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TRANSLATABILITY OF (HEMI)ORCHIDECTOMIZED MOUSE MODEL FOR MALE HYPOGONADISM: A REVISION ON CLINICAL CHEMISTRY WITH AN EMPHASIS ON XANTHINE OXIDASE OF SELECTED TISSUES

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Male hypogonadism is a prevalent syndrome with various clinical manifestations and ambiguity in its etiology and therapy. We investigated translatability and xanthine oxidase (XO) activity in a mouse model of hypogonadism. In this experimental study, 24 NMRI male mice were allocated into four groups: normal control (NC), bilateral orchidectomiezed (BOX), right hemi-orchidectomiezed (ROX), and left hemi-orchidectomiezed (LOX) mice. After eight weeks, plasma clinical chemistry and XO activities of selected tissues were measured. Plasma testosterone levels and bone mass density tended to decrease in (hemi)orchidectomized mice. The atherogenic index and coronary heart disease risk factor have not been altered in hemi-orchidectomized mice while it increased in orchidectomized mice. Plasma cholesterol has been increased in LOX (P=0.011) and BOX (P=0.041) compared to NC. Plasma calcium and phosphorus levels have not been changed in (hemi)orchidectomized mice as compared to NC. The globulin levels raised significantly in ROX (P=0.043) compared to NC while its levels tended to increase in BOX and LOX compared to NC. The activities of aspartate transaminase, alanine transaminase, and lactate dehydrogenase, and plasma and bone alkaline phosphatase were not different among groups. The XO activity tended to decrease in all tissues however, it significantly decreased in muscles of ROX (P=0.040) and BOX (P=0.047) with respect to NC. The XO activity has also decreased significantly in the heart tissues of LOX (P=0.045) and BOX (P=0.043) as compared to that of NC. It seems that more than 8 weeks post-orchidectomy time is required to translate male hypogonadism. Furthermore, XO activities were reduced in selected tissues following bilateral or unilateral orchidectomy. ***Biomed Rev*** **2021; 32: 71-78**

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INTRODUCTION

Male hypogonadism is a clinical syndrome defined by low testosterone and low sperm production (1, 2). It might etiologically lie on hypothalamic, pituitary, testicular, or target organ disorders which can be congenital (e.g., Klinefelter syndrome) or acquired (e.g., posttraumatic; 1, 3-5). Male hypogonadism manifests as numerous symptoms which reflect various physiological functions of testosterone in men. Hypogonadism is commonly associated with decreased muscle mass and libido, erectile dysfunction, sexual problems, low stamina, decreased strength and/or endurance, and metabolic disorders like hyperuricemia, osteoporosis, and even metabolic syndrome (MetS; 1, 4, 6-12).

Male hypogonadism can alter several hormones, however, among androgens, testosterone might be the most important one, which is produced by interstitial cells of Leydig of testes (1, 11). This 19-carbon steroid hormone plays an array of essential roles in the body. More specifically, the level of this hormone is decreasing during aging (8, 13, 14), although the level of testosterone pathologically changes in conditions that include cardiovascular diseases (CVDs), MetS, diabetes mellitus, stress, osteoporosis, anemia, obesity, and sexual and fertility problems (1, 4, 15).

It is well established that androgens exert marked effects on bone homeostasis and testosterone potentially affects bone metabolism by regulating inflammatory cytokines and osteoblasts, as well (10, 16). Indeed, alteration in testosterone level causes an increase in bone inflammation and bone loss (15). One possible explanation for these findings is that testosterone has the dominant effect on osteoblast formation besides cytokines such as interleukin 6 and tumor necrosis factor α. Therefore, orchidectomy or generally decreased testosterone levels can cause bone loss and ultimately osteoporosis. In this context, alkaline phosphatase assay is an appropriate prognostic and diagnostic tool due to its role in the bone healing process (15, 17). Physiologically, testosterone results in an increased bone matrix, protein anabolism, and calcium turnover (18).

The rate of death due to CVDs is high among old men and there are several involved factors for this incidence. The reactive oxygen species (ROS) are involved in vasculature thickening and its production is increased following cardiac and/or cerebral strokes and ischemia (11, 19, 20). The uric acid (UA) besides H_2O_2 are principal products of xanthine oxidase (XO) activity, however, ROS are considered as byproducts of XO activity (19, 20). According to the oxidative stress induced by ROS, it is rational to expect ROS overload in inflammatory diseases such as MetS, CVDs, T2DM, and strokes (11, 21) and the interesting point of this topic is the role of testosterone in oxidative stress and XO activity (15, 19).

In this essence, MetS is another mentioned hypogonadism-related sequel that has different diagnostic clues, such as an increase in fasting blood glucose or insulin resistance, obesity, dyslipidemia, and hypertension (11). It is crystal clear that MetS increases the risk of CVDs and more noticeably, the androgens are involved in both pathologic conditions, i.e. MetS and CVDs, besides other complications. Accordingly, decreased testosterone causes XO increment and intensifies aging-related oxidative stress; the aged-old men's quality of life can be improved at the molecular level by XO inhibition and its related drugs. For this purpose, the aim of this study was to investigate the XO activity in the mouse model of hypogonadism.

MATERIALS AND METHODS

Animal subjects

All animal procedures were approved by the local ethics committee on animal care at Razi University, Kermanshah, Iran. Adult Male Naval Medical Research Institute (NMRI) mice*, Mus musculus*, (3-4 months; 25-30 g) were maintained under 45–55% relative humidity, 12 h light: 12 h dark photocycle, and temperature 24 ± 2 °C. The animals were housed in colony cages with free access to pelleted feed (Gharbdaneh Co., Iran) and tap water and divided into 4 groups (n = 6 for each), namely; sham-operated normal mice (NC), right hemi-orchidectomized mice (ROX), left hemi-orchidectomized mice (LOX), and bilateral orchidectomized mice (BOX).

Clinical chemistry

On the last day of the experiment (day 56), after 14-16 hours of fasting, whole blood samples were taken via cardiac puncture under deep anesthesia induced by an intraperitoneal injection of ketamine (100 mg/kg)/xylazine (30 mg/kg) cocktail and collected into tubes containing heparin as an anticoagulant. The plasma was separated by centrifugation at 1,400 × *g* at 4 °C for 15 min, and stored at –20 °C until analysis. The plasma levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triacylglycerol (TGs) were assayed using a diagnostic kit (ELI TECH Diagnostic, French). Plasma high-density lipoprotein cholesterol (HDL-C) was determined by the immuno-inhibition method (ELI TECH Diagnostic, French). Very low-density lipopro-

tein-cholesterol (VLDL-C) was calculated by the formula: VLDL-C = TGs/5 (22). Atherogenic index (AI) was calculated according to the following equation: AI = LDL-C/HDL-C (23). Coronary heart disease (CHD) risk factor was defined according to the following equation: CHD=TC/HDL-C (24).

Plasma samples were thawed and the levels of albumin (ALB), total protein (TP), calcium (Ca), and phosphorus (P) were determined by biochemical kits (Pars Azmoon, Tehran, Iran) using an auto-analyzer (Heitachi902, Germany). The Ca/P ratio was also calculated. The level of globulin (G) was obtained by subtracting albumin concentration from plasma TP, then the albumin/globulin (A/B) ratio was calculated.

Plasma testosterone level was measured using an enzyme-linked immunosorbent assay (ELISA) kit (IBL International GmbH, Hamburg, Germany) with intra- and inter-assay coefficients of variation less than 6.8% and a sensitivity of 0.083 ng/ml.

Enzyme assay

All reagents were purchased from Sigma-Aldrich, UK. After blood collection, selected tissues were excised, labeled individually, and snap-frozen in liquid nitrogen at -80 °C as described previously (25). Tissues were homogenized in 5-10 volumes of potassium phosphate buffer (pH 7.4) containing 5 mM ethylenediaminetetraacetic acid disodium salt (EDTA-Na) and 1 mM phenylmethanesulfonyl fluoride (PMSF) using a WiseTis homogenizer (HG-15D, Korea). The homogenate was centrifuged at 12,000 × *g* at 4 °C for 15 min. The supernatant fraction was centrifuged at 12,000 × *g* at 4 °C for 15 min once again and the supernatant fraction was used to detect XO activity as described elsewhere (25). In short, the test reaction was started by adding 0.1 ml of the supernatant in 3.9 ml of a phosphate buffer solution (pH 8.0, 50 mM, containing 1mM EDTA-Na) with 1 ml of xanthine (500 μM, final concentration 100 μM) as the substrate. The mixture (total 5 ml) was incubated at 37 °C for 30 min. The reaction was stopped by adding 0.5 ml 0.58 M HCl. We considered a blank reaction containing all components of the test reaction but stopped by the addition of 0.5 ml 0.58 M HCl at once. Then the blank mixture (total of 5.5 ml) was incubated at 37 °C for 30 min. The production of UA was measured by determining the UV absorbance (A) at 290 nm ($A = A_{\text{Test tube}} - A_{\text{Blank tube}}$). One unit of XO enzyme activity was calculated as the amount of enzyme required to convert 1 nmol of xanthine to UA per minute per mg protein at 37 °C using a standard curve of UA.

Plasma samples were thawed and aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) enzyme activities were determined by biochemical kits (Pars Azmon Co., Tehran, Iran) using an auto-analyzer (Hitachi 896, Germany). Each assay was performed in triplicate and protein concentration was determined using bovine serum albumin as the standard (26).

Digital bone densitometry

The mice were killed at the end of the study (56th day) with a lethal injection of a mixture of ketamine hydrochloride (300 mg/ml) and xylazine hydrochloride (30 mg/mL). The femurs were surgically removed and trimmed and the bone mass density was evaluated by radiographic densitometry via Digital Flash Beam (DMS Lexxos DR; Diagnostic Medical Systems Group, French)**.** Digital Flash Beam was designed in a way that uses an amorphous silicon flat-panel detector. The radiographs have been submitted to Digora for Windows 2.8™ software (SOREDEX, Finland) for detection of density, and mean ± standard errors of the mean (SEM) of bone density of each femur have been recorded. After calibration of the Digital Flash Beam for mice, the bone density has been reported as an arbitrary T-score which, following cutoff levels has been considered to compare bone densities of studied groups. This score indicates the comparison of the mean mass bone of each subject with matured youngsters with peak bone mass. If the T-score was between zero and -1, it is normal and if it was between -1 and -2.5, it is the indicator of osteopenia or the first phase of bone loss. Now if this score was less than -2.5, it shows osteoporosis.

STATISTICAL ANALYSIS

Data were expressed as mean ± SEM. The comparison of means between groups was carried out using a one-way analysis of variance (ANOVA) and *post hoc* Tukey's HSD test. Statistical analyses were performed using SPSS version 16 software for Windows (SPSS, Chicago, IL, USA). The P-value < 0.05 was considered statistically significant.

RESULTS

The results of bone densitometry did not show significant differences among the studied groups, although bone density tended to decrease by 44%, 22%, and 62% in ROX (P=0.728), LOX (P=0.126), and BOX (P=0.897), respectively as compared to NC mice (Table 1, Fig. 1).

Table 1. *The effects of unilateral or bilateral orchidectomy on plasma lipid and lipoprotein profiles of male mice*

	NC	ROX	LOX	BOX
TC (mg/dl)	108.17±6.72^{ab}	127.67±7.45^{ab}	147.17±7.70^{b}	140.50±9.40^{b}
HDL-C (mg/dl)	67.66±3.64^{ab}	78.00±5.57^{b}	91.66±3.43^{b}	66.16±4.48^{ab}
LDL-C (mg/dl)	29.10±4.57^{a}	36.72±8.23^{ab}	35.10±6.17^{ab}	57.50±7.71^{b}
VLDL-C (mg/dl)	11.40±0.89	19.43±3.21	20.40±2.66	16.83±2.38
TGs (mg/dl)	57.00±4.47	97.16±16.05	102.00±13.33	84.16±11.92
BD (T-score)	-0.70±-0.12	-1.75±-0.44	-2.33±-0.33	-1.83±-0.40
AI	0.60±0.07^{a}	0.67±0.14^{a}	0.60±0.07^{a}	1.13±0.08^{b}
CHD	1.60±0.07^{a}	1.67±0.14^{a}	1.60±0.07^{a}	2.13±0.08^{b}

Note: Normal control (NC), right hemi-orchidectomized (ROX), left hemi-orchidectomized mice (LOX), and bilateral orchidectomized (BOX) mice. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), Very low-density lipoprotein cholesterol (VLDL-C), Triacylglycerol (TGs), Bone density (BD), Atherogenic index (AI), and Coronary heart disease index (CHD). Results were shown as mean ± SEM. Values with various letters in rows were statistically different, at $P \leq 0.05$.

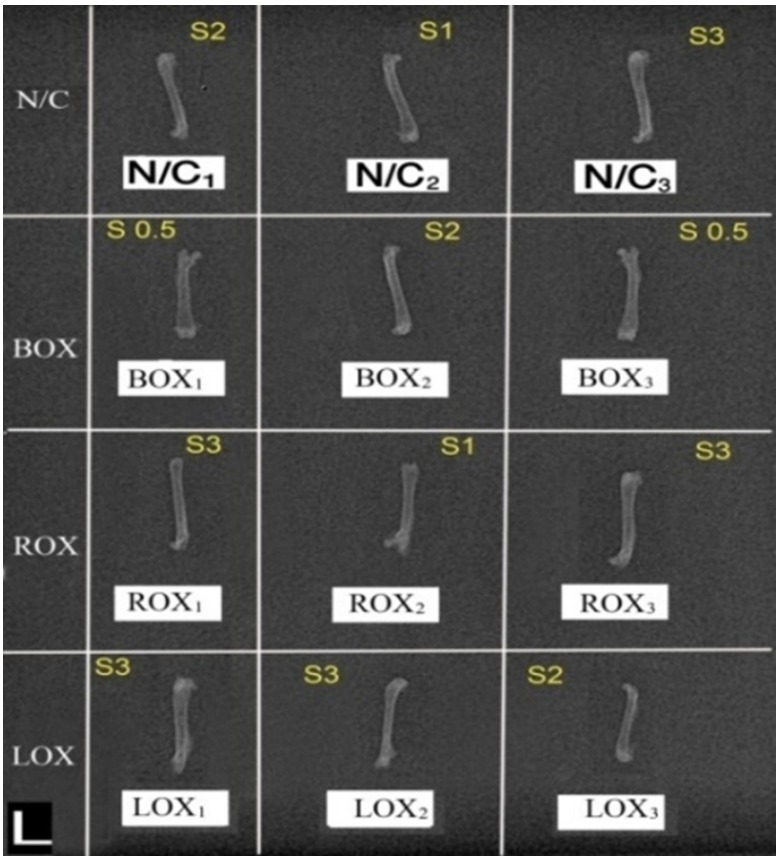

Figure 1*. The radiographs of femurs of normal control (N/C), right hemi-orchidectomized (ROX), left hemi-orchidectomized mice (LOX), and bilateral orchidectomized (BOX) mice*

The TC was increased significantly in LOX (P=0.011) and BOX (P=0.041) compared to the NC group, although it tended to increase by 15% in ROX (P=0.328) as compared to NC (see Table 1). The HDL-C level was increased significantly in ROX (P=0.008) and LOX (P=0.006). Moreover, the LDL-C (P=0.030) was increased significantly in the BOX in comparison to NC, although TGs and VLDL-C levels did not show any significant differences among these groups. In sum, based on the results of the lipid profile, the AI (P=0.005) and CHD (P=0.005) showed a significant increase in the BOX group as compared to the NC group. The AI and CHD did not change in LOX and ROX groups with respect to NC.

The TP levels were increased significantly in ROX (P=0.020), LOX (P=0.004), and BOX (P=0.004) as compared to that of the NC group (Table 2).

The ALB level was significantly increased in LOX (P=0.011) and BOX (P=0.004) compared to NC, while its level in ROX (P=0.172) tended to increase as compared to that of NC. The G levels were increased significantly in the ROX group (P=0.043) compared to NC, while its levels were not different in BOX (P=0.059) and LOX (P=0.323) compared to NC. The unilateral or bilateral orchidectomy did not alter TP, ALB, and G levels and A/G ratios among (hemi)orchidectomized mice. The A/G ratio was also not different in NC mice compared to (hemi)orchidectomized mice.

The Ca value tended to decrease by 3%, 7%, and 3% in ROX (P=0.978), LOX (P=0978), and BOX (P=0.981) respectively, as compared to NC mice (Table 2). The P-value also tended to decrease by 53%, 49%, and 2% in ROX (P=0.042), LOX (P=0.057), and BOX (P=0.998) respectively, as compared to NC mice. Generally, the Ca/P value tended to increase by 25% and 22% in ROX (P=0.303) and LOX (P=0.446) while it decreased by 2% in BOX (P=1.000) respectively, as compared to NC (see Table 2).

The plasma testosterone level was decreased by trends of 43% in all selected groups as compared to NC (Table 3) but they were not significant (Table 4). The plasma levels of AST, ALT,

Table 2. *The effects of unilateral or bilateral orchidectomy on plasma protein profile and phosphorus and calcium values of male mice*

	NC	ROX	LOX	BOX
TP (g/dl)	4.06±0.18[a]	4.91±0.21[b]	4.85±0.12[b]	5.10±0.17[b]
ALB (g/dl)	1.44±0.12[a]	1.70±0.31[ab]	1.86±0.04[b]	1.91±0.10[b]
GLB (g/dl)	2.62±0.10[a]	3.21±0.19[b]	2.98±0.14[ab]	3.18±0.08[ab]
A/G ratio	0.55±0.04	0.53±006	0.63±0.04	0.60±0.02
Ca (mg/dl)	7.83±0.61	7.53±0.42	7.30±0.67	7.53±0.32
P (mg/dl)	10.29±1.24	6.71±0.27	6.90±0.38	10.08±1.13
Ca/P ratio	0.83±0.13	1.12±0.04	1.07±0.12	0.81±0.12

Note: Normal control (NC), right hemi-orchidectomized (ROX), left hemi-orchidectomized mice (LOX), and bilateral orchidectomized (BOX) mice. Total protein (TP), albumin (ALB), Globulin (GLB), calcium (Ca), and phosphorus (P). A/G ratio stated ALB to Globulin ratio, Ca/P ratio. Results were shown as mean ± SEM. Values with various letters in rows were statistically different, at $P \leq 0.05$.

Table 3. *The effects of unilateral or bilateral orchidectomy on enzyme and testosterone profile of male mice*

	NC	ROX	LOX	BOX
Plasma AST (IU/l)	230.83±52.47	348.33±46.31	270.33±33.87	235.17±62.98
Plasma ALT (IU/l)	3.83± 0.79	33.50±7.07	21.66±2.95	35.33±23.36
Plasma LDH (IU/l)	996.00±155.15	1780.8±115.23	1808.5±126.5	1635.0±122.9
Plasma ALP (IU/l)	269.33±30.07	158.33± 14.00	180.50 ±9.92	213.50 ±1415
Bone ALP (IU/l)	0.019±0.005	0.007±0.023	0.075±0.0213	0.125±0.059
Testosterone (ng/ml)	0.035±0.008	0.020±0.000	0.020±0.000	0.020±0.000

Note: Normal control (NC), right hemi-orchidectomized (ROX), left hemi-orchidectomized mice (LOX), and bilateral orchidectomized (BOX) mice. Aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP). Results were shown as mean ± SEM. Values with various letters in rows were statistically different, at $P \leq 0.05$.

Table 4. *The effects of unilateral or bilateral orchidectomy on xanthine oxidase activity (nanomoles of uric acid/min.mg protein) in selected tissues of male mice*

	NC	ROX	LOX	BOX
Heart	0.69± 0.02[a]	0.65± 0.04[a]	0.48±0.01[b]	0.55±0.03[b]
Brain	1.53± 0.08	1.47±0.12	1.57±0.04	1.58±0.08
Muscles	8.79±0.34[a]	6.74 ±0.023[b]	8.00 ±0.34[ab]	8.08 ±0.80[b]

Note: Normal control (NC), right hemi-orchidectomized (ROX), left hemi-orchidectomized mice (LOX), and bilateral orchidectomized (BOX) mice. Xanthine oxidase (XO), alanine transaminase (ALT), and alkaline phosphatase (ALP). Results were shown as mean ± SEM. Values with various letters in rows were statistically different at $P \leq 0.05$.

LDH, and ALP were not different among the groups (Table 3). The LDH enzyme tended to increase by 44%, 44%, and 39% in ROX, LOX, and BOX respectively, as compared to NC. The plasma and bone ALP levels did not show any significant differences between the groups (Table 3). However, plasma ALP tended to decrease by 70%, 49%, and 26% in ROX, LOX, and BOX respectively, as compared to NC. The bone ALP tended to decrease by 61% and 60% in ROX and LOX respectively, as compared to that of NC mice. The bone ALP tended to increase by 34% in BOX as compared to that of NC (see Table 3).

The XO activities in heart tissues of LOX ($P=0.045$) and BOX ($P=0.043$) groups were decreased significantly compared to that of NC, however, XO activities in ROX and NC groups were not different ($P=0.078$; Table 4). The XO activities in muscle tissues have been decreased in ROX ($P=0.040$) and BOX ($P=0.047$) significantly compared to NC, while XO activity was not different between LOX and NC ($P=0.059$; Table 4). The muscle XO activities were not different among (hemi)orchidectomized mice. However, brain XO tended to increase by 3.9% and 3.2% in LOX and BOX, respectively. Moreover, its increasing trend in LOX was 2.6% as compared to that of the NC group (see Table 4).

DISCUSSION

Physiologically, the testosterone level and its functions are gradually decreasing through aging. For instance, the maintenance of *levator ani* muscle is controlled by testosterone in rats, which is diminished during aging (27). In the present study, the level of testosterone decreased in (hemi)orchidectomized mice, however it was not significant as compared to normal mice. It seems that more time is required after orchidectomy to trigger and translate the changes which physiologically occur in male hypogonadism. More specifi-

cally, the identical levels of testosterone in (hemi)orchidectomized mice may be a technical limitation to estimating low levels of testosterone. We proposed detecting testosterone with more sensitive kits or techniques. It is clinically suggested to confirm senile hypogonadism which is caused by low bioavailability of testosterone, clinical signs like changes in logical functioning, mood, lean body mass, and hair, skin, and fat distribution should all be considered. Furthermore, dual-energy x-ray absorptiometry (DEXA) can be used to confirm decreases in bone mineral density (28). The bone density tended to decrease more than 22% in (hemi)orchidectomized mice as compared to normal mice and this animal model can be reliable to be employed in experimental studies focused on hypogonadism-induced osteoporosis. In sum, the present study guarantees diminished bone density following (hemi)orchidectomy.

It is highly accepted that low testosterone is a risk factor for the development of atherosclerosis, especially among elderly men (reviewed in 29). The AI and CHD have not been altered in hemi-orchidectomized mice as compared to normal mice, while orchidectomized mice showed a considerable increase in these atherogenic indices. Lipid and lipoprotein profiles indicate an increased cholesterol biosynthesis in (hemi)orchidectomized mice as compared to normal mice. It seems that more time is needed to induce overt dyslipidemia and atherosclerosis in hemi-orchidectomized mice, however, mice are HDL-C animals and physiologically resistant to dyslipidemia and atherosclerosis.

The plasma levels of Ca and P and their ratio have not changed significantly in (hemi)orchidectomized mice as compared to normal mice 56 days of post-operation. The trend of Ca decrement and significant increase of TP in (hemi)orchidectomized mice as compared to normal mice can be explained by considering the effect of testosterone on increasing Ca turnover and protein anabolism (18). Generally, testosterone results in the increased bone matrix, and protein anabolism and affects calcium levels in response to increased protein (18). The G levels increased significantly in the ROX group compared to NC, while its levels tended to increase in BOX and LOX compared to NC. If we rule out aging dehydration (reviewed in 30), the mechanisms of increased G, Alb, and TP need more investigations. In sum, it should be mentioned that although there was no significant difference between LOX and ROX, although through considering the more alterations in CHD, AI, bone density, LDL, P, AST, ALT, plasma and bone ALP, it can be hypothesized that the ROX causes more pathological changes than LOX.

Plasma levels of clinically relevant enzymes including AST, ALT, LDH, and ALP were not different among groups. This means more time is needed to induce clinical manifestation of internal organ dysfunctions in (hemi)orchidectomized mice. The bone ALP level also did not show significant differences among groups. More cautiously, the increasing trend of bone ALP in bilateral orchidectomized mice may show that more time is needed to induce clinical osteoporosis in the orchidectomized mouse model.

In this study, XO activities showed significant reductions in selected tissues following uni- or bi-lateral orchidectomy. In this regard, XO activity significantly decreased in the muscles of ROX mice compared to normal mice. The XO activity also decreased significantly in the heart tissues of LOX mice with respect to normal mice. The XO activity also decreased significantly in the heart and muscle tissues of BOX mice with respect to normal mice. As mentioned before, XO is considered a principal enzyme in several body functions, including immune, metabolic and oxidative stress processes. Moreover, it is known as an important factor in CVDs regarding its roles in cardiac calcium level, UA, and ROS production and inducing more oxidative stress, therefore affecting cardiac muscle efficiency (31, 32). Inconsistent with our results, it has been shown that testosterone depletion through orchidectomy may protect mice from heat-induced multiple organ damage and lethality (33). Similar to our results in bilateral orchidectomized mice, the XO levels of heart and brain tissues did not significantly change following bilateral ovariectomy in mice (20). Powers *et al* (34) showed that the XO can cause increased UA production and muscle loss. Furthermore, several studies proved that muscular oxidative stress in diabetic rats increases XO and H_2O_2 (20, 34, 35). Therefore, XO might be a therapeutic target for muscular oxidative stress state in aging (35). In this line, Kumagai *et al* (36) reported that XO inhibitors abolish cryptorchidism-induced apoptosis of testicular germ cells.

CONCLUSION

To the best of our knowledge, this is the first study that shows XO alternations in the mouse model of hypogonadism. It seems that the orchidectomy is the simplest, fastest, and most economical model for modeling hypogonadism in a short time. However, simplicity does not always lead to very precise translated outcomes. However, the present model could induce some aspects of hypogonadism acceptably. More spe-

cifically, testosterone and bone density tended to decrease in (hemi)orchidectomized mice and this animal model guarantees diminished bone density following (hemi)orchidectomy. The lipid and lipoprotein profiles were also disturbed in this mouse model of hypogonadism. However, more time is needed to induce overt dyslipidemia and atherosclerosis in hemi-orchidectomized mice, since mice are physiologically resistant to dyslipidemia and atherosclerosis. The increased levels of G, Alb, and TP in this mouse model of hypogonadism need more investigation. The plasma levels of clinically relevant enzymes including AST, ALT, LDH, and plasma and bone ALP were not altered in this mouse model of hypogonadism. In sum, more time is requested post-orchidectomy to completely translate hypogonadism into a mouse model. In this study, XO activities were declined in this mouse model of hypogonadism, and deciphering the interrelationship of testosterone depletion and XO would be the subject of further investigations in the more reliable animal model. It seems that more time (> 8 weeks) is required after orchidectomy to trigger and translate the changes which physiologically occurred in male hypogonadism.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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