Research Article

JOURNAL OF HEPATOLOGY

Glucagon-like peptide 1 decreases lipotoxicity in non-alcoholic steatohepatitis

Matthew J. Armstrong[1,*], Diana Hull[1], Kathy Guo[1], Darren Barton[2], Jonathan M. Hazlehurst[3], Laura L. Gathercole[3], Maryam Nasiri[3], Jinglei Yu[4], Stephen C. Gough[5], Philip N. Newsome[1,†], Jeremy W. Tomlinson[5,*,†]

[1]*NIHR Liver Biomedical Research Unit and Centre for Liver Research, University of Birmingham, Birmingham, UK;* [2]*CRUK Clinical Trials Unit, University of Birmingham, Birmingham, UK;* [3]*Centre for Endocrinology, Diabetes and Metabolism, Institute of Biomedical Research, School of Clinical and Experimental Medicine, University of Birmingham, Edgbaston, Birmingham, UK;* [4]*School of Sport, Exercise & Rehabilitation Sciences, University of Birmingham, Birmingham, UK;* [5]*Oxford Centre for Diabetes, Endocrinology and Metabolism, and NIHR Oxford Biomedical Research Centre, University of Oxford, Churchill Hospital, Oxford, UK*

See Editorial, pages 262–264

Background & Aims: Insulin resistance and lipotoxicity are pathognomonic in non-alcoholic steatohepatitis (NASH). Glucagon-like peptide-1 (GLP-1) analogues are licensed for type 2 diabetes, but no prospective experimental data exists in NASH. This study determined the effect of a long-acting GLP-1 analogue, liraglutide, on organ-specific insulin sensitivity, hepatic lipid handling and adipose dysfunction in biopsy-proven NASH.

Methods: Fourteen patients were randomised to 1.8 mg liraglutide or placebo for 12-weeks of the mechanistic component of a double-blind, randomised, placebo-controlled trial (ClinicalTrials.gov-NCT01237119). Patients underwent paired hyperinsulinaemic euglycaemic clamps, stable isotope tracers, adipose microdialysis and serum adipocytokine/metabolic profiling. *In vitro* isotope experiments on lipid flux were performed on primary human hepatocytes.

Results: Liraglutide reduced BMI (−1.9 *vs.* +0.04 kg/m^2; p <0.001), HbA1c (−0.3 *vs.* +0.3%; p <0.01), cholesterol-LDL (−0.7 *vs.* +0.05 mmol/L; p <0.01), ALT (−54 *vs.* −4.0 IU/L; p <0.01) and serum leptin, adiponectin, and CCL-2 (all p <0.05). Liraglutide increased hepatic insulin sensitivity (−9.36 *vs.* −2.54% suppression of hepatic endogenous glucose production with low-dose insulin; p <0.05). Liraglutide increased adipose tissue insulin sensitivity enhancing the ability of insulin to suppress lipolysis both globally (−24.9 *vs.* +54.8 pmol/L insulin required to ½ maximally suppress serum non-esterified fatty acids; p <0.05), and specifically within subcutaneous adipose tissue (p <0.05). In addition, liraglutide decreased hepatic *de novo* lipogenesis *in vivo* (−1.26 *vs.* +1.30%; p <0.05); a finding endorsed by the effect of GLP-1 receptor agonist on primary human hepatocytes (24.6% decrease in lipogenesis *vs.* untreated controls; p <0.01).

Conclusions: Liraglutide reduces metabolic dysfunction, insulin resistance and lipotoxicity in the key metabolic organs in the pathogenesis of NASH. Liraglutide may offer the potential for a disease-modifying intervention in NASH.

Introduction

Non-alcoholic steatohepatitis (NASH) incurs a significantly increased risk of both liver- and cardiovascular disease (CVD)-related morbidity and mortality [1], yet, there remains a lack of safe and efficacious pharmacological treatments [2].

Insulin resistance (IR) in both liver and adipose tissue is believed to be a key driver in the pathogenesis of NASH [3]. Detailed metabolic studies, using 'gold-standard' euglycaemic clamps and/or stable isotope tracers, have demonstrated that patients with NASH have severe adipose IR, alongside increased hepatic IR [4,5] and *de novo* lipogenesis (DNL) [6,7]. Even though collectively these contribute to excess lipid accumulation in the liver, it is widely believed that the overspill of non-esterified fatty acids (NEFA) and release of triglyceride-derived toxic metabolites from adipose tissue lipolysis, form the primary lipotoxic insult in the pathogenesis of NASH and its extrahepatic complications including increased CVD-morbidity and mortality [1,8]. In addition to driving intrinsic hepatic IR and inflammation, hepatic lipotoxicity is thought to further fuel the circulating

Keywords: Glucagon-like peptide 1; Non-alcoholic fatty liver; Insulin sensitivity; Adipose tissue; Lipolysis.
Received 1 June 2015; received in revised form 25 August 2015; accepted 28 August 2015; available online 21 September 2015
☆ DOI of original article: http://dx.doi.org/10.1016/j.jhep.2015.11.031.
* Corresponding authors. Addresses: NIHR Centre for Liver Research, 5th Floor IBR, University of Birmingham, Birmingham B15 2TH, UK (M.J. Armstrong). Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Churchill Hospital, Headington, Oxford OX3 7LJ, UK (J.W. Tomlinson).
E-mail addresses: mattyarm2010@googlemail.com (M.J. Armstrong), jeremy.tomlinson@ocdem.ox.ac.uk (J.W. Tomlinson).
† These authors share senior authorship.

pro-inflammatory milieu and IR status in NASH, which in turn contributes to the cycle of worsening adipose dysfunction and lipolysis [8]. Therefore, identifying pharmaceutical options that can target both liver and adipose IR as well as reduce hepatic exposure to the effects of lipotoxic metabolites may provide the best strategy to halt the progression of NASH and its clinical consequences.

Glucagon-like peptide-1 (GLP-1) analogues have been shown to improve glycaemic control, weight loss and in retrospective studies, liver enzymes in patients with type 2 diabetes [9], making them an attractive therapeutic option in NASH. Recent animal studies of NASH have supported these findings by demonstrating improvements in hepatic steatosis following GLP-1 therapy [10–13], which in some cases was accompanied by reductions in oxidative stress [11,14,15] and fibrosis [16]. In particular, using euglycaemic clamp techniques, murine studies have shown that chronic GLP-1 administration improves insulin sensitivity and reduces hepatic glucose production [13,17,18]. Similar findings have been reported with short-durations of GLP-1 treatment ranging from single infusions up to 6-weeks in healthy volunteers [19] and in patients with type 2 diabetes [20]. Importantly, no such studies have been performed in the context of patients with NASH. The effects of GLP-1 on muscle insulin sensitivity in humans have been inconsistent albeit with most studies showing improvements in glucose disposal [19,21,22]. To date, there are no data that have examined the impact of GLP-1 treatment on human adipose insulin action *in vivo*.

The effect of GLP-1 analogues on metabolic dysfunction, most notably tissue-specific IR and hepatic DNL, in patients with biopsy-confirmed NASH is currently unknown. We therefore incorporated functional measures of lipid and carbohydrate flux at baseline and 12 weeks into the treatment regimen of our phase II, double-blinded, placebo-controlled randomised clinical trial, entitled 'Liraglutide Efficacy and Action in NASH (LEAN) [23]'. Our aims were to determine the effect of 12-weeks treatment of liraglutide on tissue-specific IR (adipose, muscle, liver), hepatic DNL and adipose tissue function in patients with biopsy-defined NASH. In addition, we performed isotope tracer studies on primary cultures of human hepatocytes to establish if GLP-1 analogues have direct lipid-lowering effects, independent of changes in weight and glycaemic control as reported *in vivo*.

Methods and materials

Clinical study

The full clinical protocol of the LEAN trial (clinicaltrials.gov NCT01237119) has previously been described [23]. This was an investigator-initiated/led study, with charitable (Wellcome Trust, NIHR) and pharmaceutical (Novo Nordisk Ltd) support, with the University of Birmingham (UK) acting as the sole sponsor. The National Research Ethics Service (NRES) East Midlands – Northampton committee (UK) and the Medicines and Healthcare products Regulatory Agency (MHRA) approved all versions of the study protocol. All adult subjects gave informed written consent prior to participation.

Study participants

Consecutive adult patients from the Queen Elizabeth University Hospital Birmingham trial site only (UK), who met the eligibility criteria for the LEAN trial, were given the option of participation in the current experimental metabolic study. With the exception of the voluntary component, other aspects of study bias were minimised by incorporating the metabolic sub-study into the first 12-weeks of the randomised, double-blinded, placebo-controlled LEAN trial. The full eligibility criteria are listed in the published trial protocol [23]. All participants had a definitive diagnosis of NASH on liver biopsy within 6 months of the study, as defined by two independent liver histopathologists [2]. The participants were of adult age (18–70 years) and had a body mass index (BMI) $\geqslant$25 kg/m^2. Patients with co-existing type 2 diabetes were diet-controlled or were on a stable dose of metformin ± gliclazide for a minimum of 3 months prior to the study and had a HbA1c <9.0%. All patients with no previous diagnosis of type 2 diabetes underwent a 75 g oral glucose tolerance test.

Treatment groups

Patients who satisfied the eligibility criteria were randomly assigned on a 1:1 basis to once-daily (OD) subcutaneous injection of 1.8 mg liraglutide (Victoza®, Novo Nordisk A/S, Denmark) or liraglutide-placebo control (Novo Nordisk A/S, Denmark). To aid with gastrointestinal tolerability the dose was titrated by 0.6 mg every 7 days from a starting dose of 0.6 mg OD until the maximum dose of 1.8 mg OD was achieved.

Study design

At baseline and after 12-weeks of treatment all participants underwent paired 2-step hyperinsulinaemic euglycaemic clamps incorporating stable isotopes with concomitant subcutaneous adipose tissue microdialysis at the NIHR/Wellcome Trust Clinical Research Facility (WTCRF, Birmingham, UK) (Supplementary Fig. 1). A full description of such is detailed in the Supplementary Methods.

Participants were admitted to the WTCRF the evening (17.00 hours) before the euglycaemic clamp study. After a standardised meal, participants were fasted until completion of the clamp study, with the exception of drinking oral deuterated water (^2H_2O) to determine rates of DNL. At 08.00 hours the next morning fasting blood samples were taken and an adipose microdialysis catheter was inserted into the abdominal subcutaneous adipose tissue (SAT), prior to starting the 2-step hyperinsulinaemic euglycaemic clamp (as previously described [24]). Thereafter, microdialysate samples were collected into microvials (0.3 μl/min) every 30 minutes until the end of the clamp. After basal measurements, hepatic and peripheral ('muscle') insulin sensitivity were assessed with consecutive 2 hour infusions of insulin at 20 and 100 mU/m^2/min, respectively. Fasting glycaemic concentrations were maintained ('clamped') with a concomitant variable infusion of 20% glucose enriched with U-[^{13}C]-glucose (4%) throughout the hyperinsulinaemic phases. During the 6 hour clamp, steady state blood samples were taken at three time points in the final 30 minutes of the basal (90–120 min), low-dose (210–240 min) and high-dose insulin (330–360 min) phases.

Data collection and analysis

Participant demographics and clinical/biochemical measures were recorded at baseline and after 12-weeks of treatment (see Supplementary Methods). Serum insulin (Mercodia, Sweden), NEFA (Zen-Bio, USA) and adipocytokines (Fluorokine® Multi-Analyte Profiling; R&D Systems, United Kingdom) were measured using commercially available kits, as previously described [24]. In addition, abdominal SAT microdialysate samples were analysed using a mobile photometric, enzyme-kinetic analyser (CMA Iscus Flex, Sweden) for interstitial glycerol concentrations.

Stable Isotope Mass Spectrometry analysis

The enrichment of U-[^{13}C]-glucose in plasma (for hepatic endogenous glucose production (EGP) and glucose disposal (Gd) calculations) and deuterium (^{2}H) in the body water pool/palmitate fraction of total plasma triglycerides (for DNL calculations) were determined by gas chromatography-mass spectrometry, as previously described [24,25].

Data definitions and calculations

Hepatic (hepatic EGP) and muscle (Gd) sensitivity were calculated by using modified versions of the Steele Equations. In order to quantify whole body lipolysis and adipose tissue insulin sensitivity, the Adipose-IR index and the insulin concentrations causing half-maximal suppression of serum NEFA (INS ½-max NEFA) were calculated for each participant using regression analysis [24,25]. The INS ½-max NEFA was interpolated from the gradient in decline in circulating NEFA following the transition from basal, to low and then to high-dose insulin (maximal suppression) across the duration of the clamp. Similarly, the rate of glycerol release from SAT in response to fasting, low- and high-dose insulin was used to determine abdominal SAT lipolysis and insulin sensitivity. The percentage contribution of hepatic DNL to endogenous palmitate synthesis was determined by the incorporation of ^2H_2O in the palmitate present in the plasma total triglyceride pool, as previously described [24,25].