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COMMENTARIES

Oxytocin Thinks Globally and Acts Locally: The Oxytocinergic Regulation of Bone Mass

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Commentary on: Tamma R, Colaianni G, Zhu LL, DiBenedetto A, Greco G, Montemurro G, Patano N, Strippoli M, Vergari R, Mancini L, Colucci S, Grano M, Faccio R, Liu X, Li J, Usmani S, Bachar M, Bab I, Nishimori K, Young LJ, Buettner C, Iqbal J, Sun L, Zaidi M, Zallone A. Oxytocin is an anabolic bone hormone. *Proc Natl Acad Sci U S A*. 2009 Apr 28;106(17):7149-54.

The neuropeptide oxytocin (Oxt) is a well-known hormone triggering both central and peripheral effects including parturition, milk let-down reflex and social behavior (1;2). Besides these classical effects, recently, two novel roles of Oxt, apparently unrelated, have emerged. The first is as a regulator of energy metabolism, as suggested by the fact that murine models deficient in Oxt or its receptor (Oxtr) develop late onset obesity, hyperleptinemia, insulin resistance and low sympathetic tone (3;4). The second role is as a new player in the regulation of bone formation. In fact, it has been reported that intraperitoneal injection of Oxt negatively modulates adipogenesis while promoting osteogenesis (5). This latter effect of Oxt is exerted peripherally since the neuropeptide cannot cross the blood-brain barrier (6).

These two novel roles of Oxt are only apparently unrelated since leptin has been the first hormone linking energy and skeletal homeostasis through the activation of β2-adrenergic receptors (Adrβ2) on osteoblasts (7;8). Once activated, Adrβ2 regulates Rankl expression via ATF4, an osteoblast (OB)-specific member of the CREB family (9).

Tamma *et al*. (10) now report that mice lacking Oxt or Oxtr display increased body weight and decreased bone mass accrual with decreased expression of ATF4 and low Rankl. The authors state that these effects are mediated by peripheral Oxt thus excluding a role for leptinergic-sympathetic relay as a mediator of Oxt's effects on bone mass. However, the pathway they describe corresponds to the one expected following the inactivation of leptin-sympathetic signaling (9), as previously observed in *Oxt*(-/-) mice (11).

What is the reason for such discrepant findings? Should the difference between central and peripheral effects of Oxt on bone metabolism be further investigated? The relative contributions of central and peripheral effects of Oxt in various experimental settings may have a different impact on the bone phenotype observed in animal models.

Tamma *et al*. report that deletion of Oxt or Oxtr in knockout murine models resulted in reduced bone formation and increased body weight at the age of three and six months (10). The authors exclude a role for leptinergic/sympathetic relay as a mediator of Oxt's effects on bone formation since intracerebroventricular administration of Oxt for five days did not affect bone cell markers. However, leptin and epinephrine levels were not measured in this study. In contrast, Oxt injected intraperitoneally increased the number of osteoclasts (OCs) as well as OB formation at five weeks. Interestingly, Tamma *et al*. (10) describe the dual and direct actions of Oxt on both OBs and OCs. Indeed, the authors find that the absence of Oxt in *Oxt*(-/-) mice results in a

decreased proliferation rate in OB colonies in agreement with the fact that exogenous Oxt stimulates OB proliferation in wild type animals. The latter also promotes OB differentiation, increasing osteopontin and osteocalcin mRNA expression in calvarial OB cultures. The authors also report that the two critical transcription factors for OB differentiation, Runx2 and Osterix (Osx), are differentially modulated in *Oxt*(-/-) mice with Runx2 increased and Osx decreased.

Surprisingly, the pattern of Runx2 expression is similar in wild-type and *Oxt*(-/-) mice although wild-type cells displayed decreases in Osx mRNA while OBs from *Oxt*(-/-) mice express persistently low Osx levels with both exhibiting decreased levels as cells mature. Moreover, the authors report a lack of increase in BMP-2 expression together with a parallel reduction of the Shnurri-2 and Shnurri-3 transcription factors in OBs from *Oxt*(-/-) mice after one or two weeks in culture.

The *Oxt*(-/-) mice, besides low bone mass, display attenuated OC formation. Although apparently paradoxical, the authors explain this observation by invoking the coupling between osteoblastogenesis and osteoclastogenesis. In particular, they propose that the state of osteoporosis is due to a reduction in Rankl production. In fact, when Oxt was applied to wild type marrow stromal cells, it increased Rankl and decreased OPG. Despite the fact that Oxt may favor osteoclastogenesis, it can inhibit the resorptive function of mature OCs through a calcium signaling mechanism activated by Oxtr.

Finally, the authors state that the presence of Oxtr on bone cells together with a lack of evidence of a central neural mechanism for Oxt's skeletal action confirm the anabolic and dominant effect of peripheral Oxt on bone.

It has been shown recently that Oxt depletion results in a metabolic phenotype characterized by obesity, high plasma leptin levels, insulin resistance and glucose intolerance at the age of three and six months (3). This is also in agreement with the phenotype recently reported for mice lacking Oxtr (4). Interestingly, obese *Oxt*(-/-) or *Oxtr*(-/-) mice don't show any changes in food consumption albeit the significant increase in leptin levels (3;4). There are several possible explanations for this apparent discrepancy. The first is that Oxt may only mark the identity of neurons projecting from the paraventricular nuclei of the hypothalamus (PVN) to the brain stem (BS), but not be critical for their action in meal termination, which could be mediated by classical neurotransmitters such as GABA or glutamate. Alternatively, Oxt may be an important regulator of feeding in normal mice, but there could be a developmental mechanism that compensates for its absence in *Oxt*(-/-) mice (12).

Strikingly, Oxt depletion in *Oxt*(-/-) mice also resulted in low sympathetic tone thereby suggesting that the lack of central Oxt impairs leptin sympathetic transmission (3). In this regard, it has been shown recently that intracerebroventricular infusion of an Oxtr antagonist blocks the effect of leptin on food intake (13).

Evidence indicates that β-adrenergic signaling plays a key role in the regulation of bone remodeling (8). Sympathetic nerve fibers are present in bone and bone marrow, and Adrβ2 have been identified on OBs (9;14). In particular, the antiproliferative action of the sympathetic nervous system is mediated by hypothalamic activation of Adrβ2 on OBs that activate ATF4, an osteoblast-specific transcription factor, which then upregulates the osteoclastogenic cytokine Rankl (9).

Thus, inactivation of β2 adrenergic signaling by pharmacological intervention or leptin resistance may lead to high trabecular bone mass and decreased bone resorption by limiting ATF4-mediated regulation of Rankl expression (8;9;15). This same pathway is described by Tamma *et al*. (10) in *Oxt*(-/-) mice albeit not leading to increased bone mass accrual, but, on the contrary, to a state of profound osteoporosis. While this effect can be explained as the result of direct peripheral effects of Oxt on bone cells, the long-term impact of the central effect of Oxt

on metabolism and the skeleton cannot be fully ruled out.

It is important to consider that the contribution of trabecular bone to bone strength in certain districts of the skeleton, such as the femoral neck, is low, namely <10%. Therefore, it seems that cortical bone is the main determinant of bone strength, whereas trabecular bone seems to make only a limited contribution (16). Nevertheless, Tamma *et al*. (10), though stating that the lack of Oxt in *Oxt*(-/-) mice results in a low bone mass phenotype, did not assess cortical bone parameters.

Mice devoid of leptin (*ob/ob*) or the signaling form of its receptor (*db/db*) have markedly increased body weight and increased trabecular bone in the lumbar vertebrae (7). However, whereas *ob/ob* mice have increased trabecular bone volume, their cortical bone mass is not increased but rather is decreased (17;18), indicating that leptin deficiency has opposite effects on trabecular and cortical bone compartments or that β-adrenergic signaling may specifically affect trabecular bone structure (19). In this regard, it has been shown recently that the global absence of β-adrenergic signaling in a β-less murine model results in obesity, increased trabecular bone volume in young animals and increased cortical bone mass in older animals (20). Thus, a lack of Oxt might impair leptin sympathetic transmission, resulting in increased body weight, hyperleptinemia, low sympathetic tone and increased trabecular bone, as has been reported in preliminary findings (11;21). Moreover, leptin binding in the hypothalamus suppresses the expression of neuropeptide Y (NPY), an inhibitor of cortical bone formation (18). Then, given that the lack of Oxt impairs leptin-sympathetic transmission (3;11), one can hypothesize that *Oxt*(-/-) mice could have greater cortical bone mass due to the downregulation of NPY, and hence greater total body bone mass.

Nonetheless, it has been shown recently that intraperitoneal injection of Oxt negatively modulates adipogenesis while promoting osteogenesis in human bone marrow mesenchymal stromal cells (5). Furthermore, it has been shown that ovariectomized mice have significantly lower levels of Oxt compared to sham-operated controls. Subcutaneous Oxt injections partially rescued the phenotype of these Oxt-depleted mice, reversing bone loss and reducing adiposity (5). All of these findings may reflect peripheral effects of Oxt on bone tissue. In parallel, the lack of Oxt in *Oxt*(-/-) mice increases adipogenesis that drives leptin concentration above a critical level and this might result in a state of resistance to the hormone, obesity and high bone mass (11). Interestingly, it has been reported that patients carrying a chromosomal translocation that disrupts one allele of Sim1, a transcription factor essential for formation of the PVN, display a significant decrease in Oxt expression, as well as early-onset obesity and accelerated linear growth (22).

It is important to underscore once again that the peripheral effects of Oxt on the bone phenotype are different from its central ones since Oxt cannot cross the blood-brain barrier (6). Thus Oxt is *de facto* a molecule with two functional identities depending on its site of synthesis. It is therefore feasible to hypothesize that the lack of Oxt induces two different bone phenotypes: a central one leading to leptin resistance and high bone mass (11), and a peripheral one preventing bone formation (5) (Fig. 1). The bone phenotype observed by Tamma *et al*. (10) might result from the balance between peripheral and central effects of Oxt *in vivo*, as suggested by the complex changes observed in *Oxt*(-/-) mice of some transcription factors (*e.g*., low expression of ATF4 or high expression of Runx2) that are somehow not consistent with the osteopenic phenotype they report.

In conclusion, whereas central Oxt can contribute to the central regulation of bone metabolism mediating the antiproliferative action of sympathetic tone on bone formation, peripheral Oxt is a promising candidate for the treatment of osteoporosis. The use of further experimental approaches will help to elucidate open questions concerning the mechanisms underlying the effects of Oxt on bone metabolism.

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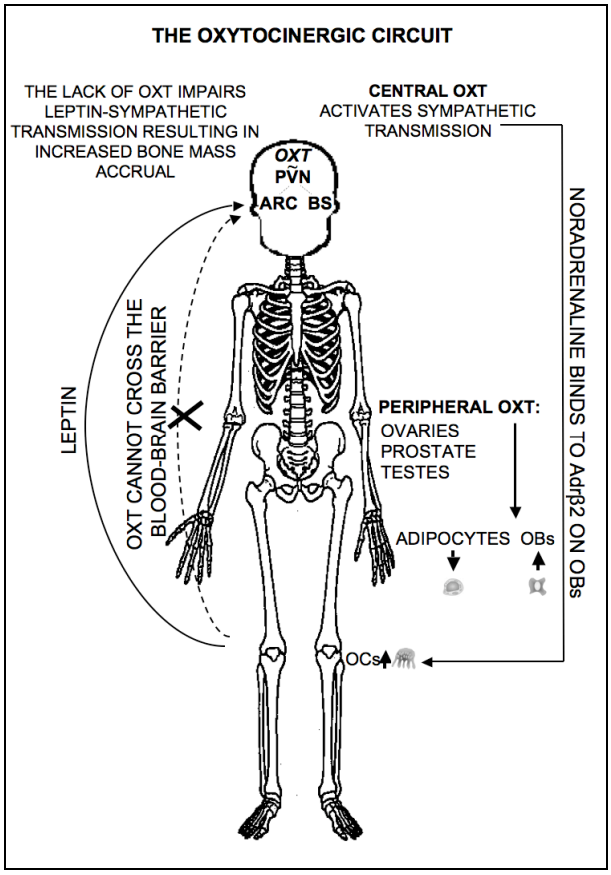

Fig. 1. Central oxytocin (Oxt) in the paraventricular nuclei (PVN) activates sympathetic tone leading to an increase in noradrenaline that binds to β2-adrenergic receptors (Adrβ2) on osteoblasts (OBs), activating ATF4 and upregulating the osteoclastogenic cytokine Rankl. Oxt produced by peripheral organs, like the ovaries and testes, negatively regulates adipogenesis while increasing osteoblastogenesis. This effect of Oxt is exerted only locally since Oxt cannot cross the blood-brain barrier. Then, in the absence of Oxt, adipogenesis increases, driving leptin concentration above a critical level that can cause a state of resistance to the hormone, obesity and high trabecular bone mass. In addition, the lack of Oxt may result in the downregulation of NPY leading to high cortical bone mass and then in increased total body bone mass.

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References

1. Kronenberg H, Melmed S, Polonsky KS, Larsen PR. *Williams Textbook of Endocrinology*. Amsterdam: Saunders Elsevier; 2007.

2. Russell JA, Leng G, Douglas AJ. The magnocellular oxytocin system, the fount of maternity: adaptations in pregnancy. *Front Neuroendocrinol.* 2003 Jan;24(1):27-61.

3. Camerino C. Low sympathetic tone and obese phenotype in oxytocin-deficient mice. *Obesity (Silver Spring).* 2009 May;17(5):980-4.

4. Takayanagi Y, Kasahara Y, Onaka T, Takahashi N, Kawada T, Nishimori K. Oxytocin receptor-deficient mice developed late-onset obesity. *Neuroreport.* 2008 Jun 11;19(9):951-5.

5. Elabd C, Basillais A, Beaupied H, Breuil V, Wagner N, Scheideler M, Zaragosi LE, Massiéra F, Lemichez E, Trajanoski Z, Carle G, Euller-Ziegler L, Ailhaud G, Benhamou CL, Dani C, Amri EZ. Oxytocin controls differentiation of human mesenchymal stem cells and reverses osteoporosis. *Stem Cells.* 2008 Sep;26(9):2399-407.

6. Wahl RU. Could oxytocin administration during labor contribute to autism and related behavioral disorders? A look at the literature. *Med Hypotheses.* 2004;63(3):456-60.

7. Ducy P, Amling M, Takeda S, Priemel M, Schilling AF, Beil FT, Shen J, Vinson C, Rueger JM, Karsenty G. Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. *Cell.* 2000 Jan 21;100(2):197-207.

8. Takeda S, Elefteriou F, Levasseur R, Liu X, Zhao L, Parker KL, Armstrong D, Ducy P, Karsenty G. Leptin regulates bone formation via the sympathetic nervous system. *Cell.* 2002 Nov 1;111(3):305-17.

9. Elefteriou F, Ahn JD, Takeda S, Starbuck M, Yang X, Liu X, Kondo H, Richards WG, Bannon TW, Noda M, Clement K, Vaisse C, Karsenty G. Leptin regulation of bone resorption by the sympathetic nervous system and CART. *Nature.* 2005 Mar 24;434(7032):514-20.

10. Tamma R, Colaianni G, Zhu LL, DiBenedetto A, Greco G, Montemurro G, Patano N, Strippoli M, Vergari R, Mancini L, Colucci S, Grano M, Faccio R, Liu X, Li J, Usmani S, Bachar M, Bab I, Nishimori K, Young LJ, Buettner C, Iqbal J, Sun L, Zaidi M, Zallone A. Oxytocin is an anabolic bone hormone. *Proc Natl Acad Sci U S A.* 2009 Apr 28;106(17):7149-54.

11. Camerino C. Oxytocin inhibits bone formation through the activation of the sympathetic tone: A new candidate in the central regulation of bone formation. *J Bone Miner Res.* 2008 Sep;23(Suppl 1):S56.

12. Kublaoui BM, Gemelli T, Tolson KP, Wang Y, Zinn AR. Oxytocin deficiency mediates hyperphagic obesity of Sim1 haploinsufficient mice. *Mol Endocrinol.* 2008 Jul;22(7):1723-34.

13. Blevins JE, Schwartz MW, Baskin DG. Evidence that paraventricular nucleus oxytocin neurons link hypothalamic leptin action to caudal brain stem nuclei controlling meal size. *Am J Physiol Regul Integr Comp Physiol.* 2004 Jul;287(1):R87-96.

14. Moore RE, Smith CK 2nd, Bailey CS, Voelkel EF, Tashjian AH Jr. Characterization of beta-adrenergic receptors on rat and human osteoblast-like cells and demonstration that beta-receptor agonists can stimulate bone resorption in organ culture. *Bone Miner.* 1993 Dec;23(3):301-15.

15. Pierroz DD, Muzzin P, Bouxsein ML, Rizzoli R, Ferrari SL. Mice null for β1β2-adrenergic receptors have low bone mass and architecture and are resistant to isoproterenol-induced inhibition of bone growth. *Bone.* 2005 Jun;36(Suppl 2):S130-31.

16. Holzer G, von Skrbensky G, Holzer LA, Pichl W. Hip fractures and the contribution of cortical versus trabecular bone to femoral neck strength. *J Bone Miner Res*. 2009 Mar;24(3):468-74.

17. Hamrick MW, Pennington C, Newton D, Xie D, Isales C. Leptin deficiency produces contrasting phenotypes in bones of the limb and spine. *Bone.* 2004 Mar;34(3):376-83.

18. Baldock PA, Allison S, McDonald MM, Sainsbury A, Enriquez RF, Little DG, Eisman JA, Gardiner EM, Herzog H. Hypothalamic regulation of cortical bone mass: opposing activity of Y2 receptor and leptin pathways. *J Bone Miner Res.* 2006 Oct;21(10):1600-7.

19. Hamrick MW, Ferrari SL. Leptin and the sympathetic connection of fat to bone. *Osteoporos Int.* 2008 Jul;19(7):905-12.

20. Bouxsein ML, Devlin MJ, Glatt V, Dhillon H, Pierroz DD, Ferrari SL. Mice lacking beta-adrenergic receptors have increased bone mass but are not protected from deleterious skeletal

effects of ovariectomy. *Endocrinology.* 2009 Jan;150(1):144-52.

21. Colaianni G, Tamma R, Patano N, Camerino C, Montemurro G, Strippoli M, Di Benedetto A, Colucci S, Grano M, Sun L, Zaidi M, Zallone A. Lack of oxytocin in KO mice results in a denser appendicular skeleton. Role of the hormone as autocrine-paracrine enhancer of osteoblast and osteoclast activity. *J Bone Miner Res.* 2005 Sep;20(Suppl 1):S27.

22. Holder JL Jr, Butte NF, Zinn AR. Profound obesity associated with a balanced translocation that disrupts the SIM1 gene. *Hum Mol Genet.* 2000 Jan 1;9(1):101-8.