

Liraglutide suppresses obesity and promotes browning of white fat via miR-27b *in vivo* and *in vitro*

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Abstract

Objective: To investigate the effect of liraglutide on the browning of white fat and the suppression of obesity via regulating microRNA (miR)-27b *in vivo* and *in vitro*.

Methods: Sprague-Dawley rats were fed a high-fat (HF) diet and 3T3-L1 pre-adipocytes were differentiated into mature white adipocytes. Rats and mature adipocytes were then treated with different doses of liraglutide. The mRNA and protein levels of browning-associated proteins, including uncoupling protein 1 (UCPI), PR domain containing 16 (PRDM16), CCAAT enhancer binding protein β (CEBP β), cell death-inducing DFFA-like effector A (CIDEA) and peroxisome proliferator-activated receptor- γ -coactivator 1 α (PGC-1 α), were detected using quantitative real-time polymerase chain reaction and Western blotting.

Results: Liraglutide decreased body weight and reduced the levels of blood glucose, triglyceride and low-density lipoprotein cholesterol in HF diet-fed rats. Liraglutide increased the levels of UCPI, PRDM16, CEBP β , CIDEA and PGC-1 α *in vivo* and *in vitro*. The levels of miR-27b were upregulated in HF diet-fed rats, whereas liraglutide reduced the levels of miR-27b. *In vitro*, over-expression of miR-27b decreased the mRNA and protein levels of UCPI, PRDM16, CEBP β , CIDEA and PGC-1 α . Transfection with the miR-27b mimics attenuated the effect of liraglutide on the browning of white adipocytes.

Conclusion: Liraglutide induced browning of white adipose through regulation of miR-27b.

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Keywords

Liraglutide, miR-27b, obesity, browning of white fat, beige adipocytes

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Introduction

Obesity is the most common metabolic disorder, which is linked to several adverse health consequences, including cardiovascular disease, hypertension, type 2 diabetes mellitus, nonalcoholic fatty liver disease and malignant disorders.^{1–3} In consideration of its importance for health, fat conversion has attracted significant attention. Traditionally, adipose tissues have been divided into two types including white adipose tissue (WAT) and brown adipose tissue (BAT).⁴ WAT is composed of white adipocytes containing one large lipid droplet and fewer mitochondria, which functions to store energy.⁵ BAT is composed of brown adipocytes, containing a large number of small lipid droplets and mitochondria, which is specialized for generating heat through the constitutively expressed uncoupling protein 1 (UCP1).⁶ Recently, a new kind of adipocytes, known as beige adipocytes, were identified in WAT.⁷ Beiging of white adipocytes can be induced by various stimuli including cold conditions, certain hormones and pharmacological agents.⁸ This beiging/browning process can be observed in subcutaneous WAT and visceral WAT (vWAT).^{9,10} Epididymal WAT (eWAT) is part of the vWAT, which plays a crucial role in preventing metabolic syndrome including type 2 diabetes mellitus and obesity.^{11,12} Additionally, a previous study concluded that chitosan and chitosan oligosaccharide can induce browning of eWAT and thereby combat obesity.¹³ Those findings indicated that the browning process might be an

effective method for the suppression of obesity.

The molecular mechanisms of browning are being further investigated. Previous research has demonstrated that the PR domain containing 16 (PRDM16) is a key transcriptional regulator in the development of brown and beige adipocytes; and cooperates with other transcriptional regulators including peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1 α), CCAAT enhancer binding protein β (CEBP β) and cell death-inducing DFFA-like effector A (CIDEA), regulates thermogenesis.^{14,15} MicroRNAs (miRNAs) are short non-coding RNAs with 19–22 nucleotides, which play an important role in the browning process, including miR-27b.¹⁶ Furthermore, recent studies have confirmed that miR-27b is closely associated with lipid metabolism and regulates the browning of WAT.^{16–18} Thus, it was speculated that miR-27b may be a target gene for the suppression of obesity. Liraglutide is a human glucagon-like peptide-1 (GLP-1) receptor agonist, which plays an important role in resisting obesity and promoting the browning process of WAT.^{19,20} Liraglutide has been shown improve a number of cardiometabolic risk factors in patients with type 2 diabetes mellitus via regulation of miR-27b.²¹ However, whether liraglutide promotes browning of white fat via regulation of miR-27b has not been reported yet.

The aim of the current study was to investigate the effect of liraglutide on the browning of white fat in high-fat (HF)

diet-induced obese rats in order to explore the interaction between liraglutide and miR-27b in this process.

Materials and methods

Animal model

A total of 40 healthy male Sprague-Dawley rats (8 weeks old, 240–260 g) were obtained from the Animal Centre of Hebei Medical University (Shijiazhuang, Hebei Province, China) and randomly divided into five groups. All rats were fed in separate cages and housed at a temperature of $23 \pm 1^\circ\text{C}$ with a 12-h light/12-h dark cycle. They had free access to food and water. Briefly, rats were fed with either a standard diet (3.48 kcal/g; 10.3% fat, 65.5% carbohydrate and 24.2% protein; control group; $n=8$) or a HF diet (5.01 kcal/g; 59.8% fat, 20.1% carbohydrate and 20.1% protein; HF group; $n=32$). After 2 weeks of feeding, the rats in the HF group were subdivided into a HF diet control group (HF-NC, $n=8$) and liraglutide intervention groups ($n=24$). Specifically, rats ($n=24$) in the liraglutide intervention groups were subcutaneously injected with 0.4 mg/kg, 0.6 mg/kg and 0.8 mg/kg liraglutide (Novo Nordisk, Beijing, China) once daily for 12 weeks; and the groups were named as HF + liraglutide-L group ($n=8$), HF + liraglutide-M group ($n=8$) and HF + liraglutide-H group ($n=8$), respectively. The rats in the control and HF-NC groups received an equivalent volume of 0.9% saline. Rats were anesthetized by intraperitoneal injection of pentobarbital sodium (40 mg/kg) and sacrificed on the 12th week. Blood samples and eWAT were collected and stored at -80°C .

Animal experiments were performed following the approval of the Ethics Committee of Hebei General Hospital, Shijiazhuang, Hebei Province, China

(no. 202053) and complied with international guidelines for animal welfare.

Cell culture and induction of adipocyte maturation

3T3-L1 pre-adipocytes were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's modified Eagle's medium (DMEM, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Corning, Glendale, AZ, USA) and 1% penicillin-streptomycin (Thermo Fisher Scientific). When cells had formed a confluent monolayer, 3T3-L1 pre-adipocytes were induced to mature into white adipocytes using a 3T3-L1 Differentiation Kit (1.5 mg/ml insulin, 1 mM dexamethasone, 500 mM 3-isobutyl-1-methylxanthine and 1 mM rosiglitazone in DMEM with 10% FBS) (Sigma-Aldrich, St Louis, MO, USA) for 3 days, followed by refreshing with maintenance DMEM medium. Oil Red O staining was used to confirm the success of cell differentiation.

Cell grouping

After successful differentiation of 3T3-L1 pre-adipocytes, mature white adipocytes were treated with 10 mM phosphate-buffered saline (PBS; pH 7.4) and 1 or 10 $\mu\text{mol/l}$ liraglutide, respectively (blank group, 1 μM liraglutide group, 10 μM liraglutide group). Mature white adipocytes were transfected with mimic-NC or miR-27b mimics (Ribobio, Guangzhou, China) using Lipofectamine[®] 2000 (Invitrogen, Carlsbad, CA, USA) for 6 h, and then treated with 1 $\mu\text{mol/l}$ liraglutide for 48 h, with the groups named as the mock group, mimics-NC group, miR-27b mimics group, mimics-NC + liraglutide group and miR-27b mimics + liraglutide group.

Quantitative real-time PCR assay

Total RNA was extracted from WAT and cells using TRIzol[®] reagent (Ambion, Austin, TX, USA). The cDNA synthesis was performed with 1 µg total RNA using a FastQuant Reverse Transcription Kit (with gDNase) (Tiangen, Beijing, China). The quantitative real-time polymerase chain reaction (qRT-PCR) was performed on an ABI PRISM 7300 RCR (Applied Biosystems, Foster City, CA, USA) using SuperReal Premix Plus (Tiangen). The primer sequences were synthesized by Shengong Company (Shanghai, China) and were as follows: UCPI (forward): 5'-GAGGTGGTGAAGGTCAGAATG-3', (reverse): 5'-TTGTAGGTCCCAGTGTA GCG-3' (74 base pairs [bp]); PRDM16 (forward): 5'-GTGAAAGAAGGTGCCTACT CC-3', (reverse): 5'-ACTCATCGCAGCG GAAC-3' (76 bp); CEBP β (forward): 5'-GCACCACGACTTCCTTTC-3', (reverse): 5'-GCTTCTTGCTCGGCTTG-3' (62 bp); CIDEA (forward): 5'-TCTTGTCATCA CAACTGGC-3', (reverse): 5'-GGTGTCC ATTTCTGTCCCTT-3' (136 bp); PGC-1 α (forward): 5'-CAAGACCAGGAAATCC GAG-3', (reverse): 5'-TTGCCATCCCG TAGTTCAC-3' (104 bp); miR-27b (forward): 5'-AGAGCUUAGCUGAUUGGU GA ACAG-3', (reverse): 5'-CTCAACTG GTGTCGTGGA-3' (22 bp); β -actin (forward): 5'-GTGCTATGTTGCTCTAGA CTTCG-3', (reverse): 5'-ATGCCACAGG ATTCCATACC-3' (174 bp); U6 (forward): 5'-CTCGCTTCGGCAGCACATATACT-3', (reverse): 5'-ACGCTTCACGAATTT GCGTGTC-3' (22 bp). The relative levels of mRNA were evaluated using the $2^{-\Delta\Delta Ct}$ method. The internal control U6 was used to quantify the miR-27b level and β -actin was used for the other mRNAs. The cycling programme involved preliminary denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and elongation

at 72°C for 30 s, followed by a final elongation step at 72°C for 10 min. The PCR products were quantified by the ABI PRISM 7300 RCR system (Applied Biosystems).

Western blotting assay

Proteins from WAT and cells were lysed using RIPA Lysis Buffer (Solarbio, Beijing, China) and the concentrations were determined using a BCA kit (Applygen, Beijing, China). The proteins (50 µg per lane) were separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA) using electroblot apparatus at 80 V for 1 h (Bio-Rad, Hercules, CA, USA). After blocking with 5% skimmed milk for 2–4 h at room temperature, the membranes were incubated overnight at 4°C with rabbit primary antibodies against the following antigens: polyclonal to UCPI (dilution 1:1000, ab10983; Abcam[®], Cambridge, MA, USA); polyclonal to PRDM16 (dilution 1:1500, ab106410; Abcam[®]); monoclonal to CEBP β (dilution 1:1000, ab32358; Abcam[®]); monoclonal to PGC-1 α (dilution 1:500, 2178; Cell Signaling Technology, Danvers, MA, USA); polyclonal to CIDEA (dilution 1:500, 13170-1-AP; Proteintech, Wuhan, China) and polyclonal to β -actin (dilution 1:1000, I102; Bioworld Technology, Bloomington, MN, USA). The membranes were washed with $1 \times$ Tris-buffered saline-Tween 20 (TBST; pH 7.5) three times, followed by incubation with goat antirabbit secondary polyclonal antibody (dilution 1:10 000, 5210-0174; SeraCare, Milford, MA, USA) for 1.5 h at room temperature. The membranes were then washed with $1 \times$ TBST (pH 7.5) three times and enhanced chemiluminescence reagent (CW BIO, Beijing, China) was added and exposed to X-radiography film. The protein

blots were captured and analysed using a UVP Biochemi System (UVP, Upland, CA, USA). β -actin was used as the internal control.

Measurement of triglyceride and total cholesterol levels

Serum was produced from fresh whole blood samples from the rats. Blood glucose concentration, triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels were measured using an automatic biochemical analysis system (cobas[®] 8000 modular analyzer series; Roche, Basel, Switzerland). All reagents were purchased from Roche and experiments were conducted according to manufacturer's instructions.

Histological examination

The samples of WAT were fixed with 4% paraformaldehyde and then embedded in paraffin. The tissues were cut into 4 μ m sections, then deparaffinized, rehydrated, and stained with haematoxylin and eosin. The tissue sections were blocked with neutral gum and images were captured using an inverted microscope (TS100F; Nikon Corporation, Sendai, Japan).

Oil Red O staining

The 3T3-L1 cells were gently washed twice with 10 mM PBS (pH 7.4) and fixed in 4% paraformaldehyde for 10 min. After removing this solution, the cells were washed twice with 10 mM PBS (pH 7.4) and then stained with 60% saturated Oil Red O dye for 15 min at room temperature. All the cells were washed three times with 10 mM PBS (pH 7.4). Images were collected using an inverted microscope (TS100F, Nikon Corporation).

The amount of TG, an index of lipid accumulation, was quantitatively measured

using a TG assay kit (E1013; Applygen, Beijing, China) according to manufacturer's instruction. Briefly, 4×10^7 cells were lysed ultrasonically for 1 min, centrifuged for 10 min at 8000 *g* at 4°C (5425; Eppendorf, Hamburg, Germany). The supernatant was collected and used for the analysis. The amount of TG was normalized to the protein content.²²

Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA). Data are expressed as mean \pm SD. The groups were compared using one-way analysis of variance followed by Tukey's *post hoc* test. A *P*-value < 0.05 was considered statistically significant.

Results

As shown in Figures 1a–1d, body weight, epididymal adipose tissue weight, blood glucose concentration and TG concentration in the HF-NC group were significantly higher than those in the control group ($P < 0.01$ for all comparisons); and significantly decreased after administration of liraglutide compared with the HF-NC group ($P < 0.01$ for all comparisons). The TC level in the HF-NC group was not significantly different compared with the control group, while it was significantly decreased following administration of 0.8 mg/kg liraglutide compared with the HF-NC group ($P < 0.05$) (Figure 1e). HDL-C levels were not significantly different among the five groups (Figure 1f). The LDL-C level in the HF-NC group was significantly higher than that of the control group ($P < 0.01$). Compared with the HF-NC group, the LDL-C level was significantly reduced in the HF + liraglutide-L and HF + liraglutide-H groups ($P < 0.05$ for both

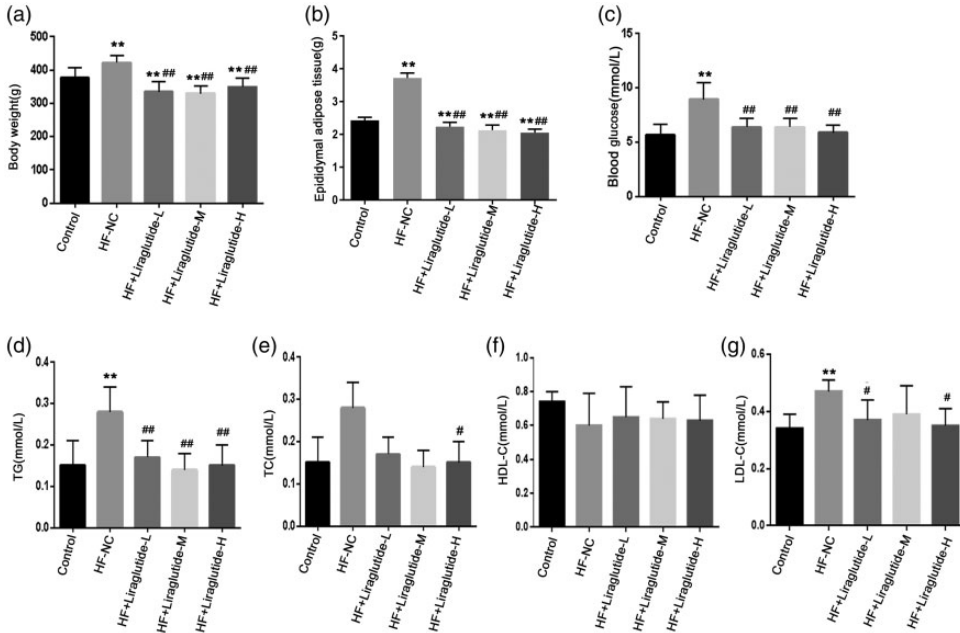


Figure 1. Effects of liraglutide treatment in high-fat diet (HF)-induced obese rats following administration of different doses of liraglutide for 12 weeks: (a) body weight; (b) epididymal adipose tissue weight; (c) blood glucose concentration; (d) triglyceride (TG) concentration; (e) total cholesterol (TC) concentration; (f) high-density lipoprotein cholesterol (HDL-C) concentration; (g) low-density lipoprotein cholesterol (LDL-C) concentration. HF-NC, high-fat diet control group; HF + liraglutide-L group, HF + 0.4 mg/kg liraglutide; HF + liraglutide-M group, HF + 0.6 mg/kg liraglutide; liraglutide-H group, HF + 0.8 mg/kg liraglutide; ** $P < 0.01$ versus the control group; ## $P < 0.05$, ### $P < 0.01$ versus the HF-NC group; one-way analysis of variance followed by Tukey's *post hoc* test. All data are shown as the mean \pm SD of eight rats.

comparisons), but not in the HF + liraglutide-M group (Figure 1g).

Haematoxylin and eosin staining showed that the size of white adipocytes was smaller after liraglutide treatment compared with the HF-NC group (Figure 2a). The mRNA levels of browning-related markers, including UCP1, PRDM16, CEBP β , CIDEA and PGC-1 α , were significantly decreased in the HF-NC group compared with the control group ($P < 0.01$ for all comparisons) (Figures 2b–2f). The levels of UCP1, PRDM16, CEBP β , CIDEA and PGC-1 α mRNA were significantly increased after liraglutide treatment compared with the HF-NC group ($P < 0.05$ for all comparisons). No significant

differences were observed among the HF + liraglutide groups.

Oil Red O staining showed the accumulation of lots of lipid droplets in the adipocytes, which confirmed the successful differentiation of mature adipocytes (Figure 3a). Mature adipocytes were used as a cellular model to further investigate the effect of liraglutide on the browning process in white adipocytes. Liraglutide inhibited lipid droplet accumulation in a concentration-dependent manner in mature adipocytes (Figure 3b), which was supported by quantitative data from the TG analysis (Figure 3c). As presented in Figures 3d–3n, the relative mRNA and protein levels of UCP1, PRDM16, CEBP β ,

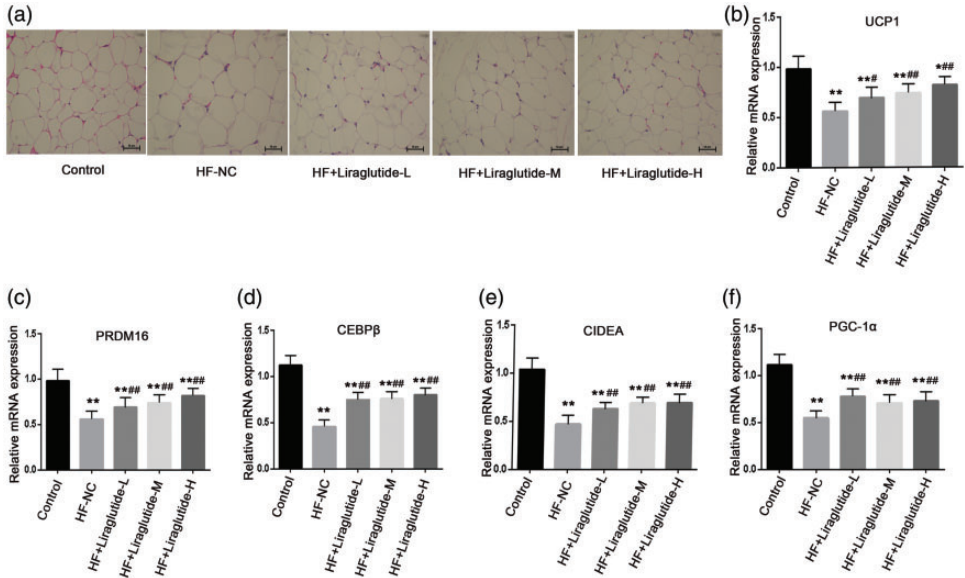


Figure 2. Effects of liraglutide treatment on white adipose tissue (WAT) browning in high-fat diet (HF)-induced obese rats following administration of different doses of liraglutide for 12 weeks: (a) histological examination with haematoxylin and eosin staining (scale bar 50 μ m); (b–f) relative mRNA levels of WAT browning-related markers, including uncoupling protein 1 (UCP1), PR domain containing 16 (PRDM16), CCAAT enhancer binding protein β (CEBP β), cell death-inducing DFFA-like effector A (CIDEA) and peroxisome proliferator-activated receptor- γ -coactivator 1 α (PGC-1 α) in epididymal WAT as measured using quantitative real-time polymerase chain reaction. HF-NC, high-fat diet control group; HF + liraglutide-L group, HF + 0.4 mg/kg liraglutide; HF + liraglutide-M group, HF + 0.6 mg/kg liraglutide; liraglutide-H group, HF + 0.8 mg/kg liraglutide; * P < 0.05, ** P < 0.01 versus the control group; # P < 0.05, ### P < 0.01 versus the HF-NC group; one-way analysis of variance followed by Tukey's *post hoc* test. All data are shown as the mean \pm SD of eight rats. The colour version of this figure is available at: <http://imr.sagepub.com>.

CIDEA and PGC-1 α were significantly increased after liraglutide (1 μ mol/l or 10 μ mol/l) treatment compared with the blank group (P < 0.05 for all comparisons).

In order to examine the interaction between liraglutide and miR-27b on the browning process in white fat, miR-27b levels were first measured *in vivo* and *in vitro*. The results showed that the relative miR-27b level in the HF-NC group was significantly higher than that in the control group (P < 0.01) (Figure 4a). The relative miR-27b levels were significantly decreased after liraglutide treatment compared with the HF-NC group *in vivo* and compared with the blank group *in vitro* (P < 0.01 for

all comparisons) (Figures 4a and 4b). Transfection experiments showed that transfection with the miR-27b mimics significantly increased the relative miR-27b levels compared with the mimic-NC group (P < 0.01) (Figure 4c). The relative miR-27b level in the miR-27b mimics + liraglutide group was significantly decreased compared with miR-27b mimics group (P < 0.01).

To verify the association between liraglutide and miR-27b in the browning process of white adipocytes, the relative mRNA and protein levels of five browning-related markers were measured. As shown in Figures 4d–4n, the mRNA and protein levels of browning-related markers,

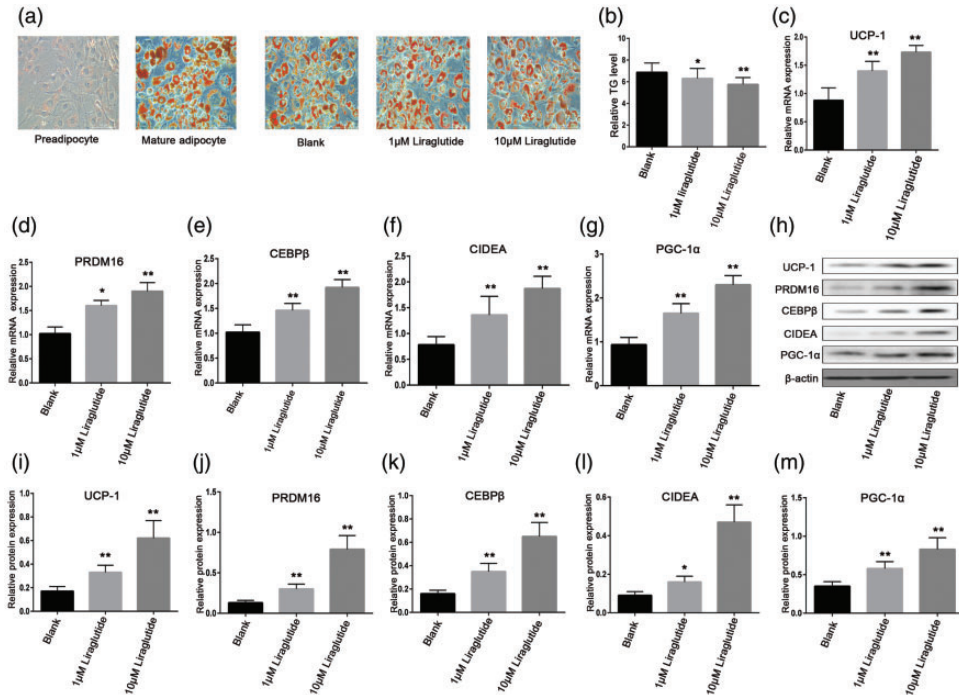


Figure 3. Effects of liraglutide treatment on the browning of white adipocytes *in vitro*: (a) representative images of pre-adipocytes and mature adipocytes using Oil Red O staining (scale bar 100 μm); (b) representative images of mature adipocytes treated with different concentrations of liraglutide (1 μmol/l or 10 μmol/l) using Oil Red O staining (scale bar 100 μm); (c) relative TG levels in each group; (d–h) relative mRNA levels of browning-related markers, including uncoupling protein 1 (UCP1), PR domain containing 16 (PRDM16), CCAAT enhancer binding protein β (CEBPβ), cell death-inducing DFFA-like effector A (CIDEA) and peroxisome proliferator-activated receptor-γ-coactivator 1α (PGC-1α) in mature white adipocytes; (i–n) relative protein levels of UCP1, PRDM16, CEBPβ, CIDEA and PGC-1α in mature white adipocytes were measured by Western blotting. * $P < 0.05$, ** $P < 0.01$ versus the blank group; one-way analysis of variance followed by Tukey's *post hoc* test. All data are shown as the mean \pm SD of three independent experiments. The colour version of this figure is available at: <http://imr.sagepub.com>.

including UCP1, PRDM16, CEBPβ, CIDEA and PGC-1α in the miR-27b mimics group were significantly lower than those in mimics-NC group ($P < 0.05$ for all comparisons), while they were significantly increased after liraglutide treatment compared with the miR-27b mimics group ($P < 0.05$ for all comparisons). There were no significant differences between the miR-27b mimics + liraglutide group and the mimics-NC group with regard to the mRNA and protein levels of adipose browning-related markers.

Discussion

Recently, inducing conversion of white adipocytes into beige adipocytes has attracted increasing attention, due to its potential use in modulating obesity.²³ This current study showed that liraglutide induced browning of white fat through regulation of miR-27b levels and thereby combated obesity.

Generally, weight loss is achieved by mechanisms including reduced adipogenesis and increased thermogenesis.²⁴ Adipogenesis is a cellular

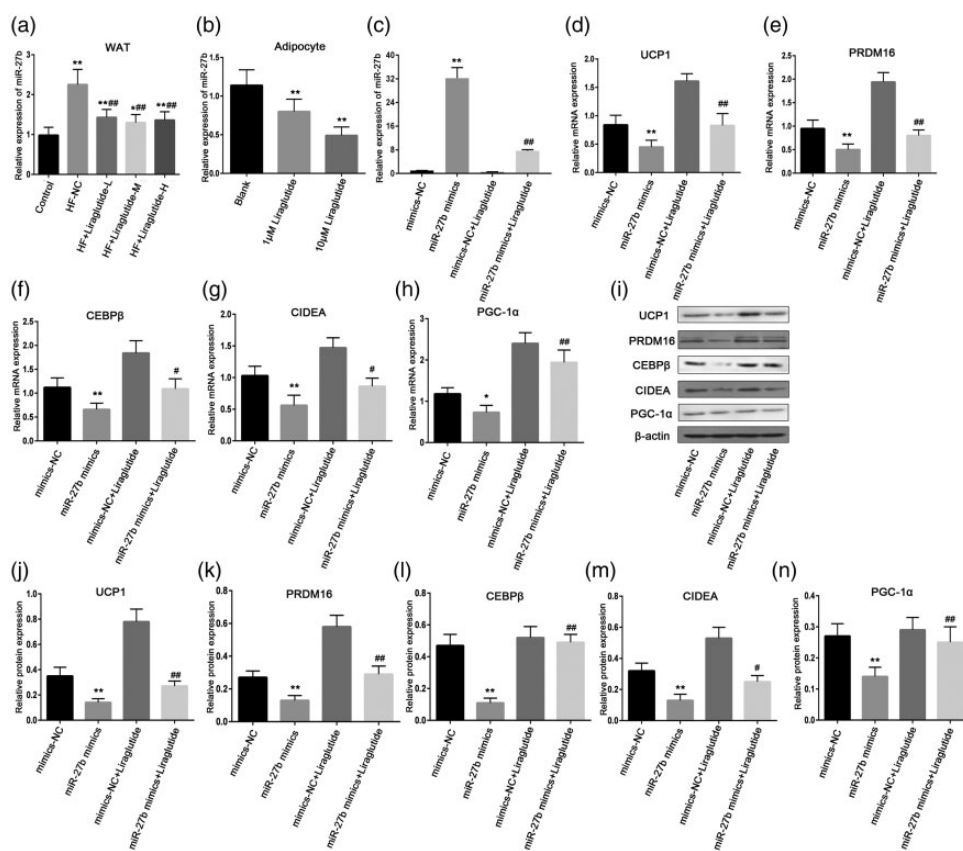


Figure 4. Role of micro-RNA (miR)-27b in liraglutide-induced browning of white adipocytes: (a) quantitative real-time polymerase chain reaction (qRT-PCR) analysis of relative miR-27b levels in epididymal white adipose tissue (WAT) in high-fat diet (HF)-induced obese rats following administration of different doses of liraglutide for 12 weeks; HF-NC, high-fat diet control group; HF + liraglutide-L group, HF + 0.4 mg/kg liraglutide; HF + liraglutide-M group, HF + 0.6 mg/kg liraglutide; liraglutide-H group, HF + 0.8 mg/kg liraglutide; * $P < 0.05$, ** $P < 0.01$ versus the control group; ### $P < 0.01$ versus the HF-NC group; one-way analysis of variance followed by Tukey's *post hoc* test. All data are shown as the mean \pm SD of eight rats; (b) qRT-PCR analysis of relative miR-27b levels in mature white adipocytes after treatment with liraglutide (1 $\mu\text{mol/l}$ or 10 $\mu\text{mol/l}$) for 2 days; ** $P < 0.01$ versus the blank group; (c) transfection efficiency was measured using qRT-PCR in mature white adipocytes transfected with mimic-NC or miR-27b mimics treated with/without 1 $\mu\text{mol/l}$ liraglutide for 48 h; (d–h) relative mRNA levels of uncoupling protein 1 (UCP1), PR domain containing 16 (PRDM16), CCAAT enhancer binding protein β (CEBP β), cell death-inducing DFFA-like effector A (CIDEA) and peroxisome proliferator-activated receptor- γ -coactivator 1 α (PGC-1 α) in mature white adipocytes transfected with mimic-NC or miR-27b mimics treated with/without 1 $\mu\text{mol/l}$ liraglutide for 48 h; (i–n) Relative protein levels of UCP1, PRDM16, CEBP β , CIDEA and PGC-1 α in mature white adipocytes transfected with mimic-NC or miR-27b mimics treated with/without 1 $\mu\text{mol/l}$ liraglutide for 48 h as measured by Western blotting. * $P < 0.05$, ** $P < 0.01$ versus the mimic-NC group; # $P < 0.05$, ### $P < 0.01$ versus the miR-27b mimics group; one-way analysis of variance followed by Tukey's *post hoc* test. All data are shown as the mean \pm SD of three independent experiments.

differentiation process that converts pre-adipocytes into mature adipocytes, however thermogenesis is induced by specific proteins, including PRDM16, PGC1 α , and UCP1.²⁵ A previous study demonstrated that browning-related markers were significantly increased after cold exposure, indicating that the browning process in WAT can be stimulated by cold exposure.²⁶ This current study showed that HF diet feeding resulted in increased body weight in rats and reduced the levels of browning-related markers including UCP1, PRDM16, CEBP β , CIDEA and PGC-1 α , findings that were consistent with previous studies. For example, a previous study demonstrated that consumption of a HF diet increased the body weight in mice, while it reduced the levels of UCP1 significantly in the WAT.²⁷ However, treatment with *Spirulina maxima* 70% ethanol extract (SM70EE) decreased the body weight and increased the UCP1 levels in the WAT compared with HF diet-fed mice.²⁷ The current study showed that the administration of liraglutide suppressed the body weight and elevated the mRNA levels of browning-related proteins including UCP-1 in the WAT from HF diet-fed rats. In addition, the UCP1 level increased with the browning of WAT, which was also demonstrated in a previous study.²⁸ A previous study demonstrated that liraglutide stimulated the expression of browning-related markers *in vivo* and *in vitro*.¹⁹ This current study showed that lipid accumulation was decreased after treatment with liraglutide *in vivo*, which was characteristic of the brown fat-like phenotype. These current findings suggest that liraglutide limited weight gain in HF diet-fed rats by inducing browning of WAT.

Liraglutide plays an important role in controlling body weight. For example, previous research demonstrated that liraglutide reduced body weight and maintained blood glucose within the normal range in rats.²⁹

In addition, liraglutide has been shown to decrease serum lipid profiles.³⁰ This current study showed that liraglutide reduced body weight, blood glucose and TG levels in HF diet-fed rats. In terms of the browning activity, a previous study demonstrated that the levels of browning-related markers were elevated to varying degrees in HF diet-fed rats after administration of SM70EE.²⁷ In this current study, the levels of browning-related mRNAs among the HF + liraglutide groups were not significantly different. The reasons were probably the low concentrations of liraglutide administered and the short duration of drug treatment. These current results suggest that treatment with liraglutide can elevate the levels of browning-related markers to a certain extent, but that the extent of the effects might depend on the concentration and duration of treatment.

MicroRNAs play an important role in adipogenesis and thermogenesis via regulation of browning-related markers. For example, previous research demonstrated that miR-192 regulated the expression levels of browning-related genes through autophagy, thereby inhibiting fat formation.³¹ In terms of miR-27b target genes, miR-27b is considered to act upstream of PRDM16 and peroxisome proliferator-activated receptor gamma (PPAR γ), as reported in previous studies;^{18,32} and as predicted by TargetScanHuman Release 7.2.³³ The overexpression of PRDM16 increases the levels of UCP1 and PGC-1 α .³⁴ Other research has shown that the increased expression of CIDEA and CEBP β was induced by PPAR γ .^{35,36} Thus, it was speculated the miR-27b may regulate the expression of browning-related markers. This current study found that miR-27b was upregulated, while the levels of UCP1, PRDM16, PGC-1 α , CIDEA and CEBP β were downregulated, in the WAT from HF diet-induced obese rats. In addition, when adipocytes were transfected with

miR-27b mimics this resulted in decreased levels of browning-related mRNA and proteins. These current results agreed with a previous study,³⁷ which demonstrated that miR-27b was upregulated in visceral adipose tissue of obese humans and negatively correlated with UCP1. Moreover, miR-27b was downregulated in the browning of WAT following exposure to colder temperatures and miR-27b inhibition has been shown to increase the browning of white adipocytes.³⁸ The current results confirmed that miR-27b was closely associated with the browning of white adipocytes via regulation of the browning-related markers.

In the current study, liraglutide was shown to affect the browning of white fat via miR-27b. Adipose tissue seems to play a central role in the pathophysiology of metabolic syndrome.³⁹ Several studies have been published on the effects of GLP-1/GLP-1 receptor agonists and miRNAs on metabolic syndrome. For example, a previous study reported that the GLP-1 receptor agonist, exendin-4, regulates the expression of miR-375 via the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) pathway.⁴⁰ GLP-1 (7–36) amide increases the expression of miR-132 and miR-212 via the cAMP/PKA pathway to stimulate insulin secretion, but miR-132 and miR-212 also mediate the action of GLP-1 (7–36) amide.⁴¹ Another similar study concluded that inhibition of miR-124a can attenuate the responsiveness of GLP-1 receptor agonist, liraglutide, in non-alcoholic fatty liver disease.⁴² These findings were consistent with those of the current study that demonstrated that miR-27b antagonizes the effects of liraglutide. To the best of our knowledge, cAMP is the second messenger that GLP-1 stimulates beta cells to release insulin.⁴³ Moreover, liraglutide promotes adipose tissue decomposition via a downstream cAMP signalling pathway.⁴⁴ Taken together, these findings suggest that liraglutide

regulated miR-27b expression via the cAMP/PKA pathway. This possible mechanism of action will be explored in future research in order to verify the relationship between liraglutide and miR-27b in the browning of white fat.

In conclusion, liraglutide induced browning of white fat via regulating miR-27b levels, which may serve as a novel therapeutic approach for the management of obesity.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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