Glucagon-Like Peptide-1 Receptor Mediates the Improvement of Glycolipid Metabolism Disorder via AKT and AMPK Signaling Pathways in L02 with Insulin Resistance

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Research

Keywords: GLP-1R, L02, Insulin Resistance, AMPK, PI3K-AKT

Posted Date: September 11th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-29811/v2>

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Abstract

Background: Glucagon like peptide 1 (GLP-1) has previously been found to regulate glycolipid metabolism in the liver by promoting insulin secretion. However, the direct regulation of GLP-1 on liver glycolipid metabolism was still controversial, due to the presence of GLP-1 receptors (GLP-1R) in human liver has not been determined. In this research, the presence and direct effect on hepatic metabolism of GLP-1R were investigated in L02 cells (normal human hepatocytes).

Methods: The insulin resistance cell model with metabolic disorder was constructed using oleic acid and fructose to simulate the metabolic syndrome caused by high-fat and high-fructose in modern diet. Glycolipid metabolism markers and the involvement of AMP-activated protein kinase (AMPK) and PI3 Kinase (PI3K)-AKT were assessed.

Results: GLP-1R was found to be expressed in L02 cells and decreased in insulin resistance cells. GLP-1R knockdown induced a dramatic decrease in glucose utilization and intracellular content, and increase in mRNA levels of genes associated with gluconeogenesis via AKT pathway independent of PI3K. Furthermore, GLP-1R knockdown increased the contents of triglyceride (TG) and total cholesterol (TC) by increasing the mRNA levels of sterol response element binding protein-1c (SREBP-1c), fatty acid synthase (FAS) and acetyl CoA carboxylase (ACC), and decreasing the mRNA level of carnitine palmitoyl transferase 1 (CPT-1) via AMPK pathway.

Conclusions: GLP-1R mediated the LG-caused promotion of glucose utilization and inhibition of lipid synthesis, as the positive effects of LG were diminished by GLP-1R knockdown. These findings demonstrated that GLP-1R is the molecular basis for GLP-1 to improve glycolipid metabolism in L02 cells.

1 Background

With the increase of sugar and fat consumption, insulin resistance, type 2 diabetes mellitus (T2DM) and obesity caused by metabolic disorders are serious public health problems in the world. Metabolic disorders are induced by the modern diet of high-fat and high-fructose, including hyperglycemia, dyslipidemia (Stanhope et al., 2009), insulin resistance (Zhao et al., 2016), 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% to 8.5% (WHO., 2016). Insulin resistance caused by metabolic disorders was susceptibility factor for obesity and T2DM (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-1R expressed in islet, intestine, heart, the central nervous systems, and blood vessels (Drucker, 2018). 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 is the molecular basis for GLP-1R to reduce the metabolism caused by IR. Furthermore, GLP-1R plays an important role in restore glucose homeostasis and reduce lipid accumulation of IR cells via AMPK and AKT pathway independent of PI3K.

2 Materials And Methods

2.1 Chemicals

Oleic acid (OA, #O1383) were obtained from Sigma-Aldrich, Inc. (St. Louis, USA). Fructose (#T0131) were purchased from Biotopped Co.Ltd. (Beijing, China). Liraglutide (LG, purity $\geq$ 98%, #MB5555) were purchased from Meilun Biotechnology Co.Ltd. (Dalian, China). FFA-free Bovine Serum Albumin (BSA, #354331) were purchased from BioDee Biotechnology Co.Ltd. (Beijing, China).

For cell culture, RPMI1640(#CM10041), Hank's Balanced Salt Solution (HBSS, #CC014) were obtained from Macgene Technology Co.Ltd. (Beijing, China). Fetal bovine serum (FBS, #10099), penicillin-streptomycin (#15140163) were purchased from Gibco Life Technologies (Grand Island, NY, USA). Pancreatin (#C0201) Enhanced BCA Protein Assay Kit (#P0012) and RIPA buffer (#P00138) were purchased from Beyotime Biotechnology Inc. (Beijing, China). Total cholesterol assay kit (#A111-1-1), Triglyceride assay kit (#A110-1-1) and Glucose Assay Kit (#F006-1-1) were purchased from Nanjing Jiancheng Bioengineering Institute.(Jiangsu, China) The adenosine triphosphate [ATP (#BU-BN11245)] Elisa kit and Adenosine monophosphate [AMP (#SU-BN10545)] were purchased form Inselisa Biotechnology Co.Ltd. (Hubei, China). Antibodies of anti-PI3K [(p110 α),#4255], anti-Akt (#2920), anti-pAkt

(4060), anti-GSK3 β (#12456), anti-pGSK3 β (#9323), anti-FOXO1 (#2880), anti-pFOXO1 (#9464), anti-AMPK (#5831)], anti-pAMPK (#2535) were purchased from Cell Signaling Technology, Inc. (Boston, USA). Antibodies of GLP-1R (#ab186051) and anti-PPAR α (#ab24509) were purchased from abcam (Cambridge, UK) and the antibodies of anti-beta actin (#AF0003) were purchased from Beyotime Biotechnology Inc. (Beijing, China). 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 4.5 g/L glucose, 10% NCS, 100 $\mu\text{g mL}^{-1}$ streptomycin and 100 U mL^{-1} penicillin) and incubated at 37 °C with 5% CO₂ /95% air atmosphere.

L02 (3111C0001CCC000035) were purchased from Biological Technology Co.Ltd. (Shanghai, China), grown in RPMI 1640 (containing 2.0 g/L glucose, 10% FBS, 100 $\mu\text{g mL}^{-1}$ 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 h as Control (C), or 100 μmol of OA and 25 mmol of fructose (IR) for 24 h as IR model, or IR and 100 nmol LG for 24 h as LG intervention (LG), or 100 nmol siRNA and IR and LG (100 nmol) for 24 h 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. At the same time set Blank (Normal L02 cells), mock transfection (Normal L02 cells incubated with transfection reagent), and positive control (Normal L02 cells incubated with siGADPH) to determine the interference and detection method. The transfection efficacy was determined by mRNA analysis (Figure S2). 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 utilization and intracellular glycogen content

Glucose utilization 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 and quantification

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). The quantification of Oil Red O using image J (Bethesda, Maryland, U.S.).

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 and AMP 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-Rad, USA). The thermal cycling conditions for PCR were 45°C for 5 min, 94°C for 30 s, 40 cycles of 94°C for 5 s, 65°C 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 (GAPDH). All the above steps follow MIQE guidelines. 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'-ATGACCGTCTTGCTTTTCGAT-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 2h at room temperature. Afterward, antibodies of anti-PI3K, anti-Akt, anti-pAkt, anti-GSK3 β , anti-pGSK3 β , anti-FOXO1, anti-pFOXO1, anti-AMPK, anti-pAMPK, anti-PPAR α 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 Immunohistochemistry

The slides of L02 cells were incubated 50-100 μ L of membrane rupture working solution for 20 min at room temperature, and washed with PBS (PH7.4) for 5 min. The slides were then incubated with a primary anti-GLP-1R antibody (#bs-1559R, Bioss, Beijing China) at 4 °C overnight. The slides were washed in PBS for 5 min and covered with secondary antibody at room temperature for 50 min. The slides were washed in PBS and incubated with 3,3'-diaminobenzidine (DAB, Servicebio, Inc. Hubei China) for 3 min. Hematoxylin was used to counterstain nuclei. AE100 automatic microscope (Nikon, Japan) was used to collect images. The nucleus of hematoxylin stained is blue, and the positive expression of DAB is brownish yellow.

2.11 Statistical analysis

Statistical analysis was performed by one-way ANOVA analysis followed by Tukey's test of variance using Origin 8.5 software (Origin Lab Corp., USA). All data are presented as the mean values $\pm$ standard deviation (SD). Differences were considered statistically significant when $p < 0.05$.

3 Results

3.1 GLP-1R expression in normal human hepatocytes L02

Studies have shown that GLP-1 has a direct regulation on hepatic lipogenesis, but the opposite view is that the existence of GLP-1R in hepatocytes remained controversial. (Ben-Shlomo et al., 2011, Drucker, 2018). Thus, the expression of GLP-1R in L02 cells was detected by qPCR and western blotting 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 cells. According to the results of immunofluorescence staining, it can be concluded that GLP-1R is expressed in HepG2 and L02 cells (Fig. 1c). In addition, the protein level of GLP-1R was induced

significantly reduction in L02 cells with IR (Fig. 1d). The protein expression of GLP-1R in different groups showed that LG treatment didn't change GLP-1R expression, and siRNA treatment seriously inhibited GLP-1R expression. (Fig. 1e).

3.2 The role of GLP-1R 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](#)). As shown in Fig. 2a, GLP-1R knockdown reversed LG-induced increase in glucose utilization. Similarly, the content of intracellular glycogen content was significantly decreased after siGLP-1R transfection. In this section, glucose utilization and glycogen content are regulated by GLP-1R independently of insulin.

3.3 GLP-1R knockdown enhances gluconeogenesis via AKT without PI3K

Regulation of gluconeogenesis is the medium of the liver to maintain glucose homeostasis. As rate-limiting enzymes for gluconeogenesis, the mRNA expression levels of PEPCK and G6Pase were assessed. GLP-1R knockdown significantly increased LG-induced reduction in the gene levels of PEPCK (Fig. 3a). As shown in Fig. 3b, GLP-1R knockdown significantly down-regulated LG-induced the phosphorylation of AKT, but the expression of PI3K at protein levels were no significant change (Fig. 3b). Furthermore, AKT was one of the regulatory factors of FOXO1 and GSK3 β . LG-caused phosphorylation of FOXO1, a switch of gluconeogenesis and GSK-3 β a regulatory of glycogen synthesis were diminished by GLP-1R knockdown (Fig. 3b). These results suggest that GLP-1R was involved in the intervention of liver glucose metabolism by LG and that these effects are mediated by AKT phosphorylation and downstream transcription mechanisms.

3.4 GLP-1R mediates the LG-caused inhibition of lipid accumulation

GLP-1 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-1 on lipid metabolism in liver was still unclear. As shown in Fig. 4a-b, the IR-induced increases in intracellular TG and TC were significantly reduced after LG treatment, and the effects of LG was reversed by GLP-1R knockdown. In addition, GLP-1R knockdown inhibited LG-caused transport of TG from intracellular to extracellular (Fig. 4c). Oil Red O staining showed that the LG-induced hepatic lipid oxidation was significantly increased by GLP-1R interfered (Fig. 4d-e). This result indicated that GLP-1R media LG-caused inhibition of fat accumulation and promotion of fat oxidation.

3.5 GLP-1R knockdown enhances lipogenesis via AMPK pathway in L02 cells

It has been reported that GLP-1 could promote fatty acid β oxidation and reduce lipogenesis ([Ben-Shlomo et al., 2011](#)). As shown in Fig. 5a, LG significantly increased the levels of typical fat synthase including, ACC, FAS, SREBP-1c, and decreased the levels of CPT-1, a rate-limiting enzyme of fatty acid β oxidation. However, compared with the LG, GLP-1R knockdown weakened the intervention of LG. Notably, Fatty acid β oxidation was strictly regulated by AMPK through activated PPAR α to promote transcription of CPT-1 in

the liver. What's more, AMPK activation 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](#)). Energy homeostasis is one of the conditions for AMPK activation([Viollet et al., 2006](#)). Next, AMP:ATP ratio was investigated in L02 cells. As shown in Fig. 5b, GLP-1R knockdown dramatically decreased LG-induced increase in AMP: ATP ratio. In addition, LG-caused increase in PPAR α and the phosphorylation of AMPK were reversed by GLP-1R interference. In conclusion, GLP-1R was an important target for GLP-1 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 utilization in glucose metabolism and modulate the decline of fat accumulation and the enhancement of fatty acid oxidation in lipid metabolism. Besides that, GLP-1 significantly increased AKT phosphorylation and reduced the levels of its downstream target genes FOXO1 and GSK3 β 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 mediates the decrease of transcription factor SREBP-1c mRNA levels and its downstream lipogenic enzymes FAS and ACC and the increase of CPT-1 mRNA levels via AMPK pathway.

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 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 GSK3 β , which in turn inhibits the gluconeogenesis rate-limiting enzymes PEPCK, and G6Pase activity and increases glycogen synthase activity ([Musmann et al., 2007](#), [Gross et al., 2009](#))([Gross et al., 2009](#)). In the current study, the phosphorylation AKT was increased by GLP-1R, causing inactivation of FOXO1 and GSK3 β , 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 GSK3 β on glycogen synthetase was weakened, resulting in the increase of intracellular glycogen synthesis.

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-1 stimulated hepatic lipid oxidation and restores hepatic signaling alteration induced by high-fat diet in patients with nonalcoholic steatohepatitis(NASH) via GLP-1R (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 on liver lipid metabolism. Our results demonstrate that GLP-1 has an insulin-independent liver metabolism regulation mechanism, which is GLP-1R mediates the improvement of glycolipid metabolism disorder via AKT and AMPK signaling pathways in L02 with insulin resistance.

5 Conclusions

In summary, GLP-1 induced metabolic balance and energy homeostasis by increasing fatty acid oxidation, and decreasing gluconeogenesis and fatty acid synthesis via AKT and AMPK pathways, which appeared to be primarily mediated by GLP-1R (Fig. 6). Therefore, GLP-1R is a potential target for the treatment of T2DM and obesity caused by liver metabolic disorders.

6 Abbreviations

GLP-1: Glucagon like peptide 1

GLP-1R: GLP-1 receptors

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

AMPK: AMP-activated protein kinase

PI3K: PI3 Kinase

CPT-1: carnitine palmitoyl transferase 1

ACC: acetyl CoA carboxylase

FAS: fatty acid synthase

SREBP-1c: sterol response element binding protein-1c

AMP: Adenosine monophosphate

ATP: Adenosine triphosphate

DAB: 3,3'-diaminobenzidine

7 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.

Funding

This work was supported by the National Key Research and Development Program (2016YFD0401303).

Authors' contributions

Qiaoji Tian wrote the manuscript, performed data collection and analysis. Kai Xia, Shenglin Duan conducted experiments. Yifeng Liu, Peng Yuan and Jia Liu participated in research design. Haizhi Li,

Xiaofeng Han and Cong Pan contributed to the writing of the manuscript. The authors read and approved the final manuscript.

Acknowledgments

None.

8 References

- BAGGIO, L. L. & DRUCKER, D. J. 2007. Biology of Incretins: GLP-1 and GIP. *Gastroenterology*, 132, 2131-2157.
- BEN-SHLOMO, S., ZVIBEL, I., SHNELL, M., SHLOMAI, A., CHEPURKO, E., HALPERN, Z., BARZILAI, N., OREN, R. & FISHMAN, S. 2011. Glucagon-like peptide-1 reduces hepatic lipogenesis via activation of AMP-activated protein kinase. *Journal of Hepatology*, 54, 1214-1223.
- DRUCKER, D. J. 2018. Mechanisms of Action and Therapeutic Application of Glucagon-like Peptide-1. *Cell Metabolism*, 27, 740-756.
- GROSS, D. N., WAN, M. & BIRNBAUM, M. J. 2009. The role of FOXO in the regulation of metabolism. *Current Diabetes Reports*, 9, 208-214.
- GUPTA, N. A., MELLS, J., DUNHAM, R. M., GRAKOU, A., HANDY, J., SAXENA, N. K. & ANANIA, F. A. 2010. Glucagon-like peptide-1 receptor is present on human hepatocytes and has a direct role in decreasing hepatic steatosis *in vitro* by modulating elements of the insulin signaling pathway. *Hepatology*, 51, 1584-1592.
- HOLST, J. J. 2007. The Physiology of Glucagon-like Peptide 1. *Physiological Reviews*, 87, 1409-1439.
- J.DRUCKER, D. 2016. The Cardiovascular Biology of Glucagon-like Peptide-1. *Cell Metabolism*, 24, 15-30.
- LEE, Y. S., SHIN, S., SHIGIHARA, T., HAHM, E., LIU, M. J., HAN, J., YOON, J. W. & JUN, H. S. 2007. Glucagon-like peptide-1 gene therapy in obese diabetic mice results in long-term cure of diabetes by improving insulin sensitivity and reducing hepatic gluconeogenesis. *Diabetes*, 56, 1671-9.
- LIU, M., LIU, H., XIE, J., XU, Q. & LIU, J. 2017. Antiobesity effects of zeaxanthin on 3T3-L1 preadipocyte and high fat induced obese mice. *Food & Function*, 8, 3327.
- MUSSMANN, R., GEESE, M., HARDER, F., KEGEL, S., ANDAG, U., LOMOW, A., BURK, U., ONICHTCHOUK, D., DOHRMANN, C. & AUSTEN, M. 2007. Inhibition of GSK3 Promotes Replication and Survival of Pancreatic Beta Cells. *Journal of Biological Chemistry*, 282, 12030-12037.
- NAIM, P., MULVIHILL, E. E., CHRISTINE, L., BERNARDO, Y., CAMPBELL, J. E., BROWN, T. J., CATHERINE, S., DIANNE, H., CAO, X. & BAGGIO, L. L. 2013. GLP-1 Receptor Activation Indirectly Reduces Hepatic Lipid

- Accumulation But Does Not Attenuate Development of Atherosclerosis in Diabetic Male ApoE^{-/-} Mice. *Endocrinology*, 1.
- NICKAVAR, B. & AMIN, G. 2004. Effects of Glucagon-like Peptide 1 on the Hepatic Glucose Metabolism. *Hormone & Metabolic Research*, 36, 837-841.
- POSTIC, C. & GIRARD, J. 2008. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. *Journal of Clinical Investigation*, 118, 829-838.
- PYKE, C., HELLER, R. S. & KIRK, R. K. 2014. GLP-1 Receptor Localization in Monkey and Human Tissue: Novel Distribution Revealed With Extensively Validated Monoclonal Antibody. *Endocrinology*, 155, 1280-1290.
- SAVAGE, D. B., PETERSEN, K. F. & SHULMAN, G. I. 2007. Disordered lipid metabolism and the pathogenesis of insulin resistance. *Physiol Rev*, 87, 507-20.
- STANHOPE, K. L., SCHWARZ, J. M., KEIM, N. L., GRIFFEN, S. C., BREMER, A. A., GRAHAM, J. L., HATCHER, B., COX, C. L., DYACHENKO, A. & ZHANG, W. 2009. Consuming fructose-sweetened , not glucose-sweetened , beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight / obese humans. *Journal of Clinical Investigation*, 119, 1322-1334.
- STEINBERG, G. R. & KEMP, B. E. 2009. AMPK in Health and Disease. *Physiol Rev*, 89, 1025-1078.
- SVEGLIATI-BARONI, G., SACCOMANNO, S. & RYCHLICKI, C. 2011a. Glucagon-like peptide-1 receptor activation stimulates hepatic lipid oxidation and restores hepatic signalling alteration induced by a high-fat diet in nonalcoholic steatohepatitis. *Liver International Official Journal of the International Association for the Study of the Liver*, 31, 1285-1297.
- SVEGLIATI-BARONI, G., SACCOMANNO, S., RYCHLICKI, C., AGOSTINELLI, L., DE MINICIS, S., CANDELAESI, C., FARACI, G., PACETTI, D., VIVARELLI, M., NICOLINI, D., GARELLI, P., CASINI, A., MANCO, M., MINGRONE, G., RISALITI, A., FREGA, G. N., BENEDETTI, A. & GASTALDELLI, A. 2011b. Glucagon-like peptide-1 receptor activation stimulates hepatic lipid oxidation and restores hepatic signalling alteration induced by a high-fat diet in nonalcoholic steatohepatitis. *Liver International*, 31, 1285-1297.
- TUSHUIZEN, M. E., BUNCK, M. C., POUWELS, P. J., VAN WAESBERGHE, J. H., DIAMANT, M. & HEINE, R. J. 2006. Incretin mimetics as a novel therapeutic option for hepatic steatosis. *Liver Int*, 26, 1015-1017.
- VIOLET, B., FORETZ, M., GUIGAS, B., HORMAN, S., DENTIN, R., BERTRAND, L., HUE, L. & ANDREELLI, F. 2006. Activation of AMP-activated protein kinase in the liver: a new strategy for the management of metabolic hepatic disorders. *The Journal of Physiology*, 574, 41-53.
- WHO. 2016. Global report on diabetes.

ZHAO, L., GUO, X., WANG, O., ZHANG, H., WANG, Y., ZHOU, F., LIU, J. & JI, B. 2016. Fructose and glucose combined with free fatty acids induce metabolic disorders in HepG2 cell: A new model to study the impacts of high-fructose/sucrose and high-fat diets in vitro. *Molecular Nutrition & Food Research*, 60, 909-921.

ZHOU, D., CHEN, Y.-W., ZHAO, Z.-H., YANG, R.-X. & XIN, F.-Z. 2018a. Sodium butyrate reduces high-fat diet-induced non-alcoholic steatohepatitis through upregulation of hepatic GLP-1R expression. *Experimental & Molecular Medicine*.

ZHOU, D., CHEN, Y., ZHAO, Z., YANG, R., XIN, F., LIU, X., PAN, Q., ZHOU, H. & FAN, J. 2018b. Sodium butyrate reduces high-fat diet-induced non-alcoholic steatohepatitis through upregulation of hepatic GLP-1R expression. *Experimental and Molecular Medicine*, 50, 157.

Figures

Figure 1

GLP-1R expression in normal human hepatocytes L02. (a-b) GLP1-R mRNA was assessed by qPCR and Western blotting in HepG2 (positive controls) and L02 cells (a. qPCR, b. WB). (c) Immunofluorescence labeling of GLP-1R in HepG2 and L02 cells. The nucleus of hematoxylin stained is blue, and the positive expression of GLP-1R is brownish yellow (red arrow), Scale bar =100 μm. (d) GLP-1R protein expression was assessed by Western blotting in Control and IR cells. (e) GLP-1R protein expression was assessed by

Western blotting in siRNA-transfected L02 cells that were cotreated with IR and LG (100 nmol) for 24h. Data are expressed as means $\pm$ SD (n = 3), *p < 0.05 compared with the control.

Figure 2

The role of GLP-1R in regulating glucose metabolism. (a-b) Glucose utilization and intracellular glycogen content in siRNA-transfected L02 cells that were cotreated with IR and LG. (a. glucose utilization, b. glycogen content) Data are expressed as means $\pm$ SD (n = 9), *p < 0.05 compared with the IR, #p < 0.05 compared with the LG.

Figure 3

GLP-1R knockdown enhances inhibition of gluconeogenesis via AKT without PI3K. (a) qPCR analysis of PEPCK and G6Pase in siRNA-transfected L02 cells that were cotreated with IR and LG. (b) Western blotting analysis of critical protein expression of PI3K/pAKT/AKT/pFOXO1/FOXO1 and pGSK3 β /GSK3 β in siRNA-transfected L02 cells that were cotreated with IR and LG (100 nmol). Data are expressed as means $\pm$ SD (n=3) *p<0.05 compared with IR, #p<0.05 compared with LG.

Figure 4

GLP-1R mediates the LG-caused inhibition of lipid accumulation. (a-c) Intracellular TG and TC and supernatant TG content in siRNA-transfected L02 cells that were cotreated with IR and LG (a. intracellular TG, b. intracellular TC, c. supernatant TG). (d-e) quantification of Oil Red O staining in L02 cells. Scale bar = 50µm. Data are expressed as means $\pm$ SD (n=3) *p<0.05 compared with IR, #p<0.05 compared with LG.

Figure 5

GLP-1R knockdown enhances lipogenesis via AMPK pathway in L02 cells. (a) qPCR analysis of CPT-1, FAS, ACC and SREBP-1c in siRNA-interfered L02 cells that were correlated with IR and LG. (b) Energy metabolism in siRNA-transfected L02 cells that were cotreated with IR and LG. (c) Western blotting analysis of pAMPK/AMPK and PPARα in siRNA-interfered L02 cells that were correlated with IR and LG. Data are expressed as means $\pm$ SD (n=3) *p<0.05 compared with IR, #p<0.05 compared with LG.

Figure 6

Mechanism of GLP-1R mediates alleviation of insulin resistance of hepatocytes L02.

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