Oxytocin Hormone as A Preventive Strategy for Treating Osteoporosis in Ovariectomized Rats

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Abstract

Background: osteoporosis (OP) represents a major public health problem worldwide. Therefore, several medications were developed to treat it in humans either by antiresorptive or anabolic agents. For most of these drugs, if not all, no outstanding results have been documented. As oxytocin (OT) has already been safely administered to patients for many indications, it represents a highly promising molecule for the effective treatment of OP. The aim of the study was to clarify the possible protective effect of OT hormone in ovariectomized osteoporotic rats.

Material and methods: 30 adult female albino rats were subjected either to bilateral ovariectomies (OVX) or to sham surgery, categorizing them into 3 groups. Two weeks after being ovariectomized, rats were received intraperitoneal OT for 7 weeks. Serum levels of oxytocin, alkaline phosphate (ALP) and Bone mineral density (BMD) were measured. Histological examination of the head of the tibial bone was performed for confirmation.

Results: Osteoporotic rats had significantly lower oxytocin, higher ALP measures, and the lowest BMD measurements. Conversely, the other treated rats with OT showed normal ALP levels and noticeably improved BMD measures.

Conclusion: OT has an intense outcome on the bone, therefore, OT can be used as a preventive strategy in post-menopausal females.

Introduction

Osteoporosis (OP) constitutes a major worldwide public health burden characterized by enhanced skeletal fragility. Bone metabolism is the combination of bone resorption by osteoclasts and bone formation by osteoblasts. Increasing in bone resorption is considered as the main contributor of bone loss that may lead to osteoporosis (1, 2). With aging, the composition of bone marrow shifts to favor adipocyte formation, increase in osteoclast activity, and decrease in osteoblast functions (3, 4). Additionally, OP is due to an abnormal quantity and/or quality of bone. Quantity is evaluated by measuring BMD. Quality is affected by many factors, including the degree of mineralization, the rate of bone remodeling, the connectivity of the bony trabeculae, the quality of the collagen fibers, and the health of the bone cells (5).

A limited number of approved therapeutic molecules capable of activating bone formation and increasing bone mass and strength has been available. It is hoped that providing more options for developing efficient therapeutic strategies targeting bone formation will allow prevention and restoration of age-related bone strength (6).

Recently, it has been shown that oxytocin (OT), a hormone produced in the neurohypophysis and also by osteoblasts, is involved in bone metabolism (7).

Osteoblasts in bone marrow produce abundant OT, suggesting that locally released OT may be an autocrine regulator of bone formation and bone mass. In this local circuit, the produced OT from osteoblasts in response to estrogen acts upon the OT receptors to stimulate further OT release, which amplifies estrogen action. Physiologically, in addition of being a downstream mediator of estrogen action on bone, the OT autocrine circuit may serve to coordinate the bone-forming activity of neighboring osteoblasts (8).

Plasma OT levels could represent a novel diagnostic marker for osteoporosis and that OT administration holds promise as a potential therapy for this disease (9).

The aim of the work was to clarify the possible protective effect of oxytocin hormone in ovariectomized osteoporotic rats.

Materials and Methods

Study design and setting:

The study materials were recruited for a duration of nearly 6 months; starting from January 2017 to June 2017 in the physiology department, Sohag University. This study was carried out in accordance with the guidelines of the University Animal Ethics and approved by Research Ethics Committee considering care and use of laboratory animals.

Eligibility criteria:

Each adult female rat with a body weight not less than 200 mg were included in this study.

Procedure:

30 adult female albino rats, about (200-250) gm weight, (75-90) days age, were obtained from the animal facility, faculty of Science, Sohag university. Animals were maintained in room temperature and in a normal light-dark cycle, they were fed a standard diet of commercial rat chow and tap water. All animals were housed in group of 5 in metal box cages (20×32×20 cm) in animal facility, faculty of Medicine, Sohag university. Animals were left one week for acclimatization prior to inclusion in the experiment. All rats were subjected either to bilateral ovariectomies (OVX) or sham surgery. Under intraperitoneal anesthesia, using sodium phenobarbital (40 mg/kg), a midline incision (about 2 cm) was made. The ovary was withdrawn out and was tied with a silk ligature. The ovary of the other side was similarly removed. The sham operated control group will have the same previous incision, but with no excision of the ovaries (10).

All animals were given oral antibiotic with postoperative care of the surgical incision.

The Rats were divided into three groups (n= 10):

- Group I, (the control group) after sham surgery, rats were received intraperitoneal injection (Ip) of physiological saline 0.1 mg/kg/day Nacl 0.9 % for 7 weeks.
- Group II, after being ovariectomized, rats were received Ip of physiological saline 0.1 mg/kg/day Nacl 0.9 % for 7 weeks.
- Group III, two weeks after ovariectomy, rats were injected Ip with (0.1 mg/kg/day of OT according to the manufacture instruction) for 7 weeks.

Prior to the scarification, BMD was assessed using DEXA machine and calibrated according to the manufacturer's instruction protocol. Measurements were obtained by positioning the rat in a prone position with knee flexed and extended hips.

At the end of the experimental period all animals were anaesthetized with thiopental sodium (40 mg/kg) intraperitoneally. Intra cardiac samples of blood were being collected, centrifuged for 10 minutes at 3000 r.p.m to separate the serum, and then the serum was stored at -20℃ until the time of biochemical analysis.

Study measurements and outcomes:

Biochemical measurements:

Serum concentration of OT (OT EIA kit) was measured using commercially available kits (WKEA Med. supplies, Changchun, China, Cat. No. WAR-671).

Moreover, ALP was determined by ALP assay kits (DALP-250, Bioassay Systems, CA, USA).

Histological measurement:

Head of the tibia were surgically removed with a sharp blade, followed by fixation and decalcification using Ethylene diamine tetra acetic acid (EDTA) 10% for 2–3 weeks. Then, the specimens were fixed in 10% Buffered Neutral Formalin for up to 5 days. Once the decalcification was completed, specimens were washed in a running tap water before being embedded in paraffin. The specimens were impregnated in soft Paraffin wax (melting point) and were put in the oven for 3hrs.Then, emersion in hard Paraffin waxes (melting point) was done.

Measurements were obtained, using the image analyzer (Leica version; 3.7.2005-2010) counting the numbers of osteoclast and osteoblast lining the endosteum under x 400 magnification in three sections from each slide.

Statistical analyses:

Continuous variables were presented as means and standard deviation, while categorical variables were expressed as percentages. Quantitative variables were compared using the Student's t-test. For qualitative variables, Pearson Chi-square tests were used after assumptions have been verified. A 95% confidence interval (CI) was reported for both measures. A P value < 0.05 was considered statistically significant. All statistical tests were performed using IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp, Version 20.

Results

a) ***Measures of serum level of ALP:***

In this work, serum ALP was increased in the ovariectomized group II and was noticeably decreased in group III after being treated with OT. As summarized in table 1, the highest level of ALP was observed in group II compared to other groups. Marked increase was noticed in group II compared to group I (154.3±30.3 vs 57.0±6.0, P=0.002, respectively).

When OT therapy was introduced 2 weeks after ovariectomy in group III, it resulted in a decrease in the serum ALP in this group compared with group II, (51.1±9. 2 vs, 154.3±30.3 P=0.009, respectively).

No significant difference was obtained on measuring the ALP level when comparing each of group III with group I (G III vs G I: 51.1±9.2 vs 57.0±6.0, P=0.065), respectively.

b) DEXA measurements:

In this work, BMD measures was decreased in the ovariectomized group II and was noticeably increased in groups III after being treated with OT. As summarized in table 1, marked deterioration was noticed in group II in the DEXA measurements, when compared between group II and group I (0.009 ± .002 vs 0.0675±0.007, P=0.000, respectively).

A noticed improvement in the DEXA measurements showed in group III on comparing between group II (0.0492±0.009 vs 0.009±0.002, P=0.003, respectively). Also, there was an improvement noticed in group III in the DEXA measurements on comparing between group III and group I (0.0492±0.009 vs 0.0675±0.007, P=0.590).

c) Measures of serum Oxytocin level:

In this work, serum OT was increased in group III after being injected by OT for 7 weeks. There was a noticed decrease in the serum level of OT in group II compared to group I (16.3±2.7 vs 29.4±3.9, P=0.313, respectively). There was a high significant increase in the OT level in group III in comparison to group II (71.5±4.4 vs, 16.3± 2.7 P=0.000, respectively.

d) Morphometric and histological results:

The control group:

Examination of H & E stained sections from the control group showed normal architecture of the cancellous bone; the tissue arranged as trabeculae which appeared eosinophilic, numerous interconnecting bone marrow spaces; of various sizes were present between the bone tissues (Fig.1). Osteocytes were seen inside their lacunae in the bone lamellae. Osteoblasts appeared with their large size, with their cytoplasm was heavily basophilic (Fig. 2, 3).

Group II (OVX+ saline):

In group II the bone showed picture of osteoporosis in the form of; the bone lost its normal architecture, trabeculae were thin, reduced in number and sometimes fragmented and fractured. They are separated with wide marrow spaces (Fig. 4). There is marked decrease in number of osteocytes associated with bone loss in the form of appearance of cavities (Fig. 5, 6). There was a significant decrease in the number of osteocyte and osteoblast between group II and group I (5.7±1.4 vs 40.4±4.8, P=0.028), (2.0±0.8 vs 20.6±1.9, P=0.048) respectively.

Group III (OVX+ OT 2 weeks after OVX):

Treatment of ovariectomized rats with OT in group III showed many changes. The bone trabeculae, osteocytes and osteoblasts showed improvement. Trabecular bone appeared thicker than group II. The bone lamellae were mainly arranged in regular pattern (Fig. 7). Osteocytes were apparent with large nuclei located inside lacunae. The osteoblasts were seen in increased amount and appeared cuboidal in shape. The matrix appeared homogenous with small cavities (Fig. 8). Supportive results were observed with significant increase in osteocyte and osteoblast number, group III vs group II (P=0.003 and P=0.03) respectively. No significant difference was obtained on comparing group III with group I as regard the number of osteocyte and osteoblast (P=0.897 and P=0.654) respectively.

Table 1: the mean of DEXA, ALP and Oxytocin statistical results in all groups

	DEXA	***ALP***	***Oxytocin***
Group *I* ***Sham*** ***Nacl 0.9% for 7 weeks***	0.0675 ± .007	57.0 ± 6.0	29.4±3.9
Group *II* ***OVX*** ***Nacl 0.9% for 7 weeks***	0.009 ± .002 [a]	154.3 ± 30.3 [a]	16.3± 2.7
Group *III* ***OVX + OT*** ***2 weeks after surgery***	0.0492 ± .009 [b]	51.1 ± 9.2 [b]	71.5 ± 4.4 [b]

Data are represented as mean ± SD

(a) significant (P < 0.05) vs Sham Group I; (b) significant (P < 0.05) vs OVX Group II (n =10 rats per group)

Table 2: the mean of osteocyte & osteoblast numbers in section of the head of the tibia in all groups

	osteocyte	***osteoblast***
Group *I* ***Sham*** ***Nacl 0.9% for 7 weeks***	40.4±4.8	20.6±1.9
Group *II* ***OVX*** ***Nacl 0.9% for 7 weeks***	5.7±1.4 [a]	2.0±0.8 [a]
Group *III* ***OVX + OT*** ***2 weeks after surgery***	36.1±4.5 [b]	15.5± 2.27 [b]

Data are represented as mean ± SD

(a) significant (P < 0.05) vs Sham Group I; (b) significant (P < 0.05) vs OVX Group II (n =10 rats per group)

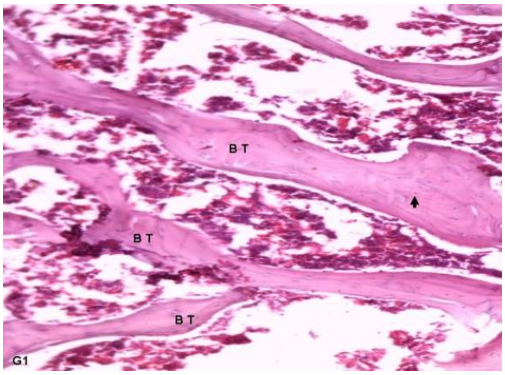

Fig. 1: A photomicrograph of a section of a control group (G I) at the head of the tibia showing: Irregular cancellous bone trabeculae (BT) of the metaphysis and bone marrow spaces in between the trabeculae. Osteocytes seen in the bone lamella (↑). H&E (X 200)

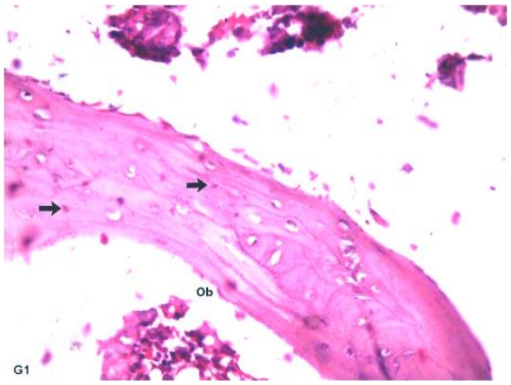

Fig. 1: A higher magnification of a section of a control group (G I) at the head of the tibia showing: Osteocytes seen inside their lacunae in the bone lamella (↑). Osteoblast (Ob) are seen in the outer surface of the lamellae. H & E (X400)

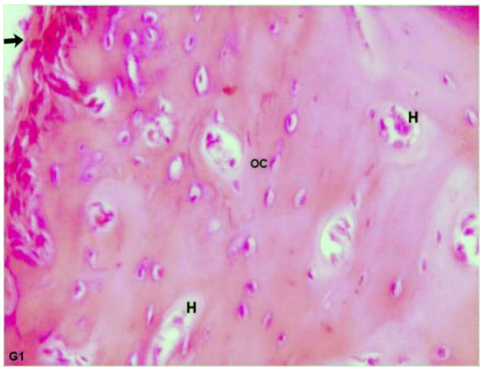

Fig. 3: A photomicrograph of a section from a control rat (G I) showing the outer part of the cortex of tibia: The bone is covered from outside by the periosteum (↑). The compact bone tissue is well organized, showing concentric lamellae arranged around the Haversian canals (H). Osteocytes (OC) inside the lacunae are shown in between the bone lamellae. (H & E X 400)

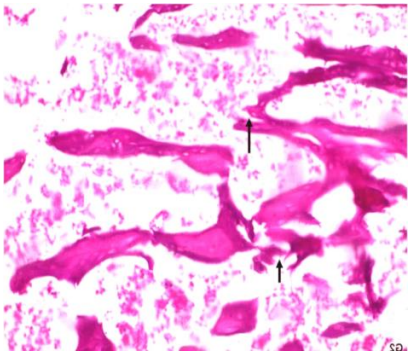

Fig. 4: A photomicrograph of a section of ovariectomized group (G II) at the head of the tibia showing: Bone trabeculae appeared thin and fragmented (↑). H&E (X200)

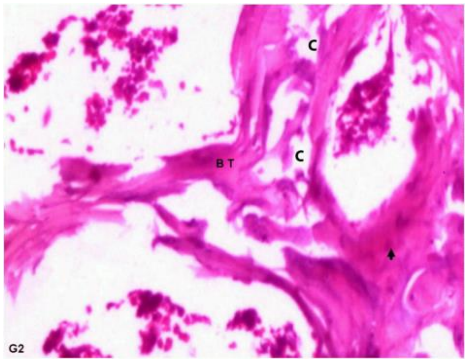

Fig. 5: A higher magnification of a section of ovariectomized group (G II) at the head of the tibia showing: Appearance of cavities (C) in the bone trabeculae near the medullary cavity. There is also apparent decrease in number of the osteocyte (↑). H&E (X400)

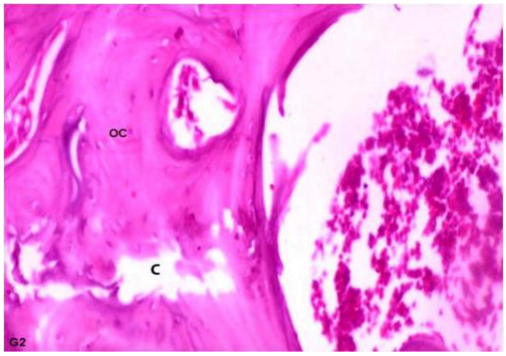

Fig. 2: A photomicrograph of a section of ovariectomized group (GII) at the head of the tibia showing: Areas of bone loss that appeared in the form of cavities (C) in the compact bone. osteocytes showing apparent decrease in number (OC). H&E X400

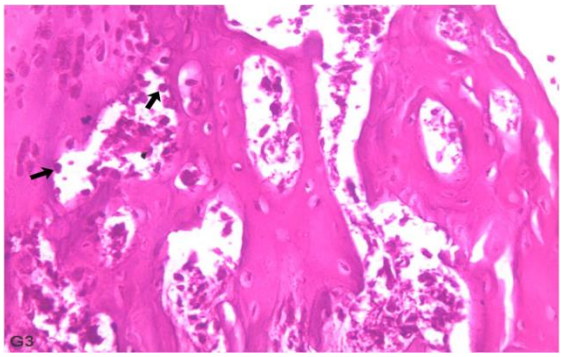

Fig. 3: A photomicrograph of a section of group III at the head of the tibia showing: Apparent thickened bone trabeculae. The osteoblasts were seen covering the trabeculae (↑). H&E X 400

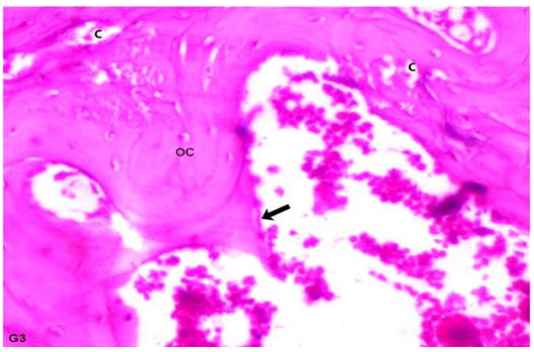

Fig. 8: A photomicrograph of a section from group III rat showing: The outer part of the cortex of tibia bone in which the number of osteocytes (OC) and osteoblasts (↑) showed improvement. The matrix appeared homogenous with less widening of marrow spaces with small cavities appeared (C). H&E (X400)

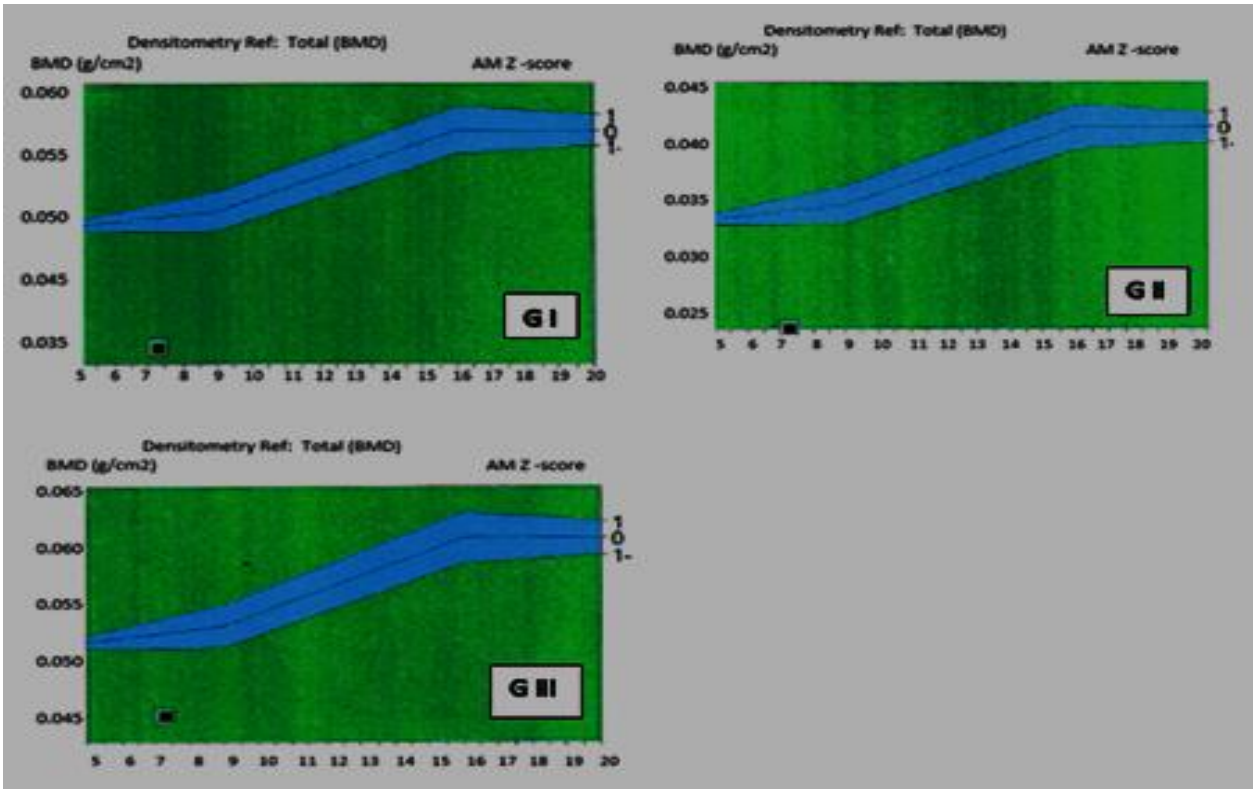

Figure 9: example of BMD measurement by DEXA scan in each group

Discussion

Bone metabolism has quickly become a topic of fascinating research. The bone, far from being a metabolically inactive tissue, is a tissue where different cell types and different molecules carry out numerous and varied functions. Bone loss is the primary risk factor for postmenopausal osteoporosis. It was reported that similar in nature disorder occurred also in ovariectomized rats (11).

OP , defined by the deterioration of bone density and microarchitecture leading to bone fragility, is a major public health problem with 6.3 million of cases expected in 2050 (12). Menopause is known to be associated with numerous physiological and biochemical changes affecting bone mineral metabolism.

In the present study, a model of osteoporosis was designed by removing both ovaries of each rat. Detection of OP was done by measuring ALP & DEXA and observing the histological changes.

In this study there was a statistically increase in serum level of the bone turnover marker (ALP) in group II in comparison to group I. These results are in accordance with (13-15) they found an elevation of serum ALP with post-menopausal females. It was noticed that ALP measurements correlate with the rates of bone mineralization. Lack of inhibiting activity of estrogen on osteoclasts caused the increase in bone resorption and increase the bone turn over marker (ALP) (13).

We confirmed that group II (OVX rats) had developed osteopenia with high level of ALP and very low BMD by DEXA scan after 7 weeks of the surgery. Histologically, the bone lost its normal architecture; the trabeculae became thin and associated with almost no osteoblastic cover, like the osteoporotic picture seen in Naim study (16). This was resulted in a highly significant difference when comparing with group I in the level of ALP and the DEXA measures.

Interestingly, the OT value was significantly decreased in the group II. These results of low OT level in group II comes in agreement with (2, 4) they found that 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 (17).

Up till now, there is no efficient treatment free from side effects that can restore bone health. Current therapies for OP mainly consist of anti-resorptive treatments, such as bisphosphonates, estrogen, selective estrogen receptor modulators, calcitonin and the only currently available anabolic treatment for osteoporosis is PTH (18). For most of these treatments, if not all, side effects have been reported, that is, osteonecrosis, dysphagia, esophagitis, headache, nausea, arthralgia, dizziness, and others.

One of the most meaningful results recently obtained in bone research has been that the posterior pituitary hormone OT have profound effect on bone (10).

OT treatment 2 weeks after surgery for 7 weeks in group III resulted in, a picture of osteopenia was noticed as a statistical significance difference in the ALP and DEXA measures when comparing group III with the group II. A high statistical significant in serum OT level was observed in this group. These results coincides with (1, 12)**,** who found that OT has the ability to restore bone homeostasis when applied 2 weeks post-OVX, when bone turnover and resorption are induced.

Confirmatory histological results showed, an apparent improvement demonstrated by apparent increased in thickness of the bone trabeculae with less cavitation. In addition, the number of osteocytes and osteoblasts was statistically significant increased.

The possible mechanism by which OT can protect against postmenopausal OP is that the action of OT on the skeleton is mainly mediated not only through its stimulation of osteoblast differentiation but also through a modulation of osteoclast formation and function. At the same time, OT stimulated osteoclast differentiation by increasing ratio of RANKL and OPG, while inhibited bone resorption by triggering cytosolic Ca^{2+} release and nitric oxide synthesis (19).

Conclusion:

These beneficial observations, which were discussed above, showed that OT is involved in regulation of bone balance and OT may provide a protective role against postmenopausal OP. Further studies are needed to support these results.

References

1. Beranger GE, Pisani DF, Castel J, Djedaini M, Battaglia S, Amiaud J, et al. Oxytocin reverses ovariectomy-induced osteopenia and body fat gain. Endocrinology. 2014 Apr;155(4):1340-52. PubMed PMID: 24506069.

2. Elabd C, Basillais A, Beaupied H, Breuil V, Wagner N, Scheideler M, et al. Oxytocin controls differentiation of human mesenchymal stem cells and reverses osteoporosis. Stem Cells. 2008 Sep;26(9):2399-407. PubMed PMID: 18583541.

3. Muruganandan S, Roman AA, Sinal CJ. Adipocyte differentiation of bone marrow-derived mesenchymal stem cells: cross talk with the osteoblastogenic program. Cell Mol Life Sci. 2009 Jan;66(2):236-53. PubMed PMID: 18854943.

4. Breuil V, Amri EZ, Panaia-Ferrari P, Testa J, Elabd C, Albert-Sabonnadiere C, et al. Oxytocin and bone remodelling: relationships with neuropituitary hormones, bone status and body composition. Joint Bone Spine. 2011 Dec;78(6):611-5. PubMed PMID: 21441053.

5. Florencio-Silva R, Sasso GR, Sasso-Cerri E, Simoes MJ, Cerri PS. Biology of Bone Tissue: Structure, Function, and Factors That Influence Bone Cells. Biomed Res Int. 2015;2015:421746. PubMed PMID: 26247020. Pubmed Central PMCID: 4515490.

6. Marie PJ, Kassem M. Osteoblasts in osteoporosis: past, emerging, and future anabolic targets. Eur J Endocrinol. 2011 Jul;165(1):1-10. PubMed PMID: 21543379.

7. Takeuchi Y. [Possible involvement of pituitary hormones in bone metabolism]. Clin Calcium. 2013 Feb;23(2):195-202. PubMed PMID: 23354086.

8. Colaianni G, Sun L, Di Benedetto A, Tamma R, Zhu LL, Cao J, et al. Bone marrow oxytocin mediates the anabolic action of estrogen on the skeleton. J Biol Chem. 2012 Aug 17;287(34):29159-67. PubMed PMID: 22761429. Pubmed Central PMCID: 3436530.

9. 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 Sep;37(8):1485-92. PubMed PMID: 23665533.

10. Fallahnezhad S, Piryaei A, Darbandi H, Amini A, Ghoreishi SK, Jalalifirouzkouhi R, et al. Effect of low-level laser therapy and oxytocin on osteoporotic bone marrow-derived mesenchymal stem cells. J Cell Biochem. 2017 Jul 06. PubMed PMID: 28681933.

11. Colaianni G, Sun L, Zaidi M, Zallone A. Oxytocin and bone. Am J Physiol Regul Integr Comp Physiol. 2014 Oct 15;307(8):R970-7. PubMed PMID: 25209411. Pubmed Central PMCID: 4200383.

12. Beranger GE, Djedaini M, Battaglia S, Roux CH, Scheideler M, Heymann D, et al. Oxytocin reverses osteoporosis in a sex-dependent manner. Frontiers in endocrinology. 2015;6:81. PubMed PMID: 26042090. Pubmed Central PMCID: 4437051.

13. Parra-Torres AY, Valdés-Flores M, Orozco L, Velázquez-Cruz R. Molecular Aspects of Bone Remodeling. In: (Ed.) DMV-F, editor. Topics in Osteoporosis: InTech; 2013. p. 28.

14. Mukaiyama K, Kamimura M, Uchiyama S, Ikegami S, Nakamura Y, Kato H. Elevation of serum alkaline phosphatase (ALP) level in postmenopausal women is caused by high bone turnover. Aging clinical and experimental research. 2015 Aug;27(4):413-8. PubMed PMID: 25534961.

15. Kuo TR, Chen CH. Bone biomarker for the clinical assessment of osteoporosis: recent developments and future perspectives. Biomarker research. 2017;5:18. PubMed PMID: 28529755. Pubmed Central PMCID: 5436437.

16. Naim MM. Histological assessment of zoledronic acid (Aclasta) in protection against induced osteoporosis in female albino rats. The Egyptian Journal of Histology. 2010;34:129–38.

17. Breuil V, Panaia-Ferrari P, Fontas E, Roux C, Kolta S, Eastell R, et al. Oxytocin, a new determinant of bone mineral density in post-menopausal women: analysis of the OPUS cohort. The Journal of clinical endocrinology and metabolism. 2014 Apr;99(4):E634-41. PubMed PMID: 24446658.

18. Yoo JH, Moon SH, Ha YC, Lee DY, Gong HS, Park SY, et al. Osteoporotic Fracture: 2015 Position Statement of the Korean Society for Bone and Mineral Research. J Bone Metab. 2015 Nov;22(4):175-81. PubMed PMID: 26713308. Pubmed Central PMCID: 4691591.

19. Colaianni G, Sun L, Zaidi M, Zallone A. The "love hormone" oxytocin regulates the loss and gain of the fat-bone relationship. Frontiers in endocrinology. 2015;6:79. PubMed PMID: 26042088. Pubmed Central PMCID: 4435037.