LRG ameliorates steatohepatitis by activating the AMPK/mTOR/SREBP1 signaling pathway in C57BL/6J mice fed a high-fat diet

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Abstract. The pathogenesis of nonalcoholic fatty liver disease (NAFLD) is the most widespread liver disease worldwide, and its incidence continues to rise (1,2). It describes a number of liver diseases including simple steatosis or NAFL with low inflammation, and can progresses to non-alcoholic steatohepatitis (NASH). NASH is the most severe form of NAFLD and is characterized by the presence of an abnormal accumulation of fat in the liver which can progress to liver cell injury (hepatocellular ballooning) and inflammation (3). NASH results from aberrant hepatic lipid accumulation, which is strongly associated with high-fat diet (HFD)-induced metabolic abnormality. A continuous intake of HFD contributes to the progression of NAFLD (4,5). However, the exact molecular mechanisms underlying NASH remain largely unknown, and currently there are no effective therapeutic strategies for NASH apart from caloric restriction (CR) and regular exercise (6,7). Therefore, further understanding of the pathology of NASH is critical in order to develop effective management strategies for NAFLD.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most widespread liver disease worldwide, and its incidence continues to rise (1,2). It describes a number of liver diseases including simple steatosis or NAFL with low inflammation, and can progresses to non-alcoholic steatohepatitis (NASH). NASH is the most severe form of NAFLD and is characterized by the presence of an abnormal accumulation of fat in the liver which can progress to liver cell injury (hepatocellular ballooning) and inflammation (3). NASH results from aberrant hepatic lipid accumulation, which is strongly associated with high-fat diet (HFD)-induced metabolic abnormality. A continuous intake of HFD contributes to the progression of NAFLD (4,5). However, the exact molecular mechanisms underlying NASH remain largely unknown, and currently there are no effective therapeutic strategies for NASH apart from caloric restriction (CR) and regular exercise (6,7). Therefore, further understanding of the pathology of NASH is critical in order to develop effective management strategies for NAFLD.

Sterol regulatory element-binding protein-1 (SREBP1) mediates the expression of lipogenesis-associated triglyceride synthesis and accumulation (8,9). SREBP1 can cause excessive triglyceride accumulation in the liver, thereby leading to NAFLD development (10). Mechanistic target of rapamycin (mTOR) is a member of the phosphatidylinositol-3-kinase-associated family of kinases and forms two distinct complexes: mTORC1 and mTORC2 (11). mTORC1 signaling stimulates cell growth via multiple mechanisms, including promoting lipid biosynthesis (12,13). In addition, mTORC1 enhances de novo lipogenesis by enhancing the nuclear localization and activity of SREBP1 (14-16). Therefore, agents targeting mTORC1 have therapeutic potential for NASH.

Glucagon-like peptide-1 (GLP-1), an incretin hormone, as well as glucose-dependent insulinotropic polypeptide, are responsible for mediating glucose-mediated insulin production.
in pancreatic β-cells (17-19). GLP-1 and its analogues also perform pleiotropic functions in extra-pancreatic organs in mammals, including hepatic lipid deposition alleviation, weight loss and appetite inhibition (20,21). GLP-1 may regulate the expression of genes associated with lipid metabolism in liver cells, thereby preventing the development and progression of NAFLD (22). Therefore, the GLP-1 receptor agonist liraglutide, may have potential for improving NASH outcomes as a novel therapeutic agent by activating the adenosine mono-phosphate-activated protein kinase (AMPK)/mTOR/SREBP1 signaling pathway.

Materials and methods

Animals. A total of 32 male C57/Bl6j mice (18-20 g), 8 weeks of age, were supplied by the Laboratory Animal Center, West China Hospital, Sichuan West China School of Medicine (Chengdu, China). The mice were housed individually in cages at 20-25°C with a constant humidity (55±5%) with a 12-h light/dark cycle. All animal experiments were approved by the Ethics Committee of Sichuan University (Sichuan, China) and were performed in accordance with guidelines of the Institutional Animal Ethics Committee and international guidelines (23,24).

Animal groups and treatments. C57BL/6J mice were randomly divided into 4 groups (n=8/group) as follows: Standard-fat diet: i) Control (8 kcal % fat, 42 kcal % protein and 50 kcal % carbohydrate); the HFD-fat diet groups: ii) the ad libitum group [61 kcal % fat, 15 kcal % protein, and 25 kcal % carbohydrate, 0.2% cholesterol, high glucose water (2% fructose plus 2.5% glucose) ad libitum], iii) the ad libitum+LRG (liraglutide, 0.6 mg/kg/day) and iv) the CR group (calorically restricted to the manufacturer's instructions).

Biochemical indices. Tail blood of mice was collected at 0, 3, 6, 9, 12 weeks and was measured using a blood glucose meter (Life Scan, Inc., USA). After sacrificing the mice at the end of week 12, the liver tissues were excised, immediately frozen in liquid nitrogen and stored at -80°C for protein extraction.

Statistical analysis. Statistical analysis was performed using GraphPad Prism 6 software (GraphPad Software, Inc., La Jolla, CA, USA). The results are expressed as the mean ± standard error. A Student's t-test or paired Student’s t-test were used to compare two groups. Comparisons among multiple groups were analyzed using one-way analysis of variance (ANOVA) followed by the Scheffe post-hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Effects of LRG treatment on body weight, energy intake and blood glucose in C57 mice. The body weight and energy changes as well as blood fasting glucose in the different groups are presented in Fig. 1. To confirm the beneficial metabolic effects of LRG treatment in vivo, the CR group was designed in the present experiments to consistently match the energy intake of the LRG-treated group, thereby avoiding the effects of long-term LRG treatment on energy intake.

The body weights of the C57 mice fed the HFD both treated with LRG (0.6 mg/kg/day) and the CR group (ad libitum+LRG and CR groups) were significantly lower than that of the ad libitum group (P<0.01 at 9 and 12 weeks);
whereas there were no significant differences in whole body weights between the ad libitum and CR groups. The energy intakes of mice treated with LRG were significantly decreased when compared with the ad libitum (P<0.01 at 9 and 12 weeks; Fig. 1B). No differences were observed in the energy intakes between the CR and ad libitum+LRG treatment groups. Furthermore, fasting blood glucose levels were significantly lower throughout the experimental period in the ad libitum+LRG group when compared with the ad libitum group (P<0.01; Fig. 1C). ACC, a downstream target of AMPK, is a key enzyme in fatty acid metabolism. Activated AMPK phosphorylates and inhibits ACC activity, leading to the inhibition of fat synthesis and increased oxidation (Fig. 4). The present study demonstrated that LRG treatment upregulated the phosphorylation of ACC and AMPK in the mice treated with HFD (Fig. 3A and C). SREBP1c promotes the synthesis of fatty acids and triglycerides (Fig. 4). As shown in Fig. 3B, the HFD-fed mice (ad libitum and CR groups) had significantly increased SREBP1 activity (P<0.01) when compared with the control group, yet this increase was significantly attenuated in the ad libitum+LRG mice (P<0.05) compared with the ad libitum group (P<0.05).
Discussion

The pathology of steatohepatitis (NASH) is largely unknown, and there are currently no effective treatments available for NASH apart from diet and physical activity. In the present study, we investigated the therapeutic effects of the GLP-1 receptor agonist liraglutide (LRG) on NASH and the underlying mechanisms. The results revealed that C57 mice with HFD-induced NASH exhibited increased body weight and increased levels of hepatic fat. In addition, LRG treatment significantly reduced the body weight, improved hepatic lipid accumulation, and suppressed the elevated levels of total cholesterol and low-density lipoprotein cholesterol in the serum of HFD-fed mice. Mechanistically, it was revealed that LRG improves NASH through the AMPK/mTOR/SREBP1 signaling pathway.

AMPK is a sensor of intracellular energy status and negatively regulates mTORC1 (26-28). Recent studies have reported that AMPK agonists (such as metformin and adiponectin) improve NASH by inhibiting lipid synthesis via mTORC1/SREBP-1c signaling (12,29-31). In addition, ACC is a downstream target of AMPK and a rate-limiting enzyme involved in the synthesis of fatty acids. Activated liver AMPK inhibits fatty acid synthesis by increasing the phosphorylation and inactivation of ACC in order to reduce the production of malonyl coenzyme A (32,33). In addition, mTORC1 triggers hepatic de novo lipogenesis to promote lipid synthesis by activating SREBP-1c (26,33,34).
Furthermore, mTORC1 regulates SREBP-1 activation by stimulating SREBP-1 mRNA expression, and promoting the nuclear localization and activity of SREBP-1 (30-32). Düvel et al (35) also demonstrated that p70S6K1 is required for the mTORC1-mediated increase and activation of SREBP1 (36-38). In the present study, LRG treatment reduced lipid accumulation in the liver by activating AMPK, thereby suppressing the mTORC1/SREBP1 signaling pathway.

The present results demonstrated that HFD inhibited the phosphorylation of AMPK in the mouse liver and significantly enhanced the phosphorylation of mTOR and 70S6K1 as well as the expression of SREBP1. However, LRG treatment activated AMPK and suppressed the mTOR-mediated activation of SREBP1, thereby blocking the transcription of target lipogenic genes involved in the liver steatosis of HFD-fed C57 mice. In addition, LRG treatment inhibited HFD-induced nuclear SREBP1 activation, thereby inhibiting SREBP1 translocation into the nucleus and the subsequent changes in liver triglyceride accumulation.
The GLP-1 receptor is widely expressed in various organs of the body and is responsible for improving islet function, suppressing appetite and reducing body weight (39-41). We hypothesized that drug-induced GLP-1 activation may reduce the body weight of HFD-fed mice. Indeed, the body weight changes between the CR group and the group fed HFD
indicating that the activation of GlP-1 by lrG directly exerts a pharmacological action on NAFLD in HFD-fed mice.

In conclusion, the GLP-1 receptor agonist LRG reduced the accumulation of hepatic lipids by regulating the AMPK/mTOR/SREBP1 signaling pathway. The present results identified a mechanism by which LRG alleviates hepatic lipid accumulation. Thus, the results of the present study may be used to develop novel therapeutic strategies for steatohepatitis.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors’ contributions

HYC carried out the molecular studies, participated in the immunoassays and drafted the manuscript. SSW carried out the immunoassays. HMT and TH conceived and participated in the immunoassays and drafted the manuscript. SSW carried out the molecular studies, participated in the design of the study, performed the statistical analysis and drafted the manuscript. HYc carried out the molecular studies, participated in the design of the study, performed the statistical analysis and drafted the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal experiments were approved by the Ethics Committee of Sichuan University (Sichuan, China) and were performed in accordance with guidelines of the Institutional Animal Ethics Committee and international guidelines.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

References


