

GLP-1 Receptor Agonist and Non-Alcoholic Fatty Liver Disease

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Non-alcoholic fatty liver disease (NAFLD), one of the most common liver diseases, is caused by the disruption of hepatic lipid homeostasis. It is associated with insulin resistance as seen in type 2 diabetes mellitus. Glucagon-like peptide-1 (GLP-1) is an incretin that increases insulin sensitivity and aids glucose metabolism. In recent *in vivo* and *in vitro* studies, GLP-1 presents a novel therapeutic approach against NAFLD by increasing fatty acid oxidation, decreasing lipogenesis, and improving hepatic glucose metabolism. In this report, we provide an overview of the role and mechanism of GLP-1 in relieving NAFLD.

Keywords: Fatty acid oxidation; Glucagon-like peptide 1; Non-alcoholic fatty liver disease

INTRODUCTION

Excess accumulation of fat in the liver is known as fatty liver disease. Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver diseases in the Western hemisphere, affecting 20% to 30% of the adult population [1]. Approximately 10% to 25% patients with NAFLD can progress to non-alcoholic steatohepatitis (NASH) and 10% to 15% patients with NASH develop hepatocellular carcinoma [2,3]. In addition, NAFLD is closely associated with metabolic syndrome, type 2 diabetes mellitus (T2DM) and cardiovascular morbidity and mortality. Despite these associated pathologies, there is still no specific treatment for NAFLD.

Glucagon-like peptide-1 (GLP-1) is an incretin secreted by L-cells in the small intestine in response to food intake [4]. The main roles of GLP-1 are stimulation of glucose-dependent insulin secretion, inhibition of postprandial glucagon release, delay of gastric emptying, and induction of pancreatic β -cell proliferation [5].

Once in circulation, GLP-1 has a short half-life (1 to 2 minutes) due to rapid degradation by the ubiquitous endogenous enzyme dipeptidyl peptidase-4 (DPP-4). To overcome this obstacle, GLP-1 receptor agonists that have increased resistance to DPP-4 (such as exenatide and liraglutide) or DPP-4 inhibitors (such as sitagliptin, vildagliptin, saxagliptin, alogliptin, and linagliptin) have been used in animal and human studies [6,7].

Recent studies have shown that exendin-4 could improve hepatic steatosis by modulation of lipid metabolism and hepatic insulin signaling in *ob/ob* mice and in human hepatocyte [8,9]. Additional studies have demonstrated that treatment with exendin-4 and liraglutide could reduce steatosis by enhancing autophagy. Treatment with exendin-4 and liraglutide leads to reduced endoplasmic reticulum (ER) stress-related apoptosis in human hepatocytes treated with fatty acids as well as in mice fed a high fat diet, respectively [10]. In this review, we present the pleiotropic effects of GLP-1 on reducing NAFLD.

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LIPID METABOLISM IN NORMAL AND NAFLD PATIENTS

The liver, as a key organ for lipid homeostasis, has roles in various aspects of energy metabolism. Hepatic lipid homeostasis is usually maintained through balance between the influx or production of fatty acids and their use for oxidation or secretion as very low density lipoprotein (VLDL) triglycerides [11-13]. Disruption of hepatic lipid homeostasis causes liver dysfunction, eventually leading to liver disease.

Hepatic steatosis is associated with the alteration of nuclear receptors, membrane transport proteins and cellular enzymes [14,15]. Sterol regulatory element binding protein 1c (SREBP-1c) transcription factor and peroxisome proliferator-activated receptor α (PPAR α) are essential regulators for steatosis in obese patients. Enhancement in the SREBP-1c/PPAR α ratio, an indication that lipogenesis is higher than fatty acid oxidation, was found in obese patients with hepatic steatosis [16]. Additional studies demonstrated that SREBP-1c *-/-* mice had impaired induction of hepatic genes coding for fatty acid biosynthesis (e.g., acetyl-CoA carboxylase, fatty acid synthase, and stearoyl-CoA desaturase) compared to wild type mice [17]. These results suggest that NAFLD is caused by both increased lipid storage (from circulating fatty acid uptake and *de novo* lipogenesis) and decreased lipid removal (via fatty acid oxidation or VLDL-triglycerides secretion).

GLP-1 SIGNALING IN NORMAL AND NAFLD PATIENTS

Pleiotropic effects of GLP-1 on glucose metabolism, appetite, weight, blood pressure, cardiovascular risk factors, cardiovascular function, and the central nervous system have been reported [18]. GLP-1 achieves its roles by binding to its specific receptor (GLP-1R) on human hepatocytes [9]. In addition, we recently reported that exendin-4 increases expression of GLP-1R in a dose-dependent manner in human hepatoma cell lines [19].

Several human and animal studies have demonstrated the therapeutic effects of GLP-1 receptor agonist in slowing the progression of NAFLD. Exenatide therapy decreased hepatic fat accumulation, insulin resistance, and the risk of cardiovascular disease in patient with T2DM [20] and also improved fatty acid β -oxidation and insulin sensitivity in the livers of rats on a high fat diet [21]. Hepatic gluconeogenesis and insu-

lin sensitivity were improved in *ob/ob* mice treated with recombinant adenovirus expressing GLP-1 (rAd-GLP-1) [22].

Protective effects of GLP-1 against hepatic steatosis were found in only diet-induced obese mice [23]. Sitagliptin, a DPP-4 inhibitor, showed a decrease in liver triglyceride content, expression of lipogenesis genes and gluconeogenesis in wild type mice [24]. Ultimately, exendin-4 may play role as a novel treatment of NAFLD via direct effect on hepatic lipid and glucose metabolism.

GLP-1 EFFECT ON FATTY ACID OXIDATION IN LIVER

In mammalian liver, fatty acids oxidation serves as a source for energy generation and occurs in both the mitochondria and the peroxisomes. Short and medium-chain fatty acids (SCFAs: <6 carbons long and MCFAs: 6 to 12 carbons long) are oxidized in the mitochondria, long-chain fatty acids (LCFAs: C12 to C20) are oxidized in both the mitochondria and peroxisomes, and very long-chain fatty acids (VLCFAs: >C20) are preferentially oxidized in the peroxisomes [25]. However, excess fatty acids can impair fatty acid oxidation by inhibiting the activities of enzymes involved in fatty acid oxidation [26]. PPAR α is a transcriptional factor regulating the expression of a number of genes involved in mitochondrial and peroxisomal fatty acid β -oxidation [27,28].

Exendin-4 significantly increases the expression of PPAR α and acyl-Coenzyme A oxidase (ACOX) mRNA in *ob/ob* mice [8]. Hepatocytes isolated from rats with NASH demonstrated reduced expression of hepatic PPAR α and its downstream target genes: ACOX and carnitine palmitoyltransferase 1A (CPT1A). ACOX is a rate-limiting enzyme involved in peroxisomal fatty acid β -oxidation and CPT1A is a key enzyme allowing the initial transport of fatty acids into mitochondria for β -oxidation. Expression of PPAR α , and subsequently ACOX and CPT1A, was improved by exendin-4 treatment [21].

GLP-1 EFFECT ON AMPK, NAMPT, AND SIRT SIGNALING

AMP-activated protein kinase (AMPK) and silent mating type information regulation 2 homolog (sirtuin, SIRT) 1 are metabolic sensors regulating energy homeostasis and various intracellular systems. These include fatty acid oxidation, lipogenesis, glucose uptake, gluconeogenesis, mitochondria biosynthe-

sis, and insulin sensitivity [29,30]. They are also involved in mediating the effect of adiponectin in inhibiting the accumulation of liver fat [31]. AMPK and SIRT1 are activated by an AMP and NAD⁺ dependent mechanism and acts through regulation of phosphorylation and deacetylation of their targets, respectively. PGC-1 α is a common target of the two metabolic sensors, and has been shown to have protective effects in patients with metabolic diseases [29].

Nampt/visfatin is a mammalian NAD⁺ biosynthetic enzyme that controls SIRT1 activity by mediating the conversion of nicotinamide (NAM) to NAD⁺ [30]. Activated SIRT1 enhances AMPK activity via an LKB1-dependent manner in human hepatocytes (Fig. 1A) [32]. However, the precise mechanism of interaction between AMPK and SIRT1 is still controversial and varies with tissue and condition. Cantó et al. [33] reported that AMPK regulates SIRT1 activity by increasing cellular NAD⁺ levels in mouse skeletal muscle (Fig. 1B).

Recent studies reported that GLP-1 increases the phosphorylation of AMPK in hepatocytes and reverses hepatic steatosis by stimulating fatty acid oxidation [8,31,32]. Moreover, we recently demonstrated that activating GLP-1 receptor by exendin-4 treatment enhances the expression of NAMPT, SIRT1,

and AMPK in mouse liver. SIRT1 inhibitor (NAM) leads to a decrease in phosphorylation of AMPK in human hepatocytes, indicating that SIRT1 stimulates AMPK (Fig. 2) [19]. Thus, GLP-1 could improve hepatic lipid metabolism by regulation of NAMPT/SIRT1/AMPK signaling.

GLP-1 EFFECT ON LIVER FUNCTION AND GLUCOSE METABOLISM IN HUMAN

The liver has a vast range functions in the body, including immunity against infection, synthesis of proteins and cholesterol, detoxification, glycogen storage, excretion of bile for fat digestion, and regulation of metabolism. However, insults such as alcohol, autoimmune malfunction, hereditary diseases, and metabolic diseases could result in liver dysfunction.

In a study investigating human hepatocytes treated with fatty acids, exenatide was shown to reduce fatty acid storage and improve hepatocyte viability and markers of autophagy [10]. A clinical trial evaluating the effects of exenatide in patients with T2DM over a period of 3 years revealed that treatment lead to significant improvements in hepatic markers, such as elevated liver enzymes, and other cardiovascular risk factors were significantly improved [34]. A case study exploring the efficacy of exenatide therapy in a 59-year-old NAFLD patient, reported a 73% reduction in hepatic fat content and significant improvements in liver enzymes 44 weeks post-treatment [20].

GLP-1 has recently been shown to increase hepatic insulin signaling and sensitivity [9]. A modified glucose clamp study demonstrated that exenatide reduces postprandial glucose by increasing the hepatic uptake of exogenous glucose [35]. Furthermore, GLP-1 in obese mice reduces hepatic gluconeogenesis by inhibiting phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) [22]. Park et al. [36] reported that exendin-4 treatment decreases hepatic glucose output at hyperinsulinemic states and promotes hepatic insulin signaling by potentiating tyrosine phosphorylation of the insulin receptor substrate-2 in 90% pancreatectomized diabetic rats fed high fat (40% energy from fat) diets.

CONCLUSION

Disruption of lipid metabolic homeostasis has been recognized as a major cause of NAFLD, which is associated with insulin resistance, T2DM, obesity, and cardiovascular disease. Although the incretin GLP-1 has been widely studied for its ef-

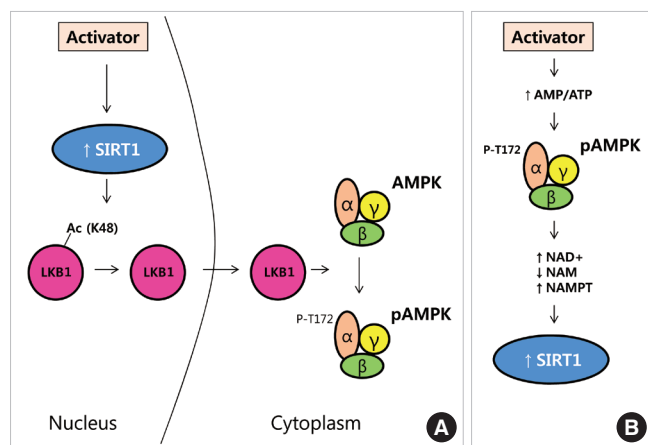


Fig. 1. Proposed regulatory mechanisms between silent mating type information regulation 2 homolog (SIRT1) and AMP-activated protein kinase (AMPK). (A) Activation of SIRT1 by activator leads to deacetylation of Lys48 residues on LKB1. LKB1 moves to the cytoplasm and then, phosphorylates and activates the AMPK. (B) The enhancement of AMP/ATP ratio stimulates AMPK activity. Activated AMPK regulates SIRT1, an NAD⁺-dependent protein deacetylase, by increasing NAD⁺ levels. NAM, nicotinamide; NAMPT, nicotinamide phosphoribosyltransferase (Adapted from Cantó et al., *Curr Opin Lipidol* 2009;20:98-105 [29]).

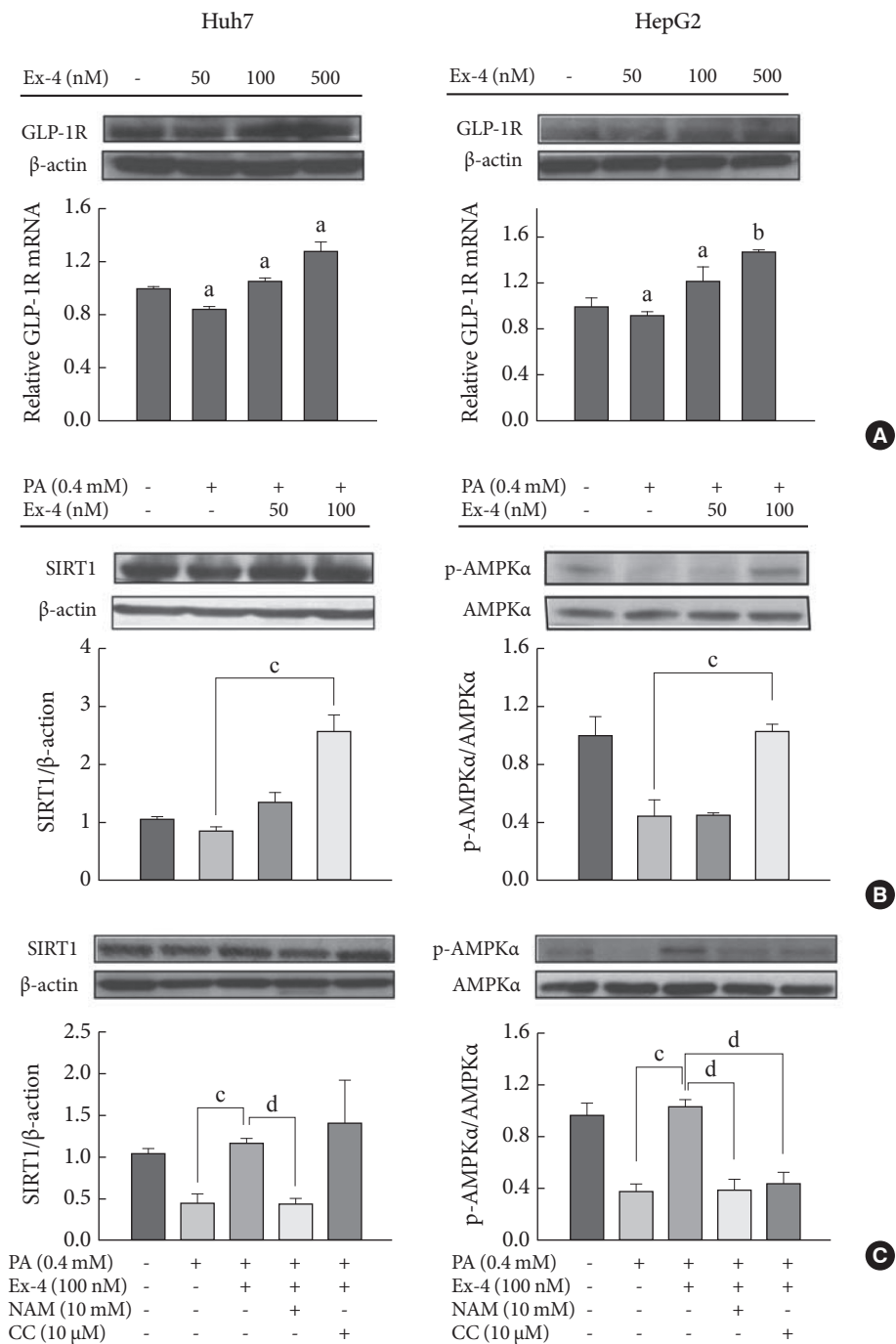


Fig. 2. Regulation of glucagon-like peptide-1 receptor (GLP-1R), SIRT1 and AMPK by exendin-4 (Ex-4) in HepG2 and Huh7 cells. (A) Cells were treated with 50, 100, or 500 nM Ex-4 for 24 hours. GLP-1R and β-actin were measured by western blot and real-time PCR. GLP-1R was normalized to β-actin. (B) Cells given 0.4 mM palmitic acid (PA) were treated with either vehicle or 50 to 100 nM Ex-4 for 24 hours. (C) Cells given 0.4 mM palmitic acid were treated with 100 nM Ex-4 in the absence or presence of 10 mM nicotinamide (NAM) or 10 μM compound C (CC) for 24 hours. (B, C) SIRT1, phosphorylated AMPKα at threonine 172, AMPK, and β-actin were measured by Western blot in HepG2 cells. SIRT1 and phosphorylated AMPKα were normalized to the β-actin and total AMPKα of each sample, respectively. ^a*P*<0.05, ^b*P*<0.01 compared with control, ^c*P*<0.05, compared with PA, and ^d*P*<0.05 compared with Ex-4 (Adapted from Lee J, et al. PLoS One 2012;7:e31394 [19]).

fects on stimulation of glucose-dependent insulin secretion in pancreatic β -cell, GLP-1 receptor agonists have other important effects in peripheral tissues. Treatment with GLP-1 mimetics improves hepatic insulin signaling in NAFLD animal models, and these effects could be invoked by direct stimulation of hepatic GLP-1 receptor. GLP-1 is expected to have pleiotropic effects on the liver, and more research is needed to explore its mechanism.

CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

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