RESEARCH

# Effects of exercise and dietary intervention on muscle, adipose tissue, and blood IRISIN levels in obese male mice and their relationship with the beigeization of white adipose tissue

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## Abstract

*Background:* Obesity is a growing problem worldwide, and newer therapeutic strategies to combat it are urgently required. This study aimed to analyze the effect of diet and exercise interventions on energy balance in mice and elucidate the mechanism of the peroxisome proliferator-activated receptor-gamma co-activator-1-alpha-IRISIN-uncoupling protein-1 (PGC-1α-IRISIN-UCP-1) pathway in the beigeization of white adipose tissue.

*Methods:* Four-week-old male C57BL/6 mice were randomly divided into normal (NC) and high-fat diet (HFD) groups. After 10 weeks of HFD feeding, obese mice were randomly divided into obesity control (OC), obesity diet control (OD), obesity exercise (OE), and obesity diet control exercise (ODE) groups. Mice in OE and ODE performed moderate-load treadmill exercises: for OD and ODE, the diet constituted 70% of the food intake of the OC group for 8 weeks.

*Results:* Long-term HFD inhibits white adipose tissue beigeization by downregulating PGC-1α-IRISIN-UCP-1 in the adipose tissue and skeletal muscles. Eight weeks of exercise and dietary interventions alleviated obesity-induced skeletal muscle, and adipose tissue PGC-1α-IRISIN-UCP-1 pathway downregulation promoted white adipose tissue beigeization and reduced body adipose tissue. The effects of the combined intervention were better than those of single interventions.

*Conclusions:* Diet and exercise intervention after obesity and obesity itself may affect the beigeization of WAT by downregulating/upregulating the expression/secretion of skeletal muscle and adipose PGC-1α-IRISIN, thereby influencing the regulation of bodyweight. The effects of the combined intervention were better than those of single interventions.

**Key Words**

- obesity
- white adipose beigeization
- food restriction
- PGC-1α-IRISIN-UCP-1

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## Introduction

Development in science and technology over the past 40 years has changed the human lifestyle from active to sedentary. In many cases, food shortage has been replaced by food abundance. Obesity, which seriously threatens human health, is increasing worldwide. Hence, exploring the mechanisms through which obesity occurs and finding effective prevention and treatment strategies are urgently required in the field of public health.

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Mammalian adipose tissue comprises white (WAT) and brown adipose tissue (BAT). White adipose cells are round or oval and have a large lipid droplet in the center of each cell, accounting for approximately 90% of the cell volume. There are fewer mitochondria in adipose cells than in other cell types (1). BAT, which contains small intracellular lipid droplets and numerous mitochondria, is the primary organ for heat production. When the sympathetic nerves of the body are excited during exercise or in a low-temperature environment, WAT transforms into a new type of adipocytes, beige adipocytes (2, 3), which have a similar thermogenic function to that of BAT.

Increased thermogenesis in skeletal muscle caused by exercise is related to peroxisome proliferator-activated receptor-gamma co-activator 1-alpha (PGC-1α), which can stimulate mitochondrial oxidative metabolism and biosynthesis (4). Bostrom *et al.* found uncoupling protein-1 (UCP-1) expression in the s.c. adipose cells of transgenic mice with high PGC-1α expression increased following exercise (5). Furthermore, *in vitro* experiments have demonstrated that PGC-1α can significantly increase the expression of fibronectin type-III domain containing 5 (*Fndc5*), *Ucp1*, and other thermogenic genes and also induce WAT beigeization (5). FNDC5 can be cleaved and modified to form the secretory muscle factor IRISIN, which acts on s.c. WAT through blood circulation to disperse large lipid droplets in adipose cells into small adipose droplets. UCP-1 is considered a hallmark protein of WAT beigeization, given its significantly elevated expression in mitochondria, whereas IRISIN is considered an important myokine regulating adipose tissue beigeization. However, reports on the relationship between obesity and IRISIN are inconsistent. Some studies have found that BMI correlates negatively with IRISIN levels (6, 7), whereas others have revealed increased blood IRISIN expression in people with obesity (8, 9). In addition, it has been speculated that IRISIN may function similarly to leptin (10) and that leptin mediates the cross-talk between adipose tissue and skeletal muscle through the regulation of FNDC5/IRISIN in both tissues in order to increase thermogenesis and stimulate weight loss (11). Nevertheless, the mechanism of action of IRISIN in obesity warrants further investigation.

Exercise and dietary interventions are effective means of reducing adipose content. Yun Lu *et al.* showed that swimming could effectively increase blood IRISIN levels in obese rats (12). IRISIN in the blood reaches the adipose cells through the blood circulation, upregulating UCP-1 in adipose cells and promoting WAT beigeization (13). Another study showed that exercise could upregulate *Fndc5* in skeletal muscle through *Pgc1α*, increase IRISIN production and secretion, upregulate *Ucp1* in WAT (5), increase energy consumption, and promote WAT conversion to beige adipose tissue, effectively achieving the goal of adipose loss (14). A study generated a mouse model with systemic loss of *Fndc5* function using DNA targeting technology (15). The mice ran at a speed of 18 m/min for 60 min/day for 5 days per week, but there was no obvious browning of WAT for 8 weeks (15), suggesting that the *Fndc5* gene may play an important role in the beigeization of WAT caused by exercise. Although few studies have explored the effects of dietary intervention on IRISIN levels in animals, a human study demonstrated that obese children had significantly lower IRISIN levels after 1 year of a lifestyle intervention program of diet and physical exercise (16); however, the mechanism of action was unclear. Previous studies have suggested that skeletal muscles are the primary source of blood IRISIN. However, other studies have found that IRISIN is not only a muscle factor but also an adipose factor (17). As an adipose factor, the expression of FNDC5 is downregulated in the visceral and s.c. adipose tissue of patients with obesity (18, 19), making the mechanism of IRISIN regulation of adipose tissue beigeization more complicated. In this study, the energy balance of mice was regulated through exercise and diet, and the changes in muscle and adipose tissue and blood IRISIN levels were analyzed under different energy balance states, as were their relationships with WAT beigeization.

## Materials and methods

### Laboratory animals

Fifty 4-week-old male C57BL/6J mice were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) (license number: SCXK (JING) 2018-0008). The mice were managed and used in accordance with the animal management regulations proposed by the Ministry of Health of the People's Republic of China in 1998. All experimental studies were approved by the Ethics Committee of the Shenyang Institute of Physical Education (ethical approval number: (2020) 20).

### Establishment and grouping of mouse obesity model

The 50 mice were randomly divided into the following groups: normal diet control group (NC, $n = 10$) fed a regular diet and a high-fat diet group (HFD, $n = 40$) fed a

high-fat diet. The ratio of total energy and nutrients in the two diets was according to the literature (Table 1) (20). The chow diet was purchased from Jianmin Company Ltd. (Shenyang, China). After 10 weeks of feeding, mice in the HFD group with bodyweight exceeding 20% of the average bodyweight of the NC group were considered obese model mice (21); three obesity-resistant mice were excluded from the HFD group. After successful modeling, the mice were randomly divided into the following four groups: obesity control (OC, $n=9$), obesity dietary intervention (OD, $n=9$), obesity exercise (OE, $n=9$), and obesity diet exercise groups (ODE, $n=10$) (Fig. 1).

## Exercise and diet intervention plan

After the obesity model was successfully established, exercise interventions for OE and ODE were carried out for 8 weeks, using treadmill running (22); the first 3 weeks constituted an incremental load adaptive training of 6 days/week, with a slope of 0%, starting speed of 10 m/min, and duration of 20 min/day. Thereafter, the training period was increased by 4 min and the running speed by 1 m/min, on average every day, based on previous reports and the state of the mice (23). The load reached 25 m/min and 90 min/day from the fourth week and was continuously applied until the eighth week.

Mice in the OC, OD, OE, and ODE were all fed an HFD throughout the whole period (18 weeks); the OC group and the OE group did not control the amount of diet and ate freely. The diets of OD and ODE mice were controlled to 70% of the food intake of OC mice (24). This type of diet control program without affecting the normal nutritional status of the mice prevents mice from fighting manically. The NC group served as a control group and ate a regular diet freely throughout the whole period (18 weeks). All groups had free access to water. Two mice died unexpectedly during the exercise intervention in ODE.

**Table 1** Composition of animal diets (20).

| Ingredients | NC | HFD |
|---|---|---|
| Sucrose, g/100 g | 34.1 | 34.1 |
| Casein acid, g/100 g | 19.5 | 19.5 |
| Canola oil, g/100 g | 6.0 | |
| Clarified butter, g/100 g | | 21.0 |
| Cellulose, g/100 g | 5.0 | 5.0 |
| Wheat starch, g/100 g | 30.5 | 15.5 |
| Minerals, g/100 g | 4.9 | 4.9 |
| Digestible energy, MJ/kg | 16.1 | 19.4 |
| Digestible energy from lipids, % | 21.0 | 40.0 |
| Digestible energy form protein, % | 14.0 | 17.0 |

HFD, high-fat diet; NC, normal control.

## Collection of specimens

Samples were obtained after 8 weeks of exercise and dietary intervention. The exercise groups had completed the last exercise 36–40 h before the samples were taken to eliminate the stress response of one-time exercise. Before sampling, each group (NC, OC, OD, OE, and ODE) was subjected to 12 h of fasting. Mice were anesthetized by i.p. injection of sodium pentobarbital (50 mg/kg bodyweight; Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). Blood was drawn from the orbital venous plexus, and the serum was centrifuged at 900 ***g*** for 20 min at 4°C, after which it was immediately stored at –80°C. After blood collection, the skeletal muscle (quadriceps femoris) and white adipose tissues (s.c. and visceral adipose tissues) were immediately separated, weighed, and stored at –80°C. Subsequently, analysis of the relevant blood parameters and Western blotting and real-time PCR was performed. Serum, skeletal muscle, and adipose tissues were harvested from eight mice in each group.

## Determination of specific serum IRISIN levels by enzyme-linked immunosorbent assay

Specific serum IRISIN levels were determined using an ELISA kit (ml058018; Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China) according to the manufacturer's instructions. Thereafter, absorbance at 450 nm was measured using a Multiskan GO 1510 microplate reader (Thermo Fisher Scientific).

## RNA extraction and RT-PCR analysis of adipose tissue (visceral adipose tissue) and skeletal muscle tissue

An RNA extraction reagent, TRIzol (Vazyme Biotech Co., Ltd, Wuhan, China), was used to extract total RNA from the mouse visceral adipose tissue (the amount of s.c. adipose tissue is very small, and the protein content of adipose tissue is low, which cannot meet the needs of the experiment; therefore, visceral adipose tissue was chosen) and skeletal muscle tissues, according to the manufacturer's instructions. The total RNA was then reverse-transcribed into cDNA using a GoScript™ RT System (Promega) in a 96-well thermal cycler (Applied Biosystems ). Thereafter, an RT-PCR kit (Promega) was used to determine the target mRNA content using an RT-PCR system (Applied Biosystems), according to the manufacturer's instructions. The primers used in this study were as follows: *Pgc1a*, 5′-TTCCTCGTGTCCTCGGCTGAG-3′

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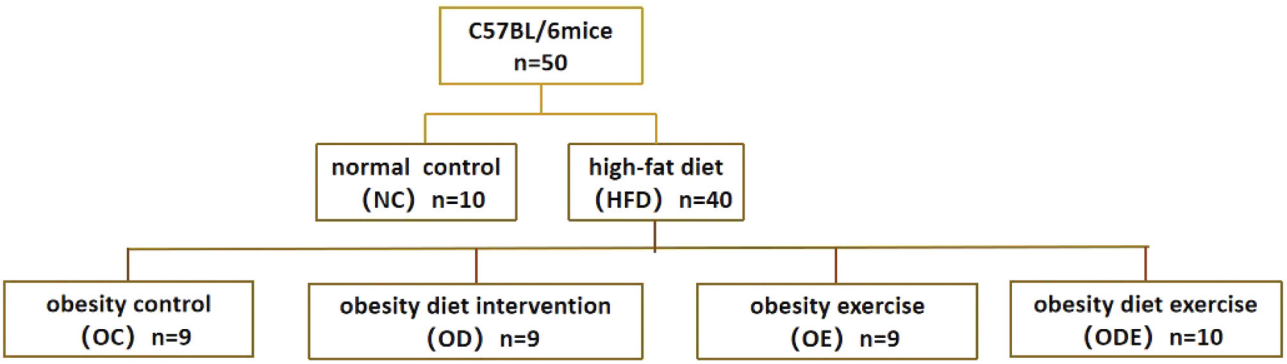


**Figure 1**
Experimental animal groups. NC, normal control; OC, obesity control; OD, obesity diet intervention; OE, obesity exercise; ODE, obesity diet exercise.

and 5′-GTGCCACCGCCAACCAAGAG-3′; *Irisin*, 5′-GAGCATCCGCACATCCTTCTTCTG-3′ and 5′-TGACCGTCCGGCACCTCAAG-3′; *Ucp1*, 5′-GCATTCTGACCTTCACGACCTCTG-3′ and 5′-ACTCAGGATTGGCCTCTACGACTC-3′; *Gapdh*, 5′-TCAAGAAGGTGGTGAAGAC-3′ and 5′-TGGGAGTTGCTGTTGAAGTC-3′. All primers were designed and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). For the RT-PCR analysis, reactions were performed in triplicate for each sample, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal reference. The results were calculated using the $2^{-\Delta\Delta Ct}$ method.

### Protein extraction from adipose tissue (visceral adipose tissue) and skeletal muscle and Western blotting

After weighing the mouse visceral adipose tissue and skeletal muscle, radioimmunoprecipitation assay buffer and the protease inhibitor phenylmethylsulfonyl fluoride were added to the tissues. Next, the tissues were homogenized on ice, centrifuged, and the supernatant was extracted. A bicinchoninic acid protein quantification kit (Beijing Dingguochangsheng Biotechnology Co., Ltd., Beijing, China) was used to quantify protein. Thereafter, protein lysates were loaded, separated by denaturing SDS-PAGE, transferred to a nitrocellulose membrane, and blocked for 1 h in a 5% skimmed milk solution. Subsequently, each nitrocellulose membrane was incubated with the corresponding primary antibodies: IRISIN (No. A1459-30T; BioVision), PGC-1α (A12348; ABclonal, Wuhan, China), FNDC5 (23995-1-AP; Proteintech, Chicago, IL, USA), UCP-1 (A5857; ABclonal), GAPDH (AC027; ABclonal), β-Actin (AC026; ABclonal) and incubated overnight (12 h) in a refrigerator at 4°C. Next, the nitrocellulose membranes were incubated with a fluorescent secondary antibody for 1 h at 25 ℃, after which they were placed into an Odyssey Infrared Imaging System (LI-COR, Lincoln, NE, USA). Finally, the Image Studio software was used to analyze the protein bands quantitatively. Thus, the ratio of a target protein content/internal control content was obtained. All data were normalized.

### Statistical analysis

All results are expressed herein as the means ± S.D. Statistical analyses were performed using SPSS v.18.0 (SPSS Inc). The Student's *t*-tests were used to compare the NC and OC groups, while comparisons between the OC, OD, OE, and ODE groups were performed using one-way ANOVA. Differences were considered statistically significant at $P < 0.05$ and $P < 0.01$.

## Results

### Bodyweight, food intake, white adipose mass, and white adipose mass/bodyweight ratio of mice in each group

As shown in Fig. 2A, C, E and F, the bodyweight, white adipose mass, and white adipose mass to bodyweight ratios of mice in OC were significantly higher ($P < 0.01$) than those of mice in NC. Furthermore, these three indicators were significantly lower in OD, OE, and ODE mice than in OC mice ($P < 0.01$ or $P < 0.05$); the reduction in ODE (combined intervention group) was more significant than that in OD and OE ($P < 0.01$).

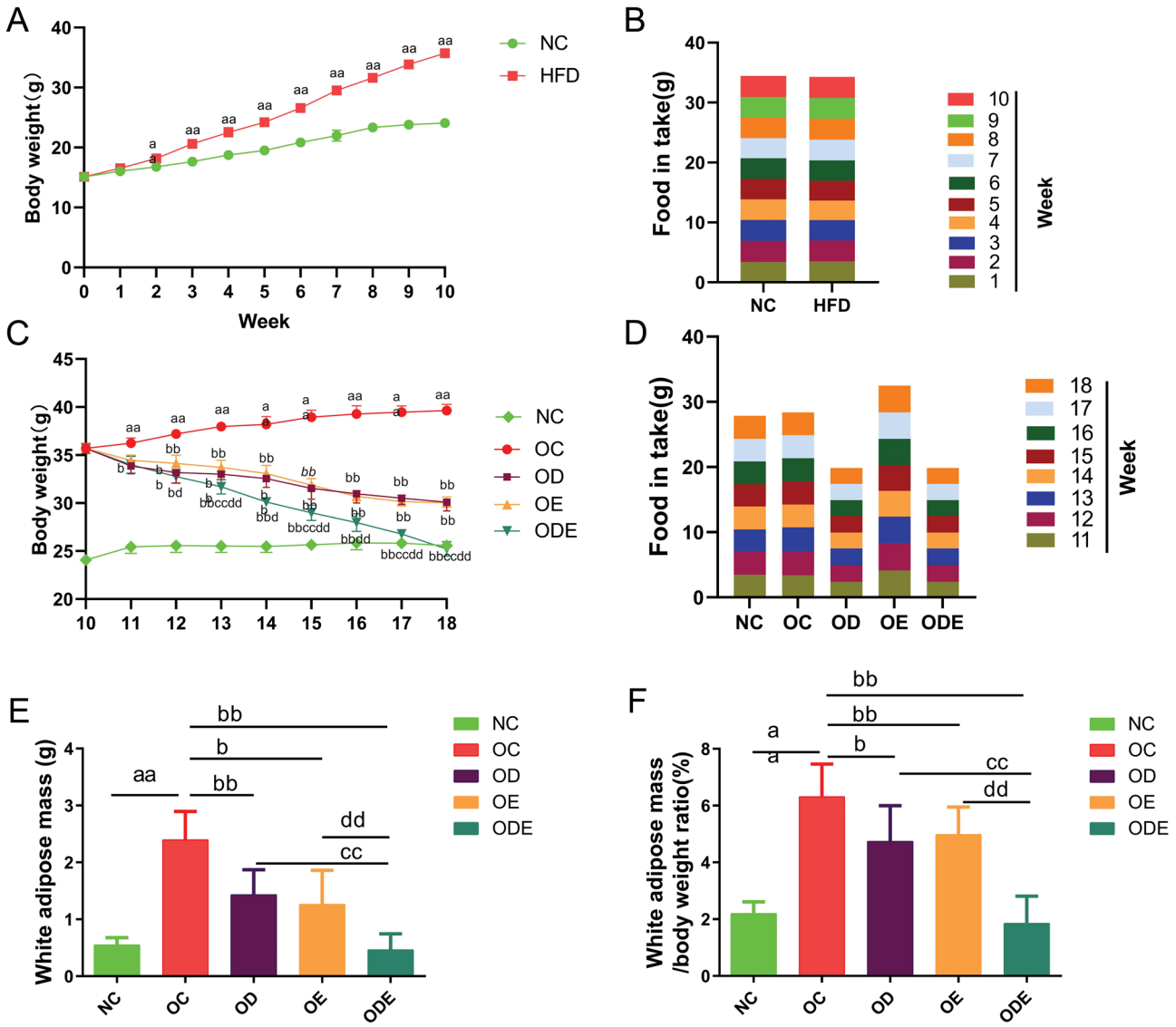


**Figure 2**

Changes in bodyweight, food intake, white adipose mass, and the adipose-to-body ratio of mice in each group; data are presented as the means ± S.D. NC, normal control; OC, obesity control; OD, obesity diet intervention; OE, obesity exercise; ODE, obesity diet exercise. vs NC: $^{aa}P < 0.01$; vs OC: $^{b}P < 0.05$, $^{bb}P < 0.01$; vs OD: $^{cc}P < 0.01$; vs OE: $^{d}P < 0.05$, $^{dd}P < 0.01$

## Changes in the relative expression of PGC-1α, IRISIN mRNA/protein level in the skeletal muscle, and specific blood IRISIN content of mice in each group

As shown in Fig. 3A, B, D, E and F, the relative expression of PGC-1α and FNDC5/IRISIN mRNA/protein in the skeletal muscle tissues was significantly lower in OC than those in NC ($P < 0.01$). After 8 weeks of exercise and/or diet intervention, the relative expression of PGC-1α and FNDC5/IRISIN mRNA/protein in OD, OE, and ODE increased significantly ($P < 0.01$ or $P < 0.05$) compared with those in OC. In addition, the relative levels of PGC-1α or FNDC5/IRISIN mRNA and protein were significantly higher in ODE than in OE or OD ($P < 0.01$ or $P < 0.05$).

As shown in Fig. 3C, after 8 weeks of exercise and/or diet intervention, the specific blood IRISIN levels of OD, OE, and ODE were significantly higher than those of OC ($P < 0.01$ or $P < 0.05$).

## Changes in the relative expression of PGC-1α, IRISIN, and UCP-1 mRNA/protein in adipose tissue of each group

As shown in Fig. 4A, B, C, D, E, F and G, the relative mRNA and protein expression of PGC-1α, FNDC5/IRISIN, and

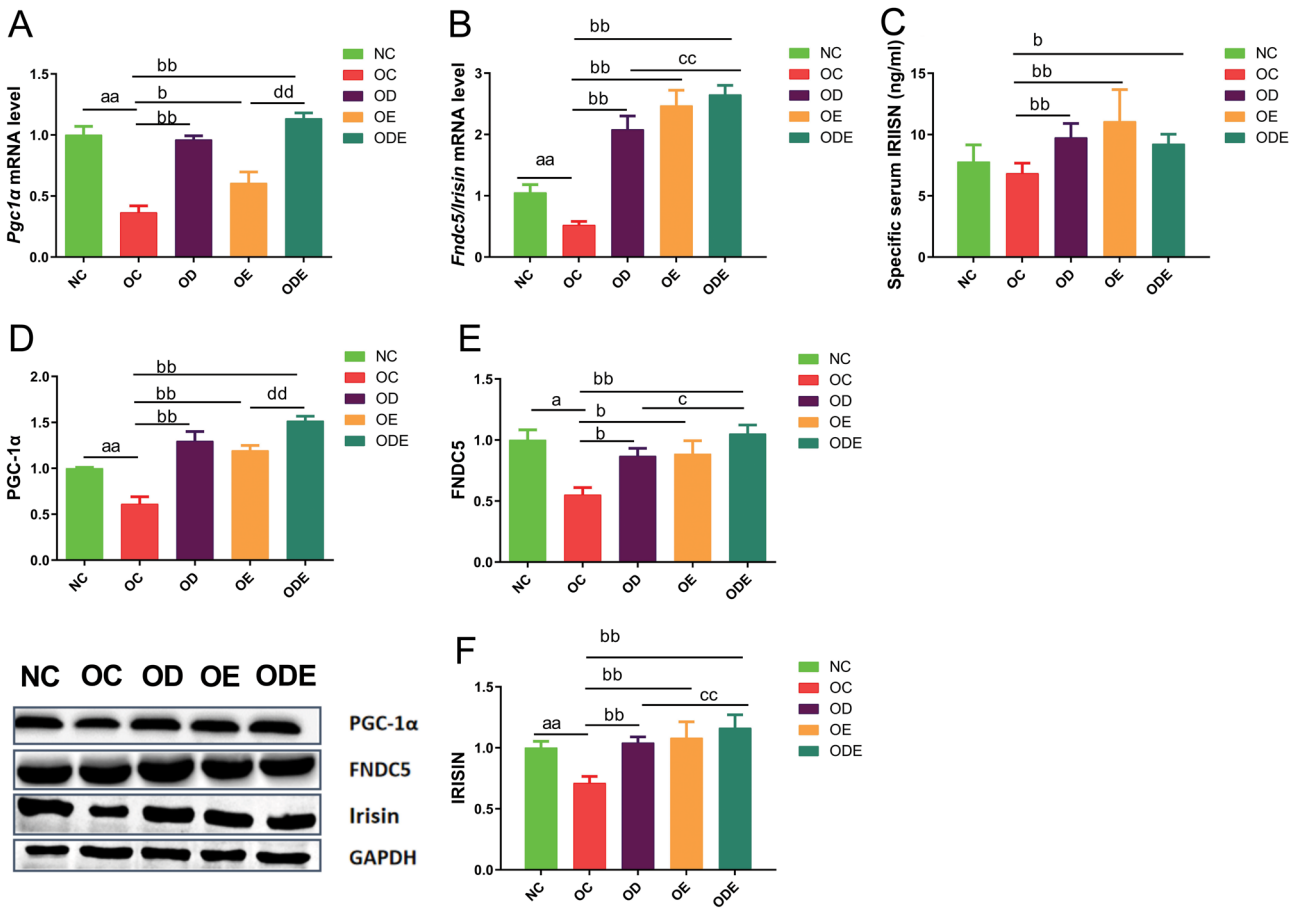


**Figure 3**
Changes in relative mRNA and protein expression of skeletal muscle IRISIN and level of blood IRISIN in each group. Data are presented as the means ± S.D. NC, normal control; OC, obesity control; OD, obesity diet intervention; OE, obesity exercise; ODE, obesity diet exercise. vs NC: $^{a}P < 0.05$, $^{aa}P < 0.01$; vs OC: $^{b}P < 0.05$, $^{bb}P < 0.01$; vs OD: $^{c}P < 0.05$, $^{cc}P < 0.01$. vs OE: $^{dd}P < 0.01$

UCP-1 in OC decreased significantly compared with that in NC ($P < 0.01$ or $P < 0.05$). After 8 weeks of exercise and/or diet intervention, the relative mRNA and protein levels of PGC-1α, FNDC5/IRISIN, and UCP-1 were significantly higher in OD, OE, and ODE mice than in OC mice ($P < 0.01$). In addition, the relative expression levels of *Pgc1a*, *Fndc5/irisin*, and *Ucp1* mRNA and PGC-1α protein were higher in ODE than in OD ($P < 0.01$ or $P < 0.05$) and OE mice ($P < 0.01$ or $P < 0.05$), while those of FNDC5/IRISIN and UCP-1 protein were higher in ODE mice than in OE mice ($P < 0.01$).

## Discussion

IRISIN has been proven to be both a muscle and an adipose factor (17). However, it is unclear whether its mechanism in promoting the beigeization of WAT is endocrine, autocrine, or both. The current study showed that the white adipose mass in obese male mice increased, whereas UCP-1 mRNA and protein expressions in the adipose tissue decreased significantly. Furthermore, PGC-1α and IRISIN mRNA protein levels in the adipose tissue and skeletal muscle decreased significantly, as did specific blood IRISIN levels. After 8 weeks of dietary and exercise interventions, the white adipose mass of obese mice decreased significantly, whereas the mRNA and protein expressions of UCP-1 in adipose tissue increased significantly. Furthermore, the mRNA and protein levels of PGC-1α and IRISIN in the adipose tissue and skeletal muscle increased significantly, as did specific blood IRISIN levels. Thus, the joint intervention effects of exercise and diet were better than those of either intervention alone.

Some studies have shown that individuals who are overweight and obese have relatively low amounts of BAT (25), and brown adipose is inversely proportional to both BMI and adipose content (26). UCP-1 in the mitochondria is a marker for brown adipose and has strong oxidizing

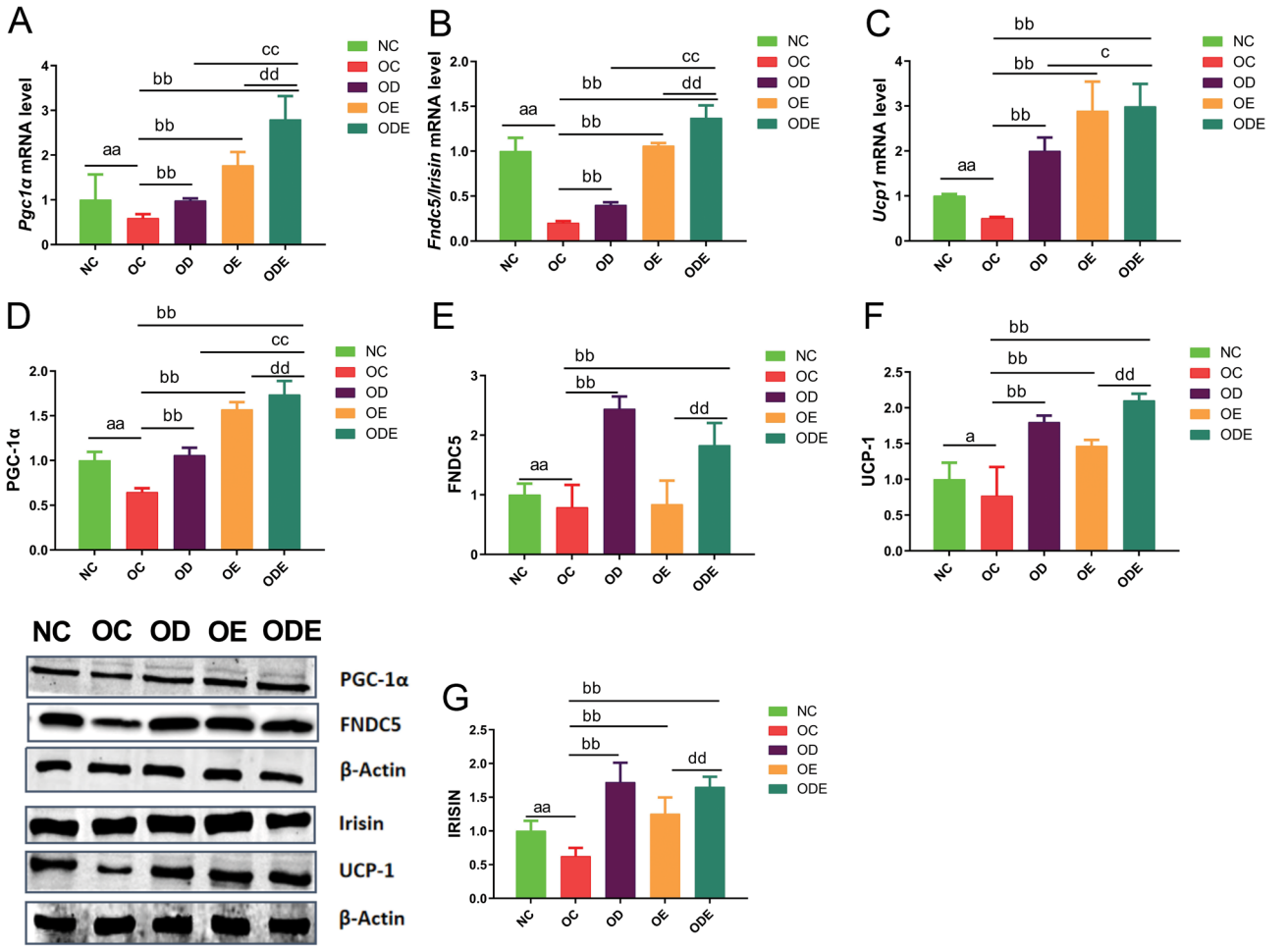


**Figure 4**

Changes in relative mRNA and protein expressions of PGC-1α, IRISIN, and UCP-1 in the adipose tissue of each group. Data presented as the means ± S.D. NC, normal control; OC, obesity control; OD, obesity diet intervention; OE, obesity exercise; ODE, obesity diet exercise. vs NC: $^{a}P < 0.05$, $^{aa}P < 0.01$; vs OC: $^{bb}P < 0.01$; vs OD: $^{c}P < 0.05$, $^{cc}P < 0.01$. vs OE: $^{dd}P < 0.01$

properties; it can uncouple the mitochondrial respiratory chain and oxidize metabolic substrates, causing electrons to form a potential difference during the transfer process. This action releases chemical energy in the form of heat (27, 28, 29), promotes lipolysis, and makes adipose cells present a brown adipose cell phenotype (30). The present study findings showed that a long-term HFD significantly decreased the expression of UCP-1 mRNA and protein in the adipose tissue of male mice, accompanied by weight gain and increased white adipose mass and adipose-body ratios. These results suggest that in obese mice, the level of beigeization is weakened, promoting the storage of adipose as white adipocytes (31). Studies have also shown that IRISIN can be secreted into the blood through the muscles and can act on adipose cells to regulate the beigeization of WAT. In a time-gradient experiment where s.c. adipocytes were incubated with 20 nM of FNDC5 protein, *Ucp1* mRNA expression increased 7–500 times. The number of *Ucp1* positive adipocytes also increased significantly, accompanied by many adipose droplets that presented brown adipocyte phenotypes (5). *In vitro* experiments revealed that using a medium containing muscle cells expressing PGC-1α, culturing primary adipocytes could upregulate *Fndc5* in adipocytes, increase *Ucp1* levels, and induce WAT beigeization (5). An HFD can cause ectopic lipid deposition (32) and decrease PGC-1α expression in skeletal muscles (33). Furthermore, IRISIN was found to be downregulated. Another study showed that the expression of PGC-1α protein was decreased in skeletal muscle tissue in patients with type 2 diabetes (34). FNDC5 expression and IRISIN levels in the blood samples of individuals and animals with obesity were also significantly reduced (35, 36). The results obtained in this study showed that the mRNA and protein expression of PGC-1α and

FNDC5/IRISIN decreased in the adipose tissue and skeletal muscle of obese mice. Adipose tissue mass and bodyweight increased significantly, and the mice became obese. Although adipose tissue and skeletal muscle *Irisin* mRNA levels were decreased, there was no statistical difference in specific serum IRISIN levels between NC and OC. In line with the results of the present study, other authors support the notion that circulating irisin remains unchanged albeit its expression is downregulated in adipose tissue and skeletal muscle of animal models of genetic and diet-induced obesity (11, 37). IRISIN is a factor secreted by multiple tissues, indicating tissue differences in IRISIN secretion levels in obesity. The concentration of IRISIN in the blood results from the joint action of multiple tissues. In addition, it may be that the number of samples is relatively small, resulting in only a downward trend in the results, with no statistical difference.

To further verify the regulatory effects of IRISIN on skeletal muscle and adipose tissue, as well as blood on adipose tissue beigeization, the energy balances of the mice bodies were altered through exercise and by controlling their caloric intake. Observing the relationship between adipose tissue beigeization and changes in adipose tissue, skeletal muscle, and specific blood IRISIN showed that after 8 weeks of exercise, dieting, or dieting and exercise combined resulted in significantly lower bodyweights, white adipose mass, and liposome ratios in obese mice. In addition, the mRNA and protein expression of UCP-1 in mouse adipose tissue increased. These findings suggest that both exercise and dietary interventions could effectively promote WAT beigeization to achieve weight loss goals. Furthermore, existing studies have shown that endurance exercises can increase the level of PGC-1α in the skeletal muscle through protein calmodulin-dependent protein kinase IV (CaMK-IV) (38, 39), which upregulates FNDC5 and promotes mitochondrial energy consumption and heat production (5, 40). Treadmill training has also been proven to increase the expression of PGC-1α (33) and FNDC5 (41) in the skeletal muscle tissue of obese mice and SD rats on an HFD. Moreover, the present study results confirmed that the mRNA and protein expression of PGC-1α and IRISIN in the skeletal muscle and adipose tissue showed a synchronous rebounding effect after 8 weeks of treadmill exercise intervention, as did IRISIN. At the same time, the body adipose tissue mass decreased, further verifying that the changes in specific blood IRISIN levels caused by long-term endurance exercise may result from the combined effect of the increased expression of PGC-1α and IRISIN in the skeletal muscle and adipose tissue.

Dietary intervention is another effective way to reduce body adipose tissue by reducing energy intake. Lopez-Legarre *et al.* demonstrated that following 8 weeks of low-energy dietary intervention, the weights of patients who were overweight and those with metabolic syndromes decreased significantly, and the levels of IRISIN circulating in their blood were significantly reduced (42). However, animal experiments have shown that after 3 months of energy restriction, *Fndc5* and *Ucp1* levels in the WAT of rats increased significantly (43), suggesting that dietary control could promote WAT beigeization. However, the relationship between PGC-1α and IRISIN in skeletal muscle and adipose tissue remains unclear. The present study showed similar exercise intervention results to those obtained after 8 weeks of 70% dietary restriction. In addition, the mRNA and protein expression of PGC-1α and FNDC5/IRISIN in the skeletal muscle and adipose tissue and blood IRISIN levels also significantly increased, accompanied by a significant decrease in body adipose content. These findings suggest that dietary intervention can also increase specific blood IRISIN levels by upregulating PGC-1α and IRISIN in the skeletal muscle and adipose tissue, promoting the beigeization of adipose and reducing body adipose content.

A combined exercise and dietary intervention was also applied to the obese mice in this study to clarify the effects of exercise and dietary intervention. The results showed that the expression of PGC-1α, FNDC5/IRISIN, and UCP-1 mRNA/protein in the adipose tissue of obese mice in the combined intervention group increased significantly, and the white adipose mass decreased with weight loss. In addition, the expression of PGC-1α, IRISIN mRNA/protein in skeletal muscle, and specific blood IRISIN was significantly increased. The effect of the 8-week combined intervention was superior to that of individual interventions. The above results suggest that IRISIN, as a muscle and adipose factor, may play a role in energy balance regulation. When the energy balance of the body is disrupted, PGC-1α regulates the expression of IRISIN in adipose tissue and skeletal muscle through autocrine and endocrine means by acting on the adipose cells, promoting adipose beigeization, increasing mitochondrial heat production, promoting adipose consumption, and reducing adipose content.

In conclusion, a long-term HFD reduced the expression of PGC-1α in skeletal muscle and adipose tissue, inhibited the synthesis and secretion of IRISIN, weakened WAT browning, promoted adipose tissue storage, and caused obesity. Furthermore, dietary and exercise intervention may reverse the decrease of PGC-1-IRISIN expression

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in skeletal muscle and adipose tissue, promote WAT browning, and effectively reduce bodyweight. Thus, a combined exercise and dietary intervention is superior to a single intervention.

**Declaration of interest**
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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**Author contribution statement**
Xuejie Yi, Bo Chang, and Shicheng Cao conceived and designed the study; Jing Li and Tao Li performed the molecular biology experiments and prepared the manuscript; Guangxuan Hu and Yongqi Ma prepared the experimental animal model; Dongyang Li conducted the blood test; Tingting Yao performed data analysis and mapping. All authors read and approved the final manuscript.

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