ORIGINAL RESEARCH

Calorie Restriction Combined with High-Intensity Interval Training Promotes Browning of White Adipose Tissue by Activating the PPARγ/PGC-1α/UCP1 Pathway

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ABSTRACT

Objective • This study was designed to survey the effect and the mechanism of action of calorie restriction combined with high-intensity interval training (HIIT) on the browning of white adipose tissue.

Methods • For the human study population, obese adult males were randomly assigned to one of three major groups: the control group (CN group), the calorie restriction combined with HIIT group (CR+HIIT group) and the HIIT group. After 3 months of training, body composition was measured. For the rodent study population, Sprague Dawley rats were randomly split into a normal diet control group (CON group) and an obesity model group. After successful obesity modeling, the latter was divided into the obesity control group (HON group), the calorie restriction plus HIIT group (ONE group) and the HIIT group (OHE group), with 8 animals in each group. A treadmill was used for training 5 days a week for 10 weeks. The messenger RNA (mRNA) expression of uncoupling protein 1 (UCP1), Prdm16 gene, and Cidea gene in visceral adipose tissue were detected with real-time polymer chain reaction (RT-qPCR), while the protein levels of UCP1, PPARγ and PGC-1α in visceral adipose tissue (VAT) were detected by western blot analysis.

Results • Body weight and body fat rate in the human experiments demonstrated that fat mass, body weight and body fat rate in the CR+HIIT group were clearly lower than in the CN group. In the rat model, the body fat rate and body weight in the HON group were significantly higher than in the CON group, which indicated that the obesity model was successfully generated. As expected, the body fat rate and body weight in the ONE and OHE groups were considerably lower than in the HON group. Moreover, the body fat rate in the ONE group was considerably lower than in the OHE group.

Further investigation indicated that the area under this curve (AUC) of oral glucose tolerance test (AUC_{OGTT}), insulin (INS) levels and fasting blood glucose (FBG) levels in the HON group were more significantly increased than in the CON group, while AUC_{OGTT} and INS levels in the ONE and OHE groups were considerably lower than in the HON group. Hematoxylin and eosin (H&E) staining showed that, compared with the CON group, the adipocyte area in the HON group was expanded, but narrowed in the ONE and OHE groups. In addition, the adipocyte area in the ONE group was apparently smaller than in the OHE group.

We also compared molecular markers among the groups. RT-qPCR analysis showed that the expression of UCP1, Prdm16 and Cidea had been downregulated in the HON group compared with CON group but upregulated in the HON group compared with the ONE and OHE groups. Western blot analysis indicated that UCP1 in the HON group was lower than in the CON group but higher than in the ONE and OHE groups. In addition, the protein level of UCP1 was upregulated in the ONE group compared with the OHE group. Furthermore, expression levels of PPARγ coactivator-1α (PGC-1α) and peroxisome proliferator-activated receptor gamma (PPARγ) were downregulated in the HON group compared with the CON group, then further downregulated in the ONE and OHE groups compared with the HON group. In addition, the PGC-1α level in the ONE group was greatly improved compared with the OHE group.

Conclusion • Calorie restriction integrated with HIIT and HIIT alone upregulates PPARγ, PGC- 1α, as well as UCP1 in VAT of obese rats, promoting the browning of visceral fat and ultimately achieving fat loss. Calorie restriction integrated with HIIT is more effective than HIIT alone for fat loss (*Altern Ther Health Med.* 2023;29(3):134-139).

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INTRODUCTION

Obesity has become a major metabolic disease that threatens human health, leading to many health problems in the 21st century, and the importance of fat loss is prominent.[1,2] As the core organ of energy metabolism, adipose tissue contributes to the maintenance of normal body energy homeostasis, lipid metabolism and insulin sensitivity (INS).[3] Types of adipocytes include beige, brown and white.[4,5] The effect of white adipocytes in adipose tissue is mainly energy storage, and excessive accumulation of white adipocytes induces obesity. Brown and beige adipocytes are the main cells for non-shivering thermogenesis mediated by uncoupling protein 1 (UCP1), and they promote energy consumption and convert energy into heat under external stimuli.[6,7] Under the conditions of exercise and cold exposure, beige adipocyte-like changes occur in white adipocytes with enhanced expression of UCP1 as well as other proteins in thermogenesis and lipolysis, which is a phenomenon known as the browning of white adipose tissue (WAT).[8,9] WAT browning burns fat and combats obesity and metabolic dysfunction.[10]

Peroxisome proliferator-activated receptor gamma (PPARγ) is critical for regulating adipogenesis[11,12] and adipocyte differentiation.[13-15] PPARγ regulates the mitochondrial function in beige adipocytes by regulating PPARγ coactivator 1α (PGC-1α), thereby promoting the browning of WAT.[13,16,17] As an activator of PPARγ, PGC-1α is vital for regulating mitochondrial biogenesis.[18,19] It has been reported that exercise induces browning of WAT and storage of brown adipocytes by stimulating the PGC-1α pathway.[20-24]

Calorie restriction (CR) is a measure of dietary adjustment. It refers to reducing the daily caloric intake to an extent that the body does not suffer from malnutrition. In general, a 25% reduction is considered CR. High-intensity interval training (HIIT) is superior to aerobic exercise in reducing adipogenesis,[25] enhancing cardiopulmonary function and improving metabolic risk factors for type 2 diabetes.[26 27] However, the effect of CR integrated with HIIT on fat loss and the browning of WAT is unclear. Our study was designed to survey the effect and mechanism of action of CR integrated with HIIT on WAT browning.

MATERIALS AND METHODS

Human Population

A total of 18 obese adult males participated in the study; average age was 33.5 ± 6.2 years and average weight was 80.6 ± 4.5 kg. There was no significant difference in their baseline conditions. All participants submitted medical certificates indicating they were in good health and took part in normal training activities. The participants were randomly assigned to the control group (CN group), CR plus HIIT group (CR+HIIT group) or the HIIT group.

The participants in the CR+HIIT group consumed 70% of the daily caloric intake of the CN group. The following HIIT program was performed 3 times a week for 3 months: running at 90% intensity of anaerobic threshold on a treadmill with a 6% slope for 30 min and an 8% slope for 20 min (total 50 min). Body composition was measured on an empty stomach in the morning to reduce the interference of food using the Inbody720 body composition tester (Inbody, Seoul, South Korea).

Rodent Population

Five-week-old specific pathogen-free male Sprague Dawley rats were bought from the Experimental Animal Center of Sun Yat-sen University. Rats were randomly divided into 2 groups: the normal diet group (CON group; n=8) and the over-fat diet group (n=60). Rats in the CON group were nourished with normal chow, while the rats in the over-fat diet group were fed over-fat chow with the main energy composition of protein containing 20% carbohydrates and 60% fat (Beijing Huafukang Biotechnology Co., Ltd.) for 10 weeks. At week 11, the top 24 rats in the over-fat diet group in descending order of body weight were selected as the obese rats[28] and divided into 3 main groups: the obesity control group (HON group), CR plus HIIT group (ONE group) and the HIIT group (OHE group), with 8 rats in each group. The rats in the ONE group were given 70% of the food intake for daily modeling without restricting their water intake.

Training Program in the Rodent Population

After 1 week of adaptive exercise, the rats in the ONE and OHE groups underwent the following exercise training program 5 times per week for 10 weeks: 4 sessions of 1-min high-intensity exercise (18 m/min) with 10.5 min interval training (9 m/min) in the first 2 weeks, and 12 sessions of 1-min high-intensity exercise (30 m/min) with 2.5 min interval training (18 m/min) in weeks 3 through 10.

Rodent Population Sampling

After anesthesia, we measured body length and weight. Perirenal, epididymal and visceral fat were gathered from the rats. A small portion of adipose tissue was fixed with 4% paraformaldehyde for hematoxylin and eosin (H&E) staining, and the remaining adipose tissue was stored in liquid nitrogen for additional analysis.

H&E Fat Staining

The epididymal fat (visceral fat) in the 4% paraformaldehyde solution was dehydrated, embedded in paraffin and sectioned at 7 μm for H&E. These sections were put in an oven at 60°C for half an hour, dewaxed, hydrated and stained with hematoxylin for 12 min. These sections were then dipped by 1% acid ethanol (1% HCl in 70% ethanol), blued by ammonia solution and counterstained with eosin dye for 5 minutes after rinsing. These sections were dehydrated with alcohol, soaked in xylene and finally sealed with neutral gum. Images were obtained using 1 microscope, and the adipocyte area was analyzed using the Image-Pro Plus® system (Media Cybernetics, Rockville, Maryland USA).

Experimental Instruments and Reagents

The primary instruments included a power supply for the electrophoresis apparatus (Liuyi Biotechnology, Beijing China), a vertical electrophoresis apparatus (Bio-Rad, Hercules, California USA), western blot transfer system (Bio-Rad), microplate reader (Bio-Rad), a quantitative polymer chain reaction (PCR) instrument (Bio-Rad) and a Siemens 1800 biochemical analyzer (Siemens, Munich Germany).The main reagents included a UCP1 antibody (ABclonal Technology, Woburn, Massachusetts USA), PPARγ antibody (ABclonal), PGC-1α antibody (Affinity Biologics, Hamilton, Ontario Canada), goat anti-rabbit secondary antibody (TDY Biotech, Beijing China), protein marker (Fermentas-Thermo Fisher Scientific, Waltham, Massachusetts USA), and Millipore polyvinylidene difluoride (PVDF) membranes (ThermoFisher Scientific).

Detection of UCP1, PPARγ, and PGC-1α Protein Expression Levels by Western Blot Analysis

Proteins were transferred to the PVDF membranes after SDS-PAGE, and the membranes were blocked with a 5% nonfat milk powder solution at room temperature for 1 hour and then incubated via diluted primary antibodies overnight at 4°C. The membranes were then incubated by diluted secondary antibody (1:5000) at room temperature for 1 hour with membrane, then incubated with enhanced chemiluminescence (ECL for 5 min), followed by exposure to X-ray-sensitive film. Gray values were analyzed using ImageJ software.

Detection of UCP1, Prdm16, and Cidea mRNA expression levels by RT-qPCR

TRIzol reagent was used to isolate total RNA from adipose tissue. After treatment with DNase I, cDNA was synthesized from RNA with a reverse transcription kit. RT-qPCR was carried out via a fluorescence quantitative PCR instrument (Bio-Rad), and the relative mRNA expression levels were measured in the method of 2-delta-delta CT. Primer sequences are shown in Table 1.

Table 1. Forward and Reverse Primer Sequences of Target Genes

Gene	Primer sequence (F)	Primer sequence (R)
UCP1	GTGAACCACCACATACTGG	GATGACGTTCCAGGATCCG
Prdm16	CCTCTGTCGACCTCTGACC	CAGATGCAGAATGTCGGGAAC
Cidea	GGAGTCTGCTGCGGTTCG	GGACCTCTGTATCTGTGATCTG
GAPDH	GGCTGGCATTGCTCTCAATG	GTCCAGGGTTTCTTACTCCTTG

Abbreviations: UCP1, uncoupling protein 1.

Table 2. Comparison of Body Weight and Body Composition in Adult Males After Training

Group	n	Body weight (Kg)	Muscle mass (Kg)	Fat mass (Kg)	Body fat rate (%)
CR+HIIT	6	76.8 ± 1.3[a]	31.7 ± 2.2	18.5 ± 1.9[b]	24.1 ± 2.2[b]
HIIT	6	78.6 ± 1.4	32.1 ± 1.1[a]	22.4 ± 1.3[a]	28.6 ± 1.7[a]
CN	6	80.3 ± 1.2	29.9 ± 1.8	24.8 ± 1.9	30.9 ± 2.4

[a]$P < .05$ compared with the CN group
[b]$P < .01$ compared with the CN group

Table 3. Comparison of Body Weight, LEE'S Index and Body Fat Rate in Rats

Group	n	Body Weight (g)	Lee's Index	Visceral fat (g)	Body Fat rate(%)
CON	8	529.38 ± 37.76[a]	8.8 ± 0.26	13.73 ± 4.12[a]	2.51% ± 0.75[a]
HON	8	635.88 ± 27.73[b]	9.02 ± 0.29	35.68 ± 7.26[b]	5.63 ± 0.12[b]
ONE	8	546.9 ± 15.93[a]	8.78 ± 0.34	14.87 ± 2.2[a]	2.6 ± 0.49[a]
OHE	8	564.2 ± 35.63[a]	8.86 ± 0.13	19.28 ± 3.93[c,d,e]	3.7 ± 0.84[c,d,e]

[a]$P < .01$ compared with the HON group
[b]$P < .01$ compared with the CON group
[c]$P < .05$ compared with the CON group
[d]$P < .05$ compared with the HON group
[e]$P < .05$ compared with the ONE group

Statistical Analysis

Data, which were expressed as mean ± standard deviation, had been analyzed and processed by SPSS 19.0 and GraphPad Prism 6.7. One-way analysis of variance was employed for inter-group analysis. $P < 0.05$ implied the significant difference.

RESULTS

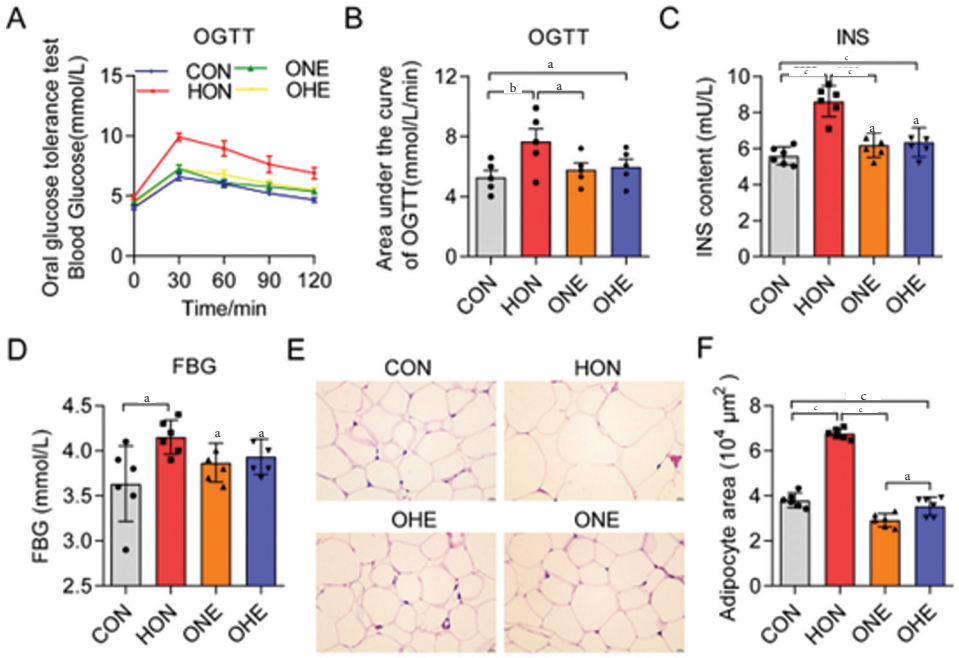
Effects of CR Integrated with HIIT and HIIT Alone on Body Weight and Body Composition in Obese Adult Males

As shown in Table 2, body weight decreased in both the CR+HIIT and HIIT groups. The fat mass, body fat rate and body weight were greatly decreased in the CR+HIIT group compared with the CN group. The muscle mass gain and weight loss were attributed to the decrease in fat mass. In the HIIT group, body weight decreased, muscle mass significantly increased and body fat rate and fat mass were considerably decreased. All the results indicated that CR integrated with HIIT is more beneficial than HIIT alone in reducing body weight and fat mass.

Effects of CR Integrated with HIIT and HIIT Alone on Body Weight, Body Fat Rate and Oral Glucose Tolerance Test (OGTT) in Obese Rats

Table 3 shows that the body fat rate and body weight in the HON group were considerably higher than in the CON group, and significantly lower in the ONE and OHE groups were significantly lower than in the HON group. In addition, the body fat rate in the ONE group was notably lower than in the OHE group. As shown in Figures 1A and 1B, the blood glucose level in the HON group was higher at each OGTT time point than in the CON group, and the area under the curve of OGTT (AUC_{OGTT}) was considerably increased

Figure 1. (**1A**) OGTT curves of rats in all groups. (**1B**) Histogram of AUC_{OGTT} of rats in all groups. (**1C**) Comparison of INS levels of rats in all groups. (**1D**) Comparison of fasting blood glucose levels of rats in all groups. (**1E**) Micrographs (H&E staining, ×400) of VAT of rats in all groups. (**1F**) Histogram of adipocyte area of VAT in all groups.



[a]$P < .05$
[b]$P < .01$
[c]$P < .0001$

Abbreviations: AUC_{OGTT}, AUC of oral glucose tolerance test; H&E, hematoxylin and eosin (staining); INS, insulin; VAT, visceral adipose tissue.

($P < .01$), indicating abnormal glucose tolerance. The AUC_{OGTT} was much lower in both the ONE and OHE groups than in the HON group. Levels of INS and fasting blood glucose (FBG) in the HON group were much higher than in the CON group (see Figures 1C and 1D). Moreover, the INS levels in the ONE and OHE groups were considerably lower than in the HON group.

Effects of CR Integrated with HIIT and HIIT Alone on the Morphology of VAT

Compared with the CON group, the number of adipocytes decreased but the adipocyte area was considerably increased in the HON group (see Figures 1E and 1F). The adipocyte area in the OHE and ONE groups was much smaller than in the HON group, and the adipocyte area in the ONE group was obviously smaller than in the OHE group.

Effects of CR Integrated with HIIT and HIIT Alone on the mRNA Expression of UCP1, Prdm16 and Cidea, as well as Protein Expression of UCP1 in the VAT of Obese Rats

Figure 2A shows that expression of thermogenesis-related genes (UCP1, Prdm16, and Cidea) in the HON group was much lower than in the CON group. Expression of Cidea and UCP1 in the ONE and OHE groups was much higher than in the HON group. To confirm these results, we detected the UCP1 protein levels in different groups. As expected, the UCP1 protein levels in HON group were much lower than in the CON group but higher in the ONE and OHE groups than in the HON group (see Figure 2B).

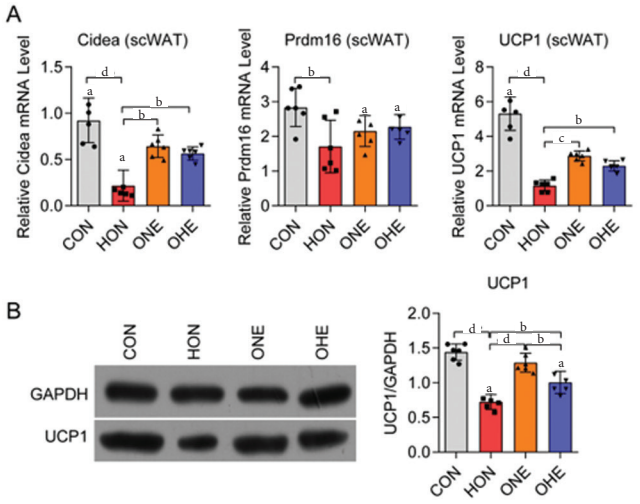
Effects of CR + HIIT and HIIT Alone on Protein Expression of PPARγ and PGC-1α in VAT in Obese Rats

Figure 3A show that expression of PPARγ in the HON group was lower than in the CON group ($P < .01$), whereas expression of PPARγ in the ONE and OHE groups were much higher than in the HON group ($P < .01$) and expression of PPARγ in the ONE group was much higher than in the OHE group ($P < .01$). Figure 3B shows that expression of PGC-1α was greatly decreased in the HON group compared with the CON group ($P < .01$). Moreover, expression of PGC-1α was considerably increased in the ONE and OHE groups compared with the HON group ($P < .01$). Expression of PGC-1α in the ONE group was higher than in the OHE group ($P < .05$).

Correlation Analysis of Protein Expression of PPARγ, PGC-1α and UCP1 with Rat Body Weight, Visceral Fat Mass and Body Fat Rate

Table 4 shows that protein expression of PPARγ, PGC-1α and UCP1 in rats were significantly negatively correlated with body weight, visceral fat mass and body fat rate.

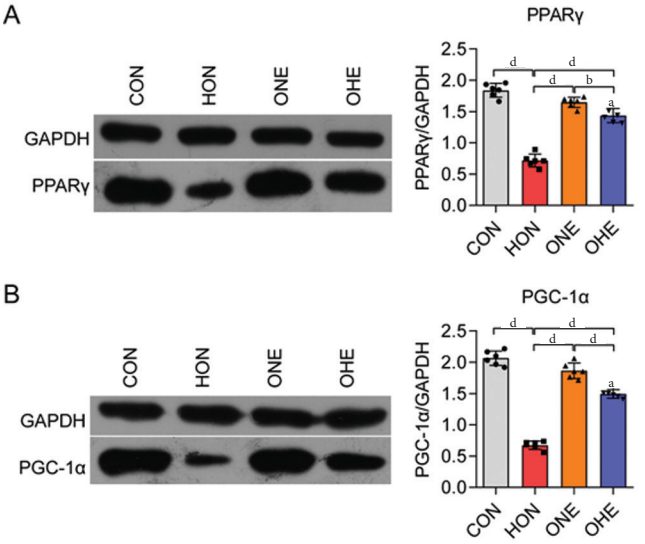
Figure 2. (**2A**) mRNA expression of UCP1, Prdm16 and Cidea in all groups as detected by RT-qPCR. (**2B**) Protein levels of UCP1 in all groups as detected by western blot analysis. GAPDH was used as an internal control.



[a]$P < .05$
[b]$P < .01$
[c]$P < .001$
[d]$P < .0001$

Abbreviations: mRNA, messenger RNA; RT-qPCR, real-time quantitative polymer chain reaction; UCP1, uncoupling protein 1.

Figure 3. (**3A**) Protein levels of PPARγ in all groups as detected by western blot analysis. GAPDH was used as an internal control. (**3B**) Protein levels of PGC-1α in all groups as detected by western blot analysis. GAPDH was used as an internal control



[a]$P < .05$
[b]$P < .01$;
[c]$P < .001$
[d]$P < .0001$

Abbreviations: PGC-1α, PPARγ coactivator; PPARγ, peroxisome proliferator- activated receptor gamma.

Table 4. Correlation Analysis of Protein Expression of PPARγ, PGC-1α and UCP1 with the Body Weight, Visceral Fat Mass and Body Fat Rate of Rats

		Body weight after exercise	Visceral fat mass	Body fat rate
PPARγ	Pearson correlation	-0.765[a]	-0.805[a]	-0.813[a]
PGC-1α	Pearson correlation	-0.901[b]	-0.816[a]	-0.804[a]
UCP1	Pearson correlation	-0.824[a]	-0.737[a]	-0.602

[a]indicates a significant correlation at the 0.05 level (2-sided)
[b]a significant correlation at the 0.01 level (2-sided)

DISCUSSION

Obesity is caused by the imbalance of energy intake and consumption, and it is an important risk factor for many diseases. Our study suggests that the negative impacts of obesity on adipose tissue can be reversed with 10 weeks of CR plus HIIT treatment.

Both human and animal research have suggested that CR effectively reduces body weight and fat rate.[29] Studies have also shown that HIIT effectively reduces body weight[30,31] and total body fat.[25,26] A previous study has shown that CR+HIIT is more effective,[32] which agrees with the findings of our study. Our human population findings showed that CR+HIIT in adult males was more conducive to reducing body weight and fat mass than HIIT alone. Our study also showed that CR+ HIIT and HIIT alone reduced body weight and body fat, as well as improved glucose tolerance and decreased visceral adipocytes in obese rats, and CR+HIIT was more effective than HIIT alone.

The specific thermogenesis of adipose tissue has been widely recognized, and the UCP1-mediated non-shivering thermogenesis of brown adipose tissue and beige adipose tissue is considered to be one of the important mechanisms by which exercise regulates adipose energy balance.[33,34] This study showed that mRNA expression of thermogenic genes (UCP1, Prdm16 and Cidea) and expression of thermogenic protein (UCP1) were decreased in the VAT of obese rats. Moreover, CR+ HIIT and

HIIT alone promoted the browning of WAT and increased mRNA expression levels of thermogenic genes (UCP1, Prdm16 and Cidea), as well as expression of thermogenic protein (UCP1), and CR+HIIT was more effective than HIIT alone.

PPARγ is abundantly expressed in adipose tissue. A previous study has shown that PPARγ promotes the transformation of WAT into a brown adipocyte-like phenotype.[35] The binding of PPARγ to rosiglitazone promotes transcription of the UCP1 gene.[36] PGC-1α, as a transcriptional coactivator, also enhances expression of UCP1 and key metabolic enzymes in the mitochondrial oxidative respiratory chain in WAT.[20,37] PGC-1α is critical for regulating mitochondrial biogenesis. A previous study found that PGC-1α deficiency inhibits the expression of thermogenesis-related genes in brown fat.[18] Multiple studies have demonstrated that exercise training emulates the PGC-1α pathway, increases the abundance of brown adipocytes and induces WAT browning by propelling release of myogenic myokines.

Our study demonstrated that the expression of PPARγ, PGC-1α and UCP1 were reduced in the adipose tissue of obese rats. In addition, CR+HIIT and HIIT alone upregulated the expression of PPARγ, PGC-1α and UCP1, and these expression levels were negatively correlated with body weight and body fat rate in rats. These findings suggested that CR plus HIIT regulates body weight and reduces body fat mass via the PPARγ/PGC-1α/UCP1 signaling pathway. Thus, the specific mechanism of 10 weeks of CR+HIIT involves the induction of PPARγ expression in adipose tissue, which regulates mitochondrial biosynthesis and oxidation function by regulating PGC-1α and enhancing the expression of the UCP1 mitochondrial thermogenic protein, thereby increasing energy consumption and achieving fat loss.

In the present study, we also demonstrated that the protein expression of PPARγ, PGC-1α and UCP1 in the adipose tissue of rats in the ONE group was much higher than in the OHE group, indicating that CR+HIIT is more effective than HIIT alone in activating the PPARγ/PGC-1α/UCP1 pathway, promoting the browning of WAT and reducing the accumulation of visceral fat in obese rats, thereby achieving fat loss.

CONCLUSIONS

Both CR+HIIT and HIIT alone achieve fat loss, and the former is more effective than the latter in terms of reducing body fat mass.

The effect of CR+HIIT on the upregulation of the PPARγ/PGC-1α/UCP1 signaling pathway and the browning of WAT in the VAT of obese rats is better than that of HIIT alone. Thus, CR may be a key variable leading to different effects on the browning of WAT.

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CONFLICT OF INTEREST

None.

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