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Original Article

Chronic Kisspeptin-10 Ameliorates Osteoporotic Changes in Orchidectomized Male Albino Rats: Involvement of Bone Morphogenic Protein-2 and Wnt/β-Catenin Signaling Pathways

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ABSTRACT

Background: Osteoporosis is a serious worldwide skeletal illness manifested by diminished bone density with increased susceptibility to fractures and increases with aging. Kisspeptin is a hypothalamic neuropeptide that regulates the gonadal hormones and was suggested to have a positive effect on bone metabolism. **Methods:** 30 male rats were divided equally in to three groups: Sham, orcidectomized (ORX), and kisspeptin-10 (kiss)-treated groups. Blood, urine, and femoral bone samples were taken for biochemical, histopathological, and gene studies. **Results:** ORX rats showed increased serum levels of alkaline phosphatase (ALP), osteocalcin, RANKL, Osteopetrogenin (OPG), RANKL/ OPG ratio, tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), while decreased serum OPG level and bone minerals associated with disruption of bone architecture compared with Sham group. Chronic treatment with kisspeptin-10 significantly enhanced bone architecture, increased bone minerals, improved RANKL/OPG ratio, up-regulated mRNA expression levels of OPG, bone morphogenic protein 2 (BMP-2), Wnt3a, and β-catenin as compared to untreated ORX rats. **Conclusions:** Chronic treatment with kisspeptin-10 enhances osteogenesis and reduces bone resorption in ORX rats via modulating RANKL/OPG ratio, BMP-2, and Wnt/β-catenin signaling pathways. So, the present study recommends kisspeptin-10 as a promising novel therapeutic agent for age-related osteoporosis in men.

Keywords: Osteoporosis; Kisspeptin; BMP-2; Wnt/β-catenin pathway.

INTRODUCTION

Osteoporosis is a global skeletal illness characterized by diminished bone quality and density with increased susceptibility to fractures [1]. The disturbed balance between bone resorption and formation is the leading cause of osteoporosis with subsequent distortion of bone microarchitecture. Deficiency of sex steroid hormones is the primary cause of this disturbance, although other risk factors and secondary causes also present [2].

Osteoporosis prevalence has increased in men with the ageing process and bone mineral density (BMD) was reported to decrease in men with the increase in age, particularly after 50 years old [3]. Also, fragility fractures in males were reported to be associated with increased rates of morbidity and mortality than in females, even though they are less frequent [4]. Male osteoporosis is closely associated with androgen deficiency and has been shown to be reversed by testosterone replacement therapy [5]. The effect of androgens on bone was attributed to a direct binding to the bone androgen receptors or through aromatization of testosterone to estrogen which binds to bone estrogen receptors [5, 6].

The existing treatments for osteoporosis have contraindications and are not effective in all patients besides the adverse effects, that limit the duration of therapy [7]. Therefore, identifying a novel effective and safe therapeutic agent for osteoporosis prevention and treatment becomes urgent.

Kisspeptin is a naturally occurring hypothalamic neuropeptide encoded by the KISS1 gene and is

critical for male and female reproduction via affecting the apex of the hypothalamic-pituitary-gonadal (HPG) axis [8]. Kisspeptin was suggested to have a positive effect on bone metabolism based on the finding of delayed bone maturation with loss of function mutation in one of the kisspeptin genes, but up-regulation of bone maturation and remodeling with Kisspeptin-activating mutations [9].

The expression of kisspeptin-1 receptor protein was demonstrated in MG-63 osteoblast-like osteosarcoma cells, human osteoprogenitor stem cells and human osteoclasts [10,11]. In vitro direct administration of kisspeptin in rodents was demonstrated to promote osteoblast differentiation via the kisspeptin receptor expressed on osteoblasts. So, it was hypothesized that kisspeptin might have valuable effects on bone homeostasis, independent of its stimulatory effect on sex steroid levels via affecting the HPG axis [12].

However, the chronic effects of kisspeptin on bone metabolism have not been studied yet, therefore we aimed to investigate the effect of chronic administration of kisspeptin-10 on osteoporotic changes in orchidectomized male albino rats and to clarify the possible involved molecular mechanisms.

METHODS

Ethical Approval: The experimental procedures were approved by the Committee for the Care and Use of Animals at the Faculty of Medicine, Zagazig University, with approval No (ZU-IACUC/3/F/406/2023).

1. Animals and experimental design

Thirty male albino rats of local strain; weighing 210–240 g and aged 26–28 weeks were obtained from Faculty of Veterinary Medicine, Zagazig University and then housed 5 animals/ steel wire cage (50 cm x 30 cm x 20 cm) in the Animal House of Physiology Department, Faculty of Medicine, Zagazig University at room temperature and under natural light/dark cycles. Rats were fed on a standard commercial diet, given food and water ad libitum, and were kept for 2 weeks before the start of experiments acclimatization for to the animal house conditions. The animals were divided randomly into three equal groups of ten rats each, as follows: (i) Sham-operated (Sham); injected with 0.2 ml saline/day intraperitoneally (ip), (ii) orchidectomized (ORX); injected with ip 0.2 ml saline/day, (iii) orchidectomized and kisspeptin (Kiss)-treated (Kiss-treated); injected with kisspeptin-10 (MyBioSource, Inc. San Diego, USA) at a dose of 400 nmole/kg/day in 0.2 ml volume of saline, i.p [13], All treatments started two days after the surgical operations and continued for eight consecutive weeks [14].

2. Induction of osteoporosis:

The overnight fasted rats of ORX and kiss-treated groups were anaesthetized ip with pentobarbital sodium (40 mg/kg body weight) then osteoporosis was induced by bilateral orchidectomy where both testes were explored and removed surgically as previously described [15]. Similarly, the rats of Sham group were anaesthetized, sham operated without excision of testes, and they served as control.

3. Blood sampling: At the end of the experiment, blood samples were collected under thiopental anesthesia (100 mg/kg, ip) from retro-orbital sinus in pre-labeled centrifuge tubes. The blood samples were centrifuged for 15 min at 3500 rpm. For biochemical analysis, serum was extracted and immediately stored at -20 ◦C.

4. Urine sampling: On the last day of the experimental period, rats were housed separately in metabolic cages in which funnels with pierced plastic discs were constructed at their bases to collect urine without the feces. The 24-hour urine was collected, the volume was estimated, and then centrifuged for 10 minutes at 3000 rpm to remove insoluble materials. The supernatant was stored at -20ºC until analysis for calcium and phosphorus concentrations.

5. Biochemical analysis:

Commercial kits (Sigma-Aldrich, St. Louis, USA) were used for analysis of serum levels of alkaline phosphatase (ALP), serum and urine concentrations of calcium, phosphorus using standard colorimetric methods according to manufacturer's guidelines. An enzyme linked immunoassay (ELISA) kits were used to analyze serum levels of testosterone (Shanghai Sunred biological technology, China), osteocalcin (Biomedical Technologies Inc., Stoughton, USA), osteoprotegerin (OPG), receptor activator of nuclear factor-κB ligand (RANKL) (Beijing North Biotechnology Co., China), tumor necrosis factor-α (TNF-α) (BioSource International Inc., California, USA) and interleukin-6 (IL-6) (Bio diagnostic- Egypt) according to the manufacturer's instruction. The ratio of serum RANKL/OPG was calculated.

6. Histological examination of femur bones:

The right femurs were removed from anesthetized rats, carefully dissected and cleaned from adherent tissues, then placed in 10 % formalin solution for one day. Thereafter, the tissues were dehydrated in ascending grades of ethanol, cleared with xylene and embedded in paraffin wax. Then,

the fixed tissues were sliced into 5 microns sections using a standard rotary microtome. Then, all sections were stained with hematoxylin and eosin (H&E) and examined by light microscope at 200× magnification to detect the histopathological changes as previously described [16]. The trabecular thickness was calculated, and the number of osteocytes, osteoclasts and resorbed bone cavities were counted per 10 HPF.

7. Bone ashing:

The femur bone was dried overnight at 100°C, then burned at 1000°C in muffle apparatus for 12 hours to get the ash. The ash weight was measured, then hydrolyzed with 6 N HCL to measure calcium and phosphorus by calorimetric methods as previously described [17].

8. Gene expression studies:

8.1. Bone preparation: The left femur bones were extracted, attached soft tissues were removed, and the bones were rinsed with cold RNAase-free phosphate-buffered saline (Sigma-Aldrich, St. Louis, USA) before being flash-frozen with liquid nitrogen (Malaysian Oxygen, Malaysia). The bones were then stored at −70°C until RNA extraction [18].

8.2. RNA Extraction and Quality Test: Purification of total RNA was done using the Rneasy Lipid Tissue Mini Kit according to the instructions of the manufacturer (Qiagen, Germany). RNA samples obtained were then stored at −70°C until polymerase chain reaction (PCR) analysis was carried out. All RNA samples were quantified using the NanoDrop ND-1000 for quality control .

8.3. Quantitative Real-Time PCR (qRT-PCR) was conducted using the iScript One-Step RT-PCR Kit with SYBR Green (Bio-Rad, USA). The PCR products were resolved on 2% agarose gel. Agarose gels were photographed for densitometry analysis using gel doc UV chamber with Quality One software (Bio-Rad, USA). The primer sequences of the studied genes are represented in **Table 1.** Relative gene expression levels were evaluated, with GAPDH serving as an internal control.

STATISTICAL ANALYSIS

The data were represented as the mean ± standard deviation (SD). SPSS software program, version 19 was used for statistical analysis **(**SPSS Inc. Chicago, IL, USA). Kolmogorov-Smirnov test was done to test the normality of data. The data were normally distributed, and one-way ANOVA followed by Tukey's post hoc test was used to identify significant differences between studied groups. $P<0.05$ was established for significance of statistical tests.

RESULTS

1. Biochemical findings:

1.1. Serum testosterone: A significant reduction in testosterone concentration was found in ORX rats as compared to Sham group, while no significant difference was found between ORX and Kiss-treated rats **(Table 3)**.

1.2. Serum, urine and bone concentrations of calcium and phosphorus and femoral bone ash weight: As expressed in **Table 2,** no significant difference was found in serum levels of calcium and phosphorus between the three studied groups. A significant increase in urine calcium and phosphorus concentrations was found in ORX rats as compared to Sham group, while Kiss-treated rats showed a significant reduction in their concentrations as compared to ORX rats, but still significantly increased as compared to Sham group. Conversely, a statistically significant decline in femur bone concentrations of calcium and phosphorus and femoral bone ash weight were found in ORX rats as compared to Sham group, while Kiss-treated rats showed a significant increase in these parameters as compared to ORX rats, but still significantly decreased as compared to Sham group.

1.3. Serum bone turnover markers: As demonstrated in **Table 3,** a significant decrease in serum OPG, while a significant increase in serum ALP, osteocalcin, RANKL and RANKL/OPG ratio were found in ORX rats as compared to Sham groups. These parameters were significantly reversed in Kiss-treated rats as compared to ORX rats, but still significantly different as compared to Sham group.

1.4. Serum inflammatory markers: As shown in **Table 3,** a significant increase in serum TNF-α and IL-6 levels was found in ORX rats as compared to Sham groups. These markers were significantly decreased in Kiss-treated rats as compared to ORX rats, but still significantly different as compared to Sham group.

2. Histopathological and morphometric findings:

Histopathological examination of the longitudinal bone sections from the distal femoral diaphysis of Sham group **(Figure 1.a)** revealed a classic bone architecture with the cortical bone comprising few acidophilic large osteoclasts and many small osteocytes. However, bone sections from ORX group **(Figure 1.b)** showed a loss of the classic bone architecture, an increase in the osteoclasts number and resorbed bone cavities and a faint cement line of subperiosteal bone deposition compared to the Sham group, indicating minor deposition of new bone matrix. Conversely, Kiss-

treated group **(Figure 1.c)** demonstrated improved bone architecture, few subperiosteal resorbed bone cavities, few osteoclasts, abundant osteocytes and obvious subperiosteal cement lines demarcating bone deposition compared to ORX group.

Statistical analyses of morphometric data **(Table 4)** revealed that the trabecular thickness and osteocytes number were significantly decreased, while the number of osteoclasts and resorbed bone cavities were significantly increased in ORX group as compared to Sham group. The former parameters were significantly reversed in Kiss-treated group in comparison to the ORX group, while still significantly different from the Sham group.

3. Gene expression findings:

As represented in **(Table 5)**, mRNA expression level of RANKL was significantly up-regulated, while those of OPG, BMP-2, Wnt3a, and β-catenin were significantly down regulated in ORX rat's femur bones as compared to Sham group. Chronic treatment of ORX rats with kisspeptin-10 significantly down regulated mRNA expression level of RANKL, while up-regulated those of OPG, BMP-2, Wnt, and β-catenin in comparison to untreated ORX rats.

Table 1: Primer sequences used for qRT-PCR

Primer sequence of the studied genes	
OPG	Forward: 5′-ACGCGGTTGTGGGTGCG-3′ Reverse: 5′-AAGACCGTGTGCGCCCC-3′
RANKL	Forward: 5′-CAGAAGATGGCACTCACTG-3′ Reverse: 5′-CACCATCGCTTTCTCTGC-3′
Bone morphogenic protein 2 (BMP-2)	Forward: 5′-TGA ACA CAG CTG GTC TCA GG-3′ Reverse: 5′-TTA AGA CGC TTC CGC TGT TT-3′
Wnt3a	Forward: 5'-TCCATgCCATCgCTTCC-3' Reverse: 5'-TCACTACAgCCACCCCACTTC-3'
B-catenin	Forward: 5'-GTCTGAGGACAAGCCACAGGACTAC-3' Reverse: 5'-AATGTCCAGTCCGAGATCAGCA-3'
GAPDH	Forward: 5′-GGAGTCTACTGGCGTCTTCAC-3′ Reverse:5′-ATGAGCCCTTCCACGATGC-3′

Table 2: Serum, urine and bone concentrations of calcium and phosphorus and femoral bone ash weight in all studied groups

	Sham	**ORX**	**Kiss-treated**
Calcium (mg/dl)	9.56±1.01	9.18±0.54	9.49±0.92
Phosphorus (mg/dl)	5.93±0.48	6.55±0.86	6.39±0.74
Urinary calcium (mmol/L)	3.12±0.13	4.34±0.2[a]	3.65±0.11[a,b]
Urinary phosphorus (mmol/L)	7.75±0.53	11.56±0.71[a]	9.42±0.51[a,b]
Femoral calcium (mg/gm ash)	152±9.13	109±5.08[a]	134±5.16[a,b]
Femoral phosphorus (mg/gm ash)	76.8±4.7	42±5.6[a]	59.8±5.7[a,b]
Femoral bone ash weight (mg/femur)	243.2±7.05	201.8±8.02[a]	227.1±4.29[a,b]

a: significant vs Sham group, b: significant vs ORX group

Table 3: Serum testosterone, bone turnover and inflammatory parameters in all studied groups

	Sham	ORX	Kiss-treated
Testosterone (ng/ml)	7.05±1	0.16±0.06[a]	0.26±0.08
ALP (U/L)	137.40±8.03	172.20±7.05[a]	150.50±7.99[a,b]
OPG (ng/ml)	5.42±0.59	2.75±0.33[a]	3.84±0.52[a,b]
RANKL (ng/ml)	1.87±0.31	4.10±0.54[a]	2.81±0.39[a,b]
RANKL/OPG ratio	0.35±0.08	1.51±0.25[a]	0.74±0.13[a,b]
Osteocalcin (ng/ml)	12.57±0.83	17.49±1.03[a]	14.50±0.76[a,b]
TNF-α (pg/ml)	21.83±1.95	39.16±3.44[a]	28.59±1.04[a,b]
IL-6 (pg/ml)	50.69±3.77	79.29±3.34[a]	62.37±4.56[a,b]

a: significant vs Sham group, b: significant vs ORX group

Table 4: Bone morphometric measurements in all studied groups

	Sham	ORX	Kiss-treated
Tr. Thickness (µ)	67.28±11.53	21.62±3.33[a]	48.35±7.29[a,b]
Osteocytes No./ 10 HPF of bone tissue	283.2±9.5	207.4±10.3[a]	242.4±8.8[a,b]
Osteoclasts No. /10 HPF of bone tissue	12.3±1.3	29.1±3.2[a]	20.1±1.6[a,b]
Bone cavities No. /10 HPF of bone tissue	2±0.7	9.4±1.1[a]	5.6±1.2[a,b]

a: significant vs Sham group, b: significant vs ORX group

Table 5: Changes in mRNA expression levels of studied genes in all groups

	Sham	ORX	Kiss-treated
OPG	0.9±0.09	0.5± 0.05 [a]	0.78±0.07 [a,b]
RANKL	0.8±0.03	1.3±0.04 [a]	0.95±0.02 [a b]
BMP-2	0.94± 0.05	0.54±0.04 [a]	0.82±0.07 [a,b]
Wnt3a	1.2± 0.03	0.71±0.01 [a]	0.91±0.03 [a,b]
β-catenin	1.1± 0.04	0.68±0.05 [a]	0.9±0.04 [a,b]

a: significant vs Sham group, b: significant vs ORX group

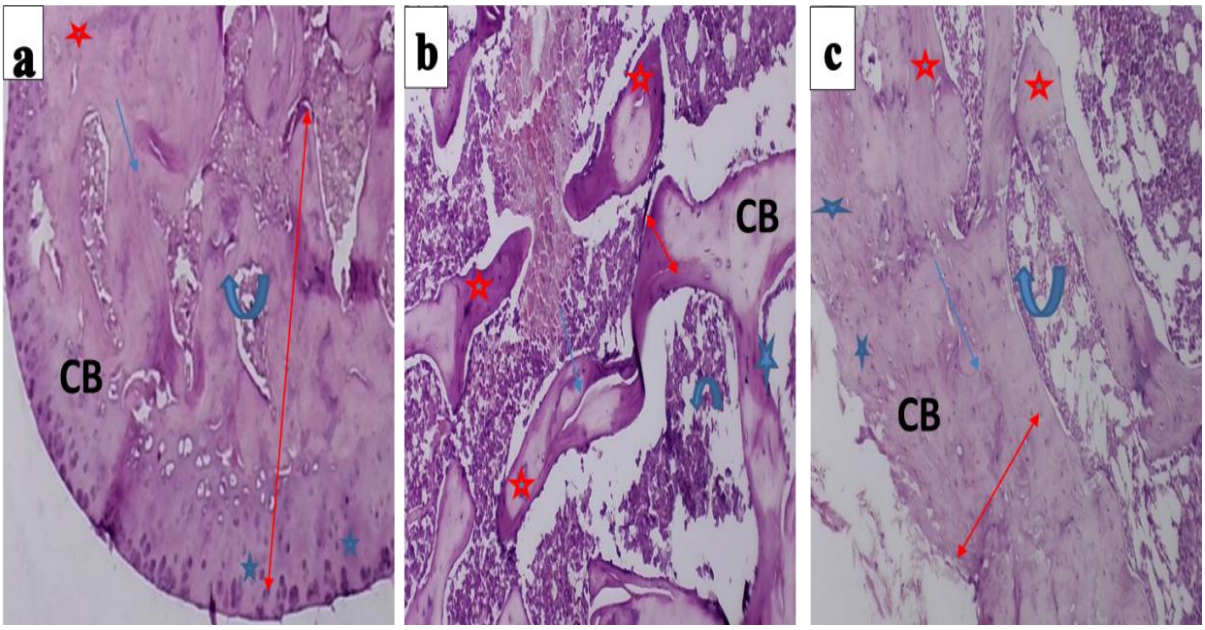

Figure 1: Representative photomicrographs from the longitudinal sections of distal femoral diaphysis of all studied groups (H&E; 200 µm). (a): The Sham group shows the classic bone architecture, outer cortical bone (CB) covered by outer smooth periosteum and lined by smooth endosteum and an apparent basophilic cement lines (blue arrow) between the older and newly formed bone matrix (b): the ORX group shows loss of the classic bone architecture with irregular periosteal surface, some osteocytes (blue star), increased number of osteoclasts (red star) and resorbed bone cavities (curved arrow). (c) The Kiss-treated group shows improved architecture of the cortical bone (CB), numerous osteocytes (blue star), few osteoclasts (red star), and apparent basophilic cement lines (blue arrow).

DISCUSSION

Osteoporosis is identified by the impaired bone architecture, gradual waning of bone mass and density and augmented risk of osteoporotic fractures [19]. Male aging was reported to be associated with increased frequency of osteoporosis and fragility fractures that was attributed to androgen deficiency [3,5]. Kisspeptin was suggested to have positive stimulatory effects on bone homeostasis, apart from its sex steroid-stimulatory effect [12]. The present study hypothesized that kisspeptin might be a potential therapeutic agent for osteoporosis-induced hypoandrogenism. Therefore, the present study investigated the chronic anti-osteoporosis effects of kisspeptin-10 against the bilaterally ORX rats as an established model of male osteoporosis.

The current histopathological results revealed marked distortion in the trabecular architecture, decreased trabecular thickness accompanied by increased number of osteoclasts and resorbed bone cavities in ORX rats. The quality of bones is significantly indicated by BMD which affects calcium and phosphorus metabolism [20,21]. Our findings revealed insignificant change in serum levels of calcium and phosphorus in ORX rats. Similar findings were reported in previous studies [14,22]. The insignificant difference in serum calcium and phosphorus levels between ORX and Sham groups was attributed to the rise in their urinary excretion levels, the increased bone turn over in ORX rats and decreased intestinal calcium absorption [22]. To further confirm our results, we measured urinary calcium and phosphorus concentrations and found a significant increase in their concentrations in ORX rats as compared with Sham group. In contrast, other studies reported decreased serum levels of the previous parameters [23,24], while others reported increased levels [17]. The successful induction of osteoporotic model in our study was ascertained by finding a significant reduction in BMD in ORX rats that was manifested by decreased femoral bone ash weight and femoral concentration of calcium and phosphorus compared with the Sham group. Similar findings were reported in other studies [14,17].

Our study revealed that chronic treatment of ORX rats with kisspeptin-10 significantly improved the cortical bone architecture, increased the bone mineral concentrations and femoral bone ash weight in comparison to the untreated ORX rats. These findings support the hypothesized therapeutic potential of kisspeptin-10 against osteoporosis in rats.

The RANKL/RANK/OPG signaling pathway has been reported to be involved in regulating bone

metabolism. OPG and RANKL have been found to be produced principally by osteoblasts and stromal cells in bone marrow. They were reported to be ligands of RANK receptor found on osteoclasts surface [25]. RANKL has been reported to interact with RANK triggering osteoclast differentiation and bone resorption, while OPG impedes this interaction and hinders osteoclasts activation. Bone resorption has been found to be regulated through the balance between RANKL and OPG expression [26]. The present study revealed a significant decrease in serum level of OPG, while serum level of RANKL and RANKL/OPG ratio were significantly increased in ORX rats as compared to Sham group. Additionally, we found that the expression of mRNA of OPG was down-regulated, while the expression of mRNA of RANKL was up-regulated in ORX rat femur bones compared with those of the Sham group. Previous studies reported similar findings [24,27]. The chronic treatment of ORX rats with kisspeptin-10 significantly downgraded serum and femur mRNA expression levels of RANKL, while upgraded those of OPG as compared to untreated ORX rats. So, the observed anti-osteoporotic effect of kisspeptin-10 in the present study was suggested to be through restoring the RANKL/ OPG ratio. Consistent with our findings, kisspeptin was reported to have a dose-dependent inhibitory effect on osteoclast activity in osteoclast monocultures and also in osteoclast/osteoblast cocultures that closely simulate the in vivo bone remodeling environment [11].

Inflammatory cytokines were reported to exacerbate osteoclastogenesis and bone resorption through hindering the mineralization of bone nodules [28]. Also, inflammatory cytokines were reported to exacerbate osteoclastogenesis via upgrading the expression RANKL mRNA in osteoblasts [29]. The current study revealed increased serum levels of TNF-α and IL-6 in the ORX rats as compared to the Sham group. These findings are in harmony with other [24,30]. The chronic treatment of ORX rats with kisspeptin-10 significantly reduced the levels of the former cytokines; a finding that signify the potential anti-inflammatory effect of kisspeptin-10 in osteoporosis. Our results are supported by a study which stated that kisspeptin-10 ameliorated the collagen induced arthritis as it suppressed lipopolysaccharide (LPS)-induced inflammatory cytokines in vitro and reduced nuclear factor kappa B (NF-κB) signaling pathway [31].

Osteoblasts were reported to express numerous bone formation markers including ALP, and osteocalcin [32]. ALP is a homodimer protein with phosphorylation properties produced during bone remodeling and frequently used as indicator for osteogenic activity and bone turnover rate [33,34]. It was reported that low BMD promotes activation of static osteoblasts to active osteoblasts resulting in bone-like tissue which cannot be mineralized and prevents transformation of osteoblasts into osteocytes. This leads to feed-back proliferation of osteoblasts which synthesize greater amounts of bone specific-ALP, and subsequently a significant increase in serum total ALP [35].

Osteocalcin; bone gamma-carboxyglutamic acid-containing protein (BGLAP); is produced by osteoblasts during osteogenic phase of bone remodeling and is used a serum indicator for bone formation and turnover rate [36]. Osteocalcin was reported to be a calcium-dependent biomarker that has a great affinity with hydroxyapatite in bone matrix accountable for bone mineralization. The calcium and phosphorus deficiency in osteoporosis results in reduced formation of hydroxyapatite crystal and therefore increased serum osteocalcin levels [37]. In the same line with the previous studies, the present study found a significant increase in serum levels of ALP and osteocalcin in ORX rats as compared to sham group. Similarly, gonadectomized rats were formerly reported to have increased levels of ALP and osteocalcin [17,38,39].

In the current study, chronic treatment of the ORX rats with kisspeptin-10 significantly diminished serum levels of ALP and osteocalcin in comparison to untreated ORX rats, a finding that indicates the potential valuable therapeutic effect of kisspeptin-10 through suppressing the high bone turnover rate found in osteoporosis. In support of our finding, in vitro incubation with kisspeptin was reported to induce osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) based on finding increased ALP activity in this human cell line which reflects increased osteoblastogenesis. Also, acute administration of kisspeptin in healthy men was reported to increase circulating osteocalcin level [11].

BMP-2; a member of transforming growth factor-β (TGF-β) family; was reported to promote osteoblast differentiation via activating SMAD signaling cascade [40]. The present study found a significant down regulation of mRNA expression level of BMP-2 in femurs of ORX rats as compared with Sham group. Similarly, several

studies formerly reported decreased gene expression level of BMP-2 in osteoporotic rat models [35,41,42]. The present study suggested that the low expression of BMP-2 in ORX rats resulted in diminished activation of BMP-2 signaling pathway and subsequently impaired osteoblast differentiation and defective bone mineralization. Impaired osteoblast differentiation into osteocytes in turn led to feedback proliferation of osteoblasts and subsequent increase in serum ALP that was detected in the present study. Consistent with our hypothesis, it was found that low expression of BMP-2 suppressed BMP signaling pathway activation, while high expression of BMP-2 activated BMP signaling pathway and promoted osteoblastic differentiation of BMSCs in osteoporotic rats [43]. A disparity in BMP signaling pathway was discovered in patients having osteoporosis where BMP-2 induced SMAD signaling was decreased in osteoporotic patients as compared to controls [44]. Also, it was reported that osteoblasts extracted from osteoporotic patients did not respond to BMP-2 stimulation based on mineralization measurements [45]. Additionally, it was reported that neither SMAD nor ERK signaling was activated in osteoblasts isolated from osteoporotic patients after BMP-2 stimulation [46].

The current study found that chronic treatment of the ORX rats with kisspeptin-10 significantly upgraded mRNA expression level of BMP-2 in comparison to untreated ORX rats. Our study is the first to explore and signify the chronic stimulatory effect of kisspeptin-10 on promoting osteoblastogenesis through increasing BMP-2 expression and subsequently activating BMP signaling pathway, increasing osteoblast differentiation and bone mineralization that was confirmed by the finding of a significant increase in femoral bone calcium and phosphorus concentrations and femoral bone ash weight in comparison to untreated ORX rats. Consistent with our finding, BMP-7 expression was reported to be regulated by kisspeptin-10/G-protein-coupled receptor 54 (GPR54) through nuclear factor of activated T-cells (NFAT) c2 and Sp1 in embryonic kidney [47]. Moreover, kisspeptin was reported to increase BMP-2 expression and osteoblast differentiation in the mouse mesenchymal stem cell line (C3H10T1/2) via GPR54/NFATc4 signaling pathway [48].

In the present study, we found that mRNA expression levels of Wnt3a and β-catenin were downgraded in femoral bones of ORX rats as compared with Sham group. The relationship between Wnt/β-catenin signaling pathway and bone remodeling was reported in several studies [49,50]. Wnt gene has been associated with MSCs growth, differentiation, and apoptosis which regulates signal transduction in osteoporosis and impacts bone remodeling [51]. β-catenin was reported to promote osteoblast differentiation either directly [52], via increasing their response to BMP-2 [53] or via activating OPG gene promoter in osteoblasts [54]. In the ordinary cellular condition, the degradation of β-catenin proteasome complex occurs by glycogen synthase kinase-3β (GSK-3β). On activation, Wnt attaches to the membrane receptor Frizzled to activate Dvl2 which in turn inhibits the interaction between GSK3β and β-catenin, resulting in phosphorylation of β-catenin and then transmitted to the nucleus where it upgrades the expression of the osteogenic marker; RUNX2 that promotes osteoblasts differentiation and activity [55].

Scarce studies demonstrated the association between kisspeptin and Wnt/β-catenin pathway. It was reported that kisspeptin inhibited the proliferation and migration of gastric cancer cells through interaction with Wnt/β-catenin signaling pathway [56]. In the present study, chronic treatment of ORX rats with kisspeptin-10 upgraded mRNA expression levels of Wnt3a and β-catenin as compared to untreated ORX rats. These findings provided additional potential molecular pathway for kisspeptin-10 induced osteoblastogenesis via activating Wnt/β-catenin pathway.

CONCLUSION

The present study revealed that experimental androgen deficiency in ORX rats disrupted bone mass and microstructure inducing osteoporosis. Chronic treatment with kisspeptin-10 enhanced bone mass and microarchitecture in ORX rats. The osteoclast-inhibiting ability of kisspeptin-10 was exerted via modulating RANKL/OPG ratio, while the osteoblast-promoting ability was exerted through BMP-2 and Wnt/β-catenin signaling pathways. The discovered dual effect of kisspeptin-10 on stimulating osteogenesis and reducing bone resorption led the present study to recommend it as a promising novel therapeutic agent for age-related osteoporosis in men.

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