doi.org/10.36721/PJPS.2024.37.6.REG.1429-1441.1

Resinacein S ameliorates the obesity in mice via activating the brown adipose tissue

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Abstract: Brown adipose tissue (BAT) is an ideal target organ for obesity treatment. Resinacein S is extracted from *Ganoderma lucidum* and can elevate Uncoupling protein 1 (UCP1) in cells, but its related effects at the animal level are not clear. The mice were fed with high-fat diet to construct obesity models and treated with Resinacein S. Fasting blood glucose and insulin concentrations were measured. The blood glucose changes were recorded by injection of glucose and insulin and the body temperature during cold stimulation were recorded. BAT was weighed and stained with HE. The blood lipid metabolism indexes were detected by kits and the UCP1 level was tested. Resinacein S could improve the obesity of mice, lower the body weight, fat weight, fat cell diameter and size, reduce fasting blood glucose, improve dyslipidemia, hyperinsulinemia and insulin resistance, enhance the tolerance to cold stimulation. After UCP1 knocking out, the body weight and blood glucose levels were raised, the blood lipid disorder was aggravated, and the heat production and BAT activity of mice were decreased, while Resinacein S up-regulated UCP1 level and activate BAT activity. Resinacein S can improve obesity in mice by up-regulating UCP1 expression to activate BAT activity.

Keywords: Resinacein S, obesity, BAT, UCP1.

Submitted on 15-08-2024 – Revised on 24-09-2024 – Accepted on 18-10-2024

INTRODUCTION

Due to the improvement living standards, high-fat and high-sugar diet is likely to cause fat accumulation in the body, causing obesity, which further leads to a series of metabolic disorders such as hyperlipidemia (Liu *et al.*, 2022). In addition, excessive fat deposition can interfere with glucose balance and promote the process of insulin resistance (Koren and Taveras, 2018). In China, the incidence of obesity is also increasing. According to the National Health and Family Planning Commission, the overweight rate and obesity rate of adults are as high as 30.1% and 11.9% respectively. The incidence of adolescent obesity in China is also increasing, which will inevitably have an immeasurable adverse impact on the physical quality of the whole people and the harmonious development of the whole society. In the long run, it will also endanger China 's national security (Cai *et al.*, 2017). Therefore, obesity has become a social problem to be solved.

Adipose tissue is important in regulating body energy, which is mainly divided into white adipose tissue (WAT) and brown adipose tissue (BAT)(Pan and Chen, 2022). WAT store energy mainly in the form of TG and their excessive accumulation and expansion can lead to obesity (Reyes-Farias *et al.*, 2021). BAT is the main source of non-shivering heat production, which is important for maintaining body temperature and energy balance (Wu *et al.*, 2012). Brown adipocytes consume energy by burning fatty acids, glucose and other substrates to produce heat (Liu *et al*., 2022). The thermogenic activity of BAT depends largely on uncoupling protein 1 (UCP1). UCP1 can catalyze the proton leakage of fatty acid oxidation, and finally release energy through oxidative phosphorylation (Chouchani *et al.*, 2019). Activated BAT has the characteristics of a large amount of glucose uptake, and is negatively correlated with age and fat content (Lidell *et al.*, 2013; Wang *et al.*, 2015). Therefore, finding small molecule compounds that can promote BAT may be an effective strategy for the treatment of obesity and its induced metabolic diseases.

Resinacein S is a triterpene compound, which is the main component of Stemless *Ganoderma lucidum*. It has been found that Resinacein S has the pharmacological effect of regulating lipid metabolism (Mao *et al.*, 2023). Resinacein S can not affect the differentiation of BAT at the cellular level, but can notably up-regulate UCP1 in BAT. It is speculated that Resinacein S has potential application value in anti-obesity and improvement of metabolic diseases (Huang *et al.*, 2020). However, it is unclear whether Resinacein S can up-regulate the expression of UCP1 in vivo and activate BAT to regulate related metabolic disorders to treat obesity.

Therefore, in this study, the obesity model of C57BL/6J and UCP1 gene knockout (UCP1 KO) mice was established to explore the effect and mechanism of Resinacein S on improving obesity. The obesity symptoms, blood glucose and lipid indexes, heat production and BAT activity of mice were detected to provide new ideas for obesity treatment.

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MATERIALS AND METHODS

Animal group and process

Thirty SPF male C57BL/6J mice, ten UCP1 KO mice and 20 littermate male WT mice, aged 6 weeks, weighing (20±2) g, were prepared and supplied by Saiye Biotechnology Co., Ltd. (Suzhou, China). The mice were feeding in usual conditions (temperature 20-24ºC, relative humidity 50% -70%, light and dark 12h/12h). This experiment has been approved by the Animal Ethics Committee.

Twenty C57BL/6J mice were fed with high-fat diet (D12492, research diets, New Jersey, USA), and 10 mice were fed with standard diet (D12450B, research diets, New Jersey, USA) as normal diet (NC) group for 18 weeks. After 11 weeks of modeling, the model mice were randomly divided into high-fat diet (HFD) group and Resinacein S (HFD+Res S) group. The HFD + Res S group was intragastrically administered with 20mg/kg Resinacein S at an interval of 48 hours, the NC group and the HFD group were intragastrically administered with the same amount of saline for 8 weeks. Meanwhile, twenty WT and ten UCP1 KO mice were fed with high-fat diet (D12492) for 18 weeks. After 11 weeks of modeling, WT mice were randomly divided into high-fat diet (WT+HFD-Vehicle) group and Resinacein S (WT+HFD-Res S) group according to body weight. UCP1 KO (UCP1 KO+HFD-Res S) group and WT+HFD-Res S group were intragastrically administered with 20 mg/kg Resinacein S once every 48 hours and WT+HFD-Vehicle was intragastrically administered with the normal saline for 8 weeks. The body weight was tested weekly from the 10th week during the feeding period. The detailed recipes for each diet are shown in table 1.

Serum test

Low-density lipoprotein cholesterol (LDL-c) kit (A113-1-1), alanine aminotransferase (ALT) kit (C009-2-1), triglycerides (TG) kit (A110-1-1), aspartate aminotransferase (AST) kit (C010-2-1), blood urea nitrogen (BUN) kit (C013-1-1), high-density lipoprotein cholesterol (HDL-c) kit (A112-1-1), creatinine (Cre) kit (C011-2-1), total cholesterol (TC) kit (A111-1-1), free fatty acids (FFA) kit (A042-2-1) was purchased from Jiancheng Bioengineering Institute (Nanjing, China). According to the kit instructions, the absorbance of serum was detected by microplate reader colorimetric method, and the contents of HDL-c, ALT, LDL-c, AST, BUN, TG, Cre, TC and FFA in serum were calculated by formula.

Determination of fat weight

After blood collection, BAT, iWAT and eWAT were dissected, weighed and recorded.

HE staining

BAT, iWAT and eWAT tissues were fixed with 4% paraformaldehyde, embedded in paraffin and then cut into 4μm thick sections. The sections were stained with HE, observed and photographed under an inverted fluorescence microscope and Imag J was used to analyze the average diameter of adipocytes.

UCP1 protein was determined by immunohistochemistry (IHC)

Paraffin sections of brown adipose tissue specimens, after conventional dewaxing, microwave for antigen repair. The slices were incubated in 3% H_2O_2 solution for 25 min and washed with PBS. 5% bovine serum albumin (V900933, Sigma-Aldrich, St. Louis, MO, USA) was added to cover the tissue evenly and the tissue was sealed for 30 min. UCP1 primary antibody (ab234430, 161000, Abcam) was added dropwise and incubated at 37ºC for 90 min. Tissues were covered with HRP-labeled goat anti-rabbit IgG secondary antibody (31460, 1610000, Invitrogen, Shanghai, China) and incubated at 37ºC for 20 min. DAB (DA1010, Solarbio) color development, tap water stop color development. Mayer hematoxylin (MHS16, Sigma-Aldrich) double staining, neutral gum sealing, observed under a microscope (OLYMPUS, Tokyo, Japan), photographed using ZEISS ZEN image acquisition software, and analyzed using Imag J software (NIH, Bethesda, MD, USA).

Determination of fasting blood glucose and insulin

Fasting blood glucose concentration was tested using a Roche blood glucose meter (Instant, ACCU-CHEK, Shanghai, China). Fasting serum insulin were measured using an insulin radioimmunoassay kit (Xinfan Biotechnology Co., Ltd., Shanghai, China) and insulin resistance index (HOMR-IR) was computed.

$$\text{HOMR - IR} = \frac{\text{Fasting blood glucose (mmol/L)} \times \text{fasting insulin level (}\mu\mu\text{U/mL}}{22.5}$$

IPGTT and IPITT detection

At the end of the experiment, intraperitoneal glucose tolerance test (IPGTT) was made. The mice were fasted 12 h in advance. The injection volume of 20% glucose solution was calculated at 2g/kg. The blood glucose was measured at 0, 15, 30, 60, 90, 120 min and the area under the curve (AUC) was computed.

After 48h, intraperitoneal injection of insulin tolerance test (IPITT) was performed and the operation was the same as IPGTT. Insulin (4 U/mL) was injected at 0.75 U/kg. The blood glucose was detected at 0, 15, 30, 60, 90, 120min and the AUC was calculated.

Determination of rectal temperature

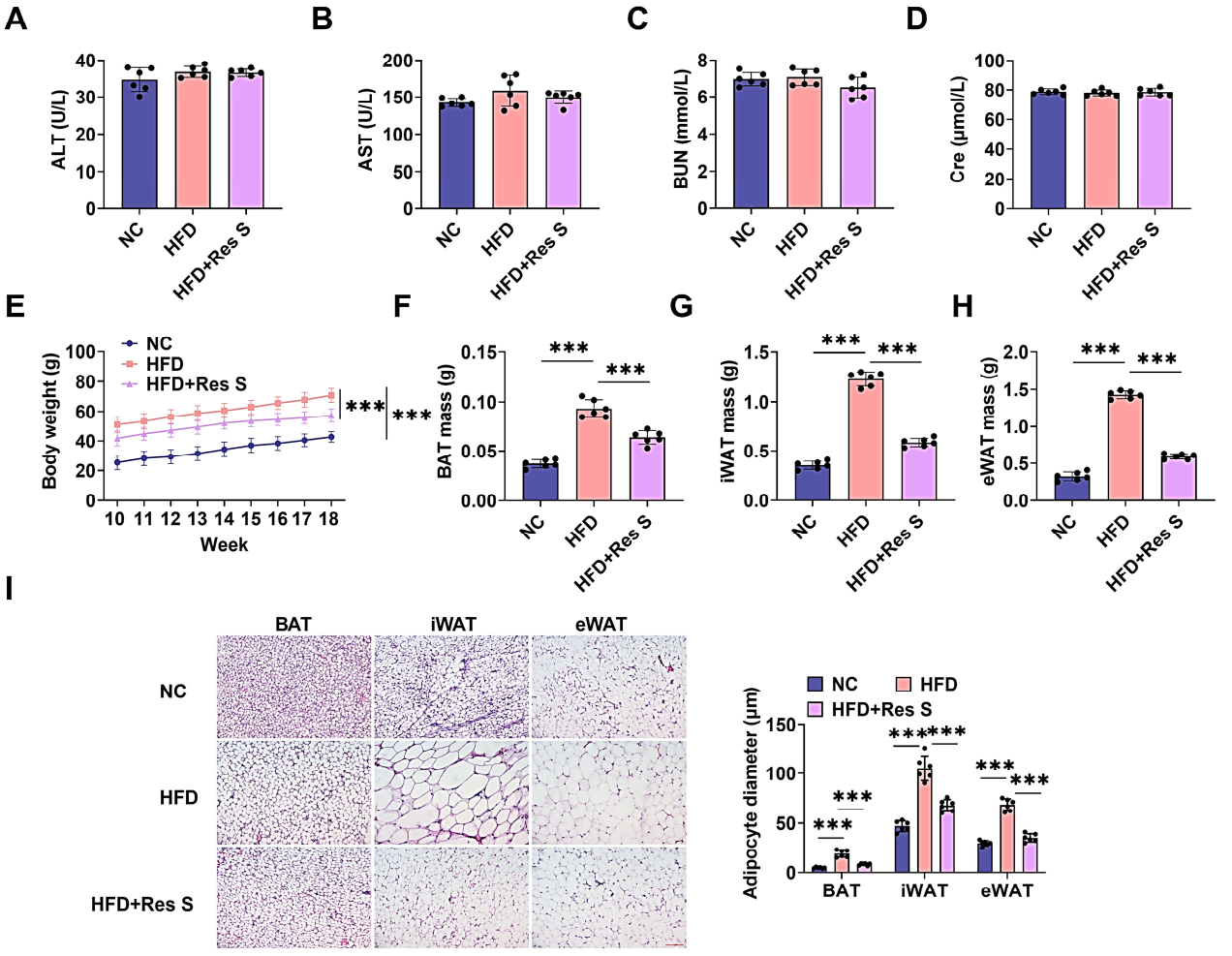
The mice were placed in a 4ºC environment for 3 h and the rectal temperature was tested every 30 min.

Measurement of O_2 consumption and CO_2 release

The oxygen consumption volume (VO_2) and carbon dioxide production volume (VCO_2) of mice were tested by TSE animal physiological telemetry system (Phenomaster, Jianyi Instrument Equipment Co., Ltd., Shanghai, China), and the monitoring data for 48h were collected.

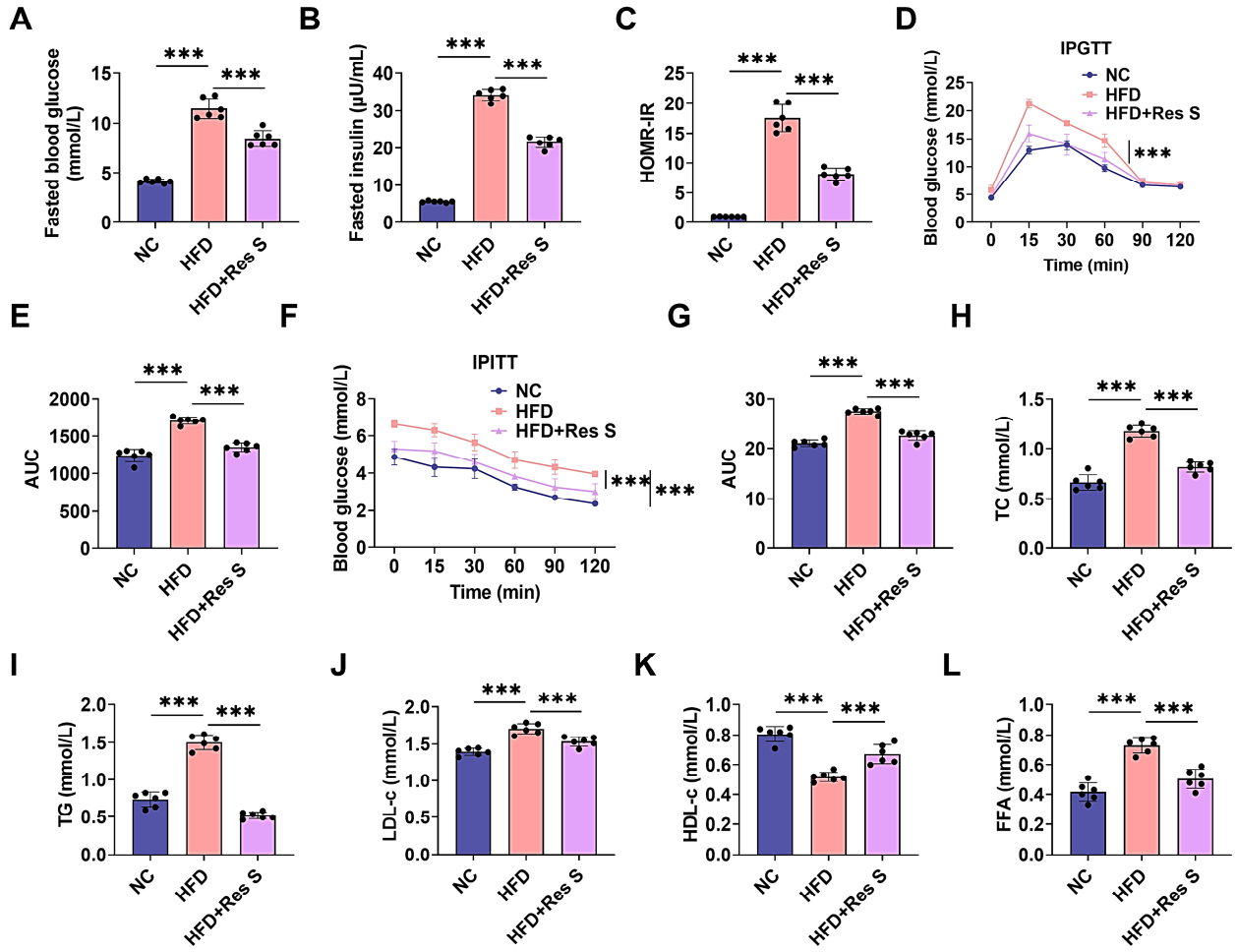
Table 1: Detailed recipes of each diet (%)

Ingredients	Standard diet (D12450B)	High-fat diet (D12492)
Corn starch	33.00	0.00
Casein	19.13	26.17
L-Cystine	0.29	0.39
Flaxseed polysaccharide	0.00	0.00
Sucrose	34.47	9.00
Cellulose BW200	4.78	6.54
Soybean Oil	2.39	3.27
Lard	1.91	32.06
Mineral Mix S10026	3.35	4.58
Vitamin Mix V10001	0.96	1.31
Choline Bitartrate	0.24	0.33
Dextrin	3.35	16.35



A-D: C57BL/6J mice were divided into NC group, HFD group and HFD + Res S group. NC group was fed with standard diet, HFD group and HFD + Res S group were fed with HFD for 18 weeks. At the 11th week, HFD + Res S group was given 20 mg/kg Resinacein S by gavage, once every 48 hours. The liver and kidney function indexes (ALT, AST, BUN, Cre) in serum of mice were detected by kit.
E: The body weight was tested weekly from the 10th week.
F-H: The weights of BAT, iWAT and eWAT were weighed.
I: HE staining BAT, iWAT, eWAT.
n=6, ***$P < 0.001$.

Fig. 1: Resinacein S can improve HFD-induced obesity in mice without affecting liver and kidney function in mice



A: The fasting blood glucose concentration was detected.
B: The fasting serum insulin level was detected by radioimmunoassay.
C: HOMR-IR.
D-E: IPGTT and the AUC.
F-G: IPITT and the AUC.
H-L: TC, TG, LDL-c, HDL-c and FFA were detected by kit.
n=6, ***$P < 0.001$.

Fig. 2: Resinacein S can improve HFD-induced fasting blood glucose, systemic insulin resistance and dyslipidemia in mice.

The mice were fed in a TSE system with a temperature of 22-25ºC, 12/12 hours of light/dark cycle, and the gas exchange volume was measured every 20 minutes. After the data collection was successful, the VO_2 and VCO_2 of each group of mice in the day and night time period were statistically analyzed.

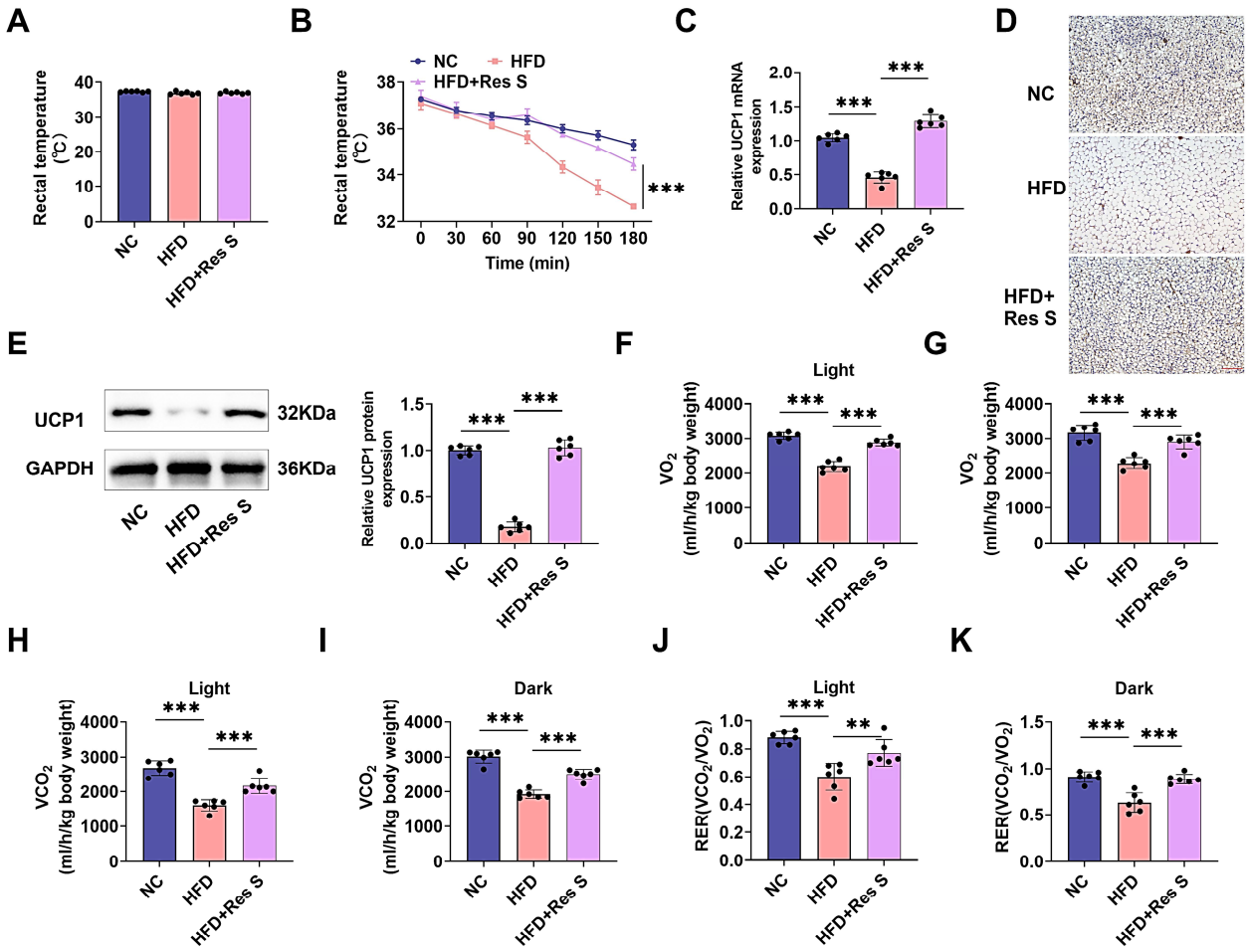
qRT-PCR assay

Adipose tissue was lysed with Trizol Up reagent (ET111-01-V2, TRANS, Beijing, China) to extract total RNA, and then AMV reverse transcriptase (2621, TAKARA, Tokyo, Japan) was added for reverse transcription to obtain cDNA. Then TB Green FAST qPCR (CN830S, TAKARA) was used for PCR amplification. The relative level of mRNA was computed by $2^{-\Delta\Delta Ct}$ method. β-actin can be used as an endogenous control.

The primer sequences: UCP1: F: 5'-GCTTTGCCTCACTCAGGATTGG-3'; R: 5'-CCAATGAACACTGCCACACCTC-3'; β-actin: F: 5'-ATCACTATTGGCAACGAGCG-3'; R: 5'-ACTCATCGTACTCCTGCTTG-3'.

Western blot assay

After the adipose tissue was collected, the supernatant was obtained by full lysis. The protein concentration was tested by the BCA kit (PC0020, Solebold). Gel preparation, electrophoresis and protein transfer to PVDF



A: Mouse basal body temperature.
B: The body temperature was changed with time after cold stimulation in a 4ºC cold storage.
C: The relative expression level of UCP1 mRNA was tested by qRT-PCR.
D: UCP1 was tested by IHC.
E: The relative expression of UCP1 protein was tested by Western blot.
F-K: Mice were housed in a TSE system at 22-25ºC for 12/12 hours of light/dark cycle, and VO_2 and VCO_2 were measured.
n=6, ***P* <0.01, ****P*<0.001.

Fig. 3: Resinacein S can increase HFD-induced thermogenesis and BAT activity in mice

membrane (YA1700, Solarbio). The cells were incubated with 5% skimmed milk powder (LP0033B, Solarbio) for 2 h. Anti-UCP1 (ab234430, 161000, Abcam) and GAPDH (TA-08, 161000, ZSGB-BIO, Beijing, China) were added and incubated in a refrigerator. On the next day, TBST buffer (T1082, Solarbio) was washed and secondary antibody (1620000) was added and incubated for 1 h. TBST buffer was washed 5 times, 5 min each time. ECL (PE0010, Solarbio) reagent was used to react for 2-3 min, and then Tanon 5200 Multi automatic chemiluminescence imaging system (Tianneng Technology Co., Ltd, Shanghai, China) was used for imaging.

Ethical approval

This study was approved by Gongli Hospital of Shanghai Pudong New Area GLYY1s2021-008.

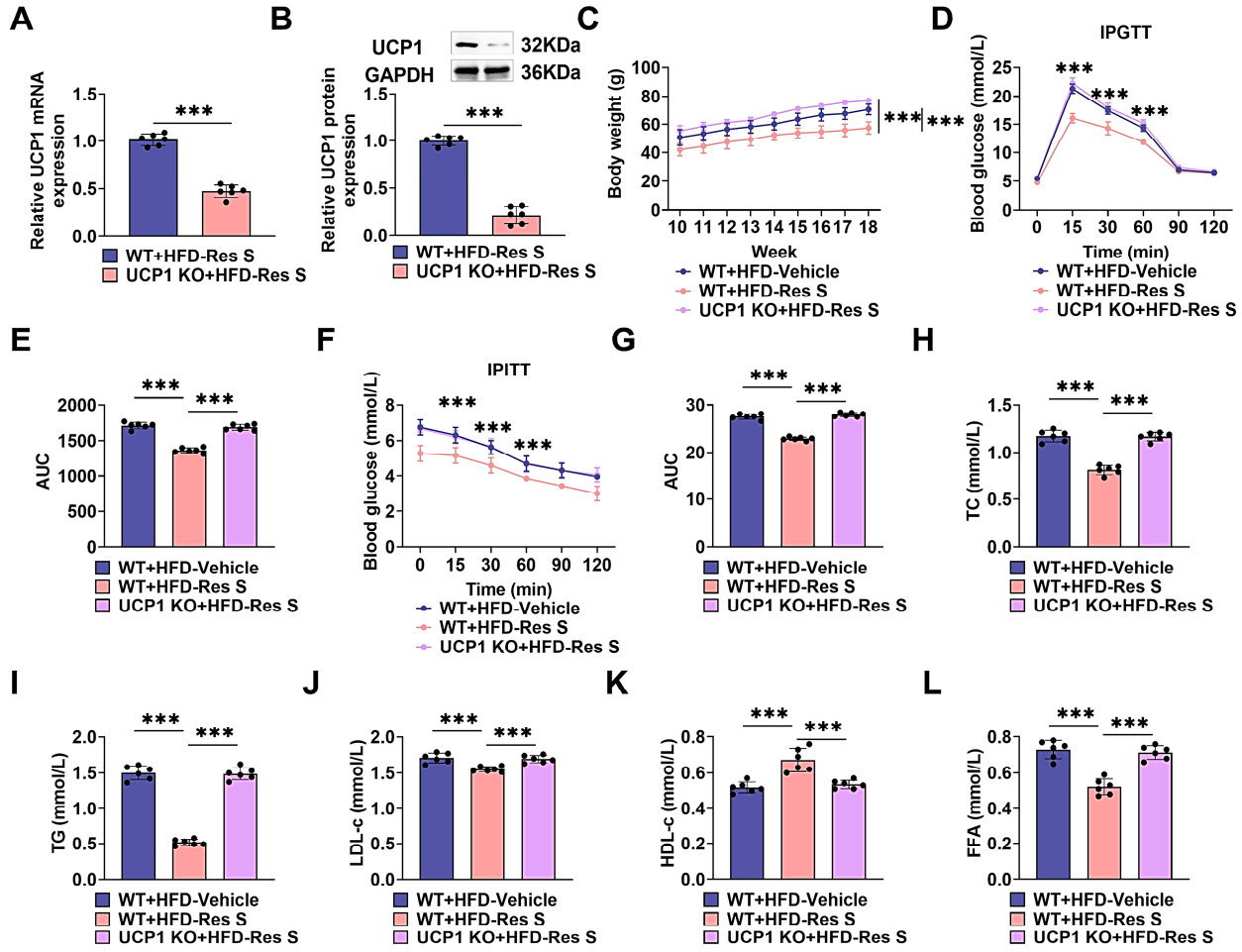
STATISTICAL ANALYSIS

All data were expressed as mean ± standard deviation. Statistical analysis and image drawing were performed using Graphpad 9.0. Student's t-test was used to analyze the difference between the two groups, and One-way ANOVA analysis was used to compare multiple groups. *P*<0.05 was considered statistically significant.

RESULTS

Resinacein S can improve HFD-induced obesity in mice without affecting liver and kidney function

We used the kit to detect the liver and kidney function indexes (ALT, AST, BUN, Cre) to explore the safety of Resinacein S. There was no remarkable difference in the contents of ALT, AST, BUN and Cre among the groups,



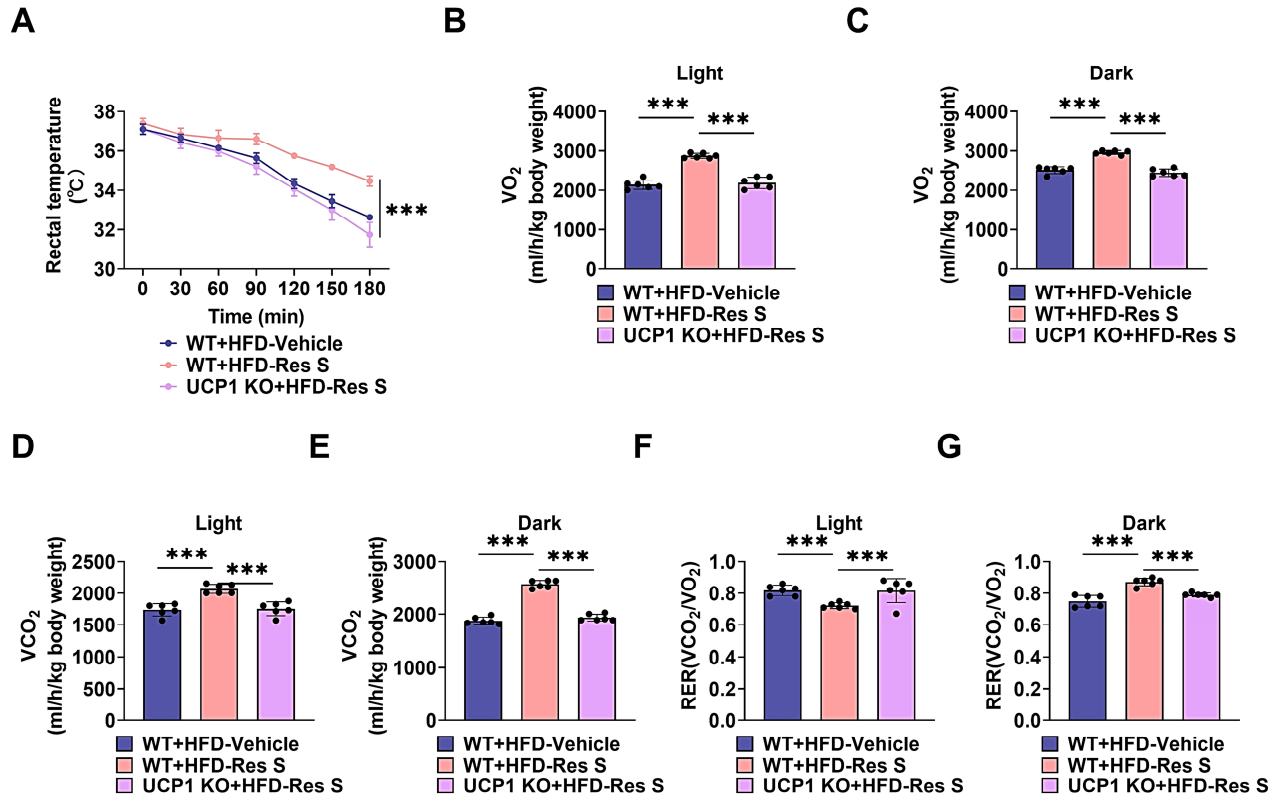
A-B: The obese mouse model was constructed by UCP1 knockout mice, and the knockout efficiency of UCP1 was detected by qRT-PCR and Western blot.
C: WT and UCP1 KO mice were fed with HFD for 18 weeks. After 11 weeks of modeling, WT mice were randomly divided into WT+HFD-Vehicle group and WT+HFD-Res S group according to body weight. UCP1 KO+HFD-Res S group and WT+HFD-Res S group were intragastrically administered with 20mg/kg Resinacein S once every 48 hours. The body weight was recorded weekly from the 10th week. After UCP1 knockout, the body weight was obviously rebounded.
D-E: IPGTT and the AUC, after UCP1 knockout showed a significant raise in blood glucose levels at 15, 30, 60 min..
F-G: IPITT and the AUC, after UCP1 knockout showed a significant raise in blood glucose levels at 15, 30, 60 min.
H-L: TC, TG, LDL-c, HDL-c and FFA were detected by kit. The dyslipidemia was observed after UCP1 knockout.
n=6, ***$P < 0.001$.

Fig. 4: Knockout BAT-specific UCP1 attenuates Resinacein S-induced improvements in HFD-induced obesity, hyperglycemia, systemic insulin resistance and dyslipidemia in mice.

indicating that the dose of Resinacein S used in the experiment had good safety (fig. 1A-D). We explored the effect of Resinacein S on obesity, we first statistically analyzed the body weight of mice. There was no obvious various in initial body weight between HFD and HFD+Res S groups. After feeding with HFD, the body weight was obviously elevated. With the augmentation of feeding time, the change of body weight was more significant (fig. 1E). Then we weighed and HE stained BAT, iWAT and eWAT. The weight and adipocyte diameter of BAT, iWAT and eWAT in HFD group were significantly increased and notably decreased after treatment with Resinacein S. (fig. 1F-I). Taken together, these findings together demonstrate that Resinacein S can improve HFD-induced obesity in mice.

Resinacein S can improve HFD-induced fasting blood glucose, systemic insulin resistance and dyslipidemia in mice

To investigate the effect of Resinacein S on blood glucose, we first measured fasting blood glucose and insulin in mice. Fasting blood glucose, insulin and HOMR-IR index were markedly rose in HFD group. After intragastric administration of Resinacein S, fasting blood glucose, insulin and HOMR-IR index were significantly decreased (fig. 2A-C). Then we carried out IPGTT and IPITT



A: The body temperature was changed with time after cold stimulation in a 4ºC cold storage. The body temperature was significantly reduced after UCP1 knockout.
B-G: UCP1 knockout mice were fed in a TSE system with a temperature of 22-25ºC, 12/12 hours of light/dark cycle and VO2 and VCO2 were detected. After UCP1 knockout, the oxygen consumption of mice was significantly reduced.
n=6, ***$P < 0.001$.

Fig. 5: Knockout BAT-specific UCP1 attenuated Resinacein S-induced decrease in HFD-induced thermogenesis and BAT activity in mice.

experiments. The results of IPGTT showed that the blood glucose at 0, 15, 30, 60 min after intragastric administration of Resinacein S was lower than that of HFD group. The results of IPITT showed that the blood glucose at 0, 15, 30, 60, 90, 120 min was lower than that HFD group. The AUC of IPGTT and IPITT in HFD group increased significantly, and reduceed significantly after treatment with Resinacein S (fig. 2D-G). Next, we used the kit to detect the contents of TC, TG, LDL-c, HDL-c and FFA. TC, TG, LDL-c and FFA were obviously rose, and HDL-c was obviously declined (fig. 2H-L). After intragastric administration of Resinacein S, the above indexes were obviously reversed, indicating that Resinacein S improved the dyslipidemia of obese mice. The above results indicate that HFD can cause raised blood glucose and dyslipidemia in mice, accompanied by hyperinsulinemia and insulin resistance in mice, while Resinacein S can improve fasting blood glucose, systemic insulin resistance and dyslipidemia in obese mice.

Resinacein S can increase HFD-induced thermogenesis and BAT activity in mice

To investigate the effect of Resinacein S on HFD-induced heat production in mice, we analyzed the body temperature changes of mice during cold stimulation. There was no remarkable inequality in the basal body temperature of mice. During the cold stimulation experiment, there was no remarkable inequality in body temperature between the groups at 0, 30, 60 min. From 90 min to 180 min of cold stimulation, the body temperature was lowered maekedly. After intragastric administration of Resinacein S, the body temperature of the HFD+Res S group was higher markedly at 90 min. The body temperature was obviously rose at 120, 150 and 180 min (fig. 3A-B), indicating that Resinacein S enhances the tolerance of high-fat diet mice to cold stimulation and increases the level of adaptive heat production in mice. BAT is a thermogenic organ in mammals. The typical feature of enhanced activity and function of BAT is the increased of UCP1 gene and increased oxygen consumption level. We measured the levels of UCP1 in BAT by qRT-PCR, IHC and Western blot. Resinacein S significantly reversed the down-regulation of UCP1 expression in HFD group (fig. 3C-E). Then, the oxygen consumption during the day and night was statistically analyzed. The VO_2, VCO_2 and RER of HFD group mice

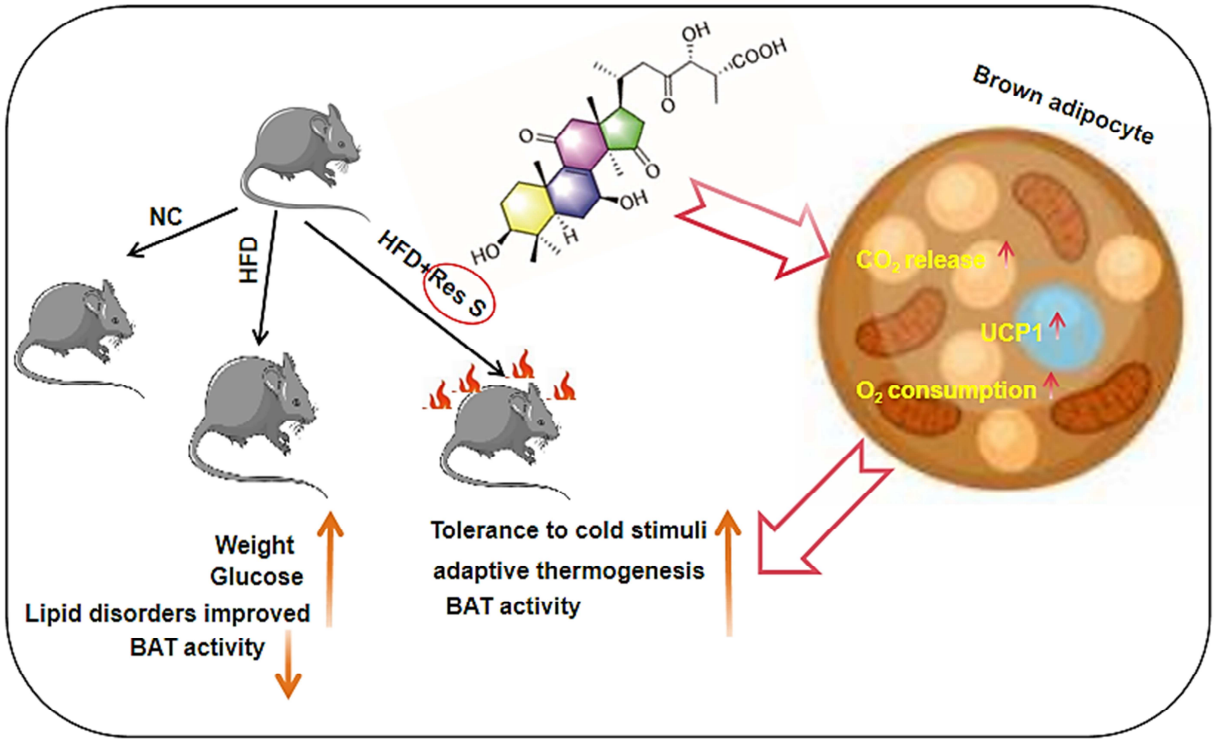


Fig. 6: Resinacein S can activate BAT activity by increasing UCP1 expression level and oxygen consumption and improve obesity in mice.

during the day and night were reduced after high-fat diet intervention. After intragastric administration of Resinacein S, the VO_2, VCO_2 and RER of mice increased (fig. 3F-K), indicating that Resinacein S can up-regulate the expression of UCP1, increase the VO_2, VCO_2 and RER, and enhance mitochondrial function. In summary, Resinacein S can improve the decrease of BAT activity induced by HFD and has the activity of activating BAT thermogenesis.

Knockout BAT-specific UCP1 attenuates Resinacein S-induced improvements in HFD-induced obesity, hyperglycemia, systemic insulin resistance and dyslipidemia in mice.

To verify the role of BAT-specific UCP1 in obese mice, we used WT and UCP1 KO mice to construct obese mouse models, treated with Resinacein S by gavage and detected related indicators. Firstly, qRT-PCR and Western blot were used to test the knockout efficiency of UCP1. The expression levels of UCP1 declined after knocking out UCP1, which confirmed that the knockdown efficiency of UCP1 was better (fig. 4A-B). Then, the body weight, IPGTT, IPITT and blood lipid indexes (TC, TG, LDL-c, HDL-c, FFA) of mice were detected. The body weight of WT+HFD-Vehicle group fed with high-fat diet increased significantly (fig. 4C), and the blood glucose level increased significantly at 15, 30, 60 min (fig. 4D-G), and dyslipidemia occurred (fig. 4H-L). The body weight and blood glucose level of WT+HFD-Res S group were obviously decreased at 15, 30, 60min and the dyslipidemia was improved. After intragastric administration of Resinacein S to UCP1 KO mice, the body weight and blood glucose level of UCP1 KO+HFD-Res S group were significantly increased at 15, 30, 60 min, and the dyslipidemia was aggravated, indicating that knockdown of BAT-specific UCP1 could attenuate Resinacein S to improve HFD-induced obesity, elevated blood glucose, systemic insulin resistance and dyslipidemia, indicating that the role of Resinacein S was related to the up-regulation of BAT-specific UCP1 expression.

Knockout BAT-specific UCP1 attenuated Resinacein S-induced decrease in HFD-induced thermogenesis and BAT activity in mice

In order to further verify the role of BAT-specific UCP1 in BAT activation, we detected the body temperature changes, VO_2 and VCO_2 of mice during cold stimulation. After intragastric administration of Resinacein S to WT obese mice, the tolerance of WT+HFD-Res S mice to cold stimulation was enhanced, the adaptive heat production level of mice was increased (fig. 5A), and VO_2 and VCO_2 were increased (fig. 5B-G). After intragastric administration of Resinacein S, the tolerance of UCP1 KO+HFD-Res S mice to cold stimulation was weakened. The level of adaptive thermogenesis in mice decreased, and VO_2 and VCO_2 decreased, indicating that knockdown of BAT-specific UCP1 attenuated Resinacein S to reduce

HFD-induced thermogenesis and BAT activity in mice, further demonstrating that Resinacein S can improve obesity in mice by up-regulating BAT-specific UCP1 expression.

DISCUSSION

The incidence of obesity in the world is increasing (Blüher, 2019), which brings inconvenience to people 's lives (Jin *et al.*, 2023). Actively exploring the mechanism of obesity and screening drugs to improve obesity on this basis has become an urgent problem to be solved by the society. Previous studies have found that Resinacein S can reduce the size of lipid droplets by regulating lipid metabolism at the cellular level, enhance BAT activity, induce mitochondrial biosynthesis and increase oxygen consumption rate, so it has potential therapeutic significance for obesity and related metabolic diseases (Huang *et al*., 2020). However, its effect and molecular mechanism at the animal level are still unknown. Therefore, this study constructed a mouse obesity model through a high-fat diet, and investigated the effects of Resinacein S on obesity, glucose and lipid metabolism, insulin resistance, thermogenesis and BAT activity, so as to explain the therapeutic effect of Resinacein S on obesity and related mechanisms. In this study, we first detected the liver and kidney function, and found that the dose of Resinacein S used in the experiment had no impact on the liver and kidney function, and had good safety, indicating that the dose could be used for subsequent experiments. Body weight and body fat content are important indicators to measure the degree of obesity(Yun *et al.*, 2018). The results showed that Resinacein S can reduce the body weight, the weight and cell size of BAT, iWAT and eWAT, indicating that Resinacein S administration can improve obesity in mice at the in vivo level, which provides a certain reference for the treatment of obesity.

Under the induction of high-fat diet, adipose tissue undergoes rapid lipid metabolism, causing free fatty acids in the blood to flow into the liver, stimulating gluconeogenesis and increasing TG synthesis (Azzu *et al.*, 2020). At the same time, the decomposition of adipocytes is increased, which aggravates the ectopic accumulation of lipids and induces lipotoxicity (Koehler *et al.*, 2012; Marcus *et al.*, 2010; Tchkonia *et al.*, 2013), thus exacerbating insulin resistance (Moreno-Navarrete and Fernández-Real, 2019; Morigny *et al.*, 2016) and eventually leading to glucose and lipid metabolism disorders (Cunha *et al.*, 2008; Unger and Zhou, 2001). Therefore, obese patients have long-term metabolic disorders, insulin resistance and other risk factors (Gutiérrez-Cuevas *et al.*, 2021). The C57BL/6J mouse strain is prone to insulin resistance and lipid metabolism disorders under HFD feeding. In this study, Resinacein S inhibited the growth of fasting blood glucose, insulin level, IPGTT and IPITT, increased the insulin sensitivity of mice, elevated the contents of TC, TG, LDL-c and FFA, decreased the content of HDL-c, and alleviated the increase of fasting blood glucose, insulin resistance and dyslipidemia induced by HFD. It is suggested that Resinacein S can improve obesity by regulating blood glucose, blood lipid and insulin metabolism disorders in obese mice.

During cold exposure, skin temperature sensors transmit signals to the preoptic area of the hypothalamus, which secretes norepinephrine, acts on the β-adrenergic receptor of BAT (van den Berg *et al.*, 2017), and stimulates BAT activity (de Las Heras *et al.*, 2018; Weiner *et al.*, 2017). BAT is dominated by the sympathetic nervous system and is mainly distributed in the scapula. Its activation can increase the consumption of white fat, reduce blood lipid levels, and alleviate obesity symptoms (Bartelt *et al.*, 2011; Kim and Plutzky, 2016; Yoneshiro *et al.*, 2013). Studies have shown that after genetic or diet-induced obese mice are implanted with normal BAT, the energy metabolism level of obese mice is increased, and insulin sensitivity is improved (Liu *et al.*, 2015; Liu *et al.*, 2013; Sugimoto *et al.*, 2022). On the contrary, BAT removal will aggravate obesity or impaired glucose tolerance (Winn *et al.*, 2017). It has also been reported that BAT is rich in multi-cavity lipid droplets and lipid droplets and mitochondria tend to gather together. UCP1 is present on the mitochondrial inner membrane and is in charge of non-shivering thermogenesis of BAT (Betz and Enerbäck, 2018; Herz and Kiefer, 2019) to prevent hypothermia (Gonzalez-Hurtado *et al.*, 2018). With the assistance of fatty acids, UCP1 transports H^+ produced during the tricarboxylic acid cycle or fatty acid β-oxidation to the mitochondrial matrix. The chemical energy contained in the proton gradient dissipates in the form of heat energy without generating adenosine triphosphate (ATP) (Bertholet *et al.*, 2022; Robinson and Kunji, 2006; Robinson *et al.*, 2008). Therefore, the UCP1 activity of BAT cells is considered to be a useful strategy against obesity. Resinacein S obviously up-regulated UCP1, enhanced mitochondrial function, and increased heat production and BAT activity. Next, to further verify the role of BAT-specific protein UCP1 in obesity in mice, we specifically knocked out UCP1 and found that knocking out UCP1 could reduce Resinacein S to improve HFD-induced obesity, elevated blood glucose, systemic insulin resistance, dyslipidemia, fever and decreased BAT activity in mice. In summary, Resinacein S may improve obesity in obese mice by up-regulating UCP1 in BAT to stimulate BAT activity and regulate the metabolic disorders of blood glucose, blood lipid and insulin in obese mice.

CONCLUSION

Based on BAT activity, our study investigated the effect and related mechanism of Resinacein S on improving obesity. It was confirmed that Resinacein S could

effectively improve the increase of fasting blood glucose, systemic insulin resistance, dyslipidemia, fever and decrease of BAT activity in obese mice after 8 weeks of administration. The mechanism was related to the activation of BAT activity by increasing UCP1 gene and oxygen consumption level (fig. 6), which offers a reliable data for targeted therapy and drug screening of obesity. However, the safety and efficacy of Resinacein S in clinical application need to be further evaluated in the future.

ACKNOWLEDGEMENT

The authors are thankful to Special Clinical Research Project of Shanghai Health Commission (202040315) and Health Commission Discipline Construction Project of Shanghai Pudong New Area- Key Department of Endocrinology and Metabolism (PWZzK2022-05).

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