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ORIGINAL ARTICLE

The effect of testosterone itself and in combination with letrozole on bone mineral density in male rats

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Abstract

Testosterone is an essential hormone to maintain bone integrity; however, the effect of aromatase enzyme in androgen-induced bone maintenance remains somewhat unclear. The present study evaluated the effect of testosterone itself and combined with letrozole, an aromatase inhibitor, on bone mineral density of male rats. Total of 48 male rats were divided into 4 equal groups ($n = 12$/group); sham group, O: orchiectomy, O + T: orchiectomized rats treated with testosterone, O + T + L: orchiectomized rats treated with combination of testosterone and letrozole. Bone density (BMD), bone markers, and vitamin D metabolism parameters were checked in all groups before and after the study. There was no significant difference in baseline values of these parameters, but at the end of the study there was a significant decrease in delta BMD at both lumbar and femor in orchiectomized rats in comparison with the sham group ($p < 0.001$, $p < 0.001$, respectively). Both testosterone and its combination with letrozole increased lumbar and femoral BMD of orchiectomized rats, with a higher increase in lumbar BMD in O + T group. CTX were higher in O group rats. The present study showed a major role for testosterone on BMD maintenance in male rats. However, testosterone has a potent effect on lumbar BMD, by the aromatization to estradiol.

Keywords Testosterone · Aromatase inhibitor · 25(OH)D · BMD

Introduction

Osteoporosis is characterized by a reduction in bone density and bone quality worsening and micro architecture that leads to bone fragility [1, 2]. In previous studies, osteoporosis was reported to be more prevalent in women compared to men. However, osteoporosis in men aged over 50 is higher than younger age group with a prevalence of 20%, which is becoming a major health problem in elderly [3]. However, worldwide there is less concern regarding the diagnosis and treatment of osteoporosis in older men than menopausal women [4, 5].

Testosterone is an essential hormone for maintaining bone integrity that plays as an anti-osteoporotic role in adult males [6, 7]. Age-related hypogonadism and testosterone deficiency is a condition caused by the decline in serum testosterone level in elderly. It has been known that low serum testosterone in conditions such as male hypogonadism can reduce bone mineral density (BMD) [8–10]. Moreover, it has been known that in male, both testosterone and adrenal androgens can be converted by aromatase enzyme to estradiol [11]. It was reported that osteoblasts and osteoclasts have cell-surface receptors for androgen and estrogen. Also, human and rodent bone osteoblasts were reported to express aromatase gene [12–14]. Both osteoblasts and osteoclasts have sex steroid hormone receptors at the mRNA level in nucleus [15]. Nakamura et al. [16] showed that estrogen induces and upregulates osteoclasts apoptosis via the Fas ligand expression in the trabecular bones.

Previous studies have shown that testosterone replacement therapy in osteoporotic men with hypogonadism can lead to increase in bone density [17]. However, it remains unclear whether testosterone administration in males with hypogonadism can directly affect the skeletal protection or can indirectly influence bone density via estrogen-mediated actions that occurs following aromatization to estradiol [18–21]. Smith et al. [11] showed that males with nonfunctional mutation in ER have low BMD, and Carani et al. [22] showed that aromatase deficiency might cause BMD decline and increase bone turnover markers in comparison

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with normal males. Aromatase inhibitors can reduce circulating estradiol level by blocking testosterone conversion in male [23, 24].

Due to lack of sufficient data on the independent or interdependent effects of estradiol and testosterone on bone health and mineral density in men, we designed the present research to investigate the mechanism of testosterone action on bone metabolism and BMD. To achieve this, we assessed the effects of daily administration of aromatase inhibitor (letrozole) on bone mineral density in orchiectomized rats treated with testosterone.

Materials and methods

Methods

This experimental animal study was carried out on 48 adult male Sprague–Dawley rats in Shiraz Endocrinology and Metabolism Research center affiliated to Shiraz University of Medical Science (SUMS).

Ethics

All authors declare that they have no conflict of interest. All procedures were approved by the local ethic and experimentation committee of Shiraz university of Medical Sciences (SUMS) and vice-chancellor of research at SUMS approved this study with ID:95-01-01-12923. This study is in accordance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines [25] on the use and care of research animals and all applicable institutional and national guidelines for care and use of animals were followed in this study.

Animals

A total of 48 adult (8 weeks old) male Sprague–Dawley rats with mean weight of 250–300 gr were enrolled in this study. Animals were housed in standard cages, four per cage, with 12:12 h light–dark cycles, at a comfortable temperature of 23 ± 2 °C and were fed normal standard rodent chow diet with free access to tap water. All rats were randomly divided into four groups of 12 rats as follows:

A. *Sham group*, which underwent sham operation, then received intramuscular (IM) injection of 0.2 ml of olive oil every week.
B. *Orchiectomy* group (O), which underwent orchiectomy operation, then received intramuscular (IM) injection with 0.2 ml of olive oil weekly.
C. *Orchiectomized rats treated with testosterone* (O + T) group, in which orchiectomized rats were given weekly 15 mcg/kg IM testosterone enanthate replacement therapy (which were diluted in 0.2 ml of olive oil), weekly for 12 weeks.
D. *Orchiectomized rats underwent treatment with combination of testosterone and letrozole* (O + T+L) group in which orchiectomized rats were given 15 mcg/kg IM testosterone enanthate per week, and received 0.1 mg/kg letrozole daily through oral gavages for 12 weeks.

Surgical procedure

General anesthesia was performed by intraperitoneal injection of ketamine/xylazine mixture (100/10 mg/kg) [26]. In orchiectomized rats after shaving the testicular area and disinfecting of the operation site with iodized alcohol, operation was performed by making an incision on subcutaneous tissues till exploration of testes, then testicular artery was ligated and testes were removed, next the testicular skin was sutured with silk suture. In sham group, after a midline incision through the ventral aspect of the scrotum, subcutaneous tissues were dissected and both testes were explored then testes were returned to the scrotum, after that skin was sutured with silk suture. Local antibiotic ointment was used over the sutured area in both sham and orchiectomized rats [26, 27]. Animals were left for 2 weeks after surgery to completely heal and achieve andropause in orchiectomized rats.

Biochemical studies

Blood samples were taken twice; first, at week 0 blood was taken from the tail vein and once again after the 12th week. All rats were euthanized at the end of the study and 4 mL of blood was collected via cardiac puncture. The blood was centrifuged at 3500 rpm for 12 min, the plasma was collected and stored at − 70 °C till further analysis.

Serum calcium (Ca) (mg/dL), phosphorous (Phos) (mg/dL), and alkaline phosphatase (ALP) (IU/L) were measured, using colorimetric assays on Biosystem SA auto-analyzer, produced by Biosystems company in Spain. 25-hydroxyvitamin D 25(OH) D levels (ng/ml) were assessed using Electrochemolumicence methods, made in Germany with Sensitivity of 2 ng/ml and intra- and inter-assay CVs of, 3.3% and 5.1%, respectively. Serum 1,25-dihydroxyvitamin D 1,25(OH)2D (pmol/ml) were checked with ELISA method produced by Bioassay Technology Laboratory in China with intra- and inter-assay CVs Sensitivity of < 8% and < 10%, respectively. Serum Parathyroid hormone (PTH) level (pg/ml) was checked with ELISA kits based on sandwich technology and coefficient of variation less than 10%, produced by MyBiosource company in USA intra- and inter-assay were < 6% and < 7%, respectively.

Assays for estradiol (pg/ml) were done by ELISA kits produced by MyBiosource company in USA with

intra- and inter-assay < 15% and < 15%, respectively. Serum Testosteron(ng/ml) were checked with ELISA kit produced by Bioassay Technology Laboratory in China with intra- and inter-assay CVs Sensitivity of < 8% and < 10%, respectively. Serum Luteinizing Hormone (LH) (mIU/ml) was checked with Immunoradiometric assay (IRMA) method produced by Institute of Isotopes. Ltd. in Hungary with intra- and inter-assay CVs Sensitivity of < 9% and < 10%, respectively.

Serum C-terminal telopeptide of type I collagen (CTX) (ng/ml) and N-terminal type 1 procollagen (P1NP) (ng/ml) were measured with ELISA kit produced by Bioassay Technology Laboratory in China with intra- and inter-assay CVs Sensitivity of < 8% and < 10%, respectively.

BMD measurement

BMD and bone mineral content (BMC), and bone area of femor, and lumbar spine of all rats were assessed by a Hologic system dual-energy X-ray absorptiometry (DXA) (Discovery W(S/N84107), USA) with small animal software in Shiraz Endocrinology and Metabolism Research center.

DXA bone densitometry was done twice, first one at the beginning of the study, as the baseline data, and the second one after 12 weeks. During the bone densitometry, all rats were anesthetized by combining Ketamine hydrochloride, (dose of 50 mg/kg) and xylazine hydrochloride, with a dose of 5 mg/kg of body weight, intramuscularly in the gluteus region.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Statistical significance was defined as p value less than 0.05. The paired-samples t test was used to analyze values within the same group at baseline and after 12 weeks. ANOVA followed by the Tukey post-hoc test was used to compare DXA data between different groups.

Results

Biochemical markers

According to Table 1, there was no significant difference in baseline body weight amongst the groups. However, the body weight of the O group became significantly lower than the other groups after the 12th week, but no significant differences were observed between other groups.

There was no significant difference between baseline 25(OH)D, 1,25(OH)2D3, Ca, Phos and ALP serum level in all four groups (Table 2).

At the end of the study, all biochemical parameters were checked again. As shown in Table 3, mean estradiol serum level was significantly lower in O group in comparison with the sham group ($p = 0.024$). Estradiol serum level was significantly lower in O + T + L group in comparison with O + T group ($p = 0.043$). However, this difference was not significant between O group and O + T + L group.

Table 1 Body weight (gr) measured for studied groups

	Week 0	Week 6	Week 12
Sham	223.8 ± 5.92	289.5 ± 22.42	370.0 ± 38.03 $p = 0.003^{O}$
Orchidectomy	226.7 ± 10.32	263.1 ± 14.68	309.8 ± 11.94
O + T	229.9 ± 14.39	293.7 ± 42.34	353.1 ± 40.40 $p = 0.038^{O}$
O + T + L	230.3 ± 11.34	283.5 ± 12.58	360.0 ± 18.03 $p = 0.048^{O}$

Values are the mean ± SD

^{O}p value of significance versus orchiectomy group

Mean serum testosterone level was significantly lower and the mean serum LH was significantly higher in O group in comparison to the other groups. However, there was no significant difference in serum LH and testosterone amongst other groups.

CTX was significantly higher in O group in comparison to the other groups. However, there were no significant differences in CTX level amongst all other groups. No significant difference was observed regarding mean serum P1NP amongst all groups (Table 3).

There were no statistically significant differences in the mean serum Ca between all the four groups. Mean 25(OH)D serum level was significantly lower in O group in comparison to sham, O + T and O + T + L group ($p = 0.009$, $p = 0.004$ and $p = 0.001$, respectively) (Table 4).

Mean Serum level of 1,25(OH)2D3 was significantly lower in O group in comparison to sham group. However, mean serum level of 1,25(OH)2D3 was significantly higher in O + T group in comparison with O + T + L group ($p = 0.032$).

Phos, ALP, and PTH serum level were significantly higher in O group in comparison to sham ($p < 0.001$, $p = 0.013$ and $p = 0.020$, respectively) and other groups (Table 4).

There were no significant differences in serum Ca, Phos, 25(OH)D, AlP, and PTH between O + T in comparison with O + T + L group (Table 4).

DXA outputs

There was no significant difference in lumbar and femoral baseline DXA values between all four groups. After 12 weeks of study, DXA outputs were rechecked. Figures 1 and 2, summarize the final and delta values of DXA outputs, in the four groups. Table 5 shows baseline analysis, after trial and delta of DXA outputs.

Table 2 Baseline biochemical parameters [Ca,Phos,ALP, 25(OH)D] measured for studied groups

Studied parameters	Sham $n=12$	O $n=12$	O+T $n=12$	O+T+L $n=12$	p value
Ca (mg/dl)	11.5 ± 0.79	11.44 ± 0.38	11.9 ± 0.53	11.5 ± 0.90	0.42
Phos (mg/dl)	8.5 ± 1.02	8.9 ± 0.57	8.7 ± 1.24	8.7 ± 1.19	0.74
ALP (IU/L)	684.1 ± 119.45	776.55 ± 198.46	663.8 ± 126.62	678.9 ± 131.30	0.35
25(OH)D (ng/ml)	12.02 ± 0.86	13.03 ± 0.82	12.8 ± 1.53	11.9 ± 2.00	0.33
1,25(OH)2D (pmol/L)	206.44 ± 22.56	225.39 ± 40.30	197.19 ± 18.20	203.25 ± 20.78	0.12

Values are reported as mean ± SD. No statistically significant differences between groups were observed

Table 3 Serum hormone and bone markers, measured at the end of trial

Studied parameters	Sham $n=12$	O $n=12$	O+T $n=12$	O+T+L $n=12$
Testosterone (ng/ml)	3.08 ± 0.50 $p<0.001^{O}$	0.016 ± 0.007	3.49 ± 0.67 $p<0.001^{O}$	3.60 ± 0.53 $p<0.001^{O}$
Estradiol (pg/ml)	159.21 ± 31.82 $p=0.024^{O}$	124.51 ± 23.75	154.26 ± 28.8 $p=0.048^{O}$	124.72 ± 8.92
LH (mIU/ml)	0.50 ± 0.12 $p<0.001^{O}$	40.03 ± 11.93	0.21 ± 0.16 $p<0.001^{O}$	0.45 ± 0.45 $p<0.001^{O}$
CTX (ng/ml)	25.85 ± 5.17 $p=0.003^{O}$	35.55 ± 4.66	23.50 ± 4.84 $p<0.001^{O}$	24.75 ± 4.92 $p=0.001^{O}$
P1NP (ng/ml)	260.57 ± 31.57	243.77 ± 54.13	289.91 ± 32.50	268.12 ± 38.71

Values are the mean ± SD

^{O}p value of significance versus orchiectomy group

Table 4 Biochemical parameters 25(OH)D, Ca, Phos, PTH, ALP and 1,25(OH)2D3 measured at end of trial for studied groups

Studied parameters	Sham $n=12$	O $n=12$	O+T $n=12$	O+T+L $n=12$
Ca (mg/dl)	10.41 ± 0.68	9.96 ± 0.45	10.12 ± 0.31	10.49 ± 0.69
Phos (mg/dl)	78 ± 1.22 $p<0.001^{O}$	12.25 ± 2.13	7.88 ± 1.04 $p<0.001^{O}$	8.45 ± 1.03 $p<0.001^{O}$
PTH (pg/ml)	469.32 ± 121.77 $p=0.02^{O}$	716 ± 192.03	421.84 ± 186.09 $p=0.006^{O}$	472.60 ± 183.11 $p=0.019^{O}$
ALP (IU/L)	447.88 ± 85.84 $p=0.013^{O}$	664.22 ± 213.59	397.8 ± 123.35 $p=0.001^{O}$	478.45 ± 119.57 $p=0.03^{O}$
25(OH)D (ng/ml)	14.54 ± 2.43 $p=0.009^{O}$	10.12 ± 1.57	14.8 ± 3.29 $p=0.004^{O}$	15.24 ± 3.20 $p=0.001^{O}$
1,25(OH)2D(pmol/L)	226.65 ± 23.65 $p<0.001^{O}$	184.64 ± 13.29	227.29 ± 17.02 $p=0.032^{T+L}$ $p<0.001^{O}$	205.70 ± 13.11 $p=0.045^{O}$

Values are the mean ± SD

^{O}p value of significance versus orchiectomy group, ^{T+L}p value of significance versus orchiectomy treated testosterone and letrozole

In O group at the end of trial, lumbar BMD was reduced in comparison to the baseline values. However, this reduction was not statistically significant ($p=0.102$). We observed a considerable reduction in femoral BMD in comparison to the baseline results in this group ($p=0.029$), (Table 3 and Fig. 1a, b).

In the sham group, after 12 weeks significant increase was observed in lumbar BMD in comparison with the baseline ($p<0.001$). Also, significant increase was detected in femoral BMD, ($p=0.001$).

In O+T group, we detected significant increase in both lumbar and femoral BMD at the end of trial (week 12) in comparison with the baseline values ($p<0.001$, $p<0.001$ respectively), (Table 3 and Fig. 1a, b).

In O+T+L group, we observed significant increase in lumbar BMD after 12 weeks in comparison with the baseline

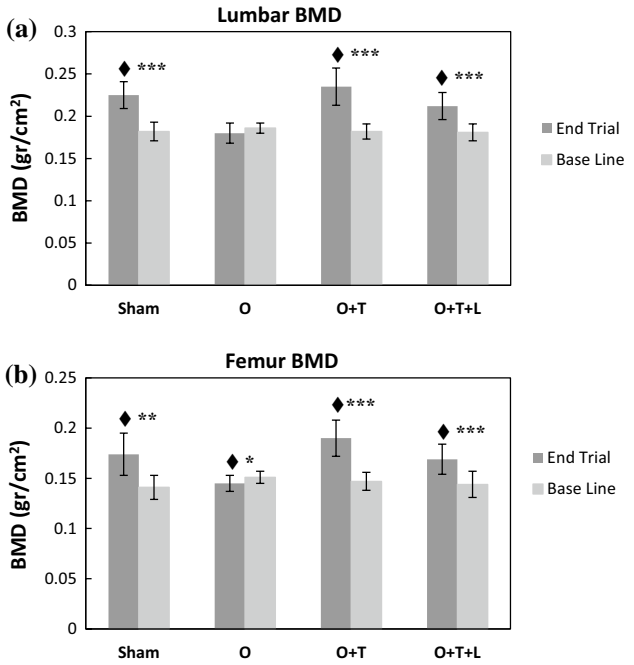

Fig. 1 End of trial BMD variation from the baseline **a** lumbar BMD, **b** femor BMD, black diamond = *P* value of significance from the baseline values * $p < 0.005$, ** $p < 0.01$, *** $p < 0.001$

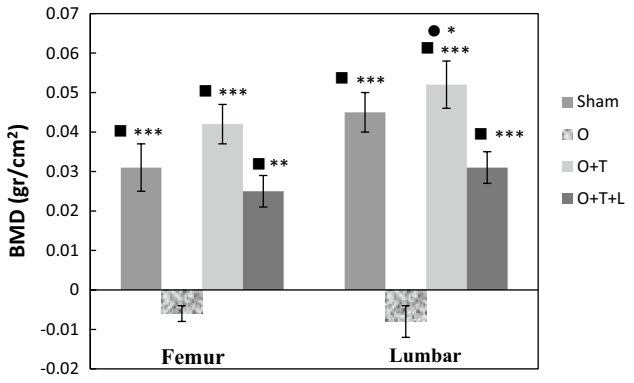

Fig. 2 Comparison of delta BMD between groups, black square = *p* value of significance versus orchiectomy group, black circle = *p* value of significance versus orchiectomy treated testosterone and letrozole *$p < 0.005$, **$p < 0.01$, ***$p < 0.001$

values ($p < 0.001$). Also, significant increase in post-trial femoral BMD in comparison with the baseline was detected ($p < 0.001$), (Table 3 and Fig. 1a, b).

By comparing BMD DXA delta output changes between the four groups, we detect a significant positive delta in both lumbar and femoral BMD in all three groups of sham, O + T and O + T + L in comparison with the negative delta changes in the lumbar and femoral BMD in the O group. However, lumbar delta BMD was significantly higher in O + T group in comparison with O + T + L group ($p = 0.037$). But no significant difference was found in delta of femoral BMD between O + T and O + T + L, as shown in Table 3 and Fig. 2.

Discussion

The present study showed a significant lower serum testosterone and higher serum LH level in the O group in comparison with the other groups. However, mean estradiol serum level was significantly lower in O and O + T + L groups in comparison with other groups. Also, we detected a lower 25-(OH)D and 1,25(OH)2D serum level, and higher Phos, ALP, and PTH serum level in the O group in comparison to the other groups after 12 weeks. However, mean serum level of 1,25(OH)2D was significantly higher in the O + T group in comparison with the O + T + L group. Also, we showed that in adult rats, orchiectomy led to a significant bone loss. Conversely, testosterone replacement therapy in rats of both O + T and O + T+L groups resulted in femoral and lumbar BMD improvement, with a higher increase in lumbar BMD in the O + T group.

Sprague–Dawley male rats reach maturity within 6 to 8 weeks of life. Researches have shown that young adult male rats reached maximum bone mass during the first 3 months of life. However, trabecular bone mass decreased by aging. The bone formation and resorption parameters fall rapidly between the 3rd and 7th month [28]. To prevent the age effect on bone density and turnover markers we preferred to select 8 weeks' adult male rats. At the end of the study, all groups had gained weight, but the orchidectomised rats did not gain weight normally, since their body weight was significantly lower than the sham group. In contrast, testosterone therapy alone or in combination with letrozole was able to maintain the weight. This difference could be due to testosterone effect on growth hormone release and maintenance of bone or muscle mass [29, 30].

Androgen effect on target organs is mediated by androgen receptor (AR), which is essential to maintain bone mass in male rats. AR-knocked out mice showed increased bone resorption in both trabecular and cortical bone [31]. Our results were in accordance with some previous studies that showed the significant role of testosterone in inducing and maintaining bone density in male. MacLean et al. [32] showed that deletion of androgen receptor gene in male mice resulted in the reduction of trabecular bone volume and cortical thickness. While low serum testosterone in hypogonadal male has a direct effect on BMD reduction, [6, 33] testosterone replacement therapy in hypogonadal male leads to improved BMD [34]. The present study showed that there was no significant difference in serum estradiol among O and O + T + L; however, serum testosterone was significantly higher in O + T+L group in comparison to O group. We had

Table 5 Baseline and end of trial BMD with delta variation changes from baseline

Studied parameters		Sham $n=12$	O $n=12$	O+T $n=12$	O+T+L $n=12$
Femor BMD (g/cm^2)	Baseline	0.141 ± 0.012	0.151 ± 0.006	0.147 ± 0.009	0.144 ± 0.013
	After trial	0.174 ± 0.021 #$p=0.001$	0.145 ± 0.008 #$p=0.029$	0.190 ± 0.018 #$p<0.001$	0.169 ± 0.015 #$p<0.001$
	Delta	0.031 ± 0.019 $p<0.001^{O}$	0.006 ± 0.007	0.042 ± 0.017 $p<0.001^{O}$	0.025 ± 0.015 $p=0.001^{O}$
Lumbar BMD(g/cm^2)	Baseline	0.182 ± 0.011	0.186 ± 0.006	0.182 ± 0.009	0.181 ± 0.010
	After trial	0.225 ± 0.016 #$p<0.001$	0.180 ± 0.012	0.235 ± 0.022 #$p<0.001$	0.212 ± 0.016 #$p<0.001$
	Delta	0.045 ± 0.017 $p<0.001^{O}$	0.008 ± 0.013	0.052 ± 0.021 $p<0.001^{O}$ $p=0.037^{T+L}$	0.031 ± 0.015 $p<0.001^{O}$

Values are the mean ± SD

^{O}p value of significance versus orchiectomy group, ^{T+L}p-value of significance versus orchiectomy treated testosterone and letrozole, #p value of significance from the baseline values in either group

hypothesized that androgens might have a significant compensatory role in the maintenance of femoral and lumbar BMD even in the conditions of suppressed estradiol action, due to significant reduction in delta femoral and lumbar BMD in O group in comparison to O + T + L. In addition, this study showed that adding letrozole to testosterone could partially reduce the effect of testosterone on lumbar BMD, which resulted in significant difference in delta lumbar BMD among the O + T and O + T + L. This suggests that aromatization of testosterone to estradiol could influence lumbar bone maintenance in male rats. Imai et al. [35] showed that estrogen could affect bone mass through estrogen receptors on both osteoclasts and osteoblasts. Kastelan et al. [36] also revealed that estrogen receptors and aromatase gene inactivation might be associated with low BMD in men. In addition, some reports considered the role of the aromatase enzyme in mediating the musculoskeletal effects of androgens and the possible role of estrogen in lumbar BMD maintenance and bone turnover markers in men [11, 37–39]. In contrast, some studies showed that testosterone has positive effect on bone density independent from estradiol action in men. Falahati et al. [40] observed the independent effects of estrogen and testosterone on bone density. Another study by Vanderschueren et al. [41] showed that androgens have bone-protective effects in orchiectomized rats, even in aromatized or non-aromatized form. Hence, these controversies could be due to differences in bone architecture and physiology amongst lumbar and femoral bone as well as the differences between human and rats bone physiology [42].

For better evaluation of BMD changes, the bone turnover markers were assessed at the end of study. Serum levels of P1NP and CTX were measured as bone formation and bone resorption marker, respectively. CTX was shown to be higher in O group in comparison with other groups. Conversely, testosterone replacement therapy either alone or in combination with letrozole, was able to reduce bone resorption marker (CTX) to normal level similar to sham group. The results confirmed that testosterone deficiency could result in bone mass reduction and increased bone resorption in orchiectomized rats [43]. At the end of study, mean serum P1NP was lower in orchiectomized rats in comparison with other groups, but this difference was not statistically significant. This could be due to gradual or non constant variation of serum P1NP in orchiectomized rats [44, 45].

We also detect that orchiectomized rats had lower 25-(OH)D serum level, which resulted in secondary increase in serum PTH and ALP in the O group. Our results suggest that orchiectomy might have an additional indirect effect on bone resorption by decline in serum vitamin D that subsequently increases serum PTH. Francis et al. [46] showed that vitamin D reduction is a major risk factor in the development of osteoporosis in hypogonadal men. We also detected higher 1,25(OH)2D serum level in the O + T group in comparison with O + T + L. Hence, it seems that aromatization of testosterone to estradiol could lead to an increase in serum 1,25(OH)2D, which could furthermore have influence on bone density in male rats. Studies have shown that active vitamin D has positive effects on bone mass through enhanced intestinal Ca absorption and inhibition of osteoclast's bone resorption. This effect is greater on mouse bones than human bones [47].

There are some important points in this study including measuring biochemistry markers at the baseline and at the end of experiment, which might have influenced bone density. BMD was measured at the baseline and at the end of experiment to have a better BMD evaluation. In addition to these strengths, we had some limitations. It was better to give an oral daily gavage to other groups rather than only to O + T + L, which received letrozole by gavage. This could reduce the effect of stress hormones during daily gavage on

bone phenotype. In addition, it would have been also better to analyze the bone phenotypes by micro CT or bone histomorphometry rather than measuring the total bone density by DXA.

Conclusions

We concluded that testosterone deficiency could lead to significant bone reduction, and testosterone replacement therapy could prevent bone loss. We hypothesized that androgens might play an important compensatory role in maintenance of femoral and lumbar BMD, even in conditions of suppressed estradiol action. We are of the opinion that aromatization of testosterone to estradiol could influence lumbar bone maintenance in male rats.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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