ORIGINAL ARTICLE

Liraglutide may affect visceral fat accumulation in diabetic rats via changes in FTO, AMPK, and AKT expression

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Abstract

Purpose The aim of this study is to explore the effects of liraglutide (LRG) on the expression of FTO, AMPK, and AKT in the visceral adipose tissues of obese and diabetic rats and the underlying mechanisms thereof.

Methods Thirty SPF-grade, male SD rats were randomly divided into the healthy control, diabetic model (DM), and DM + LRG groups. The DM and DM + LRG groups were administered normal saline and LRG (0.6 mg/kg/d), respectively. After 12 weeks, the body weight of the rats was measured, and their visceral adipose tissues were collected and weighed; the levels of serum biochemical indicators and FTO, AMPK, and AKT in these tissues were then measured using qRT-PCR and western blotting.

Results Compared to the control group, the body weight and visceral fat accumulation and blood glucose, TG, TC, and LDL-C levels increased significantly, while the HDL-C levels decreased significantly, in the DM group ($p < 0.05$). After LRG treatment, the HDL-C levels increased significantly, but the levels of the other indicators decreased significantly ($p < 0.05$). Compared to the control group, the visceral adipose tissue levels of FTO and AKT increased significantly, while the AMPK levels decreased significantly in the DM group ($p < 0.05$). After LRG treatment, the FTO and AKT levels decreased significantly, and the AMPK levels increased significantly ($p < 0.05$).

Conclusion LRG may activate and inhibit the AMPK and AKT pathways, respectively, and decrease FTO expression, thereby alleviating abdominal obesity in type 2 diabetes.

Keywords Liraglutide · Diabetes · Obesity · FTO · AMPK · AKT

Introduction

Diabetes mellitus (DM) is a common chronic disease. The prevalence of type 2 diabetes (T2DM) and obesity is rapidly increasing worldwide because of lifestyle changes and accelerated aging, posing a global public health issue [1]. Being overweight and abdominal obesity are the greatest risk factors for T2DM, which aggravate insulin resistance

and adversely affect blood glucose control [2]. The fat mass and obesity-associated (FTO) gene, discovered by Frayling et al. is abundantly expressed in adipose tissues [3, 4]. FTO is a transcription co-factor that may affect the process of obesity by modulating the growth, development, and adipogenesis of adipocytes [5]. Accumulating evidence suggests that FTO polymorphisms are closely related to obesity and T2DM [6]. AMP-activated protein kinase (AMPK), a key factor in lipid metabolism, reduces the accumulation of lipids by inhibiting the expression of FTO in skeletal muscle cells [7]. The phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signaling pathway is a classic pathway affected by the development of T2DM. The activation of PI3K/AKT signaling plays an important role in lipid metabolism and insulin resistance. In endometrial cancer, the expression of FTO is induced by the estrogen-activated pPI3K/AKT pathways in adipocytes [8].

Liraglutide (LRG) is a glucagon-like peptide-1 (GLP-1) receptor agonist (GLP-1RA). Similar to GLP-1, LRG

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promotes insulin secretion, inhibits glucagon release, and maintains blood glucose stability in a glucose concentration-dependent manner [9]. LRG may also regulate glucose metabolism by improving the constitution of intestinal flora, promoting the enrichment of short-chain fatty acid-producing bacteria (probiotic bacteria, e.g., bifidobacteria). In such a manner, it could alleviate systemic inflammation and elicit a beneficial effect on diabetes [10]. In addition, GLP-1RAs reduce energy intake by delaying gastric emptying, increasing satiety, and suppressing appetite, posing a direct impact on energy balance and body weight control [11]. Long-term combined application of LRG for the treatment of T2DM effectively regulates the level of glycated hemoglobin, markedly reduces the body weight of overweight and abdominally obese T2DM patients, alleviates hyperlipidemia, and reduces the risk of cardiovascular disease [12]. In db/db mice, LRG administration reduces body weight and visceral fat production by activating AMPK and inhibiting AKT [13].

Herein, a streptozotocin (STZ)-induced obese rat model of T2DM was used to observe LRG-induced changes in the visceral adipose tissue expression of FTO, AMPK, and AKT, and how these changes may affect metabolism and visceral fat accumulation. Our study provides an experimental basis for examining the effects of LRG on FTO, AMPK, and AKT expression and suggests a mechanism whereby LRG alleviates abdominal obesity in diabetic rats.

Materials and methods

Animals

SPF-grade male Sprague Dawley (SD) rats, weighing $180-220$ g, were provided by the Laboratory Animal Center of Xinjiang Medical University (Xinjiang, China). A high-fat and high-sugar diet was provided by Beijing Botai Hongda Biotechnology Co., Ltd (China).

Reagents and instruments

STZ and LRG were purchased from Sigma and Novo Nordisk, Denmark, respectively. Primers for the FTO, AMPK, and AKT genes were obtained from the Beijing Genomics Institute. RNA extraction kits, cDNA reverse transcription kits, and real-time PCR kits were purchased from QIAGEN, Germany. Antibodies against FTO, AMPK, and AKT were purchased from Abcam, USA. A portable blood glucose monitor and test papers were purchased from ACCU-Chek, Germany, and the enzyme-labeled analyzer was obtained from Thermo, USA. The real-time PCR machine, CFX96,

and the gel imaging and electrophoresis system were obtained from BIO-RAD, USA.

Establishment of the T2DM model

After adaptive feeding for 1 week, searching the related researches, considering the ethics and modeling rationality of animal experiments, thirty SPF-grade male SD rats were randomly divided into healthy control (n = 10) and diabetic model (DM) (n = 20) groups. Rats in the control group were fed an ordinary diet, while those in the DM group were fed with a high-fat, high-sugar diet for 8 weeks. When the average body weight of DM rats reached $400-450$ g, streptozotocin was intraperitoneally injected at a dose of 30 mg/kg, and the high-fat, high-sugar diet was continued. Blood was collected from the tail vein of all DM rats after 3 days. Blood glucose ≥ 16.7 mmol/L indicated successful induction of diabetes. Then, the twenty diabetic rats were randomly divided into two subgroups (10 per group). One group received LRG (0.6 mg/kg/d) subcutaneously twice a day, while the other was injected with saline as a control. Meanwhile, the high-fat, high-sugar diet was continued. Accordingly, a total of three groups were created, a healthy control group, a DM + LRG group, and a DM control group. All rats were anesthetized and sacrificed after 12 weeks of LRG or standard saline treatment.

Sample collection

After 12 weeks of treatment, rats were fasted overnight for 12 h and anesthetized by intraperitoneal injection of 3% sodium pentobarbital (40 mg/kg). Blood was collected from the abdominal aorta. The visceral adipose tissues were harvested, weighed, and stored at -80 °C.

Detection of serum biochemical indicators

Whole blood samples were centrifuged, and the supernatants were collected and stored on ice until analysis. For the detection of the levels of serum glucose, triglycerides, total cholesterol, and high- and low-density lipoproteins, ELISA was performed using specific kits according to the manufacturer's protocols.

Real-time PCR

Total RNA was extracted from visceral adipose tissue and reverse transcribed into cDNA. The FTO, AMPK, and AKT primer sequences used for real-time RT-PCR are shown in Table 1. The mRNA expression levels of FTO, AMPK, and AKT were determined using qPCR in a total volume of 20 μl with the following parameters: denaturation at 95 °C for

Table. 1 Primers for each gene

Gene	Forward primer	Reverse primer
FTO	5'-GACCGTCCTGCGATGATGAAGTG-3'	5'-CCTGTCCACCAAGTTCTCGTCATG-3'
AMPK	5'-ATGATGAGGTGGTGGAGCAGAGG-3'	5'-GTTCTCGGCTGTGCTGGAATCG-3'
AKT	5'-CTGCCTTCTGCCAAGCCACAC-3'	5'-ACTCCTCTGAGACCTGCCAAGATG-3'
β-actin	5'-CAACCTTCTTGCAGCTCCTC-3'	5'-CGGTGTCCCTTCTGAGTGTT-3'

10 min, annealing at 60 °C for 1 min, extension at 95 °C for 15 s, for a total of 40 cycles. β-actin was used as an internal control.

Western blotting

Liquid-frozen visceral adipose tissue was homogenized in lysis buffer containing phosphatase inhibitor and protease, sonicated, and centrifuged at 4 °C for 15 min. The supernatants were collected, and protein concentrations were determined using a BCA protein detection kit. Denatured proteins (20 μg/well) were subjected to 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and then transferred to a polyvinylidene fluoride (PVDF) membrane. The membrane was suspended in 5% BSA at ambient temperature for 2 h, left to react with the primary antibodies (FTO, 1:1000; AMPK, 1:2000; AKT, 1:1000; β-actin, 1:2000) and incubated overnight at 4 °C. After washing with TBST thrice, the membrane was incubated with an HRP-labeled secondary antibody (1:10,000) at ambient temperature for 2 h. After washing with TBST thrice, signals were detected by high-sensitivity chemiluminescence (ECL) and quantified using a gel analysis system. β-actin was used as the internal control.

Statistical analysis

Experimental data were analyzed using SPASS 25.0 software. Data were presented as $\bar{x} \pm sd$. Differences among the three groups were analyzed using ANOVA, followed by least significant difference *t*-test(LSD). Results with p values < 0.05 were considered statistically significant.

Results

Body weight and visceral fat accumulation

Compared with healthy control rats, the body weight of the DM + LRG and DM rats was significantly increased ($p < 0.05$) (Table 2, Fig. 1A). However, the body weight of the DM + LRG rats was significantly lower than that of the DM rats ($p < 0.05$) (Table 2, Fig. 1A). In addition, the weight of visceral fat in DM + LRG and DM rats was significantly higher than that in control rats ($p < 0.05$) (Table 2, Fig. 1B);

however, the visceral fat weight in DM + LRG rats was significantly lower than that in the DM rats ($p < 0.05$) (Table 2, Fig. 1B).

Glucose and biochemical indicators

Blood glucose (Table 3, Fig. 2A) and the serum triglyceride and total cholesterol (Table 3, Fig. 2B) levels were significantly higher in the DM + LRG and DM rats than in control rats ($p < 0.05$); however, these levels were significantly lower in DM + LRG rats than in DM rats ($p < 0.05$) (Table 3, Fig. 2). Moreover, serum high-density lipoprotein cholesterol (HDL-C) levels were significantly lower, while those of low-density lipoprotein cholesterol (LDL-C) were significantly higher in the DM + LRG and DM rats, than in the control rats ($p < 0.05$) (Table 3, Fig. 2C). However, the DM + LRG rats had significantly higher HDL-C levels, but significantly lower LDL-C levels, than DM rats ($p < 0.05$) (Table 3, Fig. 2C).

Expression of FTO, AMPK, and AKT in visceral adipose tissues

Compared with control rats, visceral adipocyte FTO and AKT mRNA levels were significantly higher ($p < 0.05$) (Table 4, Fig. 3A$_{(1)}$ and 3C$_{(1)}$), while those of AMPK were significantly lower ($p < 0.05$) (Table 4, Fig. 3B$_{(1)}$) in DM and DM + LRG rats. However, DM + LRG rats showed significantly lower FTO (Table 4, Fig. 3A$_{(1)}$) and AKT (Table 4, Fig. 3C$_{(1)}$) mRNA levels ($p < 0.05$), and significantly higher AMPK levels ($p < 0.05$) (Table 4, Fig. 3B$_{(1)}$) than DM rats.

Similarly, the visceral fat of DM and DM + LRG rats showed significantly higher FTO (Table 5, Fig. 3A$_{(2)}$, Fig. 4) and AKT (Table 5, Fig. 3C$_{(2)}$ and Fig. 4) protein levels, and significantly lower AMPK (Table 5, Fig. 3B$_{(2)}$ and Fig. 4)

Table 2 Body weight and visceral adipose levels

Group	Number	Body Weight (g)	Adipose Weight (g)
Control	10	222.51±22.24	6.90±1.75
DM+LRG	10	475.86±67.21*	10.58±1.76*
DM	10	596.67±36.46*#	18.14±1.94*#

Note: Control: normal group; DM+LRG: diabetes+liraglatide group; DM: diabetes group. *P<0.05 vs Control; #P<0.05 vs DM+LRG

Fig. 1 Body weight and visceral adipose levels
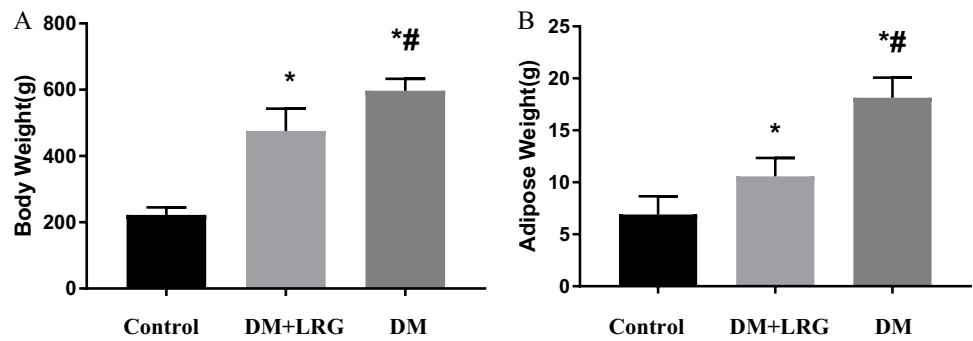
**Table 3** Blood glucose andbiochemical levels

Group	Number	Glucose (mmol/L)	TG (mmol/L)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Control	10	5.64±0.97	1.07±0.17	2.40±0.40	1.74±0.21	1.56±0.37
DM+LRG	10	8.56±0.95*	1.68±0.17*	3.44±0.44*	1.06±0.20*	2.52±0.22*
DM	10	11.16±1.29*#	2.62±0.29*#	5.25±0.89*#	0.63±0.15*#	3.90±0.59*#

Note: Control: normal group; DM+LRG: diabetes+liraglatide group; DM: diabetes group. *$P<0.05$ vs Control; #$P<0.05$ vs DM+LRG

Fig. 2 Blood glucose and bio-chemical levels
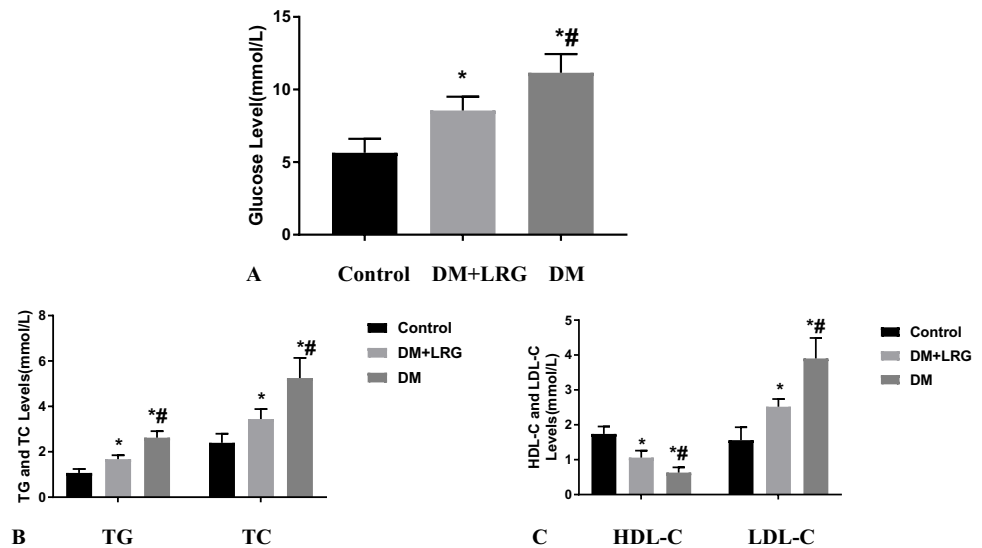
Table 4 mRNA expression of FTO, AMPK and AKT

Group	Number	mRNA FTO	mRNA AMPK	mRNA AKT
Control	10	0.64±0.22	2.42±0.29	0.37±0.14
DM+LRG	10	1.44±0.56*	1.88±0.26*	0.70±0.11*
DM	10	2.74±0.68*#	0.86±0.28*#	1.16±0.18*#

Note: Control: normal group; DM+LRG: diabetes+liraglatide group; DM: diabetes group. *$P<0.05$ vs Control; #$P<0.05$ vs DM+LRG

protein levels. However, DM + LRG rats showed significantly lower FTO (Table 5, Fig. 3A$_{(2)}$ and Fig. 4) and AKT (Table 5, Fig. 3C$_{(2)}$ and Fig. 4) protein levels, but significantly higher AMPK protein levels, than DM rats ($p < 0.05$) (Table 5, Fig. 3B$_{(2)}$ and Fig. 4).

Discussion

In this study, we used a STZ-induced diabetic obese rat model to observe LRG-induced changes in metabolism and visceral adipose tissue expression of FTO, AMPK, and AKT. Our study provides an experimental basis to study the effects of LRG on FTO, AMPK, and AKT expression and

Fig. 3 mRNA and protein expression of FTO, AMPK, and AKT
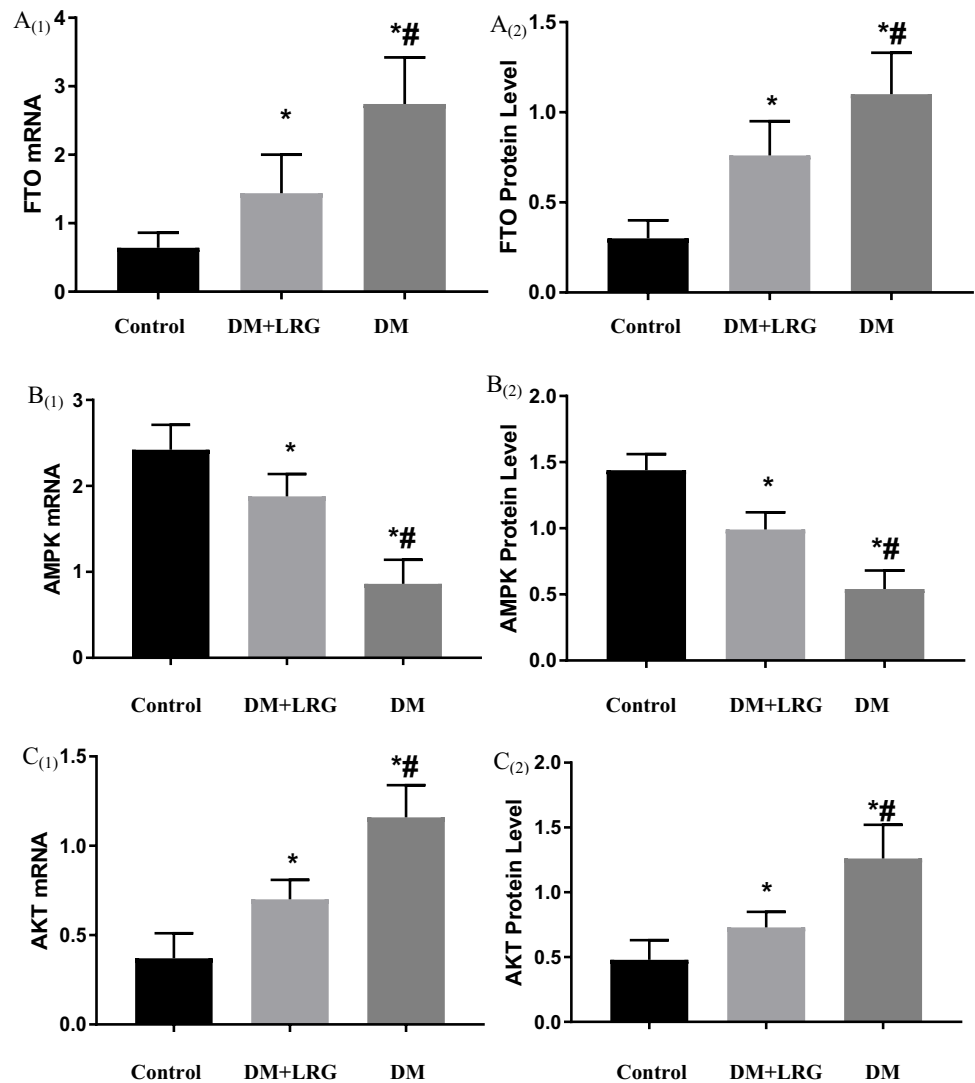
Table 5 Protein expression ofFTO, AMPK and AKT
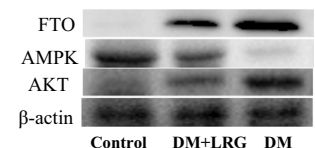
Group	Number	Protein FTO	Protein AMPK	Protein AKT
Control	10	0.30±0.10	1.44±0.12	0.48±0.15
DM+LRG	10	0.76±0.19*	0.99±0.13*	0.73±0.12*
DM	10	1.10±0.23*#	0.54±0.14*#	1.26±0.26*#

Note: Control: normal group; DM+LRG: diabetes+liraglatide group; DM: diabetes group. *$P<0.05$ vs Control; #$P<0.05$ vs DM+LRG

with hyperlipidemia, obesity, and insulin metabolism, and this association is mediated by target tissue receptors. Moreover, activation of the SIRT-AMPK signaling pathway reduces the expression of fatty acid synthases and associated transcription factors, increases the oxidation rate of fatty acids, and regulates lipid and energy metabolism [17–19]. In a high-fat-induced obesity model in C57BL/6 mice, the activation of AMPK significantly reduced liver fat accumulation and prevented diabetes by inhibiting gluconeogenesis. Insulin-sensitive PI3K/AKT signaling affects glucose and lipid homeostasis in the body [20]. The PI3K/AKT pathway is also involved in the defocused low-energy shock wave

suggests a mechanism whereby LRG may exert its effects in diabetic and obese rats. Many studies have shown that an allele at FTO, rs8050136, is closely related to insulin resistance, inflammatory factors, and obesity markers such as BMI and waist and hip circumference [14–16]. Similarly, a polymorphism at FTO rs9939609 is significantly associated

Fig. 4 Western blotting shows the expression of FTO, AMPK and AKT protein

in activated adipose tissue-derived stem cells [21]. Inhibition of the PI3K/AKT signaling pathway during liver lipid accumulation promotes hepatocyte autophagy and reduces liver steatosis in db/db mice [22]. In addition, an elevated expression of FTO has been detected in cancer cells, which may regulate cell metabolism and growth via the PI3K/AKT pathway, whereas FTO may be suppressed by the activation of AMPK [23].

Based on the abovementioned findings, the present study explored the expression of FTO, AMPK, and AKT in visceral fat tissue of diabetic obese rats. We found that body weight and visceral fat accumulation were significantly increased in diabetic rats compared to control rats ($p < 0.05$). In addition, blood glucose, triglyceride, total cholesterol, and LDL-C levels were significantly increased, while those of HDL-C were notably decreased ($p < 0.05$), in diabetic rats ($p < 0.05$). The FTO and AKT mRNA and protein levels were significantly elevated, while those of AMPK were decreased, in visceral fat of diabetic rats compared to that of control rats ($p < 0.05$). These findings suggest that increased FTO and AKT expression, and decreased AMPK levels in visceral fat, may affect fat accumulation in diabetic obese rats. LRG decreases blood sugar levels and may potentially lower apolipoprotein III and triglyceride levels, reducing the levels of lipoprotein particles in atherosclerotic lesions in patients with hyperglycemia [24]. In obese patients, LRG reduces the risk of cardiovascular events by restoring endothelial function [25, 26]. In addition, LRG could alleviate the metabolic status and vascular dysfunction of high-fat-induced obese mice by activating the PKA-AMPK pathway, improving their antioxidative capacity and effecting a protective role within the cardiovascular system [27]. Moreover, inhibition of AKT activity can decrease lipid accumulation [13]. AMPK can promote the dephosphorylation of AKT/PKB via PP2A and thereby affect the activity of AKT in MDA-MB-231 cells [28]. Treatment of diabetic and obese mice with LRG was shown to activate AMPK and inhibit AKT in visceral adipose tissue (composed of perinephric, epididymal, and omental fat), decrease visceral fat accumulation, and reduce body weight [13]. In the present study, we first found that increases in FTO and AKT expression, and reductions in AMPK levels may be involved in the visceral fat accumulation of diabetic and obese rats. Furthermore, we explored the effect of LRG treatment on metabolism and visceral fat tissue expression of FTO, AMPK, and AKT in diabetic and obese rats. We found that treatment with LRG significantly reduced body weight and visceral fat accumulation ($p < 0.05$); decreased blood glucose, triglyceride, total cholesterol, and LDL-C levels; and increased HDL-C levels in diabetic and obese rats ($p < 0.05$). Compared with diabetic control rats, the mRNA and protein levels of FTO and AKT were lower, and those of AMPK were higher, in LRG-treated diabetic rats ($p < 0.05$). These results suggest

that LRG may reduce abdominal obesity in type 2 diabetic patients by activating AMPK and suppressing AKT and FTO expression in visceral fat tissue. However, the mechanisms whereby LRG alleviates visceral fat accumulation in type 2 diabetes via the FTO, AMPK, and AKT pathways require further investigation.

In summary, changes in the expression levels of FTO, AMPK, and AKT may affect visceral fat accumulation in diabetic conditions. Mechanically, LRG activates the AMPK pathway, while inhibiting the AKT pathway and decreasing FTO expression, thereby alleviating abdominal obesity in diabetic rats. These findings provide a theoretical basis for the prevention and treatment of type 2 diabetes and abdominal obesity.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Xinjiang Medical University (Xinjiang, China), and the procedure was strictly performed according to the relevant regulations.

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