Oxytocin is an anabolic bone hormone

Roberto Tamma*,1, Graziana Colaianni*,1, Ling-ling Zhu*, Adriana DiBenedetto, Giovanni Greco, Gabriella Montemurro, Nicola Patano, Maurizio Strippoli, Rosaria Vergari, Lucia Mancini, Silvia Colucci, Maria Grano, Roberta Faccio, Xuan Liu, Jianhua Li, Sabah Usmani, Marilyn Bachar, Itai Bab, Katsuhiko Nishimori, Larry J. Young*, Christoph Buettnerr, Jameel Iqbalb, Li Sunb, Mone Zaidib,2, and Alberta Zallonea,2

*Department of Human Anatomy and Histology, University of Bari, 70124 Bari, Italy; 1Bone Laboratory, The Hebrew University of Jerusalem, Jerusalem 91120, Israel; 2Graduate School of Agricultural Science, Tohoku University, Aoba-ku, Sendai, Miyagi 981-8555 Japan; and 3Center for Behavioral Neuroscience, Department of Psychiatry, Emory University School of Medicine, Atlanta, GA 30322

Communicated by Maria Ilando New, Mount Sinai School of Medicine, New York, NY, February 19, 2009 (received for review October 24, 2008)

We report that oxytocin (OT), a primitive neurohyophyseal hormone, hitherto thought solely to modulate lactation and social bonding, is a direct regulator of bone mass. Deletion of OT or the OT receptor (Oxtr) in male or female mice causes osteoporosis resulting from reduced bone formation. Consistent with low bone formation, OT stimulates the differentiation of osteoblasts to a mineralizing phenotype by causing the up-regulation of BMP-2, which in turn controls Sclerostin and 3, Osterix, and ATF-4 expression. In contrast, OT has dual effects on the osteoclast. It stimulates osteoclast formation both directly, by activating NF-kB and MAP kinase signaling, and indirectly through the up-regulation of RANK-L. On the other hand, OT inhibits bone resorption by mature osteoclasts by triggering cytosolic Ca2+ release and NO synthesis. Together, the complementary genetic and pharmacologic approaches reveal OT as a novel anabolic regulator of bone mass, with potential implications for osteoporosis therapy.

Ox
tocin (OT), a hypothalamic nanopeptide secreted into the circulation from the posterior pituitary, is indispensable for lactation. It acts on a G protein-coupled receptor (Oxtr), the expression of which in reproductive tissues is regulated by sex steroids and OT. In humans and rodents, plasma OT levels are elevated maximally during suckling (1, 2).

Mice lacking OT or its receptor (Oxtr) are unable to lactate, despite unperturbed breast tissue and milk formation (3, 4). Most notably, newborn pups die shortly after birth in the absence of a foster mother postpartum. This effect of OT is exerted peripherally, as the i.p. administration of recombinant OT to OT+/− mice rescues milk ejection, allowing the newborn to feed normally. In contrast to the milk ejection defect, no deficits in copulation, gestation, fecundity, or parturition have been noted in either OT−/− or Oxtr−/− mice, suggesting that these mice are typically eugonadal (5). Furthermore, compound mutants with both the Oxtr and the prostaglandin F2α receptor deleted exhibit no defects in parturition, indicating significant redundancy in the birth process per se (5). However, in view of the established pharmacology of circulating OT on the uterine myometrium, the possibility of a physiological action of OT during childbirth cannot be excluded, even without a loss-of-function phenotype.

Two other key actions of OT warrant mention: effects on social behavior and on the regulation of food intake. Male OT−/− and Oxtr−/− mice show deficits in social recognition, without altered cognition or olfactory learning. That this social amnesia is a central rather than a peripheral action of OT is supported by the observation that recombinant OT injected directly into the amygdala rescues the defect (6). Compared with males, female OT or Oxtr null mice display anxiety and exaggerated stress responses, which are likewise mediated through central OT-ergic neurons (7). OT also is involved in the regulation of food (particularly carbohydrate) intake (8). The loss of OT’s anorexogenic effect leads to overfeeding, increased carbohydrate intake, and increased body weight in OT and Oxtr null mice (5). But the mice are not rendered diabetic, and serum glucose homeostasis remains unaltered (9). Thus, whereas the effects of OT on lactation and parturition are hormonal, actions that mediate appetite and social bonding are exerted centrally. The precise neural networks underlying OT’s central effects remain unclear; nonetheless, one component of this network might be the interactions between leptin- and OT-ergic neurons in the hypothalamus (10).

Considering that calcium is mobilized from the maternal skeleton during late pregnancy and lactation, we speculated that the same hormone that regulates parturition and lactation might also control skeletal homeostasis. Thus, we explored whether the deletion of OT or Oxtr in mice affects bone mass and bone remodeling. In view of OT’s known central actions, we attempted to determine directly whether intracerebroventricular (ICV) OT affects bone remodeling. We conducted further gain-of-function studies that focused on the effects of OT on osteoclast and osteoblast formation. Finally, we probed the signaling cascades that mediate OT action on bone cells.

We report a direct and dominant action of peripheral OT on the skeleton that is mediated mainly through its stimulation of osteoblast formation, with variable effects on osteoclasts. We suggest that OT, as a circulating peptide, is indispensable for basal skeletal homeostasis in both sexes and may play an additional role in the initial mobilization and subsequent restoration of the maternal skeleton during periods of calcium stress in pregnancy and lactation. We speculate that because of its skeletal anabolic action, recombinant OT or its analogs might have potential utility in therapy for human osteoporosis.

Results

Mice lacking OT or Oxtr displayed profound osteoporosis. Histomorphometry and μ-CT revealed marked reductions in trabecular volume [bone volume per trabecular volume (BV/TV)] in both OT−/− and Oxtr−/− mice, despite an expected increase in weight [supporting information (SI) Table S1] (5).

There were no significant sex differences (Fig. 1 A–D). These findings confirm that the action of OT is indispensable for maintaining optimal bone mass in both sexes. Importantly, haploinsufficiency of OT or the Oxtr reduced BV/TV in the face of high-carbohydrate intake, and increased body weight in OT and Oxtr−/− mice.


The authors declare no conflict of interest.

1R.T. and G.C. contributed equally to this work.

2To whom correspondence may be addressed. E-mail: mone.zaidi@mssm.edu or a.zallone@anatomi.uniba.it.

This article contains supporting information online at www.pnas.org/cgi/content/full/0901890106/DCSupplemental.
of unperturbed lactation and no increase in weight (Table S1), attesting to OT’s exquisitely sensitive actions on the skeleton.

We sought to determine whether OT acts directly on bone cells or indirectly via a central neural mechanism. Hypothalamic leptinergic neurons directly regulate bone formation through synaptic relay directed to osteoblasts via Adrger receptors (11). Furthermore, hypothalamic OT-ergic neurons regulate social bonding and food intake (5–8, 12). However, we found that ICV OT infusion for 5 days neither affected serum markers of osteoblast [osteocalcin (OC)] and osteoclast (C-telopeptide) function nor influenced ex vivo osteoblast or osteoclast formation (Fig. 1 E–I). At the same doses, OT stimulated food intake on all days (Fig. 1K), suggesting an action on the central nervous system that does not regulate bone metabolism. Our findings also largely exclude a role for leptinergic/sympathetic relay as a mediator of OT’s effects on bone formation and bone mass (13).

In contrast, OT injected i.p. dramatically increased the number of tartrate-resistant acidic phosphatase (TRAP)-positive osteoclasts in ex vivo marrow cultures (Fig. 1J). Importantly, the direct i.p. injection of OT into mice increased bone mineral density (BMD) as well as osteoblast (CFU-f) formation at 5 weeks (Fig. S1), confirming a peripheral skeletal action of OT, a molecule that otherwise does not cross the blood–brain barrier. Finally, it is highly unlikely that OT’s skeletal action is mediated via prolactin, and although serum prolactin levels might decline in OT deficiency, this decline will (and should not) account for an osteoporotic phenotype considering the known proresorptive actions of prolactin.

Providing further confirmation of a peripheral action of OT, Fig. 1L and M clearly show Oxtr immunolabeling on osteoblasts and osteoclasts, respectively. The bottom panels show that Oxtr localizes to the cytoplasm on exposure to recombinant OT, providing evidence not only for ligand-mediated receptor internalization, but also for ligand specificity. Overall, the data confirm original reports for Oxtr on bone cells (14, 15) and reaffirm a direct action of OT on osteoblasts and osteoclasts.

To examine whether the observed osteopenia in OT −/− mice was due to reduced bone formation, increased resorption, or both, we performed histomorphometry after 2 injections of calcine (15 mg/kg) given 7 days apart. We found a significant reduction in mineralization by OT −/− mice, with only few observable double labels (Fig. 2 A and B). We then studied the effect of OT deletion on osteoblast differentiation ex vivo by culturing calvarial osteoblasts. Von Kossa staining, used to examine mineral deposition in 21-day cultures, revealed a striking reduction in mineralization by OT −/− calvarial osteoblasts (Fig. 2C). Exposure to recombinant human OT during the same 21-day culture partially rescued the mineralization defect (Fig. 2C).

The reduction in osteoblast colonies seen in OT −/− mice could be caused by attenuated proliferation, inhibited differentiation, or both. We found that calvarial osteoblast proliferation was decreased in OT −/− mice, whereas in wild-type cells, recombinant OT enhanced osteoblast precursor proliferation in a concentration-dependent manner (Fig. 2D and E). To examine the effects of OT on osteoblast differentiation, we measured the per-cell expression of genes indicative of osteoblast maturation. We found that OT treatment increased osteopontin (OPN) and OC mRNA expression in calvarial osteoblasts (Fig. 2F). Similarly, OT −/− osteoblasts had reduced OPN and OC mRNA levels (Fig. 2G). Thus, although OT increases both osteoblast proliferation and differentiation, genetic OT ablation, expectedly, has the opposite effects.

We next explored the mechanism by which OT causes increased osteoblast differentiation. Western blot analysis revealed that the 2 critical transcription factors for osteoblast formation, Runx2 and Osterix (Osx), were differentially regulated in OT −/− mice. Runx2 protein was increased and Osx...
Statistics: Student’s t test comparing OT−/− with wild-type mice; n = 4 mice per group; *, P < 0.05. In contrast, OT (at stated doses) stimulated the proliferation (E) and differentiation (F) of wild-type murine osteoblast precursors in the MTT assay and qPCR for the osteoblast markers OPN and OC, respectively. OC and OPN mRNA were expectedly low in ex vivo murine OT−/− osteoblast cultures compared with wild-type cultures (G). This result was associated with reduced Osx protein [H; Western blot analysis, at time 0 (confluence)] and protein was decreased in OT−/− cells (Fig. 2H). Enhanced Runx2 and reduced Osx mRNA expression were confirmed by quantitative PCR (qPCR) in osteoblasts that were cultured to confluence for 2 weeks and then allowed to differentiate (Fig. 2J). The pattern of Runx2 expression was similar in wild-type and OT−/− mice, with both exhibiting decreased levels as cells mature. But whereas during differentiation, wild-type cells displayed decreases in Osx mRNA, OT−/− osteoblasts exhibited persistently low basal Oxs levels.

We next measured BMP-2 expression after 1 and 2 weeks of culture, and a lack of increase in OT−/− mice (Fig. 2J). The transcription factors of the ZAS family of zinc-finger proteins, Schnurri (Shn) 1–3, lie downstream of BMP-2 (16). Shn3 is known to physically associate with Runx2 to promote its degradation; thus, its absence in mice is known to result in retained Runx2 protein and increased bone formation (17). Therefore, we reasoned that the down-regulation of Shn3 in OT−/− cells might increase Runx2 protein levels by preventing degradation; this result indeed was the case (Fig. 2H and K).

In contrast to Shn3 deficiency, Shn2 deficiency in mice is known to reduce osteoblastic bone formation by suppressing Oxs and ATF4 expression and terminal mineralization (18). Thus, we speculated that if Shn2 also is reduced in parallel with Shn3, then Oxs and ATF4 expression will both decline, even in the face of elevated Runx2 protein expression. Fig. 2K shows that Shn2 expression was reduced in parallel with Shn3 expression in OT−/− cells, providing a single molecular explanation for the parallel elevations in Runx2 and reductions in Oxs and ATF4 compared with wild-type littermates (Figs. 2 I and J). The latter reductions were consistent with a defect in mineralization seen in OT−/− cultures (Fig. 2C); however, the increase in Runx2 mRNA expression (Fig. 2F) in OT−/− osteoblasts cannot be explained by reduced Shn3, because Shn3 participates in protein degradation, not in the modulation of mRNA expression. Finally, Shn1 expression also was reduced in OT−/− cells (Fig. 2K); however, the effects of Shn1 on the skeleton remain unknown in the absence of a Shn1 null mouse (16).

Because of coupling between osteoblastic bone formation and osteoclastic bone resorption, enhanced osteoblastogenesis usually is accompanied by increased osteoclastogenesis and vice versa. To explore whether an osteoclastic defect accompanies the reduced bone formation, we incubated osteoclast precursors from OT−/− mice for 12 days with the pro-osteoclastogenic cytokines M-CSF and RANK-L. We found significant reductions in TRAP-positive osteoclast formation (Fig. 3A), precursor proliferation (Fig. 3B), and the expression of c-fms, RANK, and downstream NF-κB subunits and inhibitors (Fig. 3C). Expectedly, recombinant OT caused increased TRAP-positive osteoclast formation through the enhancement of both proliferation and differentiation (Fig. 3 D–F).

The attenuated osteoclast formation seen in OT−/− mice could arise from decreased RANK-L production, increased osteoprotegerin (OPG) production, or reduced osteoclast precursor numbers. To explore the possibility that RANK-L and/or OPG contributed to the decreased osteoblastogenesis in OT−/− mice, we measured RANK and OPG in bone marrow stromal cells. We found low RANK-L and, surprisingly, low OPG mRNA in OT−/− osteoblasts (Fig. 3G). But when OT was applied to wild-type marrow stromal cells, we found an increase in mRNA [qPCR, at time 0 (confluence) and at 1 and 2 weeks postdifferentiation induction], but with elevated Runx2 protein and mRNA in OT−/− osteoblasts. Likewise, BMP-2 and ATF4 (J), as well as Schnurri (Shn) isoforms 1, 2, and 3 (K and L) were reduced dramatically at 0 weeks in OT−/− osteoblasts compared with wild-type controls, with the exception of Shn3 at 1 and 2 weeks. Statistics: Student’s t test comparing OT−/− with wild-type mice at every time point; *, P < 0.05; **, P < 0.01 (in triplicate).

Fig. 2. OT stimulates osteoblastic bone formation. OT deficiency reduced MAR, an index of bone formation in calcein-labeled calvaria from 7-week-old mice (A and B), as well as mineralization (C) and proliferation (D) in ex vivo bone marrow cell cultures, evident on von Kossa staining and the MTT assay, respectively. OT (10 nM) partly rescued the ex vivo mineralization defect (C). Enhanced Runx2 and reduced Oxs mRNA expression were confirmed by quantitative PCR (qPCR) in osteoblasts that were cultured to confluence for 2 weeks and then allowed to differentiate (Fig. 2J). The pattern of Runx2 expression was similar in wild-type and OT−/− mice, with both exhibiting decreased levels as cells mature. But whereas during differentiation, wild-type cells displayed decreases in Oxs mRNA, OT−/− osteoblasts exhibited persistently low basal Oxs levels.

We next measured BMP-2 expression after 1 and 2 weeks of culture, and a lack of increase in OT−/− mice (Fig. 2J). The transcription factors of the ZAS family of zinc-finger proteins, Schnurri (Shn) 1–3, lie downstream of BMP-2 (16). Shn3 is known to physically associate with Runx2 to promote its degradation; thus, its absence in mice is known to result in retained Runx2 protein and increased bone formation (17). Therefore, we reasoned that the down-regulation of Shn3 in OT−/− cells might increase Runx2 protein levels by preventing degradation; this result indeed was the case (Fig. 2H and K).

In contrast to Shn3 deficiency, Shn2 deficiency in mice is known to reduce osteoblastic bone formation by suppressing Oxs and ATF4 expression and terminal mineralization (18). Thus, we speculated that if Shn2 also is reduced in parallel with Shn3, then Oxs and ATF4 expression will both decline, even in the face of elevated Runx2 protein expression. Fig. 2K shows that Shn2 expression was reduced in parallel with Shn3 expression in OT−/− cells, providing a single molecular explanation for the parallel elevations in Runx2 and reductions in Oxs and ATF4 compared with wild-type littermates (Figs. 2 I and J). The latter reductions were consistent with a defect in mineralization seen in OT−/− cultures (Fig. 2C); however, the increase in Runx2 mRNA expression (Fig. 2F) in OT−/− osteoblasts cannot be explained by reduced Shn3, because Shn3 participates in protein degradation, not in the modulation of mRNA expression. Finally, Shn1 expression also was reduced in OT−/− cells (Fig. 2K); however, the effects of Shn1 on the skeleton remain unknown in the absence of a Shn1 null mouse (16).

Because of coupling between osteoblastic bone formation and osteoclastic bone resorption, enhanced osteoblastogenesis usually is accompanied by increased osteoclastogenesis and vice versa. To explore whether an osteoclastic defect accompanies the reduced bone formation, we incubated osteoclast precursors from OT−/− mice for 12 days with the pro-osteoclastogenic cytokines M-CSF and RANK-L. We found significant reductions in TRAP-positive osteoclast formation (Fig. 3A), precursor proliferation (Fig. 3B), and the expression of c-fms, RANK, and downstream NF-κB subunits and inhibitors (Fig. 3C). Expectedly, recombinant OT caused increased TRAP-positive osteoclast formation through the enhancement of both proliferation and differentiation (Fig. 3 D–F).

The attenuated osteoclast formation seen in OT−/− mice could arise from decreased RANK-L production, increased osteoprotegerin (OPG) production, or reduced osteoclast precursor numbers. To explore the possibility that RANK-L and/or OPG contributed to the decreased osteoblastogenesis in OT−/− mice, we measured RANK and OPG in bone marrow stromal cells. We found low RANK-L and, surprisingly, low OPG mRNA in OT−/− osteoblasts (Fig. 3G). But when OT was applied to wild-type marrow stromal cells, we found an increase in mRNA [qPCR, at time 0 (confluence) and at 1 and 2 weeks postdifferentiation induction], but with elevated Runx2 protein and mRNA in OT−/− osteoblasts. Likewise, BMP-2 and ATF4 (J), as well as Schnurri (Shn) isoforms 1, 2, and 3 (K and L) were reduced dramatically at 0 weeks in OT−/− osteoblasts compared with wild-type controls, with the exception of Shn3 at 1 and 2 weeks. Statistics: Student’s t test comparing OT−/− with wild-type mice at every time point; *, P < 0.05; **, P < 0.01 (in triplicate).
as assessed by toluidine-blue staining for pits, was sharply reduced within 48 h.

Whereas osteoclastogenesis was enhanced RANK-L through a transwell coculture experiment, in which osteoclast precursors were on the filter and osteoblasts were on the plate. In the absence of added RANK-L (i.e., with M-CSF alone; 50 ng/mL), TRAP-positive osteoclasts were on the filter, suggesting that OT-induced osteoclastogenesis may be exerted in part through the release of diffusible soluble RANK-L in response to OT. Whereas osteoclastogenesis by increasing soluble RANK-L and decreasing OPG, suggesting both direct and indirect actions of OT on osteoclastogenesis.

Whereas OT triggers osteoclastogenesis, published data indicate that when administered acutely, OT decreases serum calcium in rats (19). Because this action is mimicked by calcitonin (20), we speculated that despite increasing osteoclast formation, OT might inhibit the resorptive function of mature osteoclasts. Consequently, we explored the ability of osteoclasts plated on dentine substrate to form resorption "pits." Although TRAP-positive osteoclasts increased with OT (Fig. 3K Upper), as expected, toluidine-blue-positive pits were diminished (Fig. 3K Lower). We further confirmed this antisresorptive action by incubating titrated proline-labeled bone particles with mature osteoclasts for 48 h and separately, and measuring supernatant levels of cross-laps (a resorption marker) in osteoclast–dentine cultures. A ≈30% reduction in bone resorption was found with OT (Figs. 3L and 4I). The inhibition was fully reversed by atosiban, a specific OTR antagonist, confirming OT specificity (Fig. 3L).

We examined the specific pathways triggered by OT in osteoclasts, focusing on MAPK, NF-κB, and Ca²⁺ signaling. OT elicited rapid phosphorylation of Erk1/2 within 5 min and of Iκ-B and Akt within 15 min in osteoclast precursors (Fig. 4AC). Activation of all 3 osteoclastogenic pathways by OT likely underlies OT's stimulatory action on osteoclastogenesis and is reminiscent of pro-osteoclastogenic signals triggered by FSH (21).

To gain insight into the mechanism underlying OT-induced inhibition of bone resorption, we examined the effect of OT on Ca²⁺ signaling, because an elevated intracellular Ca²⁺ level is invariably associated with diminished osteoclast function (22). We performed single-cell measurements of cytosolic Ca²⁺ in fura-2-loaded mature osteoclasts. To separate cytosolic Ca²⁺ release from extracellular Ca²⁺ influx, we used thapsigargin and EGTA, respectively (23). OT triggered rapid elevations in cytosolic Ca²⁺, which were abolished by depleting intracellular Ca²⁺ stores with thapsigargin (Fig. 4D Middle). In contrast, the chelation of extracellular Ca²⁺ by EGTA did not alter the Ca²⁺ signal (Fig. 4D Bottom). Our findings attribute OT-induced Ca²⁺ signals to intracellular Ca²⁺ release rather than to Ca²⁺ influx.

Ca²⁺ signaling increases NO production as a mechanism to inhibit bone resorption (24, 25). Thus, we measured the expression and function of the Ca²⁺-sensitive NOS isoform eNOS, as well as, more directly, NO production in mature osteoclasts. As expected, OT stimulated eNOS mRNA and protein expression at 6 h (Fig. 4E and F). In addition, OT triggered a time-dependent increase in the production of nitrite, a surrogate for NO (Fig. 4G and H). Importantly, whereas OT induced the reduction of supernatant cross-laps, this effect was fully reversed by the NOS inhibitor L-NAME (Fig. 4F), providing evidence of a role of NO in OT-induced resorption inhibition.
In summary, OT increases osteoblastic bone formation, in decrements that likely account for the osteopenic phenotype of OT deficiency, with no discernable alterations in osteoclast numbers seen on histomorphometry (Fig. 2B). The latter finding is surprising, because OT stimulates osteoclastogenesis acutely but inhibits the resorptive activity of mature osteoclasts ex vivo. From this finding, we would expect decreasing numbers of osteoclast in OT−/− mice, particularly because osteoclastogenesis is diminished in ex vivo OT−/− cultures (Fig. 3A). That this expectation was not the case is likely due to redundancy arising in vivo from paracrine and autocrine mechanisms that keep osteoclast numbers constant despite chronic OT deficiency. Nonetheless, the inhibitory effect of OT on mature cells may in fact serve as a checkpoint for unrestricted bone resorption that otherwise may ensue from the stimulation of osteoclastogenesis. In addition, OT could be used as an anabolic stimulus in vivo to restore the maternal skeletal loss occurring during pregnancy and lactation. This anabolic action has been documented in both wild-type mice (Fig. S1) and ovariectomized mice (26).

Discussion

Our findings regarding the positive effects of OT on osteoblasts, together with the bone formation defect of OT and Oxt deficiency, suggest that OT is of fundamental importance to skeletal physiology in both sexes. The finding that haploinsufficiency induces a bone phenotype without affecting lactation, albeit considered the primary action of OT, suggests that bone is more sensitive to OT compared with the breast. Likewise, we found that haploinsufficiency of another pituitary hormone, FSH, increased bone mass without affecting the ovaries, the primary target organ of FSH (21). Moreover, haploinsufficiency of the receptor for the pituitary hormone TSH similarly caused a reduction in bone mass without affecting thyroid development or function (27). Thus, it seems that the newly identified pituitary–skeletal axis has a physiological sensitivity that surpasses, and is distinct from, that of the traditional pituitary–endocrine axis.

Indeed, pituitary hormone receptors have evolved from ancient chemical receptors widely distributed in evolution (28). TSH and FSH receptors, for example, date before the separation of vertebral ancestors from primitive animals. OT and the closely related vasopressin receptors are even more ancient, having emerged to regulate ion homeostasis in a sex-independent manner. Considering that the teleost skeleton predates the appearance of the breast, thyroid, and ovary, it would not be surprising if the hitherto unrecognized but exquisite sensitivity of the skeleton to pituitary hormones would exceed that of their respective target endocrine organs.

We speculate that in addition to maintaining bone mass in both sexes, OT might regulate maternal skeletal homeostasis during pregnancy and lactation, during which OT level rises (1, 2). The fetal skeleton is unlikely to be mineralized effectively in the absence of calcium mobilized from the maternal skeleton (29). Likewise, without adequate replenishment, the mother’s skeleton would waste. We hypothesize that elevated OT levels during pregnancy and lactation not only enhance bone resorption by increasing the number of osteoclasts to make maternal calcium available to the fetus, but also prevent unrestricted bone removal by inhibiting the activity of mature osteoclasts and, in parallel, replenish the lost skeleton by enhancing osteoblastic bone formation.

Furthermore, like OT, parathyroid hormone-related protein (PTHrP), another breast-specific peptide, displays both proreresorptive and anabolic actions. PTHrP secreted from the breast causes, at least in part, the bone loss that accompanies pregnancy and lactation (30). Thus, in mammary tissue–specific knockout mice, a reduction in serum PTHrP is associated with decreases in serum markers of resorption (31). Another primitive neuropeptide, calcitonin, also increases during both pregnancy and lactation, likely to counteract the stimulatory effects of OT (32). Finally, it also is possible that PTHrP and OT may interact with lower estrogen levels to cause the acute and rapid maternal bone

...
loss required for fetal skeletal morphogenesis and postnatal skeletal growth (33). If in certain instances, the lost bone is not replaced adequately, then severe osteoporosis ensues (34).

In conclusion, the presence of Otxr on bone cells, the lack of evidence of a central neural mechanism for OT’s skeletal action, and OT’s anabolic effects all attest to a direct action of circulating OT on bone. The findings suggest that, like intermittent PTH, OT or another bone-active analog could be used as an anabolic therapy for postmenopausal osteoporosis. Indeed, we recently showed that TSH injected i.p. once every 2 weeks restored ovariectomy-induced bone loss (35). Further studies with OT should undoubtedly explore a truly anabolic human osteoporosis.

Materials and Methods

Skeletal Phenotyping. The generation of mice lacking OT or Otxr on a C57BL/6 × 129svEv background has been reported previously (3, 4). Histomorphometry and μ-CT were carried out as described previously (21, 35). Protocols were approved by the Institutional Animal Care and Use Committee of the Faculty of Medicine, University of Bari and Mount Sinai School of Medicine.

Cytosolic Free-Ca<sup>2+</sup> Measurement. Cytosolic free-calcium concentration ([Ca<sup>2+</sup>]i) was measured in single human osteoclasts loaded with the intracellular Ca<sup>2+</sup> indicator fura-2 (Sigma Aldrich).

Nitrite Production. NO was determined with a colorimetric NO assay kit (Fluka). In this assay, nitrate, resulting from autoxidation of NO from osteoclast culture media, is converted to nitrite by nitrate reductase (0.0005 IU) and NADH (0.2 mM). The concentration of nitrite is measured as a NO-stable end-product.

Statistics. Results are given as mean ± SEM. Statistical analyses were performed by using Student t tests (with Microsoft Excel) for significant differences at P < 0.05. For additional details, see SI Methods.

ACKNOWLEDGMENTS. This work was supported by grants from the Italian Space Agency (Osteoporosis and Muscular Atrophy Project), European Space Agency (European Research in Space and Terrestrial Osteoporosis Microgravity Application Promotion project), Ministero dell’Istruzione, dell’Università e della Ricerca (to A.Z.), and the National Institutes of Health Grants DK804590, AG23176, and DK70526 (to M.Z. and L.S.).