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Research article

Possible mechanisms underlying the regulation of postmenopausal osteoporosis by follicle-stimulating hormone

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ABSTRACT

Objective: To explore the possible mechanisms by which follicle-stimulating hormone (FSH) regulates postmenopausal osteoporosis through the FSH/FSH receptor (FSHr)/G protein/C/EBPβ/heat shock protein 90 alpha (HSP90α) signalling pathways.
Methods: We measured serum FSH, luteinising hormone (LH), and HSP90α levels in the serum and adipose tissue of women of childbearing age and menopausal status. In the in vivo studies, 12 B57CL female mice were divided equally into Sham, OVX, and OVX + FSHr Blocker groups. Serum levels of alkaline phosphatase, FSH, and HSP90α, along with StRACP vitality, were determined, and femur micro-computed tomography was performed. Additionally, FSH, FSHr, G protein, C/EBPβ, and HSP90α levels were assessed using quantitative polymerase chain reaction. Finally, we divided the human multiple myeloma cell line U266 into three groups. The activity of tartrate-resistant acid phosphatase (TRAP) in the supernatant at different stages was detected, and myeloma cells were stained with TRAP.
Results: HSP90α levels in adipose tissue supernatant and serum were lower in women of child-bearing age than in menopausal women ($P < 0.05$). Serum FSH and HSP90α levels demonstrated a strong correlation. Treatment with FSHr blockers resulted in decreased FSH, FSHr, G protein, C/EBPβ, and HSP90α levels in mice. TRAP staining of osteoclast-like cells exhibited a significantly higher intensity in the M-CSF + RANKL + recombinant HSP90α group than in the M-CSF + RANKL and blank control groups ($P < 0.05$).
Conclusions: Our results indicate that FSH promotes HSP90α secretion by adipocytes via the FSHr/G protein/C/EBPβ pathway. This mechanism affects osteoclast activity and exacerbates osteoporosis.

1. Introduction

Osteoporosis is a chronic bone metabolic disease characterised by reduced bone mineral density and microstructure degradation. Approximately 10 % of the global population and 30 % of postmenopausal women aged >50 years' experience osteoporosis [1]. Osteoporosis increases bone fragility and the risk of fracture, exerting a heavy burden on social health, medical services, and the economy [2,3]. Therefore, an improved understanding of the pathogenesis, potential biomarkers, and novel therapeutic targets of osteoporosis is required to develop new diagnostic and therapeutic modalities.

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In recent years, research has demonstrated that a large proportion of tumour cells and some normal cells stimulated by stress can secrete heat shock protein 90 (HSP90) as extracellular HSP90 (eHSP90) [4]. Unlike intracellular HSP90, eHSP90 has no molecular chaperone function. Extracellular secretion is independent of ATPase activity but is regulated by p53, hypoxia-inducible factor-1 α (HIF-1 α), and hectd1 ubiquitin ligase. Moreover, eHSP90 is secreted in exosomes or transported in peptide form via the endoplasmic reticulum/Golgi apparatus. eHSP90 can be detected in the cycle [5]. As an accessory protein, eHSP90 regulates cell function by binding to other cell surface proteins, including human epidermal growth factor receptor-2 (HER2), matrix metalloproteinase-2, and extracellular matrix. Additionally, eHSP90 can also combine with receptors on the cell membrane, such as low-density lipoprotein receptor-related protein-1 (LRP-1), to activate Akt and other signalling pathways for regulating cell migration and motor function [5]. In this manner, eHSP90 can control various physiological and pathophysiological processes, such as wound healing [6], tumour angiogenesis [7], cell rearrangement during neuroembryogenesis [8], activation of monocyte macrophages and dendritic cells [9,10], and tumour invasion and metastasis [11]. In our previous study, rats treated with gonadotropin (follicle-stimulating hormone [FSH]) exhibited a significant increase in serum eHSP90 levels and a significant decrease in bone mass. Moreover, serum eHSP90α was highly correlated with the expression of osteoporosis-related proteins [12]. FSH typically exerts its effect via the FSH receptor (FSHr), whereas C/EBPβ is an important factor in adipocyte differentiation. To expand on this research, the present study explored the roles of FSH and eHSP90 and the possible mechanisms of the FSHr/G protein/C/EBPβ pathway in bone metabolism (focusing on the FSH-HSP90α-bone mass axis). This investigation aims to initiate a novel discourse regarding adipose tissue and bone, offering new theoretical perspectives into the development of postmenopausal osteoporosis and identifying potential new therapeutic targets.

2. Materials and methods

2.1. Clinical sample acquisition

The blood and abdominal adipose tissue of 20 women who underwent physical examination in the gynaecological clinic of our hospital and those hospitalised for benign diseases (such as laparoscopic exploration due to infertility, pelvic organ prolapse, hysteromyoma, and benign ovarian tumour) were collected. This study compared HSP90α levels in the serum and adipose tissue of women of childbearing age with normal bone mass and postmenopausal women with reduced bone mass. According to the diagnostic criteria recommended by the World Health Organisation, osteoporosis is defined by a bone density value that is 2.5 standard deviations below the peak bone density of healthy adults of the same sex and race, as measured using dual-energy X-ray absorptiometry.

The inclusion criteria for this study were as follows: (1) provision of informed consent before enrolment; (2) age 18–65 years; (3) body mass index (BMI) 18–25 kg/m^2; (4) normal bone mass in women of childbearing age; and (5) decreased bone mass in postmenopausal women. The exclusion criteria were as follows: (1) the presence of malignant tumours and a recent history of trauma; (2) heart, liver, lung, and kidney dysfunction; (3) autoimmune diseases; (4) acute or chronic infections; and (5) lipid metabolism abnormalities and resulting comorbidities. This study was approved by the human research ethics committee of the supporting unit.

2.2. Basic measurement

General patient data, including age, height, and weight, were collected. HSP90α levels in the serum and adipose tissue, as well as serum FSH and luteinising hormone (LH) levels, were evaluated by enzyme-linked immunosorbent assay.

2.3. Animal experiments

Twelve B57CL female mice, aged 13–15 weeks, were purchased from Shanghai SLAC Laboratory Animal Co. Ltd. The mice were housed in groups of four per cage and maintained under a 12-h light/dark cycle at a temperature of 24–26 °C and a humidity of approximately 50 %. Water and food (standard rodent pellets) were provided *ad libitum*. After one week of adaptive feeding, mice were randomly divided into three groups: Sham (n = 4), OVX (n = 4), and OVX + FSHr blocker (40 mg/kg/day × 5 days, Youning Biology Science and Technology Co., Ltd., Shanghai, China) (n = 4). The FSHr blocker (hFSH-β-(33–53)TFA) is a thiol-containing peptide that binds to the second binding domain of FSHr, thereby inhibiting the binding of FSH to the receptor.

Mice were anaesthetised by the intraperitoneal injection of 3 mg/mL pentobarbital (10 mL/kg, Veterinary Institute of Military Supplies University, Changchun, China). Serum levels of ALP, FSH, and HSP90α, as well as StrACP vitality, were measured. The supernatant of the adipose tissue was assessed for FSH, FSHr, G protein, C/EBPβ, and HSP90α using quantitative polymerase chain reaction (qPCR). The oligonucleotide primers for β-actin were as follows:

CATCCGTAAAGACCTCTATGCCAAC(F) and ATGGAGCCACCGATCCACA(R). The primers for FSH, FSHr, G protein, C/EBPβ, and HSP90α were as follows: FSH, TTGACCAACATCACCATCTCAGTAG(F) and AGCAGTAGCCCGCACACC(R); FSHr, GCCTTGCTCCTGGTCTCCTTG(F) and GAATCTCGGTCACCTTGCTATCTTG(R); G protein, CAGATCGACTTTGCTGATCCC(F) and TAAGCGGCTGAGTCATTGAGC(R); C/EBPβ, GCTGAGCGACGAGTACAAGATG(F) and TGTGCTGCGTCTCCAGGTTG(R); and HSP90α, GACGCTCTGGATAAAATCCGTT(F) and TGGGAATGAGATTGATGTGCAG(R). After extracting precursor adipocytes and culturing them into mature adipocytes, we prepared adipocyte culture medium with FSH at concentrations of 200, 400, and 600 mIU/mL and cultured the cells for 2, 4, and 6 days. Adipocytes were subsequently collected, and the expressions of FSH, FSHr, G protein, C/EBPβ, and HSP90α in the adipocytes were evaluated using qPCR.

Finally, micro-computed tomography (μCT) and haematoxylin and eosin (H&E) staining of mouse bones were performed.

All animal experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of

Laboratory Animals. The study was approved by the Animal Management and Use Committee of Zhejiang University, School of Medicine, the Second Affiliated Hospital.

2.4. Cell experiments

The human multiple myeloma cell line U266, isolated from our haematological patients, was utilised in the in vitro experiments. They can differentiate into osteoclast-like cells, secrete HSP90α, and respond to HSP90α. Cells were divided into three treatment groups: M-CSF + RANKL + recombinant HSP90α, M-CSF + RANKL, and blank control. Cells were cultured in 24-well plates, with each group occupying three wells. Tartrate-resistant acid phosphatase (TRAP) activity in the supernatant was evaluated at different stages. Finally, myeloma cells were stained with TRAP.

2.5. Statistical analysis

SPSS 22.0 (version 22.0; SPSS Inc., USA) and GraphPad Prism (version 8.0.2; GraphPad Software Inc., USA) were used for the statistical analyses. The results are expressed as the mean ± standard deviation. One-way analysis of variance and t-tests were used to compare the groups. Pearson's correlation analysis was used to assess correlations. Statistical significance was set at $P < 0.05$.

3. Results

3.1. General information and blood indicators

The BMI of women of childbearing age was similar to that of postmenopausal women; however, significant differences were observed in age and FSH and LH levels ($P < 0.05$). HSP90α levels in adipose tissue supernatant and serum were lower in women of childbearing age than in menopausal women ($P < 0.05$) (Table 1). Furthermore, serum FSH and HSP90α levels demonstrated a strong correlation (Fig. 1).

3.2. Animal experimentation

We measured the body weight of the mice at 0, 2, 4, and 6 weeks (Table 2). No statistically significant differences were observed between the groups ($P > 0.05$). Serum levels of ALP, FSH, and HSP90α, as well as StrACP activity, were measured at 2, 4, and 6 weeks (Table 3). The results revealed a statistically significant difference in HSP90α levels among the groups at 6 weeks. Further comparison revealed that the OVX group exhibited significantly higher levels than the Sham group, and the FSHr blocker group exhibited significantly lower levels than the OVX group ($P = 0.036$ and $P = 0.020$, respectively). Furthermore, no statistically significant differences were detected in HSP90α levels among the groups at 4 weeks. Further comparison revealed that the OVX group exhibited higher HSP90 levels than the Sham group, and the FSHr blocker group exhibited lower levels than the OVX group ($P = 0.049$ and $P = 0.023$, respectively). A difference in StrACP activity among the groups was observed at 6 weeks. Activity levels were higher in the OVX group than in the Sham and FSHr blocker groups ($P = 0.023$ and $P = 0.047$, respectively). Although no significant difference was observed in serum FSH levels among the groups, further comparison revealed that the OVX group displayed higher levels than the Sham and FSHr blocker groups. The differences were significant only at 4 weeks ($P = 0.029$ and $P = 0.039$, respectively).

We measured the FSH, FSHr, G protein, C/EBPβ, and HSP90α levels in the adipose tissue supernatant using qPCR (Fig. 2). The results revealed statistically significant differences in FSH and FSHr levels among the groups ($P = 0.015$ and $P = 0.024$, respectively). Further analysis demonstrated that the OVX group had significantly higher FSH levels than the Sham and FSHr blocker groups ($P = 0.005$ and $P = 0.037$, respectively). Similarly, the OVX group had significantly higher FSHr levels than the Sham and FSHr blocker groups ($P = 0.009$ and $P = 0.042$, respectively). G protein levels were also higher in the OVX group than in the Sham group ($P = 0.004$). The OVX group demonstrated significantly higher C/EBPβ levels than the Sham group ($P = 0.046$). HSP90α levels were higher in the OVX group than in the Sham and FSHr blocker groups ($P = 0.031$ and $P = 0.045$, respectively).

Finally, μCT analysis of the mouse femur revealed significant differences among the groups in Tb.Sp, Tb.Th, Tb.N, and BV/TV ($P < 0.05$) (Fig. 3a, Table 4). The OVX group had the highest Tb.Sp and lowest Tb.Th, Tb.N, and BV/TV values. Additionally, H&E staining of the mouse femur revealed that the degree of osteoporosis gradually increased across the Sham, OVX + FSHr, and OVX groups (Fig. 3b).

We determined the FSH, FSHr, G protein, C/EBPβ, and HSP90α levels in the adipocytes using qPCR (Fig. 4). On day 2, statistically

Table 1
General information and blood indicators.

	Age	BMI	FSH	LH	hsp90α	hsp90α in fat supernatant
Postmenopausal women	61.60 ± 10.69	21.77 ± 2.60	49.33 ± 12.93	27.24 ± 13.08	11.93 ± 5.50	22.00 ± 5.75
Childbearing age women	30.8 ± 5.72	23.22 ± 2.62	5.78 ± 3.51	6.84 ± 2.67	2.38 ± 1.50	5.36 ± 3.37
P	<0.05	0.406	<0.05	0.009	0.016	0.001

Table 1: General information and blood indicators. The age, FSH, LH, HSP90α, HSP90α in fat supernatant were statistically different between groups ($P < 0.05$), while the BMI had no significant difference($P > 0.05$). P: comparation between Postmenopausal and Childbearing age women groups.

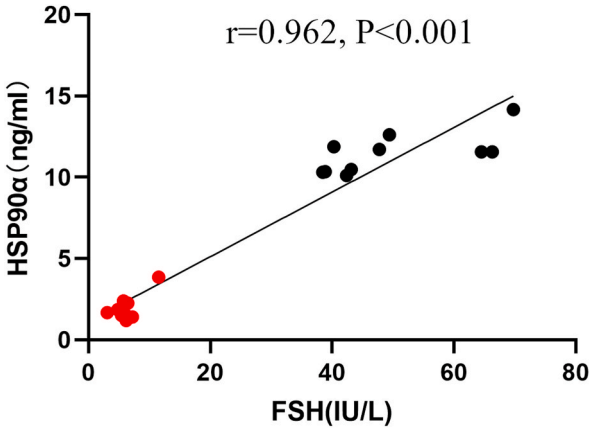

Fig. 1. Red markers: women of childbearing age. Black markers: postmenopausal women. The correlation between serum follicle-stimulating hormone and heat shock protein 90 alpha levels was statistically significant. R = 0.962, P < 0.001.

Table 2
The body weight of mice at 0,2,4 and 6 weeks.

	Weight(g)			
	0w	2w	4w	6w
Sham	21.33 ± 0.75	22.13 ± 1.12	22.85 ± 1.07	23.70 ± 0.99
OVX	21.71 ± 0.94	22.83 ± 1.28	23.70 ± 0.76	24.05 ± 0.75
FSHr blocker	21.85 ± 0.97	22.75 ± 1.14	23.15 ± 0.50	23.83 ± 0.77
P	0.643	0.668	0.364	0.841
P1	0.471	0.474	0.613	0.839
P2	0.889	0.93	0.362	0.715
P3	0.393	0.424	0.172	0.572

Table 2: The weight had no statistical difference between groups(P > 0.05). P: comparation between Sham, OVX and FSHr blocker groups; P1: comparation between Sham and OVX groups; P2: comparation between Sham and FSHr blocker groups; P3: comparation between OVX and FSHr blocker groups.

Table 3
The serum HSP90α, FSH, StrACP activity, ALP of mice at 2,4 and 6 weeks.

	HSP90α(ng/ml)			FSH(mIU/ml)		
	2w	4w	6w	2w	4w	6w
Sham	21.81 ± 3.80	15.69 ± 2.46	24.05 ± 0.86	0.11 ± 0.07	0.19 ± 0.02	0.17 ± 0.02
OVX	27.28 ± 3.97	20.36 ± 4.12	27.79 ± 3.01	0.44 ± 0.23	0.50 ± 0.26	0.33 ± 0.23
FSHr blocker	21.28 ± 1.06	14.74 ± 1.55	23.50 ± 2.02	0.31 ± 0.23	0.21 ± 0.14	0.35 ± 0.30
P	0.051	0.05	0.04	0.098	0.052	0.477
P1	0.04	0.049	0.036	0.037	0.029	0.33
P2	0.822	0.655	0.723	0.186	0.854	0.277
P3	0.027	0.023	0.02	0.335	0.039	0.901
	StrACP activity(U/L)			ALP(U/L)		
	2w	4w	6w	2w	4w	6w
Sham	110.35 ± 16.37	114.74 ± 12.68	109.37 ± 15.98	147.83 ± 10.33	140.53 ± 12.17	144.90 ± 11.07
OVX	119.7 ± 18.43	136.63 ± 22.02	162.78 ± 36.14	150.20 ± 10.05	146.8 ± 8.06	136.20 ± 10.36
FSHr blocker	123.08 ± 24.99	113.99 ± 2.42	118.15 ± 34.85	134.95 ± 11.20	139.22 ± 12.68	130.35 ± 5.30
P	0.674	0.098	0.048	0.144	0.608	0.131
P1	0.558	0.065	0.023	0.757	0.447	0.211
P2	0.397	0.944	0.662	0.118	0.873	0.051
P3	0.786	0.058	0.047	0.071	0.362	0.389

Table 3: The results of serum HSP90α, FSH, StrACP activity, ALP of mice at 2,4 and 6 weeks were showed as above. Sham: Sham group; OVX: OVX group; FSHr Blocker: OVX + FSHr Blocker group. P: comparation between Sham, OVX and FSHr blocker groups; P1: comparation between Sham and OVX groups; P2: comparation between Sham and FSHr blocker groups; P3: comparation between OVX and FSHr blocker groups.

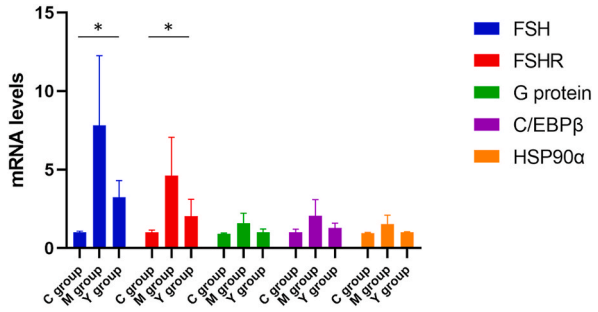

Fig. 2. Follicle-stimulating hormone (FSH), FSH receptor (FSHr), G protein, C/EBPβ, and heat shock protein 90 alpha messenger RNA levels in adipose tissue supernatant by quantitative polymerase chain reaction. C group: Sham group; M group: OVX group; Y group: OVX + FSHr blocker group.

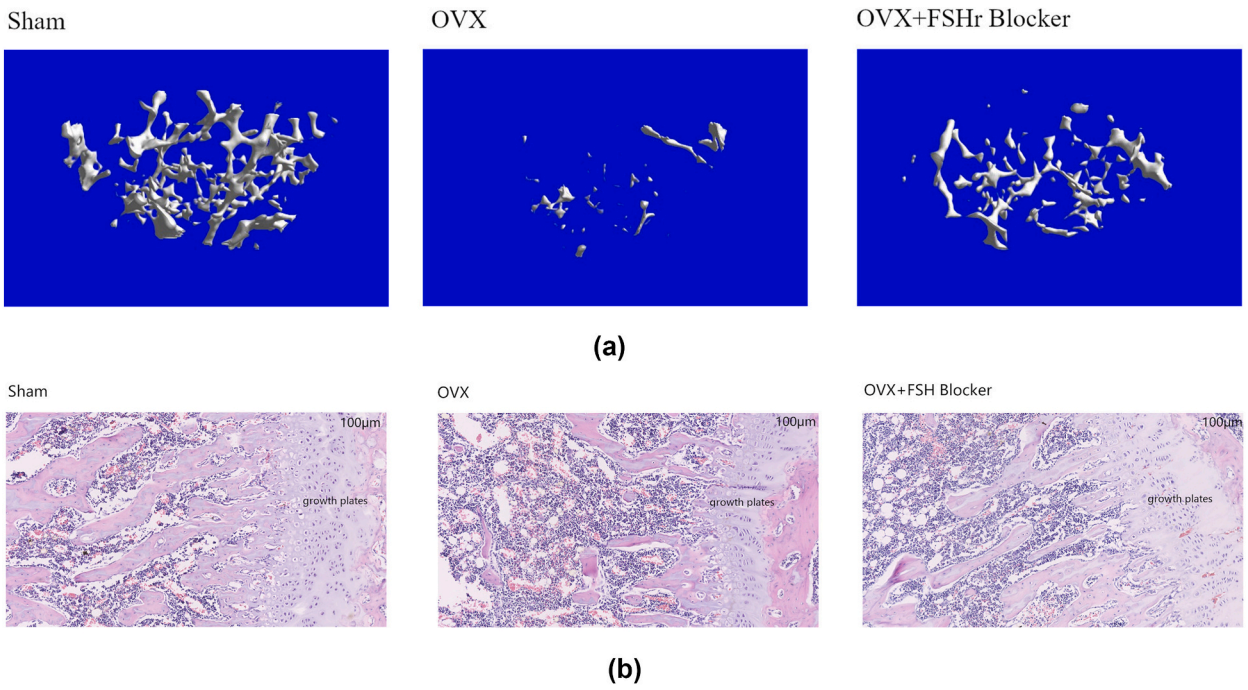

Fig. 3. a. Three-dimensional reconstruction of the mouse femur. Sham: Sham group; OVX: OVX group; OVX + FSHr blocker: OVX + FSHr blocker group.
Fig. 3b. Haematoxylin and eosin (H&E) staining of mouse femur. Sham: Sham group; OVX: OVX group; OVX + FSHr blocker: OVX + FSHr blocker group. (H&E × 100).

Table 4
μCT of mice femur.

	Tb.Sp	Tb.Th	Tb.N	BV/TV
Sham	0.311 ± 0.028	0.051 ± 0.003	0.736 ± 0.188	3.946 ± 1.025
OVX	0.447 ± 0.013	0.038 ± 0.006	0.160 ± 0.036	0.372 ± 0.162
FSHr blocker	0.418 ± 0.014	0.048 ± 0.007	0.198 ± 0.056	1.508 ± 0.647
P	<0.01	0.021	<0.01	<0.01
P1	<0.01	<0.01	<0.01	<0.01
P2	<0.01	0.36	<0.01	<0.01
P3	0.063	0.038	0.665	0.049

Table 4: It showed Tb.Sp, Tb.Th, Tb.N and BV/TV in three groups. Sham: Sham group; OVX: OVX group; FSHr Blocker: OVX + FSHr Blocker group. P: comparation between Sham, OVX and FSHr blocker groups; P1: comparation between Sham and OVX groups; P2: comparation between Sham and FSHr blocker groups; P3: comparation between OVX and FSHr blocker groups.

significant differences were observed in FSH, FSHr, and C/EBPβ levels among the groups (P = 0.039, P = 0.005, and P = 0.002, respectively). Further analysis demonstrated that the differences in FSH levels between groups 1 (FSH = 0 mIU/mL) and 3 (FSH = 400 mIU/mL) and between groups 1 and 4 (600 mIU/mL) were significant (P = 0.023 and P = 0.01, respectively). Moreover, group 1 had lower levels than the other two groups. The differences in FSHr levels between groups 1 and 4, groups 2 (FSH = 200 mIU/mL) and 4, and groups 3 and 4 were significant (P = 0.001, P = 0.004, and P = 0.014, respectively). Group 4 had higher levels than the other three groups. The differences in C/EBPβ levels between groups 1 and 3, groups 1 and 4, groups 2 and 4, and groups 3 and 4 were significant (P = 0.021, P = 0.000, P = 0.001, and P = 0.020, respectively). Group 4 had higher levels than the other three groups, and group 3 had higher levels than group 1. The differences in G protein levels between groups 1 and 4 and between groups 2 and 4 were significant (P = 0.026 and P = 0.034, respectively), with group 4 demonstrating higher levels than groups 1 and 2.

On day 4, we noted statistically significant differences in FSH, FSHr, C/EBPβ, G protein, and HSP90α levels among the groups (P = 0.004, P < 0.01, P = 0.035, P = 0.027, and P = 0.021, respectively). Further analysis demonstrated that the differences in FSH levels between groups 1 and 3, groups 1 and 4, and groups 2 and 4 were significant (P = 0.005, P = 0.001, and P = 0.015, respectively). Furthermore, group 4 had higher levels than groups 1 and 2, and group 3 had higher levels than group 1. The differences in FSHr levels between groups 1 and 3, groups 1 and 4, groups 2 and 4, groups 2 and 3, and groups 3 and 4 were significant (P < 0.01, P = 0.001, P = 0.01, P < 0.01, and P = 0.036, respectively). Group 3 had higher levels than the other three groups, and group 4 had higher levels than groups 1 and 2. The differences in C/EBPβ levels between groups 1 and 3 and between groups 1 and 4 were significant (P = 0.008 and P = 0.032, respectively). Group 1 exhibited lower levels than groups 3 and 4. The differences in G protein levels between groups 1 and 3, groups 1 and 4, and groups 2 and 3 were significant (P = 0.005, P = 0.04, and P = 0.045, respectively). Group 3 demonstrated higher levels than groups 1 and 2, and group 4 displayed higher levels than group 1. The differences in HSP90α levels between groups 1 and 3 and between groups 1 and 4 were significant (P = 0.007 and P = 0.01, respectively); group 1 had lower levels than groups 3 and 4.

On day 6, we detected statistically significant differences in FSH, FSHr, C/EBPβ, and HSP90α levels among the groups (P = 0.011, P = 0.048, P = 0.004, and P = 0.048, respectively). Further analysis demonstrated that the differences in FSH levels between groups 1 and 3, groups 1 and 4, and groups 2 and 4 were significant (P = 0.012, P = 0.003, and P = 0.016, respectively). Group 4 exhibited higher levels than groups 1 and 2, and group 3 displayed higher levels than group 1. The differences in FSHr levels between groups 1 and 3 and between groups 1 and 4 were significant (P = 0.012 and P = 0.024, respectively). Group 1 demonstrated lower levels than groups 3 and 4. The differences in C/EBPβ levels between groups 1 and 3, groups 1 and 4, groups 2 and 3, and groups 2 and 4 were significant (P = 0.004, P = 0.006, P = 0.003, and P = 0.006, respectively). Group 3 displayed higher levels than groups 1 and 2, and group 4 demonstrated higher levels than groups 1 and 2. The G protein level was lower in group 1 than in group 3 (P = 0.034). The differences in HSP90α levels between groups 1 and 3 and between groups 2 and 3 were significant (P = 0.015 and P = 0.02, respectively). Group 3 exhibited higher levels than groups 1 and 2.

3.3. In vitro analysis in U266 cells

3.3.1. U266 transformed into osteoclast-like cells

Myeloma cell lines can successfully differentiate into osteoclast-like cells following induction. TRAP staining of osteoclast-like cells exhibited a higher intensity in the M-CSF + RANKL + recombinant HSP90α group than in the M-CSF + RANKL and blank control groups (P < 0.05) (Fig. 5).

3.3.2. TRAP activity in the supernatant

TRAP activity in the supernatant was higher in the M-CSF + RANKL + recombinant HSP90α group than in the M-CSF + RANKL and blank control groups (P < 0.05) (Fig. 6).

4. Discussion

The present study demonstrated that HSP90α levels in the serum and adipose tissue supernatant were lower in women of child-bearing age than in menopausal women. Furthermore, a strong correlation was observed between serum FSH and HSP90α levels. To elucidate the intrinsic relationship between FSH, HSP90α, and osteoporosis, we investigated their levels in adipose tissue as the tissue expresses HSP90α and responds to FSH [13,14], which explains the correlation between FSH and HSP90α. Integrating our results with the PUBMED expression profile (https://www.ncbi.nlm.nih.gov/gene/3320?report=expression), we inferred that HSP90α may be secreted by adipocytes and exacerbate osteoporosis, which is consistent with the results of a previous study [15]. These results indicate that osteoporosis can be treated by targeting the regulation of HSP90α.

In vivo studies in mice demonstrated no significant differences in ALP, which represents osteoblast activity, among the three groups. Thus, we inferred that FSH and HSP90α primarily act on osteoclasts. After using FSHr blockers, we observed a significant difference in FSH levels in the other groups compared to those in the OVX group 4 weeks after surgery. This suggests that FSH may function by binding to its receptor, and the optimal effect of FSHr may be achieved by week 4. No significant differences were observed in HSP90α levels between the OVX and FSHr blocker groups at 4 and 6 weeks after surgery, suggesting that HSP90α functions downstream of FSH. StrACP activity was the highest in the OVX group, with levels exhibiting a significant difference compared to those in the FSHr blocker group 6 weeks after surgery. This indicates that FSHr blockers can reduce osteoclast activity. Consistent with the aforementioned results, μCT of the mouse femur suggested that FSHr blockers could improve osteoporosis in ovariectomised mice. However, FSHr blockers may have little impact on Tb.Sp and Tb.N; a large sample size and further investigation are needed to elucidate the underlying mechanism. The above results suggest that FSHr blockers downregulate HSP90α, potentially affecting osteoporosis in ovariectomised

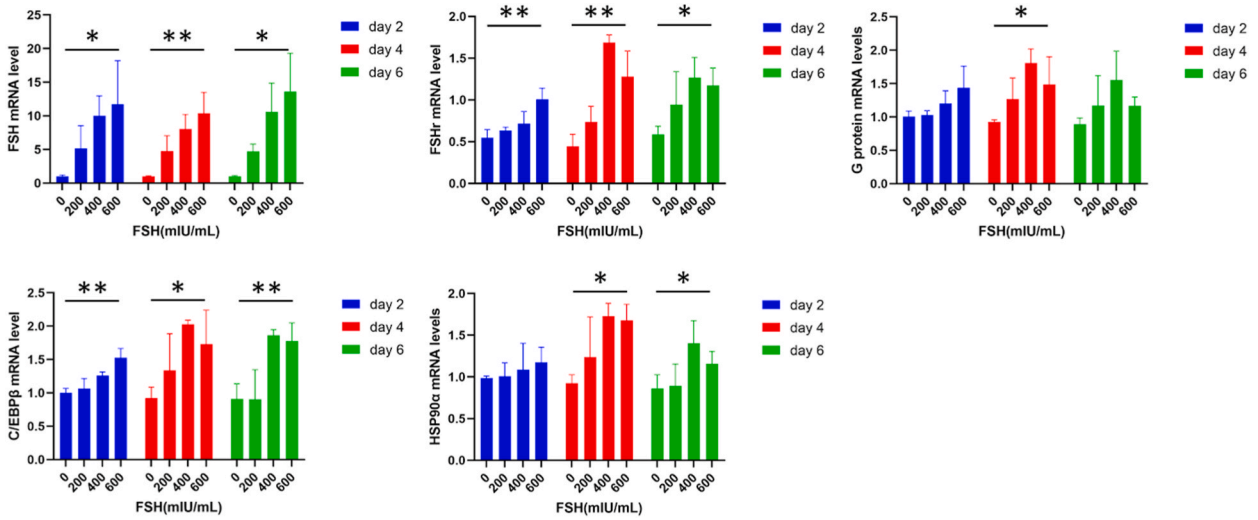

Fig. 4. Follicle-stimulating hormone (FSH), FSH receptor (FSHr), G protein, C/EBPβ, and heat shock protein 90 alpha expressions in adipocytes were tested by quantitative polymerase chain reaction. *: $P < 0.05$; **: $P < 0.01$.

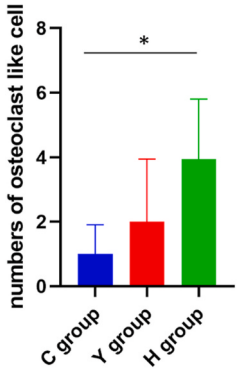

Fig. 5. The proportion of U266 cells transformed into osteoclast-like cells in the three groups. C group: blank control group; Y group: M-CSF + RANKL group; H group: M-CSF + RANKL + recombinant heat shock protein 90 alpha. *: $P < 0.05$.

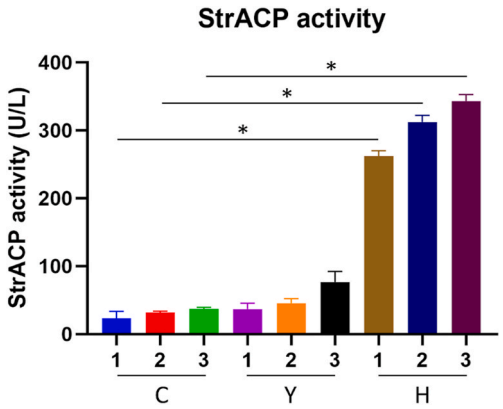

Fig. 6. TRAP activity in the supernatant of the three groups on days 1–3. C: Blank control group; Y: M-CSF + RANKL group; H: M-CSF + RANKL + recombinant HSP90α. TRAP activity in the three groups was statistically significant on days 1–3. *: $P < 0.05$.

mice by altering osteoclast activity. These results are consistent with the findings of a previous study [15,16]. However, HSP90α levels were low at 2 and 6 weeks, which was not consistent with the FSH trend observed in the Sham and OVX groups. The increase in HSP90α levels at 2 weeks may be attributed to the influence of surgical stress.

To study the intrinsic mechanisms underlying the relationship between FSH, HSP90α, and osteoporosis, we euthanised the mice at 6 weeks and assessed the FSH, FSHr, G protein, C/EBPβ, and HSP90α levels in the adipose tissue supernatant using qPCR. In general, FSHr blockers caused a decrease in the levels of FSH, FSHr, G protein, C/EBP β, and HSP90α in mouse fat supernatant compared to those in the OVX group, suggesting that FSHr blockers may regulate the secretion of HSP90α by acting on the pathways described above. We further determined that changes in FSH, FSHr, G protein, C/EBPβ, and HSP90α levels changed accordingly, reaching a peak at FSH 400 mIU/mL after 4 days of cultivation. This indicates that FSH promotes the secretion of HSP90α by adipocytes via the FSHr/G protein/C/EBPβ pathway. This may provide a new mechanism to explain the onset of postmenopausal osteoporosis. Furthermore, FSHr blockers reduced the degree of osteoporosis in mice, which is consistent with the results of previous studies [17,18]. These results provide new insights into the treatment of postmenopausal osteoporosis.

Myeloma cell lines have been shown to successfully differentiate into osteoclast-like cells upon induction. U266 cells specifically secrete HSP90α and exhibit responsiveness to this protein [19]. Therefore, we used the U266 cell line to test the function of HSP90α in osteoclasts. The TRAP activity in the supernatant was enhanced by HSP90α, indicating that HSP90α may exacerbate osteoporosis by enhancing osteoclast activity. This agrees with the results of a previous study [15] and indicates that HSP90α may be a favourable target for osteoporosis treatment.

In summary, our results demonstrate that FSH may promote the secretion of HSP90α by adipocytes via the FSHR/G protein/C/EBPβ pathway by modulating osteoclasts and exacerbating osteoporosis. This study proposes a new perspective on the role of HSP90α in regulating the relationship between adipose tissue and bone, offering new insights into the development and treatment of postmenopausal osteoporosis.

However, this study had some limitations. First, the sample size was small. Second, the study of the mechanisms underlying the relationship between FSH, HSP90α, and osteoporosis was insufficient. Further investigation with a larger sample size is warranted to improve our understanding of these mechanisms. In the future, we can study postmenopausal osteoporosis by considering the FSH-HSP90α-bone mass axis.

Disclosure statement

This study was reviewed and approved by the Animal and Human Ethics Committees of Zhejiang University, School of Medicine, the Second Affiliated Hospital, with approval numbers 2023-117 and IRB-2023-1590, respectively. No potential conflicts of interest are reported by the authors.

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Ethical approval and consent to participate

This study was reviewed and approved by the Animal and Human Ethics Committees of Zhejiang University, School of Medicine, the Second Affiliated Hospital, with approval numbers 2023-117 and IRB-2023-1590. All protocols complied with relevant biosafety regulations, and written informed consent was obtained from all participants.

Data availability statement

All data are included in the article. Data can be provided by the corresponding author upon reasonable request.

CRediT authorship contribution statement

Jianxia Huang: Writing – review & editing, Writing – original draft, Methodology. **Zhifen Zhang:** Project administration, Data curation. **Pei He:** Methodology, Investigation. **Jianwei Zhou:** Writing – review & editing, Validation.

Declaration of competing interest

No potential conflict of interest was reported by the authors.

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