GLP-1R activation is a key pathway to alleviate the metabolic disorder in L02 cells

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Abstract

Background

Glucagon like peptide 1 (GLP-1) has been shown to regulate glucose and lipid metabolism in the liver by promoting insulin secretion. However, the direct regulation of GLP-1 on liver glucose and lipid metabolism was still controversial, due to the presence of GLP-1 receptors (GLP-1R) in human liver has not been determined. The aim of this study was to investigate the presence of GLP-1R in L02 cells (normal human hepatocytes), and to explore the direct effect of GLP-1, on hepatic glucose and lipid metabolism.

Methods

The insulin resistance (IR) cell model with metabolic disorder was constructed using FFAs (including oleic acid and fructose) to simulate the metabolic syndrome caused by high-fat and high-fructose in modern diet.

Results

The results showed that GLP-1R is expressed in L02 cells, and reduced in L02 with IR. The RNA interference experiment of GLP-1R suggested that GLP-1R activation decreased glycogenesis and increased intracellular glycogen content via AKT pathway independent of PI3K, and increased fatty acid oxidation and decreased lipid accumulation via AMPK pathway.

Conclusions

These results indicate that GLP-1 could directly regulate hepatic glucose and lipid metabolism by activating GLP-1R, which could be potential therapeutic targets. GLP-1R activation leads to reduction of gluconeogenesis and lipogenesis via PI3K-independent AKT pathway and AMPK pathway.

1 Background

Sugar and fat are important factors in food taste, but with the increase of their consumption, it's harmful to human health. Metabolic disorders are induced by the modern diet of high-fat and high-fructose, including hyperglycemia, dyslipidemia(Stanhope et al., 2009), insulin resistance(IR) (Zhao et al., 2016), Type 2 Diabetes Mellitus (T2DM) and its complications (J.Drucker, 2016). According to the 2016 global report on diabetes by the WHO, globally, an estimated 422 million adults were living with diabetes in 2014, compared to only 108 million in 1980 (WHO., 2016). The global prevalence of diabetes has doubled in the past 30 years, and the prevalence of adults has risen from 4.7–8.5% (WHO., 2016). Insulin resistance (IR) caused by metabolic disorders was susceptibility factor for obesity and diabetes (Savage et al., 2007).

The pathological features of liver IR are disorder of glycolipid metabolism and imbalance of energy metabolism, which leads to accumulation of liver lipids.

GLP-1 is an incretin hormone secreted by enteroendocrine L-cells in a nutrient-dependent manner. Recent studies showed that GLP-1 receptors (GLP-1R) expressed islet, intestine, heart, the central nervous systems, and blood vessels. GLP-1 stimulates glucose-dependent insulin production and secretion, inhibit glucagon secretion, and reduce hypoglycemia via specific GLP-1R expressed on islet (Baggio and Drucker, 2007, Holst, 2007). The main pathway of GLP-1 signaling in islet β cells are the activation of adenylate cyclase and phosphatidylinositol-3-kinase/Akt pathway. In nervous system, GLP-1R agonists could increase energy expenditure and decrease peripheral lipid storage through sympathetic nervous system (SNS) pathways (Drucker, 2018). As a new kind of medicine for diabetes, GLP-1 and its analogues could reduce blood glucose and weight, minimize the risk of hypoglycemia, meanwhile, its mechanism are affected extensive attention. However, it is still controversial that GLP-1 directly regulates liver metabolism independently of increasing insulin secretion, and the expression of GLP-1R in liver is still unclear(Naim et al., 2013).

GLP-1R expressed in human liver and human hepatoma cell lines HepG2 was confirmed at gene and protein levels with human pancreas as positive control, and the researchers also found that GLP-1R activation was beneficial to stimulate liver lipid oxidation caused by nonalcoholic steatohepatitis and restore liver metabolic changes induced by high-fat diet(Svegliati-Baroni et al., 2011a). Another study showed that GLP-1 inhibits hepatic lipogenesis by activating the liver AMPK pathway (Ben-Shlomo et al., 2011). It is worth noting that a research using RNA and in situ hybridization have not detected expression of GLP-1R in hepatocytes (Naim et al., 2013, Pyke et al., 2014).

This research confirmed that GLP-1R was expressed in the normal human hepatocytes L02. The GLP-1R was activation by liraglutide (LG) and interference by siRNA to find that GLP-1R activation could improve the metabolic disorder caused by IR. Furthermore, GLP-1R activation plays an important role in restore glucose homeostasis and reduce lipid accumulation of IR by activating AMPK and AKT pathway independent of PI3K.

2 Materials And Methods

2.1 Chemicals

oleic acid (OA) were obtained from Sigma-Aldrich, Inc. (St. Louis, USA). Fructose were purchased from Biotopped Co.Ltd. (Beijing, China). Liraglutide(LG, purity ≥ 98%)were purchased from Meilun Biotechnology Co.Ltd. (Dalian, China). FFA-free Bovine Serum Albumin (BSA) were purchased from BioDee Biotechnology Co.Ltd. (Beijing, China).

For cell culture, RPMI1640, Hank's Balanced Salt Solution (HBSS) were obtained from Macgene Technology Co.Ltd. (Beijing, China). Fetal bovine serum, penicillin, and streptomycin were purchased from Gibco Life Technologies (Grand Island, NY, USA). Pancreatin□Enhanced BCA Protein Assay Kit and RIPA

buffer were purchased from Beyotime Biotechnology Inc. (Beijing, China). Total cholesterol assay kit, Triglyceride assay kit and Glucose Assay Kit were purchased from Nanjing Jiancheng Bioengineering Institute. All other chemicals used were of analytical grade.

2.2 Cell culture and treatment

HepG2 (3111C0001CCC000035) were purchased from Institute of Basic Medicine, Chinese Academy of Medical Sciences (Beijing, China), grown in DMEM (containing 10% NCS, 100 μg mL^{-1} streptomycin and 100 U mL^{-1} penicillin) and incubated at 37 °C with 5% CO_2 /95% air atmosphere.

L02 (3111C0001CCC000035) were purchased from Biological Technology Co.Ltd. (Shanghai, China), grown in RPMI 1640 (containing 10% FBS, 100 μg mL^{-1} streptomycin and 100 U mL^{-1} penicillin) and the culture conditions are same as HepG2.

L02 cells were treated with RPMI1640 medium containing 0.1% BSA for 24 hours as Control (C), or 100 μmol of OA and 15 mmol of fructose (FFAs) for 24 hours as IR model (M), or FFAs and 100 nmol LG for 24 hours as LG intervention (LG), or 100 nmol siRNA and FFAs and LG (100 nmol) for 24 hours as siGLP-1R and negative control (NC).

2.3 Small interfering RNA (siRNA) transient knockdown in L02

The L02 cells were cultured at stationary phase for 24 h, and transfected with 100 nmol siRNA targeting GLP-1R (gene ID: 2740),with a negative siRNA (NC) as control. The transfection efficacy was determined by mRNA analysis. The siRNA (siGLP-1R & NC) was mixed with the riboFECT™ CP transfection reagent. Then, siRNA was delivered into the L02 (~ 30% confluent density) and cultured in normal medium for 24 h. At the same time of transfection, the cells were cotreated with FFAs and LG (100 nmol). Then cell lysates were collected to analyze mRNA and protein levels. All siRNAs and transfection reagent were purchased from RiboBio Co. Ltd. (Guangzhou, China).

2.4 Glucose uptake and intracellular glycogen content

Glucose uptake and intracellular glycogen content were measured in different groups. Briefly, L02 were exposed to the treatments for 24 h. Afterward, the medium was removed, and the L02 were washed with HBSS. Subsequently, the medium was changed to RPMI1640 containing 2 g/L glucose for an additional 24 h. Finally, the glucose concentrations in the supernatants of each well were measured by a glucose assay kit and normalized to the total cellular protein. The intracellular glycogen contents under the treatments were measured using the glycogen assay kit and normalized to the total protein. All kits used are from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.5 Intracellular TG, TC and supernatant TG levels

After treatments for 24 h, L02 were washed with HBSS and lysed with RIPA buffer. TG, and TC levels were detected using corresponding determination kits and normalized to the total cellular protein. All kits used are from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.6 Oil Red O staining

After treatments for 24 h, L02 were washed with HBSS buffer to remove the residual culture medium. And then fixed with 4% polyformaldehyde for 30 min, followed by staining with Oil Red O solution for 10 min, at room temperature(Liu et al., 2017). The stained lipid droplets were visualized and photographed by using CKX41 microscope (CellSens, Olympus, Japan)

2.7 Intracellular AMP/ATP levels

L02 were seeded in 6-well plates. After the same treatment for 24 h, cells were washed with HBSS and lysed with RIPA buffer. The ATP (adenosine triphosphate)、AMP(Adenosine monophosphate) levels were detected by ELISA kits (Inselisa Biotechnology Co. Ltd, Hubei, China) according to the manufacturer's instructions, and the results were normalized to the total cellular protein.

2.8 Real-time Quantitative PCR

Total RNA was extracted using TransZol Up Plus RNA Kit (Beijing, China). About 1 μg of total RNA was reverse transcribed using *TransScript*®Green One-Step qRT-PCR SuperMix Kit (Beijing, China). The preparation of 20 μL reaction system,includes: 1 μg RNA template, 0.5 μL forward and reverse primers, 10 μL 2 × *TransScript*® Tip green qPCR SuperMix, 0.5 μL *TransScript*® One-Step RT/RI Enzyme Mix and some enzyme free water. SYBR fluorescent dye was used to detect the relative expression of the selected genes. Real-time quantitation was performed on the CFX Connect real-time PCR instrument (Bio-Red, USA). The thermal cycling conditions for PCR were 45℃ for 5 min, 94℃for 30 s, 40 cycles of 94℃ for 5 s;65℃ for 1 min. All measurements were performed in triplicate, and the data were analyzed with Bio-Rad CFX Manager (California, USA). All PCR data were normalized to the glyceraldehyde-3-phosphate dehydrogenase expression. The primers used are all designed on NCBI official website, and the sequence of the gene primers were as follows: GLP-1R, forward 5'- CGATGGCCCAGTCCTGAAC-3' and reverse 5'-GGACACAGTGGCACCCTGG-3'; phosphoenolpyruvate carboxykinase (PEPCK), forward 5'-AGCTGTGCCAGCCAGAGTAT-3' and reverse 5'-ATGACCGTCTTGCTTTCGAT-3'; glucose 6 phosphatase (G6Pase), forward 5'-AAAGATAAAGCCGACCTACAGA-3' and reverse 5'-GGACGAGGGAGGCTACAATA-3'; carnitine palmitoyl transferase 1(CPT-1), forward 5'-GCATGATCGCAAAGATCAGT-3' and reverse 5'-TGGTAGGAGAGCAGCACCTT-3'; acetyl CoA carboxylase (ACC), forward 5'-GCATGATCGCAAAGATCAGT-3' and reverse 5'-TGGTAGGAGAGCAGCACCTT-3'; fatty acid synthase(FAS), forward 5'-

GCAAGCTGAAGGACCTGTCT-3' and reverse 5'-AATCTGGGTTGATGCCTCCG-3'; sterol response element binding protein-1c(SREBP-1c), forward 5'-TCTGACAGCCATGAAGACAGAC-3' and reverse 5'-ATAGGCAGCTTCTCCGCATCTA-3'; GAPDH, forward 5'-GACCCGTGCTGCTTTCTTGA-3' and reverse 5'-GGGTGGAGTCGTACTGGAAC-3'.

2.9 Western blotting

After protein content determination, equal amounts of proteins from cell lysates were loaded onto an 8% SDS-PAGE gel after denaturation with SDS loading buffer and then transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% skimmed milk for 2 h at room temperature. Afterward, antibodies of anti-PI3K (p110α), anti-Akt, anti-pAkt, anti-GSK3β, anti-pGSK3β, anti-FOXO1, anti-pFOXO1, anti-AMP-activated protein kinase (AMPK), anti-pAMPK and anti-PPARα (Cell Signaling Technology, Inc.), and anti-beta actin were added overnight at room temperature. The membranes were incubated with HRP-conjugated secondary antibodies for 1 h at room temperature. The protein bands were captured and analyzed by ChemiScope (ChemiScope 6000 Touch ChemiAnalysis).

2.10 Statistical analysis

Statistical analysis was performed by one-way ANOVA analysis of variance using Origin 8.5 software (Origin Lab Corp., USA). All data are presented as the mean values ± standard deviation (SD). Statistical significance was set at $P < 0.05$.

3 Results

3.1 Glucagon-like peptide-1 receptor expression in normal human hepatocytes L02

It was well illustrated that GLP-1 has a direct regulation on hepatic lipogenesis, however the existence of GLP-1R in hepatocytes still remained controversial (Ben-Shlomo et al., 2011, Drucker, 2018). Thus, the expression of GLP-1R in L02 cells was detected by qPCR and Western blot for the first time. As show in Fig. 1a-b, GLP-1R could be detected both at gene and protein levels in L02 and HepG2 cell lines. In addition, the protein level of GLP-1R was induced significantly reduction in L02 cells with IR (Fig. 1c, d). The protein expression of GLP-1R in different groups showed that GLP-1R activation didn’t change GLP-1R expression, and siRNA treatment seriously inhibited GLP-1R expression. (Fig. 1d).

3.2 The role of GLP-1R activation in regulating glucose metabolism

Insulin sensitivity and hepatic gluconeogenesis of ob/ob mice were improved, when treated with recombinant adenovirus expressing GLP-1 (Lee et al., 2007). GLP-1 was well-illustrated to enhance insulin sensitivity, however, studies on direct regulation of glucose metabolism are rarely. As shown in Fig. 2a, GLP-1R activation reversed the FFAs-induced decrease of glucose uptake, which significantly reduced after GLP-1R was knockdown. Similarly, the content of glycogen was significantly increased in IR cells with LG treatment, and the intracellular glycogen content was significantly reduced after GLP-1R was interference in IR cells with LG treatment.

3.3 GLP-1R activation inhibited gluconeogenesis through activated AKT without PI3K

As shown in Fig. 3a, FFAs-induced increase of PEPCK was significantly reduced after GLP-1R activation. However, the gene level of PEPCK and G6Pase was significantly increased when GLP-1R was interfered. As rate-limiting enzymes for fat synthesis, the mRNA expression levels of PEPCK and G6Pase are closely related to gluconeogenesis. The expression of PEPCK and G6Pase was regulated by FOXO1 and glycogen synthesis was regulated by GSK3β. Similar to this result, FFAs-induced FOXO1 and GSK3β dephosphorylation was restored by GLP-1 activation, the phosphorylation of FOXO1 and GSK3β was decreased again when GLP-1R was interfered. AKT was one of the regulatory factors of FOXO1 and GSK3β. GLP-1R activation induced the significantly increase of AKT phosphorylation, but the protein levels of PI3K were no significant change (Fig. 3b). Taken together, GLP-1R could activate AKT and downstream pathway without PI3K. Taken together, these results suggest that GLP-1R activation is directly associated with glucose metabolism in the liver and that these effects are mediated by AKT phosphorylation and downstream transcription mechanisms.

3.4 GLP-1R activation inhibited fat accumulation caused by metabolic disorders

The ratio of AMP to ATP reflects the energy metabolism of L02 cells. As shown in Fig. 4a, The AMP:ATP ratio was decreased by IR, and the anabolism was higher than catabolism. Catabolism was increased by GLP-1R activation, and cell energy metabolism returned to normal state. This effect was inhibited after the expression of GLP-1R was interfered.

Similar with the regulation on glucose metabolism, GLP-1R activation was proved decreased hepatic fat accumulation, insulin resistance and improved fatty acid β-oxidation in the rodents and human (Tushuizen et al., 2006, Svegliati-Baroni et al., 2011b). However, the direct regulatory mechanism of GLP-1R activation on lipid metabolism in liver was still unclear. As shown in Fig. 4b-c, the FFAs-induced increases in intracellular TG and TC were all significantly reduced after GLP-1R activation, which was reversed after GLP-1R was interfered. In addition, the transport of TG from intracellular to extracellular was promoted by GLP-1R activation (Fig. 4c), and this effect was weakened after GLP-1R was interfered.

Oil Red O staining showed that the FFAs-induced hepatic lipid accumulation was significantly increased by GLP-1R interfered (Fig. 4d). This result indicated that liver fat accumulation was reduced by GLP-1R activation.

3.5 GLP-1R activation reduced lipogenesis via AMPK pathway in L02 cells

It has been reported that GLP-1 activation could promote fatty acid β oxidation and reduce lipogenesis(Ben-Shlomo et al., 2011). As shown in Fig. 5c, FFAs-induced increases of ACC, FAS, SREBP-1c was significantly reduced after GLP-1R activation, which was reversed by GLP-1R interfered. Similarly, the downregulation of CPT-1, key enzymes of fatty acid β oxidation, was significantly enhanced by GLP-1R activation, but which was still inhibited after GLP-1R was interfered. Notably, Fatty oxidation was strictly regulated by AMPK through activated PPARα to promote transcription of CPT-1 in the liver. AMPK was reduced hepatic lipogenesis, acting by phosphorylation of ACC to inactivate this enzyme, or by regulating transcription of the master transcription factor SREBP-1c, which regulated the transcription of lipogenic enzymes ACC and FAS(Postic and Girard, 2008, Viollet et al., 2006). As shown in Fig. 5b, FFAs-induced activity reduction of PPARα and AMPK activity, which resulted in the increase of lipid synthesis in L02 cells. GLP-1R activation could increase the FFAs-induced activity reduction of PPARα and AMPK, and the results were reversed by GLP-1R interference.

AMP:ATP ratio was known a sensor of energy metabolism and an activator of AMPK (Viollet et al., 2006). Similar to the results of energy metabolism, the activation of AMPK caused by IR was due to the decrease of AMP: ATP ratio, which leads to the increase of fat accumulation in L02 cells. In conclusion, GLP-1R was an important target for LG to reduce intracellular fat accumulation, increase fatty acid oxidation and restore energy metabolism balance.

4 Discussion

In this study, GLP-1R in human normal hepatocyte L02 was detected at the gene and protein levels, and which mediate the downregulation of gluconeogenesis and the increase of glucose uptake in glucose metabolism and modulate the decline of fat accumulation and the enhancement of fatty acid oxidation in lipid metabolism. Besides that, GIP-1R activation significantly increased AKT phosphorylation and this is confirmed in the present study where reduced expression of its downstream target genes FOXO1 and GSK3β was observed. In parallel, GLP-1 activation increases the AMP:ATP ratio to activate AMPK, indicating that GLP-1 induces starvation state and leads to fat oxidation in the liver. GLP-1R interference experimental showed that GLP-1R activation decreased the mRNA levels of the transcription factor SREBP-1c and its downstream lipogenic enzymes FAS and ACC and increased the mRNA levels of CPT-1 via AMPK pathway, and ultimately leads to decline fat content in L02 cells.

GLP-1 was widely used to increase postprandial insulin secretion and reduce postprandial blood glucose in preclinical studies. However, the existence of GLP-1R in the liver was still controversial. Naim et al. showed that GLP-1R mRNA transcription could not be detected from mouse hepatocytes and the indirect GLP-1R actions for the regulation of hepatic lipid accumulation(Naim et al., 2013). Nevertheless, Gupta et al. revealed that both Huh7 cells and primary human hepatocytes had GLP-1R at the protein level(Gupta et al., 2010). Zhou et al. used immunohistochemical staining to find that human liver expresses GLP-1R and the level of GLP-1R was downregulated in patients with non-alcoholic fatty liver(Zhou et al., 2018a). Moreover, the direct regulation of GLP-1 via activating GLP-1R in hepatic fat and glucose metabolism and its signaling pathway are also unclear(Holst, 2007, Nickavar and Amin, 2004).

The PI3K-AKT pathway is essential for the liver to regulate glucose metabolism by coordinating endogenous glucose output and intracellular glycogen biosynthesis. Studies have shown that AKT causes phosphorylation inactivation of downstream FOXO1 and GSK3β, which in turn inhibits the gluconeogenesis rate-limiting enzymes PEPCK, and G6Pase activity and increases glycogen synthase activity (Mussmann et al., 2007, Gross et al., 2009). In the current study, the phosphorylation AKT was increased by GLP-1R activation, causing inactivation of FOXO1 and GSK3β, which resulted in the loss of transcriptional control of the gluconeogenic genes G6Pase and PEPCK, inhibiting gluconeogenesis in the liver. In addition, the inhibition of GSK3β on glycogen synthetase was weakened, resulting in the increase of intracellular glycogen synthesis.

AMP-activated protein kinase (AMPK) plays an important role in hepatic metabolism. Activation of AMPK leads to the stimulation of fatty acid oxidation and inhibition of lipogenesis, glucose production and protein synthesis in the liver (Viollet et al., 2006). Studies have reported AMPK activation in hepatocyte by intracellular changes in AMP: ATP ratio (Steinberg and Kemp, 2009). AMPK has been shown to inhibit liver fatty acid synthesis by reducing SREBP-1C expression and down-regulating the inactivation of lipogenic enzymes such as ACC and FAS.

Some studies show that GLP-1R activation stimulated hepatic lipid oxidation and restores hepatic signaling alteration induced by high-fat diet in patients with nonalcoholic steatohepatitis(NASH) (Svegliati-Baroni et al., 2011b), and upregulation of GLP-1R expression in liver of patients with nonalcoholic fatty liver disease (NAFLD) could reduce the incidence of NAFLD induced by high-fat diet (Zhou et al., 2018b). However, these studies focused on the effect of GLP-1R activation on liver lipid metabolism. Our results demonstrate that GLP-1 has an independent mechanism of liver metabolism regulation independent of insulin, which is also an improvement of glucose and lipid metabolism disorder under the condition of insulin resistance mediated by activation of GLP-1R.

5 Conclusions

In summary, the current study suggests that GLP-1R was found in L02 cells, and the level of GLP-1R was significantly reduced in L02 cells with IR. Furthermore, GLP-1R activation leads to reduction of gluconeogenesis through activation of GLP-1R via PI3K-independent AKT pathway and reduction of

lipogenesis via AMPK pathway (Fig. 6). Therefore, GLP-1R is a potential target for the treatment of T2DM and obesity induced liver metabolic disorders.

Abbreviations

GLP-1
Glucagon like peptide 1
GLP-1R
GLP-1 receptors
IR
insulin resistance
T2DM
Type 2 Diabetes Mellitus
SNS
sympathetic nervous system
LG
liraglutide
OA
oleic acid
HBSS
Hank's Balanced Salt Solution
TC
Total cholesterol
TG
Triacylglycerol
PEPCK
phosphoenolpyruvate carboxykinase
G6Pase
glucose 6 phosphatase
CPT-1
carnitine palmitoyl transferase 1
ACC
acetyl CoA carboxylase
FAS
fatty acid synthase
SREBP-1c
sterol response element binding protein-1c

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Qiaoji Tian wrote the manuscript, performed data collection and analysis.Kai Xia, Shenglin Duan conducted experiments. Yifeng Liu, Jia Liu and Peng Yuan participated in research design. Haizhi Li, Xiaofeng Han and Cong Pan contributed to the writing of the manuscript. The author(s) read and approved the final manuscript.

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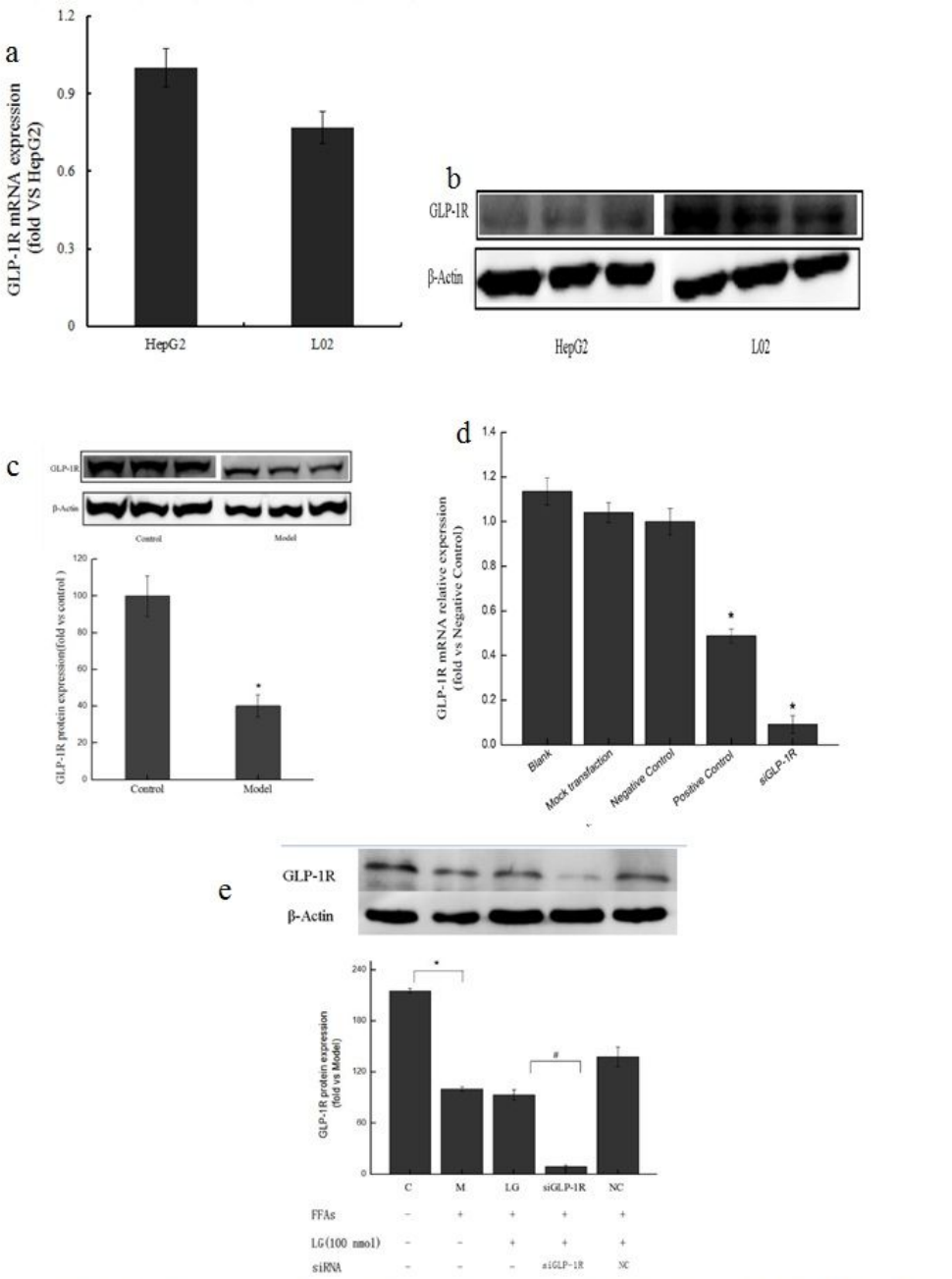
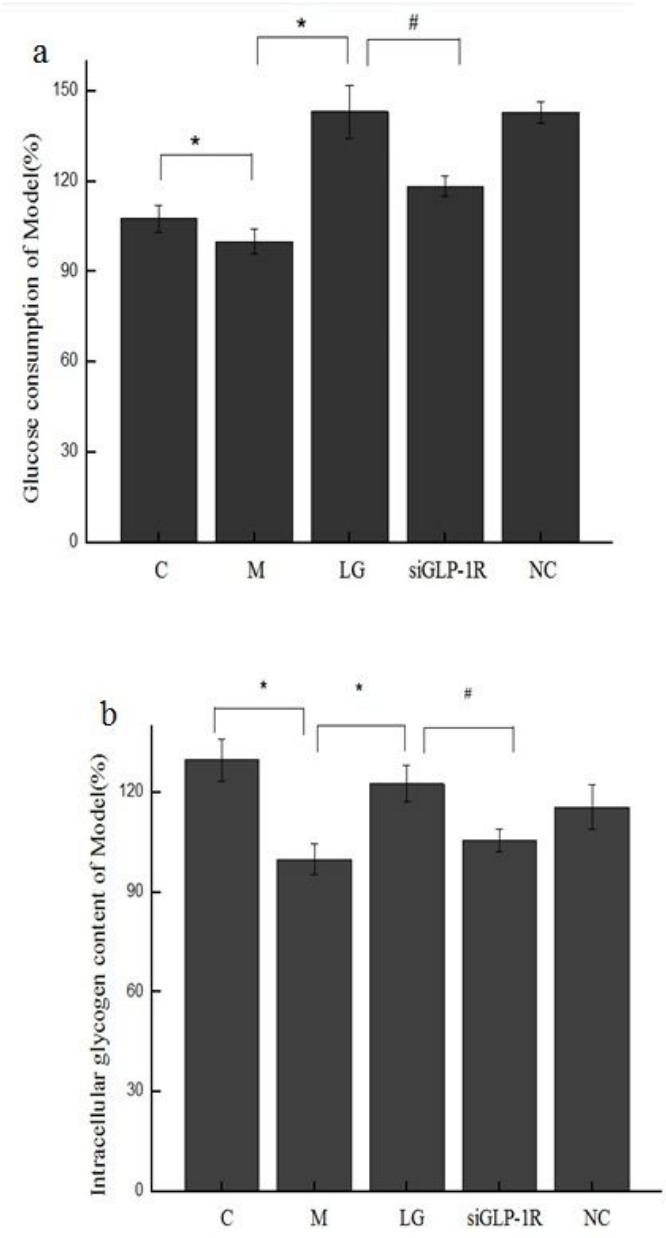
Figures



(a-b) GLP1-R mRNA was assessed by qPCR and Western blot in HepG2 (positive controls) and L02 cells (a. qPCR, b. WB). (c) GLP-1R protein expression was assessed by Western blot in Control and IR groups. (d) GLP-1R mRNA expression was assessed by qPCR in Blank, Mock transfection, Negative Control, Positive Control, siGLP-1R group. (e) GLP-1R protein expression was assessed by Western blot in siRNA-transfected L02 cells that were cotreated with FFAs and LG (100 nmol) for 24h. Data are expressed as means ± SD (n = 3), $*p < 0.05$ compared with the control.

Figure 1

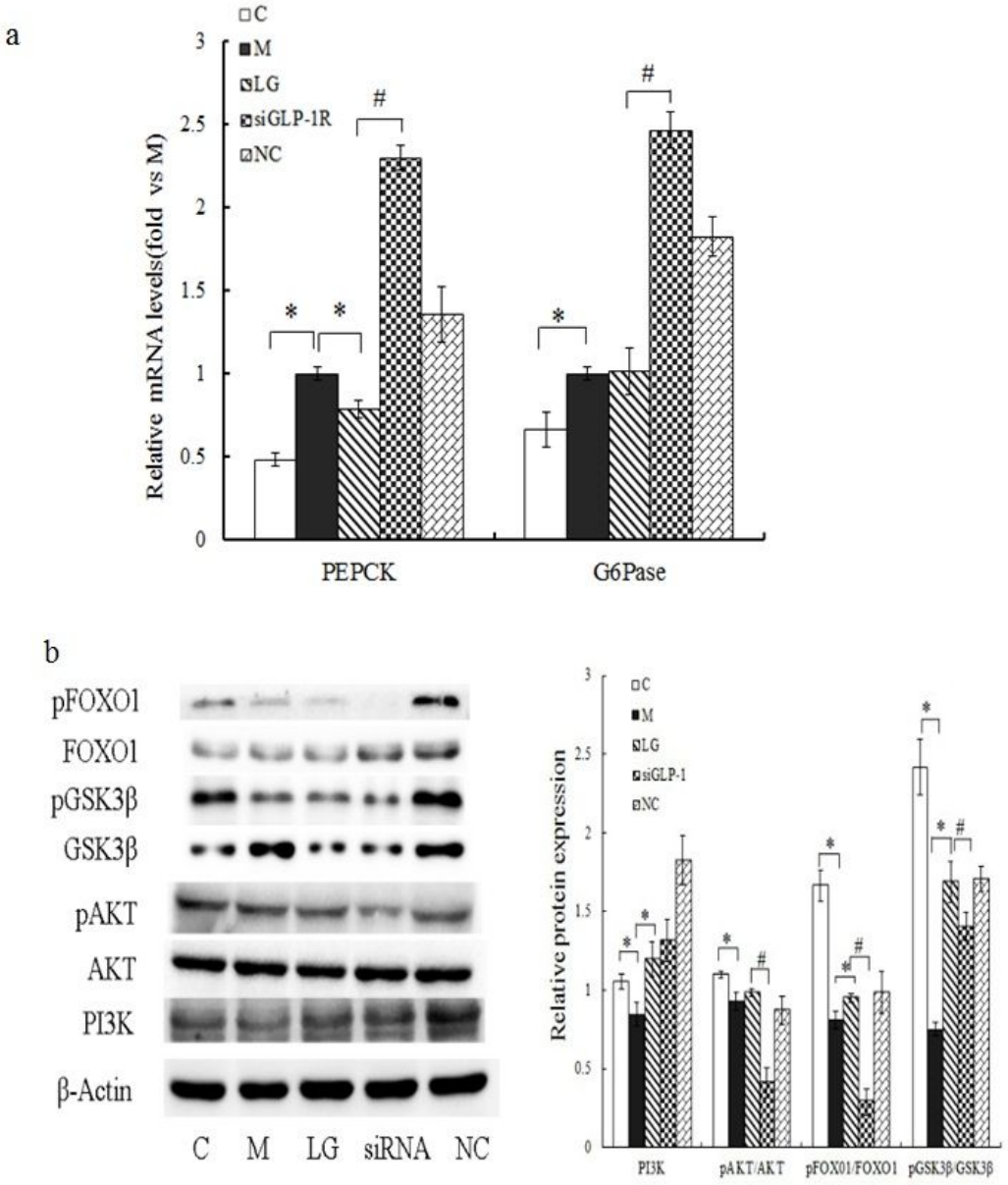
Assessment GLP-1R expression in HepG2 and L02 cells



(a-b) Glucose uptake and intracellular glycogen content in siRNA-transfected L02 cells that were cotreated with FFAs and LG. (a. glucose uptake, b. intracellular glycogen) Data are expressed as means ± SD (n = 9), $^{*}p < 0.05$ compared with the M, $^{\#}p < 0.05$ compared with the LG.

Figure 2

The Effect of GLP-1R activation on glucose uptake and intracellular glycogen content in L02 cells

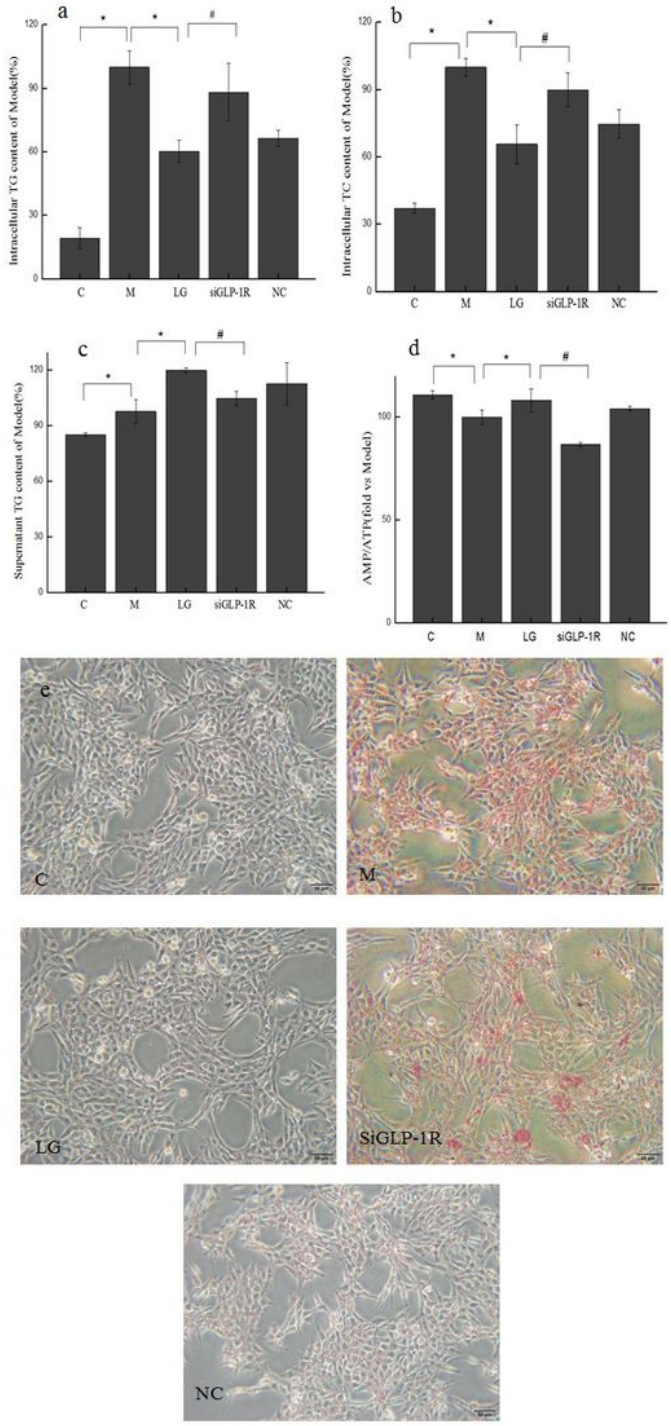


(a) qPCR analysis of PEPCK and G6Pase in siRNA-transfected L02 cells that were cotreated with FFAs and LG.

(b) Western blot analysis of critical protein expression of PI3K、pAKT/AKT、pFOXO1/FOXO1 and pGSK3β/GSK3β in siRNA-transfected L02 cells that were cotreated with FFAs and LG (100 nmol). Data are expressed as means ± SD (n=3) $^{*}p<0.05$ compared with M, $^{\#}p<0.05$ compared with LG.

Figure 3

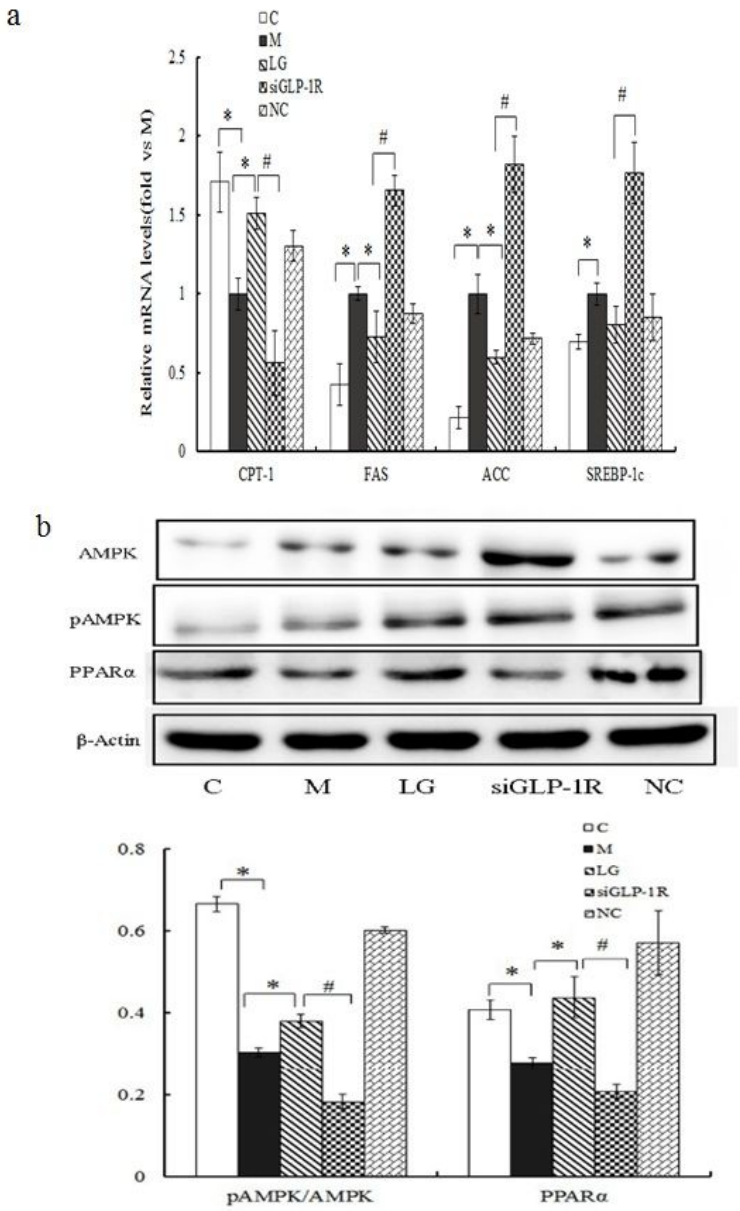
Gluconeogenesis was inhibited by GLP-1R activation via AKT



(a) Energy metabolism in siRNA-transfected L02 cells that were cotreated with FFAs and LG. (b-d) Intracellular TG and TC level and supernatant TG content in siRNA-transfected L02 cells that were cotreated with FFAs and LG (b. intracellular TG, c. intracellular TG, d. supernatant TG). (e) Oil Red O staining of L02 cells (200 ×). Data are expressed as means ± SD (n=3) *p<0.05 compared with M, #p<0.05 compared with LG.

Figure 4

Effect of GLP-1R activation on energy metabolism and lipid metabolism in L02 cells



(a) qPCR analysis of CPT-1, FAS, ACC and SREBP-1c in siRNA-interfered L02 cells that were correlated with FFAs and LG. (b) Western blot analysis of pAMPK、AMPK and PPARα in siRNA-interfered L02 cells that were correlated with FFAs and LG. Data are expressed as means ± SD (n=3) $^{*}p<0.05$ compared with M, $^{\#}p<0.05$ compared with LG.

Figure 5

GLP-1R activation reduced lipogenesis via AMPK pathway in L02 cells.

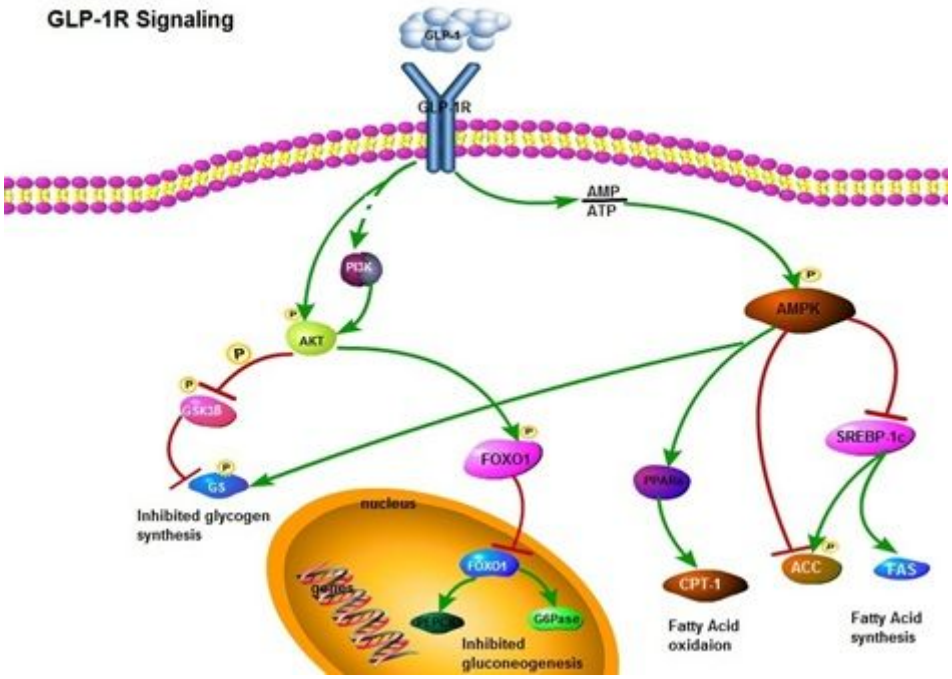


Figure 6

Mechanism of GLP-1R activation in alleviating insulin resistance of hepatocytes L02