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LIRAGLUTIDE EFFICACY AND ACTION IN NON-ALCOHOLIC STEATOHEPATITIS (LEAN): A MULTI-CENTRE, DOUBLE-BLIND, RANDOMISED, PLACEBO-CONTROLLED PHASE II TRIAL

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ABSTRACT/SUMMARY

Background: Glucagon-like peptide-1 (GLP-1) analogues reduce hepatic steatosis, liver enzymes and insulin resistance in murine models of fatty liver disease. They are licensed for type 2 diabetes, but their efficacy in patients with non-alcoholic steatohepatitis is unknown. The aim of the study was to assess the efficacy and safety of the long-acting GLP-1 analogue, liraglutide, in patients with non-alcoholic steatohepatitis.

Methods: This multicentre, double-blinded, randomised, placebo-controlled phase II trial was conducted in the UK to assess 48-weeks treatment with once-daily, subcutaneous injections of 1.8mg liraglutide or liraglutide-placebo in overweight patients with non-alcoholic steatohepatitis. Patients were randomly assigned 1:1 using a computer-generated, centrally administered procedure, stratified by trial centre and diabetes status. The trial was designed using A'Herns single arm method requiring 8/21 (38%) successes in the liraglutide arm. It incorporated a concurrently randomised placebo group to provide an unbiased assessment of outcome for this patient population. The primary outcome measure was improvement in liver histology, defined as 'resolution of definite NASH' with no worsening in fibrosis from baseline to end-of-treatment, as assessed centrally by two independent, blinded, pathologists. Analysis was by intention-to-treat. The trial was registered with ClinicalTrials.gov;NCT01237119.

Findings: Between 1st August 2010 and 31st May 2013, 26 patients were randomly

assigned to receive liraglutide and 26 to placebo. 45 (87%) of 52 patients underwent end-of-treatment liver biopsy at 48 weeks. The primary end-point was met as 9/23 (39%) patients on liraglutide had resolution of definite NASH. This was higher than the 2 (9%) of 22 responders on placebo (relative risk for all patients that had end-of-treatment biopsy; 4.30, 95% CI 1.04 to 17.74; p=0.019). Fewer patients on liraglutide (2/23; 9%) demonstrated progression of fibrosis compared to placebo (8/22; 36%) (p=0.03).

Interpretation: Liraglutide was safe, well-tolerated and led to histological resolution of non-alcoholic steatohepatitis, warranting extensive longer-term studies.

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Key words: Glucagon-like peptide 1, liraglutide, incretin mimetic, non-alcoholic fatty liver, non-alcoholic steatohepatitis, liver biopsy

Introduction

Non-alcoholic steatohepatitis (NASH) is now the commonest cause of liver disease and is predicted to be the main indication for liver transplantation by 2020.[1] Patients with NASH have an increased risk of liver and cardiovascular disease (CVD) related morbidity and mortality,[2] compared to those with non-alcoholic fatty liver (NAFL) and the general population.[3, 4] Moreover, there are currently no licensed therapies for NASH.

Lifestyle modifications are the mainstay of treatment for NASH,[5] yet most patients fail to achieve, or maintain, dietary goals and weight loss.[6] In the two largest randomised controlled trials in patients with NASH thus far treatment with pioglitazone, vitamin E (PIVENS)[7] and obeticholic acid (FLINT)[8] were associated with improvements in liver histology compared to placebo, with the findings of the PIVENS trial relevant to patients without type 2 diabetes. Concerns about the side-effects and long-term safety profile of both pioglitazone and Vitamin E has reduced enthusiasm for their use.[9] Obeticholic acid also reduced liver fibrosis in the FLINT trial and was associated with an elevated LDL cholesterol, which will be studied further in phase 3.[8]

The strong association of NASH with the metabolic syndrome, in particular obesity and type 2 diabetes, provides a compelling rationale for investigating therapies such as the gut-derived incretin hormone, glucagon-like peptide-1 (GLP-1), that induce

weight loss and insulin sensitivity. Native GLP-1 has a potent blood glucose-lowering action, mediated via its ability to induce insulin secretion and reduce glucagon secretion in a glucose-dependent manner, as well as suppressing appetite and delaying gastric emptying.[10] Endogenous GLP-1 is degraded within minutes *in vivo* by the enzyme dipeptidyl peptidase-4, whereas liraglutide is a long-acting (half-life 13 hours) human GLP-1 analogue.[11] Liraglutide has been shown to cause weight loss,[12] decrease glycated haemoglobin (HbA1c) and systolic blood pressure and improve beta-cell function,[13] and is licensed for glycaemic control in patients with type 2 diabetes.

GLP-1 analogues have been shown to reduce liver enzymes and oxidative stress as well as improving liver histology[14] in murine models of NASH.[15-17] This may reflect their effects on obesity and systemic insulin resistance, although studies have also reported that GLP-1 analogues can act directly on human hepatocytes *in vitro*, to reduce steatosis by decreasing *de novo* lipogenesis and increasing fatty acid oxidation.[15, 18, 19]

To date, human studies investigating the effect of GLP-1 analogues on liver injury have been limited to case reports,[20, 21] a case series (n=8)[22] and retrospective studies of liver enzymes in patients with type 2 diabetes.[23,24] However, these studies were retrospective and lacked histological data, therefore we designed and conducted a multi-centre randomised controlled trial of liraglutide to test its safety and efficacy in

the treatment of histologically confirmed NASH in overweight patients with and without diabetes.

Methods

Study Design:

The Liraglutide Efficacy and Action in NASH (LEAN) trial was a multicentre, double-blinded, randomised, placebo-controlled trial of 48 weeks treatment with the once daily (OD) human GLP-1 analogue, liraglutide, in patients with biopsy-proven NASH. Between 1st August 2010 and 31st May 2013, patients were recruited from 4 trial centres at hospitals in the United Kingdom (UK). 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. In addition, all recruitment sites obtained approval from their local hospital Research and Development (R&D) departments. The University of Birmingham (Birmingham, UK) acted as the sponsor of the trial. A detailed version of the LEAN protocol is published online.[25]

Participants:

All patients provided written informed consent. The trial entry criteria were based on a diagnosis of 'definite' NASH on liver biopsy obtained within 6 months of screening. Prior to randomisation, two independent liver histopathologists (SGH, RB) reviewed all of the liver biopsies to confirm whether a diagnosis of 'definite' NASH was present, as defined by macrovesicular steatosis (>5%), hepatocyte ballooning (with confirmation of the presence of Mallory's Hyaline by ubiquitin immunohistochemistry as necessary) and lobular inflammation (mixed infiltrate,

related to foci of ballooning).[26] In the event of disagreement with regards to a diagnosis of 'definite' NASH, a combined assessment was undertaken to achieve consensus. All participants had to be 18-70 years old and have a body mass index (BMI) ≥25 kg/m^2 at screening. Patients with type 2 diabetes had to have stable glycaemic control (HbA1c <9.0%) and be managed by either diet and/or a stable dose of metformin/sulphonylurea.

Patients were excluded on the basis of: a history of significant alcohol consumption (>20 g/day for women or >30 g/day for men), poor glycaemic control (HbA1c > 9.0%), Child-Pugh B/C cirrhosis, other causes of liver disease, confounding concomitant medications (including insulin, incretin mimetics, thiazolidinediones, vitamin E) and medical conditions including a history of pancreatitis and pancreatic/thyroid carcinoma [Supplementary Methods].

Randomisation and blinding:

Patients who satisfied the eligibility criteria were randomly assigned (1:1) to 48 weeks treatment with subcutaneous injections of 1.8 mg liraglutide OD (Victoza®; Novo Nordisk A/S, Denmark) or liraglutide-placebo (control; Novo Nordisk A/S, Denmark) using a computer generated, centrally administered procedure at the clinical trials unit (Birmingham). Randomisation was based on a minimisation algorithm and stratified by trial site and diabetes status. To improve gastro-intestinal tolerability patients underwent a 14-day dose titration, increasing their dose by 0.6 mg every 7 days from a starting dose of 0.6mg OD until the maximum dose of 1.8 mg

OD was achieved. Patients, investigators, clinical trial site staff and pathologists were blinded to treatment assignment throughout the study.

Procedures:

After randomisation, patients returned for study visits at weeks 4, 12, 24, 36 and 48 (end of treatment), at which time the primary outcome was assessed. The end of study was at week 60, 12 weeks after treatment finished. The schedule for the study visits and data collection is summarised in the Appendix (Supplementary methods/Table 1). All patients received standard National Health Services (NHS) care recommendations on life-style modifications, including exercise, weight reduction and dietary modification. Patients were not allowed any new prescriptions or over-the-counter therapies that may impact on NASH throughout the duration of the trial.[25] No dose reductions of liraglutide or placebo were allowed throughout the 48-week treatment period. Previous treatment with oral anti-diabetic drugs (metformin and/or sulphonylurea) was continued at the same dose in participants with type 2 diabetes at randomisation.

Two independent liver histopathologists (SGH, RB) assessed all baseline and end of treatment liver biopsies to: (i) determine a diagnosis of 'definite NASH,' 'uncertain NASH,' or 'not NASH', (ii) to assess the severity of liver disease including the NAFLD activity score and fibrosis stage. The histopathologists were blinded to study treatment allocation and clinical/laboratory information. Cases where there was

disagreement on the presence/absence of definite NASH were reviewed and consensus reached. For each case consensus was reached for the fibrosis score.

Outcomes:

The primary outcome measure was assessed using an intention-to-treat analysis of the proportion of evaluable patients achieving an improvement in liver histology between liver biopsies at baseline and after 48 weeks of treatment. Histological improvement was defined as a combination of the disappearance of steatohepatitis (disappearance of hepatocyte ballooning) and no worsening in fibrosis (defined as an increase by one stage of the Kleiner Fibrosis classification[27]). Secondary histological outcomes included changes in the overall NAS, individual components of NAS (steatosis, hepatocyte ballooning, lobular inflammation) and the Kleiner fibrosis stage.[27] Fibrosis stages 1a, 1b and 1c were considered as stage 1 for the purposes of analysis. Other secondary outcome measures included changes from baseline to 48 weeks in serum liver enzymes, non-invasive hepatic biomarkers (CK-18, ELF test), fasting lipids, glycaemic control (glucose, HbA1c), insulin resistance (HOMA-IR, ADIPO-IR), anthropometric measures (body weight, BMI, waist circumference), health-related quality of life scores (SF36v2 physical and mental components) and dietary consumption per day.

Statistical analysis

The primary aim of the study was to assess whether the efficacy and safety profile of liraglutide was worthy of further investigation. Recruiting patients into a no

treatment placebo-control group provided simultaneous unbiased assessment of comparable patient groups. Based on other pharmaceutical trials in biopsy-proven NASH, it was assumed that up to 20% of patients undergoing current standard of care (placebo) would have an improvement in NASH by week 48. To justify further investigation of liraglutide treatment, a clinically relevant improvement in liver histology was considered to be 50% of patients. The sample size was calculated using A'Hern's single stage phase II methodology, with a one-sided significance level of 0.05 (type 1 error) and power of 90% (type II error 0.10). The design required 21 evaluable patients in the treatment group. To account for withdrawal, the recruitment target was inflated from 21 to 25 patients per treatment group.[25]

All evaluable patients were analysed on an intention-to-treat basis. Evaluable patients were defined as those who underwent an end-of-treatment biopsy (week 48). Patients were categorised as either achieving the primary histological outcome (resolution of NASH) or not in each treatment group. The study A'Herns design stipulated that 8 or more evaluable patients out of 21 (38%) in the liraglutide group had to achieve histological improvement to be deemed worthy of further investigation.[25]

An unpowered pre-planned secondary analysis of the primary outcome measure was performed using the chi-squared test to test for a difference between the proportions of patients with histological improvement in each treatment group. In addition, a sensitivity analysis was performed for the primary outcome measure, in

253 which patients that did not have an end-of-treatment liver biopsy were classified as
254 'no histological improvement' and included in the analysis. A *post hoc* logistic
255 regression analysis was undertaken to determine the treatment effect when
256 adjusted for the stratification variables of trial site and type 2 diabetes, stage of liver
257 fibrosis as well as weight and glycaemic change during the trial.
258
259 Adjusted relative risks were determined using the Mantel-Haenszel test for diabetes
260 and fibrosis. Continuous secondary outcome measures were compared between
261 treatment groups using linear regression, adjusting for parameter baseline values
262 and allocated treatment (as model covariates, equivalent to ANCOVA). Multilevel
263 modelling for key continuous outcome measures was undertaken to account for
264 repeated measures within each patient. Categorical secondary outcomes were
265 compared between treatment groups using chi-squared tests or Fisher's exact test
266 where appropriate. Statistical analyses were performed using Stata Statistical
267 Software: Release 12. College Station, TX: StataCorp LP.
268
269 Compliance with the trial protocol and safety profile of liraglutide was reviewed on
270 an annual basis by an independent DMC (appendix), and no concerns were raised.
271 The trial was registered with ClinicalTrials.gov (NCT01237119).
272
273 **Role of the funding source**
274 The LEAN trial represents independent academic research funded by the Wellcome
275 Trust, Novo Nordisk Ltd and the NIHR Birmingham Liver BRU. The funders of the

LEAN trial had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had access to all data in the study and had final responsibility for the decision to submit for publication.

Results:

52 patients with histologically confirmed 'definite' NASH on central pathology review were randomly assigned to receive liraglutide (n=26) or placebo (n=26), between 1st August 2010 and 31st May 2013 [Figure 1]. Participants were recruited from UK sites as follows: Birmingham (n=31), Nottingham (n=12), Hull (n=6) and Leeds (n=3). With the exception of one patient randomised to placebo, all patients received their assigned treatment. Equal numbers of patients missed end of treatment (48-week) biopsies (n=3) and withdrew from treatment (n=5) in each group. Baseline demographic, clinical, laboratory and histological features were similar in the two groups [Table 1]. Mean NAS was 4.9 (SD 0.9) and ranged from 3.0 to 6.5. Of 52 patients, stage 3 fibrosis was present in 21 (40%) and cirrhosis in 6 (12%) on central review.

45 (87%) patients had paired (baseline, 48-week) liver biopsies, received treatment and were included in the intention-to-treat analysis of the primary outcome. The primary outcome (8 out of 21 successes (38%) for the single arm analysis) was met, as 9 (39%) out of 23 patients in the liraglutide group had resolution of definite NASH with no worsening of fibrosis [Table 2]. The alpha and power associated with 9 out of 23 successes under the same design conditions are 0.027 and 89.5% respectively.

2 (9%) out of 22 patients on placebo had histological improvement (relative risk 4.30, 95% CI 1.04 to 17.74; Chi-squared test of proportions (9/23 vs 2/22) p=0.019). A pre-

304 defined sensitivity analysis of the primary outcome measure, in which patients with
305 a missing end-of-treatment liver biopsy were defined as non-responders,
306 demonstrated that 9 out of 26 (35%) on liraglutide versus 2 out of 26 (7.7%) on
307 placebo achieved the primary outcome. This equated to patients on liraglutide
308 (versus placebo) having a relative risk of 4.5 (95% CI 1.1, 18.9; Chi-squared test,
309 p=0.017) of achieving the primary outcome. The odds ratio for the treatment effect
310 resulting from a logistic regression analysis adjusting for the stratification factors of
311 diabetes status and trial site is 7.83 (95%CI; 1.31, 46.68, p=0.024). No additional
312 analyses were performed to account for missing data as low absolute numbers of
313 dropout were observed.
314
315 Similar proportions of patients with [3 out of 8 [38%)] and without [6 out of 15
316 (40%)] type 2 diabetes achieved the primary outcome with liraglutide treatment.
317 Both patients assigned to placebo that achieved histological improvement did not
318 have type 2 diabetes at baseline. The relative risk for non-diabetic patients achieving
319 the primary end-point was 3.4 (95% CI 0.8, 14.4; p=0.11) for liraglutide versus
320 placebo. As there were no patients with diabetes that responded in the placebo arm
321 a factor of 0.5 was added to all 4 values in the contingency table for diabetic
322 patients. Using this adjustment the relative risk for diabetic patients was calculated
323 as 4.7 (95% CI 0.3, 75.0; p=0.20). There was no evidence of heterogeneity (p=0.841).
324 The relative risk of response on liraglutide compared to placebo adjusted for
325 diabetes using the stratified Mantel-Haenszel test was 3.7 (95% CI 1.0, 13.5;
326 p=0.047).

Fewer patients on liraglutide (2 of 23; 9%) demonstrated progression of fibrosis compared to placebo (8 of 22; 36%); relative risk 0.2 (95% CI 0.1, 1.0); Fisher's exact test, p=0.04). A greater proportion of patients on liraglutide had improvements in steatosis (relative risk 1.8 (95% CI 1.1, 3.0); Chi-squared p=0.01) and hepatocyte ballooning (relative risk 1.9 (95% CI 1.0, 3.8); Chi-squared p=0.05) compared to placebo and no differences were seen in lobular inflammation (relative risk 0.9 (95% CI 0.5, 1.6); Chi-squared p=0.65) and overall NAS (relative risk 1.2 (95% CI 0.8, 1.7); Chi squared p=0.46) [Table 2].

Differences at 48 weeks in serum aminotransferases with liraglutide were not significant compared to placebo, with only serum gamma-glutamyl transferase reaching significance [Figure 2; Table 3]. However, multilevel modelling (Supplementary Results) of longitudinal parameters indicated significant differences in both alanine aminotransferase and gamma glutamyltransferase between the two treatment arms thereby supporting the changes over time illustrated in Figure 2. There were also trends in the reduction of serum biomarkers of hepatocyte injury (serum CK-18; p=0.097) and fibrosis (serum ELF; p=0.05) with liraglutide compared to placebo.

Compared with placebo, 48 weeks treatment with liraglutide was associated with significant reductions in body weight and body mass index (Table 3). Most of the beneficial effects of liraglutide on weight were achieved by 12 weeks treatment and

sustained throughout treatment [Figure 2]. Patients assigned to liraglutide also had significant improvements in HbA1c compared to placebo. Improvements in weight and HbA1c were confirmed by multilevel modelling (Supplementary Table 5). Notably, weight increased and metabolic changes reverted towards baseline 12 weeks after liraglutide was discontinued [Figure 2; Supplementary Table 2]. There was no significant difference in HDL or systolic blood pressure when assessed using multilevel modelling.

Post hoc analysis was undertaken to determine the clinical/laboratory changes that occurred in patients that had resolution of NASH with liraglutide treatment (n=9; 'responder') compared to those that did not (n=14; 'non-responder') (Supplementary Table 7). Changes in weight and glycaemic control (HbA_{1c}) in patients on liraglutide were not significantly different for responders and non-responders (Figure 3a/b).

Patients on liraglutide reported significant improvements in the physical component score of the SF36vs questionnaire compared to those on placebo (4.05 (95% CI; 0.20, 7.90; p=0.04).

The majority of AEs were grade 1 (mild) to grade 2 (moderate) in severity, transient and similar in the two treatment groups for all organ classes and symptoms, with the exception of gastrointestinal disorders (Table 4). Patients on liraglutide were prone to diarrhoea (42% versus 19%), constipation (23% versus 0%) and loss of appetite

(31% versus 8%) compared to those on placebo. Patients with advanced fibrosis (F3-4) had similar rates of AE to those with milder grades of fibrosis (Supplementary Table 9). Two (8%) patients in the liraglutide group withdrew from treatment due to nausea and diarrhoea, but still underwent liver biopsy at week 48. A further three treatment withdrawals in the liraglutide group were due to needle phobia, work commitments and loss to follow-up and withdrew their consent from the study and did not undergo end-of-treatment liver biopsy.

There were two serious AEs in the liraglutide group (tuberculosis, migraines) both of which were judged to be unrelated to therapy. There were no deaths nor cases of pancreatitis, hepatitis or liver failure during the trial. No patients developed antibodies against liraglutide on testing at week 60. *Post hoc* analysis highlighted that the numbers of AEs were similar between patients with and without advanced fibrosis (F3-F4) (Supplementary Table 9).

Discussion

In this double-blind, randomised, placebo-controlled phase II trial, the long-acting GLP-1 analogue, liraglutide, met the pre-defined primary end-point and led to resolution of NASH. Moreover, improvements in weight and glycaemic control with liraglutide may have a favourable effect on the future risk of CVD and premature death in patients with NASH, although longer term outcome studies are needed to confirm this. Study withdrawal (i.e. no end-of-treatment biopsy) rates were the same in both treatment groups and had no impact on the primary end-point. Liraglutide was safe and well-tolerated, irrespective of the severity of underlying disease.

This study has a number of strengths. Firstly, this is the first randomised, placebo-controlled trial to report the effect of a GLP-1 analogue on liver histology in patients with NASH. Secondly, the study population included patients with and without type 2 diabetes and liver cirrhosis. Thirdly, in light of the documented intra- and inter variability in assessment of liver biopsies[27] we had two independent, blinded, central assessments of liver biopsies at baseline (same sections used for eligibility and impact of treatment) and at end of treatment. This avoided inclusion of patients without definite NASH, as happened in 21% and 20% of patients in PIVENS[7] and FLINT[8] trials, respectively. Fourthly, we collated detailed recording of concomitant medications (i.e. lipid-lowering and anti-diabetic medications) and dietary intake (i.e. caffeine, vitamin E, alcohol) for the duration of the trial.

Our sample size was similar to previous proof-of concept studies [28], albeit smaller

than some later stage phase 2 studies [7, 8], and patients were extensively phenotyped and well-matched for features of the metabolic syndrome with the exception of BMI. The study was appropriately powered for a hard histological end-point, and the level of histological resolution of NASH with liraglutide (9 of 23; 39%) was comparable to that previously reported with vitamin E (29 of 80; 36%), pioglitazone (33 of 70; 47%)[7] and obeticholic acid (22 of 102; 22%).[8] The reported placebo rate (9%) was slightly lower than those previously described (13-21%),[7, 8] but this is likely because in this study clearance of NASH had to be accompanied without any worsening of fibrosis (which has not been previously adopted).

Although liraglutide met the primary end-point, it did not result in significant mean changes in the composite NAS score, as reported with pioglitazone, vitamin E and obeticholic acid.[7, 8] Notably, a greater proportion of patients on liraglutide had improvements in steatosis and hepatocyte ballooning indicating that the overall pattern of changes are in keeping with a reduction in histological damage with liraglutide. With the exception of lobular inflammation, a greater proportion of patients on liraglutide improved steatosis (83% versus 45%; p=0.009) and hepatocyte ballooning (61% versus 32%; p=0.05), which would suggest that a larger study could identify significant mean changes in NAS. Liraglutide also showed evidence of efficacy in a *post hoc* analysis using the primary end-points (which utilised NAS) that were in place for the FLINT and PIVENS trials (Supplementary Table 6).

Resolution of NASH was selected as the primary end-point instead of changes in

NAS, in keeping with guidance from an expert consortium.[26] Notably, NAS score does not predict liver-related morbidity or mortality, whereas the presence of NASH (versus simple NAFL) is associated with a significant increase in liver-related outcomes and all-cause mortality.[3, 4]

Recent data have identified the importance of liver fibrosis as being the key determinant of clinical outcomes in patients with NASH.[29] Despite the relatively short duration of this trial fewer patients on liraglutide had progression of fibrosis (p=0.04) and there was also a greater reduction in serum ELF levels (p=0.05) than placebo. Whilst there was no difference in mean change in fibrosis stage (p=0.18) between the two groups this is likely a reflection of the duration of treatment, and a longer course should be evaluated. Notably, the univariate analysis suggested that patients with more severe fibrosis (F3/F4) at baseline were less likely to respond to liraglutide, although liraglutide still had a positive treatment effect after adjusting for baseline fibrosis (Supplementary Table 4).

The clearance of NASH by liraglutide is likely to be multi-factorial and a consequence of its cumulative effect on weight loss and glycaemic control. Comparison of patients with and without histological response to liraglutide, albeit limited by small numbers, demonstrates a possible continued modest reduction in weight loss in responders. *Post hoc* logistic regression (Supplementary Table 5) indicates that the effects of liraglutide are likely to be due to a combination of a direct hepatic effect (odds ratio for treatment effect adjusted for weight was 4.12 (CI 0.66-25.8; p=0.131))

462 and an effect on weight loss. This would imply that the mechanism of action of GLP-1
463 analogues in NASH is not solely explained by improvements in weight and metabolic
464 phenotype, and indeed *in vitro* studies have shown that GLP-1 analogues improve
465 the ability of the hepatocyte to handle excess NEFA and lipid production by
466 modulating lipid transport, beta-oxidation, and *de novo* lipogenesis,[16, 18, 30] all of
467 which have been implicated in the pathogenesis of NASH. These observations have
468 been confirmed in liraglutide-treated mice, in which reductions in hepatic steatosis,
469 insulin resistance (via clamp technique) and endoplasmic reticulum oxidative stress
470 occurred in the absence of weight loss[16, 31]. When this study was designed liraglutide
471 was only available at the 1.8mg dose, and since then a higher dose (3.0mg) has been
472 approved for weight management[12]. It is possible that a higher dose of liraglutide
473 could provide greater efficacy in the setting of NASH, although the level of added
474 benefit is unclear.
475
476 Currently, safety data regarding the use of GLP-1 analogues in liver disease are
477 limited to solitary case reports[20, 21] and retrospective analysis of large cohorts of
478 patients with type 2 diabetes and elevated transaminases.[23, 24] Liraglutide was
479 generally well tolerated in the study and had a similar AE profile to placebo, with the
480 exception of predictable gastrointestinal symptoms (mainly diarrhoea, constipation
481 and loss of appetite). These, however, were mainly transient and mild-to-moderate
482 in severity.
483
484 At present, there is a significant unmet need of therapies in patients with NASH

485 cirrhosis. We, therefore, elected to include patients with cirrhosis in this study to
486 pilot the efficacy, but importantly highlight the safety of liraglutide in this setting.
487 Due to the fact that cirrhosis is the final stage of the Kleiner scoring system (e.g. 4/4),
488 these patients may have been advantaged in achieving the primary end-point, as by
489 definition they could not have 'worsening of fibrosis'. However, their inclusion did
490 not inflate the histological response in the liraglutide group, as only one patient with
491 cirrhosis met the primary end-point and they received placebo.
492
493 In conclusion, the unique combination of histological efficacy and improvement of
494 the metabolic syndrome with liraglutide render it an attractive therapy for patients
495 with NASH and warrant further investigation in larger studies.
496

Panel: Research in context

Evidence before this study

Non-alcoholic steatohepatitis (NASH) is now the commonest cause of chronic liver disease and incurs a significantly increased risk of both liver- and cardiovascular disease (CVD)-related morbidity and mortality.[2, 4] Despite this, there are no licensed therapies for NASH.[5] To date, clinical trials of pioglitazone, vitamin E (PIVENS)[7] and obeticholic acid in patients with biopsy-proven NASH (FLINT)[8] have shown improvements in liver histology compared to placebo. With the exception of FLINT, these trials have excluded patients with type 2 diabetes, thus their effects in patients with diabetes are unknown. Moreover, there remain concerns about the side-effects and long-term safety of pioglitazone and Vitamin E which has reduced enthusiasm for their use.

In 2009, the long-acting glucagon-like peptide-1 (GLP-1) analogue, liraglutide, was licensed for glycaemic control in overweight patients with type 2 diabetes. Liraglutide also suppresses appetite centrally and delays gastric emptying[10] which induces weight loss[12, 13] rendering it an attractive therapeutic option for NASH. Prior to designing the LEAN trial, the published literature were reviewed by searching PubMed, between 1st January 1965 and 31st December 2009, for ['NAFLD', 'NASH', 'fatty liver', 'steatohepatitis' or 'liver injury] and ['glucagon-like peptide 1', 'GLP-1', 'liraglutide', 'exenatide' or 'incretin']. GLP-1 analogues, including liraglutide, improved liver enzymes, oxidative stress and hepatic steatosis in murine models *in vivo* and in isolated *in vitro* murine and human hepatocyte studies.[15, 16, 18, 19, 30, 31]

Human studies investigating the effect on liver injury were limited to single case reports,[20, 21] and large retrospective studies of liver enzymes in patients with type 2 diabetes.[23] An individual patient level meta-analysis of over 4000 patients with type 2 diabetes was performed, comparing 26 weeks of treatment with liraglutide to placebo. Liraglutide significantly improved liver enzymes in a dose-dependent manner, with comparable safety profiles in those patients with and without abnormal liver biochemistry.[24] These findings formed the basis for this phase II randomised, placebo-controlled trial of liraglutide for NASH. Despite extending the literature search dates to 1st April 2015, no clinical trials of GLP-1 based therapies in NASH were identified.

Added value of this study

This study is a first in class, randomised, controlled trial of GLP-1 analogue in patients with NASH. Liraglutide met the primary end-point of histological resolution of NASH with no worsening in fibrosis. In addition to improvements in histological steatosis and hepatocyte ballooning, fewer patients on liraglutide had progression of fibrosis. Uniquely for tested therapies in NASH, liraglutide improved several key components of the metabolic syndrome, including weight and glycaemic control, which is important, as cardiovascular disease accounts for the majority of deaths in cohorts of patients with NASH.

Implications of all the available evidence

Due to the growing global burden of NASH and the lack of licensed therapies there is a pressing need for effective interventions. Given the associated cardiovascular morbidity and mortality with NASH, the use of therapies such as liraglutide which improve both liver histology and many aspects of the metabolic syndrome are needed to improve outcomes for patients with NASH. Future, longer-term studies with liraglutide are needed to confirm their efficacy in patients with NASH, as well as to establish their cardiovascular benefits.

Contributors:

MJA, SG, JWT and PNN (Chief Investigator) had the original concept of the LEAN trial. MJA, DD, PG, DS, SG, JWT, RB, SGH and PNN designed the LEAN trial and wrote/reviewed all protocol versions. RB and SGH carried out the central histopathology review of all pre- and post-treatment liver biopsies**.** MJA and DB (senior trials coordinator) submitted all REC, MHRA, local R&D applications and coordinated the trial sites. PG (senior statistician) prepared the annual Data Management Committee reports and performed all the statistical analysis. MJA, GPA, GA, MAA, and PNN recruited the participants and MJA, GPA, DH, KG, DB, RP, JMH, GA, MAA, RB, SGH and PNN were responsible for data collection. MJA, PG, RB, SGH and PNN participated in data analysis and interpretation. MJA, PG and PNN wrote the manuscript and all authors participated in manuscript review. MJA and PG were responsible for preparation of the tables and figures. MJA, PG and PNN are guarantors.

*Other members of the LEAN trial group that have been instrumental in the conduct of the trial to date:

Queen Elizabeth University Hospital Birmingham/NIHR Liver BRU/CRUKCTU (Birmingham, UK): Manpreet Wilku, Christine Russell, Salma Iqbal, Dr Christopher Corbett, Michelle Yun Kyong Lee, Jennifer Keely and nursing staff at the WTCRF.

Nottingham University Hospitals NHS Trust/ Nottingham Digestive Diseases BRU (Nottingham, UK): Maggie Nicholls and Susanne Henry.

Hull Royal Infirmary (Hull, UK): Martin Lewis, Erica Dixon and Sally Myers.

St James's University Hospital (Leeds, UK): Samantha Sharman and Rebecca Bishop.

Declaration of interests:

PNN and MJA have received free trial drug supply from Novo Nordisk for conduct of the LEAN trial of liraglutide in NASH. PNN has received an educational grant and honoraria for lectures given on behalf of Novo Nordisk. SCG has served on advisory boards for Novo Nordisk, Eli Lilly, Sanofi Aventis and Takeda, and has received honoraria for lectures given on behalf of Novo Nordisk, Eli Lilly, Sanofi Aventis, Takeda and GSK. PG, GPA, RP, DS, DH, KG, JMH, GA, MA, JWT, RB, SGH have no conflict of interests to declare.

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599

Figures legends:

Figure 1 Trial profile: *One (1.9%) patient that was assigned to placebo never received treatment, as they disclosed use of an ineligible medication (Dipetidyl peptidase-IV inhibitor) 24 hours post-randomisation.** Two patients randomised to liraglutide withdrew from treatment (2, 16 weeks) due to adverse gastrointestinal events, but still proceeded with the 48 week liver biopsy. One patient randomised to placebo withdrew from treatment due to reactive hypoglycaemia (36 weeks) but still proceeded with the 48 week liver biopsy.

Figure 2. Changes from baseline in metabolic parameters and liver enzymes according to treatment group. Mean values (95% CI, error bars) of change from baseline during treatment with liraglutide (*blue line*) or placebo (*red line*) for up to 48 weeks followed by a 12 week post-treatment period are shown (*broken line*). (A) Weight, (B) HbA1c and (D) Alanine aminotransferase decreased during treatment with liraglutide with a rebound back toward baseline after discontinuation. (C) Serum γ-glutamyl transpeptidase concentrations decreased with liraglutide treatment. There was no difference in (E) HDL cholesterol and (F) systolic BP over time between liraglutide and placebo.

Figure 3. Changes from baseline in weight [a] and HbA_{1c} [b] for patients with and without a histological response to liraglutide treatment. Median values (IQR, error bars) of changes from baseline in patients with histological improvement (responder; *blue line*) and no histological improvement (non-responders; *red line*) on liraglutide

623 treatment for 48 weeks and post treatment follow-up (*broken line*) at 60 weeks.
624 Mean changes at 48 weeks and associated p-values are reported in Supplementary
625 Table 7.

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