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The hypothalamic estrogen receptor α pathway is involved in high-intensity interval training-induced visceral fat loss in premenopausal rats

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Abstract

Background Visceral adipose tissue (VAT) is strongly associated with metabolic diseases. Both high-intensity interval training (HIT) and moderate-intensity training (MIT) reduce VAT effectively; however, HIT might mediate greater VAT loss in females. The estrogen receptor α (ERα) pathway may play a key role. The aim of the present study was to confirm the role of adipose/hypothalamic ERα in HIT/MIT-mediated VAT loss, as well as the associated hypothalamic electrophysiology and body catabolism changes in pre- and post-menopausal animal models.

Methods Ovariectomy (OVX) or sham surgeries were conducted to establish pre/postmenopausal female rat models. After distance-matched long-term HIT and MIT interventions, ERα expression in hypothalamic/VAT, as well as food intake, spontaneous physical activity (SPA), VAT mass and morphology, local field potential (LFPs) in paraventricular nuclei (PVN) and excessive post-exercise oxygen consumption (EPOC), were observed. A target chemical block during the post-exercise recovery period was executed to further verify the role of the hypothalamic ERα pathway.

Results HIT enhanced the expression of ERα in the hypothalamus rather than VAT in the pre-, but not the postmenopausal group, which was accompanied by elevated LFP power density in α and β bands, enhanced EPOC and larger VAT loss than MIT. Chemical blockade of ERα suppressed EPOC and VAT catabolism mediated by HIT.

Conclusion During the post-exercise recovery period, the hypothalamic ERα pathway involved in HIT induced EPOC elevation and VAT reduction in premenopausal female rats.

Keywords ERα, HIT, Visceral adipose tissue, Hypothalamus, Female

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Introduction

Obesity has become a serious threat to human health. For decades, it has been established that central obesity, rather than total body fat, is a significant risk factor for adverse health outcomes. The excessive accumulation of visceral adipose tissue (VAT) constitutes a significant component of metabolic syndrome and is strongly associated with a range of adverse health outcomes, including cardiovascular disease, non-alcoholic fatty liver disease and certain cancers [1–3]. However, few common obesity interventions (diet control, drugs, bariatric surgery etc.) have been confirmed as capable of preferentially targeting VAT reduction [4]. While the impact on body mass or total fat reduction is relatively weak, convincing evidence has shown that exercise training has an independent effect on VAT loss [3, 5, 6], which could provide significant health benefits.

It should be noted that wide heterogeneity exists among different types of sports exercise. Moderate- to high-intensity exercise has been proven effective in reducing VAT [1, 7–9]. Among various training protocols, both moderate-intensity training (MIT) and high-intensity training (HIT) showed the largest effect sizes in reducing VAT [10], thereby highlighting the significance of glycolysis. Interestingly, although HIT mainly depends on an energy supply of glycogen and consumes significantly less fat than aerobic training during exercise, several high-quality studies have shown that HIT could reduce more VAT than MIT with a matched training volume [11–14]. The mechanism is typically regarded as the stronger innervation of the sympathetic nervous system (SNS, the primary regulator of adipose catabolism) to VAT [2, 15, 16], since there is a positive correlation between SNS activity and exercise intensity. It should be noted that recent meta-analyses did not verify that HIT had a higher VAT loss effect than MIT [1, 7, 10]. However, when analysing only a subgroup of female participants, the effect size of HIT in decreasing VAT exceeded that of MIT by more than 20% [10]. In studies involving females, MIT and HIT exhibited comparable VAT-reducing effects at higher exercise volumes and longer training durations [17, 18]. However, when considering lower volumes and shorter durations [14, 19], HIT showed a stronger VAT loss effect, implying a more sensitive metabolic response to HIT. It has been demonstrated that VAT exhibited a more pronounced catabolic response in females [20], suggesting that sexual dimorphism may affect the efficacy of HIT in reducing VAT.

The estrogen and estrogen receptor (ER) pathways are significant in the context of fat distribution [21, 22], which exhibits clear sexual dimorphism. In premenopausal women, fat is predominantly stored on the hips and thighs, with a lesser proportion of VAT than in men and postmenopausal women, and ERs are deeply involved in this process [22]. The current prevailing view is that HIT-induced fat loss is related to the post-exercise acceleration of fat catabolism, which is regulated by the hypothalamus-SNS-adipose axis [16]. In the hypothalamus, the distribution of ERα is more extensive than that of other receptors of the ER family. Furthermore, it has been demonstrated that oestrogen enhances SNS outflow and adipose catabolism, primarily through the ERα pathway [23]. Therefore, the hypothalamic ERα pathway may be involved in the process of HIT-induced VAT reduction.

HIT could induce greater excessive post-exercise oxygen consumption (EPOC) on the time scale of 10 h or longer [24], which is linked to an augmentation in post-exercise lipid catabolism [25]. Notably, during the recovery phase following MIT, female subjects exhibit lower levels of lipid metabolism than males [26]. Furthermore, our past work confirmed that HIT enhanced post-exercise VAT lipolysis in female rats [15]. Therefore, it was hypothesized that elevated ERα located in the hypothalamus or adipose (regulatory centre and effector, respectively) might enhance VAT catabolism after HIT. These results may, for the first time, identify a key research target to explain sexual dimorphism in exercise-induced fat loss and provide a theoretical foundation for developing tailored exercise prescriptions for gender-specific populations.

Due to obvious differences in the metabolic profiles of pre/postmenopausal subjects, both normal and ovariectomised (OVX) female rats were involved. This study consisted of three separate experiments. Firstly, the effects of HIT/MIT on ERα expression in the hypothalamus and VAT, as well as food intake, spontaneous physical activity (SPA), VAT mass and morphology, were observed to compare the effects of HIT/MIT on pre/postmenopausal subjects. Secondly, to confirm whether increased ERα expression in normal rats resulted in alterations in hypothalamic electrical activity and the metabolic rate, local field potentials (LFPs) in the paraventricular nuclei (PVN, a key regulator of lipolysis) and 1–24 h EPOC were collected. Finally, to further verify this hypothesis, an experiment was conducted in which hypothalamic ERα was blocked using intra-cerebroventricular injection (ICV). These novel findings may contribute to the elucidation of the potential pathways involved in exercise-induced VAT reduction in women and female animals.

Materials and methods

Study design

The study comprised three parts (Exp. 1–3), and a total of 75 rats were involved. Randomization was executed by computer-generated random numbers. The purpose of Exp. 1 was to compare the effects of long-term HIT/MIT on ERα expression in the hypothalamus and VAT

of pre- and post-menopausal rats. A total of 42 female rats were randomly divided into six groups (NH, NM, NC, OH, OM and OC) and received sham surgery (N-groups) or ovariectomy (OVX and O- groups) to establish pre- and post-menopausal models, respectively. After recovery from surgery, rats received 10-week interventions (equivalent to 6 human years) of HIT/MIT/sedentary control (-H, -M and -C groups). Hypothalamus and VAT ERα expression, as well as food intake, SPA and VAT morphology were tested. At the end of Exp. 1, an increase in hypothalamic ERα expression was found in the pre- (but not post-) menopausal model only. Therefore, to further explore neural and metabolic changes induced by ERα elevation, only normal (but not OVX) female rats were involved in Exp. 2 and 3. The objective of Exp. 2 was to test whether HIT could elicit a more pronounced hypothalamic electrical activity and larger EPOC than MIT. A total of 15 SD female rats were randomly divided into three groups (EH, EM and EC) and received microelectrode implantation (IE) in PVN. On the second last training day of the 4-week (equivalent to 2.5 human years) HIT/MIT/sedentary interventions, LPFs was collected 12 h after exercise. Subsequently, the EPOC was tested 1–24 h after the final training session. The purpose of Exp. 3 was to further verify whether ERα inhibition could diminish the post-exercise catabolism and VAT loss induced by HIT. A total of 18 SD female rats were randomly assigned to three groups (C, H and H + AZD) and underwent implantation of a third ventricular catheter (ICV). Four-week HIT/MIT/sedentary interventions were carried out, and AZD9496 (a selective ERα inhibitor) administration was executed during the period of elevated EPOC after each training session. After training, EPOC and the phosphorylation of hormone-sensitive lipase (HSL, a key rate-limiting lipolyase) were assessed.

It should be noted that due to animal dropout, the sample sizes were not consistent between experiments ($n = 7$, 5 or 6 in Exp. 1, 2 or 3). Some of the implanted electrodes and catheters became dislodged due to the lengthy intervention period and the growth of the skull. Nevertheless, the sample size remained sufficient to meet the requirements of previous studies ($n > 4$) [16]. Due to the limited number of monitoring channels, as well as technical reasons for electrode and catheter implantation, it was impossible to conduct the three experiments concurrently. Consequently, variations in experimental procedures and animal batches could potentially lead to discrepancies in the growth cycle, metabolism or behaviour among groups. Therefore, inter-experimental sensitivity tests were performed by combining the effect sizes for the primary outcome indicators (VAT mass, using RevMan 5.3). The results demonstrated that the exclusion of a single experiment did not impact on the statistical significance of the overall study, thereby affirming the acceptable robustness (see Fig. 1).

Animal models and OVX surgical procedure

Seven-week-old female wild Sprague-Dawley rats (Changsheng Biotech, Liaoning, China), after 1 week of adaptive feeding with standard chow, were fed (single cage, 24 ± 2 °C, 12-hour light/dark photoperiod) with a high-fat diet (HFD) throughout all experiments to ensure the earliest and longest HFD exposure possible, which was associated with VAT accumulation and brain dysfunction [27, 28]. Given that the beneficial effects of HIT, particularly in reducing VAT, have been demonstrated in HFD models but are less pronounced in subjects consuming a normal diet [29–31], and in accordance with the 3R principles of animal research (Reduction, Refinement, and Replacement), the groups that consumed a standard diet were not included during exercise interventions. The feed formulations and rearing conditions have been previously described in detail in other studies [15, 16].

To establish the postmenopausal model, OVX rats underwent bilateral ovariectomy with a double dorsolateral approach under anaesthesia with i.p. injection of sodium pentobarbital (Solarbio, Beijng, China), while others underwent sham surgery and were set up as the premenopausal model. Animals underwent a 4-week recovery after surgery. Between the second and third weeks, vaginal smear observations were carried out over 7 days to confirm the establishment of a postmenopausal model. In the fourth week, rats adapted to the running treadmill (see details in the training protocol).

To minimize heterogeneity among Exp. 1, 2 and 3, all surgeries (OVX, IE and ICV) were performed in the same week (after a 6-week adaptation period) of the animals' life cycle. For the premenopausal groups, the rats underwent sham surgeries at 13 weeks of age and were euthanized at 27 weeks of age. This timeline was chosen to ensure that the experimental period fell within the maturity to middle-aged range for SD rats.

IE and electrophysiology analysis

For electrophysiology testing of hypothalamic PVN, IE was carried out before training in Exp. 2. The rat's head was fixed with a stereotaxic apparatus. Silver wire electrodes were implanted unilaterally into the left hypothalamic PVN (AP: −1.7–1.9 mm, ML: 0.2–0.4 mm, DV: 7.9–8.1 mm). After the appearance of at least three channels of stable neuroelectric signals, the skull was closed using bio-silicone, and the electrodes were fixed with dental cement. Then, the animals were given a 2-week recovery before training. LFP data were collected during the recovery period (12 h after the last training session), and rats were in a resting, awake state. More surgery

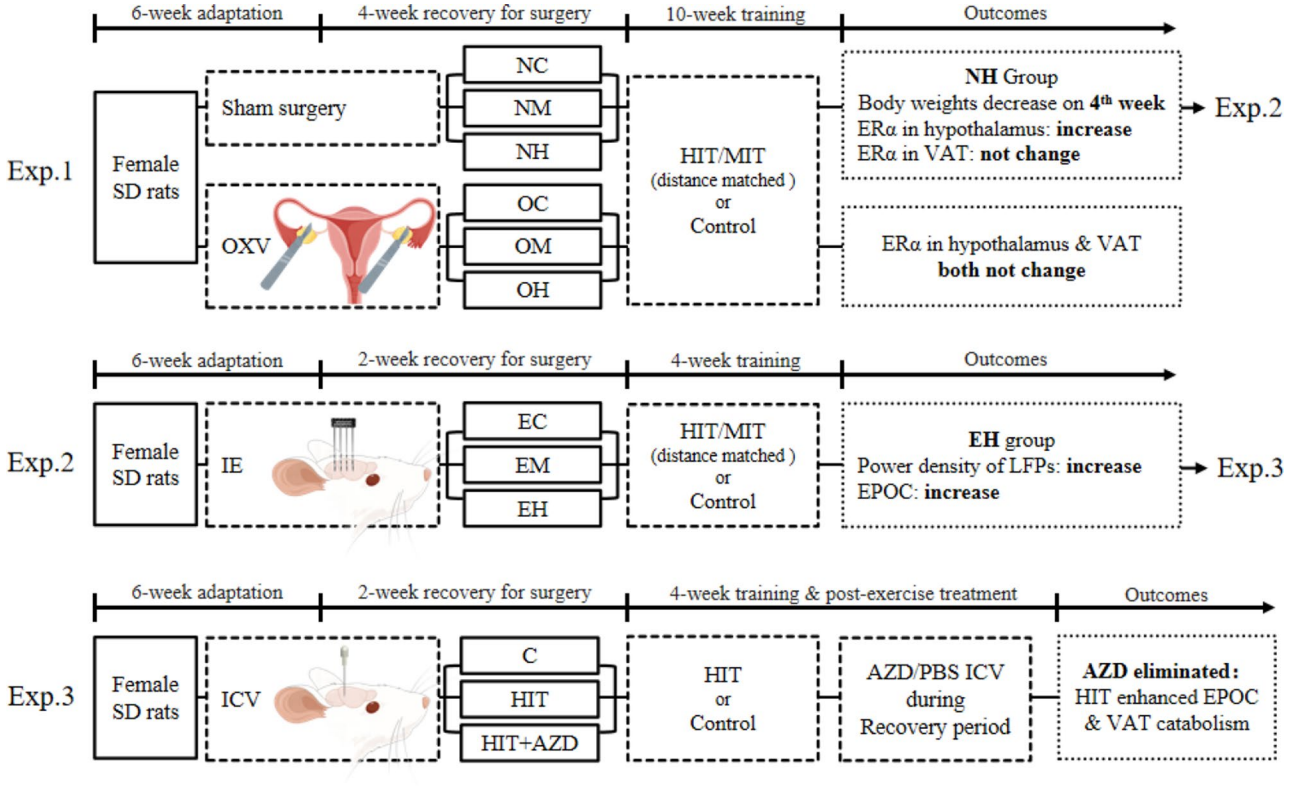

Fig. 1 Study design. *Note* OVX, Ovariectomy surgery; VAT, Visceral adipose tissue; IE, Micro-electrode implantation; LFPs, Local field potentials; EPOC, Excessive post-exercise oxygen consumption; ICV, third ventricular catheter implantation; AZD: AZD-9496, a selective ERα inhibitor

details can be found in a previous study [32]. The electrical signals of the PVN were collected by the CerePlex Direct multi-channel electrophysiological data acquisition system (Blackrock Neurotech, UT, USA). The sampling frequency was set at 2 kHz, and a low-pass filter at 250 Hz was applied to obtain LFPs. The recording was conducted for a period of 30 min. Following the completion of recording, a 5-minute segment of the data was extracted and imported into the NeuroExplorer software. Notch filtering was employed to remove 50 Hz power line interference from the data for power spectral density analysis. Frequency bands were classified as follows: delta (1–4 Hz), theta (4–8 Hz), alpha 1 (8–10 Hz), alpha 2 (10–13 Hz), beta 1 (13–19 Hz) and beta 2 (19–35 Hz) [33].

ICV and inhibitor administration

For executed hypothalamic ERα chemical blocking, ICV was carried out before training in Exp. 3. The rat's head was fixed with a stereotaxic apparatus, and the drug delivery catheters were implanted in accordance with the localization of the third ventricle (AP: + 1.0 mm, ML: ± 0.0 mm; DV: −6.8 mm). The skull was closed using biosilicone and reinforced with dental cement. A 2-week recovery period was allowed for the animals prior to the commencement of training. AZD-9496 (a targeted inhibitor of ERα, Spark Jade, Shandong, China) infusions were executed 3 h after each training session, when the EPOC elevation had not yet appeared in Exp. 2, with a dose of 0.025 mg/kg body weight, and the flow rate of 0.25 ul/min using a microinjection pump. The control and HIT groups received equal volumes and rates of phosphate buffered saline (PBS).

Training protocol

Firstly, the rats in the training groups underwent a 3-day adaptive training period (0–10° uphill treadmill running, 12 m/min, 5–15 min), 1 day for the graded incremental exercise test (GXT, to test running capacity) and 1 day of rest. Subsequently, the long-term training (5 days of treadmill running and 2 days of rest each week) was carried out. To avoid errors in metabolism monitoring caused by circadian rhythms, all training sessions were conducted at a fixed time (18:00–19:00). Rats were given distance-matched 10° uphill treadmill running MIT or HIT. One MIT contained 45 min of continuous medium-speed running (increased from 18 to 20 m/min during the training period), while each HIT contained numbers of 1-min high- and 2-min medium-speed cycles. The number of HIT cycles was adjusted to ensure equal average running distances to MIT groups. Maximum speeds of HIT (increased from 28 to 34 m/min) were determined according to the GXT, which was carried out

every 2 weeks to accommodate the animal's increasing exercise capacity. To ensure that the intensity of the HIT was sufficiently high, blood lactate levels were randomly tested throughout the training period (Biosen C-Line lactate analyser, EKF, Berlin, Germany), which were approximately 4 mmol/L in HIT groups and 1 mmol/L in MIT groups immediately after training. Further details of the protocol can be found in previous studies [15, 16].

SPA testing

The voluntary wheel running test was used to assess SPA. Each rat was subjected to adaptive wheel running six times before the training period. The tests were carried out on the second rest day of the testing week. The rats were housed singly with a running wheel (diameter 35 cm, circumference 110 cm) for a period of 24 h, and the total revolutions were recorded using electronic counters. Tests were performed at baseline and weeks 4 and 9 of Exp. 1 and at baseline and week 4 of Exp. 3.

Metabolic monitoring 1–24 h after exercise

Metabolic monitoring following exercise was conducted with the animal energy monitoring system (CLAMS, Columbus Instruments, OH, USA). As the rapid component (0–1 h post training) had shown no difference between HIT and MIT [24], and in order to avoid the immediate stress of entering the monitor chamber, rats were given 30 min of rest and 30 min of adaptation to chambers. Therefore, only the slow component (1–24 h post training) EPOC was collected. Both the respiratory quotient and the rate of fat oxidation (using the nonprotein respiratory quotient, fat oxidation rate = $1.695 \times VO_2 - 1.701 \times VCO_2$) during the same time period were also calculated [34]. To eliminate the potential confounding effects of circadian rhythms, all tests were executed during the same time period (20:00 to 19:00 of the next day).

Sample collection

Tissue sample collection in Exp. 1 and 3 was performed 48 h after the last training, which was under 12 h of fasting and anaesthesia (I.P. pentobarbital sodium). Blood was collected from the ventricle, and the serum was separated by centrifugation at 4 ℃ and stored at −80 ℃. Periuterine fat pads were weighed and collected for VAT histological observation and protein expression assessment. One animal from each group was randomly selected, and the brain was isolated for histochemical observation in Exp. 1. The hypothalamus and VAT of other rats were isolated and frozen for subsequent protein expression assessment.

Blood lipid and serum oestrogen assessment

Serum levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were measured using established enzymatic assay kits (Jiancheng Bio, Nanjing, China). Six randomly selected samples were measured twice, and all intraclass correlation coefficients were > 0.75 (good reliability).

Histological observation and assessment

For assessing visceral adipocyte volume, the haematoxylin and eosin (H&E) staining procedure was performed according to our previous study [15, 16]. Paraffin-embedded VAT was cut into 5-μm sections and subjected to the H&E procedure. After being photographed, 200 adipocytes per group were randomly selected for cell area analysis using ImageJ 1.51.

For localization observation of ERα expression, a fluorescent immunohistochemistry (IHC) procedure was performed. Then, the whole brain was obtained, fixed in 4% paraformaldehyde, frozen in optimal cutting temperature compound (OCT) and cut into 10-μm coronal sections. After dewaxing, hydration, antigen repair with EDTA antigen retrieval solution (Solarbio, Beijing, China) and blocking with normal goat serum (Solarbio, Beijing, China), tissue sections were incubated with primary antibody (1:200; ABclonal, Wuhan, China) overnight at 4 ℃ and secondary antibody (1:200; Immunoway, TX, USA) for 100 min at room temperature and washed with PBS. DAPI dye solution was dropped onto the sections, which were incubated, and the fluorescence was quenched. Image acquisition and analysis were performed with a fluorescence microscope (Nikon, TKY, Japan) and a tissue cytometry system (TissueFAXS, Tissue Gnotics, Vienna, Austria). For more details, refer to previously published research [15, 16].

ERα protein and HSL-ser660 expression assessment

The expression of ERα protein in the hypothalamus and VAT was evaluated through western blot analysis, as previously described in detail [15, 16]. Hypothalamic and VAT proteins were extracted using RIPA buffer (Solarbio, Beijing, China) and boiled in sample loading buffer. An equal amount of protein from each sample was separated by 8% SDS-PAGE, transferred onto polyvinylidene difluoride membranes (Millipore, MA, USA) and subjected to a standard immunoblotting procedure, including incubation with primary antibody (1:2000; ABclonal, Wuhan, China) overnight and secondary antibody (1:5000; Immunoway, TX, USA) for 2 h. Actin was used as the loading control (1:5000; Bioworld, MT, USA). The ECL (Solarbio, Beijing, China) excited luminescence was analysed by using a gel imaging system (VILBER LOURMAT, Paris, France). Western blotting of HSL-ser660 phosphorylated protein expression was performed as above, with the primary antibody (1:2500; CST, Boston,

USA) incubated overnight and the secondary antibody (1:10000; Abclonal, Wuhan, China) incubated for 2 h.

Statistical analyses

Data were analysed by SPSS 25.0 (IBM Corp., Armonk, NY, USA). Different trends over time in body weight and wheel running activity were analysed by repeated-measures ANOVA between groups. Differences in VAT mass, adipocyte volume, food intake, blood lipids, serum oestrogen and ERα expression between normal/OVX models were analysed by two-way ANOVA. Differences between training groups within the same model were analysed using one-way ANOVA and post-hoc pairwise comparisons using LSD. Main effects and LSD post hoc tests were used to compare the differences when no significant interaction ($P > 0.05$) was found. According to the data of VAT mass in previous experiments [15, 16], the minimum sample size was four ($\alpha = 0.05$, power = 0.8).

Results

Body weight, food intake and SPA before and during exp. 1

In Exp. 1, following a 5-week period of adaptive feeding with a HFD, subjects underwent either an ovariectomy (OVX) or a sham surgery. Post-surgery, the OVX rats swiftly surpassed the sham-surgery subjects in body weight (Figs. 2A and 20% heavier) and maintained this elevation until the end of training (Figs. 2C and 20% heavier). Compared to the NC group, postmenopausal rats exhibited a notable food intake increase following surgery (Figs. 2B and 14% increase), and this elevated pattern persisted throughout the entire training period (Figs. 2D and 10% increase).

In premenopausal animals, the body weight gain induced by a HFD was suppressed by HIT starting from the fourth and by MIT from the fifth training week, with no difference between the two training intensities. After 10 weeks of training, NH and NM rats exhibited a weight reduction of 12% and 10% less than NC rats, respectively (Fig. 2C). In postmenopausal animals, HIT suppressed the weight gain induced by HFD starting from the second week, resulting in an 11% lower weight (Fig. 2C) compared to the OC group at the end of the training period. Conversely, MIT did not exhibit an inhibitory effect on weight gain.

Lipid profile, VAT mass and adipocyte volume after exp. 1

Compared to premenopausal rats (NC), postmenopausal animals exhibited significantly elevated levels of TC, TG and LDL-C (Figs. 3A, B and D and 17%, 74% and 32% higher), along with a greater accumulation of VAT weight (Figs. 3E and 66% higher).

Both HIT and MIT were found to reduce LDL-C levels (Figs. 3D and 27–45% decrease); however, only HIT was effective in decreasing TG levels (Figs. 3B and 40% decrease), indicating that HIT exerted a more profound influence on systemic lipid metabolism. Furthermore, the VAT weight was decreased in postmenopausal rats across both the HIT and MIT groups (Figs. 3E and 54% and 39% lower than OC), whereas only the NH (but not the NM) group exhibited VAT reduction (Figs. 3E and 40% lower than NC), indicating that the effect of HIT on VAT loss was more pronounced compared to MIT in premenopausal subjects. After exercise, only postmenopausal animals exhibited a decrease in the volume of visceral adipocytes (Fig. 3E, G), implying that the underlying pattern (cell number or cell volume) of VAT loss differs between pre- and postmenopausal models.

ERα expression in the hypothalamus and VAT after exp. 1

To ascertain whether HIT and MIT exhibit distinct influences in VAT and hypothalamic ERα expression, western blot analysis was conducted to measure the ERα protein content in these two tissues. Regardless of the animal model, neither training regimen had an impact on ERα expression in VAT (Fig. 4F). However, in premenopausal animals, HIT significantly increased hypothalamic ERα expression (Figs. 4E and 78% increase). Conversely, this effect was not observed in postmenopausal animals. To further locate the specific brain regions where ERα levels were elevated, IHC was conducted. The fluorescence images (Fig. 4A-D) revealed that, in the premenopausal animal model, HIT significantly increased the immunofluorescence brightness of ERα in the arcuate nucleus (ARC), ventral medial hypothalamus (VMH) and PVN of the NH group and VMH. By contrast, in the postmenopausal model, only a modest enhancement of ERα fluorescence brightness was observed in the VMH.

Post-exercise metabolic rate, fat oxidation and hypothalamic neurophysiology after exp. 2

In Exp. 2, LFPs during the recovery period (24 h post-exercise) pre-intervention and during the second and fourth weeks were measured to assess the impact of HIT and MIT on hypothalamic electrophysiology. No statistically significant differences in power spectral density were observed between the groups, except for the HIT rats during the fourth week (Fig. 5A-C). Notably, the LFPs of the EH group exhibited a significant reduction in power of the δ-band and an increase in the α and β bands (Fig. 5C-E). By contrast, neither the control nor the MIT group demonstrated these changes. These findings suggest that a 4-week regimen of HIT, unlike MIT, had the potential to modify the functional pattern of the PVN, which is the most characterized hypothalamic interface linking adipose tissue and muscle.

Rats of the EH group, accompanied by electrophysiological alterations in the hypothalamus, exhibited a notable increase in oxygen consumption and fat oxidation

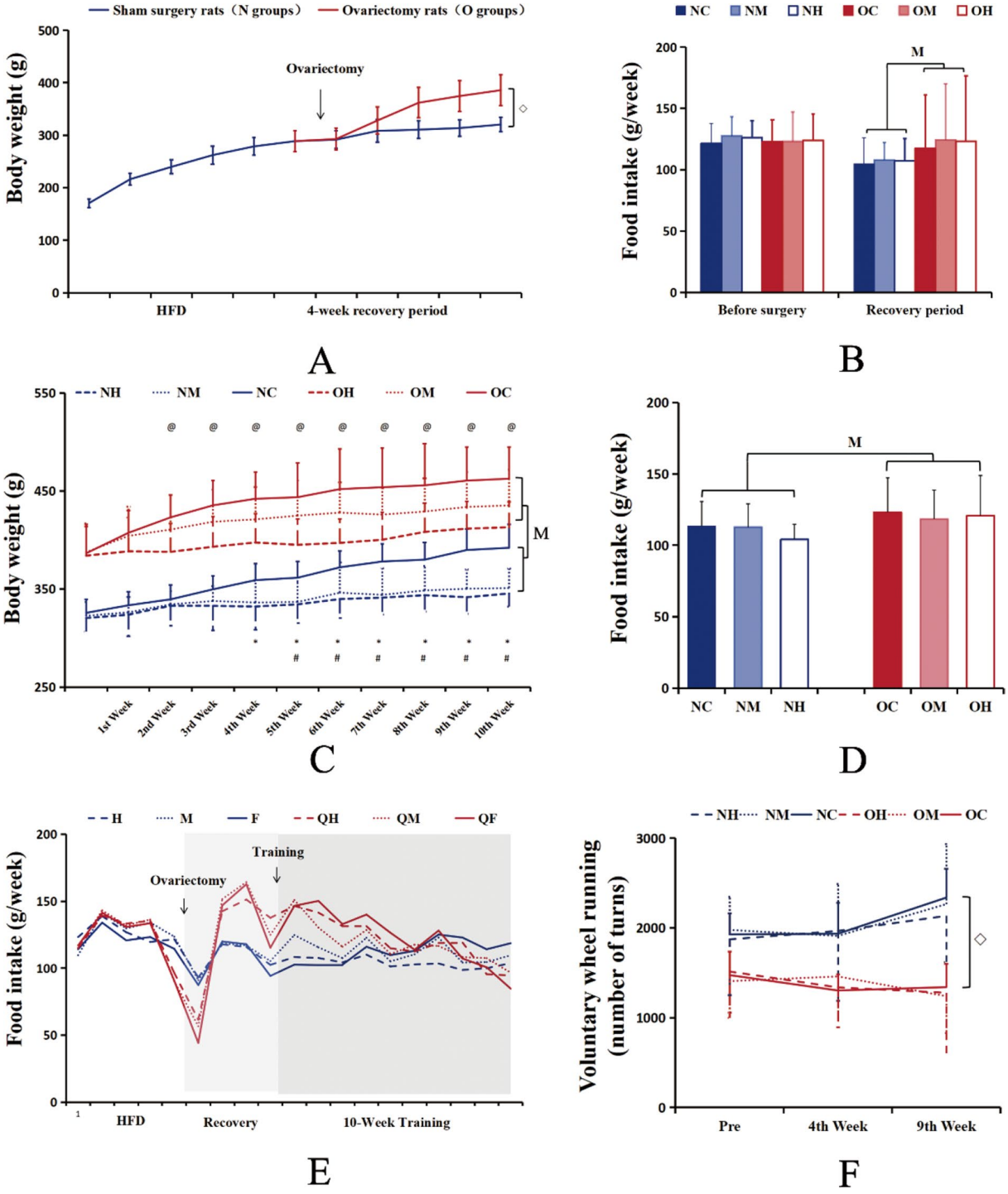

Fig. 2 Body weights and food intakes during model establishment and training period. *Note* **A**, body weight changes before/after surgery; ◇, interaction effect of time × animal model, F = 16.134, $P < 0.001$. **B**, average food intake before surgery and during recovery period; M, main effect of animal model, F = 5.657, $P = 0.02$. **C**, body weight changes during 10-week training; M, main effect of animal model F = 7.685, $P < 0.001$; *, NH vs. NC, F: 2.833 ~ 10.45, P: 0.001 ~ 0.043; #, NM vs. NC, F: 3.737 ~ 10.45, P: 0.002 ~ 0.04; @, OH vs. OC. F: 2.402 ~ 4.220, P: 0.026 ~ 0.011. **D**, average food intake during 10-week training; M, main effect of animal model F = 9.805, $P = 0.005$. **E**, trends in mean food intake during surgery and training periods. **F**, trends in Spontaneous physical activity (SPA) during training; ◇, interaction effect of time × animal model, F = 3.09, $P = 0.05$

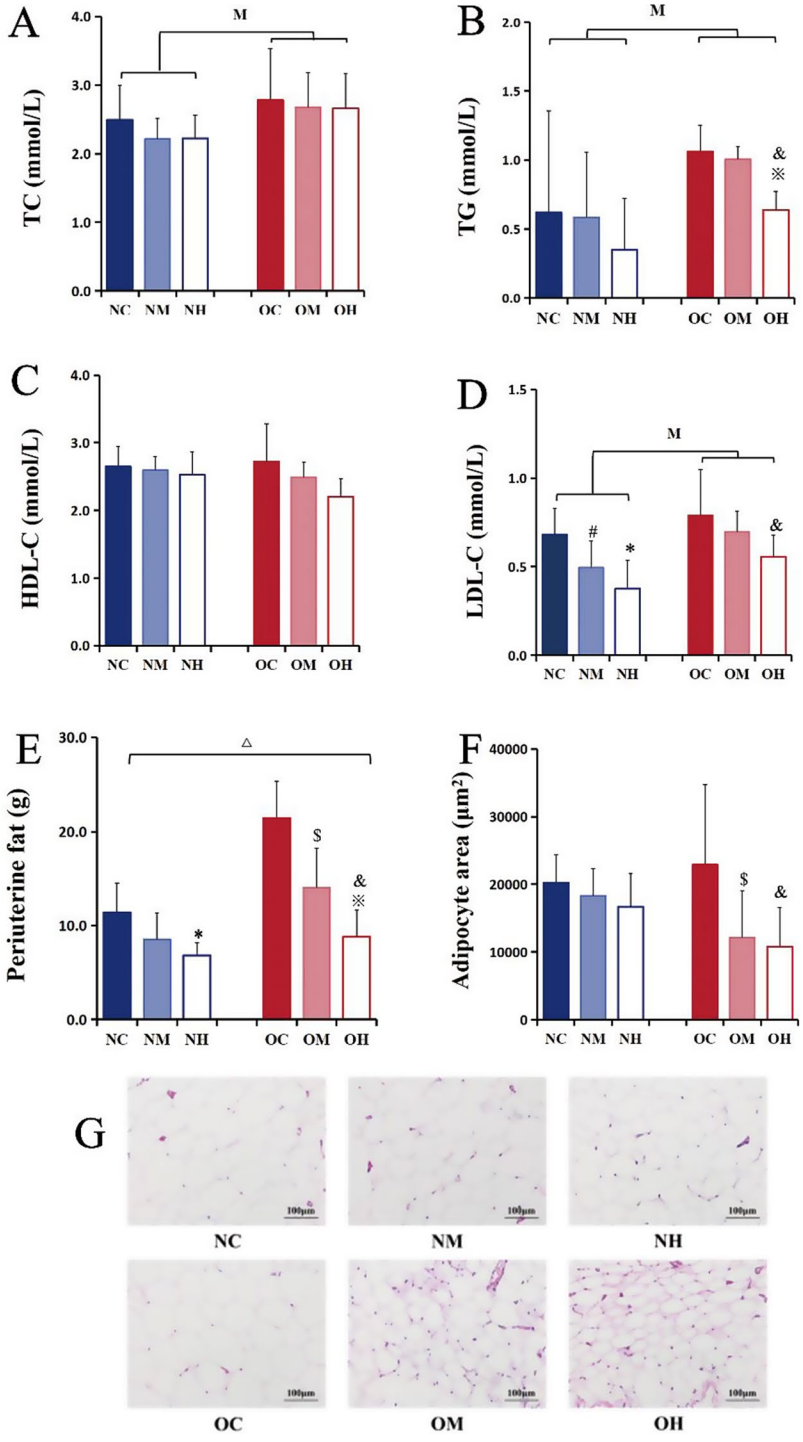

Fig. 3 Visceral fat mass, adipocyte area and blood lipids after 10 weeks of training. *Note* **A**, TC; M, main effect of animal model, F = 6.064, *P* = 0.019. **B**, TG; M, main effect of animal model, F = 7.942, *P* = 0.008; &, OH vs. OC, F = 6.231, *P* = 0.005; ※, OH vs. OM, F = 6.231, *P* = 0.013. **C**, HDL-C. **D**, LDL-C; M, main effect of animal model, F = 6.609, *p* = 0.015; *, NH vs. NC, F = 6.215, *P* = 0.003; #, NM vs. NC, F = 6.215, *P* = 0.05; &, OH vs. OC, F = 2.601, *P* = 0.039. **E**, periuterine fat mass; △, interaction effect of animal model × training type F = 3.380, *P* = 0.047; *, NH vs. NC, F = 4.880, *P* = 0.007; &, OH vs. OC, F = 17.729, *P* < 0.001; $, OH vs. OC, F = 17.729, *P* = 0.003; ※, OH vs. OM, F = 17.729, *P* = 0.027. **F**, adipocyte areas; &, OH vs. OC, F = 3.588, *P* = 0.027; $, OH vs. OC, F = 3.588, *P* = 0.046. **G**, H&E staining of adipocytes

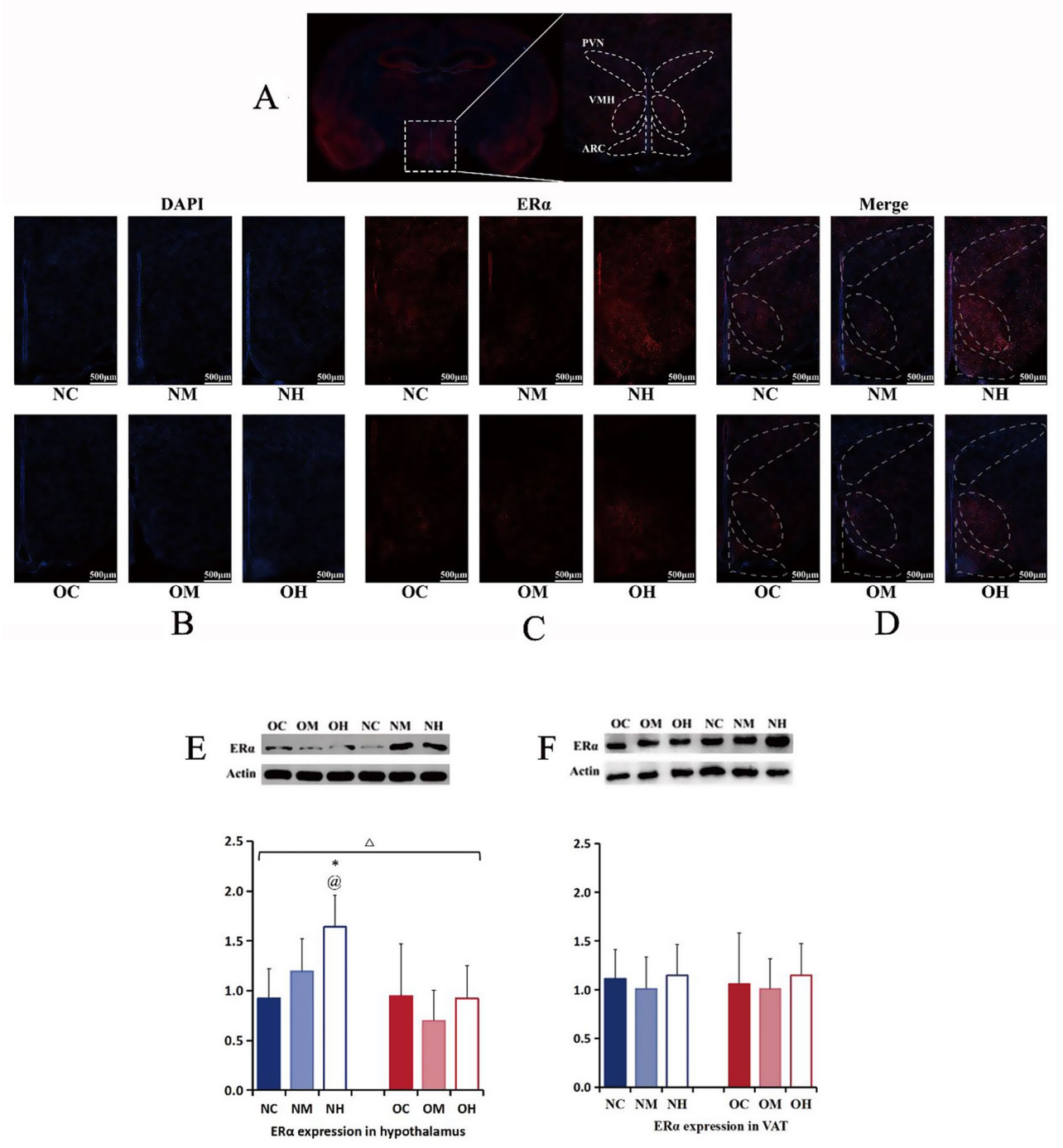

Fig. 4 Serum oestradiol and ERα expression in the hypothalamus and VAT. *Note* **A**, IHC overall views of hypothalamic DAPI (blue) and ERα (red). **B-D**, merged images of DAPI and ERα in the right hypothalamus at large scale. **E**, ERα protein expression in the hypothalamus; △, interaction effect of animal model × training type F = 3.380, $p = 0.047$; *, NH vs. NC, F = 7.994, $P = 0.001$; @, NM vs. NH, F = 7.994, $P = 0.027$. **F**, ERα protein expression in VAT

rates and a decrease in the respiratory quotient within 24 h post-exercise. This phenomenon was particularly evident during the 5–12 h post exercise, leading to a significant increase of 12% in total oxygen consumption and 50% in fat oxidation within 24 h (Fig. 5G, K).

Effects of blocking ERα on EPOC and VAT catabolism in exp. 3

In Exp. 3, rats underwent the same 4-week HIT protocol as in Exp. 2. Meanwhile, administration of AZD via ICV was performed during the early stages of each recovery period (3 h post training) to affirm the role of ERα

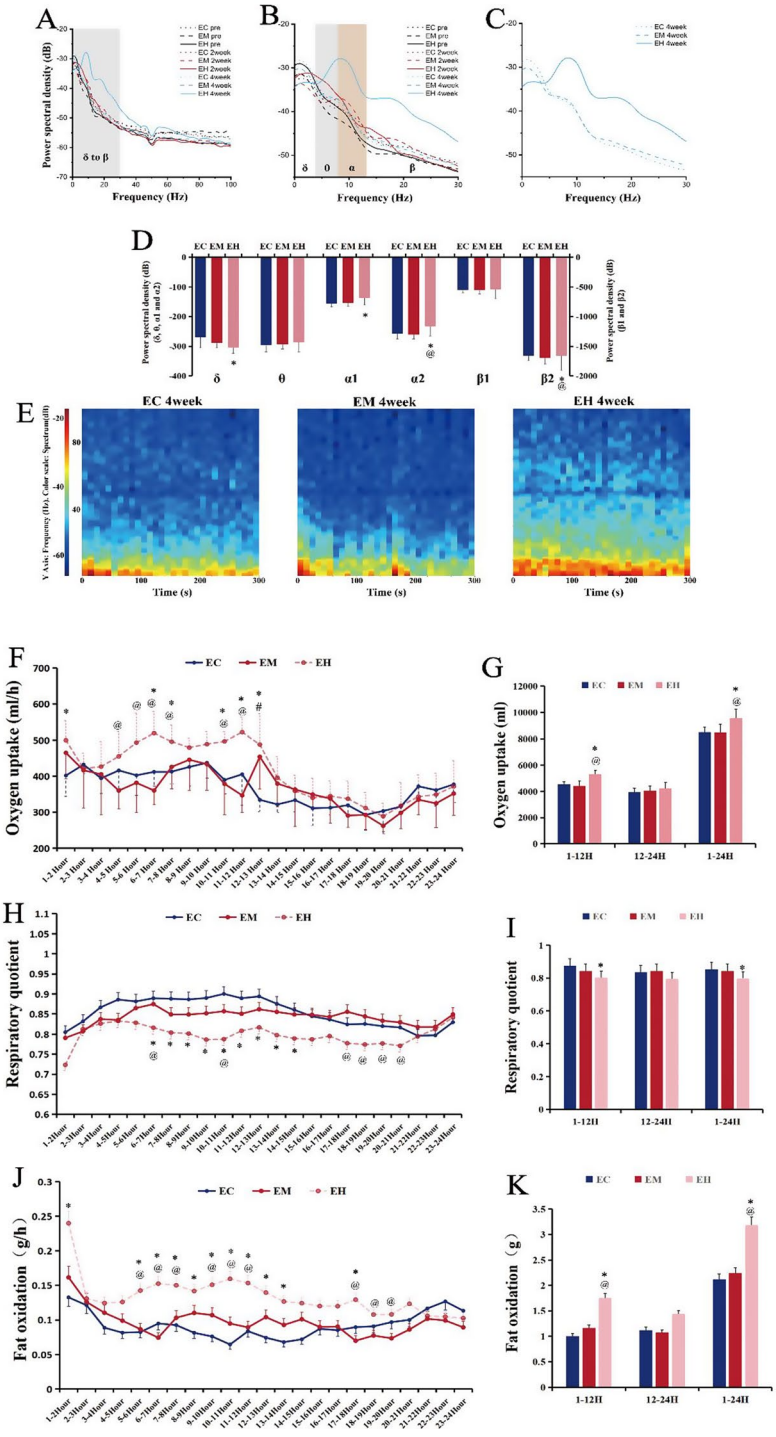

Fig. 5 LFPs of PVN and 1–24 h EPOC in Exp. 2. *Note* **A**, complete power spectrums (0–100 Hz, one representative spectrum of each group was shown). **B**, δ to β band (0–30 Hz). **C**, difference between groups on the fourth week. **D**, comparison between groups within each band; *, EH vs. EC, δ: F = 6.195, *P* = 0.003; α1: F = 5.468, *P* = 0.012; α2: F = 6.341, *P* = 0.015; β2: F = 9.483, *P* = 0.002;@, EH vs. EM, α2: F = 6.341, *P* = 0.005; β2: F = 9.483, *P* = 0.002. **E**, power spectrum for 300 s at 24 h after exercise on the fourth week. **F**, oxygen uptake of EPOC (1–24 h post training); *, EH vs. EC, F: 3.802 ~ 17.360, *P*: 0.001 ~ 0.014; #, EM vs. EC, F = 7.157, *P* = 0.013; @, EH vs. EM, F: 4.201 ~ 17.360, *P*: 0.001 ~ 0.011. **G**, oxygen uptake (1–12, 12–24 and 1–24 h post exercise); *, EH vs. EC, 1–12 H F = 14.617, *P* < 0.001, 1–24 H F = 6.488, *P* = 0.008; @, EH vs. EM, 1–12 H F = 14.617, *P* < 0.001, 1–24 H F = 6.488, *P* = 0.006. **H**, respiration quotient (1–24 h post training); *, EH vs. EC, F: 2.720 ~ 6.279, *P*: 0.002 ~ 0.046 @, EH vs. EM, F: 4.543 ~ 6.279, *P*: 0.009 ~ 0.047. **I**, respiration quotient (1–12,12–24 and 1–24 h post exercise); *, EH vs. EC, 1–12 H F = 3.969, *P* = 0.013, 1–24 H F = 3.473, *P* = 0.025. **J**, fat oxidation rate (1–24 h post training); *, EH vs. EC, F: 3.975 ~ 13.504, *P*: 0.001 ~ 0.047; @, EH vs. EM, F: 2.633 ~ 13.504, *P*: 0.001 ~ 0.040. **K**, fat oxidation rate (1–12,12–24 and 1–24 h post exercise); *, EH vs. EC, 1–12 H F = 6.598, *P* = 0.004, 1–24 H F = 5.278, *P* = 0.010; @, EH vs. EM, 1–12 H F = 6.598, *P* = 0.016, 1–24 H F = 5.278, *P* = 0.019

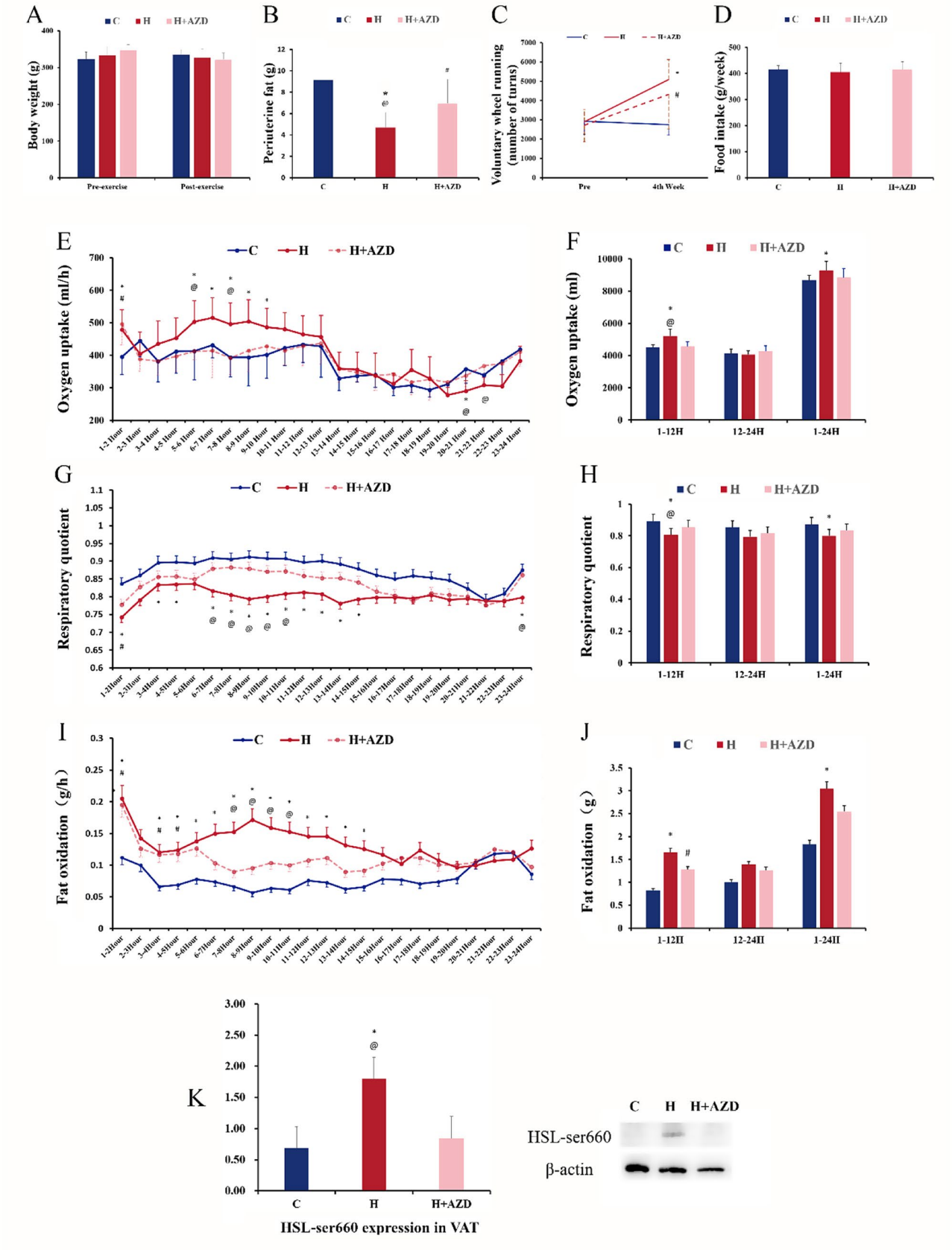

Fig. 6 (See legend on next page.)

(See figure on previous page.)

Fig. 6 Body weights, VAT mass, EPOC and HSL phosphorylation in Exp. 3. *Note* **A**, body weight. **B**, VAT mass; *, H vs. C, F = 11.1, $P < 0.001$; #, H + AZD vs. C, F = 11.1, $P = 0.034$; @, H vs. H + AZD, F = 11.1, $P = 0.031$. **C**, SPA; *, H vs. C, F = 5.440, $P = 0.006$; #, H + AZD vs. C, F = 5.440, $P = 0.046$. **D**, total food intake. **E**, oxygen uptake of EPOC (1–24 h post training); *, H vs. C F: 3.729 ~ 6.069, *P*: 0.006 ~ 0.039; #, H + AZD vs. C, F = 4.913, $P = 0.01$; @, H vs. H + AZD, F: 3.810 ~ 6.069, *P*: 0.008 ~ 0.041. **F**, oxygen uptake (1–12,12–24 and 1–24 h post exercise); *, H vs. C, 1–12 H F = 9.407, $P = 0.001$; 1–24 H F = 2.580, $P = 0.044$; @, H vs. H + AZD, 1–12 H F = 9.407, $P = 0.003$. **G**, respiration quotient (1–24 h post training); *, H vs. C F: 2.386 ~ 10.620, *P*: 0.001 ~ 0.046;#, H vs. H + AZD F = 10.620, $P = 0.013$; @, H vs. H + AZD, F: 6.530 ~ 10.152, *P*: 0.010 ~ 0.040. **H**, respiration quotient (1–12,12–24 and 1–24 h post exercise) *, H vs. C, 1–12 H F = 7.932, $P = 0.001$; 1–24 H F = 4.375, $P = 0.010$ @, H vs. H + AZD, 1–12 H F = 7.932, $P = 0.041$. **I**, fat oxidation rate (1–24 h post training); *, H vs. C F: 2.798 ~ 17.316, *P*: 0.001 ~ 0.047; #, H + AZD vs. C F: 4.058 ~ 17.316, *P*: 0.001 ~ 0.033; @, H vs. H + AZD, F: 5.716 ~ 12.072, *P*: 0.006 ~ 0.043. **J**, fat oxidation (1–12, 12–24 and 1–24 h post exercise); *, H vs. C, 1–12 H F = 8.220, $P = 0.001$; 1–24 H F = 5.601, $P = 0.006$ #, H + AZD vs. C, 1–12 H F = 8.220 $P = 0.042$. **K**, HSL-ser660 western blot; *, H vs. C, F = 5, $P = 0.007$; @, H vs. H + AZD, F = XX, $P = 0.018$

in facilitating VAT reduction through 4-week HIT. HIT mitigated the accumulation of VAT induced by HFD (Figs. 6B and 49% less than C) and augmented SPA, whereas the administration of AZD partially abolished the VAT reduction mediated by HIT (47% more than H) but exerted no influence on SPA.

As the HIT intervention remained the same as in Exp. 2, rats in the H group exhibited similar heightened oxygen consumption and fat oxidation roughly 6–12 h post training in Exp. 3 (Fig. 5E, I). To verify the involvement of the ERα pathway in facilitating this process, rats were administered AZD (ERα inhibitor) 3 h after each training session. The results showed that within approximately 1–2 h following AZD administration (4–5 h post training, Fig. 5I), the heightened fat oxidation was almost abolished. Additionally, AZD administration hindered the HIT-induced HSL phosphorylation post exercise in VAT. These data suggested that ERα was involved in the augmentation of VAT catabolism following HIT.

Discussion

The accumulation of VAT, rather than total body fat, is closely associated with metabolic disease. Exercise had an independent effect on VAT reduction, and HIT showed a stronger VAT loss effect than MIT in several studies that involved female subjects. In the past, the general consensus was that MIT was the most effective method of inducing fat loss, as it resulted in the greatest fat consumption during exercise. However, recent research has shown that HIT can have an equal or even more potent effect in reducing VAT, particularly in studies involving female participants. A typical HIT session includes multiple sets of short, high-intensity sprints (above 80% of VO2max, involving glycolytic metabolism), interspersed with medium-intensity intervals (50–60% of VO2max, involving aerobic metabolism) [35]. During HIT, adipose catabolism is inhibited due to the high levels of lactate produced by glycolysis. Consequently, many researchers believe that the fat loss benefits of HIT are related to the increased EPOC and lipolysis that occur after exercise, which help to meet the recovery needs following vigorous exercise [36]. Since female participants have lower levels of lipid metabolism during the recovery period of MIT compared to males [26], enhanced post-exercise fat catabolism may be a crucial mechanism by which HIT efficiently reduces VAT in women.

The aim of this study was to determine the role and mechanism of the adipose and hypothalamus ERα pathway in HIT/MIT-induced VAT reduction. To account for the significant inter-individual differences in lipid metabolism, the study employed both pre- and postmenopausal rat models. The findings revealed that the VAT-reducing effect of HIT was more pronounced than that of MIT in both normal and OVX rats. In the OVX group, HIT/MIT did not induce ERα elevation in the VAT or brain. Nevertheless, a higher level of hypothalamic ERα expression was observed in premenopausal rats, which was accompanied by an enhanced EPOC and electrophysiological alteration. Subsequent experimentation demonstrated that ERα blockade in the brain abolished the HIT-induced elevation of EPOC and VAT lipolysis, thereby identifying a novel target for exercise-related fat loss and health promotion.

The distribution of body fat is a heritable trait. A large-sample study has demonstrated a pronounced sexual dimorphism in 20 of the 49 genes identified as associated with the waist-to-hip ratio [37, 38]. This finding suggests that sex hormones play a pivotal role in regulating VAT metabolism. Given the significant alterations in endocrine function and metabolism that occur during menopause, an investigation was conducted to examine the disparate responses to exercise in VAT, OVX and sham surgery. The administration of a HFD throughout the course of the experiments resulted in a notable increase in body weight, VAT mass and food intake, as well as a reduction in SPA, in the rats subjected to omentopexy. These findings agree with those of previous studies [38–40].

Compared with caloric restriction, exercise has been shown to promote VAT catabolism with a lesser impact on total fat mass [2, 6]. However, given the considerable inter-individual variability in the exercise response, it remains unclear which training method is more effective [7, 16]. A recent large-sample meta-analysis has shown sexual dimorphism in the effect of HIT compared to MIT on reducing VAT. Specifically, when the subgroup analysis was confined to female participants, the combined effect size of HIT in decreasing VAT was approximately

20% greater than that of MIT (−0.84 vs. −0.69). Conversely, when the subgroup analysis included only male subjects, the difference in effect sizes between HIT and MIT narrowed (−0.96 vs. −0.93) [10]. In studies with high training volumes (400 kJ work load) [17] and a prolonged training duration (16 weeks) [18], both HIT and MIT exhibited comparable effects on VAT reduction. However, in studies featuring a lower exercise volume (200 kJ work load) [14] or short duration (6 weeks) [19], HIT was found to mediate a more pronounced loss of VAT in females. This seems to indicate that, in females, VAT responds more sensitively and rapidly to HIT. Even with a decrease in training volume or weeks, HIT still contributes to the reduction of VAT, although the maximum effect achieved by both protocols is comparable.

Notably, despite significant differences in lipid metabolism, HIT has been observed to induce greater VAT reduction than MIT in both pre- and postmenopausal individuals [11, 12, 14, 17], which is consistent with the present results (Fig. 3E-G). When comparing the two models used in the present study, it appeared that the same MIT protocol evokes a more pronounced VAT response in OVX rats, while HIT could reduce VAT in both pre- and postmenopausal animals. To our knowledge, only one meta-analysis [41] has compared the exercise response of abdominal (or visceral) fat to HIT in both pre- and postmenopausal women, by synthesizing effect sizes across multiple studies. This review revealed that when pre- and postmenopausal women were analysed separately, the impact of HIT on total and abdominal fat mass was statistically significant only among premenopausal women, aligning with the present findings. By contrast, the fat loss effects of HIT in postmenopausal women may be influenced by a multitude of factors, including training protocols and population-specific characteristics.

The 10-week training program did not alter food intake, suggesting that the primary target of exercise in reducing VAT is more closely related to fat metabolism than to feeding behaviour. Given that the enhancement of the hypothalamic ERα pathway on SPA has been well established, the decline in SPA observed in the postmenopausal model in Exp. 1 was consistent with previous findings [42]. However, an unexpected result emerged: neither MIT nor HIT altered SPA in Exp. 1 (Fig. 2F), whereas HIT did increase SPA in Exp. 3 (Fig. 6C). This discrepancy may potentially be attributed to individual variations stemming from distinct animal batches, given that the baseline SPA in Exp. 3 was roughly 50% higher than those in Exp. 1 (about 3,000 rounds vs. 2,000 rounds). Indeed, even within the same rodent strain, substantial individual variability arises due to a multitude of influences [43]. Besides the hypothalamus, factors such as the dopamine reward circuitry, various hormones and genetic polymorphisms can all exert an influence on SPA [42, 44]. Voluntary wheel running does not encapsulate the full spectrum of physical activity but rather signifies a tendency towards 'playful' exercise. Meanwhile, current studies do not support an association between SPA and VAT loss [45–47]. Given the lack of consensus on the association between HIT fat loss effects and SPA, more studies with larger sample sizes are needed in the future to reveal the underlying patterns. In summary, HIT caused a greater reduction in VAT than MIT without changing food intake in female rats. Additionally, given the extensive involvement of oestrogen and ERα in the regulation of fat distribution, observing the effect of exercise on this pathway is important to explain the underlying mechanisms.

Unlike MIT, HIT appears to rely primarily on glycogen, rather than fat, as a source of energy during exercise. This seems to contradict the comparable or even stronger VAT reduction effect that has been reported. It is generally believed that the fat loss effect of HIT is related to post-exercise catabolism and long-term adaptive changes in adipose [48]. The catecholamine and β3 adrenergic receptor (β3AR) pathways, which are controlled by the SNS, are well known as the primary regulators of adipose catabolism. After HIT, in order to meet the needs of gluconeogenesis and tissue repair, the SNS releases catecholamines to adipocytes via nerve endings and adrenal glands and increases lipolysis and fatty acid release via β3AR [16, 36]. Our previous studies confirmed that long-term HIT increased the sensitivity of visceral adipocytes to catecholamines and activated lipolysis during the recovery period [15, 16]. Since studies confirmed that oestrogen and ERs are key regulators of SNS and β3AR [49, 50], it was a reasonable explanation that HIT inhibited the accumulation of VAT by activating the oestrogen-ER pathway. Exogenous oestrogen supplementation can alleviate central obesity in menopausal women, but this treatment is controversial because of the resulting increased incidence of breast cancer [22]. A substantial body of evidence has confirmed that exercise generally decreases circulating oestrogen [51], suggesting that the VAT-reducing effects of HIT may be achieved by increasing ER expression or function rather than increasing oestrogen secretion.

In addition to its role in reproduction, oestrogen exerts a wide range of metabolic regulatory functions by interacting with ERs expressed in the brain, fat, liver, muscle and other sites. Oestrogen deficiency (menopause, lack of aromatase etc.) leads to central obesity, whereas oestrogen supplementation could reduce weight gain and alleviate metabolic abnormalities in rodent obesity models induced by HFD, OVX or leptin deficiency [22]. The ER family includes two nuclear receptors (ERα, ERβ) and a membrane receptor, the G-protein-coupled oestrogen

receptor (GPER), all of which are involved in regulating glycolipid metabolism. Studies have shown that ERα knockout leads to doubling of visceral fat mass, while ERβ pure-hybrid deletion mice showed a similar body composition to wild-type mice [23, 52]. Results of the GPER knockout were inconsistent, but it appeared to have a closer relationship with total but not visceral fat [52, 53]. In the hypothalamus, the upper centre of the SNS, ERα is expressed at much higher levels than the other two receptors and plays a greater role in influencing energy metabolism, food intake and physical activity [23]. In peripheral adipose tissue, ERα has a complex interaction with β3AR during fat catabolism [49]. These outcomes suggest a more prominent impact of ERα on VAT compared with the other two receptors. Based on these findings, this study focused on the effect of HIT/MIT on the ERα of adipose tissue and the hypothalamus, as well as their relationship with VAT reduction.

The ERα increase in adipose tissue facilitates VAT reduction. The fat pad mass is determined by a dynamic combination of lipogenesis and lipolysis. For VAT, on the one hand, oestrogen can inhibit the mRNA expression of lipoprotein lipase (LPL) through ERα [54], preventing the entry and accumulation of free fatty acids in adipocytes (anti-lipogenesis). On the other hand, the elevated ratio of ERα/ERβ could activate β3AR-related lipolysis and mitochondrial oxidation [52]. Unfortunately, there is insufficient evidence to demonstrate that exercise could affect adipose ERα. In a study involving overweight/obese postmenopausal women, marginally increased ($P = 0.08$) adipose ERα gene expression was observed with higher fat loss during training [55]. However, no evidence has confirmed that exercise enhances the protein expression of ERα in postmenopausal subjects. In an animal study, researchers found that adipose ERα protein expression was affected only by diet but not by exercise [56]. This study revealed that neither MIT nor HIT influenced ERα protein expression in adipose tissue, aligning with the aforementioned findings. Whether HIT influences the peripheral adipose ERα at the levels of gene expression, regulatory factors or hormone sensitivity requires confirmation by future studies.

Hypothalamic ERα is another possible pathway that may mediate VAT loss. Adipose metabolism is tightly controlled by the hypothalamic-SNS-catecholamine axis, which includes several key hypothalamic nuclei, such as the ARC, VMH, dorsomedial hypothalamus (DMH), lateral hypothalamus (LH) etc [57]. Their projections converge on the PVN, which plays an integrative role in regulating lipid redistribution between muscle and adipose through the SNS and adrenergic receptors [58]. A significant gender difference in the exercise response of the SNS has been found [59]. Several studies confirmed that HIT had a stronger effect on the hypothalamus and related adipose thermogenesis in females than in males [60, 61]. With high expression in the ARC, VMH, LH and PVN, ERα may be a key target for HIT to achieve VAT reduction. A previous study had demonstrated that high-speed running could increase the amount of hypothalamic ERα-positive neurons by more than low-speed running [62]. However, whether HIT can affect the hypothalamic ERα and further mediate VAT loss was not yet clear. The results of this study showed that in the premenopausal group, 10-week HIT significantly increased hypothalamic ERα expression. IHC showed that the locations of increased ERα were in the ARC, VMH and PVN, implying an enhanced impact of oestrogen on these nuclei, which are involved in energy homeostasis regulation. Currently, the precise mechanism underlying how exercise, particularly HIT, enhances ERα expression in the brain remains unclear. However, the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) pathway probably mediates this process. Studies have demonstrated co-expression of PGC-1α and ERα in the hypothalamus, with both in vitro and in vivo research confirming PGC-1α's role as an upstream regulator of ERα [63]. Notably, long-term exposure to a HFD can induce hypothalamic inflammation and systemic metabolic deficits in males and postmenopausal females, but not in premenopausal females. The hypothalamic PGC-1α/ERα pathway has been identified as a pivotal neuroprotective mechanism in this process [64]. As a well-recognized target of exercise, PGC-1α is activated more strongly by HIT (or even lactate administration) compared to MIT and further mediates neuroplasticity in the cortex and hippocampus [65, 66]. Regrettably, research on the impact of HIT on hypothalamic PGC is relatively scarce. Future studies could focus on alterations within the PGC-1α/ERα pathway across various neuronal subpopulations within the hypothalamus during HIT, aiming to further elucidate the underlying VAT loss mechanisms.

As previously stated, the fat loss resulting from HIT is mediated by the SNS during the recovery period. To further elucidate whether augmented ERα can modify neuronal operational patterns and augment EPOC, in Exp. 2, rats were implanted with microelectrodes in the PVN and subsequently trained for 4 weeks. The LFPs data were collected 12 h after the final training session to observe the neurophysiological processes occurring during the recovery period. The data demonstrated that HIT may result in a reduction in the power spectral density within the δ-band and an increase in the α- and β-bands. Conversely, MIT did not elicit these changes, suggesting that HIT may enhance or inhibit specific neuronal subpopulations. Changes in hypothalamic α- and β-bands were identified as being associated with altered energy homeostasis [67], but the effects of exercise are currently

unclear. Since many projections to the SNS converge on the PVN, this change in electrophysiological pattern may imply an enhancement of post-exercise catabolism in premenopausal rats. Data from 1 to 24 h of metabolic monitoring confirmed this speculation, which suggested that HIT mediated a larger EPOC and fat oxidation than MIT and control, especially within 12 h after exercise.

However, despite the current view that ERα, but not other ER family members, was the predominant metabolic regulator [68], it was still necessary to confirm the necessity of the α-subtype pathway for the neuroplasticity and fat loss effects of HIT. In Exp. 3, ERα was chemically inhibited by the third ventricular AZD-9496 (a selective ERα inhibitor) infusion during the post-exercise recovery period. AZD administration completely eliminated HIT-induced EPOC and fat oxidation elevation, VAT lipolytic enzyme phosphorylation and VAT loss. In particular, it is worth noting that the elimination of elevated fat oxidation began precisely 1–2 h after AZD administration, thereby confirming the involvement of ERα in mediating VAT catabolism. Inhibition of ERα did not seem to affect feeding or HIT-induced SPA increase, which might be related to the short duration of AZD infusion. In summary, these results suggested that enhanced hypothalamic ERα pathway played a key role in the HIT-mediated post-exercise VAT catabolism process.

Although the precise mechanism by which the hypothalamic ERα contributes to the HIT-induced VAT decrease in females remains unknown, lactate generated through anaerobic metabolism synergistically interacts with the ERα to boost brain-derived neurotrophic factor (BDNF, an important regulator of neuroplasticity) expression, potentially representing one of the key mechanisms involved. The notable characteristic of HIT is the capacity to generate substantial amounts of lactate, which, while suppressing lipolysis during exercise, facilitates synaptic plasticity and neurogenesis through the BDNF pathway in the hours subsequent to exercise. An intriguing study [69] revealed that vigorous-intensity continuous training (85% VO2max) also elicited heightened EPOC and post-exercise fat oxidation, hinting at the significance of lactate generated through glycolysis. On the one hand, lactate can independently mediate neuroplasticity through the NMDAR-BDNF pathway [70]. Additionally, intracerebral perfusion of lactate has been found to replicate the effect of HIT in elevating PGC-1α [66], the vital upstream signal that promotes ERα expression. Considering that ERα also contributes to the enhancement of BDNF expression [71], it is plausible that lactate stimulates the PGC-1α/ERα axis, ultimately amplifying the BDNF level and neuroplasticity in premenopausal animal models. A recent study by our team validated the important role of lactate in HIT in reducing VAT in female rats. Notably, lactate infusion added to MIT alone partially mimicked the HIT-induced training effects, including the increase in the hypothalamic neuronal firing rate, elevated BDNF expression, enhanced post-exercise fat oxidation and decreased VAT [72]. However, the specific neuron types and molecular pathways involved remain incompletely understood. In recent years, the scientific community's perspective on lactate has evolved from considering it a metabolic waste to recognizing it as the exerkine that mediates training adaptations [73]. Moreover, lactate, utilized solely as a nutritional supplement, can exhibit similar effects to HIT in suppressing appetite and enhancing fat metabolism [74]. Regrettably, this study did not explore in detail whether HIT activated the PGC-1α and BDNF pathways in the hypothalamus via lactate, nor did it specify the particular nuclei and neuronal subpopulations involved in this process. Given the apparent sexual dimorphism in fat metabolism, further elucidation of the role of the ERα pathway in the hypothalamus in integrating peripheral metabolites and messenger molecules, as well as regulating VAT catabolism through neural projections and hormone secretion, represents promising avenues for future research.

Study strengths and limitations

HIT has gained widespread popularity as a fitness protocol, but its superiority over moderate-intensity training (MIT) in reducing VAT remains debated. In this study, it is demonstrated for the first time that the ERα pathway plays a key role in HIT-mediated hypothalamic plasticity and enhanced post-exercise fat catabolism. Utilizing in vivo electrophysiology and targeted blocking methods, these findings provide new evidence elucidating the mechanisms behind the sexual dimorphism observed in exercise-induced weight loss.

Several unavoidable limitations in this study should be acknowledged. Firstly, due to skull growth, Exp. 2 and 3 could not be executed in exactly the same intervention period as Exp. 1; additionally, due to technical limitations of electrode and catheter implantation, hypothalamic electrophysiological observations and chemical inhibition of ERα and were accomplished in two separate experiments. Secondly, the limited number of monitoring channels in the metabolic and electrophysiological equipment prevented the three experiments from being conducted simultaneously. Variations in animal batches could contribute to discrepancies in SPA results observed between Exp. 1 and 3, which need to be confirmed by future studies with larger sample sizes. Thirdly, given the differences in higher brain functions between humans and rodents, the applicability of SPA and feeding behaviour results from this study to humans should be approached with caution.

Conclusion

In conclusion, compared to MIT, HIT could more strongly elevate ERα expression in several hypothalamic nuclei in pre- but not postmenopausal animal models. In premenopausal female rats, HIT-mediated PVN electrophysiologic changes, enhanced EPOC and VAT catabolism required the involvement of the hypothalamic ERα pathway.

The findings of this study affirmed that hypothalamic ERα played a pivotal role in the VAT loss process among premenopausal subjects, implying that HIT may not be a one-size-fits-all fitness protocol for the entire population. This discovery provides a theoretical foundation and a theoretical basis for tailoring optimal exercise prescriptions to individuals of varying genders and ages.

Furthermore, it warrants further exploration whether lactate, a crucial byproduct of intense exercise, collaboratively influences hypothalamic functional plasticity in conjunction with ERα. Uncovering the associated mechanisms could pave the way for developing novel exercise detection markers and nutritional supplement strategies.

Abbreviations

VAT	Visceral adipose tissue
MIT	Moderate-intensity training
HIT	High-intensity training
SNS	Sympathetic nervous system
ER	Estrogen receptor
ERα	Estrogen receptorα
EPOC	Excessive post-exercise oxygen consumption
OVX	Ovariectomised
SPA	Spontaneous physical activity
LFPs	Local field potentials
PVN	Paraventricular nuclei
ICV	Intra-cerebroventricular injection
IE	Microelectrode implantation
HSL	Hormone-sensitive lipase
HFD	High fat diet
TC	Total cholesterol
TG	Triglycerides
LDL-C	Low-density lipoprotein cholesterol
HDL-C	High-density lipoprotein cholesterol
IHC	Immunohistochemistry
VMH	Ventral medial hypothalamus
ARC	Arcuate nucleus
GPER	G-protein-coupled estrogen receptor
LPL	Lipoprotein lipase
DMH	Dorsomedial hypothalamus
LH	Lateral hypothalamus
WB	Western blotting
OCT	Optimal cutting temperature compound
PBS	Phosphate buffered saline

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Author contributions

JW and ST collected and analyzed the experimental data. ST, JD and SD performed the surgical and training procedures. YL, WC and JW designed the experiments. All authors read and approved the final version of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethical approval

All experimental procedures for this study were approved by the Ethics Committee of Hebei Normal University (2021LLSC031) and carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80 – 23) revised 1996. All efforts were made to minimize the number of animals used and their suffering.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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