A 25-Year-Old Woman with Type 2 Diabetes and Liver Disease

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Key Words
Glucagon-like peptide-1 receptor · Non-alcoholic fatty liver disease · Non-alcoholic steatohepatitis · Type 2 diabetes

Abstract
A 25-year-old female nurse was referred to our diabetes outpatient clinic with poorly controlled type 2 diabetes, obesity and elevated liver function tests (LFTs). Following a liver biopsy she was diagnosed with non-alcoholic steatohepatitis (NASH) and liver fibrosis. Treatment with subcutaneous injections of the glucagon-like peptide-1 receptor (GLP-1R) agonist liraglutide was initiated. After 46 weeks of treatment the patient had lost 16 kg, glycemic control was excellent and LFTs had normalized. Repeat liver biopsy and ultrasound showed reduction in hepatic fat content and inflammatory cells. The biopsy no longer fulfilled the criteria for NASH. The liver biopsies did not express hepatic GLP-1Rs using quantitative polymerase chain reaction. Our case suggests that liraglutide may benefit patients with NASH.

Introduction
Elevated liver function tests (LFTs) in obese patients either with or without diabetes are a common clinical problem. The most frequent cause is non-alcoholic fatty liver disease (NAFLD), which represents both a diagnostic and therapeutic challenge. NAFLD is defined as a total liver fat content of more than 5%. The diagnostic gold standard is histology. The...
diagnostic criteria also include absence of significant alcohol intake (women: >20 g per day; men: >30 g per day) and use of steatogenic drugs (e.g. amiodarone and glucocorticoids) [1]. NAFLD is associated with an increase in mortality due to cardiovascular disease, irrespective of diabetes [2]. The prevalence is closely associated with obesity and type 2 diabetes. Up to 70% of patients with type 2 diabetes have NAFLD. The underlying pathophysiology of the development from simple steatosis to non-alcoholic steatohepatitis (NASH) is not fully understood. Excess hepatic fat infiltration seems to cause lipid-induced mitochondrial dysfunction and oxidative stress (lipotoxicity), which result in inflammation and fibrosis [3]. Studies have shown that NASH is associated with cirrhosis and hepatocellular carcinoma and is predicted to be the leading cause of liver transplantation by 2020 in the US [1].

Weight loss is an effective treatment of NASH, but is difficult to maintain for a majority of patients [2]. Current medical interventions are limited and seem to be associated with risk of side-effects. We present a case of severe liver disease in a young woman with type 2 diabetes. She was treated with the glucagon-like peptide-1 receptor (GLP-1R) agonist liraglutide with remarkable results on glycemic control, LFTs and liver histology.

Case Report

A 25-year-old female nurse was referred to our diabetes outpatient clinic with poorly controlled type 2 diabetes, obesity and elevated LFTs. Her general practitioner had initiated treatment with metformin (1,000 mg twice-daily) and simvastatin (40 mg once-daily), but compliance was limited. At her first visit to our outpatient clinic her body weight was 90 kg and her body mass index 32.6 kg/m². She was asymptomatic and clinical examination, fundus photography, filament testing and albumin/creatinine ratio revealed no signs of complications related to type 2 diabetes. Blood samples showed a glycated hemoglobin A₁c (HbA₁c) of 8.9% (74 mmol/mol) and a fasting plasma glucose of 7.3 mmol/l. LFTs showed an alanine aminotransferase (ALT) of 196 U/l (reference range 10–45 U/l), an aspartate aminotransferase (AST) of 132 U/l (reference range 15–35 U/l) and an alkaline phosphatase (ALP) of 127 U/l (reference range 35–105 U/l). Lipids were elevated: total cholesterol 4.5 mmol/l, high-density lipoprotein 0.84 mmol/l, low-density lipoprotein 2.4 mmol/l and triglycerides 2.86 mmol/l. Bilirubin, international normalized ratio and albumin were within normal ranges. The patient had no history of alcohol abuse nor did she take any herbal drug. Abdominal ultrasound revealed increased echogenicity and poor visualization of the intrahepatic vessel walls, suggesting diffuse hepatic steatosis.

Statins were discontinued and the patient was strongly encouraged to be compliant with her metformin treatment (1,000 mg twice-daily). In addition neutral protamine Hagedorn insulin was initiated. The daily dose of basal insulin was gradually increased to 30 IU once daily.

After 8 weeks on metformin and insulin, HbA₁c had markedly improved to 6.3%, but LFTs except for ALP remained elevated (ALT 133 U/l, AST 76 U/l, ALP 69 U/l). The patient was then scheduled for a liver biopsy, which showed hepatic fat infiltration involving more than 66% of hepatocytes, ballooned hepatocytes, lobular inflammation as well as pericellular and periportal fibrosis. The histological diagnosis was NASH with a NAFLD activity score of 5 (score range 0–8) and a fibrosis score of 2 (score range 0–4) (fig. 1a). Treatment with subcutaneous injections of the GLP-1R agonist liraglutide was initiated. The initial dose was 0.6 mg once daily subcutaneously. The dose was increased with weekly increments of 0.6–1.8 mg once daily during the following weeks. Liraglutide was well tolerated with no side
effects such as nausea or vomiting. Insulin was gradually reduced and discontinued after 7 weeks.

After 46 weeks of treatment with liraglutide, total weight loss was 16 kg, LFTs were in the lower normal range (ALT 29 U/l, AST 25 U/l, ALP 67 U/l), glycemic control was excellent (HbA1c 5.6%) and the lipid profile was normalized without statin treatment. Repeat abdominal ultrasound showed diminished echogenicity, suggesting an overall reduction in steatosis. A repeated liver biopsy confirmed decreased hepatic fat infiltration (involving 40–50% of hepatocytes), no ballooned hepatocytes and only distinct lobular inflammation (fig. 1b). The histology was no longer consistent with NASH, but pericellular and periportal fibrosis were still present (NAFLD activity score 3, fibrosis score 2).

To investigate the expression of hepatic GLP-1Rs in the present case, the baseline and the post-treatment liver biopsy were compared to liver tissue from another patient with type 2 diabetes but without steatosis, using quantitative polymerase chain reaction. No biopsies showed expression of GLP-1Rs.

Discussion

The prevalence of obesity and type 2 diabetes is increasing, and obese patients with type 2 diabetes and elevated LFTs are regularly referred to outpatient clinics. This case illustrates the necessity for both diabetologists and hepatologist to be aware, and together take part in the diagnosis and treatment of NAFLD.

Abdominal ultrasound is recommended as a first-line evaluation if LFTs are elevated, but has high inter-observer variability and hepatic fat infiltration fat must exceed 20–30% to be detected. Magnetic resonance spectroscopy is expensive and not widely available, but can identify >5.5% hepatic fat infiltration. Transient elastography enhanced with controlled attenuation parameter can quantify liver steatosis, but has not been validated in large trials [4]. Imaging techniques cannot distinguish between simple steatosis and NASH. The final diagnosis requires histological assessment of a representative liver biopsy. The NAFLD fibrosis score can help identify patients with a high risk of NASH and fibrosis and, thus, eligible for liver biopsy. The score is based on body mass index, age, presence of diabetes and blood levels of ALT, AST, platelets and albumin [5]. If coexisting liver diseases to NASH are suspected, liver biopsy should always be considered [2].

The distinction between simple steatosis and NASH is of both prognostic and therapeutic value. The natural history of simple steatosis is benign from a 'liver standpoint' and should be managed by treating comorbidities such as obesity, hyperlipidemia and insulin resistance. On the other hand, NASH per se increases the risk of liver-related death [6] and management should include liver specific treatment(s). In patients with NASH the primary goal is to reverse oxidative stress and reduce hepatic fat infiltration, thereby reducing insulin resistance and further reverse hepatic inflammation [2]. Weight loss and glycemic control are effective measures in NASH, but can be difficult to achieve and maintain for a majority of patients. Nevertheless, if weight loss can be obtained it improves insulin sensitivity and reduces LFTs. A weight loss of 3–5% improves steatosis and a 10% decrease in body weight reduces hepatic inflammation. In line with these considerations, bariatric surgery should be considered in patients who are overweight and have type 2 diabetes as well as NASH [7].

Metformin increases hepatic and muscular insulin sensitivity, but does not improve LFTs or liver histology in NASH. Pioglitazones reduce hepatic steatosis but are rarely used due to adverse events including heart failure, bladder cancer and loss of bone density [2]. One trial suggests that vitamin E 800 IU per day improves LFTs, steatosis, ballooning and
inflammation, and vitamin E is recommended for NASH in non-diabetic subjects. However, emerging evidence suggests that vitamin E may increase mortality [2].

The use of GLP-1R agonists for patients with type 2 diabetes is increasing. These drugs are based on the incretin hormone GLP-1, which is released from enteroendocrine cells in response to food ingestion. Native GLP-1 acts via the GLP-1R (a G protein-coupled receptor expressed in several tissues) and plays an essential role in the maintenance of normal glucose homeostasis and regulation of appetite and food intake. The effects of the native hormone have been exploited through the development of stable GLP-1R agonists. These drugs have sustained effects on glucose levels, increase insulin secretion and reduce glucagon secretion, satiety, food intake and body weight [8]. Furthermore, a recent post hoc analysis concluded that liraglutide was well tolerated and safe to use in patients with type 2 diabetes and elevated LFTs [9]. In rodents, GLP-1R agonists reduce hepatic steatosis by suppressing enzymes involved in hepatic lipogenesis through activation of the 5′ adenosine monophosphate-activated protein kinase, and have been suggested to reduce hepatic expression of pro-inflammatory mediators [10]. Nevertheless, initial observations of the GLP-1R on human hepatocytes [11] have not been confirmed in subsequent studies [12, 13].

As previously described, no liver biopsies from this case had expression of GLP-1R. The patient in this report showed remarkable results after 46 weeks of treatment with a GLP-1R agonist. She lost 16 kg of body weight (from a baseline of 90 kg) and achieved normalized LFTs and lipid profile. Her hepatic fat infiltration was reduced by ~30%, a decreased number of inflammatory cells were observed and, thus, she no longer fulfilled the criteria for NASH. Since no GLP-1Rs were found in the liver tissue, these improvements may be explained by indirect results of glucose metabolic improvement and weight loss induced by treatment with the GLP-1R agonist. In spite of the fact that we were unable to identify GLP-1Rs in liver tissue in the present case, some evidence suggests that GLP-1R agonists may have direct effects on hepatic steatosis (in vitro models) [10, 11]. Furthermore, one other paper has investigated the effect of GLP-1R agonist on liver histology in NAFLD. Kenny et al. [14] presented a case series with eight biopsy-proven NAFLD patients who received exenatide 10 mg twice daily for 26 weeks. Repeat liver biopsies showed improved NAFLD activity scores of 1–2 in four patients, but no changed in fibrosis score. The patients also lost body weight and glycemic control improved. Cuthbertson et al. [15] treated 19 patients with exenatide 10 mg twice-daily and 6 patients with liraglutide 1.2 mg once daily for 6 month. Patients had a 42% relative reduction in intrahepatic lipid content assessed by magnetic resonance spectroscopy, independent of body weight loss but in correlation with a decrease in HbA1c. However, the study was open-labeled, without a control group, and did not asses liver histology. Thus, there is some evidence suggesting that there may be a direct effect of GLP-1R agonists on hepatocytes, but this case does not allow for any conclusion regarding the potential direct or indirect effect of GLP-1 on the liver.

Options for the treatment of NASH are limited and hold risk of severe side effects. In this case, the GLP-1R agonist liraglutide was well tolerated and markedly improved LFTs and histology in this patient with type 2 diabetes and NASH. Randomized controlled trials are needed to evaluate whether the present findings hold promise for the treatment of NASH.

**Disclosure Statement**

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References

1. Musso G, Gambino R, Cassader M, Pagano G: Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. Ann Med 2011;43:647–649.

2. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ: The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology 2012;55:2005–2023.

3. Lomonaco R, Sunny NE, Bril F, Cusi K: Nonalcoholic fatty liver disease: current issues and novel treatment approaches. Drugs 2013;73:1–14.

4. Festi D, Schiumerini R, Marzi L, Di Biase AR, Mandolesi D, Montrone L, Sciolli E, Bonato G, Marchesini-Reggiani G, Colecchia A: Review article: the diagnosis of non-alcoholic fatty liver disease – availability and accuracy of non-invasive methods. Aliment Pharmacol Ther 2013;37:392–400.

5. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bika JP, Lindor K, Sanderson SD, Lenzi M, Adams LA, Kench J, Therneau TM, Day CP: The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 2007;45:846–854.

6. Farrell GC, van Rooyen D, Gan L, Chitturi S: NASH is an inflammatory disorder: pathogenic, prognostic and therapeutic implications. Gut Liver 2012;6:149–171.

7. Chavez-Tapia NC, Tellez-Avila FL, Barrientos-Gutierrez T, Mendez-Sanchez N, Lizardi-Gervera J, Uribe M: Bariatric surgery for non-alcoholic steatohepatitis in obese patients. Cochrane Database Syst Rev 2010;1:CD007340.

8. Vilsbøll T, Christensen M, Junker AE, Knop FK, Gluud LL: Effects of glucagon-like peptide-1 receptor agonists on weight loss: systematic review and meta-analyses of randomised controlled trials. BMJ 2012;344:e7771.

9. Armstrong MJ, Houlihan DD, Rowe IA, Clausen WH, Elbrønd B, Gough SC, Tomlinson JW, Newcombe PN: Safety and efficacy of liraglutide in patients with type 2 diabetes and elevated liver enzymes: individual patient data meta-analysis of the LEAD program. Aliment Pharmacol Ther 2013;37:234–242.

10. Ben-Shlomo S, Zibel I, Shnoll M, Shlomai A, Chepurko E, Halpern Z, Barzilai N, Oren R, Fishman S: Glucagon-like peptide-1 reduces hepatic lipogenesis via activation of AMP-activated protein kinase. J Hepatol 2011;54:1214–1223.

11. Gupta NA, Mells J, Dunham RM, Grakoui A, Handy J, Saxena NK, Anania FA: 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 2010;51:1584–1592.

12. Panjwani N, Mulvihill EE, Longuet C, Yusta B, Campbell JE, Brown TJ, Streutker C, Holland D, Cao X, Baggio LL, Drucker DJ: GLP-1 receptor activation indirectly reduces hepatic lipid accumulation but does not attenuate development of atherosclerosis in diabetic male ApoE(-/-) mice. Endocrinology 2013;154:127–139.

13. Pyke C, Heller RS, Kirk RK, Ørskov C, Reedtz-Runge S, Kaastrup P, Hvelplund A, Bardram L, Calatayud D, Knudsen LB: GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. Endocrinology 2014;155:1280–1290.

14. Kenny PR, Brady DE, Torres DM, Raguzzino L, Chalasani N, Harrison SA: Exenatide in the treatment of patients with non-alcoholic steatohepatitis: a case series. Am J Gastroenterol 2010;105:2707–2709.

15. Cuthbertson DJ, Irvin A, Gardner CJ, Daousi C, Purewal T, Furlong N, Goenka N, Thomas EL, Adams VL, Pushpakom SP, Firoz Mohamed M, Kemp GF: Improved glycaemia correlates with liver fat reduction in obese, type 2 diabetes, patients given glucagon-like peptide-1 (GLP-1) receptor agonists. PLoS One 2012;7:e50117.
Fig. 1. a Microscopic view of liver tissue (hematoxylin-eosin, ×100). Liver tissue with hepatic fat infiltration including >66% of hepatocytes, ballooning cells and lobular inflammation (NAFLD activity score 5) consistent with NASH. Pericellular and periportal fibrosis (fibrosis score 2). b Microscopic view of liver tissue (hematoxylin-eosin, ×100). Liver tissue with hepatic fat infiltration including 40–50% of hepatocytes, sparse lobular inflammation and no ballooning cells (NAFLD activity score 2) consistent with simple steatosis. Pericellular and periportal fibrosis (fibrosis score 2).