Jian Pi Tiao Gan Yin alleviates obesity phenotypes through mTORC1/SREBP1 signaling *in vitro* and *in vivo*

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Background: Obesity has been considered as a leading cause of multiple metabolic syndromes, such as type 2 diabetes and hypertension cardiovascular diseases. Jian Pi Tiao Gan Yin (JPTGY), a Chinese herb preparation, is used to treat obesity of liver qi stagnation and spleen deficiency. The mechanism of action of JPTGY in obesity remains unclear. This study evaluated the effect of JPTGY on obesity.

Methods: The mechanism of action of JPTGY on obesity was investigated in high-fat diet (HFD)-induced obese mice and palmitic acid-treated 3T3-L1 cells. Lipid droplet accumulation was detected using oil red O staining. Factors associated with lipid accumulation were detected by western blotting.

Results: Treatment with JPTGY reduced HFD-induced adiposity and body weight gain. JPTGY increased the levels of brown adipose tissue biomarkers in obese mice and palmitic acid-treated 3T3-L1 cells, including peroxisome proliferator-activated receptor gamma coactivator-1-alpha (PGC-1α) and uncoupling protein-1 (UCP-1). Meanwhile, the protein expression of white adipose tissue biomarkers, such as AGT, primary subtalar arthrodesis (PSTA), and endothelin receptor type A (EDNRA), was decreased in obese mice and palmitic acid-treated 3T3-L1 cells. JPTGY affects browning of 3T3-L1 cells through mechanistic target of rapamycin complex 1 (mTORC1) signaling. JPTGY decreased the expression levels of key adipogenic-specific proteins and lipogenic enzymes, including peroxisome proliferator-activated receptor γ (PPARγ), CCAAT/enhancer binding protein α (C/EBPα), sterol regulatory element binding protein (SREBP), and FAS. Treatment with the mTOR activator MHY reversed JPTGY-mediated protein expression.

Conclusions: We concluded that JPTGY relieved obesity phenotypes through mTORC1/SREBP1 signaling *in vitro* and *in vivo*. JPTGY may benefit the attenuation of obesity.

Keywords: Obesity; Jian Pi Tiao Gan Yin (JPTGY); mechanistic target of rapamycin complex 1 (mTORC1); sterol regulatory element-binding protein 1 (SREBP1)

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Introduction

Obesity is a prevalent phenomenon in modern society and is characterized by the accumulation of body fat due to chronic excessive energy intake against energy expenditure (1,2). Obesity is considered a serious risk factor for multiple metabolic syndromes and chronic diseases, such as type 2 diabetes (3), hypertension (4), cardiovascular diseases (5), and arthritis (6), and emerging evidence even implies a correlation between obesity and poor prognosis of cancer patients (7). Therefore, it is a primary issue to decipher mechanisms for obesity thoroughly and explore effective treatments.

Rapamycin (mTOR) mainly functions by interacting with different subunits, raptor and rictor, to form the mechanistic target of rapamycin complex 1 (mTORC1) and mTORC2 complexes, respectively (8). It has been reported that depletion of raptor abolishes mTORC1 activity (9). Accumulating studies have revealed that mTORC1 participates in various metabolic processes, such as lipogenesis (10), protein synthesis (11), autophagy (12), and energy metabolism (13). Peterson and colleagues reported that inhibition of mTORC1 in the liver could impair SREBP (sterol regulatory element-binding protein) function and cause resistance to high-fat and high-cholesterol diet-induced mouse hepatic steatosis and hypercholesterolemia (14). Notably, mTORC1 is notably activated in adipose tissues from high-fat diet (HFD)-fed and obese mice (15,16). Moreover, multiple studies have indicated that excessive activation of the mTORC1 complex leads to obesity (17,18), and inhibition of mTORC1 results in the browning of white adipose tissue (WAT), promotes thermogenesis and alleviates HFD-induced insulin resistance and obesity in a mouse model (19,20). Therefore, targeting mTOR signaling in adipose tissue is a promising approach for the treatment of obesity.

The dialectical mode of traditional Chinese medicine diagnosis of obesity includes stomach and intestine excess heat, spleen deficiency, liver qi stagnation, spleen and kidney yang deficiency, and liver and kidney yin deficiency (21). Jian Pi Tiao Gan Yin (JPTGY), a Chinese herb preparation, is used to treat obesity of liver qi stagnation and spleen deficiency. The JPTGY prescription consists of Huang-Qi [Radix Astragali, roots of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao or *Astragalus membranaceus* (Fisch.) Bge., 30 g], Chai-Hu (Radix Bupleuri, the root of *Bupleurum falcatum Linne*, 12 g), Fu-Ling [*Poria cocos* (Schw.) Wolf., 15 g], Bai-Shao (Radix Paeoniae Alba, the root of *Paeonia lactiflora Pall*, 15 g), Yi-Mi (Coix seed, seed of *Coix lacryma-jobi*, 15 g), Dan-Shen (*Salvia miltiorrhiza*, the root and rhizome of *Salvia miltiorrhiza* Bge, 15 g), Pei-Lan (Herba Eupatorii, *Eupatorium fortunei* Turcz, 15 g), Jue-Ming-Zi [Semen Cassiae, the seed of *Senna obtusifolia* (L.) H. S. Irwin & Barneby, 15 g], Ze-Xie [Rhizoma Alismatis, the tuber of *Alisma orientale* (Sam.) Juzep (Alismaceae), 12 g], Shu-Da-Huang (Cooked Rhubarb, Radix et Rhizoma Rheum, the root and rhizome of *Rheum palmatum*, *Rheum tanguticum* and *Rheum officinale*, 6 g), and Shan-Zha (Crataegi Fructus, the fruit of *Crataegus pinnatifida* Bge. var. major N.E.Br., 12 g). In our previous study, JPTGY has a good weight loss effect on patients with simple obesity, which can increase the level of nesfatin-1 in serum and effectively improve lipid metabolism (22). JPTGY plays a therapeutic role by adjusting the intestinal flora in obese mice, its mechanism being related to the gut microbiota-fat signal axis (23). The mechanism of action of JPTGY in obesity remains unclear.

In this work, we applied JPTGY to a HFD obesity mouse model and found that JPTGY promoted the browning of WAT and thermogenesis through mTORC1/SREBP1 signaling, which led to alleviated obesity. Our work provides novel supporting evidence for applying traditional Chinese medicine in the prevention and therapy of obesity. We present the following article in accordance with the ARRIVE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-685/rc).

Methods

Mice model

To establish the obesity mouse model, C57BL/6J mice (3–4 weeks old, n=40) were obtained from Charles River Laboratory (Beijing, China). The experiment was carried out according to our previous study (24). Mice were randomly divided into 2 groups: the control group (n=10), which was fed a normal diet; and the obesity group (n=30), which was fed a standard HFD (60% HFD, Research Diet, D12492, USA). After eight weeks, a calculated obesity degree $[(W_{obesity} - W_{control})/W_{control}]$ over 20% was regarded as a successful model. After that, the mice were divided into the following groups and treated as indicated for 4 weeks: control (treatment with saline), obesity (treatment with saline), and JPTGY (treatment with JPTGY at 12 g/6 mL/kg). JPTGY was obtained from Shandong Provincial Qianfoshan Hospital. Saline and JPTGY were

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administered by gavage twice a day at 8 am and 4 pm, respectively. Mice in the MHY group were intraperitoneally injected with mTOR agonist (MHY1485, MCE, USA) twice a week (2 mg/kg) at 12 am on Wednesday and Sunday. The experiment was performed in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals (25) and was approved by Affiliated Hospital of Shandong University of Traditional Chinese Medicine (No. AWE-2020-046). A protocol was prepared before the study without registration.

Physiological observations

The body weight, food and water intake, and rectal temperature were recorded manually. Brown adipose tissue (BAT) and abdominal WAT were isolated from mice after euthanasia and weighed.

Serum parameters

Blood samples were collected at the end of experiments. Blood glucose was measured by an automatic biochemical analyzer (AU5800, Beckman, USA). Serum insulin, total cholesterol (TC) and total triglyceride (TG) were measured using a mouse insulin ELISA kit (SEKM-0141, Solarbio, Beijing China), a mouse TC ELISA kit (SP14914, Saipei, Wuhan, China) and a mouse TG ELISA kit (SP14979, Saipei, Wuhan, China), respectively.

Histological analysis

Liver tissues were isolated from mice after experiments, fixed in 4% polyformaldehyde, dehydrated, embedded in paraffin, and dissected into 4-μm slices. The tissue slices were stained with hematoxylin and eosin for 30 sec and Oil Red O working solution (C0157S, Beyotime) for 30 min. Images of five randomly selected sections of each group were photographed using an optical microscope (Olympus, Japan).

Cell culture and treatment

3T3-L1 cells were obtained from Cobioer Biosciences Co., Ltd. (Nanjing, China) and maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) supplemented with 10% NBCS (Gibco). To induce cell differentiation into adipocytes, cells were grown to 100% confluence and cultured with a mixture of hormones consisting of 0.5 mM 3-isobutyl-1-methylxanthine (Sigma-Aldrich, St. Louis, MO, USA), 1 mg/mL insulin (Sigma) and 1 mM dexamethasone (Sigma-Aldrich) for 2 days. Subsequently, the cells were maintained in DMEM containing insulin (1 mg/mL) for another 6 days for absolute differentiation, and the medium was changed every 2 days. For cell transfection, differentiated 3T3-L1 cells were placed in 6-well plates and transfected with siSREBP, pcDNA3.1-SREBP or the corresponding negative controls (Gene Pharma, China). Cells were treated with palmitic acid (0.6 mM, Sigma-Aldrich) for 24 h to induce lipid accumulation.

Western blotting

Proteins were extracted from cells and abdominal adipose tissue using ice-cold RIPA buffer. Total proteins (30 μg) were separated by SDS–PAGE and then transferred onto PVDF membranes. The protein bands were incubated with primary antibodies against the following proteins: PGC-1α (ab188102, 1:1,000), UCP-1 (ab234430, 1:1,000), AGT (ab213705, 1:1,000), PSAT (ab232944, 1:1,000), EDNRA (ab117521, 1:1,000), mTOR (ab134903, 1:1,000), p-mTOR (ab109268, 1:1,000, Abcam), Raptor (ab40768, 1:1,000, Abcam), SREBP (ab28481, 1:1,000, Abcam), FAS (ab82419, 1:1,000, Abcam), PRDM16 (PA5-20872, 1:1,000, Thermo), PPARγ (ab272718, 1:1,000, Abcam), C/EBPα (ab32358, 1:1,000, Abcam), and GAPDH (ab8245, 1:1,000, Abcam). The next day, the bands were incubated with anti-mouse (ab6728) or anti-rabbit (ab6721) secondary antibodies for 1 h at room temperature. The visualization of proteins was detected using an ECL kit and a gel imaging system (Tanon Science & Technology Co., Ltd., China).

Serum preparation

Serum containing JPTGY was prepared for the treatment of 3T3L1 cells. In brief, male C57BL/6J mice (n=20) were fed a normal diet for two days and then randomly separated into two groups (control and JPTGY groups). The mice in the JPTGY group were administered JPTGY (12 g/6 mL/kg) through gavage at 8 am and 4 pm every day for 3 days, followed by fasting for 12 h. Thirty minutes after gavage with JPTGY again, the abdomen was opened, and aortic blood was collected. The blood samples were left to set for 2 h, followed by centrifugation at 3,000 rpm for 10 min. Then, the serum was collected and stored at −70 ℃ for further experiments. For *in vitro* study, cells were treated with 2.5%, 5%, or 10% JPTGY drug-containing serum (JPTGY serum) or serum obtained from control (control serum).

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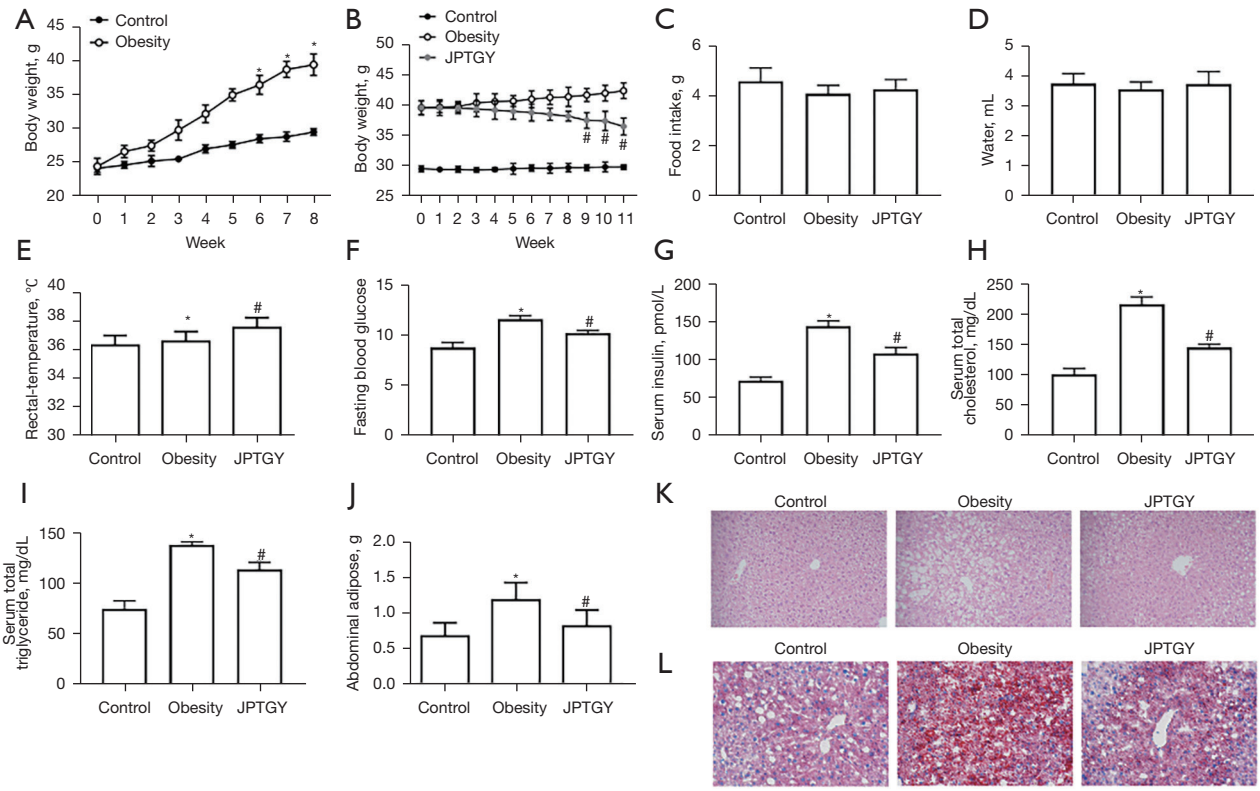

Figure 1 JPTGY treatment ameliorates HFD-induced fat accumulation and enhances the basal metabolic rate in mice. (A) An HFD-induced obesity mouse model was constructed and characterized by body weight. The mice in the established model were administered JPTGY or saline as a control, and the body weight (B), food intake (C), water intake (D) and rectal temperature (E) were recorded. (F) Blood glucose was detected using an automatic biochemical analyzer. (G-I) Serum insulin, TC and TGs were measured by ELISA. (J) The weight of abdominal adipose. (K,L) The HE (×400) and Oil Red O staining (×400) of liver tissues. *P<0.05 *vs.* control; #P<0.05 *vs.* obesity. JPTGY, Jian Pi Tiao Gan Yin; HFD, high-fat diet; TC, total cholesterol; TGs, total triglycerides; ELISA, enzyme linked immunosorbent assay.

Statistical analysis

All data are shown as the mean ± standard deviation (SD) of triplicate experiments. The statistical analysis was conducted using GraphPad Prism 7 (GraphPad Software, Inc.). Data were statistically analyzed using Student's *t*-test and one-way analysis of variance (ANOVA). P values <0.05 were considered significant.

Results

JPTGY treatment ameliorates HFD-induced fat accumulation and enhances the basal metabolic rate in mice

To assess the effect of JPTGY on obesity, we constructed an HFD-induced obesity mouse model and observed the enhanced body weight of obese mice compared with the control group (*Figure 1A*). We then validated that body weight (*Figure 1B*), food intake (*Figure 1C*), and water intake (*Figure 1D*) were slightly affected by JPTGY treatment in the mice. Interestingly, rectal temperature was increased in obese mice, and JPTGY treatment further induced this phenotype (*Figure 1E*). Blood glucose was enhanced in obese mice, and JPTGY attenuated the enhancement in the model (*Figure 1F*). Moreover, the levels of serum insulin (*Figure 1G*), TC (*Figure 1H*), and TG (*Figure 1I*) were increased in obese mice, while JPTGY treatment reversed these effects in the model. Similarly, the weight of abdominal adipose tissue was enhanced in obese mice, and JPTGY blocked this enhancement (*Figure 1J*). Consistently, fat degeneration, along with fat accumulation in liver tissues, was attenuated by JPTGY treatment in obese mice (*Figure 1K,1L*). Collectively, these results indicate

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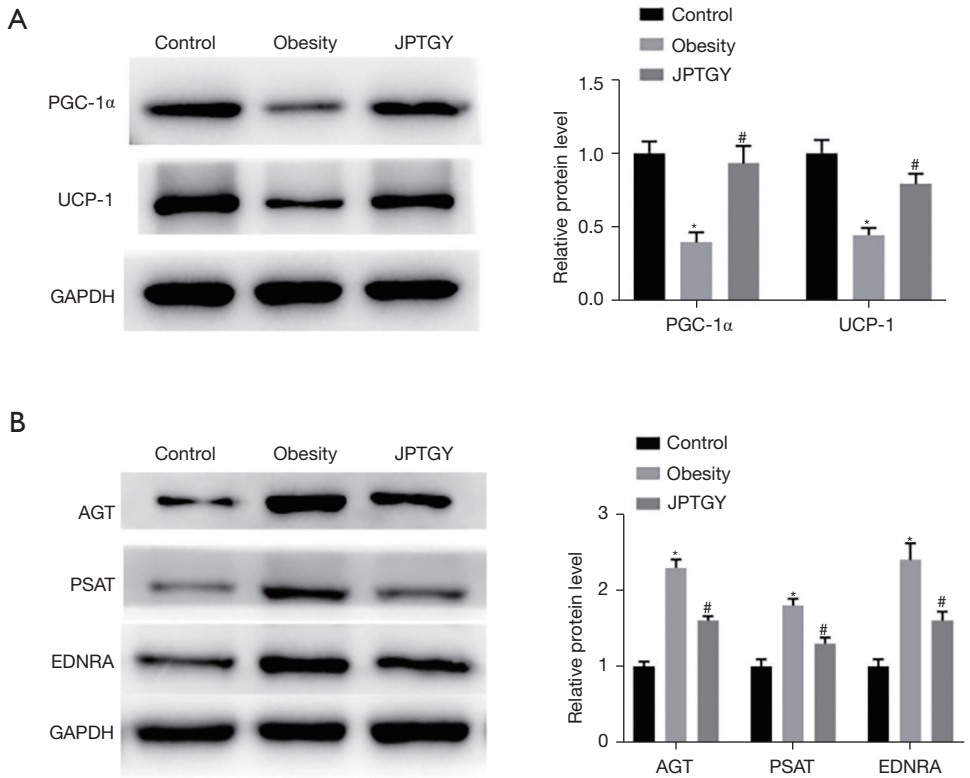

Figure 2 JPTGY treatment alters the protein expression levels of biomarkers of BAT and WAT in mice after treatment with JPTGY. (A) Protein levels of the BAT biomarkers PGC-1α and UCP-1; (B) protein levels of WAT biomarkers, including AGT, PSTA, and EDNRA, in abdominal adipose tissue. The relative protein levels were calculated and are presented in histograms. *P<0.05 *vs.* control; #P<0.05 *vs.* obesity. JPTGY, Jian Pi Tiao Gan Yin; WAT, white adipose tissue; BAT, brown adipose tissue; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator-1-alpha; UCP-1, uncoupling protein-1; AGT, angiotensinogen; PSTA, primary subtalar arthrodesis; EDNRA, endothelin receptor type A.

that JPTGY treatment ameliorates HFD-induced fat accumulation and enhances the basal metabolic rate in mice.

JPTGY treatment alters the protein expression levels of biomarkers in BAT and WAT in mice after treatment with JPTGY

Next, we further evaluated the impact of JPTGY on biomarkers of BAT and WAT. Significantly, the protein levels of BAT biomarkers, including PGC-1α and UCP-1 (*Figure 2A*), were repressed in obese mice, and treatment with JPTGY rescued the levels of BAT biomarkers in the mice. Meanwhile, the protein expression of WAT biomarkers, such as AGT, PSTA, and EDNRA, was induced in obese mice, while JPTGY treatment attenuated this induction (*Figure 2B*).

JPTGY suppresses mouse obesity through mTORC1 signaling in vivo

We then explored the potential mechanism of JPTGY-mediated obesity in mice. Given that mTORC1 signaling has been identified to regulate obesity, we investigated the effect of cotreatment of JPTGY with the mTOR activator MHY on obesity-related phenotypes in an HFD-induced obesity mouse model. The enhanced body weight of obese mice was repressed by JPTGY but increased by MHY (*Figure 3A*). Food intake (*Figure 3B*), water intake (*Figure 3C*), and rectal temperature (*Figure 3D*) were slightly affected by JPTGY treatment in the mice. Interestingly, the increased levels of fasting blood glucose (*Figure 3E*), serum insulin (*Figure 3F*), total cholesterol (*Figure 3G*), total triglycerides (*Figure 3H*), and abdominal adipose

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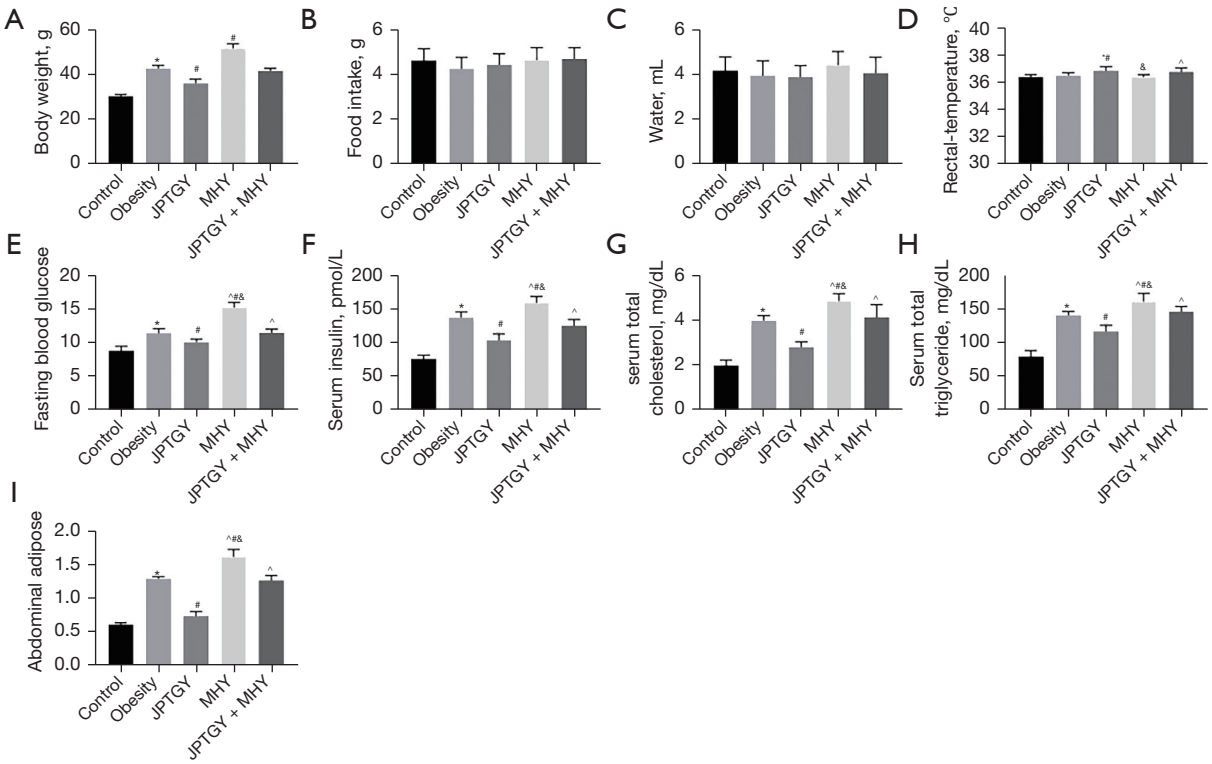

Figure 3 JPTGY suppresses mouse obesity through mTORC1 signaling *in vivo*. Mice were administered an HFD to induce obesity and were then treated with the JPTGY and/or mTOR agonist MHY. Body weight (A), food intake (B), water intake (C) and rectal temperature (D) were recorded. (E) Blood glucose was detected using an automatic biochemical analyzer. (F-H) Serum insulin, TC and TGs were measured by ELISA. (I) The weight of abdominal adipose. *P<0.05 *vs.* control; #P<0.05 *vs.* obesity; &P<0.05 *vs.* JPTGY; ^P<0.05 *vs.* MHY. JPTGY, Jian Pi Tiao Gan Yin; HFD, high-fat diet; TC, total cholesterol; TGs, total triglycerides, mTORC1, mechanistic target of rapamycin complex 1; mTOR, mechanistic target of rapamycin; ELISA, enzyme linked immunosorbent assay.

tissue (*Figure 3I*) in obese mice were suppressed by JPTGY treatment but enhanced by MHY treatment, in which MHY reversed the effect of JPTGY in the model.

JPTGY regulates biomarkers of BAT and WAT through mTORC1 signaling in vivo

We also observed that the reduced expression of UCP-1 and PGC-1α was rescued by JPTGY treatment and was further inhibited by MHY treatment, in which MHY cotreatment attenuated the effect of JPTGY in the mice (*Figure 4A*). Meanwhile, the enhanced expression of AGT, PSTA, and EDNRA was inhibited by JPTGY treatment and was further increased by MHY treatment, while MHY cotreatment relieved the effect of JPTGY in the model (*Figure 4A*). In addition, mTOR phosphorylation and raptor, SREBP, and FAS expression levels were increased in obese mice, and JPTGY treatment inhibited but MHY upregulated the phenotypes of the mice, while MHY cotreatment blocked the impact of JPTGY in the model (*Figure 4B*).

Serum containing JPTGY affects browning of 3T3-L1 cells

We then verified the function of JPTGY in the modulation of JPTGY in 3T3-L1 cells. To this end, 2.5%, 5%, or 10% serum containing JPTGY was used to treat 3T3-L1 cells, and the biomarkers of browning were analyzed in the cells. Significantly, the expression levels of UCP-1, PGC-1α, and PRDM16 were repressed and the expression levels of PARPγ and C/EBPα were enhanced in 3T3-L1 cells under palmitic acid treatment, while JPTGY treatment dose-

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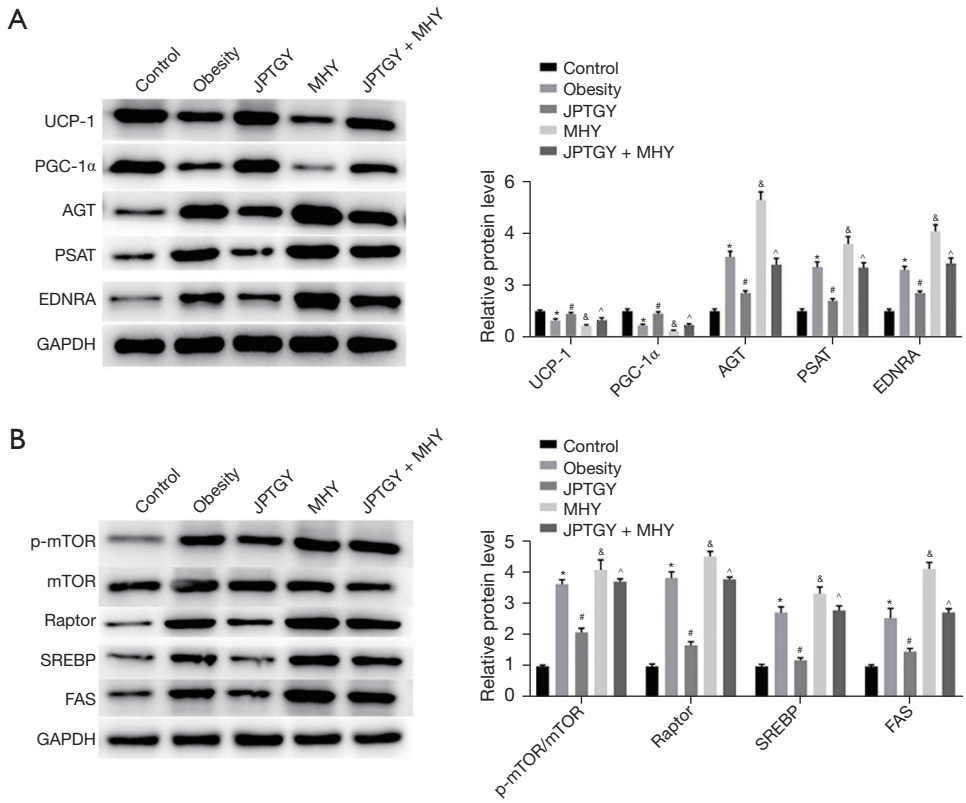

Figure 4 JPTGY regulates biomarkers of BAT and WAT through mTORC1 signaling *in vivo*. Mice were administered an HFD to induce obesity and were then treated with the JPTGY and/or the mTOR agonist MHY. Protein levels of BAT biomarkers, WAT biomarkers (A), and mTORC1/SREBP (B) in abdominal adipose tissue were detected by western blotting assay. The relative protein levels were calculated and are presented in histograms. *P<0.05 *vs.* control; #P<0.05 *vs.* obesity; &P<0.05 *vs.* control and JPTGY; ^P<0.05 *vs.* MHY. JPTGY, Jian Pi Tiao Gan Yin; HFD, high-fat diet, BAT, brown adipose tissue; WAT, white adipose tissue; mTORC1, mechanistic target of rapamycin complex 1; mTOR, mechanistic target of rapamycin; SREBP, sterol regulatory element binding protein.

dependently reversed these results in the cells (*Figure 5*).

Serum containing JPTGY affects browning of 3T3-L1 cells through mTORC1 signaling

We found that the expression levels of UCP-1 and PGC-1α were inhibited in palmitic acid-treated 3T3-L1 cells and that JPTGY treatment rescued the expression, but MHY further repressed the expression in the cells, in which MHY cotreatment attenuated the effect of JPTGY in the system (*Figure 6A*). In addition, the phosphorylation of m-TOR and the expression levels of raptor, SREBP, and FAS were upregulated in palmitic acid-treated 3T3-L1 cells; treatment with JPTGY blocked their expression, but MHY further enhanced their expression, while cotreatment with MHY relieved the impact of JPTGY in the cells (*Figure 6B*).

JPTGY regulates 3T3-L1 cell function via SREBP

Interestingly, we further observed that the overexpression of SREBP failed to affect the expression of UCP-1 and PGC-1α in control and palmitic acid-treated 3T3-L1 cells, while the overexpression of SREBP blocked JPTGY-induced expression of UCP-1 and PGC-1α in 3T3-L1 cells (*Figure 7A*). Moreover, overexpression enhanced SREBP and FAS expression in control and palmitic acid-treated 3T3-L1 cells and rescued JPTGY-inhibited expression of SREBP and FAS in the cells (*Figure 7B*).

Consistently, the depletion of SREBP by siRNA enhanced the expression of UCP-1 and PGC-1α in palmitic acid-treated but not control 3T3-L1 cells and increased JPTGY-induced expression of UCP-1 and PGC-1α in 3T3-L1 cells (*Figure 8A*). In addition, the silencing of SREBP suppressed the expression of SREBP and FAS in control

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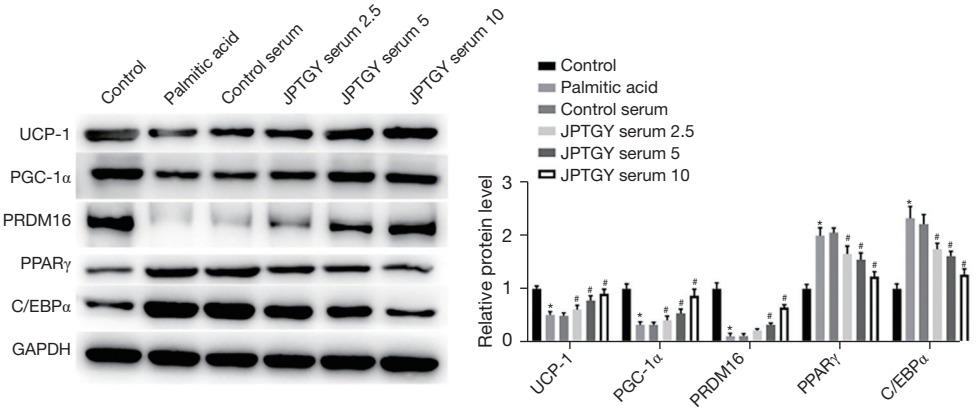

Figure 5 Serum containing JPTGY affects browning of 3T3-L1 cells. Serum was extracted from mice after treatment with JPTGY or saline. 3T3-L1 cells were treated with serum containing JPTGY at a ratio of 2.5%, 5%, or 10% or palmitic acid. The protein levels of BAT biomarkers and WAT biomarkers were detected by western blotting assay. *P<0.05 *vs.* control; #P<0.05 *vs.* control serum. JPTGY, Jian Pi Tiao Gan Yin; BAT, brown adipose tissue; WAT, white adipose tissue.

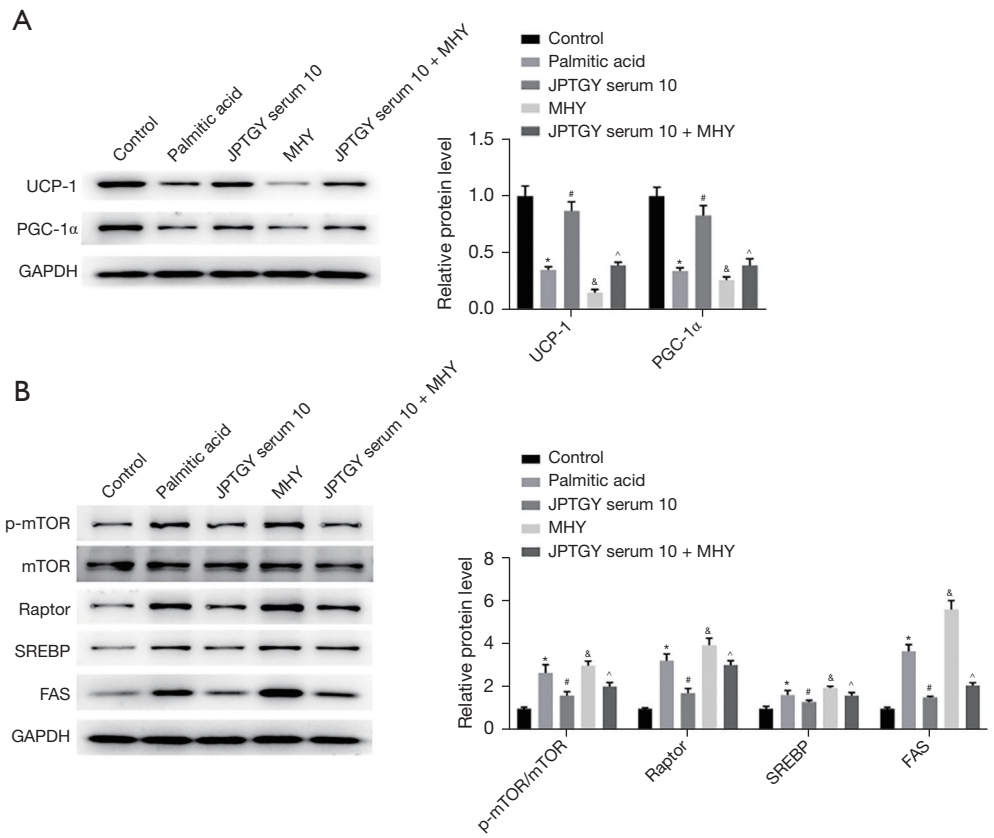

Figure 6 Serum containing JPTGY affects the browning of 3T3-L1 cells through mTORC1 signaling. Serum was extracted from mice after treatment with JPTGY or saline. 3T3-L1 cells were treated with 10% serum containing JPTGY and/or MHY. The protein levels of BAT biomarkers (A) and the mTORC1 signaling pathway (B) were measured by western blotting assay. The relative protein levels were calculated and are presented in histograms. *P<0.05 *vs.* control; #P<0.05 *vs.* palmitic acid; &P<0.05 *vs.* control and JPTGY serum; ^P<0.05 *vs.* MHY. JPTGY, Jian Pi Tiao Gan Yin; mTORC1, mechanistic target of rapamycin complex 1; BAT, brown adipose tissue.

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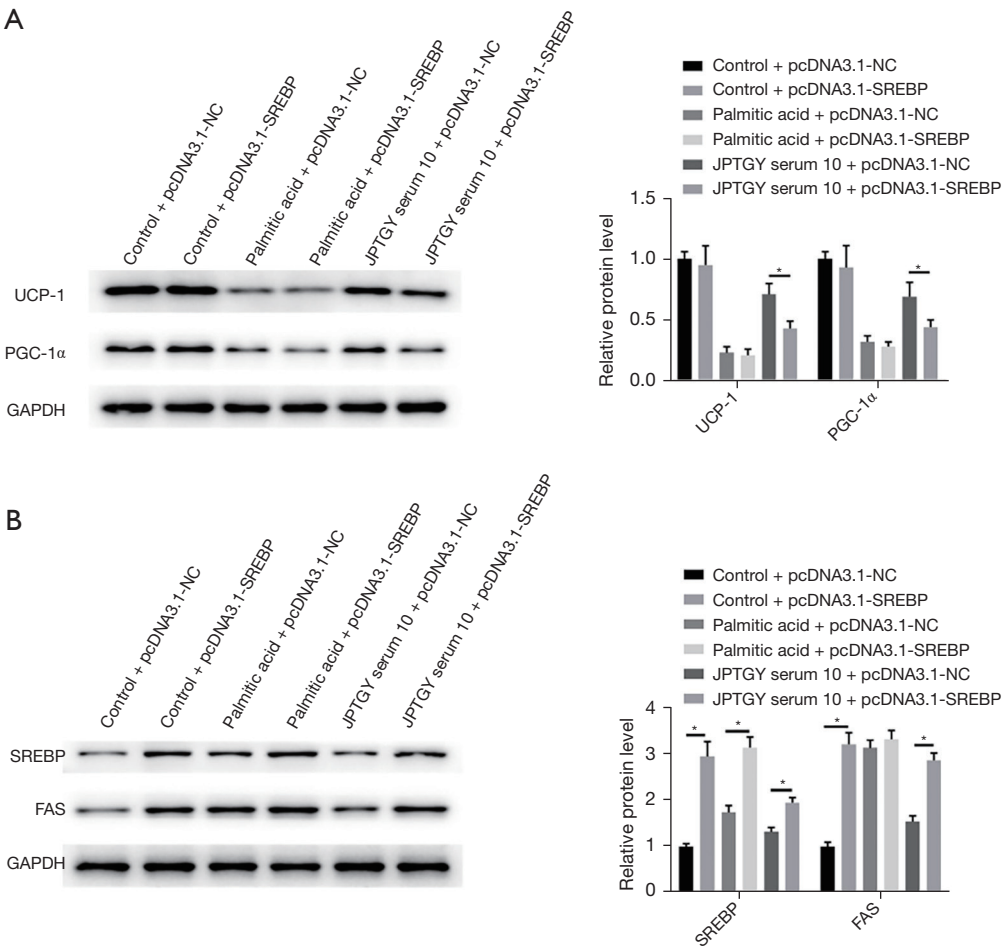

Figure 7 JPTGY regulates 3T3-L1 cell function via SREBP. 3T3-L1 cells were transfected with SREBP-overexpressing vector and treated with palmitic acid or 10% serum containing JPTGY. The protein levels of BAT biomarkers (A) and SREBP signaling (B) were evaluated by western blotting assay. The relative protein levels were calculated and are presented in histograms. *P<0.05. JPTGY, Jian Pi Tiao Gan Yin; SREBP, sterol regulatory element binding protein; BAT, brown adipose tissue.

and palmitic acid-treated 3T3-L1 cells and further inhibited JPTGY-repressed SREBP and FAS expression in the cells (*Figure 8B*).

Discussion

Multiple traditional Chinese preparations have been identified to modulate obesity. For example, Jiao-Tai-Wan regulates insulin resistance and inflammation in an obesity rat model with chronic partial sleep deprivation (26). Shaofu Zhuyu decoction ameliorates obesity-induced systemic inflammation and hepatic steatosis by regulating inflammatory cytokine and adipokine levels in the circulation and various tissues (27). In the present work, JPTGY suppressed obesity in HFD-induced obese mice.

The mechanical investigation of this study showed that the enhanced levels of fasting blood glucose, serum insulin, TC, TG, and abdominal adipose tissue in obese mice were suppressed by JPTGY treatment. Adipose tissue mainly consists of WAT, BAT, and beige adipose tissue (28). Obesity occurs due to the accumulation of WAT (29). Lipogenesis involves fatty acid synthesis and TG synthesis (30). In BAT, PGC1-α, which coactivates members of PPARγ that modulate the expression of UCP1, enhances thermogenesis (31). A previous study showed that *spirulina maxima* 70% ethanol extract treatment reduces obesity by upregulating the

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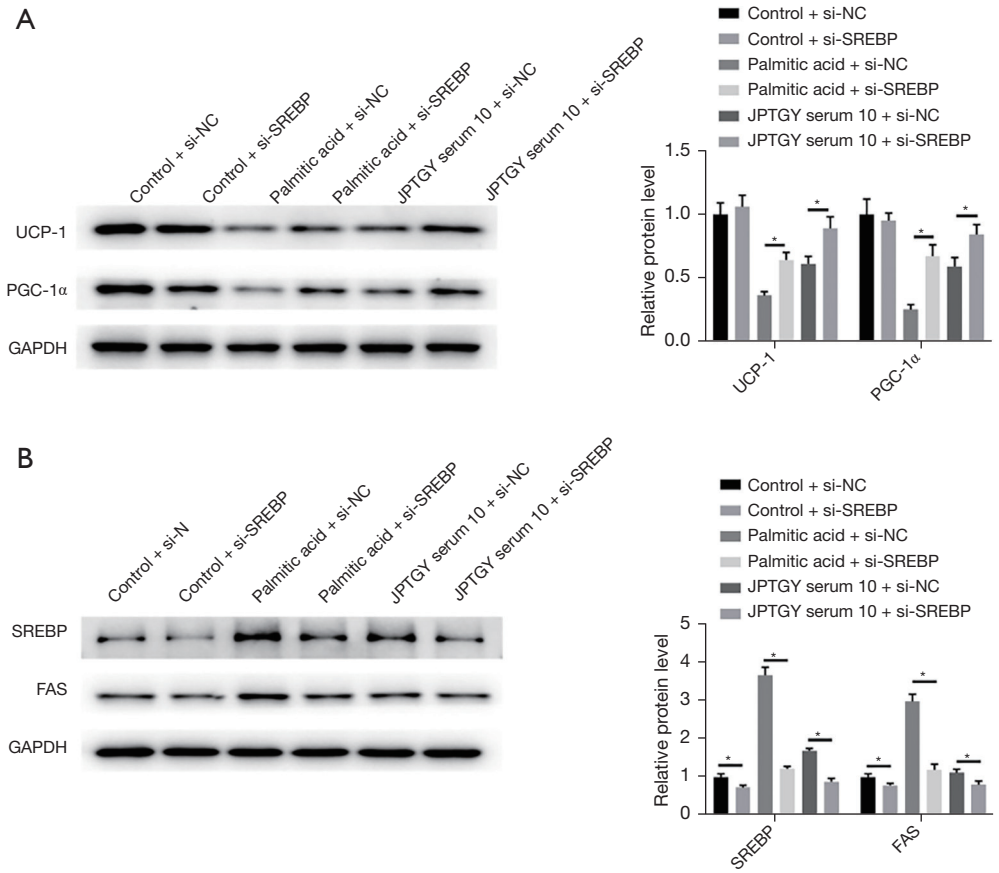

Figure 8 JPTGY regulates 3T3-L1 cell function by SREBP. 3T3-L1 cells were transfected with si-SREBP and treated with palmitic acid or 10% serum containing JPTGY. The protein levels of BAT biomarkers (A) and SREBP signaling (B) were evaluated by western blotting assay. The relative protein levels were calculated and are presented in histograms. *P<0.05. JPTGY, Jian Pi Tiao Gan Yin; SREBP, sterol regulatory element binding protein; BAT, brown adipose tissue.

expression of proteins in the thermogenic program, including PRDM16, PGC-1α, and UCP1, in WAT and BAT (32). Our findings were consistent *in vitro* and *in vivo*. The increased expression levels of adipogenic-specific proteins, including SREBP, C/EBPα, and PPARγ, and the lipogenic enzyme FAS, are associated with HFD-induced obesity (33). In the present work, the expression levels of SREBP, PPARγ, C/EBPα, and FAS were decreased in JPTGY-treated 3T3-L1 cells. Similarly, muscat bailey A grape stalk extract attenuated adipogenic differentiation by downregulating C/EBPα and PPARγ (34). Diphlorethohydroxycarmalol regulates lipid metabolism by reducing the expression levels of adipogenic-specific proteins and lipogenic enzymes, including PPARγ, C/EBPα, SREBP-1c, FABP4, and FAS, in epididymal adipose tissue (35).

Targeting mTOR may potentially relieve the development of obesity (36). Empagliflozin relieves obesity-associated heart dysfunction by modulating sestrin2-regulated mTOR signaling in an HFD-induced obesity mouse model (37). Rapamycin attenuates age-related obesity by modulating mTOR signaling (38). Rubrofusarin-6-β-gentiobioside represses weight gain and lipid accumulation by targeting mTOR signaling (39). Maternal obesity is associated with the stimulation of mTOR signaling and placental insulin in a mouse model (40). In the present work, mTOR phosphorylation was increased in obese mice, and JPTGY treatment was inhibited, while MHY cotreatment blocked the impact of JPTGY *in vitro* and *in vivo*. Further research is needed to clarify the anti-obesity signaling pathway of JPTGY.

We identified that JPTGY relieved obesity phenotypes through mTORC1/SREBP1 signaling *in vitro* and *in vivo*.

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JPTGY may benefit the attenuation of obesity. We concluded that JPTGY possesses strong *in vitro* and *in vivo* anti-obesity activities and it could be used as a potential therapeutic agent for attenuating obesity.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The experiment was performed in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals and approved by Affiliated Hospital of Shandong University of Traditional Chinese Medicine (No. AWE-2020-046).

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