Hyperbaric oxygen and treadmill exercise partially prevented bone loss and bone microarchitecture deterioration in ovariectomized rats

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Abstract
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Introduction: Previous studies have demonstrated the beneficial effects of treadmill exercise (EX) on osteoporosis, and of hyperbaric oxygen (HBO) on osteoblast and osteoclast formation *in vitro*. We investigated the effects of HBO and the combination of HBO and EX on osteoporosis in ovariectomized rats.
Methods: Forty 3-month-old female Sprague-Dawley rats were randomly divided into 5 groups (n = 8): a sham control group (Control); an ovariectomy group; an ovariectomy with treadmill exercise treatment group; an ovariectomy with HBO treatment group; and an ovariectomy with HBO treatment combined with treadmill exercise group. The HBO exposures were 203 kPa, 85–90% O_2, 90 min and the exercise regimen was 20 m·min^{-1}, 40 min·day^{1}, 5º slope. Both treatments were administered once daily, five days a week for 12 weeks until the rats were sacrificed.
Results: All three treatments (HBO, exercise, and both combined) significantly promoted the expression of the osteoblast-related gene and oxidative metabolism-related gene (PGC-1α). They also exerted significant inhibitory effects on the osteoclast-related mRNA expression (RANKL) and bone resorption marker CTX-I. Additionally, exercise and the combination exercise-HBO treatment increased serum superoxide dysmutase (SOD) and sclerostin expression. No significant between-group difference was observed.
Conclusions: Hyperbaric oxygen, exercise, and the combination ameliorated bone microarchitecture deterioration and ovariectomy-induced bone loss in rats, and these inhibitory effects may be associated with the increased SOD and up-regulated PGC-1α.

Introduction

Osteoporosis, a systemic bone disease characterised by bone mass reduction and bone microstructure destruction, can increase bone fragility and predispose to fractures. With the aging population, the incidence of osteoporosis is increasing which in turn increases the medical expenditure on fractures worldwide.[1] It is expected that the number of osteoporotic fractures will double by 2050.[2] Therefore, the prevention and treatment of osteoporosis is clinically important.

Osteoporosis can be treated by drug and non-drug therapies. In drug therapy, bone resorption inhibitors and bone formation promoters are commonly applied. Though used widely with good results in clinical practice, drug therapy has certain disadvantages, such as long medication cycles, high treatment costs, poor patient compliance, and adverse drug reactions.[3,4] Therefore, developing effective non-drug therapies for osteoporosis, such as pulsed electromagnetic field, ultrasound, extracorporeal shock wave, electroacupuncture, exercise therapy and hyperbaric oxygen (HBO) treatment, has become a focus of current research.

One study showed that a combination of mildly elevated pressure and inspired oxygen fraction (133 kPa, 40% O_2) exerted protection against osteoporosis induced by hindlimb unloading.[5] Ultra-early HBO (223 kPa, 97–99% O_2) can promote bone conversion, bone formation, improve bone mass and inhibit bone resorption in rats with osteoporosis induced by complete spinal cord injury.[6,7] Osteogenic-differentiating mesenchymal stem cells exposed to HBO (243 kPa, 100% O_2) under *in vitro* simulated inflammatory conditions exhibited enhanced differentiation towards the

osteogenic phenotype.[8] Clinically, 95–100% O_2 at 203 kPa (2 atmospheres absolute [atm abs]) is a commonly used HBO protocol in humans. The effects of HBO may vary if evaluated with different protocols.

Exercise therapy, another well-studied non-drug treatment in recent years, has been shown to exert a positive influence on the skeleton, and subsequently prevent or improve osteoporosis.[9] Exercise (EX) has been shown to benefit bone modeling by promoting bone marrow mesenchymal stromal cells, and to improve the bone metabolism of ovariectomized rats with osteoporosis.[10,11] Several studies[12,13] have investigated the effects of combining physical exercise and drugs for the treatment of osteoporosis. However, reports regarding the effects and mechanism of treadmill EX plus HBO, or HBO alone, on osteoporosis in ovariectomized rats are limited.

The purpose of this study was to evaluate the effect of HBO (203 kPa, 85–90% O_2, 90 min), EX (20 m·min^{-1}, 40 min·day^{-1}, 5-degree slope) and combining HBO and EX on osteoporosis in ovariectomized rats and to explore the mechanism of any benefit to provide a mechanistic basis for the clinical application of these therapies.

Methods

These procedures were carried out in accordance with the Animal Protection Law of the People's Republic of China (2019/315) and approved by the Ethics Committee of Sichuan Provincial People's Hospital (protocol code: 2019/315, date of approval 28 November 2019).

EXPERIMENTAL ANIMALS AND OVARIECTOMIZED MODELS

Forty 3-month-old female Sprague-Dawley rats, each weighing about 270 g, were purchased from Si Chuan Chengdu Dasuo Experimental Animal Co. Ltd. (license key: syxk (chuan) 2013-110). Prior to the experiment, all rats were housed in cages at room temperature (20–25°C) in an atmosphere of 60–70% humidity under a 12/12 h light/dark cycle for one week to minimise the physiological and psychological effects which might result from a new environment. Access to water and food was unrestricted. According to a random digit table, the rats were divided into five groups (n = 8 each): a sham-operated control group ('control'), ovariectomy group ('OVX'), ovariectomy with treadmill exercise treatment group ('OVX+EX'), ovariectomy with HBO treatment group ('OVX+HBO'), ovariectomy with HBO treatment combined with treadmill exercise group ('OVX+HBO+EX').

All rats underwent either a sham surgery or bilateral ovariectomy following a standard protocol.[14] All treatments were carried out 14 days post-ovariectomy. The rats were weighed monthly and euthanised at the end of the intervention. Blood was collected from the abdominal aorta prior to sacrifice. The uterus was carefully dissected and weighed for each rat to evaluate the estrogen agonistic activity.

HYPERBARIC OXYGEN (HBO) TREATMENT

The animal hyperbaric oxygen chamber (Yantai Hong Yuan Hyperbaric Oxygen Chamber Co. Ltd., China) was provided by Sichuan Provincial People's Hospital. The rats were compressed over 30 minutes to a pressure of 203 kPa (2 atm abs) where they remained for 40 minutes before being depressurised over 20 minutes. Rats breathed 85% oxygen for the duration of the treatment cycle (90 minutes).

The HBO exposure was applied once per day, 5 days per week for a total of 12 weeks. Rats not receiving hyperbaric treatment received air at atmospheric pressure.

EXERCISE PROTOCOLS

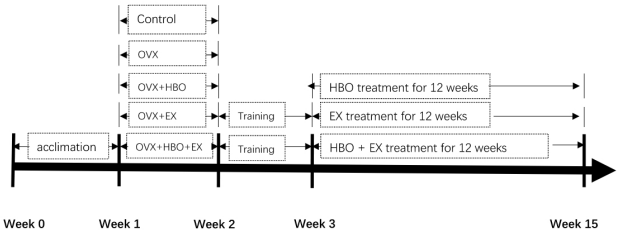
All animals ran on a six-channel motor-drive treadmill (Anhui Zheng Hua Co. Ltd, China) at a speed of 12–16 m·min^{-1} for 40 min·day^{-1} for the first week in order to reduce stress during the training period. The rats did not receive any electric stimulus to run, but manual stimulation was applied. The exercise groups participated in a running program at a constant speed of 20 m·min^{-1} for 40 min, five days per week, with a 5-degree angle for 12 weeks, 14 days post-ovariectomy. The nontrained rats were placed on the switched-off treadmill with the same duration. The experimental flowchart of the present study is shown in Figure 1.

SERUM PARAMETERS FOR BONE METABOLISM

Serum bone formation biomarker procollagen type I N-terminal propeptide (PINP) (Elabscience, China) and bone resorption biomarker C-terminal telopeptides of

Figure 1

The experimental flowchart of the present study; Week 0 – rats acquired; Week 1 – modeling rats and allocation to five groups (n = 8 each); week 15 – euthanasia at the end of the intervention; Control – sham-operated control group; OVX – ovariectomy group; OVX+EX – ovariectomy with exercise treatment group; OVX+HBO – ovariectomy with hyperbaric oxygen treatment group; OVX+HBO+EX – ovariectomy with hyperbaric oxygen treatment combined with exercise group



type I collagen (CTX) (Elabscience, China) were used to evaluate bone turnover. Serum superoxide dismutase (SOD) (Elabscience, China) was used to evaluate antioxidant capacity.

MICRO-CT FOR BONE MASS AND MICROSTRUCTURE

The left femurs of the rats were fixed with 10% formalin for 24 h and scanned using a laboratory micro-CT scanner (Quantum GX; PerkinElmer, Waltham, MA). The basic parameters of the scanner were as follows: X-ray energy, 80 kV; current intensity, 88 μA; scan time, 14 min; pixel size, 50 μm. A total of 512 slices were scanned for each femur. Then, three-dimensional images were reconstructed by PerkinElmer Analyze 12.0 software (PerkinElmer, Waltham, MA) and a series of planar cross-sectional images were generated. Regions of interest were manually selected to define the subchondral bone plate and subchondral trabecular bone of the femur. The bone microstructure parameters included bone volume to tissue volume ratio (BV/TV), trabecular number (Tb. N), trabecular thickness (Tb. Th), and trabecular thickness separation (Tb. Sp).

BONE HISTOLOGICAL ANALYSIS

Following micro-CT scan, the left femurs were decalcified for 2–3 months in 20% ethylene diamine tetra-acetic acid, processed and embedded in paraffin wax. Coronal sections from the middle of the femur (5 μm) were stained with hematoxylin eosin (H&E) and tartrate-resistant acid phosphatase (TRAP) (Sigma-Aldrich, St. Louis, MO) for histological analysis. TRAP-positive multinucleated cells (three or more nuclei as osteoclasts) were observed in the regions 2 mm beneath the growth plate.

REAL-TIME POLYMERASE CHAIN REACTION (PCR) FOR BONE-RELATED GENE EXPRESSION

After bone marrow removal, total RNA was extracted from the distal metaphyses of right femurs using Trizol reagent, following the protocol of the Eastep Super Total RNA Extraction Kit (Promega, Shanghai, China). Then, the PrimeScript RT reagent kit (Takara-Bio, Otsu, Japan) was used to synthesize cDNA from RNA. Polymerase chain reaction tests were conducted using an ABI 7300 Real-Time PCR system using the SYBR Premix Ex Taq II kit (Takara-Bio, Otsu, Japan). The primers were synthesized (Qinke Biotech, Beijing, China) and the sequences are listed in Table 1. Each RNA quantification was carried out in triplicates in a 96-well plate, and the operation was performed on each sample three times. The relative mRNA expression levels were normalised to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) for each sample and analysed using the $2-\Delta\Delta Ct$ relative quantification method.

STATISTICAL ANALYSES

All data were expressed as mean (standard deviation [SD]) and statistical analyses were performed using IBM SPSS Statistics 19 software and GraphPad Prism 8 (GraphPad Software, San Diego, CA). Statistically significant differences were assessed by one-way analysis of variance (ANOVA). A P-value of < 0.05 was considered significant.

Results

EFFECTS OF HBO AND EX ON BODY WEIGHT AND UTERINE MASS IN OVARIECTOMIZED RATS

The body weight of rats in all groups during the experimental period is shown in Figure 2. No significant differences in

Table 1

Sequence of primers for real-time fluorescence quantitative polymerase chain reaction; GAPDH–glyceraldehyde-3-phosphate dehydrogenase; OCN – osteocalcin; PGC-1α – peroxisome proliferator-activated receptor γ coactivator 1α; RANKL – receptor activator of nuclear factor κB ligand; SOST – sclerostin

Gene	(5′–3′)	Sequence
GAPDH	Forward	TGC ACC ACC AAC TGC TTA G
	Reverse	GGA TGC AGG GAT GAT GTT C
OCN	Forward	ACC CTC TCT CTG CTC ACT CTG CT
	Reverse	GCT GGG GCT CCA AGT CCA TT
SOST	Forward	GAG TAC CCA GAG CCT CCT CA
	Reverse	AGC ACA CCA ACT CGG TGA
RANKL	Forward	TGC TCA CCT CAC CAT CAA TGC
	Reverse	GTT GCT TAA CGT CAT GTT AGA GAT C
PGC-1α	Forward	CGA TGA CCC TCC TCA CAC CA
	Reverse	TTG GCT TGA GCA TGT TGC G

Figure 2

Changes in metabolic parameters (n = 8 each group); data are mean (SD); A – monthly body weight of OVX rats; B – uterus weight after sacrifice; C – serum N-terminal propeptide of type 1 procollagen (P1NP); D – C-terminal cross-linked telopeptides of type I collagen (a bone resorption marker) (CTX-I); E – serum superoxide dismutase (SOD); a – $P < 0.05$ or $^*P < 0.01$ versus Control; b – $P < 0.05$ or $^*P < 0.01$ versus OVX; Control – sham operated control group; OVX – ovariectomy group; OVX+EX – ovariectomy with exercise treatment group; OVX+HBO – ovariectomy with hyperbaric oxygen treatment group; OVX+HBO+EX – ovariectomy with hyperbaric oxygen treatment combined with exercise group

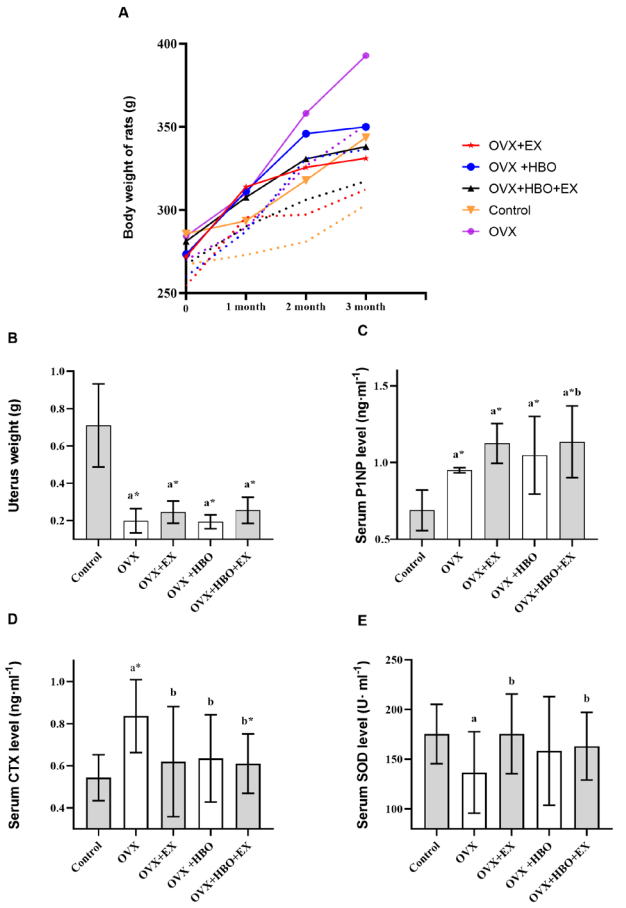
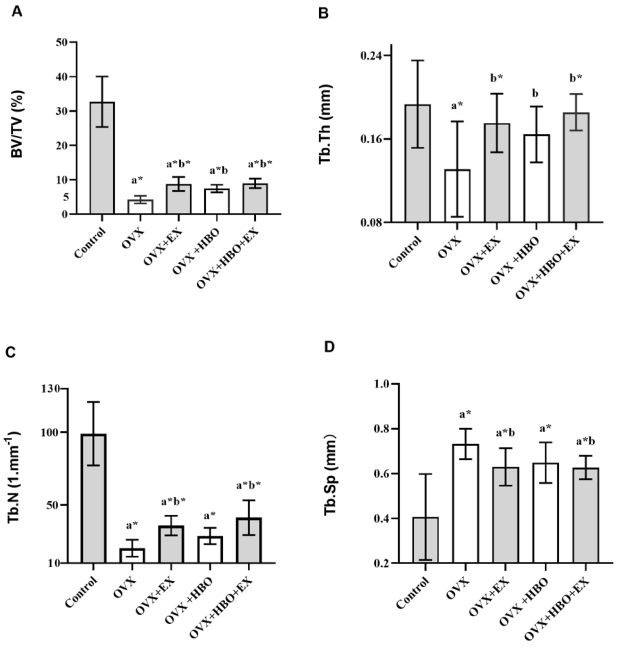


Figure 3

Changes of micro-CT parameters of subchondral trabecular bone in left femur; (n = 8 each group); data are mean (SD); A – bone volume fraction (BV/TV); B – trabecular thickness (Tb.Th); C – trabecular number (Tb.N); D – trabecular separation (Tb.Sp); a – $P < 0.05$ or $^*P < 0.01$ versus Control; b – $P < 0.05$ or $^*P < 0.01$ versus OVX; Control – sham operated control group; OVX – ovariectomy group; OVX+EX – ovariectomy with exercise treatment group; OVX+HBO – ovariectomy with hyperbaric oxygen treatment group; OVX+HBO+EX – ovariectomy with hyperbaric oxygen treatment combined with exercise group



body weight were found among groups at beginning of the experiment. The body weight of rats in the OVX group was significantly increased at two months (11.24%, 358.1 (31.8) g vs. 317.9 (36.9) g, $P < 0.05$; Figure 2A) and 3 months (14.33% 392.9 (41.7) g vs. 343.6 (40.5) g, $P < 0.05$; Figure 2A) after the ovariectomy compared with the control group. However, an inhibitory effect on OVX-induced body weight gain was found in all the intervention groups when compared to the OVX group at 3 months, and the differences were significant ($P < 0.01$ Figure 2A). Furthermore, significantly lowered uterine weight was found in groups receiving ovariectomy compared to the control group ($P < 0.01$; Figure 2B), indicating the successful establishment of the estrogen withdrawal model.

SERUM BIOCHEMICAL ANALYSIS

The serum biomarkers for bone formation and bone resorption are shown in Figures 2C and 2D. Ovariectomy resulted in an increase in serum P1NP (a bone formation marker) ($P < 0.01$) compared with the control. After the 12-week intervention, the serum P1NP was significantly increased in the OVX+HBO+EX combination group ($P < 0.01$, Figure 2C). Although the levels of P1NP in the HBO or EX rats were higher than that in the OVX rats, no significant differences were observed. Furthermore, the serum CTX-I was significantly higher in the OVX group than in the control group ($P < 0.01$, Figure 2D). The OVX rats subjected to HBO, EX and the combination therapy showed significantly decreased serum CTX-I concentration compared with the OVX group ($P < 0.05$, $P < 0.05$, $P < 0.01$, respectively, Figure 2D). The serum SOD, an important scavenger of oxygen free radicals, was significantly decreased in OVX rats compared with the control group ($P < 0.05$, Figure 2E), but increased in the groups receiving EX and the combination therapy compared with the OVX group ($P < 0.05$ Figure 2E). No significant difference was observed between the HBO and OVX rats ($P > 0.05$, Figure 2E).

Figure 4

Histological appearance (hematoxylin and eosin staining) of the left femur samples; all images are shown at ×100 magnification; Control – sham operated control group; OVX – ovariectomy group; OVX+EX – ovariectomy with exercise treatment group; OVX+HBO – ovariectomy with hyperbaric oxygen treatment group; OVX+HBO+EX – ovariectomy with hyperbaric oxygen treatment combined with exercise group

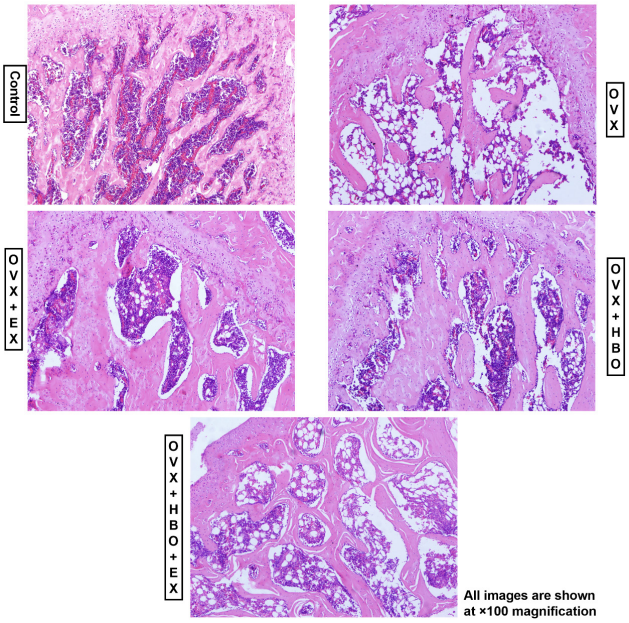
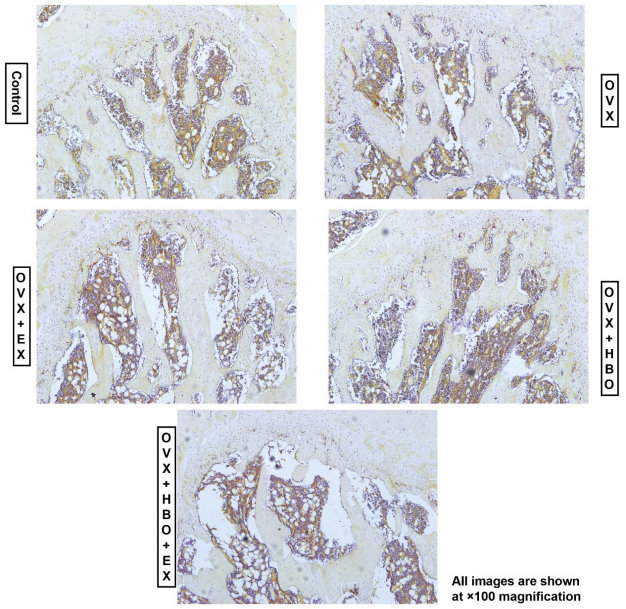


Figure 5

Histological appearance (tartrate-resistant acid phosphatase staining) of the left femur samples; all images are shown at ×100 magnification; Control – sham operated control group; OVX – ovariectomy group; OVX+EX – ovariectomy with exercise treatment group; OVX+HBO – ovariectomy with hyperbaric oxygen treatment group; OVX+HBO+EX – ovariectomy with hyperbaric oxygen treatment combined with exercise group



MICRO-CT ANALYSIS

Micro-CT analysis showed that the OVX group had an 87.1% reduction in BV/TV ($P < 0.05$, Figure 3A), a 32.2% reduction in Tb.Th ($P < 0.05$, Figure 3B), a 79.5% reduction in Tb.N ($P < 0.01$, Figure 3C), and a 180.1% growth in Tb. Sp ($P < 0.05$, Figure 3D) after the surgery, in comparison to the control group. However, the parameters Tb.Th were not altered in all the intervention groups. In comparison to the OVX group, the OVX+EX, OVX+HBO, and OVX+HBO+EX groups showed 2.07-, 1.77- and 2.12-fold higher values in BV/TV respectively ($P < 0.01$, $P < 0.05$, $P < 0.01$). Similar changes were seen in Tb.Th (1.34-, 1.25-, 1.42-fold higher, $P < 0.01$, $P < 0.05$, $P < 0.01$ respectively) and Tb.N (1.77-, 1.42-, and 2.04-fold higher, $P < 0.01$, $P > 0.05$, $P < 0.01$, respectively), but Tb.Sp was lower in the OVX+EX, OVX+HBO, and OVX+HBO+EX groups (0.86-, 0.89-, and 0.86-fold lower, $P < 0.05$, $P > 0.05$, $P < 0.05$ respectively) (Figure 3).

HISTOLOGICAL ANALYSIS

Hematoxylin and eosin staining (Figure 4) showed that in the OVX group, under the growth plate, trabeculae were thinner, less abundant, and spaced at greater distances. In the control group, the trabeculae appeared normal. In all the intervention groups, the trabeculae exhibited a near-normal histological appearance with increased trabecular bone area and trabecular number and decreased marrow cavity.

Tartrate-resistant acid phosphatase staining (Figure 5) showed significantly increased number and size of TRAP-positive multinucleated cells in the OVX group ($P < 0.01$) compared with the control group. In contrast, the number of TRAP-positive multinucleated cells in the rats from the intervention groups was less than that of OVX group (Figure 6), and the differences between them were obvious ($P < 0.01$). Moreover, a significant difference was observed in the TRAP-positive multinucleated cells between the EX group and OVX+HBO+EX group ($P < 0.01$).

REAL-TIME PCR ANALYSIS

The quantification results of rat femur gene expression via real-time PCR analysis are shown in Figure 7. The OVX group showed increased mRNA levels of osteocalcin (OCN) compared with the control group (Figure 7A). All the intervention groups demonstrated significantly upregulated femur OCN gene expression compared with the OVX group. We also found a significantly increased mRNA expression of receptor activator of nuclear factor κB ligand (RANKL) and sclerostin (SOST) in the OVX group compared with the control group (Figure 7B–C), and all the intervention groups had significantly downregulated femur SOST and RANKL gene expression compared with the OVX group. As for the expression of the peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) gene, a significant decrease was observed in the OVX group compared with the control group, while all the intervention groups showed

Figure 6

Numbers of tartrate-resistant acid phosphatase staining positive cells per unit bone sample by treatment group; a – $P < 0.05$ or *$P < 0.01$ versus Control; b – $P < 0.05$ or *$P < 0.01$ versus OVX; c – $P < 0.05$ or *$P < 0.01$ versus OVX+HBO+EX; Control – sham operated control group; OVX – ovariectomy group; OVX+EX – ovariectomy with exercise treatment group; OVX+HBO – ovariectomy with hyperbaric oxygen treatment group; OVX+HBO+EX – ovariectomy with hyperbaric oxygen treatment combined with exercise group

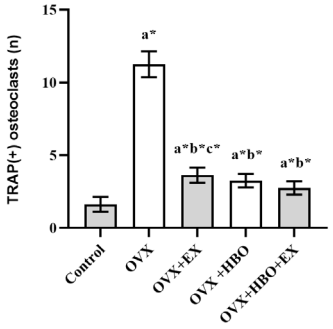
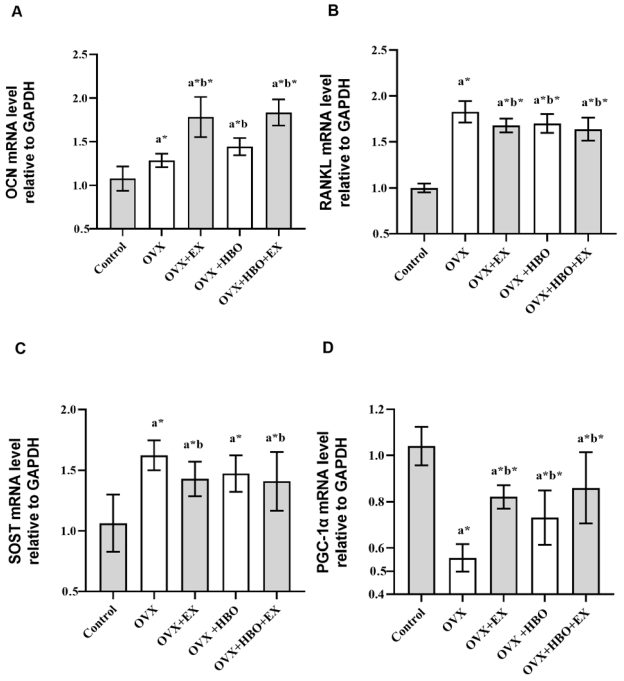


Figure 7

Relative mRNA expressions in the distal metaphyses of right femurs with bone marrow removal in OVX rats via real-time PCR analysis; A – Osteocalcin (OCN); B – receptor activator of nuclear factor kB ligand (RANKL); C – sclerostin (SOST); D – Peroxisome Proliferator-Activated Receptor γ coactivator 1α (PGC-1α); Control – sham operated control group; OVX – ovariectomy group; OVX+EX – ovariectomy with exercise treatment group; OVX+HBO – ovariectomy with hyperbaric oxygen treatment group; OVX+HBO+EX – ovariectomy with hyperbaric oxygen treatment combined with exercise group



significantly upregulated PGC-1α gene expression compared with the OVX group.

Discussion

In this study, we investigated whether HBO, EX, and a combination of HBO and EX could inhibit ovariectomy-induced bone loss and osteoporosis in rats and explored the underlying mechanisms. We found that HBO, EX and the combination treatment significantly ameliorated the deterioration of bone microarchitecture and the ovariectomy-induced bone loss in OVX rats. This inhibitory effect may be associated with the increased SOD and up-regulated PGC-1α. Exercise has been reported to inhibit bone loss after ovariectomy in rats.[15,16] Hyperbaric oxygen has also been reported to exert similar inhibitory effects on osteoporosis rats,[5–7] but in these studies, different osteoporosis models and HBO parameters were used. One study examined the effects of 'mild HBO' (134 kPa [1.32 atm abs] pressure with 40% oxygen) on unloading-induced osteoporosis rats,[5] and another group used rats with complete spinal transection as osteoporosis models with HBO delivered at 223 kPa (2.2 atm abs) with an oxygen concentration of 97–99%.[6,7] In the current study, we used 3-month-old ovariectomized rats as the postmenopausal model because these rats are reproductively mature and capable of responding appropriately to estrogen deficiency. The experimental intervention lasted for three months to establish the standard osteoporotic animal model.[17] We performed interventions at 14 days after OVX, because bone loss consistently occurred at 14 days after OVX.[43] Hyperbaric oxygen was applied at 203 kPa (2.0 atm abs) and an oxygen concentration of 85–90% because these parameters are commonly used in studying and treating clinical indications for HBO. We sampled the left femur rather than the lumbar spine for Micro-CT and histological analyses because weight-bearing bones such as tibia and femur have a higher sensitivity to treadmill exercise than the lumbar spine in rats.[18–22] Treadmill exercise was applied at 20 m·min^{-1}, 40 min·day^{-1} for 12 weeks, because previous studies have shown that moderate exercise can prevent the bone loss of the tibia and femur in ovariectomized rats.[22,23]

In our study, marked body weight gain was observed in OVX rats in the 12-week experimental period, which was consistent with the previous reports on OVX animal models.[24,25] This significant OVX-induced weight gain can be attributed to decreased energy consumption and lipid metabolism and increased fat deposition in adipose tissues in OVX animal models.[26–28] Our study also revealed that HBO, EX, and the combined intervention of HBO and EX could suppress the OVX-induced weight gain in rats. This result was in line with previous findings.[28,29] Exercise can inhibit the significant increase in body weight induced by OVX in rats. Therefore, our findings indicate that the 3-month HBO and/or EX intervention after ovariectomy may partially prevent OVX-induced weight gain by regulating energy metabolism. Furthermore, we found that endogenous estrogen production was ceased by ovariectomy, which was manifested as marked uterine atrophy in the OVX group, indicating that estrogen withdrawal was successfully

achieved in the OVX model. Comparatively, the uterine mass of OVX rats in the three intervention groups was not affected, suggesting that the interventions can inhibit bone loss without affecting the uterus after ovariectomy.

The maintenance of bone mass depends on continuous bone remodeling activity, which involves the balanced effects of osteoblastic bone formation and osteoclastic bone resorption. Yet, ovariectomy can disturb this balance, biasing the process towards bone resorption and thus leading to bone loss.[30,31] Previous *in vitro* studies reported that HBO (243 kPa, 97% O_2, 90 min, 14 HBO sessions) can accelerate human osteoblast differentiation, promote bone formation, and suppress human osteoclast formation and bone resorption in hypoxic conditions.[32,33] Another study suggests that *in vivo* HBO (243 kPa, 100% O_2, 90 min, 25 HBO sessions) suppresses osteoclast formation and bone resorption from circulating human monocytes.[34] Our study showed that osteogenesis-related gene (OCN) and serum P1NP levels were increased in the OVX group while HBO alone, EX alone and their combination increased bone formation after the 12-week intervention. Furthermore, serum CTX-I markers, the indicators of bone resorption, were significantly upregulated in OVX rats, which was consistent with the results of a previous study.[17,35] Increased expressions of osteoclastogenesis-related genes (SOST, RANKL) were also found in OVX rats. The results in the intervention groups implied that HBO alone, EX alone and their combination can significantly inhibit bone resorption. Together, our results showed that the levels of bone formation markers were significantly higher and the levels of bone resorption markers were markedly lower in the intervention groups than in the OVX group, implying that HBO alone, EX alone and their combination can enhance osteoblast genesis and inhibit osteoclast genesis in OVX rats. This was also indicated by the decreased marrow cavity, increased trabecular bone area, trabecular number, and increased Tb-N, BV/TV, and decreased Tb-Sp in intervention groups in comparison to the OVX group. The values of the combined group were superior to those of the single intervention group but without statistical difference. The above findings suggest that EX alone, HBO alone and their combination are effective in preventing ovariectomy-associated bone loss in rats, and therefore are promising alternatives for postmenopausal osteoporosis management.

Oxygen free radicals play an important role in the pathogenesis of osteoporosis by inhibiting osteoblast genesis and activating osteoclast differentiation. Therefore, the application of antioxidants might be beneficial for bone health.[36] Superoxide dismutase can scavenge oxygen free radicals and thus block the damage they cause. It has been shown that ovariectomized rats have significantly reduced estrogen and antioxidant enzyme activity, remarkably increased oxidative stress response, and thus the balance of bone metabolism is disturbed.[37] Hyperbaric oxygen has a strong antioxidant capacity and can significantly increase the activity of antioxidant enzymes in the serum.[38–41] The anti-oxidative effect of exercise can counter the aging progress in aged skeletal muscles.[42] Our study found that serum SOD level in the OVX group was significantly lower than that in the sham group ($P < 0.05$). After the 12-week intervention, the serum SOD level in the EX and combination groups was significantly increased compared to the OVX group ($P < 0.05$). PGC-1α, a master regulator of oxidative metabolism (including oxidative enzyme activity), plays a critical role in bone metabolism, and PGC-1α deficiency reduces bone mass.[5,44,45] In this present study (203 kPa, 85–90% O_2), the level of PGC-1α mRNA in the OVX group was significantly lower than that in the control group ($P < 0.01$). After a 12-week intervention, the levels in the HBO, EX and combination groups were significantly increased compared to that in the OVX group ($P < 0.05$). The results regarding SOD and PGC-1α mRNA suggest that HBO, EX and their combination can up-regulate PGC-1 α to increase SOD, improve the oxidative capacity, and thus inhibit bone loss.

Conclusions

Our findings demonstrate that HBO (203 kPa, oxygen concentration of 85–90%) and EX (20 $m \cdot min^{-1}$, 40 $min \cdot day^{-1}$, five days per week with a 5-degree slope for 12 weeks) could partially prevent bone loss and bone microarchitecture deterioration in OVX rats, and the mechanism may be associated with the increased SOD and up-regulated PGC-1α.

However, the present study failed to detect any significant difference in beneficial effects on osteoporosis between the combination treatment and monotherapy. Further investigations are needed in the future to explore the beneficial effects and underlying mechanism by the combination treatment on osteoporosis.

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