When GLP-1 hits the liver: a novel approach for insulin resistance and NASH

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NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) encompasses a spectrum ranging from simple steatosis to steatohepatitis (NASH), increasing fibrosis and eventually, cirrhosis (22). Importantly, NASH accompanied by fibrosis and severe inflammation is the most relevant predictor for disease progression to cirrhosis and hepatocellular carcinoma (21). NAFLD is tightly associated with obesity, insulin resistance (IR), and overt type 2 diabetes (T2D) and represents a manifestation and driver of the metabolic syndrome (5, 7, 20). While the best therapy to improve insulin sensitivity is diet combined with exercise, these are difficult to achieve for the majority of patients, requiring at least adjunctive pharmacological therapies (5, 20). A novel class of pharmacological agents that appear to be devoid of unwanted side effects, including weight gain, are drugs that increase the action of glucagon-like peptide-1 (GLP-1) directly (GLP-1 mimetics, such as exenatide) or indirectly via inhibition of dipeptidyl peptidase-4 (DPP-4), an enzyme that is ubiquitously expressed on the cell surface and that rapidly degrades GLP-1 to an inactive peptide. Exenatide and DPP-4 inhibitors have been widely validated to treat T2D, and their potential to effectively treat NAFLD and NASH attracts increasing attention (8, 22, 23). Moreover, experimental models that mimic human NAFLD and especially NASH are constantly improved to serve as a preclinical platform to evaluate novel therapeutic strategies. Mouse models of NAFLD are based on genetic mutations, such as ob/ob or db/db mice, or diet, such as the methionine/choline-deficient (MCD) diet (1). However, none of these fully reflect the phenotype of human NAFLD and especially NASH. The more recent models that exploit either a high-fat/trans-fat-enriched and high fructose diet (28) or a high-fat diet with low-dose streptozotocin (which induces mild insulin deficiency not matching insulin demand) (14) much better resemble human NASH pathogenesis and histopathology including fibrosis.

The study by Trevaskis et al. (29) examined another modification of these novel NASH models, i.e., mice deficient in leptin-signaling (ob/ob) or their wild-type controls fed a high trans-fat (HTF), high fructose, and high cholesterol diet. The authors also assessed the beneficial effect the GLP-1R agonist AC3174 in this model. They found that ob/ob mice fed this diet developed more severe pathophysiologic features of fibrotic NASH than wild-type mice. This is unexpected, since intact leptin signaling is considered important for the activation of fibrogenic hepatocyte stellate cells and myofibroblasts (here jointly termed HSC) (10), and leptin deficient ob/ob mice are resistant to hepatic fibrosis. Thus leptin has been shown to induce TNF-α and IL-6 in the regenerative response to hepatic injury (12) and to promote HSC activation via hedgehog signaling (4). Accordingly, ob/ob mice in which fibrogenesis was induced with thioacetamide (TAA), carbon tetrachloride, or an MCD diet, displayed an attenuated hepatic profibrogenic TGF-β and procollagen type I mRNA expression, but these transcript levels were restored to increased levels after injection of leptin (11). Similarly, a leptin antagonist ameliorated TAA-induced liver fibrosis (6).

However, hepatic fibrogenesis can be triggered and maintained by several chemokines and cytokines produced by immune and other cells, and does not solely rely on leptin signaling (18). In the present model, fibrogenesis could have been accelerated by a more excessive fat accumulation due to leptin deficiency and an unopposed generation of oxidative stress, which is considered a central driver of hepatocyte apoptosis and fatty liver inflammation (20). Notably, the present diet contained HTF, an important contributor to the development of hepatic steatosis and steatohepatitis (28), and high cholesterol (2%). The addition of 0.15–2% cholesterol to a steatogenic diet has previously been shown to exacerbate hepatic free cholesterol accumulation, which leads to severe steatosis, hepatocyte injury, macrophage recruitment, and liver fibrosis (3, 24, 27). Recently, Teratani et al. (27) provided a functional link between high dietary cholesterol and enhanced inflammation and fibrosis, demonstrating that in BL6 mice dietary cholesterol (1%) upregulated Toll-like receptor 4 on HSC, which suppressed the inhibitory TGF-β pseudoreceptor Bambi, leading to increased TGF-β signaling, HSC activation, and fibrosis. Notably, the postulated mechanism was Kupffer cell- and leptin-independent. This may explain the development of fibrosis in leptin-deficient ob/ob mice by the HTF, high-fructose and high-cholesterol diet in the present paper. Taken together, reflecting the rare prevalence of leptin deficiency in man and the multiple and often divergent activities of leptin signaling in obesity and NASH, the relevance of ob/ob mice as preclinical model for human NASH is limited, and a model based on life style changes and metabolic alterations, such as a steatogenic, and, if possible, also fibrogenic diet is desirable.

The model described by Trevaskis et al. (29) appears to come a step closer to such a humanized NASH model. However, a more rigorous fibrosis assessment would have been welcome, including hydroxyproline determination as a quantitative measure of collagen accumulation, picrosirius red staining for collagen, collagen morphometry on representative liver sections, and a broader quantitative PCR analysis of transcripts related to fibrogenesis, and fibrolysis, as well as more biochemical, histological, and transcript markers of lipid metabolism and inflammation (2, 17).

Another novel aspect of the present paper is the effect of AC3174, a GLP-1 receptor (GLP-1R) agonist, in the novel NASH model applied to wild-type, ob/ob and GLP-1 receptor deficient (GLP-1RKO) mice. AC3174 is a peptide analogue of the GLP-1R agonist exenatide, with a leucine for methionine at position 14. GLP-1 lowers plasma glucose (9), mainly via its insulinotropic activity (29) coupled to improved insulin sensi...
tivity (13). The GLP-1/GLP-1R system is complex and little studied in liver, and agonistic drugs may affect NAFL or NASH either by addressing the liver directly or indirectly (Fig. 1). AC3174 treatment significantly attenuated weight gain and especially the liver-to-body weight ratio, both in wild-type and ob/ob mice, but to a minor degree also in mice on a low-fat diet and in GLP-1RKO mice, indicating some unexplained off-target effect of this compound. This was accompanied by a significant though relatively modest effect on ALT and hepatic triglycerides. Collagen, as assessed by collagen Western blot analysis from liver extracts, a method that is unreliable, was highly significantly suppressed in AC3174-treated mice. The improvement of hepatic steatosis and presumably inflammation is likely due to activation of GLP-1R on hepatocytes, since others showed that exenatide stimulates hepatocyte expression of genes, such as PPAR-α and PPAR-γ, that improve hepatic fatty acid oxidation, lipid export, and insulin sensitivity (8, 25).

Overall, the authors mainly used weight-based readouts in a limited number of animals, to assess the effect of AC3174 in fibrotic NASH, which requires confirmation, e.g., by use of a comparator drug, such as exenatide, and more on-target molecular biomarkers. Furthermore, the use of tissue (or cell)-specific conditional KO mice would provide more information on the utility of GLP-1R agonism in fatty liver disease. In addition to the already described beneficial aspects of exenatide on fibrogenesis (15, 26), GLP-1R agonists may also or predominantly modulate activated cholangiocytes (cells resembling hepatic progenitors) that are implicated in the progression of NASH as well as other liver diseases toward cirrhosis (16, 17, 19). Thus GLP-1 receptors are highly expressed on these cells, and GLP-1R activation blocks their Bax-induced mitochondrial apoptosis (15). Furthermore, GLP-1R agonists may protect the liver from apoptotic signals from hepatocytes, signals that can induce the production of hedgehog ligand, a

Fig 1. Action of glucagon-like peptide-1 (GLP-1) and expression of GLP-1 receptors (GLP-1Rs). GLP-1 is induced postprandially and mainly secreted from intestinal L-cells and the neurons in the caudal regions of the nucleus of the solitary tract (NTS). The NTS then releases GLP-1 into the hypothalamus to control food intake. In the pancreas, activation of GLP-1R by intestinal GLP-1 stimulates insulin secretion, increases insulin sensitivity, and inhibits glucagon release. Elevated insulin combined with improved insulin signaling in glucose-regulated organs such as liver, adipose tissue, and muscle enhances glucose uptake and metabolism, which in turn reduces hepatic gluconeogenesis and improves steatosis. GLP-1 also dampens inflammatory immune responses, in part, by directly addressing immune cells, which finally ameliorates systemic (metabolic) inflammation and steatohepatitis. However, GLP-1 secreted by L-cells is rapidly inactivated by dipeptidyl peptidase-4 (DPP-4), which is ubiquitously expressed on most blood and tissue cells, underlining the utility of proteolytically stable GLP-1 mimetics or DPP-4 inhibitors to treat type 2 diabetes, the metabolic syndrome and likely nonalcoholic steatohepatitis (NASH). NAFLD, nonalcoholic fatty liver disease; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; RAGE, receptor of advanced glycation end products; MCP-1, monocyte chemotactic protein-1; PPAR, peroxisome proliferator-activated receptor; GLUT 2, glucose transporter 2; TLR-4, toll-like receptor-4, SOCS-3, suppressor of cytokine signaling-3; BDL, bile duct ligation; FAP, fibroblast activation protein; FFA, free fatty acids, EROS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase.

AJP-Gastrointest Liver Physiol • doi:10.1152/ajpgi.00078.2012 • www.ajpgi.org
mediator that can elicit and maintain HSC activation and fibrogenesis (26). Finally, GLP-1R is also found at nerve terminals in the portal vein, a site close to the origin of the major GLP-1 secretion site in the small intestine, and its inhibition was shown to promote insulin resistance (30).

Overall, the study by Trevaskis et al. (29) can be commented on, as it addresses two topics that are highly relevant in current hepatological research: 1) a further improved dietary NASH model (addition of cholesterol to the diet); and 2) a novel therapeutic approach (GLP-1 agonism). However, some aspects need to be studied more in depth, e.g., using a more rigorous assessment of inflammation and fibrosis, preferably in wild-type mice, to assess the full potential of GLP-1 agonistic treatment in NAFLD, NASH, and the metabolic syndrome.

GRANTS
D. Schuppan receives funding from European Union, the State of Rhineland-Palatinate, and the German Ministry of Education and Research.

DISCLOSURES
No conflicts of interest are declared by the author(s).

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