

Comparison of Visceral Fat Lipolysis Adaptation to High-Intensity Interval Training in Obesity-Prone and Obesity-Resistant Rats

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Abstract

Background/objectives: Visceral obesity is one of the key features of metabolic syndrome. High-intensity interval training (HIIT) could effectively reduce visceral fat but its effects show strong heterogeneity in populations with different obesity degree. The mechanism may be related to the differential adaptation to training between obesity phenotypes, namely obesity prone (OP) and obesity resistant (OR). The aim of the present study was to compare adaptive changes of visceral adipose lipolysis adaptation to HIIT between OP and OR animals and further explore the upstream pathway.

Methods: OP and OR Sprague Dawley rats were established after feeding a high-fat diet for 6 weeks; they were then divided into HIIT (H-OP and H-OR) and control (C-OP and C-OR) groups. After 12 weeks of HIIT or a sedentary lifestyle, animals were fasted for 12 h and then sacrificed for histology as well as gene and protein analysis. Visceral adipocytes were isolated without fasting for catecholamine stimulation and β 3-adrenergic receptor (β 3-AR) blockade *in vitro* to evaluate the role of upstream pathways.

Results: After training, there were no differences in weight loss or food intake between OP and OR rats ($P > 0.05$). However, the visceral fat mass, adipocyte volume and liver lipid of OP rats decreased more than that of OR rats ($P < 0.05$). Meanwhile, the cell lipolytic capacity and the increase in the expression of β 3-AR was higher in the OP compared with OR groups ($P < 0.05$). Although training did not increase sympathetic nervous system activity ($P > 0.05$), the cell sensitivity to catecholamine increased significantly in the OP compared with OR groups ($P < 0.05$). After blocking β 3-AR, the increased sensitivity disappeared.

Conclusion: With HIIT, OP rats lost more visceral fat than OR rats, which was related to stronger adaptive changes in lipolysis. Increased β 3-AR expression, rather than altered sympathetic nerve activity, mediated this adaption.

Introduction

Because of the more pronounced association of visceral adipose tissue (VAT) with metabolic syndrome, a loss of VAT rather than total fat may provide more health benefits. High-intensity interval training (HIIT) is a type of protocol that alternates high-intensity training periods with active/passive intervals, which can enable participants to complete high-intensity exercise with a higher training volume. Over the past decade, HIIT has become a hot topic of health promotion: compared with classic aerobic training, it has shown similar or better effects on visceral and liver fat loss, improving insulin resistance and cardiovascular function while being more time-efficient^{1–4}.

Numerous studies have shown that HIIT can effectively reduce visceral fat^{2, 5–7}; however, other experiments have found no change in VAT mass after training^{5, 8–13}. Moreover, even in the studies that have confirmed HIIT reduces VAT, individual differences in the reduction are huge (standard deviations up to ten times larger than means) among different subjects with same protocol². Different responses to the

same intervention indicate that individual differences can strongly affect the VAT-reducing effect of HIIT. In their meta-analysis, Maillard et al.⁵ confirmed that HIIT significantly reduces visceral fat in subjects with overweight and obesity but has no effect on normal weight people, suggesting that individual differences in the obesity degree and lipid metabolism may be the key factors affecting the VAT reducing effect of HIIT.

Due to genetic polymorphisms, there are obvious individual differences regarding gaining fat mass in subjects exposed to obesity-causing factors (diet, sedentary lifestyle, environment, etc.). The different metabolic phenotypes of fat that is easy or hard to accumulate are called obesity prone (OP) and obesity resistant (OR), respectively^{14, 15}. The causes of OP and OR are related to differences in how feeding behaviour, physical activity (PA) and the sympathetic nervous system (SNS) regulate adipose catabolism. With the same high-fat diet (HFD), compared with OR subjects, OP subjects tend to show higher food intake and lower PA^{16, 17}, lower secretion of norepinephrine (NE, the main central messenger promoting lipolysis)¹⁸, a higher respiratory quotient (lower lipid oxidation)¹⁹ and a tendency for triglycerides (TG) to accumulate in adipose tissue rather than muscle.¹⁸ The exercise responses and adaptations of OP and OR subjects are also different. While the mechanisms by which food intake and PA change are still debated, after the same aerobic training, OP subjects seem to show a greater leptin reduction and improvement in insulin resistance compared with OR subjects^{20–22}. However, few studies have been published that compare the differential adaptation to HIIT between OP and OR individuals, especially VAT metabolism.

Because the main energy substrate during exercise is glycogen, the mechanism by which HIIT can reduce fat is generally suspected to be related to the ‘post-exercise’ changes in adipose catabolism^{23, 24}. Catecholamine release by the SNS and adrenal glands can activate lipolysis in adipocytes through β3-adrenergic receptors (β3-AR), which are the major pathway of adipose catabolism²⁵. In previous studies, we and other teams have found that long-term HIIT could cause several adaptive changes in this pathway. HIIT increases the levels of β3-AR messenger RNA (mRNA)²⁶, hormone-sensitive lipase (HSL, a catecholamine-regulated lipolytic enzyme)²⁷ after NE stimulation *in vitro*. Moreover, the visceral adipocytes of mice subjected to HIIT show stronger HSL phosphorylation and TG hydrolysis²⁸, which means that VAT could be mobilised more easily during hunger, cold or stress. However, whether HIIT has different effects on OP and OR individuals remain unclear. A recent large-sample survey found that increased energy consumption during exercise would lead to a decrease in the basal metabolic rate during the recovery period. Furthermore, this ‘compensation’ phenomenon is stronger in subjects with obesity than in normal-weight people²⁹, which means people with obesity would have greater difficulty losing fat by exercising. If HIIT could provide greater post-exercise catabolism in OP compared with OR individuals, it should be recommended as an efficient protocol that better alleviates the ‘energy compensation’ for the OP population.

Based on the above considerations, we compared the fat loss effect and influence of HIIT on VAT lipolysis and the NTS pathway between OP and OR rats. First, Sprague Dawley rats were fed a HFD for 6

weeks to establish an OP/OR animal model, followed by 12 weeks of HIIT. After sacrificing the rats, we examined the morphology of adipocytes, hepatocytes and mitochondria and the mRNA expression of TBX1 (a marker of thermogenesis); the protein expression of tyrosine hydroxylase (TH, a marker of SNS transmitter release), β 3-AR, HSL, and HSL phosphorylated at serine 660 (HSLser660, a marker of HSL activation). We found that β 3-AR expression rather than SNS regulation might play the key role in the increased VAT catabolism of OP rats. Therefore, we performed an *in vitro* experiment of catecholamine stimulation and β 3-AR blockade. The findings confirmed that the greater fat lipolysis of OP rats was related to increased β 3-AR expression.

Materials And Methods

Study Design

We performed two parallel experiments: experiment 1 (Exp. 1) to compare fasting-induced lipolysis *in vivo* and experiment 2 (Exp. 2) to examine the receptor sensitivity to catecholamines *in vitro* after excluding neural control (Fig. 1). In Exp. 1, after feeding Sprague Dawley rats a HFD for 6 weeks, the rats were separated into OP and OR groups based on the model criteria and then randomly divided into control (C-) and training (H-) groups. After 12 weeks of HIIT or a sedentary lifestyle, the rats were rested for 48 h, fasted for 12 h and then sacrificed for analysis. In Exp. 2, the rats received the same feeding and exercise intervention, but they were not fasted before sacrifice. Then, visceral adipocytes were isolated for *in vitro* stimulation and receptor blocking.

Establishment of the OP/OR Rat Model

The OP/OR model was established according to classic methods^{14, 15}. Forty-eight specific-pathogen free male Sprague Dawley rats (7 weeks old; Changsheng Biotech) were housed under controlled conditions ($24 \pm 2^\circ\text{C}$, humidity of $50\% \pm 5\%$, 12-h photoperiod). After 1 week of feeding with an adaptive standard chow, the rats were fed a HFD for 6 weeks; it comprised 60% standard chow, 16% sugar, 5% fat, 18% egg yolk powder and 1% sodium cholate. Subsequently, the rats were sorted by body weight. The first quartile ($n = 12$) of rats was selected as the OP model and the fourth quartile ($n = 12$) was selected as the OR model. HFD feeding continued throughout the next training period.

Exercise Protocol

After 3 days of adaptive training (10 min/day, 5 m/min, 0° incline), the exercise groups were subjected to 12 weeks of 25° uphill treadmill HIIT with 5 days of training and 2 days of rest every week. Each HIIT contained multiple 1 min high running speed–1 min medium running speed cycles. Over 12 weeks, the medium running speed gradually increased from 12 to 20 m/min, and the high speed gradually increased from 22 to 34 m/min, which was adjusted based on an incremental exercise test (IET). The distance of each HIIT was equal to 45 min of running at medium speed. See our previous study for more details²⁸.

Sample Collection

After 48 h of rest, rats of Exp. 1 were fasted for 12 h and then sacrificed after anaesthesia (intraperitoneal pentobarbital sodium, Solarbio, China), while rats of Exp. 2 were sacrificed without fasting. The epididymal fat pads were collected to examine VAT. After weighing, the central part of the fat pads were removed and fixed in 4% paraformaldehyde or 4% glutaraldehyde for optical microscopy or transmission electron microscopy (TEM). The remaining tissues were frozen at -80°C. Fat pads of rats in Exp. 2 were minced and put into tubes containing phosphate-buffered saline for adipocyte isolation; they were not frozen.

Histological Observation of Adipocyte Volume, Liver Lipids and Mitochondria

The adipocyte volume was observed by haematoxylin and eosin (H&E) staining. Briefly, after fixation in 4% paraformaldehyde for 4 h, tissue was embedded in paraffin, cut into 5 µm sections, dewaxed, rehydrated, stained with haematoxylin (5 min), washed with water and 0.6% ammonia and finally stained with eosin (2 min). After dehydration and sealing, the tissue was observed under an optical microscope. Two hundred adipocytes per group were randomly selected for cell areas analysis using ImageJ 1.51.

Liver fat deposition was analysed with Oil red O staining. Briefly, fixed liver was stained with Oil red O (9 min), equilibrated in 60% isopropanol, stained with haematoxylin (3 min), equilibrated in 60% alcohol, washed in tap water and then observed under a microscope. The gray value of the red signal was analysed with ImageJ 1.51.

To observe the number and distribution of mitochondria, after fixation in glutaraldehyde, the tissue was washed with 0.07 M phosphate buffer, fixed with 1% osmic acid (2 h), dehydrated with an acetone gradient at 4°C, infiltrated and sealed with epoxy resin at 37°C and then allowed to stand at 60°C for 48 h. Subsequently, the tissue was sliced into 70 nm ultrathin sections, dyed with uranyl acetate and lead citrate and photographed by using a transmission electron microscope (HITACHI H-7650, Japan).

Protein and RNA Expression

The protein expression of HSL, phosphorylated HSL-ser660, TH and β3-AR was examined by western blot. Briefly, the tissue was chopped and homogenised with radioimmunoprecipitation assay (RIPA) buffer (Solarbio, China) with phosphatase inhibitor (Thermo Fisher, USA) diluted 1:100, centrifuged at 12,000 rpm for 20 min at 4°C and then boiled at 98°C for 5 min. The samples were subjected to sodium dodecyl sulphate–polyacrylamide gel electrophoresis using 10% gels. The separated protein was then transferred onto polyvinylidene fluoride membranes. The membranes were incubated with the following primary antibodies: anti-HSL (1:2000; Cell Signaling Technology), anti-HSL-ser660 (1:2000; Cell Signaling Technology, USA), anti-TH (1:2000; Cell Signaling Technology, USA), and anti-β3-AR (1:1000; Abcam, USA). Actin was used as loading control (1:5000; Bioworld, China). After incubation with the secondary antibody (1:5000; Solarbio, China), luminescence was analysed by using a gel imaging system (Fusion Fx5, VILBER LOURMAT, France).

TBX1 mRNA expression was examined by using quantitative real-time polymerase chain reaction (PCR). Briefly, tissue was homogenised with TRIzol reagent (Thermo Fisher) (1:10 ratio). Total RNA was separated after extraction and precipitation. A portion of the isolated RNA was reverse transcribed using a complementary DNA (cDNA) transcription kit (Servicebio, China). The reactions were run on a Bio-Rad real-time PCR system (USA) and the relative expression of TBX1 was calculated by using the $2^{-\Delta\Delta CT}$ method, in which $\Delta\Delta CT = [(CT_{TBX1} - CT_{\beta\text{-actin}})_{\text{treatment}}] - [(CT_{TBX1} - CT_{\beta\text{-actin}})_{\text{nontreatment}}]$.

Primary Adipocyte Isolation and Catecholamine-Induced Lipolysis In Vitro

Primary visceral adipocytes were isolated from tissue after incubation with collagenase (Solarbio, China) for 30 min at 37°C. The isolated adipocytes were stimulated with an isoproterenol (ISO) gradient (0.1, 1 and 10 µM, Sigma-Aldrich, USA). To determine whether ISO-induced lipolysis is activated through β3-AR, SR59230a (MedChemExpress, USA, 100 nM), a selective antagonist, was added alongside 10 µM ISO. The methods of isolation and *in vitro* stimulation have been published previously^{28,30}. The amount of glycerol released by cells – a marker of lipolysis – was measured by using an enzyme labelling kit (Jiancheng Bio, China). The intraclass correlation coefficient (ICC) was calculated for quality control. Twenty randomly selected samples were measured twice and the ICC was 0.814 (ICC > 0.75 indicates good reliability).

Statistical Analysis

The data are presented as the mean ± standard deviation (SD). Body weight changes over time among the groups (OP/OR, HIIT/sedentary) were analysed by using repeated-measures analysis of variance (ANOVA), with time and group as the main factors as well as the time × group cross-effect. Food intake during the model establishment period and cell glycerol release with or without β3-AR blockade were analysed by using independent samples t-test. After 12 weeks of training, the influences of HIIT between OP and OR rats regarding body weight, food intake during training, fat weight, adipocyte area, protein/gene expression and glycerol release were compared with 2 × 2 two-way ANOVA (cross-effect: HIIT/sedentary × OP/OR). When the cross-effect was significant, simple effect analysis was used to compare the differences between groups. Due to the non-normal distribution, TBX1 expression was analysed by using the Kruskal–Wallis test. Statistical significance was set at $p < 0.05$. Based on the fat pad mass data from previous experiments, the minimum sample size was 4 ($\alpha = 0.05$, power = 0.8).

Results

Body Weight and Food Intake during the Model Establishment Period

During the 6-week model establishment period, body weight increased steadily and was always higher in the OP compared with the OR group ($P < 0.05$, Fig. 2A). In week 6, OP and OR rats were selected based on

their final weight (Fig. 2B). The total food intake of OP rats after 6 weeks was significantly higher than the food intake of OR rats ($P < 0.05$, Fig. 2C).

Body Weight and Food Intake during and after 12 Weeks of HIIT

During the training period, HIIT reduced the body weight in both exercise groups (time \times HIIT/sedentary $P < 0.01$) and the difference between C-OP and C-OR was still increasing (time \times C-OP/C-OR $P < 0.01$, Fig. 3A). HIIT seemed to suppress the increasing trend of body weight in the H-OP relative to the H-OR group (time \times H-OP/H-OR $P = 0.089$, Fig. 3A). However, the data from week 12 showed that HIIT had no effect on reducing the body weight of OP and OR rats (-23.00% and -22.82%, respectively, HIIT/Sedentary \times OP/OR $P > 0.05$, Fig. 3B). Regardless of whether they were subjected to HIIT or a sedentary lifestyle, OP rats consumed more food than OR rats (HIIT/Sedentary \times OP/OR $P > 0.05$, main effect of OP/OR $P < 0.01$, Fig. 3C).

Fat Mass, VAT, Liver and Mitochondrial Histology and TBX1 expression

Data showed that 12 weeks HIIT could effectively reduce the epididymal fat mass, the visceral adipocyte volume and liver lipid deposition in both the H-OP and H-OR groups (all $P < 0.01$, Fig. 4A–C) and it should be noted that OP rats showed a greater decline than OR rats (HIIT/Sedentary \times OP/OR $P < 0.05$, Fig. 4A–C). Although due to the large individual differences, there were differences between groups regarding TBX1 mRNA expression, 12 weeks of HIIT increased the number of mitochondria gathered near lipid droplets in both exercise groups (Fig. 5C). In summary, HIIT showed a stronger effect on reducing VAT and liver lipids in OP rats.

Expression of TH, β 3-AR, HSL and phosphorylated HSL-ser660 Protein

Lipolysis is mainly activated by sympathetic nerves that release NE through adrenergic receptors and the cyclic adenosine monophosphate (cAMP)–protein kinase A (PKA) pathway. Hence, we examined the expression of two proteins that are part of the upstream pathway: TH, a marker of NE release, and β 3-AR, the major NE receptor of adipocytes as well as HSL (key lipolysis enzyme) total and phosphorylated protein expression. TH expression was not affected by the obesity phenotype or training ($P > 0.05$, Fig. 5A). By contrast, HIIT increased β 3-AR expression in both groups ($P < 0.05$, Fig. 5B). The increase in OP rats was greater than in OR rats (HIIT/Sedentary \times OP/OR $P < 0.05$, Fig. 5B). After 12 weeks of HIIT, neither the obesity phenotype nor training affected HSL expression ($P > 0.05$, Fig. 4A). OP rats showed higher HSL phosphorylation during starvation than OR rats ($P < 0.01$, Fig. 4B). These data suggest that when facing fasting pressure, the OP phenotype responds more strongly. HIIT significantly increased phosphorylated HSL-ser660 in all exercise groups ($P < 0.01$, Fig. 4B), and this training-induced increase was similar

regardless of the obesity phenotype (HIIT/Sedentary × OP/OR $P > 0.05$, Fig. 4B). In summary, these results suggest that OP rats exhibit a stronger adaptive change to HIIT due to increased β 3-AR expression rather than increased SNS activity.

Lipolytic Activity of Adipocytes Induced by **In Vitro** Catecholamine Stimulation and Target β 3-AR Blockade

To evaluate whether altered β 3-AR expression leads to greater sensitivity to catecholamines, visceral adipocytes of rats submitted to 12 weeks of training and not fasted before sacrifice were isolated and stimulated by an ISO gradient (0.1–10 μ M, to imitate hunger) *in vitro*. Released glycerol (a product of lipolysis) was measured to represent the downstream activation of β 3-AR. An overview of each group is presented in Fig. 6A. Glycerol release was similar in the two control groups ($P > 0.05$, Fig. 6B). However, the lipolysis of the H-OP group was stronger than that of the H-OR group ($P < 0.05$, Fig. 6C), suggesting that OP rats became more sensitive to catecholamines after training. To further examine whether the elevated sensitivity was related to β 3-AR, a selective antagonist (SR59230a) was added to the 10 μ M ISO stimulant. With this combination, the increased lipolysis of H-OP was inhibited ($P < 0.05$, Fig. 6D), indicating that greater β 3-AR expression in OP rats plays a key role in mediating the increased catecholamine sensitivity.

Discussion

HIIT has been confirmed to reduce VAT and relieve metabolic syndrome effectively^{2, 5–7}. However, individual differences between subjects are large². A previous meta-analysis found that the effect of HIIT on reducing VAT is evident in subjects with obesity or overweight but weak in normal weight subjects⁵. This phenomenon may be related closely to the difference between obesity phenotypes in adipose catabolism. Previous experiments have found that the fat loss effect of HIIT is related to the activation of lipolysis^{26–28, 31}. Because gene polymorphisms underlie individual differences in food intake, PA and adipose metabolism, we hypothesised that OP and OR individuals show different adaptive changes to HIIT, especially in lipolysis regulated by the SNS.

We compared how 12 weeks of HIIT affected VAT loss in OP and OR rats, as well as how this training affected catecholamine-regulated lipolysis pathways. We found: (1) HIIT could reduce HFD-induced body weight gain, liver fat and VAT mass in both OP/OR rats, and the influence in OP rats was even stronger. (2) After training, VAT of OP rats was more sensitive to catecholamines and showed stronger lipolysis compared with OR rats. (3) Increased β 3-AR expression, rather than increased SNS activity, plays a key role in mediating these adaptive changes.

HIIT Had a Stronger Influence on Body Weight, VAT mass and Liver Lipids in OP Compared with OR Rats

Similarly to the heterogeneity of human obesity, rodents also show two different obesity phenotypes, namely OP and OR^{14, 15}. Although the baseline weights of these rats are the same, marked differences in

food intake, PA, central regulation and peripheral tissue metabolism lead OP rats to become obese after several weeks of receiving a HFD, while OR rats tend to remain at a normal weight^{32–34}. We established a model of OP and OR rats by using a classic programme involving a HFD^{14, 15, 34}. While establishing the model, the food intake and weight gain of OP rats were significantly higher than for OR rats, indicating that the model was established successfully.

Studies have shown that when the environment changes, OR individuals often exhibit a steady state of energy metabolism, while OP individuals respond more strongly to caloric restriction or food surplus^{35, 36}, suggesting that the OP phenotype has a survival advantage when energy is insufficient but is harmful during periods of excess food. Previous comparative studies of OP and OR have often focussed on aerobic exercise rather than HIIT^{20–22, 35, 36}. Levin¹⁸ found that OP rats lose more body weight and fat during aerobic exercise than OR rats, and Jen et al.²³ and Zachwieja et al.²⁴ showed that aerobic exercise has similar effects on the weight loss of OP and OR rats, but a stronger effect on reducing VAT mass in OP subjects. However, differently from aerobic exercise, HIIT does not consume fat during exercise, and whether it has different effects on different metabolic phenotypes requires new evidence. To our knowledge, this study compared the fat-loss effects of HIIT on OP and OR subjects for the first time. We found that HIIT had a stronger effect on reducing VAT in OP than OR rats, indicating that the differential adaptation of OP and OR subjects may be the reason for the strong heterogeneity of the HIIT fat-loss effect.

Because the 12 weeks of training had no effect on the food intake, the heterogeneity of the VAT decrease between OP and OR rats may be more related to altered peripheral metabolism than feeding regulation by the central nervous system. The VAT mass in adulthood is more related to the volume rather than the number of adipocytes, and the main contributor (> 95%) of the cell volume comes from TG accumulation in lipid droplets^{37, 38}. We found that after 6 weeks of a HFD, the adipocyte area of OP rats was larger than that of OR rats, a finding that is characteristic of the classic OP animal model^{39, 40}. After 12 weeks of HIIT, the cell area had decreased in both OP and OR rats, although the reduction was greater in OP rats, accompanied by an increase in the number of mitochondria (mean higher local lipid oxidation), suggesting that HIIT reduces the TG content of VAT in OP rats more strongly than OR.

It is well known that VAT accumulation is associated with metabolic syndrome, non-alcoholic fatty liver disease and cardiovascular disease⁴¹. Weight reduction by energy restriction or exercise is still the preferred method to alleviate liver steatosis rather than drugs⁴². A previous study confirmed the close relationship between VAT mass and hepatic lipid deposition⁴³, and the exercise-induced decrease in VAT is often accompanied by decreased hepatic TG content⁴⁴. Our results are consistent with previous findings: very similarly to VAT, the decrease in the liver lipid level in OP rats was larger than in OR rats, indicating greater health benefits in the former group. In summary, HIIT showed a greater influence on the VAT mass, cell volume and liver lipid deposition of OP compared with OR rats, findings that imply a stronger catabolic change in OP individuals.

VAT of OP Rats Showed Greater Lipolytic Potential than OR Rats after HIIT

Based on the data that the VAT of OP rats decreased more than OR rats after training, we hypothesised that heterogeneity in VAT changes between OP and OR rats is related to the different adaptations of adipocyte lipolytic pathways. Lipolysis is the primary step of TG decomposition, which refers to the process by which TG are hydrolysed into glycerol and non-esterified fatty acids (NEFA, the released form of TG). This process is mainly regulated by AR in the SNS^{45, 46}. The SNS releases NE and epinephrine through nerve endings and adrenal glands, which can activate second messenger pathways (involving G proteins and cAMP) through AR, ultimately leading to PKA-mediated phosphorylation of HSL and activation of lipolysis⁴⁷. Catecholamine release is correlated with exercise intensity⁴⁸. Aerobic exercise mediates moderate secretion, which enhances lipolysis through β-AR of adipocytes to meet fat consumption during exercise, while high-intensity exercise induces excessive secretion of catecholamines and inhibits lipolysis through a negative feedback mechanism involving α-AR^{49, 50}.

Because minimal fat is burned during exercise, it is generally believed that HIIT can reduce fat based on post-exercise TG consumption^{23, 24, 51}. A commonly mentioned view is that HIIT could increase excess post-exercise oxygen consumption (EPOC), but there are still controversies among existing results⁵². Another reasonable hypothesis is that due to the intense load of HIIT, during the recovery period, adipose tissue would release more NEFA for tissue healing or reconfiguration in muscle, lung and other tissues²⁴, which suggests HIIT promotes adaptive catabolism of adipose tissue. Studies have verified the increase in β-AR and lipolysis in adipocytes after long-term HIIT^{26, 28}, supporting the hypothesis of adaptative changes. As mentioned earlier, when facing catabolic stress, OP individuals are more likely to show adaptation (fat is lost easily), while OR individuals are more likely to maintain homeostasis^{35, 36}. Therefore, a reasonable explanation why the VAT mass of OP rats decreases more than that of OR rats after HIIT is the visceral adipocytes of the former group generate a stronger adaptation to HIIT, and the lipolytic pathways are easier to start-up to ensure an energy supply when facing fasting, cold, exercise or other stress.

To test this hypothesis, we evaluated the expression and phosphorylation of HSL (the rate-limiting enzyme of lipolysis) as well as glycerol release (lipolysis marker) of adipocyte *in vitro*. We found that after 12 h of fasting (inducing catecholamine release), there was no difference in HSL expression between the groups, but the level of phosphorylated HSLser660 in the exercise groups was higher than in the control groups, indicating an activating effect of HIIT on VAT lipolysis. Our *in vitro* experiment confirmed greater lipolysis in the H-OP group after catecholamine stimulation, suggesting that there is stronger catabolism in the OP rats. In summary, these results suggest greater adaptation of lipolysis pathways in OP compared with OR rats.

Increased β3-AR Expression May Play a Key Role in Mediating Adaptive Change to HIIT

Although VAT lipolysis and NTS in the H-OP group showed stronger adaptations to HIIT, the upstream mechanisms that mediate these changes remained unknown. Adipose tissue is regulated extensively by the neuroendocrine network. Although the SNS plays a primary role (Bartness et al. 2014),⁵³ parasympathetic nerves, natriuretic peptides, glucocorticoids and parathyroid hormone are also involved in regulating lipolysis⁵⁴. Even downstream of the SNS, catecholamines and AR are not the only ‘transmitter–receptor’ pathway. Recent studies have reported other transmitters, receptors or intermediary cells such as neuropeptide Y (NPY), G-protein coupled receptor 3 (GPR3) and adipose mesenchymal cells (MSCs) involved in the SNS-mediated regulation of adipose catabolism^{55–57}. Due to the existence of many pathways, the role of the SNS and β3-AR needed to be further confirmed in this study.

Because the hypothalamus is the major regulator of energy homeostasis and directly controls the SNS and food intake^{53, 58}, we speculated that the hypothalamus of OP rats could adapt to HIIT, thereby changing the feeding behaviour and SNS-regulated adipose metabolism. Unexpectedly, similarly to the food intake, 12 weeks of training did not influence the TH expression of VAT, indicating that HIIT does not promote NE release of sympathetic nerves. However, β3-AR expression of the exercise groups was increased significantly, and the increase was greater in the H-OP compared with the H-OR group, indicating that HIIT could increase more strongly the receptor number and the exercise adaptation of OP rats. To determine whether higher β3-AR expression produced stronger sensitivity to catecholamines, isolated adipocytes were stimulated by an ISO gradient (0.1–10 μM, to imitate levels of catabolism pressure) *in vitro*. The glycerol release of the H-OP group was significantly higher than the H-OR group, suggesting that adipocytes of OP rats are more sensitive to catecholamines and could produce more NEFA to export or local oxidation under the same SNS signal.

So far, three beta isoforms of AR have been reported (β1, β2 and β3). Although β3-AR predominates in white adipose tissue⁵⁹, studies have confirmed that gene polymorphisms of all three isoforms could affect the fat catabolism induced by aerobic exercise^{60–62}, although the relationship between HIIT and different isoforms had been unclear. To determine whether the VAT adaptation to HIIT is only related to β3-AR or also to other isoforms, we blocked this receptor with SR59230a, a selective antagonist, concomitantly with 10 μM ISO stimulation. Without the participation of β3-AR, the increased glycerol release in the H-OP group disappeared. These data confirm that increased expression of β3-AR, rather than β1-AR or β2-AR, plays a key role in increasing the sensitivity of adipocytes to catecholamine. In summary, after 12 weeks of HIIT, greater β3-AR expression in OP rats led to an elevated sensitivity to catecholamines compared with the OR rats. This change underlies the greater VAT lipolysis capabilities of OP rats.

Limitations

Our study had some limitations. First, due to species differences of β-AR, additional studies are needed to confirm whether the conclusions of this study are applicable to humans. Second, because of equipment

limitations, basal or fasting metabolic monitoring as well as body fat percentage measurement of rats were not carried out.

Conclusion

HIIT provides notable advantages in improving metabolic syndrome and is more time-efficient than aerobic exercise, but the fat-loss effects in different populations are highly heterogeneous, and the reason may be related to different adaptive changes in VAT lipolysis between obesity phenotypes. We found that 12 weeks of HIIT reduced visceral fat more in OP compared with OR rats. After training, the VAT lipolytic capabilities of OP rats were stronger than OR rats, indicating that the OP phenotype shows greater catabolic adaptation to HIIT. In addition, increased β 3-AR expression played a key role in mediating this process. Future research should verify whether the HIIT-induced higher lipolysis could alleviate the ‘energy compensation’ in people with obesity, an endeavour that should help find more effective methods for obesity control.

Abbreviations

HIIT

High-intensity interval training

OP

obesity prone

OR

obesity resistant

β 3-AR

β 3-adrenergic receptor

VAT

visceral adipose tissue

SNS

sympathetic nervous system

HFD

high-fat diet

NE

norepinephrine

TG

triglycerides

TH

tyrosine hydroxylase

HSL

hormone-sensitive lipase

ISO

isoproterenol

EPOC

post-exercise oxygen consumption

Declarations

Ethics approval and consent to participate

All experimental procedures were approved by the Ethics Committee of Hebei Normal University (2021LLSC031).

Consent for publication

All co-authors have approved the manuscript for submission.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors declare no potential competing interests.

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Authors' contributions

Yang Liu and Haifeng Zhang designed the study and analysed the data. Yang Liu, Yu Li, Baishuo Cheng, Shige Feng and Xiangui Zhu collected the data. Yang Liu drafted the manuscript. Haifeng Zhang and Wei Chen revised the manuscript.

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Figures

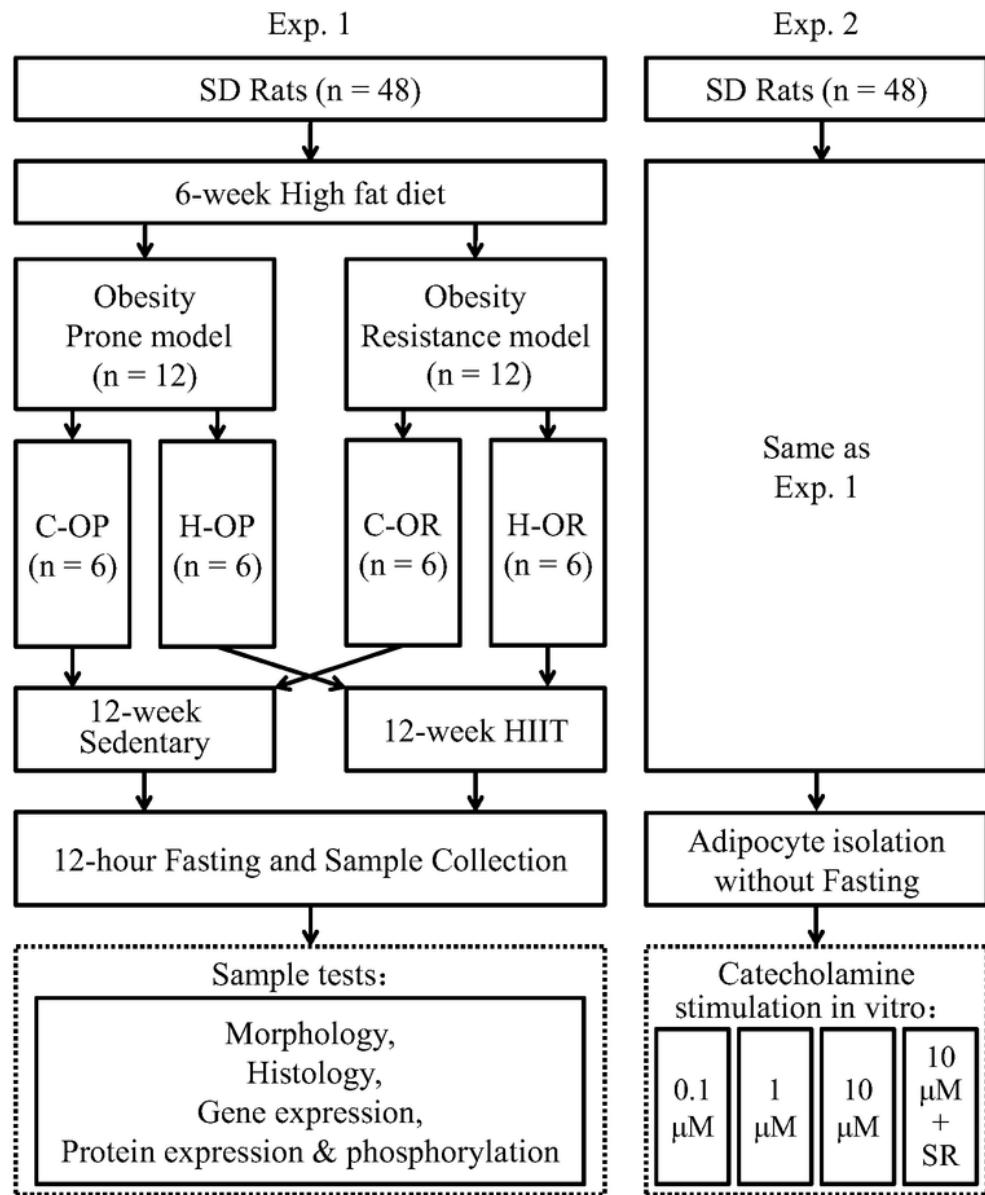


Figure 1

Study design

Notes: C- indicates the control groups; H- indicates the groups subjected to high-intensity interval training; OP indicates obesity prone; OR indicates obesity resistant; 0.1, 1 and 10 μ M indicate the gradient of isoproterenol stimulation; SR indicates SR59230a, a selective antagonist of β 3-adrenergic receptors.

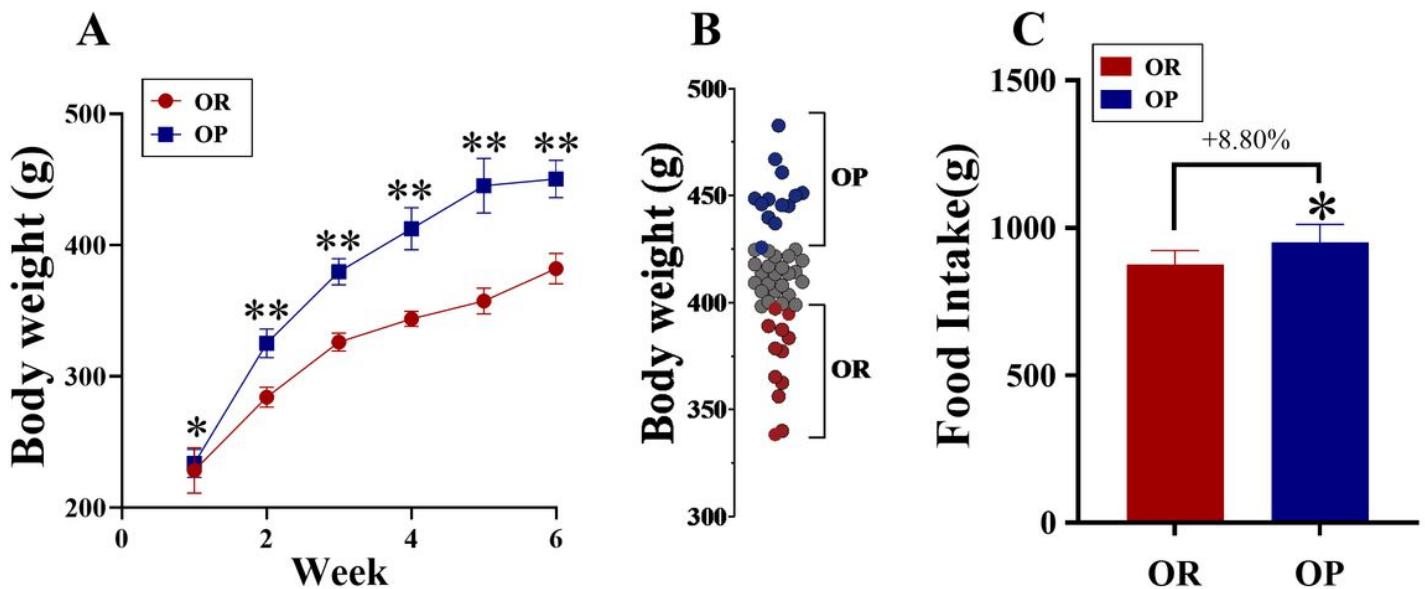


Figure 2

Body weight and food intake during the model establishment period

Notes: (A) Body weight changes during the 6-week model establishment period, analysed by repeated measures analysis of variance. (B) Body weight distribution after 6 weeks, with the fourth and first quartiles ($n = 12$ each) of rats selected as obesity prone (OP) and obesity resistant (OR) rats, respectively. (C) The total food intake during the 6-week model establishment period, analysed by t-test. * $P < 0.05$, ** $P < 0.01$.

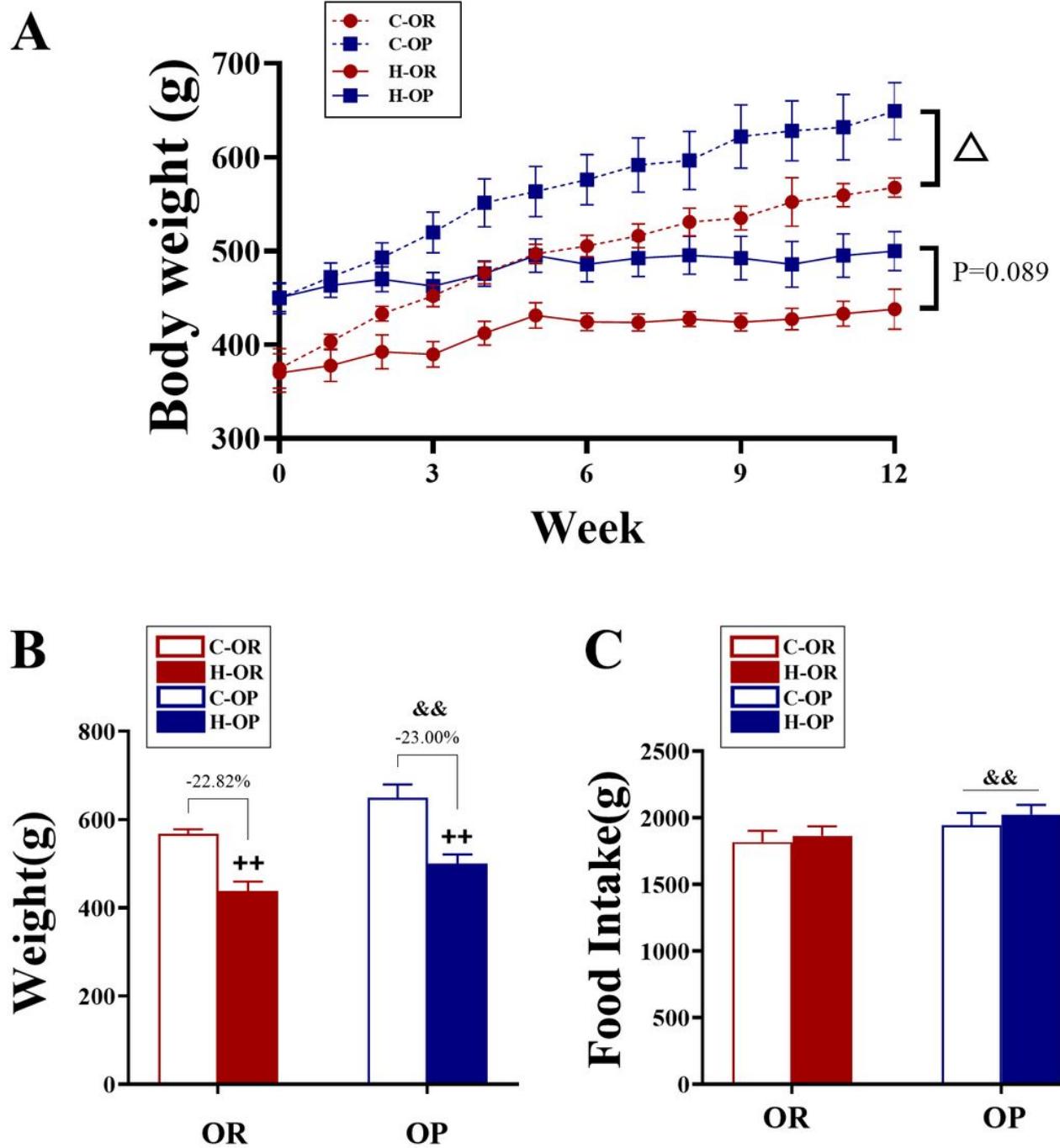


Figure 3

Body weight and food intake during and after 12 weeks of high-intensity interval training (HIIT)

Notes: (A) Body weight changes during 12 weeks of HIIT, analysed by repeated measures analysis of variance (ANOVA); \triangle , time \times OP/OR $P < 0.05$. (B) Body weight at week 12; ++, main effect of HIIT/sedentary $P < 0.01$, analysed by two-way ANOVA. (C) The total food intake during training; &&, main effect of OP/OR $P < 0.01$, analysed by two-way ANOVA.

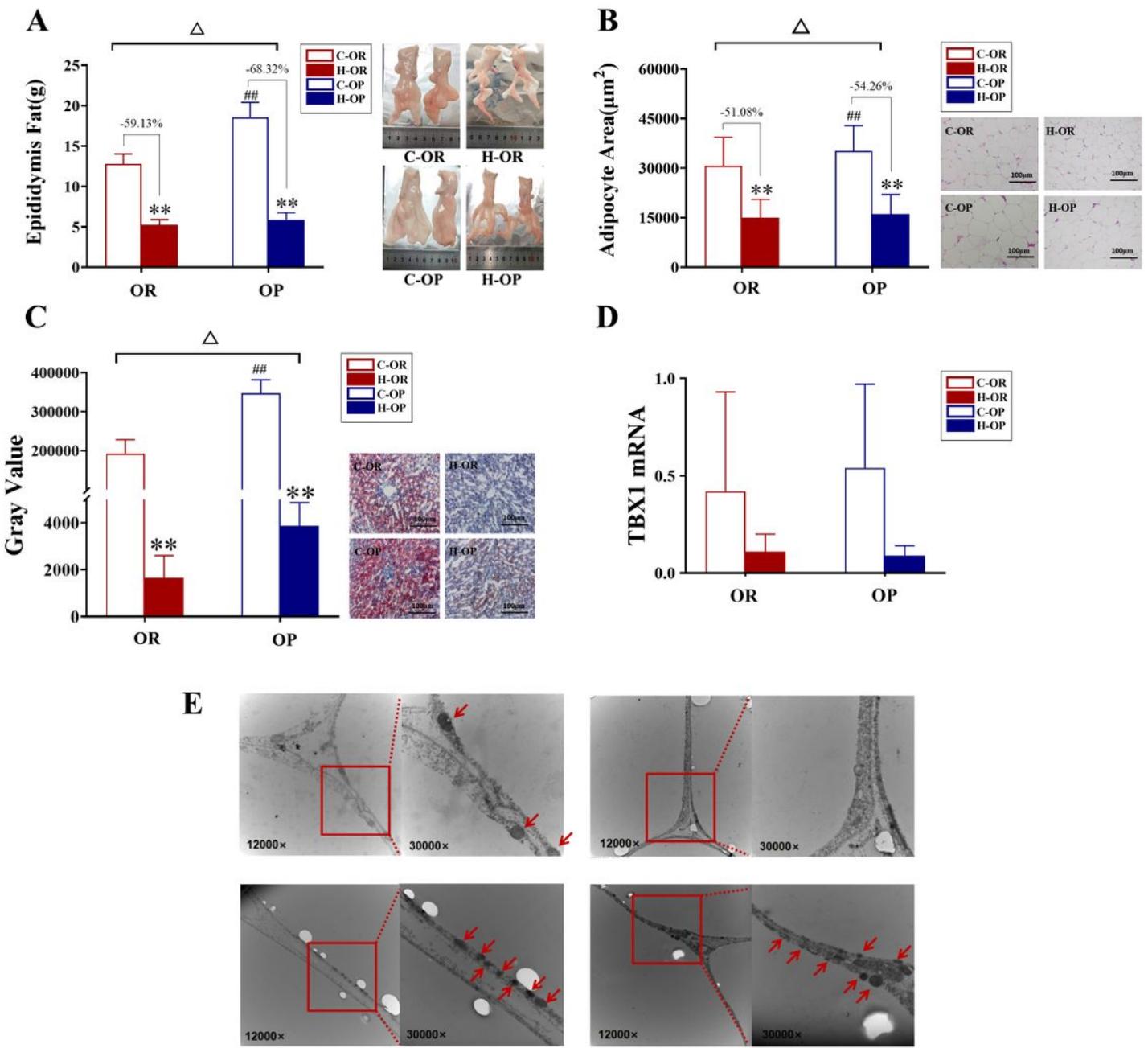


Figure 4

Fat Mass, VAT, Liver and Mitochondrial Histology and TBX1 expression

Notes: (A) Epididymal fat mass. (B) Visceral adipocyte area. (C) Lipid deposition of live (gray value of Oil red O). (D) TBX1 mRNA expression. (E) Transmission electron micrographs showing mitochondria in adipocytes (denoted by red arrows). For (A-D) Δ , HIIT/Sedentary \times OP/OR $P < 0.05$. ** H-group vs C-group $P < 0.01$; ## C-OP vs C-OR $P < 0.01$, analysed by simple effect.

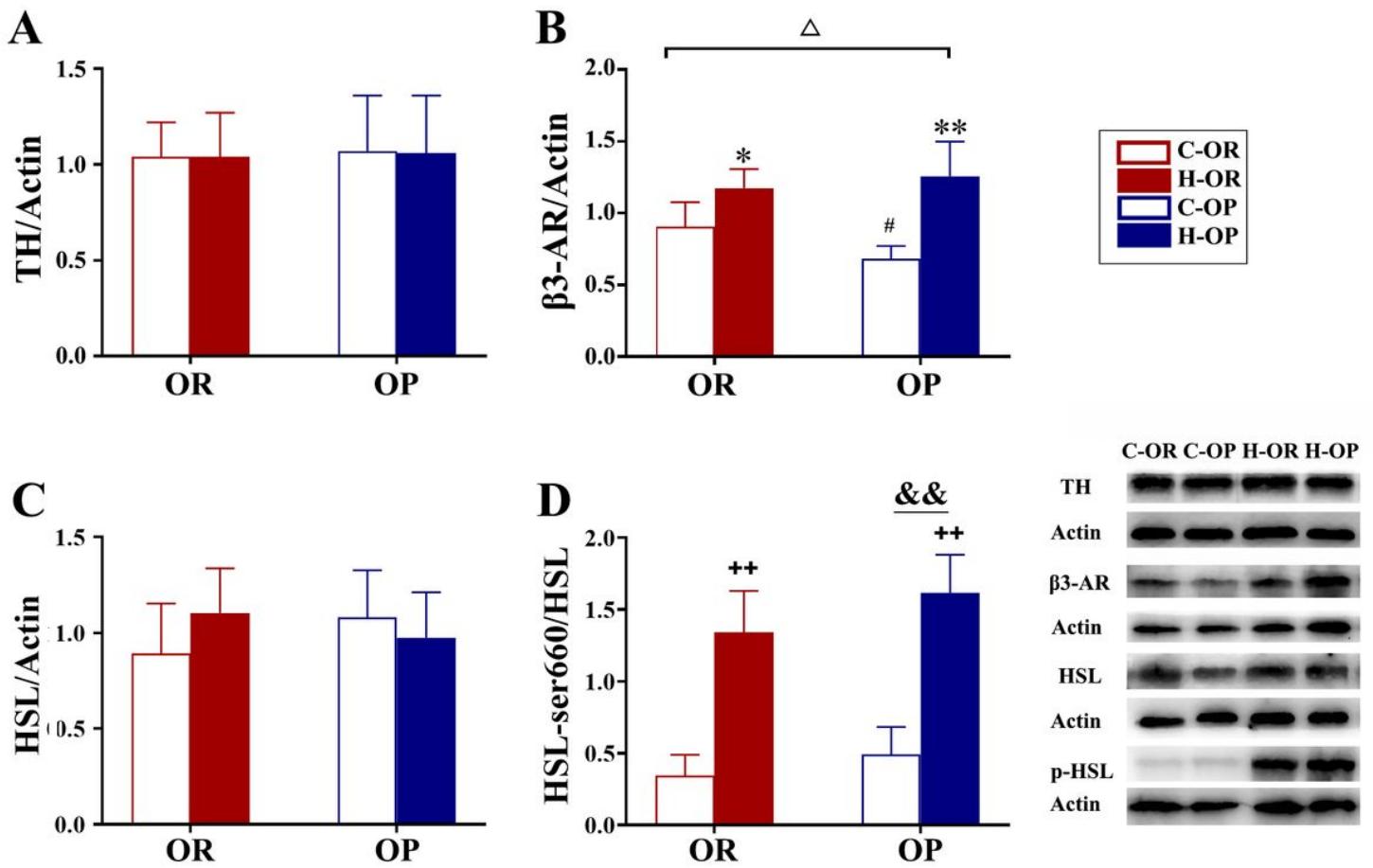


Figure 5

Expression of hormone-sensitive lipase (HSL) and phosphorylated HSL-ser660

Notes: (A) TH protein expression. (B) $\beta 3\text{-AR}$ protein expression. (C) HSL protein expression. (D) Phosphorylated HSL-ser660 protein expression. \triangle , HIIT/Sedentary \times OP/OR P < 0.05, analysed by two-way analysis of variance. #, C-OP vs C-OR P < 0.05; *, H-OR vs C-OR P < 0.05; and **, H-OP vs H-OR P < 0.01, analysed by simple effect; ++, main effect of HIIT/sedentary P < 0.01; &&, main effect of OP/OR P < 0.01, analysed by two-way analysis of variance.

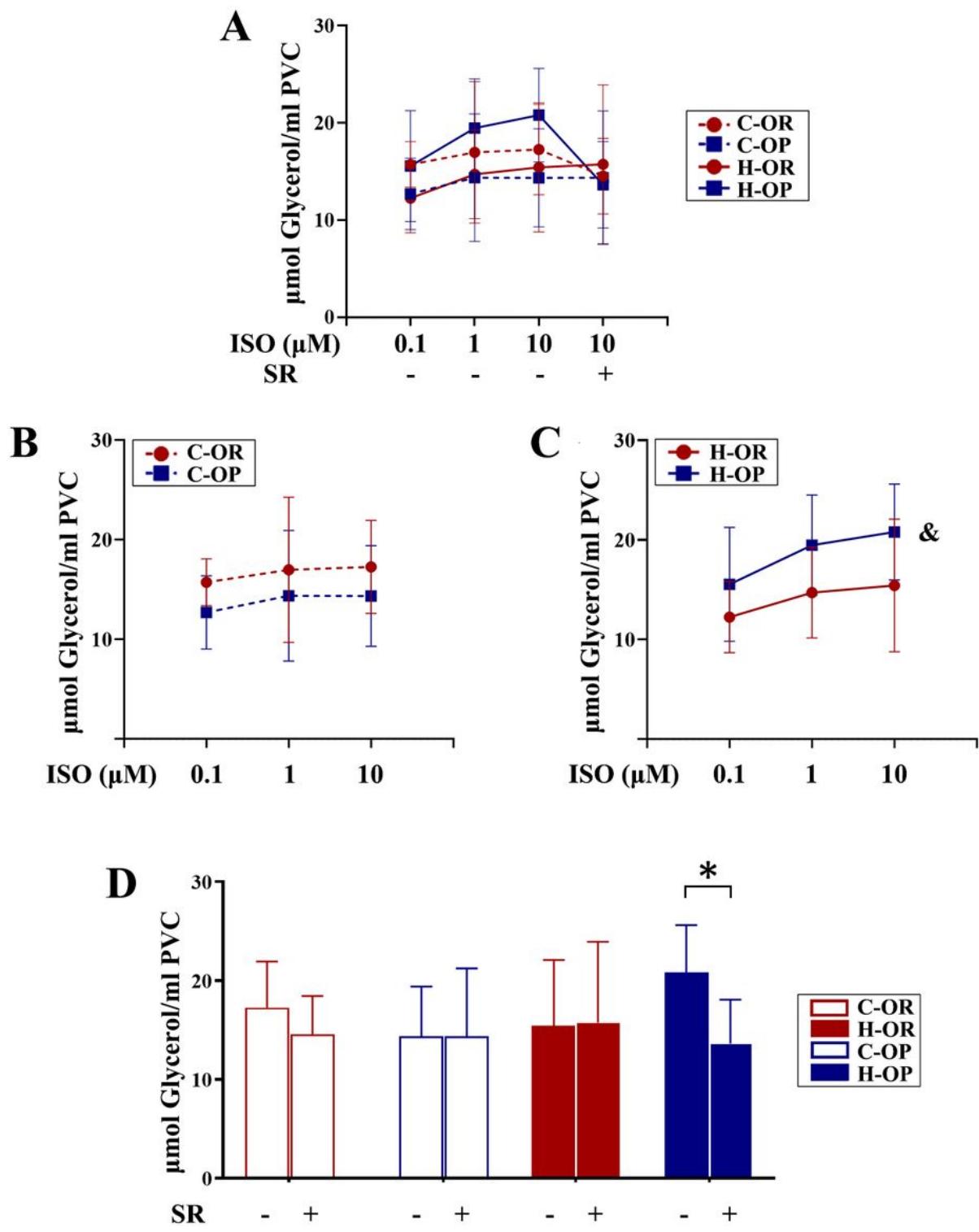


Figure 6

Glycerol release from adipocytes stimulated by isoproterenol (ISO) *in vitro*

Notes: (A) Overview of the glycerol release of all adipocyte groups. (B) Comparison between the control (C) groups. (C) Comparison between the exercise (H) groups; &, main effect of OP/OR $P < 0.05$, analysed

by two-way analysis of variance. (D) Changes in glycerol release with or without β 3-adrenergic receptor blockade with SR59230a; *, P < 0.05, analysed by t-test; ISO: isoproterenol, SR: SR59230a.