

# PA and OA Induce Abnormal Glucose Metabolism by Inhibiting KLF15 in Adipocytes

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## Research Article

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# Abstract

**Background:** Obesity-induced elevated serum free fatty acids (FFAs) levels result in the occurrence of type 2 diabetes mellitus (T2DM). However, the molecular mechanism remains largely enigmatic. This study was to explore the effect and mechanism of KLF15 on FFAs-induced abnormal glucose metabolism.

**Methods:** Levels of TG, TC, HDL-C, LDL-C, and glucose were measured by different assay kits. qRT-PCR and Western Blot were used to detect the levels of GPR120, GPR40, phosphorylation of p38 MAPK, KLF15, and downstream factors.

**Results:** KLF15 was decreased in visceral adipose tissue of obesity subjects and high-fat diet (HFD) mice. In HFD mice, GPR120 antagonist significantly promoted KLF15 protein expression level and phosphorylation of p38 MAPK, meanwhile reduced the blood glucose levels. While, blocking GPR40 inhibited the KLF15 expression. In 3T3-L1 adipocytes, 1500  $\mu$ M PA inhibited KLF15 through a GPR120/P-p38 MAPK signal pathway, and 750  $\mu$ M OA inhibited KLF15 mainly through GPR120 while not dependent on P-p38 MAPK, ultimately resulting in abnormal glucose metabolism. Unfortunately, GPR40 didn't contribute to PA or OA-induced KLF15 reduction.

**Conclusions:** Both PA and OA inhibit KLF15 expression through GPR120, leading to abnormal glucose metabolism in adipocytes. Notably, the inhibition of KLF15 expression by PA depends on phosphorylation of p38 MAPK.

## Background

Nowadays, the incidence rate of obesity is gradually increasing in the world <sup>[1]</sup>. Epidemiological data show that obesity is an essential cause for insulin resistance (IR) and type 2 diabetes mellitus (T2DM) <sup>[1]</sup>. Most notably, elevated serum free fatty acids (FFAs) levels induced by obesity result in the occurrence of T2DM <sup>[2]</sup>. It has been widely acknowledged that FFAs can not only bring energy to the body, but also act as signal factors participating in numerous physiological activities depending on different carbon chain length <sup>[3]</sup>. FFAs can be divided into three categories, that is, short chain fatty acids (SCFAs) that are no more than 6 carbons, medium chain fatty acids (MCFAs) that are 6-12 carbons, and long chain fatty acids (LCFAs) that are more than 12 carbons <sup>[3]</sup>. Palmitic acid (PA, C16:0) and oleic acid (OA, C18:1) belonging to LCFAs are the main component of total FFAs and account for 27% and 31% respectively <sup>[4]</sup>.

A large body of literature suggests that increased PA is a key risk factor for IR and T2DM <sup>[4-6]</sup>. A marked increase in synthesis of harmful complicated lipids, cell organelle dysfunction, and inflammatory reaction contribute to PA-induced IR and T2DM. Interestingly, the effects of OA on IR or T2DM are controversial. It has been reported that the OA emerges protective effects on insulin sensitivity <sup>[4]</sup>. While, these results are inconsistent with another study in which insulin sensitivity and glucose uptake were both abolished in 3T3-L1 adipocytes treated with PA and OA <sup>[7]</sup>. Similar results obtained Xie W *et al.* showed that decreased glucose transporter 4 (GLUT4) levels and impaired phosphorylation of serine/threonine protein kinase

(AKT) were found in 3T3-L1 differentiated with OA, suggesting resistance to insulin in fat cells<sup>[8]</sup>. Often, G-protein-coupled receptor (GPR) 40 and GPR120 can act as nutrient sensors that are both activated by saturated or unsaturated LCFAs<sup>[9]</sup>. However, whether PA/OA-induced disordered glucose metabolism is GPR40/120 dependent is not very clear.

The Krüppel-like factors (KLFs) are vital regulators of cell development, differentiation, and activation<sup>[10]</sup>. KLF15 is expressed in numerous tissues, such as liver, adipose tissue, spleen, and skeletal muscle, and has significant effects in glucose and lipid metabolism in cells<sup>[10,11]</sup>. It has been reported that KLF15 regulates the expression of genes related to glucose uptake and adipogenesis, such as *GLUT4* and *peroxisome proliferator activated receptor  $\gamma$*  (*PPAR $\gamma$* )<sup>[10,12]</sup>. Moreover, ADIPOLIN, an insulin-sensitizing and anti-inflammatory adipokine, could be transcriptionally activated by KLF15 in adipocytes<sup>[13]</sup>. Our results formerly demonstrated that compared with lean individuals, KLF15 mRNA expression levels of adipose tissue were markedly decreased in obese individuals, and had a negative correlation with body mass index (BMI), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), while had a positive correlation with high density lipoprotein cholesterol (HDL-C), suggesting downregulated KLF15 expression may relevant to lipid metabolism triggered by obesity<sup>[14]</sup>. It has been reported that activation of P38 mitogen activated protein kinase (p38 MAPK) is integral to inhibit KLF15 expression in cardiac myocyte<sup>[15,16]</sup>. Additionally, p38 MAPK is constantly regarded as signal downstream of Gq proteins that coupled with GPR120 or GPR40, suggesting Gq-p38 activation may be mediated by GPR120 or GPR40<sup>[17]</sup>. However, the function of GPR120(40)/p38 MAPK/KLF15 in the development of FFA-related disordered glucose homeostasis remains ambiguous.

In the present study, we provide evidence that KLF15 was decreased in visceral adipose tissue of obesity subjects and high-fat diet (HFD) mice. Moreover, in HFD mice, we found that GPR120 antagonist significantly promoted KLF15 protein expression level, while blocking GPR40 inhibited the KLF15 expression. In 3T3-L1 adipocytes, KLF15 improved PA-induced abnormal glucose metabolism. Notably, PA inhibited KLF15 expression through GPR120/P-p38 MAPK signal pathway, and OA inhibits KLF15 expression mainly through GPR120 while not dependent on P-p38 MAPK, ultimately resulting in abnormal glucose metabolism of 3T3-L1 adipocytes.

## Methods

### Study subjects

60 patients with elective abdominal surgery in Xinjiang Kashgar people's Hospital from October to December 2012 and from April to September 2013 were collected. The 60 patients were separated into normal control (NC) group ( $18.5 \text{ kg/m}^2 \leq \text{BMI} \leq 24 \text{ kg/m}^2$ ,  $n=30$ ) and obesity (OB) group ( $\text{BMI} \geq 28 \text{ kg/m}^2$ ,  $n=30$ ). General information of each subjects was collected and levels of fasting plasma glucose (FPG), HDL-C, LDL-C, TG, and total cholesterol (TC) were tested before surgery. Patients who underwent diabetes mellitus, tumors, acute inflammation, liver, and kidney disease, and took medicine in the period to treat

diseases of glucose and lipid metabolism were excluded. The patients were all signed informed consent, and ethics was supplied by the Medical Ethics Committee at First Affiliated Hospital of Medical College of Shihezi University (Approval number: 2017-049-01).

### **Experimental mouse models**

36 four-week-old male C57BL/6 mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. After acclimated for a week, they were randomly fed with normal diet including 10% fat ratio (ND, n=8) and high fat diet including 60% fat ratio (HFD, n=28) (Medicience Ltd, Jiangsu, China). The weight and body length of mice were recorded weekly. After 8 weeks of HFD feeding, 8 ND mice and 10 HFD mice were euthanized. The visceral adipose tissue and biochemical indicators were collected. The Lee's index was calculated by  $[(\text{body weight (g)} \times 10^3)/\text{body length (cm)}]^{1/3}$ . The other 18 HFD mice were start intraperitoneally injected with dimethylsulfoxide (DMSO, n=6, Solarbio, Beijing, China), AH7614 (n=6, 5mg/kg, Tocris, Bristol, UK) or GW1100 (n=6, 5mg/kg, MCE, Monmouth, USA) every other day for four weeks. After the 18 HFD mice were put to execution, visceral tissue samples and biochemical indicators were collected. The experimental animal ethics were supplied by the Medical Ethics Committee at First Affiliated Hospital of Medical College of Shihezi University (Approval number: A2017-115-01).

### **Cell lines and culture conditions**

Mouse 3T3-L1 preadipocytes, purchased from the Chinese Academy of Sciences cell bank, were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, California, USA) with 10 % bovine calf serum (Biological Industries, Beth Haemek, Israel) and 1% penicillin at 37°C under 5 % CO<sub>2</sub>. After the 3T3-L1 preadipocytes were growing to the contact inhibition for 48 hours, the culture medium was replaced with differentiation medium, which containing 0.5 mM isobutylmethylxanthine (IBMX, Sigma-Aldrich, St. Louis, USA), 5 µg/ml insulin (Sigma-Aldrich, St. Louis, USA), and 1 µM dexamethasone (Sigma-Aldrich, St. Louis, USA). 48 hours later, the differentiation medium was replaced with DMEM supplemented 10% FBS and 5 µg/ml insulin. After another two days, the medium was replaced with DMEM containing 10% FBS for two more days. Lipid droplets in mature adipocytes were stained with Oil red O.

### **Reagent preparation**

40 mM PA (Sigma-Aldrich, St. Louis, USA) or OA (Sigma-Aldrich, St. Louis, USA) solution: PA (0.0307g) or OA (30.08µL) were mixed to 3 mL of 0.1 mol/L sodium hydroxide (Fuyu Fine, Tianjin, China) solution, after that placing the mixture in a fully saponified water bath at 75°C for about 30 minutes until the liquid becomes clear. Then, the mixture was immediately put into 3mL of 40% bovine serum albumin solution without fatty acid (ZSGB-BIO, Beijing, China). 100 mmol/L AH7614 solution: 10 mg AH7614 was dissolved in 285 µL DMSO. 10 mmol/L GW1100 solution: 5 mg GW1100 was dissolved in 0.96 mL DMSO. 10 mmol/L SB203580 (MCE, Monmouth, USA) solution: 10 mg SB203580 was dissolved in 1.32 mL DMSO.

### **Quantitative reverse transcription PCR (qRT-PCR)**

Total RNA was extracted by Trizol (Thermo Fisher, New York, USA). In the reverse transcription (Eppendorf AG, Hamburg, Germany) system, 1 µg RNA was used to obtain 20 µL cDNA. The procedure set up with 42°C for 60 minutes, afterwards 70°C for 15 minutes. The mRNA expression levels of related genes were tested using the instrument Rotor-Gene Q (QIAGEN, Hilden, Germany). The procedure was as follows: 95°C for 3 minutes, 45 cycles at 95°C for 10 seconds, and 60°C for 30 seconds. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) or β-actin was used as control for normalization. The record was represented by cycle threshold (CT) values, and the  $2^{-\Delta Ct}$  or  $2^{-\Delta\Delta Ct}$  was used for analysis. Table S1 displays the primers of each genes.

### **Western Blot**

Cell lysates containing 1% phenylmethylsulfonyl fluoride (PMSF, Solarbio, Beijing, China) and radio-immunoprecipitation assay (RIPA) lysis buffer (Solarbio, Beijing, China) were used to lyse the cells and extract the total intracellular protein. The protein samples were mixed with 4× loading buffer (Thermo Fisher, New York, USA) in a ratio of 3:1, and heated at 100°C for 10 min. The gel-separated proteins were transferred onto the nitrocellulose (NC) filter membranes and then incubated with Tris buffered saline tween (TBST) buffer including 5% BSA for 2 hours at ambient temperature. NC membranes were incubated at 4°C overnight with antibodies to KLF15 (42 kDa; Abcam, 60B1220.1, 1:1000), GPR120 (42 kDa; Abcam, 60B1220.1, 1:1000), GPR40 (40 kDa; Thermofisher, D23G1, 1:1000), P-p38 MAPK (42 kDa; Cell Signaling D1F2, 1:1000), GAPDH(36 kDa; ZSGB-BIO, TA-08, 1:1000), β-Tubulin (51 kDa; ZSGB-BIO, TA-09, 1:1000), β-actin (42 kDa; ZSGB-BIO, TA-10, 1:1000). The secondary antibody (ZSGB-BIO, 1:10,000) was incubated at ambient temperature for 2 hours. The protein band results were identified by FluorChem HD2 (Thermo Scientific, Waltham, USA).

### **Biochemical indicator test**

Levels of TG, TC, HDL-C, LDL-C, and glucose were measured by TG assay kit (A110-1-1, Nanjing Jiancheng Bioengineering Institute, China), TC assay kit (A111-1-1, Nanjing Jiancheng Bioengineering Institute, China), HDL-C assay kit (A112-1-1, Nanjing Jiancheng Bioengineering Institute, China), LDL-C assay kit (A113-1-1, Nanjing Jiancheng Bioengineering Institute, China), and glucose assay Kit (F006-1-1, Nanjing Jiancheng Bioengineering Institute, China).

### **Statistical analysis**

Data are expressed as the mean ± SEM. Statistical significance was assessed using SPSS17.0 with the appropriate test: 2 tailed *t*-test or one-way ANOVA. A  $P < 0.05$  was considered statistically significant.

## **Results**

# **KLF15 is decreased in visceral adipose tissue of OB subjects**

General information and biochemical indexes of NC subjects (n = 30) and OB subjects (n = 30) were showed in Table S2. We found that the weight, waist circumference (WC), Hip- circumference (HC), Waist-Hip Ratio (WHR), BMI, and TG were all significantly higher in OB group compared with NC group ( $P < 0.01$ ), while the Age, FPG, HDL-C, LDL-C, and TC had no significant difference. Significance was also observed for KLF15 mRNA and protein levels of visceral adipose tissue in OB subjects compared to NC subjects ( $P < 0.05$ ) (Fig. 1A). KLF15 levels were then compared to general information and biochemical indexes to evaluate degree of correlation. The results showed that KLF15 levels were significantly and negatively correlated with WC, WHR, weight, BMI, and TG, suggesting KLF15 levels were correlated with obesity state ( $P < 0.05$ ) (Table S3).

## Reduced KLF15 expression of visceral adipose tissue is controlled by HFD

As shown in Fig. 2A, the HFD-fed mice gained more weight than the ND-fed mice. In the ninth week, the Lee's index and visceral adipose tissue weight were significantly higher in HFD group compared with ND group ( $P < 0.001$ ) (Fig. 2B,C). In serum biochemical assays as shown in Fig. 2D, HFD group exhibited significant increase in serum fasting blood glucose (FBG), TG, TC levels, and significant decrease in serum LDL-C level compared to ND groups ( $P < 0.05$ ). Compared to the ND group, the KLF15, ADIPOLIN and GLUT4 mRNA expression levels of visceral adipose tissue were dramatically decreased in mice receiving HFD ( $P < 0.05$ ), while GPR120 and GPR40 mRNA expression had no significant difference (Fig. 2E). Moreover, in HFD group, the GPR120 protein expression level and the phosphorylation of p38 MAPK of visceral adipose tissue were significantly increased ( $P < 0.05$ ), and the KLF15 protein expression level was significantly decreased ( $P < 0.05$ ) (Fig. 2F,G). More importantly, spearman correlation analysis revealed that KLF15 mRNA expression level was negatively relative with weight, Lee's index, visceral adipose tissue weight, TG, TC, and HDL-C ( $P < 0.05$ ), and significantly positive with ADIPOLIN and GLUT4 ( $P < 0.01$ ) (Table S4).

## KLF15 is upregulated by GPR120 antagonist and downregulated by GPR40 antagonist in HFD mice

To explore whether KLF15 expression was regulated by GPR40 or GPR120, the HFD mice received an intraperitoneal injection of GPR40 antagonist GW1100 or GPR120 antagonist AH7614 (Fig. 3A). As a result, GW1100 or AH7614 did not affect the whole body weight and visceral adipose tissue weight (Fig. 3B,C). The random blood glucose (RBG) levels in the HFD + AH7614 group were significantly decreased compared with the HFD group ( $P < 0.05$ ) (Fig. 3D). The mRNA expression levels of KLF15, ADIPOLIN, GLUT4 in the HFD + AH7614 or HFD + GW1100 group were comparable to those observed in the HFD group (Fig. 3E). Of note, the KLF15 protein expression levels were significantly increased and the phosphorylation of p38 MAPK was significantly decreased in the HFD + AH7614 group ( $P < 0.05$ ) (Fig. 3F,G). Conversely, the KLF15 protein expression levels were significantly decreased in the HFD + GW1100 group ( $P < 0.05$ ), while the phosphorylation of p38 MAPK had no significant difference

(Fig. 3H,I). Overall, these results exhibit that HFD-induced lower KLF15 expression of visceral adipose tissue is mainly regulated by GPR120.

## **PA inhibits KLF15 expression through GPR120/P-p38 MAPK, resulting in abnormal glucose metabolism of 3T3-L1 adipocytes**

3T3-L1 adipocytes were dealt with different concentrations of PA to determine its effect on GPRs/P-p38 MAPK/KLF15 levels and glucose consumption. The results showed that compared with the 0  $\mu$ M PA-treated group, the GPR40 and GPR120 mRNA expression levels were both significantly promoted and the KLF15 mRNA expression level was significantly reduced in 1500  $\mu$ M PA-treated group ( $P < 0.05$ ) (Fig. 4A). Western Blot results also discovered that 1500  $\mu$ M PA markedly promoted the GPR40 and GPR120 expression and obviously inhibited the KLF15 expression. Also, in the presence of 1500  $\mu$ M PA, p38-MAPK phosphorylation was increased (Fig. 4B). Moreover, the glucose consumption of 3T3-L1 adipocytes was obviously abrogated in 1500  $\mu$ M PA-treated group ( $P < 0.001$ ) (Fig. 4C).

Next, the expression of KLF15 was then overexpressed or silenced by transfection with overexpression plasmid (OE-KLF15) or interference fragment (si-KLF15) to investigate the role of KLF15 effects on PA-induced abnormal glucose metabolism. The results showed that KLF15 overexpression could rescue PA-mediated reduction of ADIPOLIN and GLUT4 expression ( $P < 0.05$ ) (Fig. 4D). Moreover, KLF15 overexpression could significantly improve the effects of PA on impaired insulin sensitivity and glucose consumption ( $P < 0.05$ ) (Fig. 4E,F). As expected, KLF15 silencing could significantly exacerbate the stimulatory effects of PA on reduced ADIPOLIN and GLUT4 expression, impaired insulin sensitivity and inhibited glucose consumption ( $P < 0.05$ ) (Fig. 4G-I).

To further investigate whether PA inhibited KLF15 expression and glucose consumption through a GPRs/P-p38 MAPK pathway, AH7614, GW1100 and SB203580 were respectively added into 1500  $\mu$ M PA treated cells to block GPR120, GPR40 and phosphorylated p38 MAPK. We found that PA-induced suppressed levels of KLF15 and elevated P-p38 MAPK, were overturned in 3T3-L1 adipocytes when cotreated with AH7614 (Fig. 5A,B). While, the levels of P-p38 MAPK and KLF15 were not affected by GW1100 (Fig. 5A,C). Moreover, KLF15 expression levels were obviously increased when blocking phosphorylated p38 MAPK ( $P < 0.05$ ) (Fig. 5A,D). Meanwhile, the glucose consumption was partly but significantly improved in PA cotreated with AH7614, GW1100 and SB203580 ( $P < 0.05$ ) (Fig. 5E). The above results suggest that PA inhibits KLF15 expression through GPR120/P-p38 MAPK, resulting in abnormal glucose metabolism of 3T3-L1 adipocytes (Fig. 5F).

## **OA inhibits KLF15 expression mainly through GPR120 while not dependent on P-p38 MAPK, resulting in abnormal glucose metabolism of 3T3-L1 adipocytes**

3T3-L1 adipocytes were dealt with different concentrations of OA to determine its effect on GPRs/P-p38 MAPK/KLF15 pathway and glucose consumption. The results indicated that compared with the 0  $\mu$ M OA-

treated group, the GPR120 mRNA and protein expression levels were significantly increased in 750  $\mu$ M OA-treated group ( $P < 0.05$ ) (Fig. 6A,B). Moreover, in 750  $\mu$ M OA-treated group, although GPR40 mRNA expression level was slightly but significantly decreased ( $P < 0.05$ ), GPR40 protein expression level was slightly increased (Fig. 6A,B). In addition, KLF15 mRNA and protein expression levels were both obviously abrogated in 750  $\mu$ M OA-treated group ( $P < 0.05$ ) (Fig. 6A,B). While, the p38-MAPK phosphorylation had no significant difference (Fig. 6B), which suggested that OA had no effect on p38-MAPK phosphorylation. Moreover, the glucose consumption of 3T3-L1 adipocytes was also obviously abrogated in 750  $\mu$ M OA-treated group ( $P < 0.001$ ) (Fig. 6C).

To further investigate whether OA inhibited KLF15 expression and glucose consumption through GPR120 or GPR40, AH7614 and GW1100 were respectively added into 750  $\mu$ M OA treated cells to block GPR120 and GPR40. We found that OA-induced suppressed KLF15 levels and glucose consumption were overturned in 3T3-L1 adipocytes when cotreated with AH7614 ( $P < 0.05$ ) (Fig. 6D,E,G). In contrast to cotreated with AH7614, OA-induced suppressed KLF15 levels were exacerbated when cotreated with GW1100 ( $P < 0.05$ ) (Fig. 6D,F), which indicated that GPR40 may partly promoted KLF15 expression. But unfortunately, the glucose consumption didn't alter in OA cotreated with GW1100 group compared with OA-treated group (Fig. 6G). Whatever in OA cotreated with AH7614 or GW1100 group, p38-MAPK phosphorylation had no significantly difference. These results suggest that OA inhibits KLF15 expression mainly through GPR120 while not dependent on P-p38 MAPK, resulting in abnormal glucose metabolism of 3T3-L1 adipocytes (Fig. 6H).

## Discussion

T2DM is associated with increased total FFAs concentrations, which induced by obesity<sup>[18]</sup>. While, the specific mechanisms responsible for these effects of FFAs are not well understood. It has been reported that PA and OA are the main component of total FFAs and account for 27% and 31% respectively<sup>[4]</sup>. Previous studies indicate that PA-induced GPR40 activation weakens insulin sensitivity in N43/5 hypothalamic neuronal cells<sup>[19]</sup>. By contrast, PA-stimulated GPR40-deficient beta cells exhibited less insulin secretion<sup>[20]</sup>. In adipocytes from morbidly obese subjects, tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and Interleukin-6 (IL-6) expression were significantly increased under PA stimulation, while the effects were reversed by silencing GPR120<sup>[21]</sup>. Moreover, OA can act as GPR120 agonists and an OA-abundant diet ameliorated whole-body IR in obese mice by inhibiting inflammatory reaction of adipose tissue, liver, and skeletal muscle, reducing macrophage infiltration, and promoting IL-10 expression level, while all these influences were blocked by GPR120 silencing<sup>[22, 23]</sup>. In addition, OA increases insulin sensitivity through a GPR40-calcium signaling pathway in HepG2 cells<sup>[24]</sup>. These findings indicate that both PA and OA can activate GPR40 and GPR120. More importantly, it's not hard to speculate that PA or OA may have a different behavior in response to different GPRs. Meanwhile, GPR40 or GPR120 may play different biological functions due to different FFAs.

Consistently, our data demonstrated that both PA and OA can upregulate GRP40 and GPR120 expression. Moreover, our findings also showed that both PA and OA caused glucose consumption reduction of 3T3-L1 adipocytes through GPR120, which was in contradiction with previous research indicating that a GPR120-selective activator could improve IR and chronic inflammation in HFD mice [25, 26]. One possible reason is that GPR120 is usually the functional receptor for  $\omega$ -3 FFAs, which can exhibit potent effects on resisting inflammation and improving insulin sensitivity [25, 26]. While, when its ligand is PA or OA, the different effects may be produced. Additionally, our data also showed that PA-mediated glucose consumption reduction of 3T3-L1 adipocytes also relied on GPR40. While, GPR40 antagonist didn't influence the OA-induced glucose consumption reduction although OA promoted the GPR40 expression, suggesting OA inhibited glucose consumption not dependent on GPR40. In large part, the reason could be that OA had slightly promoting effects on GPR40, which is expressed in adult  $\beta$  cells of rodents with little constitutive expression in adipose tissue [20]. Therefore, the effect on phenotype was not obvious although we blocked GPR40. Besides, it has been reported that the effect of FFAs on promoting insulin secretion had positive correlation with chain length and saturation level [27]. Thus, the saturation degree of PA and OA may lead to different physiological effects, while further experiment remains to be elucidated.

In this study, we also assessed the molecular mechanism of PA/OA-mediated abnormal glucose metabolism. Here, we showed that KLF15 is decreased in omental adipose tissue of obesity subjects. More importantly, KLF15 levels were significantly and negatively correlated with WC, WHR, Weight, BMI, and TG, suggesting KLF15 levels were correlated with obesity state. Moreover, it has been reported that KLF15 can regulate glucose uptake, and activation of p38 MAPK is essential to reduce KLF15 expression [15, 16]. Thus, it is logical to speculate that high levels of FFAs, particularly PA or OA, may regulate glucose metabolism through P-p38 MAPK/KLF15 in adipose tissue. Obviously, this study demonstrated that KLF15 protein expression was substantially increased and the phosphorylation of p38 MAPK was significantly decreased after blocking GPR120 in obesity animal model. Moreover, PA-induced abnormal glucose metabolism through a GPR120/P-p38 MAPK/KLF15 signal pathway in 3T3-L1 adipocytes, while GPR40 had no effects on P-p38 MAPK under PA stimulation. Interestingly but inconsistently, it has been reported that Gq-p38 activation was mediated by GPR40 rather than GPR120 involving in autophagy of breast cancer cells [28]. Probably, the activation mechanism of p38 MAPK may be distinct in different cell type or pattern.

Unfortunately, OA-induced abnormal glucose metabolism through GPR120/KLF15 signal pathway in 3T3-L1 adipocytes, while not depend on P-p38 MAPK. This may be associated with other important factors that can also mediate KLF15 effects on glucose consumption when OA stimulate 3T3-L1 adipocyte, such as inflammatory factors TNF- $\alpha$  that reduced the expression of KLF15 in chondrocytes and vascular endothelial cells [29, 30]. Moreover, whatever PA or OA stimulation, GPR40 didn't contribute to the elevated phosphorylation of p38 MAPK and reduced KLF15 expression. In sharp contrast, blocking GPR40 inhibited the KLF15 expression in *vivo* and *vitro*. In other words, GPR40 may promoted KLF15 expression in 3T3-L1 adipocytes, while the possible mechanism needs to be further investigated. Overall, these

results indicated that PA inhibited KLF15 expression through GPR120/P-p38 MAPK signal pathway, and OA inhibits KLF15 expression mainly through GPR120 while not dependent on P-p38 MAPK, ultimately resulting in abnormal glucose metabolism of 3T3-L1 adipocytes.

## Conclusions

This work identifies PA and OA promotes abnormal glucose metabolism by inhibiting KLF15 expression in 3T3-L1 adipocytes.

## Abbreviations

IR: insulin resistance

T2DM: type 2 diabetes mellitus

FFAs: free fatty acids

SCFAs: short chain fatty acids

MCFAs: medium chain fatty acids

LCFAs: long chain fatty acids

PA: palmitic acid

OA: oleic acid

GLUT4: glucose transporter 4

AKT: serine/threonine protein kinase

GPR: G-protein-coupled receptor

KLFs: Krüppel-like factors

PPAR $\gamma$ : peroxisome proliferator activated receptor  $\gamma$

BMI: body mass index

TG: triglyceride

LDL-C: low density lipoprotein cholesterol

HDL-C: high density lipoprotein cholesterol

p38 MAPK: P38 mitogen activated protein kinase

FPG: fasting plasma glucose

TC: total cholesterol

DMSO: dimethylsulfoxide

DMEM: Dulbecco's modified Eagle's medium

IBMX: isobutylmethylxanthine

PMSF: phenylmethylsulfonyl fluoride

RIPA: radio-immunoprecipitation assay

TBST: Tris buffered saline tween

TNF- $\alpha$ : tumor necrosis factor  $\alpha$

IL-6: Interleukin-6

## Declarations

- **Ethics approval and consent to participate**

The patients were all signed informed consent, and ethics was supplied by the Medical Ethics Committee at First Affiliated Hospital of Medical College of Shihezi University (Approval number: 2017-049-01).

The experimental animal ethics were supplied by the Medical Ethics Committee at First Affiliated Hospital of Medical College of Shihezi University (Approval number: A2017-115-01).

- **Consent for publication**

Not applicable.

- **Availability of data and materials**

The data and material used to support the findings of this study are available from the corresponding author upon request.

- **Competing interests**

The authors declare that they have no competing interests.

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- **Authors' contributions**

Conception and experiment design: Jun Zhang and Yongsheng Chang. Experiment performance: Cuizhe Wang, Xiaolong Chu, Yuchun Deng. Data analysis: Cuizhe Wang, Jingzhou Wang and Yongsheng Chang. Contribution of reagents/materials/analysis tools: Cuizhe Wang, Xiaolong Chu, Yuchun Deng, Tongtong Qiu, Jiaojiao Zhu, Xin Yang, Chongge Pan, Jianyu Xiong, Jianxin Xie. Wrote the paper: Jun Zhang, Cuizhe Wang, and Xiaolong Chu.

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Not applicable.

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## Figures

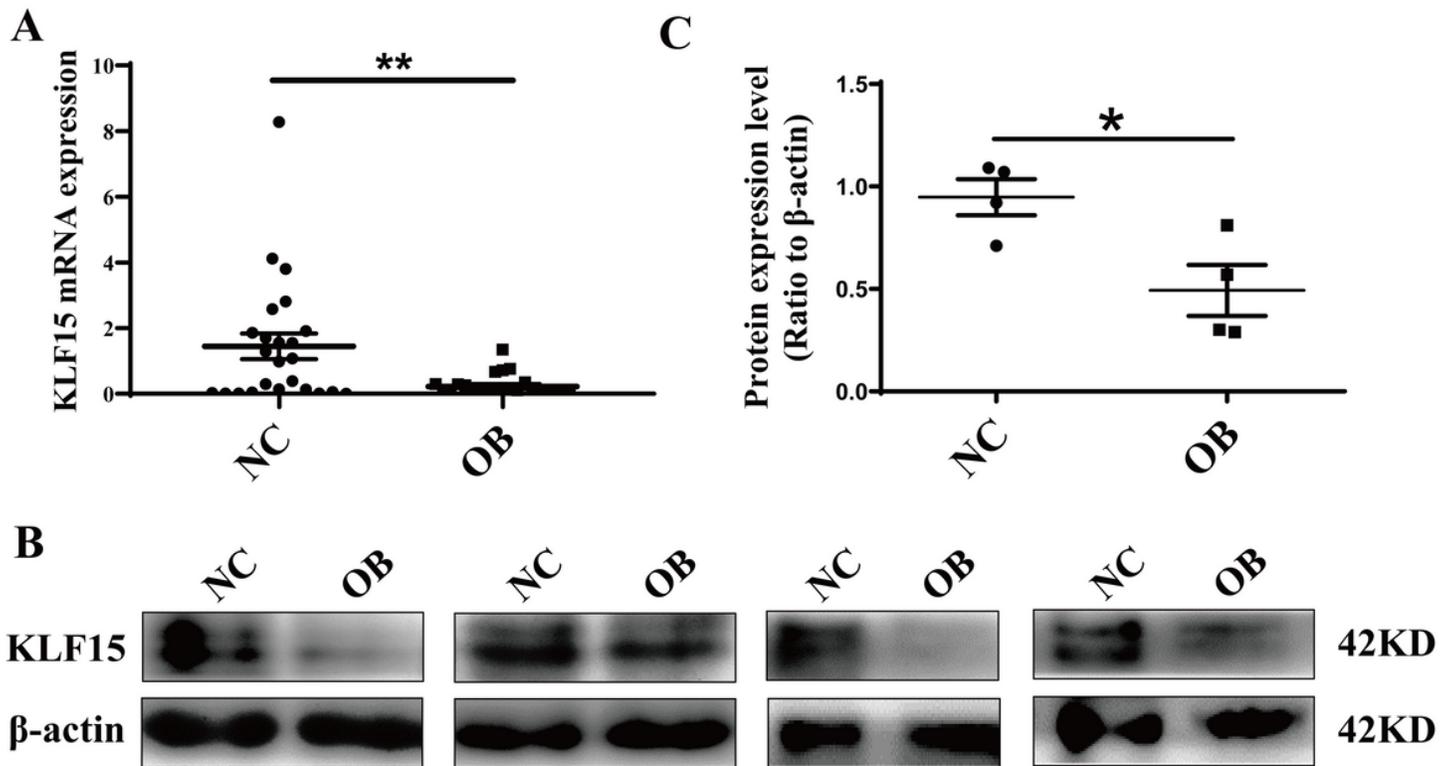
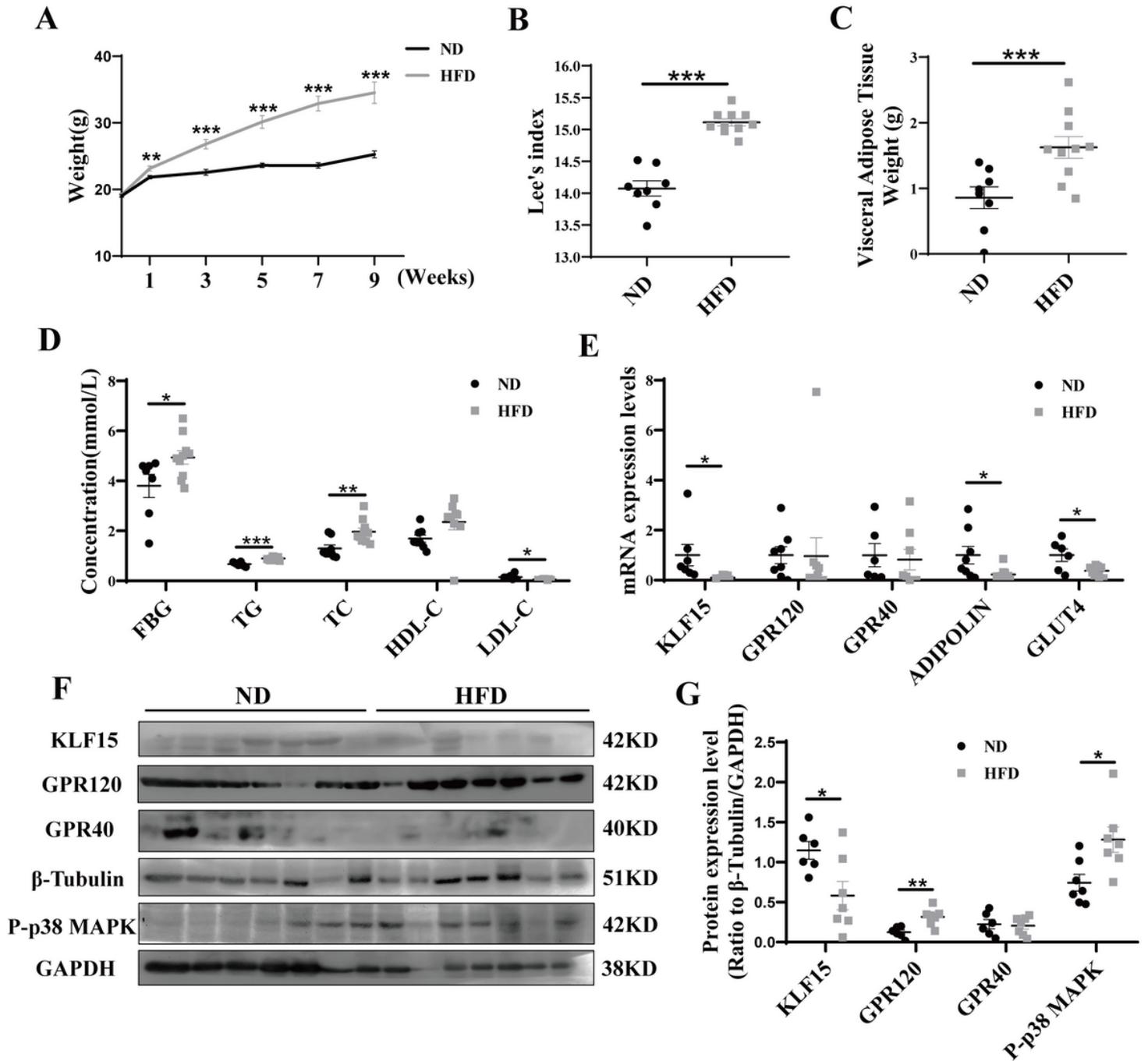


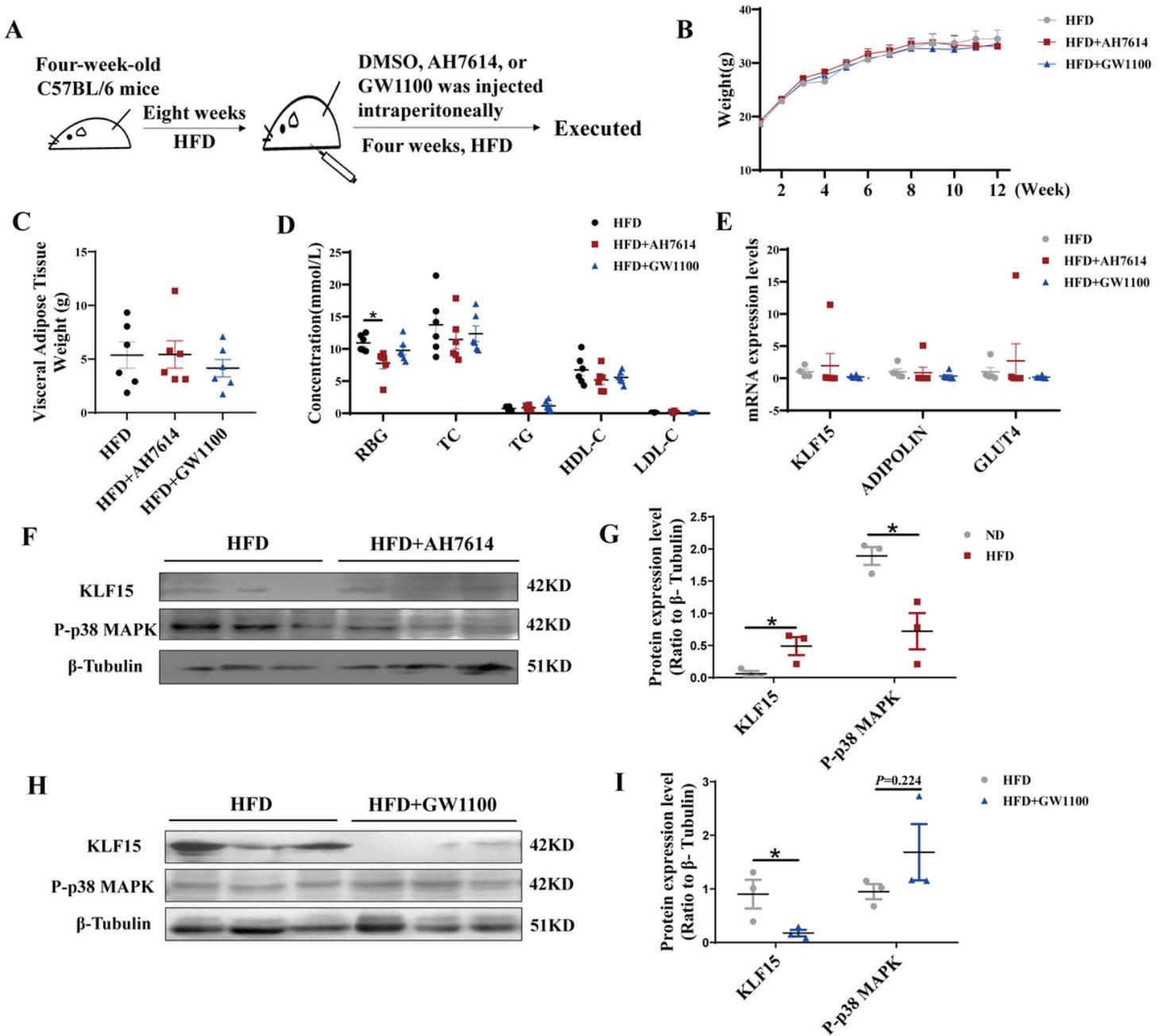
Figure 1

KLF15 mRNA and protein levels in visceral adipose tissue. mRNA expression levels (A) and protein expression levels (B) were detected. (C) Western Blot gray scale. Results represent mean  $\pm$  SEM. t test, \* $P < 0.05$  difference was statistically significant.



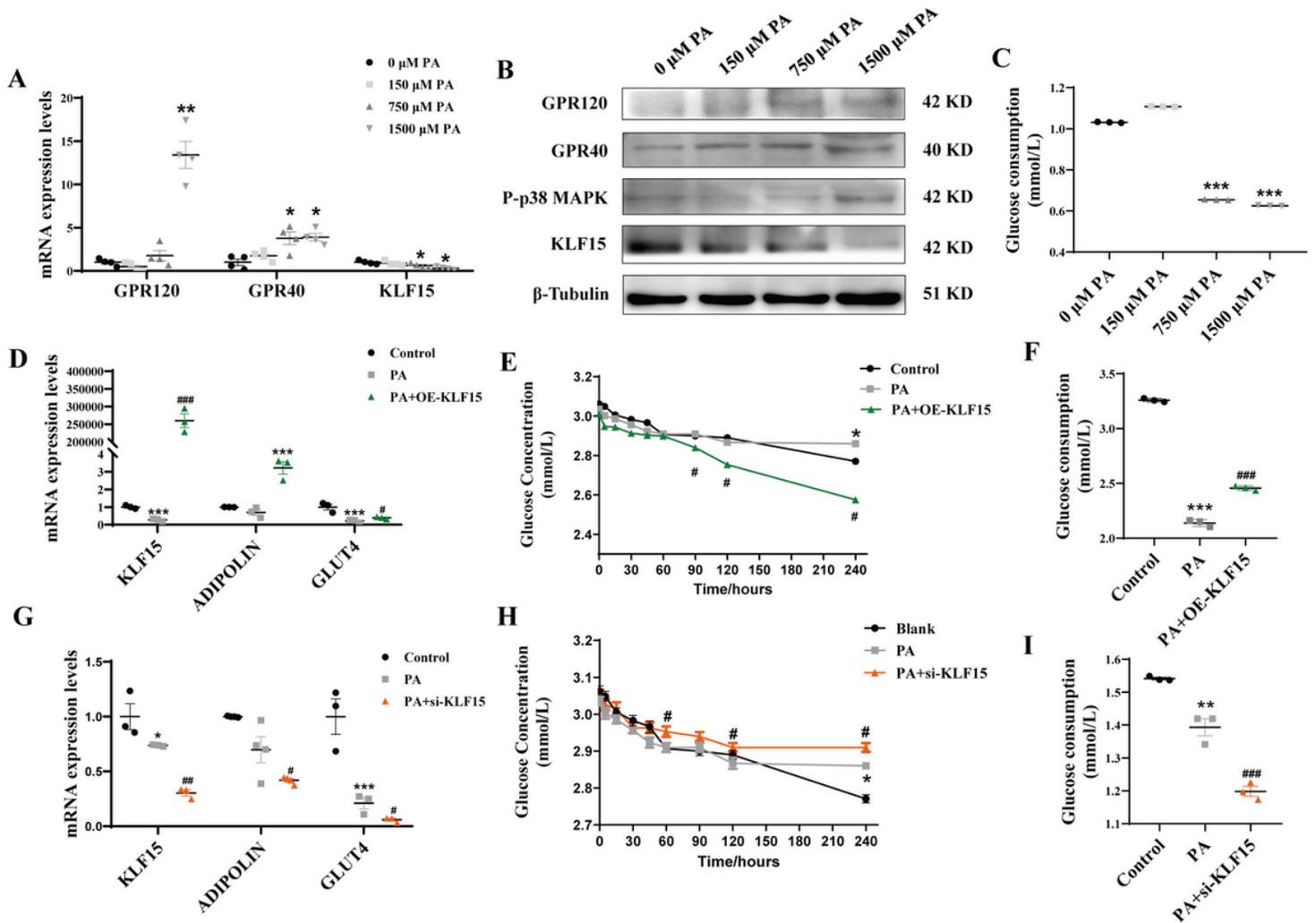
**Figure 2**

Mouse models of obesity was constructed and related genes expression were detected. Body weight (A) of ND and HFD mice was detected weekly. Lee's index (B), visceral adipose tissue weight (C), biochemical indexes (D), mRNA expression level of related genes (E) and protein expression level of related genes (F) were detected in the ninth week. (G) Western Blot gray scale. Results represent mean  $\pm$  SEM. t test, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  difference was statistically significant.



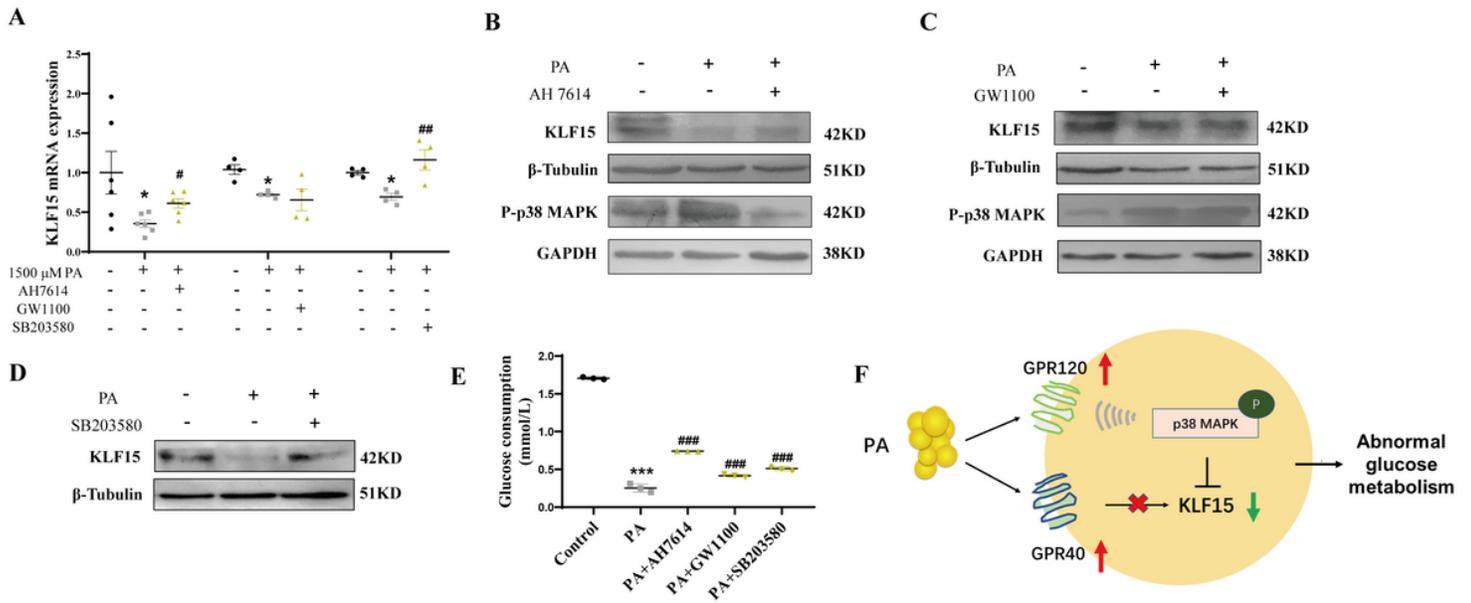
**Figure 3**

The effects of GPR120 and GPR40 antagonist in HFD mice. After HFD for eight weeks, the mice received an intraperitoneal injection of DMSO, GW1100 or AH7614 every other day for four weeks. The body weight (B) was detected in different weeks. In the twelfth week, the visceral adipose tissue weight (C), biochemical indexes (D), mRNA expression levels (E) and the protein expression levels (F,H) of related genes were detected. (G,I) Western Blot gray scale. Results represent mean  $\pm$  SEM. t test and One-way ANOVA, \* $P < 0.05$  difference was statistically significant.



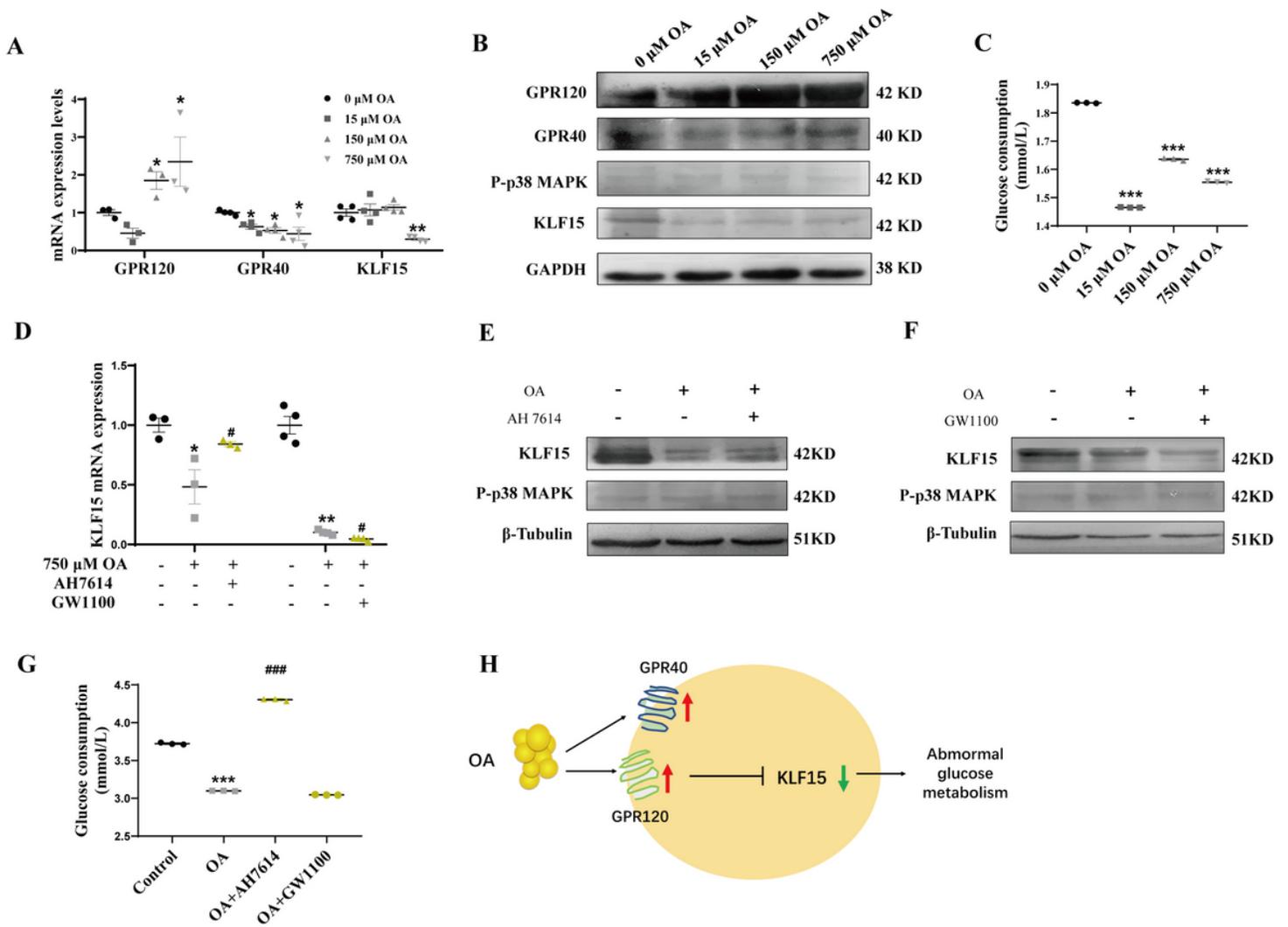
**Figure 4**

KLF15 improves PA-induced abnormal glucose metabolism in 3T3-L1 adipocytes. 3T3-L1 adipocytes were treated with different concentrations of PA (0, 150, 750, and 1500  $\mu\text{M}$ ) for 48 h. The mRNA (A) and protein (B) expression levels of GPR120, GPR40, P-p38 MAPK, and KLF15 were detected; Glucose consumption (C) of 3T3-L1 adipocytes was tested. The expression of KLF15 was then overexpressed or silenced by transfection with overexpression plasmid (OE-KLF15) or interference fragment (si-KLF15). The mRNA (D,G) expression levels of KLF15, ADIPOLIN and GLUT4 were detected; Insulin sensitivity (E,H) and glucose consumption (F,I) were tested. Results represent mean  $\pm$  SEM. One-way ANOVA, \* $P < 0.05$ , \*\* $P < 0.01$  versus the control group, # $P < 0.05$ , ## $P < 0.05$ , ### $P < 0.001$  versus the PA-treated group, difference was statistically significant.



**Figure 5**

PA inhibits KLF15 expression through GPR120/P-p38 MAPK, resulting in abnormal glucose metabolism of 3T3-L1 adipocytes. AH7614, GW1100 and SB203580 were respectively added into 1500 μM PA treated cells to block GPR120, GPR40 and p38 MAPK. The mRNA (A) and protein(B-D) levels of P-p38 MAPK, KLF15 and glucose consumption (E) were detected. Pattern diagram (F) were presented. Results represent mean ± SEM. One-way ANOVA, \*P<0.05, \*\*\*P<0.001 versus the control group, #P<0.05, ##P<0.05, ###P<0.001 versus the PA-treated group, difference was statistically significant.



**Figure 6**

OA inhibits KLF15 expression mainly through GPR120 while not dependent on P-p38 MAPK, resulting in abnormal glucose metabolism of 3T3-L1 adipocytes. 3T3-L1 adipocytes were treated with different concentration OA (0, 15, 150, and 750  $\mu$ M) for 48 h. The mRNA (A) and protein (B) expression levels of GPR120, GPR40, P-p38 MAPK, and KLF15 were detected; Glucose consumption (C) of 3T3-L1 adipocytes was tested. AH7614 and GW1100 were respectively added into 1500  $\mu$ M PA treated cells to block GPR120 and GPR40. The mRNA (D) and protein (E-G) levels of P-p38 MAPK, KLF15 and glucose consumption (H) were detected. Pattern diagram (I) were presented. Results represent mean  $\pm$  SEM. One-way ANOVA, \* $P$ <0.05, \*\* $P$ <0.01 versus the control group, # $P$ <0.05, ### $P$ <0.001 versus the OA-treated group, difference was statistically significant.

## Supplementary Files

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