interfering molecules and concentrate the sample before analysis. Sample extraction was performed using a solid-phase extraction recommended by RIA kit assay manufacturers and as described by Szeto et al (20). Briefly, solid-phase extraction of samples was performed using 200 mg C18 Sep-Pak columns (Phoenix Pharmaceuticals). The columns were equilibrated with 1 mL buffer B and washed three times with 3 mL of buffer A. Then 0.8 mL of serum was mixed with an equal volume of buffer A, centrifuged at 17 000 × *g* for 20 minutes at 4°C, and the clarified serum was then applied to the column. The flow-through fraction was discarded; the columns were washed twice with 3 mL of buffer A. Oxytocin was eluted with 3 mL of buffer B. The solvent was evaporated under a stream of nitrogen gas. For immunoassay, the samples were reconstituted in 100 μL of assay buffer provided by the RIA kit. OT quantification was done in duplicate using 40 μL of reconstituted samples. Extraction efficiency was approximately 95% as determined by adding various amounts of OT to serum samples before extraction.

Leptin serum levels were measured using a TECO leptin ELISA kit (TECOmedical Group) according to the manufacturer's instructions. The detection limit was 0.2 ng/mL, and the coefficients of variation within and between assays are, respectively, 6.1% and 7.6%. Estradiol was measured using ADVIA-Centaur XP (Siemens), a highly sensitive chemiluminescence assay. The limit of detection was 11.8 pg/mL and the coefficients of variation within and between assays were 10% and 6.7%, respectively.

Serum type I collagen C-terminal telopeptide (CTX), a bone resorption marker, and procollagen type I N-terminal propeptide (PINP) and bone alkaline phosphatase (ALP), bone formation markers, were measured using an immunoassay analyzer iSYS (IDS). Blood samples were collected at the same time of the day to mitigate the effects of diurnal variation. Coefficients of variation were less than 8.1% (serum CTX), less than 1.1% (PINP), and less than 3.5% (bone ALP).

Inclusion and exclusion criteria for analysis

Subjects were included in this analysis if they were postmenopausal, had at least 1 mL of serum available at the 6-year follow-up visit, and a valid dosage of OT. These criteria were met by 1097 subjects.

Statistical analysis

Pearson's simple correlation coefficient (Spearman's correlation coefficient where necessary) was used to assess the correlation between OT serum level and sBMD measurements at the three sites and correlation between bone turnover markers (CTX, PINP, bone ALP) and, respectively, OT serum level and sBMD at all sites. We further studied the relation between BMD status, defined according to lumbar spine T-score, total hip T-score, and global T-score as normal, osteopenic or osteoporotic, and OT serum level. We first assessed differences across all BMD status conditions for each T-score parameter using the Kruskal-Wallis' test. If statistically significant, we then compared the conditions by pairs using the Wilcoxon rank sum test. Here, to account for multiple comparisons, we used the Bonferroni method, and only values of $P \leq .017$ were considered as statistically significant.

We then studied the relation between OT serum level and sBMD using multivariate linear regression models adjusted for age (years), estradiol serum levels (picograms per milliliter), leptin serum levels (nanograms per milliliter), and body mass index (BMI; continuous variables) and parental hip fracture, current smoking (>20 cigarettes/d), alcoholism (> 20 g/d), previous treatment by bisphosphonates, steroids (>3 months), or hormone replacement therapy (binary variables) because they are known potential confounders in analyses of sBMD. These adjusted models are referred to as model 1 in the text. We also adjusted the relationship between OT serum level and sBMD at all sites for bone turnover markers and finally introduced bone turnover marker variables in model 1. We, a priori, decided to repeat our analyses stratifying on estradiol, using as a cutoff the detection limit (11.79 pg/mL), and then on leptin with the median (19.1 ng/mL) as a cutoff value according to the associations already described in the literature with OT secretion.

We finally compared OT serum levels according to the presence of osteoporotic fractures (vertebral and or nonvertebral) using the Wilcoxon rank sum test.

All statistical analyses were performed using the SAS software package version 9.1.3 (SAS Institute).

Results

The clinical characteristics of the population are listed in Table 1, biological characteristics in Table 2, and bone status (BMD and fractures) in Table 3. The OT serum level was below 13 pg/mL in all subjects except three (values

Table 1. Clinical Characteristics of the Study Population

	Study Population (n = 1097)	
		%
Age, y (median, IQR)	1097	70.8 (66.0–76.9)
BMI, kg/m^2 (median, IQR)	1085	26.2 (23.6–29.6)
Parental fracture hip	123	11.3
Smoking > 20 cigarettes/d	27	2.5
Past or current steroid use more than 3 months	83	7.7
Alcohol 3 U/d or more	19	1.8
Hormone replacement therapy ever use	354	38.8
Bisphosphonate therapy ever use	135	12.6

Values are n and percentage unless otherwise stated.

Table 2. Biological Characteristics of the Study Population

Biological Markers	Study Population (n = 1097)	
	n	Serum Values
OT, pg/mL (median, IQR)	1097	0.4 (0.3–1.1)
High sensitive estradiol, pg/mL (median, IQR)	1083	11.8 (11.8–11.8)
Leptin, ng/mL (median, IQR)	1089	19.1 (11.1–31.7)
PINP, ng/mL (median, IQR)	1078	42.4 (29.4–57)
Bone ALP, μg/L (median, IQR)	1091	14.9 (11.8–19.4)
CTX, ng/mL (median, IQR)	1088	0.3 (0.2–0.5)

Values are n.

higher than 25 pg/mL), suggesting a possible interference such as stress or technical artifact; thus, these three women have been excluded and analyses performed on the 1094 remaining women. OT measurements were below the limit of detection (≤0.3 pg/mL) for 463 women and were set to 0.3 pg/mL. Estradiol measurements were below the limit of detection (≤11.8 pg/mL) in 971 subjects (89.6%) and were set to 11.8 pg/mL. As expected in a random sample of the general population, a large majority of the women were osteopenic, more than one third had normal BMD, and 16% had low BMD, fulfilling the WHO criteria of OP (Table 3); fragility fractures were observed in 313 women (28.6%), including 158 osteopenic women.

OT, BMD, and bone remodeling

Univariate analysis showed a positive weak correlation between OT serum level and sBMD (milligrams per square centimeter) at all sites (Table 4). As shown in Figure 1, the OT serum level was significantly associated with bone status defined by T-score: lower in osteopenic than in healthy women at all sites and lower in osteoporotic than in osteopenic women at the lumbar spine and global T score, but this difference did not reach the significance at the total hip. We also observed a significant positive association between OT serum level and low bone turnover (Table 4) and, as expected, a significant negative correlation between bone turnover markers and sBMD at all sites (data not shown).

There was no association between age and OT serum level, but a positive correlation with BMI ($r = 0.25$, $P < .0001$) was observed. There was a positive correlation between OT and estradiol ($r = 0.34$, $P < .0001$), OT, and leptin ($r = 0.23$, $P < .0001$) but no correlation between estradiol and leptin. In a multivariate analysis, the association between OT and leptin remains significant after adjustment on BMI ($P = .03$, $\beta = .007$).

In a multivariate analysis, after adjustment for known determinants of sBMD, estradiol, leptin, age, BMI, parental hip fracture, current smoking, alcoholism, previous

Table 3. Bone Status of the Study Participants

Characteristics	Study Population (n = 1097)	
	n	%
Lumbar spine sBMD, mg/cm^2 (median, IQR)	1071	1026 (914–1148)
Femoral neck sBMD, mg/cm^2 (median, IQR)	1085	722 (648–811)
Total hip sBMD, mg/cm^2 (median, IQR)	1088	839 (751–932)
Lumbar spine BMD status	1071	
Normal	519	48.4
Osteopenia	411	38.4
Osteoporosis	141	13.2
Total hip BMD status	1088	
Normal	587	54.0
Osteopenia	445	40.9
Osteoporosis	56	5.1
Global BMD status (global T-score)[a]	1096	
Normal	390	35.6
Osteopenia	531	48.4
Osteoporosis	175	16.0
Vertebral fracture	170	15.7
Nonvertebral fracture	212	19.5
Subject with at least one fragility fracture (vertebral and/or nonvertebral)	313	29.1

Values are n and percentage unless otherwise stated.

[a] Global T-score is defined by the lower T-score at both sites.

Table 4. Correlation Coefficients of OT Serum Level With BMD (Milligrams per Square Centimeter) and Bone Turnover Markers

	Correlation Coefficient	*P* Value[a]
Spine sBMD (n = 1068)	0.12	.0002
Femoral neck sBMD (n = 1082)	0.13	<.0001
Hip sBMD (n = 1085)	0.21	<.0001
Bone ALP (n = 1088)	−0.07	.02
PINP (n = 1075)	−0.13	<.0001
CTX (n = 1085)	−0.18	<.0001

[a] Pearson's simple correlation coefficient (Spearman's correlation coefficient where necessary) was used to assess correlations.

treatment by bisphosphonates, steroids, and hormone replacement therapy, the relation between OT and sBMD was no longer significant.

In a separate multivariate analysis, after adjustment for bone turnover markers, the relationship between OT and sBMD was still significant at all sites (spine $\beta = 11.47$, $P = .007$; femoral neck $\beta = 9.70$, $P = .0006$; total hip $\beta = 18.01$, $P < .0001$).

OT, BMD, and bone remodeling according to estradiol and leptin levels

We observed, in the subgroup of women with an undetectable serum estradiol level (n = 776 subjects) that the OT serum level correlated positively with BMD (milligrams per square centimeter) at all sites (spine, r = 0.07, $P = .04$; femoral neck, r = 0.09, $P = .005$; hip, r = 0.17, $P < .0001$) and negatively with bone turnover markers (bone ALP, r = −0.07, $P = .01$; PINP, r = −0.13, $P < .0001$; CTX, r = −0.15, $P < .0001$). Meanwhile, in the subgroup with high estradiol (n = 93), OT was only weakly correlated with hip sBMD (r = 0.18, $P = .05$) and not with bone markers. In a multivariate analysis (model 1 without estradiol), in the low estradiol subgroup, OT serum level remained associated only with total hip sBMD (Table 5), but this relationship was no longer significant after adjustment on bone turnover markers.

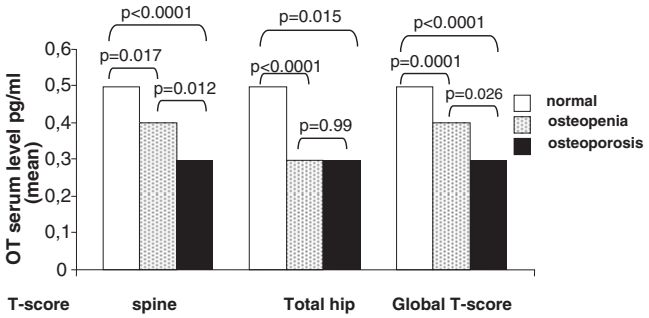

Figure 1. OT serum level according to BMD status. Pairwise comparisons of OT serum level according to BMD status (defined by T-score at lumbar spine, total hip, and global T-score) were performed using the Wilcoxon rank sum test. To account for multiple comparisons, only values of $P \leq .017$ were considered as statistically significant.

OT serum level was positively correlated with sBMD at all sites, regardless of leptin subgroup (data not shown). OT levels were negatively correlated with bone markers in the subgroup with high leptin (bone ALP, r = −0.11, $P = .007$; PINP, r = −0.13, $P = .001$; CTX, r = −0.17, $P < .0001$), whereas in the subgroup of low leptin, only CTX was significantly correlated with OT (r = −0.13, $P = .001$). In a multivariate analysis (model 1 without leptin), in the subgroup of high leptin (n = 423 subjects), the OT serum level was significantly associated with total hip sBMD (Table 5), and this relation remained weakly significant after further adjustment on bone turnover markers ($\beta = 7.11 \pm 3.58$, $P = .048$). In the subgroup of low leptin, the relationship between OT and sBMD was no longer significant (Table 5).

Relationships of OT and fracture

OT serum levels were not significantly different in women with prevalent fractures (vertebral and/or nonvertebral) and women without fractures, neither in the subgroup with low estradiol nor in the subgroup previously or currently treated with hormone replacement therapy or bisphosphonates. However, OT serum levels were significantly lower in women with prevalent fracture and low BMD (T-score < −2.5 SD at least at one site) compared with women with normal BMD at all sites and no previous fragility fracture [respectively, n = 76, median OT, 0.33 pg/mL (interquartile range [IQR] 0.30–0.71) vs n = 303, 0.46 pg/mL (IQR 0.30–1.36), $P = .02$].

Discussion

This study is the first demonstration, in a large cohort of postmenopausal women recruited from the general population, that OT serum level is correlated to BMD. Furthermore, in women with undetectable estradiol serum levels, OT is a determinant of hip BMD.

The neurohypophyseal hormone OT has been thought for a long time to be solely a modulator of lactation and social bonding; however, recently its potential effects on bone metabolism have been investigated. After the description of OT receptors on bone cells, in vitro and in vivo studies raised evidence that OT promotes bone formation (11–13, 21). Since then, only few human data on the role of OT in bone metabolism are available, performed on small groups of selected populations (12, 14, 22, 23).

One of the key points of this study was to use a reliable assay to determine OT serum level. Different, but concordant, methods for measuring plasma OT have been de-

Table 5. Multivariate Analysis of sBMD (Hip Site) and OT Serum Level Associations (Regression Coefficients β With SE) in Estradiol and Leptin Subgroups[a]

Subgroups	n	β (SE)	*P* Value	OT Serum Level, pg/mL, Median (IQR)	Total Hip sBMD, mg/cm^2, Median (IQR)
Estradiol					
≤11.79 pg/mL	776	7.42 ± 3.82	.05	0.4 (0.3–0.9)	826.5 (747–919.5)
>11.79 pg/mL	93	3.81 ± 5.97	.52	0.7 (0.3–2.1)	925 (842–997)
Leptin					
< 19.1 ng/mL	450	−1.93 ± 6.33	.76	0.3 (0.3–0.7)	803.5 (709–901)
≥19.1 ng/mL	423	9.28 ± 3.65	.01	0.5 (0.3–1.4)	876 (797–966)

[a] Adjusted for age, BMI, parental hip fracture, current smoking, alcoholism, previous treatment by bisphosphonates, steroids, hormone replacement therapy, and leptin or estradiol.

veloped over the past 4 decades, but since 2004 several commercially available methods have been favored in research with humans. Evaluation of these methods reveals that they lack reliability when used on unextracted samples of human fluids and that they tag molecules in addition to OT (24). In the present study, we used a reliable technique to measure peripheral OT, which includes extraction and concentration steps combined with a RIA as recently described by Szeto et al (20). The use of unextracted samples gives abnormally high OT levels due to the presence of multiple immunoreactive species, 10–100 times higher-than-expected concentrations. Extraction, using reverse-phase C18 Sep-Pak columns (Phoenix Pharmaceuticals), allows the removal of these nonspecific immunoreactive products and concentration of the samples (eight times) increasing the chances to have detectable values.

Our study shows that, in postmenopausal women, OT serum levels are positively correlated with BMD at the spine and hip in univariate analysis. BMD is known to have multiple determinants and after adjustment on most of them, the association between OT and BMD was no longer significant. One major determinant of BMD in postmenopausal women is the residual estrogen serum level; women with lower estradiol serum levels are known to be at higher risk of osteoporotic fractures (25–27). Moreover, estrogen and OT are closely related in the central nervous system, and OT and its receptors are known to be positively regulated by estrogen; at the peripheral level, it has been shown that human osteoblasts produce OT, and this production is under the control of estrogen by a nongenomic mechanism (21). In a murine model, bone marrow OT mediates the anabolic action of estrogen in skeleton (2, 3, 28, 29). For all these reasons, we performed an analysis according to estrogen status; in the women with undetectable estradiol (89.6% of our study population), the OT serum level was significantly associated with total hip BMD (milligrams per square meter), even after multiple adjustments (Table 5).

This finding is in agreement with data from Lawson et al (22, 23), who studied OT in two types of women characterized by their hypo estrogenic status; in amenorrheic athletes, after controlling for estradiol, a decrease in nocturnal OT secretion is strongly associated with alteration of bone architecture, assessed by high-resolution peripheral quantitative computed tomography; in women with anorexia nervosa, a decrease in nocturnal OT levels is associated with low BMD assessed by DXA at all sites of measure. Our results suggest that the effect of OT on bone metabolism is particularly crucial when the major regulating factor, namely estrogen, is very low, suggesting that OT may plays a role of rescue. The mechanism of OT action on bone metabolism is not yet fully understood, associating indirect effects through estrogen and estrogen independent effects on bone metabolism. Indeed, on the one hand, Colaianni et al (29) recently reported that OT mediates the anabolic effect of estrogen on the skeleton. On the other hand, we and others showed that OT had an in vitro and ex vivo direct effect on osteoblasts and osteoclasts, in the absence of estrogen (12, 13). Moreover, in ovariectomized mice, OT administration was able to restore bone loss induced by estrogen deficiency, which is an argument of a direct effect of OT on bone cells, independently of estrogen anabolic action.

Leptin is recognized both as a negative regulator of hypothalamic OT secretion and as a complex regulating factor of bone metabolism (5, 30). In this study, we analyzed peripheral OT and found a positive correlation between OT and leptin, suggesting that OT secretion follows different rules at the peripheral level compared with hypothalamic secretion. In women with a high level of leptin, we observed a positive correlation between OT serum level and BMD at all sites, which remains significant at the hip in a multivariate analysis (Table 5). These findings suggest that OT and leptin may act in an opposite manner on bone metabolism in postmenopausal women.

OT has been recently described to promote bone formation, as demonstrated by its ability to promote in vitro

osteoblasts differentiation, and to have dual effects on osteoclasts, stimulating their formation and inhibiting the bone-resorbing activity of mature osteoclasts (12, 13, 31). In our study, high OT serum levels are associated with low bone remodeling assessed by bone turnover markers. We cannot compare our results with any other human study on the relationship between OT and bone turnover markers. The association between OT and BMD could be mediated by the effect of OT on bone remodeling because we also observed in the subgroup of women with low estradiol serum levels that, after adjustment on main known confounding factors, the relationship between OT and BMD, significant at the hip, was no longer significant after adjustment on bone turnover markers.

In this study, we did not find a relationship between OT serum levels and the presence of fractures. Few fractures were observed in our population, and half of them were observed in subjects with BMD above the diagnostic threshold of osteoporosis, precluding any definite conclusion.

Conclusion

This study conducted in postmenopausal women shows that high OT serum levels are associated with high BMD, and this appears to be a direct effect on bone cells independent of estradiol OT-mediated action. The involved mechanism might be explained by the effect of OT on bone turnover, high OT levels being associated with low bone turnover markers. These results reinforce the concept that OT plays a role in the pathophysiology of postmenopausal osteoporosis.

Acknowledgments

We thank the members of the scientific board of the OPUS Cohort Claus Gluer, Dieter Felsenberg, and David M. Reid, who gave us the access to all the clinical and bone data and provided the serum of the subjects to the specific biological analysis for this study.

The study was registered in the clinicaltrials.gov protocol registration system (identifier NCT01192893).

Address all correspondence and requests for reprints to: Véronique Breuil, MD, PhD, Centre Hospitalier Universitaire de Nice, Hôpital l'Archet 1, Service de Rhumatologie, 151 Route Saint Antoine de Ginestière, CS 23079, F-06202 Nice, France. E-mail: breuil@unice.fr.

This work was supported by the "Programme Hospitalier de Recherche Clinique Regional du CHU de Nice" (Grant 11-API-02); the "Centre National de la Recherche Scientifique"; Roche; Amgen; and the "Fondation pour la Recherche Médicale" (Grant DVO20081013470). OPUS was supported by the Sheffield Musculoskeletal BioBank, which received ethics approval from the Oxfordshire Research Ethics Committee C (Reference 10/H0606/20) and is housed in the University of Sheffield Biorepository (HTA License 12182).

Disclosure Summary: The authors declare no conflicts of interest.

References

1. **Gimpl G, Fahrenholz F.** The oxytocin receptor system: structure, function, and regulation. *Physiol Rev*. 2001;81:629–683.
2. **Nomura M, McKenna E, Korach KS, Pfaff DW, Ogawa S.** Estrogen receptor-β regulates transcript levels for oxytocin and arginine vasopressin in the hypothalamic paraventricular nucleus of male mice. *Brain Res Mol Brain Res*. 2002;109:84–94.
3. **Choleris E, Gustafsson JA, Korach KS, Muglia LJ, Pfaff DW, Ogawa S.** An estrogen-dependent four-gene micronet regulating social recognition: a study with oxytocin and estrogen receptor-α and -β knockout mice. *Proc Natl Acad Sci USA*. 2003;100:6192–6197.
4. **Amico JA, Seif SM, Robinson AG.** Oxytocin in human plasma: correlation with neurophysin and stimulation with estrogen. *J Clin Endocrinol Metab*. 1981;52:988–993.
5. **Honda K, Narita K, Murata T, Higuchi T.** Leptin affects the electrical activity of neurones in the hypothalamic supraoptic nucleus. *Brain Res Bull*. 2002;57:721–725.
6. **Yamamoto S, Morimoto I, Kai K, et al.** Centrally administered murine leptin stimulates plasma arginine-vasopressin secretion and increases the level of mRNA expression in the supraoptic nucleus of conscious rats. *Neuroendocrinology*. 1999;70:207–212.
7. **Zagoory-Sharon O, Schroeder M, Levine A, Moran TH, Weller A.** Adaptation to lactation in OLETF rats lacking CCK-1 receptors: body weight, fat tissues, leptin and oxytocin. *Int J Obes (Lond)*. 2008;32:1211–1221.
8. **Sirotkin AV, Mlyncek M, Kotwica J, Makarevich AV, Florkovicova I, Hetenyi L.** Leptin directly controls secretory activity of human ovarian granulosa cells: possible inter-relationship with the IGF/IGFBP system. *Horm Res*. 2005;64:198–202.
9. **Copland JA, Ives KL, Simmons DJ, Soloff MS.** Functional oxytocin receptors discovered in human osteoblasts. *Endocrinology*. 1999;140:4371–4374.
10. **Colucci S, Colaianni G, Mori G, Grano M, Zallone A.** Human osteoclasts express oxytocin receptor. *Biochem Biophys Res Commun*. 2002;297:442–445.
11. **Elabd SK, Sabry I, Hassan WB, Nour H, Zaky K.** Possible neuroendocrine role for oxytocin in bone remodeling. *Endocr Regul*. 2007;41:131–141.
12. **Elabd C, Basillais A, Beaupied H, et al.** Oxytocin controls differentiation of human mesenchymal stem cells and reverses osteoporosis. *Stem Cells*. 2008;26:2399–2407.
13. **Tamma R, Colaianni G, Zhu LL, et al.** Oxytocin is an anabolic bone hormone. *Proc Natl Acad Sci USA*. 2009;106:7149–7154.
14. **Breuil V, Amri EZ, Panaia-Ferrari P, et al.** Oxytocin and bone remodelling: relationships with neuropituitary hormones, bone status and body composition. *Joint Bone Spine*. 2011;78:611–615.
15. **Lawson EA, Holsen LM, Santin M, et al.** Oxytocin secretion is associated with severity of disordered eating psychopathology and insular cortex hypoactivation in anorexia nervosa. *J Clin Endocrinol Metab*. 2012;97:E1898–E1908.
16. **Gluer CC, Eastell R, Reid DM, et al.** Association of five quantitative ultrasound devices and bone densitometry with osteoporotic vertebral fractures in a population-based sample: the OPUS Study. *J Bone Miner Res*. 2004;19:782–793.
17. **Genant HK, Grampp S, Gluer CC, et al.** Universal standardization for dual x-ray absorptiometry: patient and phantom cross-calibration results. *J Bone Miner Res*. 1994;9:1503–1514.
18. **Lu Y, Fuerst T, Hui S, Genant HK.** Standardization of bone mineral

density at femoral neck, trochanter and Ward's triangle. *Osteoporos Int*. 2001;12:438–444.
19. **O'Neill TW, Cooper C, Cannata JB, et al.** Reproducibility of a questionnaire on risk factors for osteoporosis in a multicentre prevalence survey: the European Vertebral Osteoporosis Study. *Int J Epidemiol*. 1994;23:559–565.
20. **Szeto A, McCabe PM, Nation DA, et al.** Evaluation of enzyme immunoassay and radioimmunoassay methods for the measurement of plasma oxytocin. *Psychosom Med*. 2011;73:393–400.
21. **Liu X, Shimono K, Zhu LL, et al.** Oxytocin deficiency impairs maternal skeletal remodeling. *Biochem Biophys Res Commun*. 2009; 388:161–166.
22. **Lawson EA, Ackerman KE, Estella NM, et al.** Nocturnal oxytocin secretion is lower in amenorrheic athletes than nonathletes and associated with bone microarchitecture and finite element analysis parameters. *Eur J Endocrinol*. 2013;168:457–464.
23. **Lawson EA, Donoho DA, Blum JI, et al.** Decreased nocturnal oxytocin levels in anorexia nervosa are associated with low bone mineral density and fat mass. *J Clin Psychiatry*. 2011;72:1546–1551.
24. **McCullough ME, Churchland PS, Mendez AJ.** Problems with measuring peripheral oxytocin: can the data on oxytocin and human behavior be trusted? *Neurosci Biobehav Rev*. 2013;37(8):1485–1492.
25. **Finigan J, Gossiel F, Gluer CC, et al.** Endogenous estradiol and the risk of incident fracture in postmenopausal women: the OPUS study. *Calcif Tissue Int*. 2012;91:59–68.
26. **Cummings SR, Browner WS, Bauer D, et al.** Endogenous hormones and the risk of hip and vertebral fractures among older women. Study of Osteoporotic Fractures Research Group. *N Engl J Med*. 1998;339:733–738.
27. **Ettinger B, Pressman A, Sklarin P, Bauer DC, Cauley JA, Cummings SR.** Associations between low levels of serum estradiol, bone density, and fractures among elderly women: the study of osteoporotic fractures. *J Clin Endocrinol Metab*. 1998;83:2239–2243.
28. **Colaianni G, Di Benedetto A, Zhu LL, et al.** Regulated production of the pituitary hormone oxytocin from murine and human osteoblasts. *Biochem Biophys Res Commun*. 2011;411:512–515.
29. **Colaianni G, Sun L, Di Benedetto A, et al.** Bone marrow oxytocin mediates the anabolic action of estrogen on the skeleton. *J Biol Chem*. 2012;287:29159–29167.
30. **Motyl KJ, Rosen CJ.** Understanding leptin-dependent regulation of skeletal homeostasis. *Biochimie*. 2012;94:2089–2096.
31. **Colli VC, Okamoto R, Spritzer PM, Dornelles RC.** Oxytocin promotes bone formation during the alveolar healing process in old acyclic female rats. *Arch Oral Biol*. 2012;57:1290–1297.