PIBB 生物化学与生物物理进展
Progress in Biochemistry and Biophysics
2025,52(6):1386~1400
www.pibb.ac.cn

Hypoxia Exercise Mediates The miR−27/PPARγ Pathway to Improve Lipid Metabolism in Obese Rats at Target Genes and Protein Levels*

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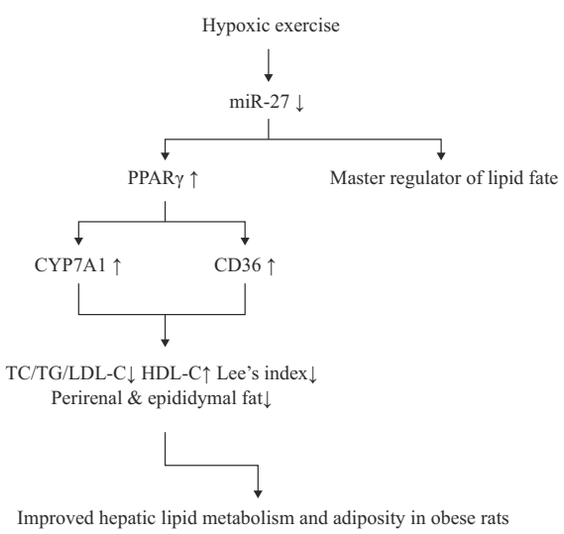
Graphical abstract



Abstract **Objective** To explore the sequential effects of hypoxic exercising on miR-27/PPARγ and lipid metabolism target gene and protein expression levels in the obesity rats' liver. **Methods** 13-week-old male diet-induced obesity rats were randomly divided into three groups (n=10): normal oxygen concentration quiet group (N), hypoxia quiet group (H), hypoxic exercise group (HE). Exercise training on the horizontal animal treadmill for 1 h/d, 5 d/week for a total of 4 week, and the intensity of horizontal treadmill training was 20 m/min (hypoxic concentration was 13.6%). Comparison of the weights of perirenal fat and epididymal fat

* This work was supported by a grant from China Postdoctoral Science Foundation (2017M622161).
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Received: May 21, 2025 Accepted: June 13, 2025

in rats across different groups and calculation of Lee's index based on body weight and body length of rats in each group were done. And the serum concentrations of total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) levels were detected. RT-PCR and Western Blot were used to detect the levels of miR-27, PPARγ, CYP7A1 and CD36. **Results** Hypoxic exercise decreased the expression levels of miR-27 in the obese rats' liver, however, the expression level of PPARγ was gradually increased. The expression levels of miR-27 in HE group were significantly lower than N group (P<0.05). The expression levels of *PPARγ* mRNA in N group were significantly lower than H group (P<0.05), especially lower than HE group (P<0.01). The protein expression of PPARγ protein in N group was significantly lower than that other groups (P<0.01). The expression of lipid metabolism-related genes and proteins increased in the obese rats' liver. The expression of *CYP7A1* mRNA in N group was significantly lower than H group (P<0.05), especially lower than HE group (P<0.01). The expression of CYP7A1 protein in the obese rats' liver in N group was extremely lower than H group and HE group (P<0.01). The protein expression of CD36 in N group was significantly lower than that in HE group (P<0.05). Hypoxia exercise improved the related physiological and biochemical indexes of lipid metabolism disorder. The perirenal fat weight of obese rats in HE group was extremely lower than N group and H group (P<0.01), and the perirenal fat weight in N group was significantly higher than H group (P<0.05). The epididymal fat weight in N group was significantly higher than H group (P<0.05), and extremely higher than HE group (P<0.01). The Lee's index in HE group was extremely lower than N group and H group (P<0.01). The serum concentration of TC in obese rats in HE group was extremely lower than N group and H group (P<0.01). The serum concentration of TG in HE group was extremely lower than N group and H group (P<0.01). The serum concentration of LDL-C in N group was extremely higher than HE group (P<0.01). The serum concentration of HDL-C in N group was extremely lower than H group (P<0.01). **Conclusion** Hypoxia and hypoxia exercise may negatively regulate the levels of PPARγ by inhibiting miR-27 in the obese rats' liver, thereby affecting the expression of downstream target genes CYP7A1 and CD36, and promoting cholesterol, fatty acid oxidation and HDL-C transport in the liver, and ultimately the lipid levels in obese rats were improved. The effect of hypoxia exercise on improving blood lipid is better than simple hypoxia intervention.

Key words hypoxia exercise, miR-27, peroxisome proliferators-activated receptor gamma, obese rat
DOI：10.16476/j.pibb.2025.0249 **CSTR**：32369.14.pibb.20250249

According to data released by the World Health Organization (WHO) in 2020, global obesity rates have tripled since 1975. Approximately 25% of the world's population is now classified as overweight, with obese individuals accounting for up to 50% of this overweight population. Obesity and its complications pose a significant threat to human health. Research findings, both domestically and internationally, consistently demonstrate that hypoxic training not only aids in weight and fat loss[1-2], but also improves physiological function. It helps prevent and manage obesity-related cardiovascular and lipid metabolism disorders while reducing disease risk[3-4]. Recent studies have highlighted the close relationship between miRNAs and lipid metabolism. By regulating the expression of key enzymes involved in lipid synthesis, transport, breakdown, and oxidation at the post-transcriptional level, miRNAs have become a key focus in lipid metabolism research[5-9]. Among these miRNAs, miR-27 modulates downstream lipid metabolism-related genes and proteins by regulating peroxisome proliferators-activated receptor gamma (PPARγ), thereby influencing the body's lipid metabolism efficiency[10-12]. Given the liver's central role in lipid metabolism, most studies on miR-27-mediated lipid regulation have focused on hepatic tissue. However, few studies have investigated how hypoxic training affects miR-27 expression and its subsequent role in PPARγ-mediated lipid metabolism regulation. In this study, we adopted obese rats to 4 weeks of hypoxic training and assessed the relative expression levels of miR-27/PPARγ and downstream lipid metabolism-related genes and proteins in the liver. Our goal was to elucidate the mechanism by which hypoxic training mediates lipid metabolism regulation through the miR-27/PPARγ pathway in obesity. The research results not only reveal the molecular mechanism of lipid metabolism regulation by hypoxia exercise but also provide a theoretical basis for artificially creating a hypoxia exercise environment to intervene in chronic diseases such as obesity. This enriches the methods of strengthening non-medical health interventions proposed in the "Healthy China 2030 Planning Outline" and leverages

the positive role of scientific fitness in the prevention of chronic diseases. Consequently, it will promote the improvement of people's quality and level of healthy life and implement the national strategy of "Implementing Healthy China".

1 Materials and methods

1.1 Laboratory animals

200 Sprague Dawley (SD) rats (male, 5 weeks old, SPF grade, (176.47±10.75) g) were purchased from Beijing Viton Lihua Laboratory Animal Technology Co. Ltd., with the production license SCXK (Beijing) 2012-0001 and experimental animal practitioner's induction certificate 1115032300009. The SD rats were randomly divided into two groups: the regular feed group (n=20, (176.26±10.62) g, fed with rodent breeding material) and the high-fat feed group (n=180, (176.54±10.80) g, fed with D12451 high-fat feed from Research Diets, Inc., USA). There was no significant difference in body weights between the two groups. The animal model for this experiment was constructed and trained in the animal room of the Institute of Sports Science of the State General Administration of Sport (ABSL-3), where the room temperature was maintained at (22±1)ºC and the humidity at (55±2)%. The animal room was adjusted to the circadian rhythm of the rats, adopting a daytime darkness and nighttime light mode with lighting alternated every 12 h to ensure that the rats were awake during daytime training. Animal experiments were conducted in accordance with the ethical policies and procedures approved by the Ethics Committee of Qufu Normal University (approval No. 2020046).

1.2 Construction of obesity animal model

After 8 weeks of feeding, 20 rats from the high-fat feed group and 20 rats from the normal feed group were randomly selected for comparison and analysis to determine the success of modeling. The criteria for modeling success were as follows: the average body weight, fat body ratio, and Lee's index of rats in the high-fat feed group were significantly higher than those in the regular feed group; serum total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) were significantly higher in the high-fat feed group compared to the regular feed group, whereas high-density lipoprotein cholesterol (HDL-C) was significantly lower in the high-fat feed group. Rats in the high-fat feed group whose body weight exceeded the average body weight of the normal feed group by more than 20% were considered nutritionally obese and could be used as research subjects in this experiment.

1.3 Experimental grouping and exercise program

A total of 97 nutritionally obese SD rats were successfully modeled. A 1-week adaptive training period was conducted to assess the exercise ability of the rats, during which the speed was incrementally increased from 16 m/min to 25 m/min and the exercise time from 20 min/d to 60 min/d. Based on the adaptation of the model animals to the treadmill training, 17 SD rats were eliminated. The remaining 80 rats were then randomly divided into 8 groups. Five of these groups were used in other experimental designs, while the remaining 3 groups were utilized in the present experiment (n=10; there was no significant difference in the body weights of the animals across groups). These groups were: the normoxic sedentary group (group N), the hypoxic sedentary group (group H), and the hypoxic training group (group HE). All training groups underwent endurance training on a horizontal treadmill at an intensity of 20 m/min, with continuous exercise for 1 h/d, 5 d/week. The hypoxic concentration was set at 13.6%, equivalent to an altitude of 3 500 m.

1.4 Experimental sampling

After all the animals were trained and allowed to recover for 24 h, sampling was conducted. The rats were fasted for 12 h before sampling and then anesthetized by intraperitoneal injection of 2% pentobarbital sodium hydrate at a dose of 50 mg/kg. Body length and weight were measured. The experimental animals were fixed on an ice-cooled sampling plate on the experimental bench, and the abdominal cavity was opened to collect blood from the abdominal aorta for subsequent separation of blood and serum. The right lobe of the liver was excised from its upper edge and divided into two equal portions. The left portion of the liver was used for quantitative real-time PCR, while the right portion was used for Western blot detection. The right portion of the liver was rinsed in pre-cooled saline to remove blood, blotted on filter paper, frozen in liquid nitrogen, and stored in an ultra-low-temperature refrigerator at −80° C for further testing. The right perirenal fat and epididymal fat were also excised,

quickly rinsed in pre-cooled saline to remove blood, drained on filter paper, and weighed on an electronic balance.

1.5 Physiological and biochemical indices

The body length of the rats was measured using a soft ruler, and their weight was determined using an electronic scale. The Lee's index of the rats was calculated according to the following formula. The concentrations of TC, TG, LDL-C, and HDL-C were detected using a semi-automatic biochemical analyzer (Beckman, AU480).

Lee's index = $\sqrt[3]{(\text{weight/g})}/(\text{length/cm})\times 10^3$

1.6 Real−time PCR

Liver tissue (100 mg) was retrieved from the ultra-low temperature refrigerator, crushed in a mortar containing liquid nitrogen, and total RNA was extracted strictly according to the Trizol method. The purity of the extracted total RNA was assessed using the A_{260}/A_{280} ratio, and the integrity of the RNA was evaluated by agarose gel electrophoresis.

1.6.1 Detection of mRNA expression

Reverse transcription was performed to generate cDNA strictly according to the instructions of the kit (RR370A, Takara Bio). The reaction conditions were: 37℃ for 15 min, 85℃ for 5 s, followed by maintenance at 4° C, and the samples were stored at −20° C for further testing. Using the synthesized cDNA as the template and β-actin as the internal reference, three replicate wells were designed for each sample. The assay was carried out in a real-time fluorescence quantitative PCR instrument (ABI 7300) strictly according to the instructions of the reagent kit (RR8200A, Takara Bio). The reaction conditions for quantitative PCR were as follows: pre-denaturation at 95°C for 30 s; 95°C for 5 s followed by 60°C for 31 s, for a total of 40 cycles of PCR reaction.

1.6.2 Detection of miRNA expression

The process of reverse transcription to generate cDNA was carried out strictly according to the instructions of the kit (purchased from Beijing Tiangen Biochemical Technology Co., Ltd.). The synthesized cDNA was used as the template, and U6 was used as the internal reference. Three replicate wells were designed for each sample, and the detection was performed in a real-time fluorescence quantitative PCR instrument (ABI 7300) following the instructions of the kit (purchased from Beijing Tiangen Biochemical Technology Co., Ltd.). The reaction conditions were as follows:

(1) Pre-denaturation at 94°C for 2 min.

(2) PCR reaction at 94°C for 20 s, 63°C for 20 s, and 72°C for 30 s, repeated for a total of 5 cycles.

(3) PCR reaction at 94° C for 20 s and 60° C for 34 s, repeated for a total of 40 cycles.

Real-time fluorescence quantitative PCR was used to obtain the values of each sample to be tested. The average cycle threshold (C_t) value of 3 replicates was calculated, and the $\triangle\triangle C_t$ value was then calculated using the formula: $\triangle\triangle C_t = (C_t$(experimental group target gene)$-C_t$(experimental group internal reference gene)) − (C_t(control group target gene) − (C_t(control group internal reference gene)). The $2^{-\triangle\triangle C_t}$ value of each sample to be tested was then calculated, representing the relative expression of each sample.

The primers used in the experiment were designed and synthesized by Shanghai Bioengineering Co., and their sequences are shown in Table 1.

Table 1 Primer sequences for RT−PCR

Gene		Primer sequence
miR-27	5P	AGAGCUUAGCUGAUUGGUGAACAG
U6	5P	CATTGCTGACAGGATGCAGAAG
PPARγ	upstream	CGGTTGATTTCTCCAGCATT
	downstream	GGACGCAGGCTCTACTTTGA
CYP7A1	upstream	ACACGCTCTCCACCTTTGAC
	downstream	GAGGCTGCTTTCATTGCTTC
CD36	upstream	TGGCAAAGAATAGCAGCAAG
	downstream	CAGTGAAGGCTCAAAGATGG
β-actin	upstream	CATTGCTGACAGGATGCAGAAG
	downstream	GAGCCACCAATCCACACAGAGT

1.7 Western blot

Approximately 100 mg of liver tissue was retrieved from the ultra-low temperature refrigerator, crushed in a mortar containing liquid nitrogen, and a protein lysate and protease inhibitor (purchased from Biyuntian Biotechnology Co., Ltd.) were added. After centrifugation, the protein concentration of the samples was determined using the BCA method. The concentration of all samples was adjusted to 4 g/L with deionized water and buffer, and the proteins were denatured by heating in boiling water for 10 min and then stored in the refrigerator at −20° C for further measurement.

A 10% gel was used for 1.5 h at a constant voltage of 120 V, followed by transfer to a PVDF membrane (Millipore) at a constant current of 200 mA for 1 h. The membrane was blocked with 5% skimmed milk powder (BD Company, USA) for 1 h, and the primary antibody (PPARγ polyclonal antibody 1∶2 000; CYP7A1 polyclonal antibody 1∶5 000; CD36 polyclonal antibody 1∶1 000) was added and incubated overnight on a shaker at 4℃. After washing with TBST, the secondary antibody (1∶10 000) was added and incubated at room temperature for 1 h. The membrane was washed again with TBST, and a luminescence solution was added to the membrane for development and fixation in a darkroom. The film was scanned, and the grayscale values of the protein bands were analyzed using Quantity One software to determine the relative expression of the target protein compared to the internal reference protein.

1.8 Statistical analysis

All data in this study were statistically analyzed using SPSS 22.0 software. Initially, the parameters were tested for normality using P-P plots and the Shapiro-Wilk test. If the data were not normally distributed, they were transformed to achieve a normal distribution. Between-group differences in the means were analyzed using one-way ANOVA. Results are expressed as mean±standard deviation (mean±SD). $P<0.05$ indicates a significant difference, and $P<0.01$ indicates a highly significant difference. The experimental data were plotted using GraphPad Prism 6.0 software.

2 Results

2.1 Hypoxia and hypoxia training downregulate miR−27 expression and upregulate PPARγ expression

According to Figure 1, the experimental results showed that the hepatic miR-27 expression in obese rats of the HE group was significantly lower than that in the N group ($P<0.05$). The hepatic *PPARγ* mRNA expression in obese rats of the N group was significantly lower than that in the H group ($P<0.05$) and was highly significantly lower than that in the HE group ($P<0.01$). The hepatic PPARγ protein expression in obese rats of the N group was highly significantly lower than that in the other groups ($P<0.01$).

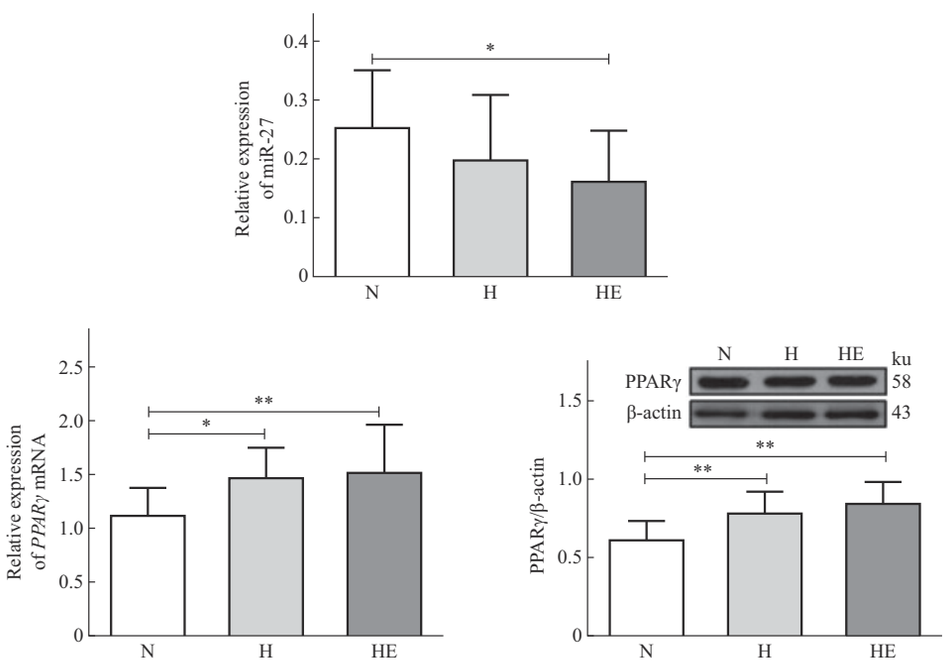

Fig. 1 Relative expression of miR−27 and PPARγ in the obesity rat liver

* Indicates that the difference in means between the two groups is significant ($P<0.05$). ** Indicates that the difference in means between the two groups is highly significant ($P<0.01$).

2.2 Hypoxia and hypoxia training promote the expression of PPARγ downstream target genes in the liver of obese rats

2.2.1 Hypoxia and hypoxia training upregulate hepatic CYP7A1 expression in obese rats

According to Figure 2, the hepatic *CYP7A1* mRNA expression in obese rats of group N was significantly lower than that in group H ($P<0.05$) and highly significantly lower than that in group HE ($P<0.01$). The hepatic CYP7A protein expression in obese rats of group N was highly significantly lower than that in groups H and HE ($P<0.01$).

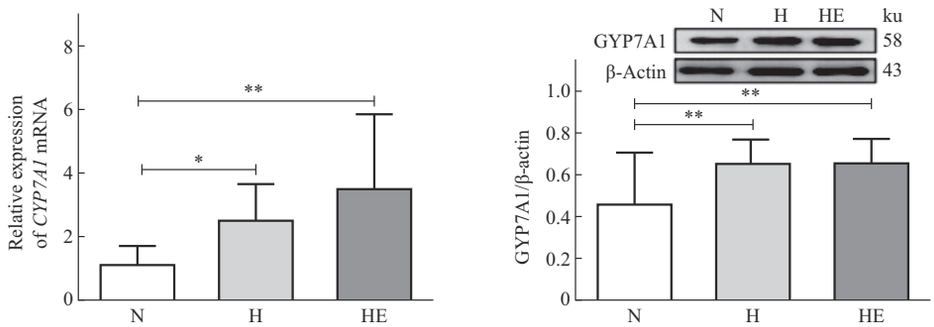

Fig. 2 Relative expression of CYP7A1 in the obesity rat liver

* Indicates that the difference in means between the two groups is significant ($P<0.05$); ** Indicates that the difference in means between the two groups is highly significant ($P<0.01$).

2.2.2 Hypoxia and hypoxia training upregulate hepatic CD36 expression in obese rats

According to Figure 3, the experimental results showed that the hepatic CD36 protein expression of obese rats in the HE group was significantly higher than that in the N group ($P<0.05$).

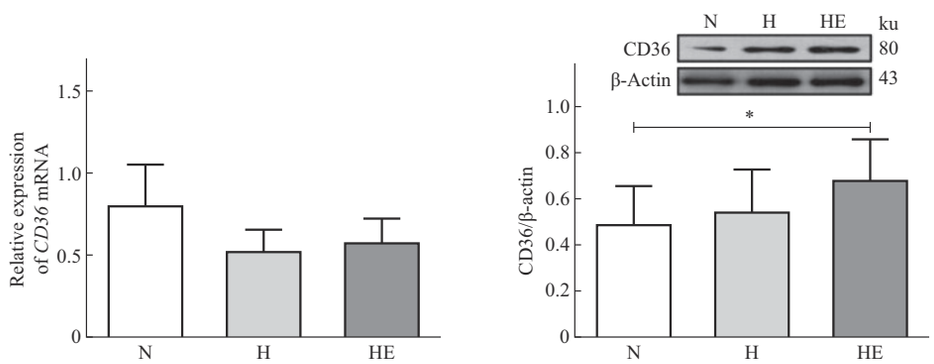

Fig. 3 Relative expression of CD36 in the obesity rat liver

* Indicates that the difference in means between the two groups is significant ($P<0.05$).

2.3 Effects of hypoxic and hypoxic training on physiological and biochemical indexes of obese rats

According to Table 2, the perirenal fat weight of experimental animals in the HE group was significantly lower than that in the N and H groups ($P<0.01$), and the perirenal fat weight of obese rats in the H group was significantly lower than that in the N group ($P<0.05$). The epididymal fat weight of obese rats in the N group was significantly higher than that in the H group ($P<0.05$), and it was extremely significantly higher than that in the HE group ($P<0.01$). Lee's index in the HE group was extremely significantly lower than that in the N and H groups ($P<0.01$).

Table 2 Physiological and biochemical characteristics of obese rats

Group	*n*	Perirenal fat/g	Epididymal fat/g	Lee's index	TC/(mmol·L^{-1})	TG/(mmol·L^{-1})	LDL-C/(mol·ml^{-1})	HDL-C/(mol·ml^{-1})
N	10	2.80±0.83	8.44±1.63	171±6	1.75±0.23	1.08±0.39	0.22±0.06	0.87±0.18
H	10	2.30±0.29*	7.13±0.86*	169±3	1.68±0.24	0.95±0.13	0.17±0.06	1.22±0.24**
HE	10	1.49±0.36$^{**,\#\#}$	6.13±1.60**	163±4$^{**,\#\#}$	1.23±0.21$^{**,\#\#}$	0.66±0.09$^{**,\#\#}$	0.12±0.06**	1.03±0.27

$^{*}P$<0.05, $^{**}P$< 0.01, compared with group N; $^{\#\#}P$<0.01, compared with group H.

The serum TC concentration of obese rats in the HE group was significantly lower than that in the N and H groups (P<0.01). The serum TG concentration of obese rats in the HE group was significantly lower than that in the N and H groups (P<0.01). The serum LDL-C concentration of obese rats in the HE group was significantly lower than that in the N group (P< 0.01). The serum HDL-C concentration of obese rats in the H group was significantly higher than that in the N group (P<0.01).

3 Discussion

Domestic and international research results consistently demonstrate that hypoxic training not only aids in reducing body weight and body fat[13], but also helps adjust bodily functions, prevent and treat obesity-induced cardiovascular and lipid metabolism disorders, and lower disease risk. The primary mechanism involves hypoxia training influencing the expression of genes related to lipid metabolism[5-6,14-16]. This influence subsequently regulates the levels of lipid metabolism-related enzymes and proteins encoded by these genes[17]. Such regulation accelerates cholesterol metabolism and efflux, promotes fatty acid catabolism and oxidation, and inhibits fatty acid synthesis. These changes improve lipid metabolism levels, ultimately contributing to the prevention and treatment of diseases associated with lipid metabolism disorders[18].

It is now reported that miR-27 is closely related to lipid metabolism. It regulates the expression of key enzymes involved in lipid synthesis, transport, catabolism, and oxidation at the post-transcriptional level, making it a hot spot in the study of lipid metabolism mechanisms[19-20]. Luciferase reporter gene analysis has shown that miR-27 specifically binds to the 3' untranslated region (3'UTR) of PPARγ and inhibits its expression, demonstrating that PPARγ is a target gene of miR-27[21-22]. In one study, Western diet (high-fat diet, HFD/HFD-HF) was shown to potentially inhibit PPARγ through upregulation of miR-27b-5p, thereby promoting the progression from simple fatty liver to fibrosis. These findings suggest that miR-27b-5p could serve as an early diagnostic marker or therapeutic target, but this study focused on the study of the targeted disease, primarily focused on disease pathogenesis[23]. Our current study similarly observed significantly reduced hepatic PPARγ protein expression in obese rats of the control group (N) compared to other experimental groups (P<0.01). Additionally, our investigation employed hypoxic/hypoxic training intervention, which notably enhanced PPARγ protein expression and consequently improved lipid metabolism. PPARγ can affect lipid metabolism by regulating the expression of multiple target genes. For example, it can influence lipid metabolism by regulating the expression of the target gene CYP7A1[24], thereby affecting the lipid metabolism process in the body[25-26]. It has also been shown that PPARγ can affect fatty acid transport and metabolism by regulating the expression of the target gene CD36[27].

In summary, hypoxia training may affect the PPARγ level in the liver of obese rats by regulating the expression of miR-27, which in turn regulates the expression of downstream lipid metabolism-related target genes. This ultimately influences the lipid metabolism process in obese rats[28-29]. Therefore, based on the experimental results, we analyzed and discussed the changes in each key index during the regulation of lipid metabolism by hypoxia and hypoxia training. This analysis aims to compare the effects of hypoxia and hypoxia training on lipid metabolism, on the one hand, and to reveal the pathways through which hypoxia training regulates lipid metabolism, on the other hand.

3.1 Effects of hypoxia and hypoxia training on the expression of miR−27 and its target gene PPARγ in the liver of obese rats

Numerous studies have shown that miR-27 is closely associated with obesity, and down-regulation of miR-27 can increase lipid accumulation in

hepatocytes[21-30]. However, research findings differ regarding the trend of hypoxia affecting the expression level of miR-27. Some studies have shown that hypoxic stimulation induces up-regulation of miR-27 expression[31]. When preadipocytes were placed in 1% O_2 (simulating the oxygen concentration in the adipose tissue of obese mice), this stimulation led to a 2-fold and 1.5-fold increase in preadipocyte miR-27a and miR-27b, respectively. Lin *et al*. [32] demonstrated that after adipocytes were placed in 21% O_2 for 24 h, the levels of miR-27a and miR-27b expression decreased, whereas the expression of miR-27a and miR-27b remained high under 1% O_2 conditions, which is consistent with the inhibition of adipogenesis by hypoxia[33-34].

The results of the present experiment showed that hypoxia stimulation led to a decrease in the expression of miR-27 in the liver of obese rats ($P>0.05$), while the expression level of miR-27 in the liver of obese rats with hypoxia training intervention was significantly decreased ($P<0.05$). These results suggest that both hypoxia and hypoxia training can down-regulate the expression of miR-27 in the liver of obese rats, with hypoxia training having a more significant effect on the expression of miR-27 in the liver of obese rats, which differs subtly from the findings of Lin *et al*. The reasons for this difference may be based on the following three factors: (1) Lin *et al.*[32] experimented with cultured cells, whereas the present study was conducted with living tissues; (2) Lin *et al*.[32] exposed cells to 1% O_2, whereas the present study was conducted with obese rats placed in a simulated hypoxic environment with 13.6% O_2; and (3) Lin *et al*. [32] detected miR-27 expression in adipocyte precursors, whereas the present study examined the expression of miR-27 in the liver tissues of obese rats. It has also been shown that hypoxia inhibits PPARγ/fatty acid synthase (FASN), promotes glycolysis, reduces fatty acid oxidation, and induces cardiomyocyte apoptosis through upregulation of miR-27/miR-195 in cardiomyocytes, therefore, targeted inhibition of miR-27/miR-195 may partially restore cardiomyocyte viability and reduce apoptosis. In contrast to our conclusions, our results showed that the hypoxic environment also caused a significant decrease in miR-27 expression levels, but fat was more vigorously metabolized in liver tissues, and the reason for this different result may be related to the different hypoxic conditions used and whether there is any specificity between tissues[35].

PPARγ serves as a target gene of miR-27[21], and when hypoxia and hypoxia training affected the expression level of miR-27, the expression level of PPARγ in the liver of obese rats was subsequently altered. Szostak *et al*. [36] randomly divided high-fat chow-fed male ApoE(−/−) mice into an exercise group and a control group, and detected changes in the expression levels of PPARα, PPARβ/δ, and PPARγ in the endothelium of the aorta of the mice after 3 months of training. They found that training significantly increased the expression level of PPARγ in the mouse aorta. Chu *et al*.[37] simulated myocardial infarction by culturing h9c2 cells under hypoxic conditions and found that the expression level of PPARγ in the hypoxic group of h9c2 cells was significantly increased, effectively inhibiting the inflammatory response of the myocardium. Liu *et al*. [38] found that 30 d of autonomic training could significantly increase the expression level of PPARγ in the colon of C57Bl/6J mice. Spangenburg *et al*. [39] used acute platform running exercise and 12 weeks of platform running training to conduct exercise intervention in 5−8-month-old female SD rats to explore the effect of the two training methods on the expression level of *PPARγ* mRNA in different skeletal muscles. The results showed that 24 h after recovery from a single acute table running exercise, there was no significant difference in the *PPARγ* mRNA expression levels in the rat metatarsal and gastrocnemius muscles compared to before the exercise. However, the expression levels of *PPARγ* mRNA in the metatarsal and gastrocnemius muscles of the rats in the long-term training group were both significantly elevated ($P<0.05$). Song *et al*. [40] experimentally found that PPARγ gene and protein expression levels in rat liver were significantly elevated in both 4-week training and 1-week pre-acclimatization ($P<0.05$). Meng *et al*. [41] constructed an obesity model in male SD rats and, after 8 weeks of training, found that all three intensities of training could elevate the concentration of PPARγ in rat plasma. The *PPARγ* mRNA expression levels in the adipose tissues of rats in the three different training intensity groups were all significantly higher than those of the control group ($P<0.05$). Slivka *et al*. [42] recruited 11 male volunteers and took the lateral femoral muscle of the volunteers by muscle biopsy before exercise. The lateral femoral muscle was taken

again after 1 h of exercise at 60% maximal power and 4 h of recovery. The results showed that training induced a significant increase in the level of PPARγ in the lateral femoral muscle. Consistent with the results of most current studies, the results of this experiment showed that both hypoxia and hypoxia training could cause a highly significant increase in hepatic *PPARγ* mRNA and protein expression in obese rats (P<0.01). This experiment also found that hypoxia significantly increased the hepatic *PPARγ* mRNA expression in obese rats (P<0.05) and caused a highly significant increase in the expression of PPARγ protein (P<0.01). The experimental results suggested that the regulation of *PPARγ* mRNA and protein expression in the liver of obese rats by training and hypoxic training showed the same elevated trend. The elevated expression of PPARγ regulated the efficiency of fatty acid oxidation and cholesterol transport, enabling more lipids to be oxidized and transported out of the liver into the blood. This not only reduced the accumulation of fat in the liver and prevented the generation of fatty liver but also reduced the body's fatty acid content and reduced lipogenesis, thus improving blood and body fat levels.

In addition, this experiment showed the following results. (1) The liver miR-27 expression of obese rats in the hypoxia group was reduced, though not significantly. In contrast, the liver *PPARγ* mRNA and protein expression of obese rats in the hypoxia group was extremely significantly elevated (P<0.01). This may be due to the fact that PPARγ is regulated by multiple miRNAs simultaneously, and miR-27 synergized with other miRNAs to significantly elevate the *PPARγ* mRNA and protein expression levels. (2) The liver miR-27 expression in obese rats in the hypoxia group was lower than that in the control group, and the hypoxia training group had a significantly lower expression than the control group (P<0.05). Meanwhile, the liver *PPARγ* mRNA expression of the target gene of miR-27 in obese rats in the hypoxia group was significantly higher than that in the control group, and the hypoxia training group had an extremely significant increase compared to the control group (P<0.01). The expression of the target gene of miR-27 in the liver of obese rats in the hypoxia and hypoxia training groups was significantly higher than that in the control group (P<0.01). The expression of PPARγ protein in the liver of obese rats in the hypoxia training group was significantly higher than that in the quiet control group. These results indicate that the expression of miR-27 and *PPARγ* mRNA and protein in rat liver showed a negative correlation, which is consistent with current research findings.

3.2 Effects of hypoxia and hypoxia training on the expression of target genes downstream of PPARγ in the liver of obese rats

Hypoxia and hypoxia training elevated *PPARγ* mRNA and protein expression levels in the livers of obese rats, which inevitably induced changes in the expression of downstream target genes of PPARγ, thereby affecting the process of lipid metabolism[43]. Studies have shown that PPARγ primarily influences lipid metabolism by regulating two pathways: cholesterol metabolism and fatty acid metabolism. The cholesterol metabolism pathway affects lipid metabolism by regulating the expression of target genes *ABCA1* and *CYP7A1* through PPARγ[44], while the fatty acid metabolism pathway affects lipid metabolism by regulating the expression of target genes *CD36*, *ATGL*, *LPL*, *L-FABP*, and *SREBP1* through PPARγ[27-45]. The end result of both pathways can cause changes in lipid metabolism.

3.2.1 Effects of hypoxia and hypoxia training on hepatic cholesterol metabolism in obese rats

CYP7A1 plays an important role in cholesterol synthesis and metabolism and is the most important regulatory gene in this process[46-48]. It is positively regulated by PPARγ[49-50]. Pinto *et al*. [51] experimentally found that the hepatic *CYP7A1* mRNA expression in rats in the aerobic exercise group was 1.7-fold higher than that in the control group. Côté *et al*. [52] found that the hepatic *CYP7A1* mRNA expression in the training group was higher than that in the control group, although the difference between the means of the two groups was not significant (P>0.05).

In the present experimental results, the expression of *CYP7A1* mRNA and protein in the liver tissues of obese rats in the training group was also higher than that in the control group, but the difference was not significant, which was consistent with the experimental results of Côté *et al*.[52] The results of the present experiments showed that the expression of *CYP7A1* mRNA in the livers of obese rats in the hypoxic group was significantly higher than that in the control group (P<0.05), and the expression

of CYP7A1 protein was highly significantly higher than that in the control group. Additionally, the expression of *CYP7A1* mRNA and protein in the hypoxia training group was significantly higher than that in the control group (P<0.01).

These findings suggest that hypoxia and hypoxia training can promote the expression of CYP7A1 in the same tissue by up-regulating the expression level of PPARγ in the liver of obese rats. This, in turn, affects the cholesterol production and transportation in liver tissues. This process not only adjusts the cholesterol content of liver cells but also influences the production of cholesterol in liver tissues and affects the transport of cholesterol produced by liver tissues, which in turn elevates the level of HDL-C in the blood[53].

3.2.2 Effects of hypoxia and hypoxia training on hepatic fatty acid metabolism in obese rats

PPARγ can not only regulate hepatic cholesterol metabolism but also hepatic fatty acid metabolism through target genes[54]. Numerous studies have shown that PPARγ can positively regulate downstream target genes such as *CD36*, *ATGL*, *LPL*, *L-FABP*, and *SREBP1*[45, 55-56]. Elevated PPARγ expression in the organism promotes the expression of many of these target genes, which in turn promotes fatty acid metabolism and reduces fatty acid content in tissues[57]. CD36 affects the efficiency of fatty acid oxidative metabolism by regulating the transmembrane transport of fatty acids[58-59]. Talanian *et al*. [60] found that 6 weeks of high-intensity intermittent endurance training increased the capacity of fatty acid transport in skeletal muscle, where the expression of CD36, a closely related transport carrier, was significantly increased (P<0.05), allowing more fatty acids to enter the mitochondria for oxidative energy supply. Kurosaka *et al*.[61] observed in their study of the relationship between adipose tissue lipolysis and hepatic fat uptake that hepatic fatty acid translocase (FAT)/CD36 protein expression was 2.09-fold higher in the dietary-restricted group compared to the obese group (P<0.01). The modification of FAT/CD36 expression induced by exercise prevented dietary-restriction-induced hepatic lipid accumulation in obese rats. Tunstall *et al*. [62] found that after 9 d of moderate-intensity power cycling training, the expression of *CD36* gene in human skeletal muscle tissues was significantly elevated (P<0.05). Chen *et al*.[63] in investigating the protective effect of hypoxic pre-adaptation against lipotoxicity in H9c2 cardiomyocytes and neonatal rat cardiomyocytes, found that short-term hypoxia-treated cardiomyocytes showed a significant increase in CD36 protein expression. Mwaikambo *et al*. [64] experimentally revealed that with the prolongation of hypoxia, the level of CD36 expression in human retinal pigment epithelial cells increased linearly. Ortiz-Masià *et al*. [65] obtained similar results when studying the effect of hypoxia on macrophages, finding that hypoxia could mediate the up-regulation of HIF-1 and CD36 expression.

Some scholars also studied the effects of 2 weeks of high-intensity intermittent training[66] and continuous hypoxia[67] on the expression of CD36 in rats and found that the expression of CD36 was similarly significantly elevated (P<0.05). Holloway *et al*.[68] found that after 2 h of cycling at 60% VO_{2max}, the content of CD36 in skeletal muscle increased significantly, and similarly, the efficiency of oxidized fatty acids in skeletal muscle and mitochondria was also significantly increased, with both changes showing a consistent trend.

The results of this experiment concluded that the liver *CD36* mRNA and protein expression of obese rats in the training group was extremely significantly higher than that of the N group (P<0.01), which was consistent with the results of most current studies. In the present experiment, the intergroup difference between the liver *CD36* mRNA expression of the hypoxia training group and the mean value of the control group was not significant, but the protein expression of liver CD36 in the hypoxia training group was significantly higher than that of the control group. This suggests that this process is a very important factor in the development of liver CD36 protein expression. However, the expression of CD36 protein in the liver of obese rats in the hypoxia training group was significantly higher than that in the control group, suggesting that there may be post-transcriptional regulation in this process, which in turn elevated the expression level of CD36 protein in the liver of obese rats.

3.3 Effects of hypoxia and hypoxia training on physiological and biochemical indexes of obese rats

Currently, serum levels of TC, TG, LDL-C, and HDL-C are commonly used indicators to study

abnormalities in blood lipid metabolism. Numerous studies have shown that obesity is often accompanied by elevated blood TC, TG, and LDL-C levels, as well as a decrease in HDL-C concentration[69-70]. However, training, hypoxia, and hypoxia training can cause different degrees of improvement in blood lipid levels[71].

The results of this experiment showed that after 4 weeks of training, the concentrations of serum TC, TG, and LDL-C in rats in the H, NE, and HE groups showed significant decreases, and the concentrations of serum HDL-C in rats in the hypoxia and training groups were higher than those in the control group (P< 0.05), which was similar to the findings of Li *et al*.[72]. Peng *et al*.[73] performed chronic intermittent hypoxia intervention in rats and found that in the hypoxia intervention group, the serum levels of TC and TG appeared to be significantly reduced in rats. Jovandaric *et al*. [74] compared the study of 50 perinatally hypoxic oxygen-requiring neonates with 50 unoxygenated healthy neonates and determined TC, HDL, LDL, and TG levels in the two groups after hypoxia classification. It was found that the levels of TC, TG, LDL, and HDL were significantly lower than those of the control group. This phenomenon may be due to the oxidation and peroxidation of lipids in the circulation of hypoxic neonates and their removal from the peripheral circulation. Guo *et al*. [75] experimentally proved that 16 weeks of aerobic exercise could affect cholesterol metabolism and lipid metabolism in obese patients and improve dyslipidemia, with a significant decrease in serum TC, TG, and LDL-C, but no significant difference in HDL-C, which is highly consistent with the changes in the hypoxic training group in this experiment.

These findings suggest that hypoxia training mainly changes blood lipid levels by regulating the concentrations of TC, TG, and LDL-C[76]. There is no evidence that HDL-C plays a role in this process. The reason for this phenomenon may be that the body's blood levels of TC and TG are reduced, and the HDL-C requirement used to transport the two to maintain a normal level of both to meet the body's needs does not show significant changes.

Hypoxia and hypoxia training induced long-term changes in plasma TC, TG, LDL-C, and HDL-C in obese rats, ultimately leading to corresponding changes in the physiological indices of the rats. Li *et al*. [72] experimentally found that body weight[75], perirenal fat, and epididymal fat in the hypoxia group were significantly lower than those in the control group (P<0.05). Both the exercise group and the intermittent hypoxia training group showed highly significant decreases relative to the control group (P< 0.01). Lei *et al*.[77] experimentally found that the lipid levels of obese rats were improved after 4 weeks in both the normoxic training group, the hypoxic quiet group, and the hypoxic training group. Among these, the Lee's index of obese rats in the 4-week group of intermittent hypoxic training was significantly decreased (P<0.05). This presented the same changes as the results of this experiment.

4 Research limitations

(1) This study was limited to PCR and Western blot for protein expression analysis in obese rat livers, lacking alternative experimental approaches (*e. g*., immunohistochemistry or mass spectrometry) to cross-validate the findings. Additionally, while miR-27 was investigated, comparative analyses of its isoforms (*e. g*., miR-27a-3p/-5p) were not conducted, which may provide further mechanistic insights.

(2) Although we demonstrated that exercise-mediated modulation of the miR-27/PPARγ pathway improves lipid metabolism-related protein and gene expression, the study did not employ miR-27 inhibitors or gene interference techniques. Future cellular-level experiments are warranted to confirm the causal relationship.

5 Conclusion

Both hypoxia and hypoxia training can negatively regulate PPARγ levels by inhibiting hepatic miR-27, thereby affecting the expression of downstream target genes *CYP7A1* and *CD36*. This process promotes hepatic cholesterol and fatty acid oxidation and enhances HDL-C transporter activity, ultimately inducing changes in physiological indexes related to lipid metabolism disorders in obese rats and improving lipid levels. In this process, the effect of hypoxia training was superior to that of hypoxia alone. Therefore, hypoxia training can be used as an effective intervention for the prevention and control of lipid metabolism-related diseases.

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低氧运动介导miR-27/PPARγ通路改善肥胖大鼠脂代谢靶基因和蛋白质水平的研究*

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摘要 目的 探讨低氧和低氧训练对肥胖大鼠肝脏中miR-27/PPARγ及其下游脂代谢相关基因、蛋白质表达水平的影响。**方法** 13周龄雄性SD肥胖大鼠随机分成3组（n=10）：常氧安静组（N组）、低氧安静组（H组）和低氧训练组（HE组）。水平跑台训练强度20 m/min（低氧浓度13.6%），持续运动1 h/d、5 d/周，共4周。称量肾周脂肪、附睾脂肪重量，称体重、量体长计算Lee’s指数；检测血清中总胆固醇（TC）、甘油三酯（TG）、低密度脂蛋白胆固醇（LDL-C）和高密度脂蛋白胆固醇（HDL-C）水平；实时荧光定量PCR和Western blot检测miR-27、PPARγ、CYP7A1和CD36相对表达量。**结果** 低氧训练降低了肥胖大鼠肝脏miR-27表达而升高了PPARγ表达。HE组肥胖大鼠肝脏miR-27表达量显著低于N组（P<0.05）；N组肥胖大鼠肝脏*PPARγ* mRNA表达量显著低于H组（P<0.05），且极显著低于HE组（P<0.01）。N组肥胖鼠肝脏PPARγ蛋白表达量极显著低于其他组（P<0.01）。肥胖大鼠肝脏脂代谢相关基因和蛋白质的表达增加。N组肥胖大鼠肝脏*CYP7A1* mRNA表达量显著低于H组（P<0.05），且极显著低于HE组（P<0.01）。N组肥胖大鼠肝脏CYP7A1蛋白表达量极显著低于H组和HE组（P<0.01）。N组肥胖大鼠肝脏CD36蛋白表达量显著低于HE组（P<0.05）。低氧训练改善了脂代谢紊乱相关生理生化指标。HE组肥胖大鼠肾周脂肪重量极显著低于N组和H组（P<0.01），N组肥胖大鼠肾周脂肪重量显著高于H组（P<0.05）；N组肥胖大鼠附睾脂肪重量显著高于H组（P<0.05），且极显著高于HE组（P<0.01）；HE组肥胖大鼠Lee’s指数极显著低于N组和H组（P<0.01）。HE组肥胖大鼠血清TC浓度极显著低于N组和H组（P<0.01）；HE组肥胖大鼠血清TG浓度极显著低于N组和H组（P<0.01）；N组肥胖大鼠血清LDL-C浓度极显著高于HE组（P<0.01）；N组肥胖大鼠血清HDL-C浓度极显著低于H组（P<0.01）。**结论** 低氧和低氧训练可能通过抑制肥胖大鼠肝脏miR-27而负调控PPARγ水平，进而影响下游靶基因*CYP7A1*和*CD36*的表达、促进肝脏胆固醇、脂肪酸氧化和HDL-C转运，最终改善肥胖大鼠脂质水平。低氧训练改善血脂效果优于单纯低氧干预。

关键词 低氧训练，miR-27，PPARγ，肥胖大鼠
中图分类号 G804.2 **DOI**：10.16476/j.pibb.2025.0249 **CSTR**：32369.14.pibb.20250249

* 中国博士后科学基金（2017M622161）资助项目。
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收稿日期：2025-05-21，接受日期：2025-06-13