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Article

Effect of aerobic exercise combined with dietary intervention on fat loss effect and the regulatory mechanism of serum irisin in adolescent obese rats based on aerobic exercise combined with dietary intervention

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Abstract: Introduction: Obesity not only affects the physical health of adolescents, but may also lead to psychological and social problems. Treatment strategies for adolescent obesity have become particularly important. Increasing evidence suggests that exercise training, especially aerobic exercise, can not only improve obesity, but may also affect obesity and metabolic diseases by regulating hormone levels in the blood. **Objectives:** The relationship between aerobic exercise combined with dietary intervention and irisin was analyzed by observing the effects of aerobic exercise combined with dietary intervention on body weight, body fat, skeletal muscle, and adipose tissue protein expression in obese rats. **Methods:** Eighty Sprague-Dawley rats were randomly divided into high-fat diet quiet, low-fat diet quiet control, low-fat diet aerobic exercise, high-fat diet aerobic exercise. During the intervention period, the high-fat group continued to be fed high-fat diet and the low-fat group was fed low-fat diet. Aerobic exercise was performed in the exercise group, and the relevant indexes were tested at the end of the intervention. **Results:** Rats in the aerobic exercise and low-fat eating intervention group had considerably lower body weights and body fat percentages than rats in the low-fat feeding calm group ($P < 0.01$). Serum irisin levels were higher in the aerobic exercise intervention group than in the quiet group ($P < 0.05$). Compared to the quiet group, the aerobic exercise intervention group's soleus muscle showed a significantly greater expression level of associated proteins ($P < 0.05$). Moreover, rats in the aerobic exercise group had significantly higher levels of associated protein expression in their white fat at the perirenal area than rats in the quiet group ($P < 0.05$). **Conclusion:** Lower dietary fat content significantly reduced body weight and body fat percentage in rats, and the fat loss effect was more obvious when combined with aerobic exercise. Therefore, the combination of aerobic exercise and dietary intervention can be used as an effective fat loss modality for adolescent obese adolescents.

Keywords: aerobic exercise; diet; intervention; adolescent obesity; rats; weight loss; serum; irisin; regulatory mechanism

1. Introduction

In addition to having an impact on adolescents' growth and development, adolescent obesity is becoming a major global public health concern. It is also intimately linked to a number of chronic illnesses, including cardiovascular disease and type 2 diabetes [1,2]. With the improvement of economic level and the change of lifestyle, the incidence of adolescent obesity is increasing, which poses a serious threat to the physical and mental health of adolescents. Therefore, it is important to explore effective interventions to control and prevent adolescent obesity [3,4]. Aerobic exercise (AE) and dietary intervention (DI) are two common methods for weight loss. AE can improve cardiopulmonary function, increase energy expenditure and promote

fat oxidation. By altering the dietary composition and lowering the consumption of foods high in calories, a reasonable DI aids in weight control [5]. AE combined with DI can more effectively improve body composition, oxidative stress and serum biochemical indices in obese adolescents. Irisin, a muscle-derived cytokine produced by exercise-induced production, has been associated with browning of adipose tissue (AT), increased energy expenditure, and weight loss [6,7]. Irisin promotes UCP1 expression by activating the p38 MAPK and ERK signaling pathways. It induces electron transfer and uncoupling of ATP production in mitochondrial oxidative respiration, thus promoting energy expenditure. To explore the effect of AE combined with DI on fat loss in rats and the regulatory mechanism of serum irisin in the related process, this study analyzes the above issues by integrating multidisciplinary research methods such as exercise physiology and molecular biology.

The novelty of the study lies in the simultaneous investigation of the impacts of dietary fat content and individual exercise status on individual serum irisin expression levels. By analyzing serum irisin expression levels, effective means of fat reduction in adolescents were identified. The significance of this study is that by analyzing the regulatory mechanism of serum irisin, adolescents who are obese during puberty will be able to adopt a reasonable and effective method to reduce fat and ensure their normal growth and development.

2. Literature review

AE positively affects fat loss through multiple mechanisms. To explore the effects of exercise modalities on elderly obese patients while dieting, Waters et al. [8] assessed physical function, gait speed, and knee strength by measuring the vo2 peak test. According to the findings, the best way to improve ectopic fat deposition and body metabolic performance in senior obese patients was to combine weight loss with aerobic and resistance training. The experimental group had AE combined with low-intensity resistance training, while the control group received standard preoperative care, according to Picó-Sirvent et al. [9] The findings revealed that some of the negative side effects of preoperative treatment could be mitigated by combining AE with mild resistance training. In an attempt to determine if there was a significant effect of aerobic mixed impact exercise modalities versus tandem yoga on body fat (BF) reduction, Chinta et al. [10] used saturated census sampling and performed adiposity measurements using an Omron tool. The findings demonstrated that both tandem yoga and mixed impact AE had an impact on BF reduction. Hassan et al. [11] employed a randomized controlled trial design to ascertain the impact of an exercise regimen on vitamin D-induced weight loss. The study took measurements of vitamin D, blood lipids, and body weight (BW). The findings demonstrated that vitamin D improved the body's general functioning state.

Irisin is strongly linked to human metabolism. Aladag et al. [12] examined how the hormone affected energy metabolism and metabolic syndrome. The findings demonstrated that irisin increased brown AT, which in turn increased energy expenditure. White AT was changed to brown AT by the irisin protein. Maak et al. [13] examined how irisin's mode of action varied throughout studies. The findings demonstrated that the use of faulty techniques to measure irisin concentration raised

doubts about numerous research linking plasma irisin levels with physiological parameters. To investigate the mechanisms by which exercise promotes cardiovascular health, Zhu et al. [14] analyzed the relevant literature by summarizing it. The results showed that exercise secreted hundreds of muscle factors by stimulating muscles to enter the blood circulation directly and target cardiac tissues. Among them, irisin was a newly discovered muscle factor. Momenzadeh et al. [15] analyzed the potential molecular mechanisms of peptides produced during physical exercise in humans. The findings demonstrated that the peptide's circulating levels were linked to improvements in immunomodulatory or regenerative mechanisms as well as metabolism-related issues, establishing it as a peptide with therapeutic significance. Aydin et al. [16] proposed the use of fructose and uric acid to induce a rat MetS model in response to the formation and intervention of metabolic syndrome (MetS), and evaluated the effects of irisin and exercise. The results showed that the combination of 10% fructose and 2% uric acid induced MetS the fastest. Whereas, irisin combined with exercise significantly reversed the decrease in adropin and the increase in betatropin, making it the best method for preventing MetS. Paczkowska et al. [17] investigated the relationship between muscle factor levels and metabolic parameters in PCOS patients by measuring serum irisin and Metrnl levels in 31 PCOS women and 18 healthy subjects. The results showed that there was no significant difference in the concentrations of irisin and Metrnl between the PCOS group and the control group, but irisin was negatively correlated with BMI, body fat mass, etc. Metrnl showed no correlation with metabolic parameters.

In summary, adolescent obesity seriously affects the normal growth and development and physical and mental health of adolescents. To explore the connection between irisin and fat reduction, the study was conducted by combining AE with DI in obese rats, and the related indexes were measured to provide reasonable reference for scientific fat reduction in adolescents.

3. Methods and materials

3.1. Experimental technology road

The rats were fed differently in two groups: the standard control group and the high-fat modeling group. The rats were divided into 4 groups for 8 weeks of AE and DI. After 8 weeks, the rats in each group were tested for relevant indexes. The technical route of the experiment is shown in **Figure 1**.

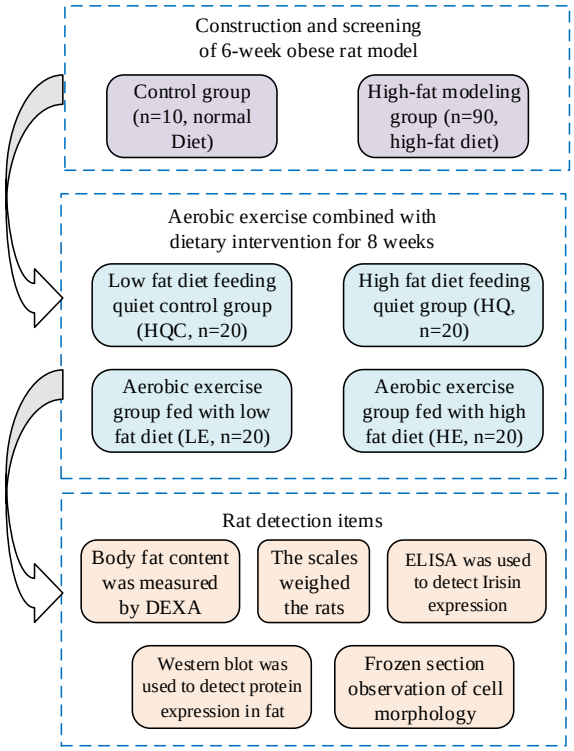


Figure 1. Technology road map.

3.2. Experimental program

3.2.1. Source of animal samples

The obesity characteristics of dietary obese rats were highly comparable to those of human obesity in the animal model of obesity that was used as the subject of the study. One hundred specific pathogen free (SPF) grade Sprague-Dawley male pups weighing 85 ± 5 g and 5 weeks old were proposed for the study. They were purchased from Shanghai Slaughter Laboratory Animals Co. This experiment was approved by the local hospital ethics committee and complies with animal welfare guidelines. There was no animal mistreatment during the experiment.

3.2.2. Establishment of an obese rat model

Ten rats were randomly selected from the rats as the normal control group and fed normal chow and the rest were fed high-fat chow. Free feeding and drinking were allowed, and the feed supply was 15 g per pup per day. The success criteria for modeling were weight gain ≥ 20%, and the Lees index of the rats was greater than that of normal rats by more than 30.

3.2.3. Animal grouping

After 6 weeks, 80 rats were selected from the successful modeling and randomly divided into 4 groups of 20 rats each. The groups were high-fat diet quiet (HQ), low-fat diet quiet control (LQC), low-fat diet aerobic exercise (LE), and high-fat diet aerobic exercise (HE). The HQ and HE groups were fed SYHF60-1 diet, while the LQC and LE groups were fed TP26342 diet. The HQ and LQC groups were restricted to an area of 10 × 10 × 20 cm after feeding, while the HE and LE groups were restricted to an area of 50 × 50 × 50 cm after feeding, with climbing and running equipment installed in the activity area. During the experiment, when the HE and LE group mice

were stationary, the experimenters stimulated them with sterile cotton swabs and other objects to prevent them from being in a stationary state. Both HE and LE group mice are required to maintain at least 2 h of exercise. The basic information of each group of mice is shown in **Table 1**.

Table 1. Comprehensive health assessment of rats before grouping.

Group	Hemoglobin (g/L)	Total bilirubin (μmol/L)	Creatinine (μmol/L)	Strain	Age (weeks)
LQC	145.3 ± 5.6	2.1 ± 0.3	25.6 ± 2.1	SD	5
HQ	143.8 ± 6.2	2.3 ± 0.4	26.3 ± 2.4	SD	5
LE	146.1 ± 4.9	2.0 ± 0.2	25.1 ± 1.9	SD	5
HE	144.5 ± 5.8	2.2 ± 0.3	26.0 ± 2.3	SD	5
P	0.654	0.332	0.608	/	/

3.2.4. AE combined with dietary intervention and outcome analysis

In the experiment, the intervention means were different for each group of rats, which were mainly reflected in the dietary fat content and the amount of exercise. After 8 weeks of experimental intervention, the individual changes of rats were observed to evaluate the changes of BF in different groups of rats. Serum irisin expression level was detected by ELISA method, and skeletal muscle irisin expression level and visceral fat content were detected by Western blot method.

3.3. Additional detection methods and reproducibility assessment

3.3.1. Mass spectrometry for serum irisin level detection

To further validate the reliability of serum irisin level detection, mass spectrometry (MS) was used as an additional method. MS analysis was performed using a high-resolution liquid chromatography-tandem mass spectrometry (LC-MS/MS) system. The following steps were performed as followed. Sample preparation: Serum samples were centrifuged at 3000 rpm for 10 min to remove debris. The supernatant was then subjected to protein precipitation with cold acetone.

LC-MS/MS analysis: The precipitated proteins were digested with trypsin, and the resulting peptides were separated on a C18 column. MS analysis was performed in positive ion mode, and irisin-specific peptides were identified based on their mass-to-charge ratio (m/z).

Quantification: Peak areas of irisin-specific peptides were quantified and compared to a standard curve generated using synthetic irisin peptides.

3.3.2. Reproducibility assessment for ELISA and Western blot experiments

To evaluate the reproducibility of the ELISA and Western blot experiments, each assay was repeated three times under the same conditions. The following steps were performed as followed. ELISA reproducibility: Serum samples from each group were tested in triplicate and the intra-assay and inter-assay coefficients of variation (CV) were calculated.

Western blot reproducibility: Protein samples from each group were analyzed in triplicate, and the band intensities were quantified using ImageJ software.

4. Research method

Dual-energy *X*-ray absorptiometry was used to measure the rats' changes in BW and form. After calibration of the relevant instruments, the rats were anesthetized and placed flat on the scanning table.

ELISA for serum irisin level: (1) Coating: the preset concentration of irisin antibody was added into 96-well plate, the plate was sealed and incubated at 4 °C overnight. (2) Wash: using an ELISA plate washer, the plate was cleaned three times with 0.05% PBST buffer. (3) Closure: after closing the plate with 100–200 μL/well of PBS buffer containing 3% BSA, it was incubated for an hour at room temperature (RT). (4) Wash again: the washing was performed according to step 2. (5) Addition of primary antibody (PA): the primary detection antibody conjugated to irisin was added, the plate was blocked and incubated at RT for 1 h. (6) Wash again: the washing was performed according to step 2. (7) Addition of secondary antibody: the HRP labeled secondary antibody was added, diluted in 0.05% PBST buffer containing 0.5% BSA, plate was blocked and incubated at RT for 1 h. (8) Wash again: the solution was removed and the plate was washed six times with 0.05% PBST buffer using an ELISA plate washer. (9) Color development: after adding the TMB substrate solution, the mixture was incubated at RT for seven min, or until the appropriate level of color intensity was achieved. (10) Termination of reaction: the reaction was terminated by adding 2 M H2SO4 solution to each well [18,19].

Western blot protein blotting test: (1) Sample preparation: the concentration of protein samples was determined according to the experimental needs. (2) SDS-PAGE electrophoresis: the protein samples were mixed with the up-sampling buffer, denatured by heating, and then loaded into a polyacrylamide gel for electrophoretic separation. (3) Transfer: the electrophoresed proteins were transferred from the gel to the solid phase carrier. (4) Closure: the closure solution was used to close the non-specific binding sites on the membrane. (5) PA incubation: the membrane was incubated with the specific PA in the appropriate diluent. (6) Wash: to get rid of the unbound PA, the membrane was cleaned using a washing buffer (such as TBST). (7) Secondary antibody incubation: the horseradish peroxidase was used to bind the PA. (8) Wash: the membrane again was cleaned with wash buffer. (9) Assay development: a fluorescence detection system was used to visualize the target protein bands [20,21].

Frozen section to detect cell morphology: (1) Quick-freezing: a tiny bit of skeletal muscle was flattened out in a specific box or a soft plastic bottle cap. The tissue was quickly frozen into blocks in ten to twenty seconds after the unique box was carefully lowered flat into a tiny cup filled with liquid nitrogen. (2) Fixation: the sample tray was coated with a layer of OCT embedding adhesive, and the frozen tissue was placed on it and pre-cooled at 4 °C for 5–10 min to allow the OCT adhesive to soak into the tissue. The tissue was removed and placed on a tin foil or slide. Tissue was placed on a sample tray, another layer of OCT adhesive was added to it, and it was placed on a quick-freezing rack for 30 min. (3) Sectioning: the sectioning was performed using a thermostatic freezer sectioning machine with a RT of −15 °C~−20 °C. After sectioning, the slices were fixed in acetone at 4 °C for 5–10 min and oven dried for 20 min. After sectioning, it was left at RT for 30 min and then fixed by adding 4 °C acetone for 5–10 min and oven dried for 20 min. PBS was washed for 5 min × 3 times for antigenic

thermal repair. The incubation was done in 3% H_2O_2 for 5–10 min. (4) Staining and observation: dropwise addition of reagents A, B and C and observation of color development [22,23].

5. Result

5.1. Changes in body weight of rats after AE combined with dietary intervention

Rats' BWs were weighed and recorded every week for the duration of the 8-week intervention. **Table 2** displays the statistical findings. Rats in the HQ group saw a gradual decline in BWs, while rats in the LQC, LE, and HE groups saw a gradual gain. Rats in the LQC, LE, and HE groups had significantly lower BW than the HQ group at the end of the 8-week intervention ($P < 0.01$). Rats in the LE group had a lower BW than those in the HE group ($P < 0.05$). The weight loss was most significant in the LE group, indicating that a low-fat diet combined with AE had the best effect on weight control. Despite the lower weight loss rate observed in the HE group compared to the LE group, it remained significantly higher than that of the HQ group. These findings suggest that AE can effectively contribute to weight reduction in high-fat diet contexts. Overall, the combination of DI and AE has a synergistic effect on weight management.

Table 2. Changes in rat body weight (g).

	LQC	HQ	LE	HE
Pre-adaptation	630.54 ± 12.85	628.36 ± 20.04	630.32 ± 10.35	629.75 ± 8.62
1	625.54 ± 12.36	630.52 ± 24.31	610.74 ± 18.62	625.54 ± 18.34
2	605.61 ± 20.35	635.56 ± 14.65	600.32 ± 20.94	610.47 ± 24.21
3	591.73 ± 18.37	641.78 ± 23.64	580.48 ± 14.69	601.18 ± 19.32
4	582.34 ± 23.45	648.86 ± 13.24	568.69 ± 20.34**	592.67 ± 18.67
5	570.43 ± 24.37	653.79 ± 12.45**	561.37 ± 19.34**	580.76 ± 14.21**
6	565.14 ± 12.96	655.63 ± 23.64	555.57 ± 14.68	575 ± 12.57**
7	559.74 ± 23.16	658.58 ± 13.57**	545.64 ± 15.68**	568.59 ± 13.98
8	555.43 ± 14.39	661.52 ± 15.45**	530.76 ± 20.54**	561.56 ± 23.65

Note: * $P < 0.05$, ** $P < 0.01$, compared with the LQC group.

5.2. Changes in BF in rats after AE combined with dietary intervention

This research was conducted to count the weekly changes in BF content of the rats in each group and the results are shown in **Table 3**. Rats in the HQ group had a considerably higher BF content ($P < 0.01$). Both the LQC and LE groups experienced a significant drop in BF ($P < 0.01$). At the end of the eighth week, the BF content of the LE group decreased by about 5 g compared to that of the LQC. The BF content of the HE group also decreased but only by about 8g compared to that of the LQC and the LE. The BF content fluctuated from the third to the eighth week around 42–40 g without a significant decreasing trend. The LE group demonstrated the most substantial weight reduction, suggesting that a low-fat diet in conjunction with AE is optimal for weight management. While the weight loss rate in the HE group was lower than that in the LE group, it was nevertheless significantly higher than that in the HQ

group, indicating that AE can effectively reduce weight even in high-fat diet contexts. In summary, the combination of DI and AE exhibits a synergistic effect on weight management.

Table 3. Changes in BF content in rats (g).

	LQC	HQ	LE	HE
1	45.32 ± 5.40	50.52 ± 4.31	40.74 ± 1.47**	48.54 ± 3.85**
2	39.61 ± 1.35	52.56 ± 4.65	35.95 ± 1.96	45.47 ± 4.21
3	37.86 ± 4.35	54.78 ± 1.57**	34.48 ± 4.67**	42.18 ± 1.87**
4	35.87 ± 2.34	57.86 ± 3.24	32.36 ± 5.25**	40.67 ± 5.42
5	34.43 ± 4.32	58.79 ± 2.45**	30.52 ± 2.98**	39.76 ± 4.21
6	33.14 ± 2.96	60.63 ± 4.63	29.57 ± 4.96	40.18 ± 2.57**
7	34.74 ± 3.15	63.58 ± 3.57**	28.46 ± 5.68**	42.59 ± 3.97
8	33.43 ± 4.25	64.52 ± 5.45**	28.76 ± 4.37**	40.56 ± 5.49

Note: * $P < 0.05$, ** $P < 0.01$, compared with the LQC.

5.3. Serum irisin levels in rats after AE combined with dietary intervention

Figure 2 displays the serum irisin content statistics for each group of rats. **Figure 2a** displays the statistics of serum irisin content in LQC and HQ groups. **Figure 2b** shows the statistics of serum irisin content in LQC and LE groups. **Figure 2c** shows the statistics of serum irisin content in LQC, HE group.

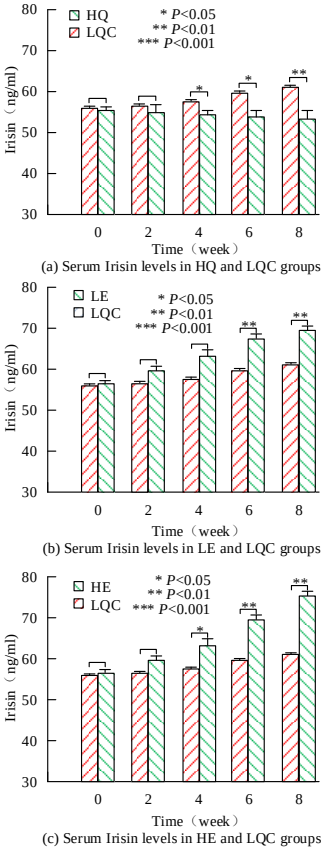


Figure 2. Serum irisin content in each group before and after intervention.

The gradual increase in serum irisin levels in HE and LE groups during the intervention was basically the same. After the 6 weeks, there was a significant difference (SD) ($P < 0.01$) in the irisin levels in both groups and the LQC group. irisin levels gradually decreased in the HQ group and were significantly different in both groups compared to the LQC group after week 8 ($P < 0.01$). The level of irisin in the HQ group underwent a gradual decrease, indicating that a high-fat diet may inhibit the secretion of irisin. The LE group exhibited the highest increase in irisin levels, suggesting that a low-fat diet combined with AE had the most significant promoting effect on irisin. The increase in irisin levels exhibited a positive correlation with the decrease in body weight and body fat, indicating that irisin may play an important role in the process of weight loss.

5.4. Changes in rat soleus muscle and fat wet weight after AE combined with dietary intervention

The fat wet weight of rat soleus muscle is shown in **Table 4**. The muscle wet weight of HE and LE groups was slightly increased compared to HQ group, while LQC group was slightly decreased compared to HQ group ($P > 0.05$). Rats in the HE, LE, and HQ groups had visceral fat wet weights that were considerably lower than those in the LQC group ($P < 0.01$). AE can effectively reduce visceral fat accumulation. The wet weight of visceral fat in the HQ group was significantly higher than that in other groups, indicating that a high-fat diet significantly increases visceral fat content. Overall, AE has a significant effect on reducing visceral fat, and a low-fat diet can further enhance this effect.

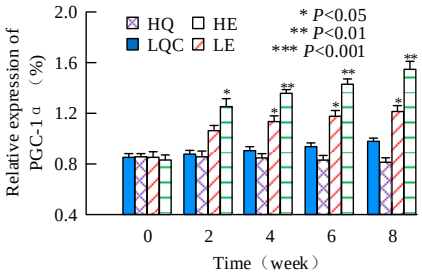
Table 4. Rat soleus muscle and fat wet weight (g).

		LQC	HQ	LE	HE
Soleus muscle	Left leg	0.28 ± 0.02	0.29 ± 0.01	0.29 ± 0.02	0.30 ± 0.02
	Right leg	0.28 ± 0.05	0.28 ± 0.03	0.30 ± 0.03	0.29 ± 0.01
Fat	Epididymis	10.86 ± 1.35	19.7 ± 4.57**	6.07 ± 2.67**	10.05 ± 1.87**
	Scapular	0.24 ± 0.034	0.60 ± 0.024**	0.25 ± 0.065**	0.35 ± 0.084**
	Perirenal	9.43 ± 1.32	29.79 ± 7.45**	5.06 ± 2.98**	14.28 ± 2.21**

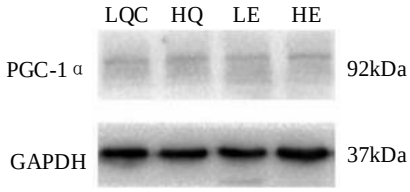
Note: * Compared with LOC, $P<0.05$** Compared with LOC, $P<0.01$*** Compared with LOC, $P<0.001$.

5.5. Expression of skeletal muscle-related proteins in rats after AE combined with dietary intervention

The statistics of the content of rat soleus muscle-related proteins in each group are shown in **Figure 3**. **Figure 3a** PGC-1α protein expression. **Figure 3b** shows the graph of Western Blot experiment results of PGC-1α protein.



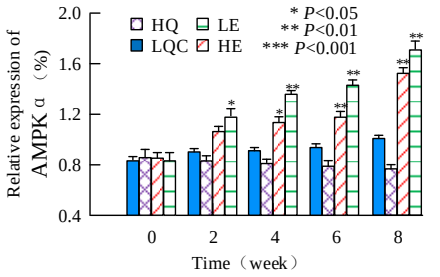
(a) Expression of PGC-1α protein in rat soleus muscle



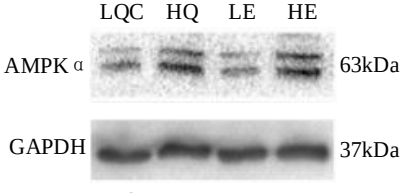
(b) Detection of PGC-1α protein expression in rat soleus muscle

Figure 3. Expression of PGC-1*α* protein in rat soleus muscle.

After week 8, the PGC-1*α* protein content in the HE group grew significantly and was significantly different from that in the LQC ($P < 0.01$). The protein content in the HQE group decreased and the difference between the HQE and LQC groups was not significant. The present study demonstrates that AE can significantly promote the expression of PGC-1*α*. The decreased expression of PGC-1*α* protein in the HQ group suggests that a high-fat diet may inhibit the expression of PGC-1*α*. PGC-1*α* is a key regulatory factor in mitochondrial biosynthesis and energy metabolism. Moreover, its increased expression is closely related to the promoting effect of AE on energy metabolism. **Figure 4** displays the expression of the AMPK*α* protein in rat soleus muscle. **Figure 4a** displays the expression of AMPK*α* protein, and **Figure 4b** shows the results of Western Blot experiment of AMPK*α* protein.



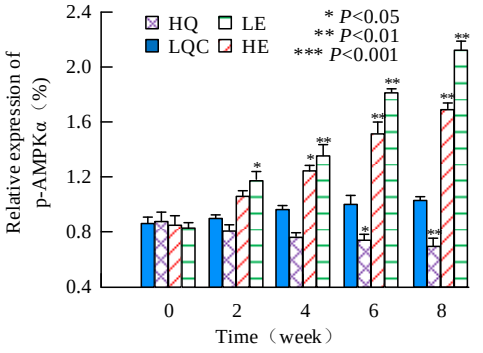
(a) Expression of AMPK α protein in rat soleus muscle



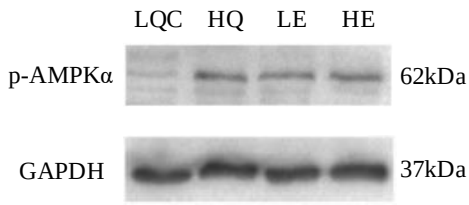
(b) Detection of AMPK α protein expression in rat soleus muscle

Figure 4. Expression of AMPK*α* protein in rat soleus muscle.

In the exercise group, the level of skeletal muscle AMPKα protein expression was significantly higher ($P < 0.01$). The level of AMPKα protein in the HQ was decreased compared with that in the LQC. AE can significantly activate the AMPKα signaling pathway. The reduced expression of AMPKα protein in the HQ group indicates that a high-fat diet may impede the activity of AMPKα. AMPKα is a pivotal regulatory factor in energy metabolism, and its augmented expression is associated with the stimulatory effect of AE on fat oxidation and energy expenditure. The p-AMPKα protein expression in rat soleus muscle is shown in **Figure 5**. **Figure 5a** shows the expression of *p*-AMPKα protein, and **Figure 5b** shows the graph of the results of Western Blot experiments of *p*-AMPKα protein.



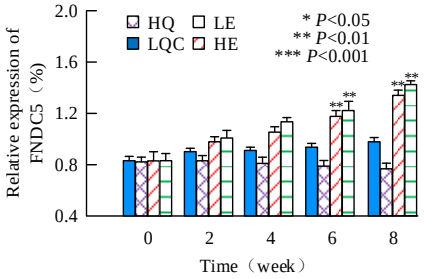
(a) Expression of PGC-1α protein in rat soleus muscle



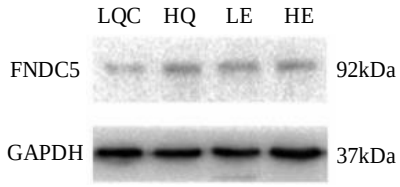
(b) Detection of p-AMPKα protein expression in rat soleus muscle

Figure 5. Expression of *p*-AMPKα protein in rat soleus muscle.

The *p*-AMPKα protein level was significantly higher in the LE and HE groups than in the LQC group ($P < 0.01$). In comparison to the LQC group, the HQ group's AMPKα protein level was lower ($P < 0.01$). This indicated that AE could significantly activate the phosphorylation of AMPKα. The decline in *p*-AMPKα protein levels observed in the HQ group indicated that a high-fat diet may impede AMPKα phosphorylation. The activation of *p*-AMPKα was associated with enhanced energy metabolism, thereby corroborating the promoting effect of AE on fat oxidation and energy expenditure. FNDC5 protein expression is shown in **Figure 6**. **Figure 6a** shows the FNDC5 protein expression, and **Figure 6b** shows the results of Western Blot experiment of FNDC5 protein.



(a) Expression of FNDC5 protein in rat soleus muscle



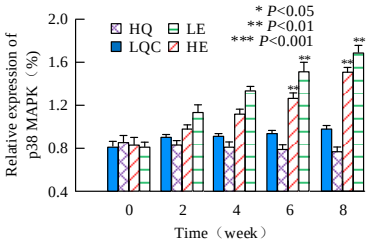
(b) Detection of FNDC5 protein expression in rat soleus muscle

Figure 6. Expression of FNDC5 protein in rat soleus muscle.

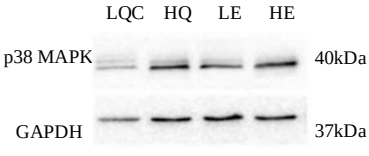
There was a significant increase in AMPKα and *p*-AMPKα protein content in the LE and HE groups, and both were significantly different compared to the LQC ($P < 0.01$). The AMPKα and *p*-AMPKα protein content in the HQ group was decreased compared to the LQC, and there was no significant difference. This indicated that AE could significantly promote the expression of FNDC5. The decreased expression of FNDC5 protein in the HQ group suggested that a high-fat diet could inhibit the expression of FNDC5. FNDC5 was a precursor protein of irisin, and its increased expression was closely related to the increase in serum irisin levels, further supporting the role of irisin in the process of weight loss.

5.6. Expression of adipose-related proteins in rats after AE combined with dietary intervention

Figure 7 displays the perirenal adiponectin expression in the rat adipose kidney. **Figure 7a** displays the expression of p38 MAPK protein. **Figure 7b** shows the results of Western Blot experiment of p38 MAPK protein.



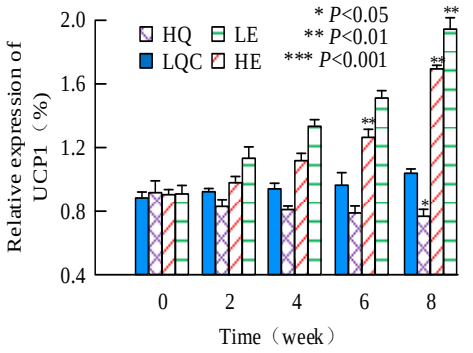
(a) Expression of p38 MAPK protein in rat soleus muscle



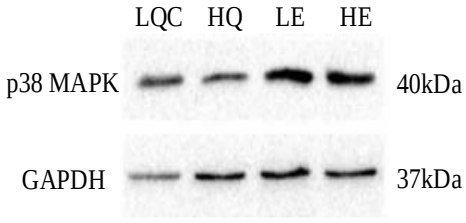
(b) Detection of p38 MAPK protein expression in rat soleus muscle

Figure 7. Expression of p38 MAPK protein in perirenal white fat in rats.

Compared with LQC, there was a SD in p38 MAPK protein levels in HE and LE groups ($P < 0.01$), and the increase was more obvious in LE group. Moreover, the p38 MAPK protein level was decreased in the HQ ($P > 0.05$). This indicated that AE could significantly activate the p38 MAPK signaling pathway. This indicated that AE could significantly promote the expression of FNDC5. The decreased expression of FNDC5 protein in the HQ group suggested that a high-fat diet could inhibit the expression of FNDC5. FNDC5 was a precursor protein of irisin. Moreover, its increased expression was closely related to the increase in serum irisin levels, further supporting the role of irisin in the process of weight loss. UCP1 protein expression in white fat at the periphery of the rat kidney is shown in **Figure 8**. **Figure 8a** shows UCP1 protein expression. **Figure 8b** is a graph of the results of Western Blot experiments of UCP1 protein.



(a) Expression of UCP1 protein in rat soleus muscle



(b) Detection of UCP1 protein expression in rat soleus muscle

Figure 8. Expression of UCP1 protein in perirenal white fat in rats.

UCP1 protein level was significantly increased in LE and HE groups ($P < 0.01$). The increase in UCP1 protein level was higher in the LE group. Rats in the HQ had lower levels of the UCP1 protein in their fat ($P < 0.05$). This indicated that AE could significantly promote the expression of UCP1. The decrease in UCP1 protein levels in the HQ group suggested that a high-fat diet could inhibit UCP1 expression. UCP1 was a mitochondrial uncoupling protein, and its increased expression was closely associated with browning and energy expenditure in AT, further supporting the beneficial effects of AE on fat metabolism.

5.7. Frozen sections of rat adipose cells

Frozen sections of AT at the epididymis of rats in each group were performed at the end of the intervention. The mean value of adipose cell size was measured for each

group of rats and expressed as the number of pixel dots. Frozen sections of rat AT are shown in **Figure 9**.

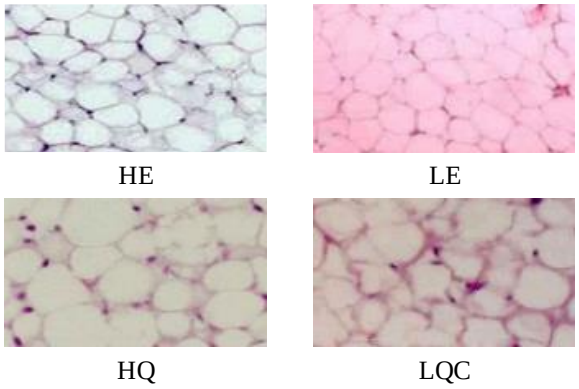


Figure 9. Frozen section of rat adipose cells.

The mean values of adipose cell size in LQC, HQ, LE, and HE groups were 6.34 mm^2, 7.56 mm^2, 4.69 mm^2, and 5.32 mm^2, respectively. There was a significant difference in the adipose cell size in HE and LE groups compared to LQC group ($P < 0.01$). There was an SD in cell size in the HQ compared to the LQC ($P < 0.05$). A high-fat diet could significantly increase the volume of fat cells. The decrease in adipocyte size was closely related to the decrease in body fat content, further supporting the fat-burning benefits of AE.

5.8. Reproducibility of ELISA and Western blot experiments

The results of the MS analysis were in agreement with the ELISA results and showed a strong correlation ($R^2 = 0.92$, $P < 0.01$). This confirms the reliability of the ELISA method for the determination of serum irisin levels. The reproducibility of the ELISA and Western blot experiments was evaluated by repeating each assay three times. The results are summarized in **Table 5**.

Table 5. Reproducibility of ELISA and Western Blot Experiments.

Assay		Intra-Assay CV (%)	Inter-Assay CV (%)	Correlation (R^2)	*P*
ELISA		4.2	6.8	0.92	< 0.01
Western Blot	PGC-1α	5.1	7.2	0.89	< 0.01
	AMPKα	4.8	6.9	0.91	< 0.01
	p-AMPKα	5.3	7.5	0.88	< 0.01
	FNDC5	4.9	7.1	0.90	< 0.01

In **Table 5**, the intra-assay CV was 4.2%, and the inter-assay CV was 6.8%, indicating high reproducibility. The intra-assay CV for PGC-1α, AMPKα, *p*-AMPKα, and FNDC5 were 5.1%, 4.8%, 5.3%, and 4.9%, respectively. The inter-assay CVs for these proteins were 7.2%, 6.9%, 7.5%, and 7.1%, respectively, indicating consistent results across repeated experiments. The reproducibility data were included in the results section (Section 5) to provide a comprehensive evaluation of the experimental methods. The reproducibility data demonstrate that both ELISA and Western blot

methods are highly reliable for the detection of serum irisin levels and protein expression in skeletal muscle and AT.

6. Conclusions

Numerous variables promote adolescent obesity, including including genetics, dietary nutrition and eating behavior, physical activity and static lifestyle, and sleep behavior. Moreover, adolescence is the stage of the most active adipose development and the most rapid proliferation of adipose cells, which is a physiologically sensitive period prone to obesity [24]. Adolescent obesity can cause metabolic abnormalities such as hypertension, dyslipidemia, hyperglycemia, hyperuricemia, and fatty liver [25]. Conventional weight loss methods include two types, one is to increase the body's energy expenditure through AE and the other is to reduce calorie intake through DI. AE burns more calories by increasing the body's energy needs through higher heart rate and respiratory rate. A calorie is a unit of energy in the body. When calories burned exceed calories ingested, the body uses stored fat to replenish energy, resulting in weight loss. Additionally, AE induces fat cells to release fatty acids, which are used as an energy source.

Irisin, an exercise-induced myokine, exerts its effects through multiple molecular pathways, including the p38 MAPK and ERK signaling pathways. In this study, it observed that AE significantly increased the expression of p38 MAPK and UCP1 in perirenal white AT, suggesting that irisin promotes browning of white AT and increases energy expenditure. The activation of p38 MAPK by irisin triggers a cascade of events leading to mitochondrial biogenesis and increased thermogenesis, as evidenced by the upregulation of PGC-1α and AMPKα in skeletal muscle. These findings are consistent with previous studies showing that irisin activates the p38 MAPK and ERK pathways, which are critical for regulating energy metabolism and fat oxidation. In addition, the increased expression of FNDC5, the precursor of irisin, in skeletal muscle further supports the role of exercise in promoting irisin secretion. The activation of AMPKα and its phosphorylated form (p-AMPKα) in the exercise groups highlights the importance of this pathway in mediating the metabolic benefits of AE. These molecular changes not only enhance fat oxidation, but also improve insulin sensitivity, which is critical for preventing metabolic disorders such as type 2 diabetes and cardiovascular disease.

With the improvement of people's living standards, DIs have gained popularity as a form of fat loss. Kim et al. [26] stated that low-fat diet improved cardiometabolic diseases in obese individuals. Although the low-fat feed-fed quiet group in the study had significantly higher levels of FNDC5 expression, there was no discernible difference or increase. This could not be determined to be due to increased feed fat content. It can be concluded that low-fat feeding could lead to an increase in FNDC5 content in skeletal muscle, which in combination with AE could improve the fat loss effect of the organism. Perirenal white fat UCP1 protein levels in rats were significantly increased in the low-fat chow-fed group. The degree of increase in UCP1 protein level was not as significant as in the AE group and both BW and BF content of the rats were significantly reduced. Merawati et al. [27] found that visceral fat FNDC5 and UCP1 levels were similarly significantly increased in rats after reducing

energy intake. This suggested that AE or DI could increase the level of UCP1 in white AT of rats while reducing BW.

By demonstrating that AE combined with DI significantly increases serum irisin levels and activates p38 MAPK and AMPKα pathways, this study provided new insights into the molecular mechanisms of fat loss. Unlike previous studies that focused on single interventions, this study highlighted the synergistic effects of AE and DI, providing a more effective strategy to combat adolescent obesity. The detailed analysis of molecular pathways, including PGC-1α, AMPKα, *p*-AMPKα, and FNDC5, provided a deeper understanding of the metabolic benefits of AE and DI. The use of MS for irisin detection and reproducibility assessment of ELISA and Western blot experiments increased the reliability and scientific impact of the results.

The results of this study have broader implications for the prevention and treatment of adolescent obesity and related metabolic disorders. By elucidating the role of irisin in regulating energy metabolism, this study opens new avenues for the development of targeted therapies that mimic the effects of exercise. For example, pharmacological agents that activate the p38 MAPK or AMPKα pathways could potentially replicate the fat-reducing effects of AE and DI. Furthermore, the combination of AE and DI could be incorporated into public health programs to promote healthy lifestyles among adolescents. In conclusion, this study demonstrates that the combination of AE and DI is an effective strategy to reduce body weight and body fat in adolescent obese rats, with serum irisin playing a central role in this process. The activation of p38 MAPK and AMPKα pathways by irisin provides new insights into the molecular mechanisms underlying the fat-reducing effects of exercise and diet. These findings not only advance our understanding of adolescent obesity, but also offer practical strategies for its prevention and treatment.

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