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Sertraline's deleterious effect on bone turnover in ovariectomized rats: The potential role of leptin, independent of estrogen.

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Abstract

Background: Selective serotonin reuptake inhibitors (SSRIs) are considered as a principal treatment for depression. They might exhibit a skeletal effect due to the existence of functioning serotonergic pathways in the bone evidenced by its harmful effects in the postmenopausal period. It was shown previously that leptin is involved in bone turnover regulation. **Objective:** The purpose of the present study was to investigate the effects of sertraline (an SSRI) on bone turnover of female rats, leptin involvement and its independence of that effect on estrogen. **Method**: Groups of bilaterally ovariectomized (performed 4weeks before starting treatment) & non-ovariectomized Wistar rats received sertraline (10&20mg/kg) for 4 weeks. Serum estradiols, serum osteocalcin, urinary hydroxyproline, mineral content of femur, serum leptin, and hypothalamic leptin receptor mRNA expression as well as histopathological studies were assessed. **Results**: The results showed sertraline increased bone turnover and decreased bone mineral content in both non-ovariectomized and ovariectomized rats. It also decreased the level of leptin peripherally as well as the hypothalamic leptin receptor mRNA expression in both OVX & NOVX groups. **Conclusion**: These findings suggest that SSRIs, which are frequently prescribed as antidepressants, have an undesirable impact on bone, which is probably not dependent on estrogen. Moreover, sertraline modulates leptin level centrally and peripherally, indicating its mechanistic involvement.

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Introduction

Bone formation & bone resorption are the two main pillars that regulate bone metabolism through the action of osteoblasts and osteoclasts consecutively. Osteoporosis, along other effects, is considered as sequelae of estrogen falling off after menopause interfering with estrogen's direct action on bone (Anolagas and Jilka, 1995).

Serotonin, a centrally acting neurotransmitter, was found to play a role in bone metabolism (Westbroek et al., 2001; Battaglino et al., 2004). Previous experimental studies investigated the interaction between the serotonergic system and bone. Serotonin had been suggested to play a role in regulating bone cell proliferation, differentiation, and activation in vitro (Gustafsson et al., 2006a). Femoral bone strengthening was witnessed with extended serotonin administration in rats (Gustafsson et al., 2006b). Additionally, the serotonin transporter was located in the different types of cells in the major bones (Bliziotes et al., 2001). Therefore, disruption of serotonergic balance with serotonin-interacting drugs might affect the normal bone metabolism (Gustafsson et al., 2006a).

From another prospective, fat-bone interaction is governed by endocrinal and paracrinal determinants. The adipose tissue produces local cytokines among which is leptin. It was discovered in 1994 by Friedman and colleagues; many years later were linked to bone mineral density (BMD) control (Reid, 2002; Zhang et al., 1994). This 16-kDa hormone, is chiefly expressed in the fat cells (Coen, 2004) and circulates either freely or bound to proteins. As for the leptin receptor, it belongs to the IL-6 receptors that are made of extracellular-binding, single transmembrane and cytoplasmatic signaling domains (Tartaglia et al., 1995). Serum leptin, bone mass index and fat mass crosstalk occurs after leptin crosses the blood–brain barrier, and reaches the hypothalamus, where it interferes with feeding being a pivotal regulator for that (Krysiak et al., 2012). Leptin exhibits profound effects in other versatile areas such as bone regulation (Patel et al., 2007). To date, little is known as regards the effect of sertraline on leptin expression.

Serotonin resembles leptin, in employing the sympathetic system in bone regulation (Elefteriou et al., 2005) through the ventromedial hypothalamic neurons. Multiple evidence points that leptin prevents bone mass accrual through serotonin inhibition in the brain (Yadav et al., 2009, Oury and Karsenty, 2011). Studies show the inconsistency of the effects of leptin and serotonin on the bone mass.

Given SSRI's remarkable selectivity for 5HT receptor, they have progressively been preferred over other antidepressants because they decrease the risk for comorbidities (Orleans et al., 2014). Sertraline, a selective

serotonin-reuptake inhibitor, is the usually prescribed drug in the management of depression and other psychological illnesses including postmenopausal depression. The net-worth ramification of inhibition the serotonergic system on bone mass is expectedly complex which could be attributed to the serotonin receptors and transporter being expressed on osteoclasts as well as osteoblasts (Valverde et al., 2005). In context, this explicates why in vivo research came up with different results (Battaglino et al., 2006; Warden et al., 2005). There are scanty experimental studies on SSRI's skeletal effects, mostly fluoxetine. As far as we can tell, the effects of other SSRIs on bone were not satisfyingly investigated in experimental studies. Taken all these points into consideration, the aim of this study was to explore how sertraline, an SSRI, influence female rats' bone turnover, peripheral leptin level, hypothalamic leptin receptor expression and whether their possible effects are estrogen dependent.

Material and Methods

Drugs and chemicals: Sertraline (Multi apex Pharma, Egypt), white crystalline powder; dissolved in distilled water was supplied as a gift from Professor Dr. Ahmad Abdel Salam (Professor of pharmacology, Ain Shams University). Sodium pentobarbital was purchased from Sigma-Chemicals (USA).

Animals:

Forty-two female Wistar rats weighing ~220 grams were supplied by the "Holding Company for Biological Products & Vaccines VACSERA, Helwan, Egypt". Rats were left to acclimatize for 1 week to the lab conditions. Food and water were provided as needed. Temperature was adjusted at 25˚C. A 12:12 light–dark cycle was ensured. All procedures were according to the guidelines of the Institutional Ethics Committee for the Faculty of Medicine, Ain Shams University.

Study Design:

Rats were allocated into 7 groups, (6 rats/each); group 1 was non-ovariectomized (NOVX) control group, 2 and 3: were treated orally with sertraline in a dose of 10 & 20 mg/kg, respectively. The dose of sertraline was selected according to the previous studies (Mos et al., 1999; Ozturk et al., 2013; Mukherjee et al., 2015). These doses correspond to 0.5 and 1 times the recommended human dose on a mg/m2 basis. Group 4 was sham operated, groups 5, 6, 7 were ovariectomized (OVX). Group 6 & 7 received sertraline 10 and 20mg/kg respectively for 4 weeks. Treatments were started 4 weeks after ovariectomy.

Ovariectomy:

"Ovariectomy was performed under sodium pentobarbital anesthesia (40 mg/kg, i.p.) (Gaertner et al., 2008). A dorsal single incision was done in the skin and through it two side incisions, in the muscle layer were made, approximately half a centimeter below each kidney to reach the peritoneal cavity. Through the incision, the ovaries were pulled out, ligated, and removed. The start of treatment was 4 weeks after a convalescence period. The same procedure was done for the sham-operated animals without the ovary being removed". Recording of the rats' weight was done at the beginning and weekly till the end of the study. In the last week of the experiment, urine samples were collected using metabolic cages. Urine was acidified with 2 ml of Hydrochloric acid (1 M) and centrifuged for a10-minute duration to remove any sediments; aliquots were stored at- 20^{o} C until they were worked on (Xie et al., 2005).

Eight weeks post-ovariectomy, serum samples were kept at -80^{o}C until being assayed. Dissection of the left tibia as well as the femur was performed. Removal of the brain, dissection of the hypothalamus on ice was done fast, that was stored at -80°C for later. The left femur was thawed, then autoclaved for 15 minutes at 110^{o} c and divested of any soft tissue for calcium and phosphorus measurement. The left tibia was fixed in 10% formalin for histopathological examination.

Outcome Measures:

Urine Analysis: Bone resorption marker, urinary hydroxyproline, was measured using Hydroxyproline Colorimetric Assay Kit from BIOVISION, INC.

Serum Analysis: Bone formation marker; serum osteocalcin (OC) was measured using a rat OC EIA Kit (Biomedical Technologies Inc., USA) based on the instructions of the manufacturer. Serum leptin & estradiol (E2) were measured using Mouse/Rat Estradiol ELISA from Calbiotech Inc. and Rat Leptin ELISA kit from Abcam, respectively.

Bone Mineral content: "The removed left femur underwent ashing at 700 °C for 7 hours, which was then dissolved in 6 M hydrochloric acid (1 ml of acid for 50 mg of ash), to prepare it for measuring calcium and phosphorus in the bone. After neutralization with sodium hydroxide and suitable dilution, calcium was measured by spectrophotometer using the calcium assay kit (Cayman chemical company, USA). Phosphorus was measured by spectrophotometer". The method described previously by Fisher and Higgins (1994), using Quantichrom phosphate assay kit (quantitative colorimetric phosphate determination).

Leptin receptor m-RNA expression in hypothalamus: "Extraction of the total RNA from the hypothalamus was done. To summarize, tissues were homogenized using Tissue Lyser LT (Qiagen) as per the manufacturer's instructions. The RNA concentration and purity were determined by spectrophotometry at 260- and 280 nm- wavelengths. The mRNA was transcribed to cDNA with Revert Aid First Strand cDNA Synthesis Kit (Fermentas) using 1 µg of total RNA and Oligo (dT) primers supplied in a kit. The samples were normalized using the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) Primers used in the PCR reaction were as follows: GAPDH, forward: 5'-TGCTGGTGCTGAGTATGTCG -3', reverse: 5'-TTGAGAGCAATGCCAGCC-3'; leptin receptor, forward: 5'– GAGAGGCTGCTGAAATCGTC-3', reverse: 5'- CTCCAGACTCCTGAGCCATC-3'. The gene expression was evaluated using quantitative realtime PCR. The measurements were done with SYBR Green chemistry with step one plus real-time PCR detection system (Applied Biosystem). The PCR program started with 3 min in 95°C. The 40 cycles of the PCR program consisted of 10 s at 95°C, annealing

for 10 s and extension at 72°C for 10 s. These were followed by the last step of 2 min at 72°C. Data were analyzed and quantified using the v1·7 Sequence Detection Software from PE Biosystems (Foster City, CA). Relative expression of studied genes was calculated using the comparative threshold cycle method. All values were normalized to the GAPDH genes" (Livak and Schmittgen, 2001).

Histopathological assessment: "Fixation of the left tibias in 10% neutral formalin, decalcification in 5% ethylenediamine tetracetic-acid for a 7 day- period, embedding in paraffin was done before being cut into longitudinal sections of 5 mm in thickness. Sections were stained with hematoxylin and eosin to be examined by the light microscope" (Mankin et al., 1971).

Statistical analysis

The statistical analyses of data were performed using Prism graphpad software. Results are expressed as mean ± SEM. One-way ANOVA, followed by Tukey's post hoc test was performed to analyze the difference between groups. $P < 0.05$ was considered as statistically significant.

Results

The effect of sertraline on body weight:

All the OVX groups whether treated or untreated, showed a significant ($p<0.05$) increase in body weights of animals recorded at the end of the experiment compared to the sham operated animals. There were no statistical significant difference in weight observed between either of the OVX groups that received sertraline and that of the untreated OVX control group (Fig 1).

The effect of sertraline on serum estradiol:

As depicted in table 1, following ovariectomy, serum estradiol level dropped significantly from 29.98 in the sham operated rats to 3.3 pg/ml (89% decrease), denoting an estrogen deficiency status. Sertraline treatment, in either of its doses, had no significant effect on serum estradiol level whether in OVX or in NOVX groups compared to their appropriate control groups.

The effect of sertraline on urinary hydroxyproline and serum osteocalcin:

Table 1 also shows that ovariectomy caused an increase in bone turnover manifested by an increase in both bone resorption and bone formation cursors. This could be evidenced by a significant increase ($p<0.001$) in OVX urinary hydroxyproline as well as serum osteocalcin compared to sham operated rats, with a 4.02- and 4.25-folds increase respectively.

Interestingly, both doses of sertraline significantly increased both markers in NOVX and OVX rats. Sertraline administration in OVX rats, in comparison to the OVX control group, dose dependently increased urinary hydroxyproline significantly (1.09-fold at 10mg/kg and 1.24 folds at 20mg/kg), likewise increased serum osteocalcin (2.19 folds at 10mg/kg and 3.58 folds at 20mg/kg). While Sertraline administration in NOVX rats resulted in a dose dependent significant increase in serum osteocalcin of 2.92 & 4.91 folds ($p<0.001$) and urinary hydroxyproline 3.57 and 2.63 folds at 10 & 20 mg/kg respectively compared to the NOVX control group.

Effect of sertraline on left femur bone mineral content:

Table 2 shows that ovariectomy significantly decreased the bone mineral content of calcium and phosphorous by 17%, 19% respectively compared to sham rats. Oral administration of sertraline significantly decreased calcium and phosphorus content in NOVX as well as OVX rats. Sertraline administration in OVX rats, as compared to OVX control group, decreased calcium content 12.5% & 8.3%, and decreased phosphorus 15.8% & 31.6% at 10 & 20 mg/kg, respectively. In parallel, in NOVX rats, not only did sertraline administration decrease calcium content 10.3% & 17.2%, but also decreased phosphorus by 19.23% & 30.7% at 10 & 20 mg/kg, respectively, in comparison to NOVX control animals.

Effect of sertraline on peripheral serum leptin and leptin receptor-mRNA expression in hypothalamus:

As shown in table 3, ovariectomy significantly increased the serum leptin level 20.8%, while it decreased leptin receptor (LR)-mRNA expression in hypothalamus by 40.6%. Oral administration of sertraline significantly decreased both serum leptin and LR-mRNA expression in hypothalamus whether in NOVX and OVX rats as compared to the corresponding controls. Sertraline administration in OVX rats decreased serum leptin by 18.75% & 33.33% in high dose, LR-mRNA expression in hypothalamus by 29.7% & 42.7% at 10 & 20 mg/kg, respectively, as compared to OVX rats. Furthermore, sertraline administration in NOVX rats significantly decreased serum leptin by 8.3% & 16.6%, as well as LR-mRNA expression in hypothalamus by 14.5% & 29.1% at 10 & 20 mg/kg, respectively, as compared to NOVX control animals.

Effect of sertraline on bone histopathology of left tibia:

Examination of sections of the NOVX control group showed the compact bone with its outer, interstitial, and inner bone lamellae in addition to Haversian systems. Osteocytes inhabited their lacunae in between the lamellae. The shell of compact bone was seen covered by its periosteum and lined by the endosteum. Examining the OVX-rats' sections revealed the outer cortical bone thinning in comparison to the Sham operated animals, cracks in bony cortex and empty bone lacunae and osteoblasts proliferation were also detected in some areas. Sections in the OVX treated rats showed almost the same histopathological findings as those of the OVX-rats. The thickness of the cortical bone was remarkably thinned analogous to the OVX group (Fig 2).

Table (1): The effect of oral administration of sertraline (10&20mg/kg) on urinary hydroxyproline, serum osteocalcin and serum estradiol levels in non -ovariectomized and ovariectomized female Wistar rats:

Animal groups (n=6)	Urine hydroxyproline (mg/dl)	Serum Osteocalcin (ng/ml)	Serum estradiol (pg/ml)
Grp 1: Normal control	13.9±0.4572	14±0.11	30.33±0.97
Grp 2: Sert10mg	49.67±1.52^{aaa}	40.88±0.94^{aaa}	28.18±1.51
Grp 3: Sert20mg	36.58±0.77^{aaa}	68.83±1.47^{aaa}	35.48±2.87
Grp 4: Sham operated	14.87±0.82	14.50±0.32	29.98±0.73
Grp 5: OVX	59.78±1.14^{bbb}	61.65±1.30 ^{bbb}	3.3±0.33^{bbb}
Grp 6: OVX+Sert10mg	65.62±1.51^{c}	135.5±3.55^{ccc}	3.6±0.24
Grp 7: OVX+Sert20mg	74.2±2.16^{ccc}	220.7±4.57 ^{ccc}	7.8±0.48

Data are presented as mean±SEM, One way ANOVA followed by Tukey's Multiple Comparisons test: $^{aaa}p<0.001$, compared to normal control group.$^{bbb}p<0.001$ compared to sham operated group, $^{c}p<0.05$, $^{ccc}p<0.001$ compared to the OVX group, Sert=sertraline, OVX= ovariectomized.

Table (2): The effect of oral administration of sertraline (10&20mg/kg) on left femur bone mineral content in non -ovariectomized and ovariectomized female Wistar rats:

Animal groups (n=6)	Femur phosphorus (mg/g ash)	femur calcium (mg/g ash)
Grp 1: Normal control	6.22±0.2	13.14±0.46
Grp 2: Sert10mg	5.01±0.12^{a}	12.24±0.45^{a}
Grp 3: Sert20mg	4.44±0.2^{a}	11.52±0.3^{a}
Grp 4: Sham operated	5.88±0.19	12.96±0.26
Grp 5: OVX	4.66±0.15^{b}	10.81±0.73 ^{b}
Grp 6: OVX+Sert10mg	4±0.24^{c}	10.07±0.4^{c}
Grp 7: OVX+Sert20mg	3.54±0.32^{c}	10.3±0.55 ^{c}

Data are presented as mean±SEM, One way ANOVA followed by Tukey's Multiple Comparisons test: $^{a}p<0.05$, compared to normal control group.^{b} $p<0.05$ compared to sham operated group, $^{c}p<0.05$ compared to the OVX group, Sert=sertraline, OVX= ovariectomized.

Table (3): The effect of oral administration of sertraline (10&20mg/kg) on peripheral serum leptin and leptin receptor-mRNA expression in hypothalamus in non -ovariectomized and ovariectomized female Wistar rats:

Animal groups (n=6)	S. leptin (ng/ml)	Hypothalamic leptin expression/GAPDH
Grp 1: Normal control	10.08 ±1.05	0.75±0.05
Grp 2: Sert10mg	9.36±0.25	0.62±0.02^{a}
Grp 3: Sert20mg	8.28±0.86^{a}	0.53±0.09^{a}
Grp 4: Sham operated	7.95±0.93	0.62±0.07
Grp 5: OVX	9.9±0.54^{b}	0.37±0.05 ^{b}
Grp 6: OVX+Sert10mg	8.06±0.82^{c}	0.25±0.0.04^{c}
Grp 7: OVX+Sert20mg	10.8±0.35^{c}	0.2±0.03 ^{c}

Data are presented as mean±SEM, One way ANOVA followed by Tukey's Multiple Comparisons test: $^{a}p<0.05$, compared to normal control group.$^{b}p<0.05$ compared to sham operated group, $^{c}p<0.05$ compared to the OVX group, Sert=sertraline, OVX= ovariectomized.

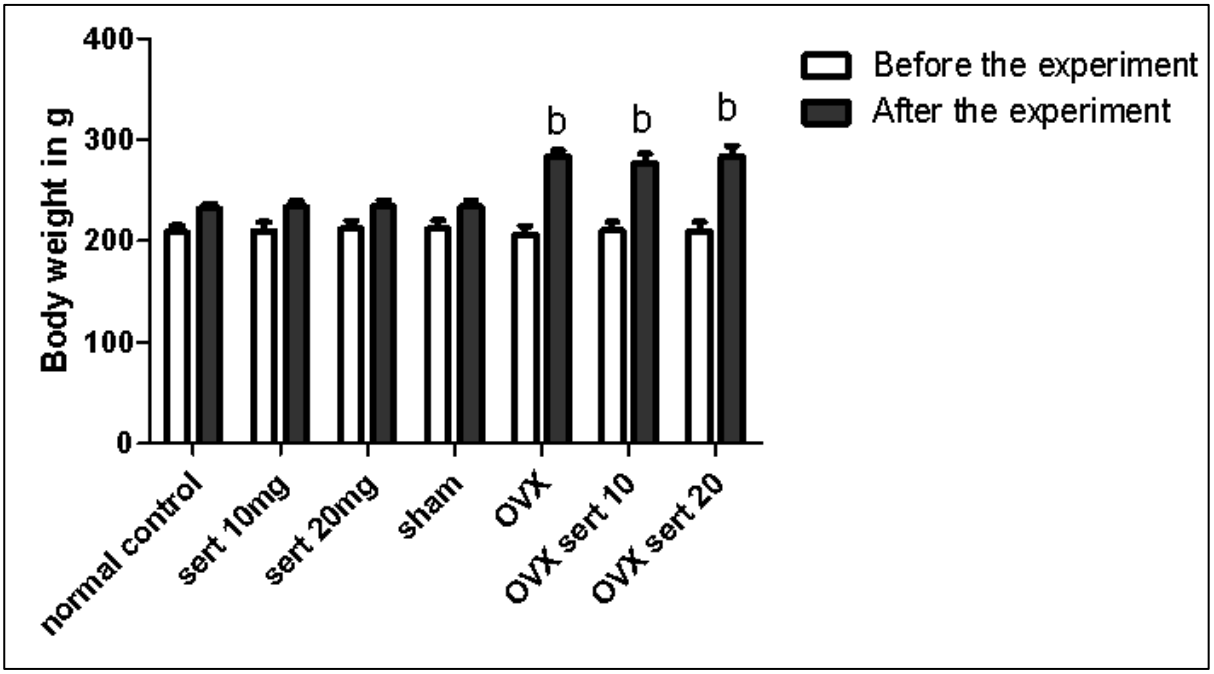

Figure (1): The effect of sertraline treatment (10 & 20m/kg) on the body weight in OVX and NOVX female rats. Data are expressed as mean±SEM. (n=6). One way ANOVA was used followed by Tukey's Multiple Comparisons test: [b:] $p<0.05$, compared to sham operated group. OVX= ovariectomized, NOVX= non-ovariectomized, sert=sertraline.

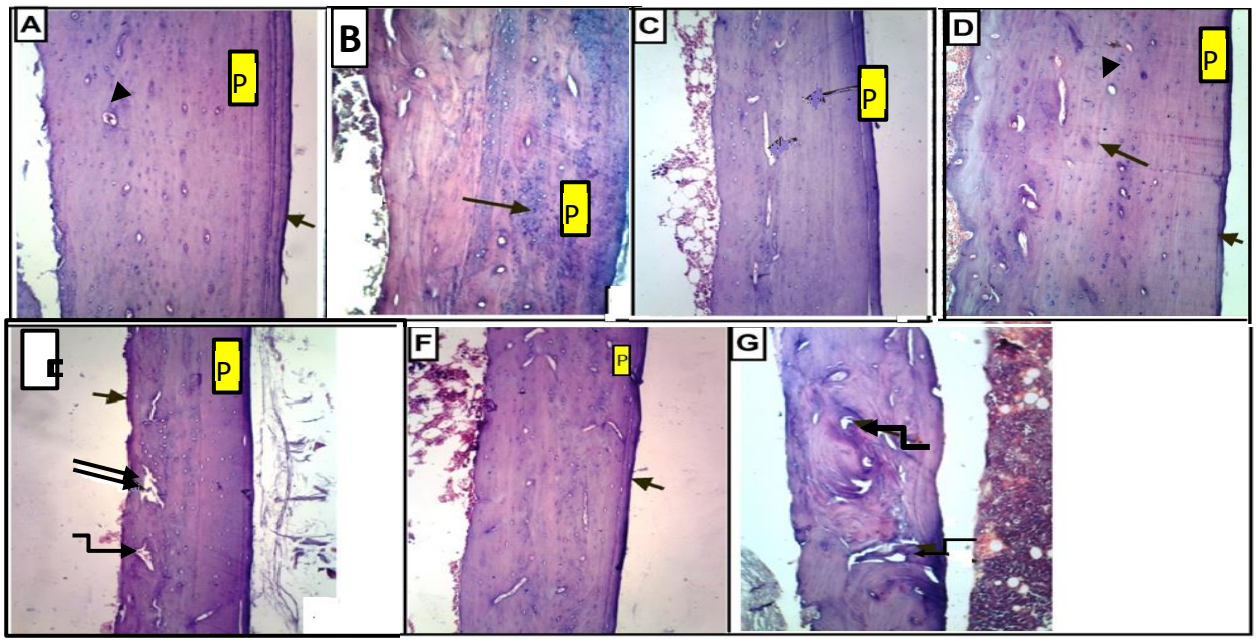

Figure (2): H&E-stained sections (X 100) showing cortical bone from Wistar rats of different groups: A) control group: showing cortical bone covered with thin periosteum (↑). Haversian canals (▲) and osteocytes lacunae are seen; B) NOVX sertraline (10mg/kg) treated group: showing an apparent increased thickness of periosteum (↑) with proliferation of osteoblasts in the inner osteogenic layer.; C) NOVX sertraline (20mg/kg) treated group: showing an apparent decreased thickness of cortical bone; D) Sham group: showing cortical bone covered with periosteum (↑). Haversian canal (▲) and osteocytes lacunae are seen. Notice cortical thickness; E) OVX non treated: showing thin periosteum covering the cortical bone. Dilated haversian canal (elbow arrow) and irregular wide cavity (↑↑) are seen in the cortical bone near the endosteum; F) OVX sertraline (10mg/kg) treated group: showing thin cortical thickness; G) OVX sertraline (20mg/kg) treated group: showing marked osteoporotic changes (thin cortical bone, and widened haversian canal (elbow arrow).

Discussion

Serotonin, glutamate, and dopamine are neurotransmitters released by the central nervous system, which participate in governing bone homeostasis. Serotonin receptors are allocated in most bone cells, suggesting the importance of the neuroendocrinal system in bone (Rizzoli et al., 2012). The different bone cells express G protein-coupled receptors of variant subtypes for serotonin, which includes receptors such as 5-HT1A & 5-HT2A, serotonin transporter (5-HTT) and the enzyme involved in serotonin synthesis, creating the serotoninergic system in bone (Bliziotes et al., 2006). Intriguingly, the exact mechanism by which serotonin regulates bone activity still needs further studying and elucidation (Gerosa et al., 2021).

With the dramatic increase in antidepressants' use on top of which are SSRIs, adverse events are being encountered, including the growing risk of fractures and decreased bone mineral density in youngsters. Moreover, post-menopausal females, who are considered as one of the high-risk groups, which are on SSRIs were correlated with increased risks of fractures superseding glucocorticoids and proton pump inhibitors (Howie et al, 2018).

Previous clinical research points out an intricate interaction between osteoporosis, fractures, depression, and antidepressants. The current approach for evaluating drug effect on osteoporosis is directed mainly at both basic processes of bone remodeling (resorption and formation) (Xie et al., 2005).

Among the types of primary osteoporosis, type I; also known as postmenopausal osteoporosis, is considered the most common type of the disease. In this matter, ovariectomy is considered as a comparable model for studying type I osteoporosis because of the alikeness in their pathophysiology in the bone insult (Frost and Jee, 1992). The underlying mechanism by which estrogen deficiency results in bone loss remains unsettled and several postulations have been proposed (Komm and Bodine, 2001; Avenell et al., 2014).

Evaluation of drug effect on bone osteoporosis is increasingly needed, especially with the extended periods of drugs' administration. Hence, the present study aimed at investigating the effect of sertraline on bone turnover of female Wistar rats, and whether its possible effects are subject to estrogen. This study was done on NOVX and OVX rats in an attempt to clarify this. It is well known that ovariectomy induces weight gain (Wellberg, 2022). Similarly in the present study, bilateral ovariectomy resulted in an increase in the body weight of rats, which was not affected by the 4-week administration of sertraline. Estrogen insufficiency is thought to be responsible, to a large extent, for a rise in fat during menopause, which being consistent with a previous study of an estrogen insufficiency animal model which displayed an ongoing increase in fat content (Heine et al., 2000; Curtis et al., 2018).

Determining the activity of osteoclasts and osteoblasts biochemically reflects bone resorption and formation respectively and is considered as bone metabolism sensitive markers (Weisman and Matkovic 2005). Most of the osteocalcin, which is synthesized by osteoblasts, is entangled into hydroxyapatite, while a small fraction escapes into blood, thus mirroring bone formation. (Dogan and Posaci 2002). However, during bone resorption, some is released from bone, hence it is an indicator of bone turnover generally. The bone collagen breakdown products have been designated to evaluate bone breakdown, of those, hydroxyproline which is eliminated in urine. Therefore, urinary hydroxyproline is viewed as an echoing of bone resorption (Seibel, 2005; Kuo et al., 2017).

Urinary hydroxyproline and serum osteocalcin showed a significant increase in OVX rats. Treatment with sertraline significantly and dose dependently added to the elevated osteocalcin and urinary hydroxyproline induced by estrogen deficiency OVX rats, indicating the increase in demolition. Furthermore, those markers showed similar outcomes in NOVX rats. These changes were associated with decrease bone mineral content as evidenced by reduction of calcium content, ascertaining its role in bone loss. Comparable results were observed with fluoxetine and Fluvoxamine (Folwarczna et al., 2009; Ortuño et al., 2016). Along with that, Park et al in 2018, stated that 5-HT 6 receptor dysregulation, through using an osteoporosis model on a ($5\text{-HT}_6\text{R}$) knock out mice, accelerated osteoclasts maturation causing bone losing. On the contrary, a 5HT 2A/2C antagonist or a selective 5-HT 2B receptor antagonist raised the compact bone calcium content, but not in the cancellous bone. However, the femoral neck's strength was significantly decreased in NOVX (Folwarczna et al., 2010). This might be related to the type and site of the examined bone or differences in drug selectivity to affect brain and gut serotonin (Gerosa et al., 2021). Our study also showed a deterioration of bone by sertraline being independent of estrogen as it was manifest in NOVX rats as well. Therefore, serotonin is a molecule with two dissimilar functions depending on its site of synthesis (Walther et al., 2003; Yadav et al., 2008). Thus, skeletal effects of drugs affecting serotonergic system should be considered separately.

In the present study, estrogen deficiency in OVX rats is plausible to be the reason behind bone mineral content diminishing (Deyhima et al. 2003; Wen et al., 2018). In a number of studies, estrogen paucity led to a significant rise in osteoclasts' number, increasing the risk of osteoporosis (Ishimi et al. 1990; Rahnama 2004; Zhang et al. 2009). Worth mentioning that estrogen deficiency was not affected in our study by the chronic administration of sertraline.

Leptin, an adipocyte-derived protein/cytokine, which is well known to help in the regulation of energy balance as well as food intake and, it does this through its widespread receptors (White et al., 1997; Gerosa et al., 2021), under the control of variant genes (Chung et al., 1997). Among those, the long form, which is the most, represented one in the brain particularly in the hypothalamus (Baskin et al., 1999). Peripherally, it is

expressed in the liver, skeletal muscles, and bone (Hamrick & Ferrari, 2008). Leptin interacts with brain derived serotonin in the midbrain favoring negative effect on bone (Yadav et al., 2009). In this context, sertraline was proposed to exert its effect by leptin modulation. In the current study, leptin level increased peripherally, and a downregulation of the hypothalamic leptin receptor was noticed in the OVX group. Whereas treatment with sertraline decreased both leptin level peripherally and downregulated hypothalamic leptin receptor in NOVX and OVX rats.

Leptin is thought to regulate bone resorption via two antagonistic mechanisms. It inhibits bone resorption as evident by reducing bone loss in OVX rats by subcutaneous injection of leptin (Burguera et al., 2001). In another study, leptin treatment inhibited the osteoclastic differentiation through osteoprotegerin upregulation and receptor activator of nuclear factor-kappa B ligand (RANKL) downregulation (Holloway et al., 2002). On the other hand, injection of leptin intracranially resulted in bone mass loss in normal mice (Takeda and Karsenty, 2001). Further, leptin deficiency or its receptors resulted in an increased bone mass phenotype in mice (Karsenty, 2001). Another study stated that leptin boosted differentiation of osteoclasts via its hypothalamic receptors (Elefteriou et al., 2005).

The results showed a decrease in the hypothalamic leptin receptor expression by sertraline, suggesting a diminished brain leptin signaling. Thus, sertraline governs leptin signaling by two aspects. On one hand, it decreased peripheral leptin levels in NOVX and OVX. On the other hand, it decreased hypothalamus receptor levels with overall negative effect on the bone metabolism.

Further concurring of the deleterious effect that OVX and/or sertraline had on the bone, histological examination of the bones showed that OVX rats showed thinning of the periosteum covering the cortical bone, dilated haversian canal and irregular wide cavities in the cortical bone near the endosteum, with decreased thickness of the cortex. Variant degrees of this finding were seen in the sertraline treated OVX & NOVX rats being more profound with the higher dose of sertraline (20 mg/kg).

Conclusion

These data suggest that a frequently prescribed SSRI; sertraline, has a damaging impact on female bones which is probably independent of estrogen hormone deficiency. Moreover, sertraline modulates leptin level peripherally as well as its hypothalamic receptor gene expression, indicating its role in sertraline's effect on bone. These results support clinical data denoting SSRI use leads to faster postmenopausal bone loss and spotlights the role of leptin in explaining the skeletal effects of SSRIs.

Conflict of Interest

No conflict of interest to declare.

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تأثير عقار سيرترالين الضار على دورة العظام في الفئران بعد استئصال المبيض: الدور المحتمل للبتين، المستقل عن الإستروجين

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الملخص العربي

المقدمة: تعتبر مثبطات امتصاص السيروتونين الانتقائية (SSRIs) علاج رئيسي للاكتئاب. ويظهر لهم تأثيرًا هيكليًا بسبب وجود مسارات هرمون السيروتونين العاملة في العظام واللذي يتضح عن طريق آثارها الضارة في فترة ما بعد انقطاع الطمث. وقد تبين سابقًا أن اللبتين يلعب دور في تنظيم دورة العظام. **هدف الدراسة:** الغرض من هذه الدراسة هو دراسة تأثير عقار سيرترالين (مثبطات استرداد السيروتونين الانتقائية) على دوران العظام فى إناث الفئران ، ودور اللبتين واستقلال هذا التأثير عن هرمون الاستروجين. **الطريقة:** مجموعات الفئران منقسمين ما بين فئران مستأصلة المبيض ثنائي الجانب (أجريت 4 أسابيع قبل بدء العلاج) وفئران بدون استئصال للمبيض، وتعطى فئران ويستار سيرترالين (10 و 20 ملجم / كجم) لمدة 4 أسابيع. تم تقييم استراديول وأوستيوكالسين فى الدم، هيدروكسي برولين البولي، المحتوى المعدني لعظم الفخذ، اللبتين فى الدم وكذلك تعبير الRNA المرسال لمستقبل اللبتين تحت المهاد وكذلك الدراسات النسيجية المرضية. **النتائج:** أظهرت النتائج زيادة السيرترالين لمعدل دورة العظام وانخفاض محتوى المعادن في العظام في كل من الفئران المستأصلة المبيض وغير المستأصلة. كما أنه قلل من مستوى اللبتين فى الدم وكذلك تعبير الRNA المرسال لمستقبل اللبتين تحت المهاد في مجموعتي OVX و NOVX. **الخلاصة:** تشير هذه النتائج إلى أن مثبطات استرداد السيروتونين الانتقائية ، التي توصف كثيرًا كمضادات للاكتئاب ، لها تأثير غير مرغوب فيه على العظام، والذي ربما لايعتمد على الإستروجين.

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