Original article

Effect of pioglitazone on skeletal muscle lipid deposition in the insulin resistance rat model induced by high fructose diet under AMPK signaling pathway

Lixin Tan [a,1], An Song [b,1], Luping Ren [c], Chao Wang [c], Guangyao Song [a,d,*]

[a] Department of Internal Medicine, Hebei Medical University, Shijiazhuang, Hebei 050017, PR China
[b] Department of Endocrinology, Key Laboratory of Endocrinology, National Health and Family Planning Commission, Peking Union Medical College Hospital, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100191, PR China
[c] Hebei Key Laboratory of Metabolic Disease, Shijiazhuang, Hebei 050051, PR China
[d] Department of Endocrinology, Hebei General Hospital, Shijiazhuang, Hebei 050051, PR China

A B S T R A C T

To study the changes of lipid deposition in skeletal muscle of insulin resistance rat and the effect of pioglitazone intervention on the expression of AMPK pathway related genes in rat, a rat model of insulin resistance was induced and constructed by high fructose diet as an test group, and normal rats were used as a control group. First, the effect of pioglitazone intervention on serum lipids-related indicators and mRNA expression levels of fat-related genes in skeletal muscle in rats was investigated. Then skeletal muscle sections were made and stained with oil red O to investigate the effect of pioglitazone intervention on lipid deposition in skeletal muscle of rats. Finally, the effects of pioglitazone intervention therapy on the mRNA and protein expression of related genes in the AMPK signaling pathway in skeletal muscle tissue of rat were explored by real-time quantitative PCR (qRT-PCR) and Western-blotting technology. The results showed that the blood glucose (BG), insulin (INS), adiponectin (ADPN), free fatty acid (FFA), triglyceride (TG), and cholesterol (TC) levels in serum of the test group were higher than the control group ($P < 0.05$); the visceral fat weight and abdominal fat index of the test group were significantly higher than the control group ($P < 0.01$); after the pioglitazone intervention, all blood lipid-related indexes in the rat model were significantly lower than before the intervention ($P < 0.05$); skeletal muscle section staining results showed that the number of lipid droplets in skeletal muscle of rat model was significantly reduced after pioglitazone intervention; and pioglitazone intervention can significantly increase the mRNA and protein expression levels of p-ACC, GLUT7, PGC-1α, and CPT1 genes in the skeletal muscles of experimental rats ($P < 0.05$). Accordingly, it can be concluded that pioglitazone can play a role in treating insulin resistance by regulating the expression of related genes of AMPK, ACC, etc. in the AMPK signaling pathway.

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1. Introduction

Obesity is a chronic metabolic disease with a high incidence, and most patients with obesity are caused by improper diet (Yamauchi et al., 2011). Obesity causes many diseases, such as diabetes and coronary heart disease. In type 2 diabetes patients, insulin resistance is up to 70%, which is the biggest risk to human health. Insulin resistance refers to the reduced ability of the body's insulin (INS) to acquire or clear glucose. The formation mechanism of insulin resistance is very complex, which is influenced by many factors including genetics and living environment. The main reason is that the accumulation of excessive adipose tissue in the body increases the secretion of INS, and the number of INS receptors is

fixed, which can't accept excessive INS, ultimately leading to reduced efficiency of INS (Fallah et al., 2017). Building a model of insulin resistance is an excellent way for people to study insulin resistance. The main construction methods are divided into the obesity model caused by heredity, the obesity model caused by some drugs and the obesity model caused by high fructose food. Among them, the obesity model caused by high fructose food is relatively stable, easy to obtain, and most close to human diseases, so it is one of the most ideal methods to build the model. Positive glucose - hyperinsulinism clamp test is an important indicator to evaluate whether the insulin resistance model is qualified (Qu et al., 2017). The drugs currently used to treat diabetes diseases include thiazolidinediones, such as pioglitazone. However, excessive doses of these drugs cause side effects in patients.

Studies have shown that SOD and GLUT4 in the body's muscles play an important role in sensitizing INS and improving insulin resistance (Mei et al., 2011). Moreover, FFA produced after adipose tissue decomposition and peroxysome proliferation active receptor (PPAR) gene specifically expressed in fat are closely related to insulin resistance. Skeletal muscle is an important target tissue of INS and plays a crucial role in glucose metabolism. In obese patients, the ability of ADPN to oxidize FFA will be reduced, and the increase of TG content will lead to the increase of fat deposition in skeletal muscle, eventually leading to insulin resistance (Song et al., 2013). Therefore, TG content plays an important role in insulin resistance. The most important target tissue of INS is skeletal muscle, and AMPK pathway plays an important role in regulating skeletal muscle energy metabolism (Chen et al., 2017; Ruderman et al., 2013). ACC is an important fatty acid synthetase and an enzyme that controls the rate of synthesis of malonyl COA. Malonyl COA is an inhibitor of the CPT1 gene, which regulates lipid performance. PGC-1α can also increase the sensitivity of INS and reduce lipid deposition in skeletal muscles (Freyssin et al., 2012). Insulin resistance in skeletal muscles may be associated with increased fat deposition in muscles.

Previous studies have confirmed that pioglitazone can reduce lipid deposition in skeletal muscle of insulin resistance model. In this study, high fructose diet was used to induce the establishment of insulin resistance rat model, and the differences of obesity physiological indicators between two groups were compared. In addition, the insulin resistance model constructed by high fructose diet was used as the experimental object to explore the differences of pioglitazone on lipid deposition in skeletal muscle and expression levels of related proteins and mitochondrial related genes in AMPK signaling pathway in model rats. It is expected to provide a molecular basis for further treatment of insulin resistance.

2. Materials and methods

2.1. Animals and breeding

In this study, 40 healthy male SD rats with uniform body weight (average weight of about 142 g) were purchased from Shanghai Slack Animal Experiment Co., Ltd. SD rats were randomly divided into two groups according to the ratio of 1:3 normal feed (ND) group (n = 10) and high-fat feed (HFD) group (n = 30). Rats in ND group were fed a common diet containing 10% fat, 25% protein and 65% carbohydrate, which met the requirements of GB 14924.3-2010. Rats in the HFD group were fed a high fructose diet containing 60% saccharides, 20% protein, 13.5% carbohydrate, 6% fat and 0.5% salt. All rats were fed at 23 ± 2℃, 55 ± 15% humidity and 12 h light cycle. During the experiment, they were free to drink water and eat. All animal procedures were approved by Ethics Committee of Hebei Medical University, and all experimental methods were conducted in accordance with approved guidelines.

2.2. Construction of animal models

Rats in the ND group and the HFD group were fasted for 13 h after 6 weeks of feeding. Six rats were randomly selected from the ND group, as were the HFD group, and 3% pentobarbital sodium (40 mg/kg) was intraperitoneally injected into the rats on an empty stomach, after which catheters were inserted into the right cervical vein and artery. The catheter was extracted subcutaneously through the back of the neck, and heparin (100U/mL) was used for anticoagulant sealing and ligation and fixation. The rats were then raised independently and fed freely. After the rats returned to the normal state, they were deprived of food overnight for another 12 h, and the hyperinsulinism-normal glucose clamp experiment was conducted the next day. The basic value of blood glucose in the arteries of rats was firstly detected. After the blood glucose reached a stable state, insulin was injected at the rate of 5 mU·kg^{-1}·min^{-1}, and then blood glucose was detected every 8 min. When the blood glucose value was lower than the basic blood glucose value, 30% glucose was injected with a speed of 8 mg·kg^{-1}·min^{-1}, keeping the blood glucose value of the rats at 5 ± 0.5 mmol/L. The average value of GIR under stable state was used to evaluate the INS sensitivity of rats, so as to determine the construction of insulin resistance model in the high fructose group.

2.3. Detection of various indicators and collection of organization

Rat abdominal aortic serums were isolated after fasting for 6 weeks and 14 weeks overnight for 13 h. At the same time, blood glucose (BG) levels and insulin (INS) levels in serum of rats in these two periods were detected, and then ADPN, TG, TC and FFA levels in rats at 14 weeks were detected. Among them, BG was detected by glucose oxidase method. INS, FFA and ADPN were tested with rat serum ELISA kit (Nanjing Senbega Biotechnology Co., Ltd., China). And TC and TG were determined by automatic biochemical analyzer. After 6 weeks of the experiment, 6 rats were randomly selected from ND group and HFD group for rapid blood bleeding to kill the rats, the quadriceps femoris muscle samples were taken, and the normal saline was rinsed and preserved. The remaining 12 rats in the HFD group were divided into 2 groups: control group and pioglitazone intervention group. After 8 weeks, the rats were sacrificed by rapid bloodletting, and the quadriceps muscle samples were taken and washed with physiological saline. All tissues were kept in triplicate, one for oil red O staining (cryopreservation), one for RNA extraction, and one for standby, which was stored in liquid nitrogen.

2.4. Extraction and detection of total RNA in tissues

RNA was extracted from skeletal muscle tissue of rats by traditional Trizol method. 80 mg of tissue was placed in a high-pressure sterilized mortar soaked overnight with 0.1% DEPC water. A small amount of liquid nitrogen was added to grind into a powder, and then placed in a centrifuge tube containing 1 mL Trizol without RNA enzyme. After the sample was placed at room temperature for 20 min, 200 μL chloroform was added and shaken violently for 15 s. The supernatant was centrifuged at 12,000 RPM at 4 °C for 15 min. After mixing with isopropanol of equal volume, it was allowed to stand for 15 min, centrifuged at 12,000 RPM at 4 °C for 10 min, and the supernatant was discarded. 800 μL of 75% ethanol prepared by 0.1% diethylpyrocarbonate (DEPC) water under high pressure was added to the sample, and the precipitate was washed twice. After standing for 15 min, 30 μL of 0.1% DEPC after high pressure was added to dissolve the precipitate. The sample was dispensed 2 μL on ice for detection of RNA concentration, and the remainder was stored in a −80 °C refrigerator. RNA was

diluted with 1 mL of ultrapure water, and the purity and concentration were detected by a nucleic acid detector (BIO-RAD, USA). Among then, the OD_{260}/OD_{280} value was about 1.9, and the sample with a concentration greater than 300 ng/μL was considered qualified.

2.5. Real-time fluorescent quantitative PCR (qRT-PCR)

Total RNA extracted from rat skeletal muscle tissues was used for cDNA reverse transcription according to PrimeScript™ RT reagent Kit with gDNA Eraser from TAKARA company. The volume of RNA used was calculated by 1000/total RNA concentration. All steps were performed on ice, and the cDNA obtained by reverse transcription was stored in the refrigerator at −20 °C. Primer Premier 5.0 software was used to design AMPK, ACC, GLUT7, NRF1, COXIV, CPT1, ACADM, PPARα, PPARγ, PGC-1α, ADIPOR1, ADIPOR2 and β-actin quantitative primers. Among them, β-actin was used as the internal reference primer to calculate the expression levels of each gene. The primer information is shown in Table 1.

Quantitative testing of these genes was performed in accordance with the specifications for the TB Green® Premix Ex Taq™ II fluorescence quantitative kit manufactured by TAKARA. Eight connecting pipes were placed on ice, and 0.5 μL of upstream and downstream primers, 10 μL of TB Green I reagent, 1 μL of cDNA, and 20 μL total reaction system containing 8 μL of ddH2O were added. After vortex mixing, they were placed in a fluorescence quantitative PCR instrument, and quantitative analysis was performed at 95 °C for 5 min, 95 °C for 15 s, 60 °C for 20 s, and 72 °C for 30 s (40 cycles).

2.6. Western blotting

100 mg rat skeletal muscle tissue was placed in a sterile centrifuge tube to which 1 mL of tissue lysate was added, homogenized in an ice bath, allowed to stand for 30 min, and fully lysed and centrifuged at 4 °C and 12,000 rpm for 5 min, and the supernatant was taken and stored at −20 °C after split charging. Protein levels (μg/μL) were measured by a BCA kit (Invitrogen, USA). After

that, 10% SDS-PAGE separation gel was prepared. The prepared protein sample solution to be tested was put into the hole, and the same amount of protein was separated by electrophoresis. The strip of the target protein was then electroporated onto the PVDF membrane, washed three times with distilled water, and blocked overnight at 4 °C. PVDF membrane was incubated with diluted primary antibodies of AMPK, P-AMPK, ACC, P-ACC, CPT1, GLUT4, PGC-1α, and β-actin (Invitrogen, USA), respectively. And horseradish peroxidase-labeled goat anti-rabbit or goat anti-mouse IgG secondary antibody (Invitrogen, USA) was added after 2–3 h. UVP software was used to calculate the protein expression of the target gene after development and fixation.

2.7. Statistical analysis

Beta-actin gene was used as the control group, and the mRNA expression level of each target gene was calculated by $2^{-\triangle\triangle Ct}$ method. The target gene IOD value/β-actin gene IOD value was used to obtain the protein expression of each target gene. SPSS19.0 software was used for statistical analysis, multiple comparisons in one-way ANOVA were conducted among multiple groups, and t-test was used for comparison between the two groups.

3. Results

3.1. Changes of various indexes in rats after HFD feeding

Rats were fed with normal diet and high fructose diet, and their body weight was recorded weekly. The change trend of body weight after 6 weeks was shown in Fig. 1. It could be concluded that there was little difference in weight between the ND and HFD groups in the first 3 weeks (296.13 ± 5.58 g vs 307.90 ± 6.52 g); from week 3 to week 6, the weight of rats in ND group increased rapidly (417.30 ± 6.56 g); the ND group showed a slow increase in body weight from week 6 (417.30 ± 6.56 g) to week 14 (470.91 ± 5.17 g); the rats in the HFD group showed a continuous upward trend from week 3 (299.15 ± 4.29 g) to week 14 (659.37 ± 7.96 g); and at week 6, after intervention with pioglitazone in some of the HFD rats, it was found that the rate of weight gain of the rats after intervention was slower than that of the HFD group, and at 12 W, the weight of Pioglitazone group was 531.20 ± 6.39 g.

After 6 weeks of feeding, the weight, blood glucose levels, and insulin levels of the ND group and the HFD group were measured. The results showed that the weight (491.63 ± 7.09 vs 417.30 ± 6.56), serum glucose (8.53 ± 0.31 vs 4.22 ± 0.32), and insulin (20.2 ± 1.03 vs 12.4 ± 0.92) of the HFD group were significantly higher than those of the ND group (P < 0.05).

After 8 weeks of feeding on the previous basis, it was found that the weight of the HFD group was significantly higher than that of the ND group (652.78 ± 8.61 vs 470.91 ± 5.17) (P < 0.05); the content of TG and FFA was significantly higher than that of ND group (0.52 ± 0.02 vs 0.32 ± 0.01) (P < 0.05); the ADPN content was significantly lower than that of ND group (13.74 ± 1.12 vs 18.02 ± 1.03) (P < 0.05); and TC content was higher than ND group (1.43 ± 0.04 vs 1.05 ± 0.09) (P < 0.05). In this study, the results showed that feeding rats with high fructose diet increased the TC content in rats, deepened the hidden dangers of the disease, and eventually caused the deposition of skeletal muscle fat to result in obesity. After pioglitazone intervention, the weight (541.00 ± 9.50 vs 470.91 ± 5.17) and FFA content of the rats were significantly lower than that of the HFD group (0.77 ± 0.06 vs 1.37 ± 0.06) (P < 0.05), while the ADPN content was significantly higher than that of the HFD group (23.05 ± 0.98 vs 13.74 ± 1.12) (P < 0.05).

Table 1
Primer information.

Gene name	Primer sequence	Product size (bp)
AMPK	F: CAAACACCAAGGCGTACG	91
	R: TGCTCTACACACTTCTGCCAT	
ACC	F: ACTAATAATACTTTCGGAAACG	96
	R: ACATAAACCTCCAGGGACGC	
GLUT7	F: ATACTCATTCTCGGACGGTTC	84
	R: CCCACATACATAGGCACCAA	
NRF1	F: ACGGTTGCTTCGGAAACTC	144
	R: CGCACCACATTCTCCAAAG	
COXIV	F: GAGCTTCGCTGAGATGAACA	85
	R: AAGCGCAGTGAAGCCGATGA	
CPT1	F: AGCTTCCAAACATCACTG	111
	R: CATGCGGAAATAGGCTTCGT	
ACADM	F: CGAGTTGACGGAGCAGCAGA	125
	R: CTCTCTTGATGAGAGGGAA	
PPARα	F: AGGCAGAGGTCCGATTCTTC	128
	R: CAGCAAGGTAACCTGGTCATTC	
PPARγ	F: TGCCATTCTGGCCCACCAAC	79
	R: GATGTCAAAGGAATGGGAGTG	
PGC-1α	F: GCTCCAAGACCAGGAAATC	108
	R: TTGCCATCCCGTAGTTCAC	
ADIPOR1	F: ATCTTCCGCATCCACACAG	145
	R: ACATCCCGAAGACCACCTT	
ADIPOR2	F: TGTAAGGTGTGGGAAGGTCG	112
	R: GGAAAGAAGGCATAGGAGGC	
β-actin	F: TGTGATGGTGGGTATGGGT	69
	R: AGGATGCCTCTCTTGCTCTG	

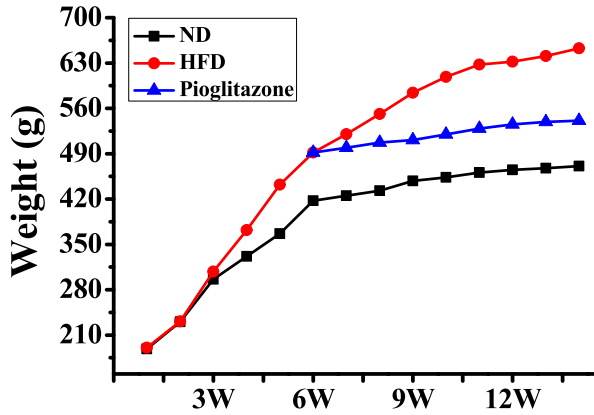**Fig. 1.** Curves of weight changes in different groups of rats.

Fig. 2. Changes of visceral fat weight and abdominal fat index in each group of rats (** indicated that the difference was extremely significant compared with ND group *(P < 0.01)*; and ## indicated that the difference was extremely significant compared with HFD group *(P < 0.01)*).

3.2. Changes of visceral fat weight and abdominal fat index in rats

At week 14, fat weight and abdominal fat index of visceral tissues of rats in each group were detected and compared, as shown in Fig. 2. It was found that the visceral fat weight and abdominal fat index in ND group were lower than those in HFD group and pioglitazone group. The visceral fat weight and abdominal fat index in the HFD group were significantly higher than those in the ND group *(P < 0.01)*, while those in the pioglitazone group were significantly lower than those in the HFD group *(P < 0.01)*.

3.3. Analysis of oil red O staining in skeletal muscle of rat

After collecting the skeletal muscle tissues of rats at 14 weeks and staining with oil red O, it can be observed from Fig. 3A and B that the red lipid droplets in the skeletal muscle tissues of rats in the HFD group were significantly more than those in the ND group. However, pioglitazone intervention can improve the situation. Therefore, compared with figure B, the number of lipid droplets in skeletal muscle tissues of rats in figure C was significantly reduced, but the number of lipid droplets was more than that in ND group.
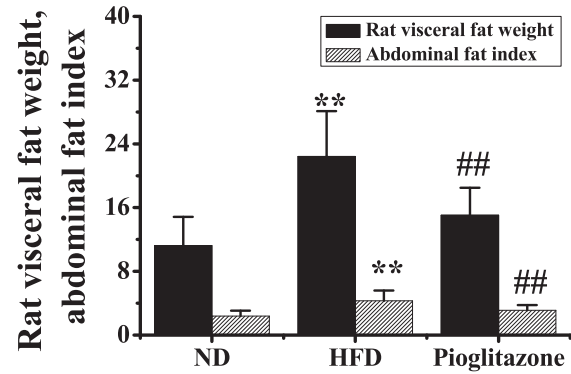
3.4. Analysis of the expression of target genes in skeletal muscle

The mRNA expressions of ADIPOR1, ADIPOR2, COXIV, NPF1, ACADM, PPARα and PPARγ genes in skeletal muscle tissues of different groups were detected by qRT-PCR. It can be observed from Fig. 4 that the expressions of ADIPOR1 and ADIPOR2 genes in skeletal muscle of HFD group were significantly lower than those in ND group and pioglitazone group. mRNA expression of ACADM, PPARα and PPARγ genes in skeletal muscle tissues of rats in ND group and pioglitazone group was significantly higher than that of rats in HFD group *(P < 0.05)*. The mRNA expression of COXIV gene and NPF1 gene in ND group was significantly higher than that in HFD group *(P < 0.05)*, while the expression of mRNA in skeletal muscle of COXIV gene and NPF1 gene after pioglitazone intervention was not different from that in HFD group *(P < 0.05)*.

MRNA and protein expression levels of AMPK and ACC genes in skeletal muscles of rats in each group, and protein expression levels of P-AMPK and P-ACC genes were shown in Fig. 5. As shown in Fig. 5A, B and D, there was no difference in the mRNA expression and protein expression of AMPK and ACC genes in ND group, HFD group and pioglitazone intervention *(P > 0.05)*. However, according
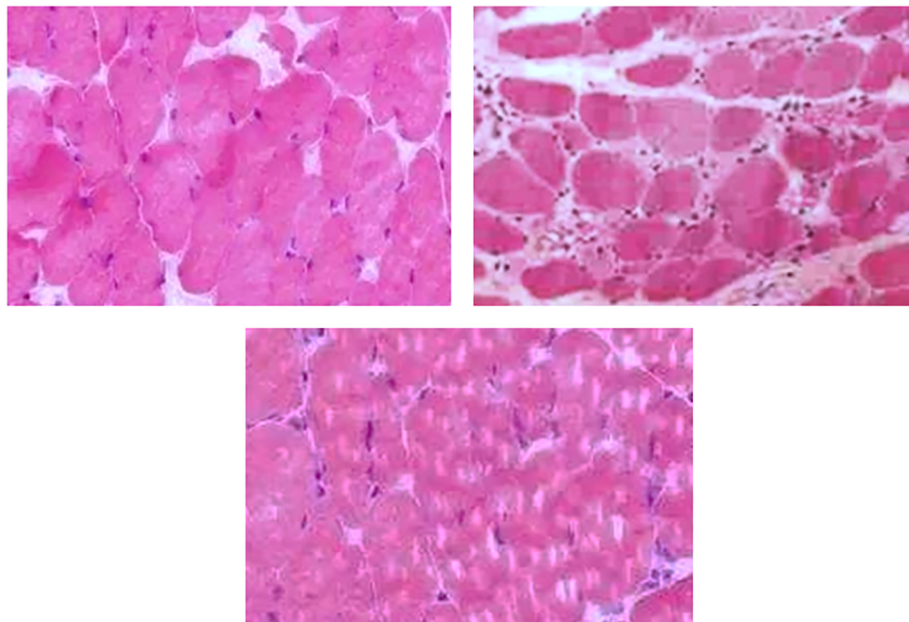

Fig. 3. Rat skeletal muscle fat drop oil red O staining (A. ND group; B. HFD group; C. Pioglitazone intervention group, ×400).
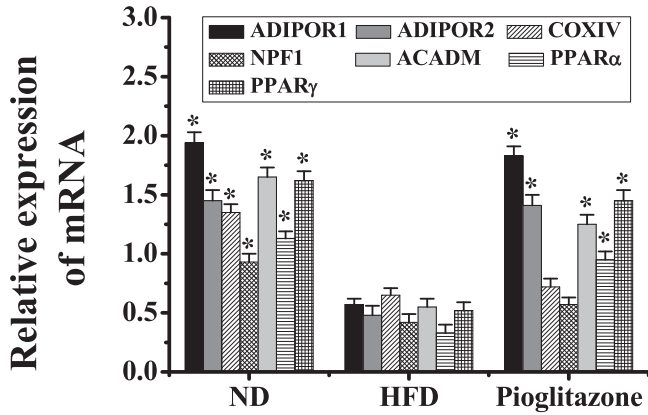
Fig. 4. mRNA expression levels of target genes in skeletal muscle of rats (* indicates significant difference in expression compared with the HFD group, *P* < 0.05).

to Fig. 5C and D, protein expression of P-AMPK and P-ACC genes in skeletal muscle tissues of rats in ND group and after intervention with pioglitazone was significantly higher than that of rats in HFD group (*P < 0.05*).

Subsequently, the mRNA and protein expression levels of GLUT7, PGC-1α, and CPT1 genes in skeletal muscle of each group were examined. It can be observed from Fig. 6A and D that the mRNA and protein expression of GLUT7 gene in skeletal muscle tissue of ND group and after the intervention with pioglitazone was significantly higher than that of HFD group *(P < 0.05)*. In Fig. 6B and D, mRNA and protein expression of PGC-1α gene in skeletal muscle tissues of rats in ND group and after intervention with pioglitazone were significantly higher than those in HFD group (*P < 0.05*). In Fig. 6C and D, mRNA and protein expressions of CPT1 gene in skeletal muscle tissues of rats in ND group and after

intervention with pioglitazone were significantly higher than those of rats in HFD group (*P < 0.05*).

4. Discussion

Rats fed a high fructose diet developed insulin resistance and fat deposits in skeletal muscles. In this study, it was found that feeding rats with high fructose diet would increase their body weight, blood glucose and insulin content, and the increase of blood glucose and insulin content would cause the increase of possibility of insulin resistance. Due to the insulin resistance effect, abnormal lipid metabolism and other problems may occur (Woodie and Blythe, 2017). In this study, the TC content in the insulin resistance rat model induced by high fructose diet increased. The fat content in the body is closely related to the formation of insulin resistance, and visceral fat is significantly related to insulin resistance, hypertension and other diseases. Previous studies have shown that the basic symptom of insulin resistance patients is the high content of visceral fat, while FFA and TG are produced the most during the decomposition of visceral fat (Liao et al., 2017), which is the same as the findings of this study that the FFA and TG content of HFD group rats is significantly higher than that of ND control group. The commonly used drugs to reduce blood glucose in western medicine are mainly thiazolidinediones, etc. Pioglitazone is one of the drugs, which is an agonist of PPARγ. When pioglitazone is used to treat diseases in clinical practice, ADPN content in patients' plasma increases significantly, while INS level in fasting state significantly decreases, which is consistent with the results of this study, possibly because pioglitazone regulates the expression of some mitochondrial oxidative phosphorylation genes (Jin et al., 2015). Adiponectin (ADPN) is an endogenous bioactive protein secreted by adipocytes, which has been shown to be related to insulin sensitization (Priyadarshini and Anuradha, 2017).
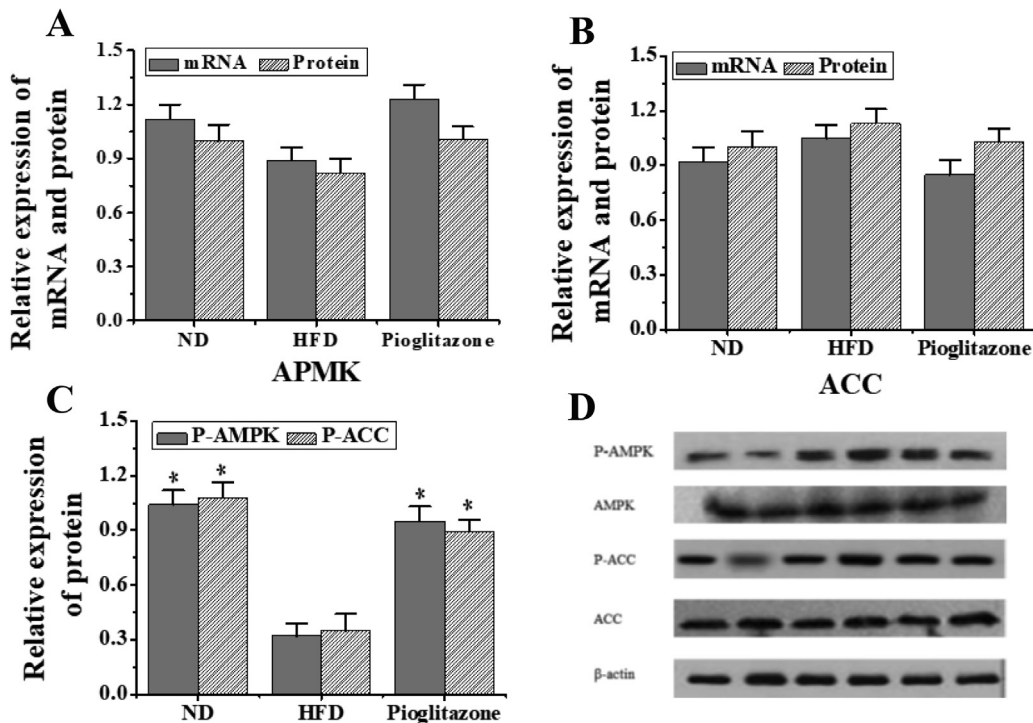

Fig. 5. mRNA and protein expression levels of AMPK and ACC genes in skeletal muscle of rats (A. Relative expression levels of mRNA and protein of APMK gene in skeletal muscle of rats under the intervention of ND, HFD, and pioglitazone; B. Relative expression levels of mRNA and protein of ACC gene in skeletal muscle of different groups of rats; C. Relative expression levels of protein of P-AMPK and P-ACC gene in skeletal muscle of different groups of rats; D. Western blotting map of P-AMPK, AMPK, P-ACC, ACC, and β-actin; * indicated that the expression was significantly different from the HFD group, *P* < 0.05).
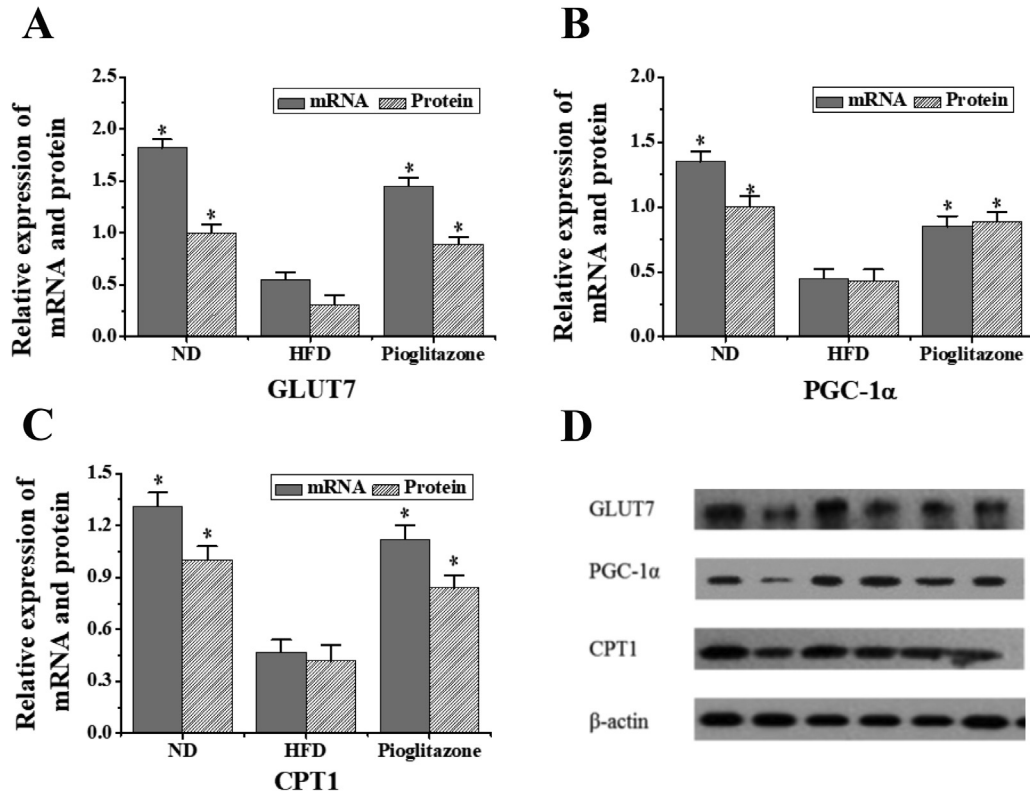
Fig. 6. mRNA and protein expression levels of GLUT7, PGC-1α, and CPT1 genes in rat skeletal muscle (A. Relative expression levels of mRNA and protein of GLUT7 gene in rat skeletal muscle under the intervention of ND, HFD and pioglitazone; B. Relative expression levels of mRNA and protein of PGC-1α gene in skeletal muscle of rats in different groups; C. Relative expression levels of mRNA and protein of CPT1 gene in skeletal muscle of rats in different groups; D. Western blotting figure of GLUT7, PGC-1α, CPT1 and β-actin; * indicated that the expression was significantly different from that of the HFD group, $P < 0.05$).

The size and shape of lipid droplets have a direct impact on cell metabolism and the secretion of important hormones in the body, and the content of saturated fatty acids in high fructose foods plays an important role in the formation of lipid droplets (Hsu et al., 2017). At present, the main drugs for the treatment of diabetes diseases are the hypoglycemic drugs of thiazolidinediones, and pioglitazone belongs to these drugs. Studies have shown that pioglitazone can accelerate the phosphorylation of AMPK and ACC, thereby reducing fat deposition in skeletal muscle and improving insulin resistance (Kunasegaran et al., 2017). In this study, it was found that the intervention of pioglitazone reduced the rate of weight gain in HFD rats, decreased the visceral fat weight and abdominal fat index in rats, and increased the expressions of PPARα, PPARγ, P-AMPK, P-ACC, PGC-1α and CPT1 genes. This may be because the expression of P-AMPK is up-regulated when the body consumes too much glucose and ADNP levels rise. Activation of AMPK pathway would increase oxidation of fatty acids, and increased phosphorylation of AMPK would inhibit ACC activity. The increased phosphorylation of AMPK also up-regulates the expression of GLUT4 gene (Rui et al., 2015). PGC-1α gene can regulate mitochondria, improve INS sensitivity, and ultimately improve insulin resistance. Phosphorylation of AMPK can reduce the content of malonyl COA and reduce the activity of malonyl COA to inhibit the expression of CPT1 gene, thereby up-regulating the expression of this gene. PPARα and PPARγ are two subtypes of peroxisome proliferator-activated receptors. PPARα reduces TG levels, while PPARγ increases glucose metabolism. These two factors are activated by decreased expression of mitochondrial protein-related genes in diabetes-resistant patients (Ghorbanzadeh et al., 2016) (see Tables 2 and 3).

Table 2
Changes of weight and serum related indexes in rats at 6 weeks.

	Weight (g)	BG (mmol/L)	INS (mU/L)
ND	417.30 ± 6.56	4.22 ± 0.32	12.4 ± 0.92
HFD	491.63 ± 7.09*	8.53 ± 0.31*	20.2 ± 1.03*

* Note: indicated significant difference between the two groups ($P < 0.05$).

Table 3
Changes of weight and other related indexes in rats at 14 weeks.

	Weight (g)	ADPN (ng/mL)	TG (mmol/L)	TC (mmol/L)	FFA (mmol/L)
ND	470.91 ± 5.17	18.02 ± 1.03	0.32 ± 0.01	1.05 ± 0.09	0.72 ± 0.03
HFD	652.78 ± 8.61*	13.74 ± 1.12*	0.52 ± 0.02*	1.43 ± 0.04*	1.37 ± 0.06*
Pioglitazone	541.00 ± 9.50#	23.05 ± 0.98#	0.25 ± 0.02#	1.01 ± 0.05	0.77 ± 0.06#

Note:
* Indicated significant difference compared with ND group ($P < 0.05$).
# Indicated significant difference compared with HFD group ($P < 0.05$).

In summary, feeding rats with high fructose diets causes insulin resistance in rats. Pioglitazone intervention can improve the status of insulin resistance in rats, reduce the level of various indicators and the deposition of fat in skeletal muscle, which may play an improving role by regulating the expression of related gene mRNA and protein in AMPK pathway. However, in this study, only the effects of pioglitazone intervention on mRNA and protein expression of related genes in AMPK signaling pathway in skeletal muscle of rats were examined. There are many factors that interfere with gene expression, so further regulation studies are needed in combination with non-coding RNA such as miRNA. In conclusion, the results of this study can lay the foundation for the research of pioglitazone in the treatment of insulin resistance caused by obesity.

References

Chen, X., Zhao, X., Lan, F., et al., 2017. Hydrogen sulphide treatment increases insulin sensitivity and improves oxidant metabolism through the camkkbeta-ampk pathway in pa-induced ir c2c12 cells. Sci. Rep. 7 (1), 13248.

Fallah, H., Akbari, H., Abolhassani, M., et al., 2017. Berberis integerrimaameliorates insulin resistance in high- fructose-fed insulin-resistant rats. Iranian J. Basic Med. Sci. 20 (10), 1093–1101.

Freyssin, C., Verkindt, C., Prieur, F., et al., 2012. Cardiac rehabilitation in chronic heart failure: effect of an 8-week, high-intensity interval training versus continuous training. Arch. Phys. Med. Rehabil. 93 (8), 1359–1364.

Ghorbanzadeh, V., Mohammadi, M., Dariushnejad, H., et al., 2016. Effects of crocin and voluntary exercise, alone or combined, on heart vegf-a and homa-ir of hfd/stz induced type 2 diabetic rats. J. Endocrinol. Invest. 39 (10), 1–8.

Hsu, M.C., Wang, M.E., Jiang, Y.F., et al., 2017. Long-term feeding of high-fat plus high-fructose diet induces isolated impaired glucose tolerance and skeletal muscle insulin resistance in miniature pigs. Diabetol. Metabolic Syndr. 9 (1), 81.

Jin, X., Zhang, Y.F., Bei, Y., et al., 2015. Effect of jinlida on cholesterol-related genes in skeletal muscle in fat-induced insulin resistance apoe~(-/-) mice. Chinese Pharmacol. Bull. 38 (6), 1237.

Kunasegaran, T., Mustafa, M.R., Achike, F.I., et al., 2017. Quercetin and pioglitazone synergistically reverse endothelial dysfunction in isolated aorta from fructose-streptozotocin (F-STZ)-induced diabetic rats. Eur. J. Pharmacol. 799 (1), 160.

Liao, N., Zheng, Y., Xie, H., et al., 2017. Adipose tissue-derived stem cells ameliorate hyperglycemia, insulin resistance and liver fibrosis in the type 2 diabetic rats. Stem Cell Res. Ther. 8 (1), 286.

Mei, Z.G., Zeng, Y.B., Liu, X.J., et al., 2011. Efficacy of auricular acupuncture on inflammatory markers and insulin resistance in type 2 diabetes model of rats: In light of cholinergic anti-inflammatory pathway. In: International Conference on Human Health & Biomedical Engineering.

Priyadarshini, E., Anuradha, C.V., 2017. Glucocorticoid antagonism reduces insulin resistance and associated lipid abnormalities in high-fructose-fed mice. Can. J. Diabetes 41 (1), 41.

Qu, W., Han, C., Li, M., et al., 2017. Anti-tnf-α antibody alleviates insulin resistance in rats with sepsis-induced stress hyperglycemia. J. Endocrinol. Invest. 41 (6), 455–463.

Ruderman, N.B., Carling, D., Prentki, M., et al., 2013. Ampk, insulin resistance, and the metabolic syndrome. J. Clin. Invest. 123 (7), 2764–2772.

Rui, B., Chen, H., Jang, L., et al., 2015. Melatonin upregulates the activity of ampk and attenuates lipid accumulation in alcohol-induced rats. Alcohol Alcohol. 51 (1), 11.

Song, R.S., Wei, P., Zhang, Y., et al., 2013. Central role of E3 ubiquitin ligase MG53 in insulin resistance and metabolic disorders. Nature 494, 375–381.

Woodie, L., Blythe, S., 2017. The differential effects of high-fat and high-fructose diets on physiology and behavior in male rats. Nutrit. Neurosci. 21 (5), 1–9.

Yamauchi, T., Kamon, J., Waki, H., et al., 2011. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat. Med. 8 (8), 941–946.