Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study

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Summary

Background **Glucagon-like peptide-1 (GLP-1) analogues reduce hepatic steatosis, concentrations of liver enzymes, and insulin resistance in murine models of fatty liver disease. These analogues are licensed for type 2 diabetes, but their efficacy in patients with non-alcoholic steatohepatitis is unknown. We assessed the safety and efficacy of the long-acting GLP-1 analogue, liraglutide, in patients with non-alcoholic steatohepatitis.**

Methods **This multicentre, double-blinded, randomised, placebo-controlled phase 2 trial was conducted in four UK medical centres to assess subcutaneous injections of liraglutide (1·8 mg daily) compared with placebo for patients who are overweight and show clinical evidence of 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'Hern's single-group method, which required eight (38%) of 21 successes in the liraglutide group for the effect of liraglutide to be considered clinically significant. Patients, investigators, clinical trial site staff, and pathologists were masked to treatment assignment throughout the study. The primary outcome measure was resolution of definite non-alcoholic steatohepatitis with no worsening in fibrosis from baseline to end of treatment (48 weeks), as assessed centrally by two independent pathologists. Analysis was done by intention-to-treat analysis, which included all patients who underwent end-of-treatment biopsy. The trial was registered with ClinicalTrials.gov, number NCT01237119.**

Findings **Between Aug 1, 2010, and May 31, 2013, 26 patients were randomly assigned to receive liraglutide and 26 to placebo. Nine (39%) of 23 patients who received liraglutide and underwent end-of-treatment liver biopsy had resolution of definite non-alcoholic steatohepatitis compared with two (9%) of 22 such patients in the placebo group (relative risk 4·3 [95% CI 1·0–17·7]; p=0·019). Two (9%) of 23 patients in the liraglutide group versus eight (36%) of 22 patients in the placebo group had progression of fibrosis (0·2 [0·1–1·0]; p=0·04). Most adverse events 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 in 21 (81%) of 23 patients in the liraglutide group and 17 (65%) of 22 patients in the placebo group, which included diarrhoea (ten [38%] patients in the liraglutide group *vs* five [19%] in the placebo group), constipation (seven [27%] *vs* none), and loss of appetite (eight [31%] *vs* two [8%]).**

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

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Introduction

Non-alcoholic steatohepatitis is now the most common cause of liver disease and is predicted to be the main indication for liver transplantation by 2020.[1] Patients with non-alcoholic steatohepatitis have an increased risk of morbidity and mortality related to liver and cardiovascular disease[2] compared with patients who have simple steatosis and the general population.[3,4] Moreover, there are currently no licensed therapies for non-alcoholic steatohepatitis.

Lifestyle modifications are the mainstay of treatment for non-alcoholic steatohepatitis,[5] yet most patients do not achieve or maintain dietary goals and weight loss.[6] In the two largest randomised controlled trials in patients with non-alcoholic steatohepatitis thus far, treatment with pioglitazone, vitamin E (PIVENS trial),[7] and obeticholic acid (FLINT trial)[8] were associated with improvements in liver histology relative to placebo, with the findings of the PIVENS trial relevant to patients without type 2 diabetes. However, 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 concentration of LDL cholesterol, which will be studied further in a phase 3 trial.[8]

The strong association of non-alcoholic steatohepatitis with the metabolic syndrome, particularly obesity and type 2 diabetes, provides a compelling rationale for the investigation of 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

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Research in context

Evidence before this study

Non-alcoholic steatohepatitis is now the most common cause of chronic liver disease worldwide and incurs a significantly increased risk of both liver-related and cardiovascular disease-related morbidity and mortality. Yet no therapies are licensed for non-alcoholic steatohepatitis. To date, clinical trials of pioglitazone, vitamin E (PIVENS trial), and obeticholic acid (FLINT trial) in patients with biopsy-proven non-alcoholic steatohepatitis have yielded results showing improvements in liver histology compared with placebo. With the exception of the FLINT trial, these trials have excluded patients with type 2 diabetes. Thus the effect of these drugs in patients with diabetes is unknown. Moreover, concerns remain about the side-effects and long-term safety of pioglitazone and vitamin E, which has reduced enthusiasm for their use.

In 2009, the longacting 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, delays gastric emptying, and induces weight loss, rendering it an attractive therapeutic option for patients with non-alcoholic steatohepatitis.

We searched PubMed for clinical studies published in English between Jan 1, 1965, and Apr 1, 2015, with terms ("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. Human studies investigating the effect of GLP-1 analogues on liver injury were limited to single case reports and large retrospective studies of liver enzymes in patients with type 2 diabetes. An individual patient-level meta-analysis of more than 4000 patients with type 2 diabetes was performed, comparing 26 weeks of treatment with liraglutide to placebo. Liraglutide significantly improved liver enzyme concentrations in a dose-dependent manner, with comparable safety profiles in patients with and without abnormal liver biochemistry. These findings informed the basis for this phase 2 randomised, placebo-controlled trial of liraglutide for non-alcoholic steatohepatitis.

Added value of this study

This study is a first-in-class, randomised, placebo-controlled trial of GLP-1 analogue in patients with non-alcoholic steatohepatitis. Liraglutide met the primary endpoint of histological resolution of non-alcoholic steatohepatitis with no worsening in fibrosis. In addition to improvements in histological steatosis and hepatocyte ballooning, fewer patients receiving liraglutide had progression of fibrosis than in the placebo group. Liraglutide improved several key components of the metabolic syndrome, including weight and glycaemic control, which is not only unique for tested therapies in non-alcoholic steatohepatitis, but also important because cardiovascular disease accounts for the majority of deaths in cohorts of patients with non-alcoholic steatohepatitis.

Implications of all the available evidence

Because of the growing global burden of non-alcoholic steatohepatitis and the scarcity of licensed therapies, there is a need for effective interventions. In view of the cardiovascular morbidity and mortality associated with non-alcoholic steatohepatitis, therapies, such as liraglutide, that improve outcomes for patients with non-alcoholic steatohepatitis are needed. Future, longer term studies with liraglutide are needed to confirm its efficacy in patients with non-alcoholic steatohepatitis and to establish the cardiovascular implications.

potent blood glucose-lowering action, mediated by its ability to induce insulin secretion and reduce glucagon secretion in a glucose-dependent manner, suppresses appetite, and delays gastric emptying.[10] Endogenous GLP-1 is degraded within minutes in vivo by the enzyme dipeptidyl peptidase-4, whereas liraglutide is a long-acting human GLP-1 analogue ($t_{½}$=13 h)[11] that has been shown to cause weight loss,[12] decrease the concentration of glycated haemoglobin (HbA_{1c}), lower systolic blood pressure, and improve beta-cell function.[13] Liraglutide 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 improve liver histology[14] in murine models of non-alcoholic steatohepatitis.[15–17] This activity might reflect the effect of these analogues on obesity and systemic insulin resistance, although studies have also reported that GLP-1 analogues can act directly on human hepatocytes in vitro, reducing 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 of eight patients,[22] and retrospective studies of liver enzymes in patients with type 2 diabetes.[23,24] However, these studies were retrospective and did not have histological data. We therefore designed and conducted a multicentre, randomised, placebo-controlled trial of liraglutide to test its safety and efficacy in the treatment of histologically confirmed non-alcoholic steatohepatitis in overweight patients with and without diabetes.

Methods

Study design and patients

The Liraglutide Efficacy and Action in NASH (LEAN) study was a multicentre, double-blind, randomised trial of 48 weeks of liraglutide versus placebo in patients with biopsy-confirmed non-alcoholic steatohepatitis. Participants were enrolled at four participating medical centres in the UK (Birmingham, Nottingham, Hull, and

Leeds), all of which obtained approval from their local hospital research and development departments. 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 LEAN study protocol, which is available online.[25]

Patients enrolled in the study were 18–70 years of age, had a body-mass index (BMI) of 25 kg/m² at screening, and had histological evidence of non-alcoholic steatohepatitis on the basis of liver biopsy obtained within 6 months of screening. Before randomisation, two independent liver histopathologists (SGH, RMB) reviewed all liver biopsies to confirm whether a diagnosis of definite non-alcoholic steatohepatitis 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 respect to a diagnosis of definite non-alcoholic steatohepatitis, a combined assessment was undertaken to achieve consensus. Patients with type 2 diabetes were eligible if they had stable glycaemic control (glycated haemoglobin [HbA_{1c}]<9·0%) and were managed by either diet or a stable dose of metformin or sulfonylurea. Patients were excluded for substantial alcohol consumption (>20 g/day for women or >30 g/day for men), poor glycaemic control (HbA_{1c}> 9·0%), Child-Pugh B/C cirrhosis, other causes of liver disease, confounding concomitant drug use (including insulin, incretin mimetics, thiazolidinediones, vitamin E), and disorders such as a medical history of pancreatitis and pancreatic or thyroid carcinoma (appendix p 5). All patients provided written informed consent.

Randomisation and masking

Centre-delegated staff telephoned randomisation officers at the Cancer Research UK Clinical Trials Unit (Birmingham, UK), who used a computer-generated, centrally administered procedure to randomly assign eligible patients (1:1) to once-daily subcutaneous injections of 1·8 mg liraglutide (Victoza; Novo Nordisk, Bagsvaerd, Denmark) or placebo. Randomisation was based on a minimisation algorithm and stratified by trial site and diabetes status. Patients, investigators, clinical trial site staff, and pathologists were masked to treatment assignment throughout the study.

Allocation concealment was achieved by packaging both liraglutide and placebo groups with a unique identification number (in keeping with the European Union's Good Manufacturing Practice for medicinal products guidelines) by the manufacturer and providing these numbers to individual centres when randomly assigning patients. A master control list of the pack identification numbers and treatment was retained at the trials unit and was accessible only by the database programmer and the statistician. The list was also provided to the contracted external provider of the emergency unblinding service.

Procedures

To improve gastrointestinal tolerability, all enrolled patients underwent a 14 day dose titration, increasing their dose of liraglutide by 0·6 mg every 7 days from a starting dose of 0·6 mg daily until the maximum dose of 1·8 mg daily was achieved. 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. Patients had routine blood tests done during each visit. The Medical Outcomes Study 36-item short-form health survey version 2 (SF-36v2) and Block Food Frequency Questionnaire (Block FFQ) were done at the initial assessment, at the end of treatment, and 12 weeks after the end of treatment. The study ended at week 60, 12 weeks after the last treatment. The schedule for the study visits and data collection is summarised in the appendix (p 10). All patients received standard National Health Service care recommendations on lifestyle modifications, including exercise, weight reduction, and dietary modification. Patients were not allowed any new prescriptions or over-the-counter therapies that might

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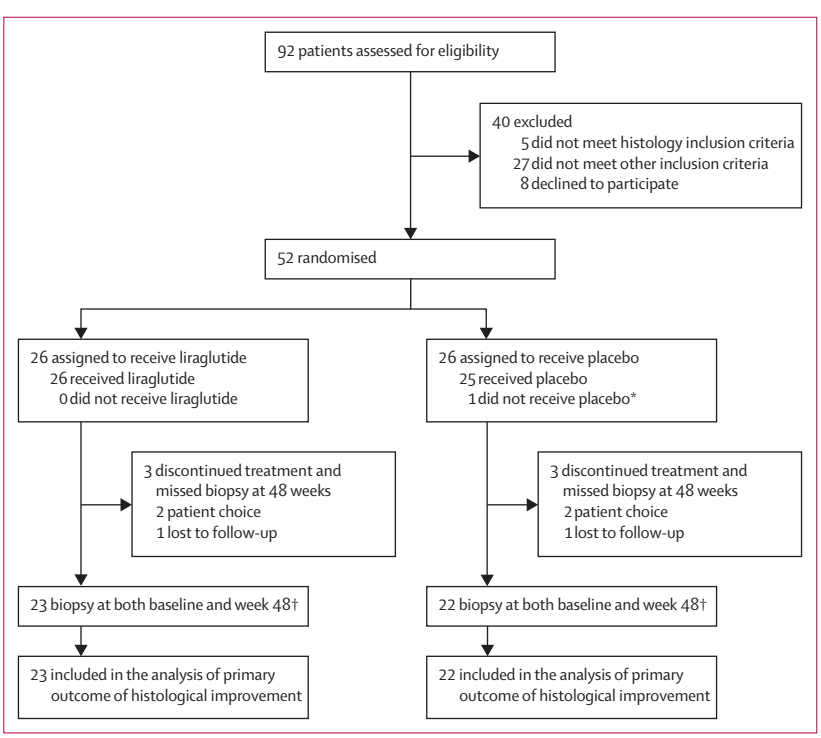

***Figure 1*: Trial profile**

*One (2%) patient assigned to placebo never received treatment, as they disclosed use of an ineligible drug (dipetidyl peptidase-4 inhibitor) 24 h post-randomisation. †Two patients assigned to the liraglutide treatment group withdrew from treatment (one patient withdrew at 2 weeks, one patient withdrew at 16 weeks) because of adverse gastrointestinal events but still proceeded with the liver biopsy at 48 weeks. One patient randomly assigned to placebo withdrew from treatment due to reactive hypoglycaemia at 36 weeks but still proceeded with the liver biopsy at 48 weeks.

affect non-alcoholic steatohepatitis throughout the duration of the trial.[25] No dose reductions of liraglutide or placebo were allowed throughout the 48 week treatment period. For participants with type 2 diabetes, previous treatment with oral anti-diabetic drugs (metformin or sulfonylurea, or both) was continued at the same dose as before randomisation. Compliance with the trial protocol and safety profile of liraglutide was reviewed on an annual basis by an independent data monitoring committee (appendix p 3), and no concerns were raised.

Two independent liver histopathologists (SGH, RMB; both of whom were blinded to study treatment allocation and clinical or laboratory information) assessed all baseline

	Liraglutide (n=26)	Placebo (n=26)
Demographics		
Age (years)	50 (11)	52 (12)
Male	18 (69%)	13 (50%)
Race		
White	23 (88%)	23 (88%)
Asian (south Asian or oriental)	1 (4%)	1 (4%)
Black (African or Caribbean)	1 (4%)	0 (0%)
Other (including mixed)	1 (4%)	2 (8%)
Comorbidities		
Type 2 diabetes	9 (35%)	8 (31%)
Hyperlipidaemia*	9 (35%)	7 (27%)
Hypertension†	15 (58%)	14 (54%)
Cardiovascular disease	0 (0%)	3 (12%)
Thyroid disease (hypothyroidism)	3 (12%)	4 (15%)
Concomitant drug use		
Anti-diabetic		
Metformin	9 (35%)	8 (31%)
Sulfonylurea	1 (4%)	1 (4%)
Anti-lipidaemic	10 (38%)	7 (27%)
Anti-hypertensive	13 (50%)	12 (46%)
Anti-platelet	5 (19%)	5 (19%)
Metabolic factors		
Glucose (mmol/L)	6·0 (1·7)	6·1 (1·5)
Insulin (pmol/L)	166 (80)	257 (289)
HOMA-IR (glucose [mmol/L] × insulin [mmol × U/L])	6·7 (4·7)	9·6 (9·8)
Glycated haemoglobin A_{1c}		
Absolute concentration (mmol/mol)	41·2 (7·8)	42·4 (9·3)
Percentage of total haemoglobin (%)	5·9% (0·7%)	6·0% (0·9%)
Non-esterified fatty acids (μmol/L)	967 (535)	836 (368)
ADIPO-IR (fasting non-esterified fatty acids [mmol/L] × insulin [mmol × U/L])	22·2 (12·7)	30·5 (42·7)
Weight (kg)	101 (18)	108 (18)
Body-mass index (kg/m²)	34·2 (4·7)	37·7 (6·2)
Waist circumference (cm)	110 (11)	120 (15)
Systolic blood pressure (mm Hg)	130 (13)	133 (12)
Diastolic blood pressure (mm Hg)	79 (11)	78 (9)
Creatinine (μmol/L)	83 (20)	71 (15)
Smoking history		
Current smoker	2 (8%)	2 (8%)
Ex-smoker	8 (31%)	13 (50%)
Never smoked	16 (62%)	11 (42%)
Lipids		
Total cholesterol (mmol/L)	4·5 (1·1)	5·0 (1·2)
HDL (mmol/L)	1·1 (0·4)	1·3 (0·2)
LDL (mmol/L)‡	2·6 (0·8)	2·9 (1·0)
Triglycerides (mmol/L)	1·9 (1·1)	1·8 (0·8)
(Continued from previous column)		
Liver function tests		
Alanine aminotransferase (U/L)	77 (34)	66 (42)
Aspartate aminotransferase (U/L)	51 (22)	51 (27)
γ-glutamyl transferase (U/L)	91 (69)	115 (174)
Alkaline phosphatase (U/L)	76 (25)	87 (41)
Total bilirubin (mol/L)	13 (5)	13 (7)
Albumin (g/L)	45 (6)	43 (5)
Non-invasive hepatic biomarkers		
Caspase-cleaved cytokeratin-18 fragment M30 (U/L)	394 (304)	352 (370)
Enhanced liver fibrosis test	9·3 (0·9)	9·4 (1·3)
SF-36 Quality of life		
Physical component	45 (11)	40 (13)
Mental component	51 (10)	45 (14)
Daily dietary consumption (Block FFQ)		
Total calories (kcal)	1885 (700)	1926 (677)
Total protein (g)	72 (34)	70 (25)
Total fat (g)	71 (30)	74 (35)
Total carbohydrate (g)	240 (87)	248 (89)
Caffeine (mg)	21 (30)	26 (45)
Alcohol (g)	6·3 (8·3)	4·8 (8·4)
Liver histology		
Definite non-alcoholic steatohepatitis	26 (100%)	26 (100%)
Total NAFLD activity score (0–8)	4·9 (0·9)	4·8 (0·9)
Hepatocyte ballooning score (0–2)	1·5 (0·5)	1·5 (0·4)
Steatosis score (0–3)	2·1 (0·7)	1·9 (0·7)
Lobular inflammation score (0–3)	1·4 (0·3)	1·4 (0·4)
Kleiner fibrosis stage (F0–F4)	2·3 (0·9)	2·3 (1·3)
Kleiner fibrosis stages F0–F2	14 (54%)	11 (42%)
Kleiner fibrosis stages F3–F4	12 (46%)	15 (58%)
Biopsy length (mm)	21·0 (7·6)	19·7 (5·7)
Number of portal tracts	18·5 (7·1)	16·2 (5·3)

(Table 1 continues in next column)

Data are n (%) or mean (SD). HOMA-IR=homoeostasis model assessment-estimated insulin resistance. ADIPO-IR=adipose tissue insulin resistance. SF-36v2=Medical Outcomes Study 36-Item Short-Form Health Survey version 2. Block FFQ=Block Food Frequency Questionnaire. *Hyperlipidaemia was defined as recorded in the past medical history, as receiving lipid-lowering drugs (eg, statin, fibrate, ezetimibe), or both. †Hypertension was defined as recorded in the past medical history, as receiving an anti-hypertensive drug, or both. ‡LDL concentration was calculated using the Friedwald formula. The non-alcoholic fatty liver disease (NAFLD) activity score and Kleiner scoring system are described in the appendix (p 12).

***Table 1*: Baseline characteristics of trial population**

and end-of-treatment liver biopsies to assign a diagnosis of definite non-alcoholic steatohepatitis, uncertain non-alcoholic steatohepatitis, or not non-alcoholic steatohepatitis and to assess the severity of liver disease, including the non-alcoholic fatty liver disease (NAFLD) activity score and fibrosis stage. Cases for which there was disagreement on the presence or absence of definite non-alcoholic steatohepatitis were reviewed and consensus was reached. Consensus for the fibrosis score was reached for each case.

Outcomes

The primary outcome was improvement in liver histology from baseline to end of treatment. Histological improvement was defined as the resolution of steatohepatitis (disappearance of hepatocyte ballooning) without worsening of fibrosis (defined as a numerical increase in the stage of the Kleiner fibrosis classification[27]). Secondary histological outcomes included changes in the overall NAFLD activity score, individual components of the NAFLD activity score (steatosis, hepatocyte ballooning, lobular inflammation), and the Kleiner fibrosis stage.[27] Fibrosis stages 1a, 1b, and 1c were considered stage 1 for the purposes of analysis. Other secondary outcome measures included changes from baseline to 48 weeks in serum liver enzyme concentrations, non-invasive hepatic biomarkers (cytokeratin 18, enhanced liver fibrosis test), fasting lipid concentrations, glycaemic control (glucose, HbA_{1c}), insulin resistance (fasting homoeostasis model of assessment of insulin resistance [HOMA-IR] and adipose tissue insulin resistance [ADIPO-IR]), anthropometric measures (body weight, BMI, waist circumference), health-related quality-of-life scores (Medical Outcomes Study 36-Item Short-Form Health Survey version 2 [SF-36v2] physical and mental components), and daily dietary consumption.

Statistical analysis

On the basis of results from other pharmaceutical trials with biopsy-proven non-alcoholic steatohepatitis,[7,8] we assumed that up to 20% of patients undergoing current standard of care (the placebo group) would have an improvement in non-alcoholic steatohepatitis by week 48. To justify further investigation of liraglutide treatment, we considered improvement in liver histology in 50% of patients to be clinically relevant. The sample size was calculated using A'Hern's single-group phase 2 method, with a one-sided type I error of 5% and power of 90%. The design required 21 evaluable patients in the liraglutide group. To account for withdrawal, the recruitment target was increased to 25 patients per treatment group.[25]

All evaluable patients who underwent an end-of-treatment biopsy at week 48 were included in the modified intention-to-treat analysis. Evaluable patients were defined as those who underwent an end-of-treatment biopsy (week 48). Patients in each treatment group were categorised as either achieving the primary histological outcome (resolution of non-alcoholic steatohepatitis) or not. The study A'Hern's design stipulated that eight (38%) or more evaluable patients out of 21 patients in the liraglutide group had to achieve histological improvement for liraglutide to be deemed worthy of further investigation.[25]

An unpowered, preplanned secondary analysis of the primary outcome measure was done using the χ^2 test of the difference between the proportions of patients with histological improvement in each treatment group. We also did a sensitivity analysis for the primary outcome measure, in which patients who did not have an end-of-treatment liver biopsy were classified as having no histological improvement and included in the analysis. A post-hoc logistic regression analysis was undertaken to determine the treatment effect when adjusted for the stratification variables of trial site and type 2 diabetes, stage of liver fibrosis, as well as weight and glycaemic change during the trial.

We calculated adjusted relative risks for diabetes and fibrosis outcomes using the Mantel-Haenszel test.

	Liraglutide	Placebo	Relative risks or mean changes (95% CI) from baseline to 48 weeks (liraglutide *vs* placebo)	p value*
Primary outcome				
Number of patients with paired liver biopsies	23	22	..	..
Patients with resolution of non-alcoholic steatohepatitis	9 (39%)	2 (9%)	4·3 (1·0 to 17·7)	0·019
Changes from baseline in histopathological parameters				
Total NAFLD activity score				
Change in score	−1·3 (1·6)	−0·8 (1·2)	−0·5 (−1·3 to 0·3)	0·24
Patients with improvement	17 (74%)	14 (64%)	1·2 (0·8 to 1·7)	0·46
Hepatocyte ballooning score				
Mean change	−0·5 (0·7)	−0·2 (0·6)	−0·3 (−0·7 to 0·1)	0·15
Patients with improvement	14 (61%)	7 (32%)	1·9 (1·0 to 3·8)	0·05
Steatosis				
Change in score	−0·7 (0·8)	−0·4 (0·8)	−0·2 (−0·6 to 0·2)	0·32
Patients with improvement	19 (83%)	10 (45%)	1·8 (1·1 to 3·0)	0·009
Lobular inflammation				
Change in score	−0·2 (0·6)	−0·2 (0·5)	−0·01 (−0·3 to 0·3)	0·97
Patients with improvement	11 (48%)	12 (55%)	0·9 (0·5 to 1·6)	0·65
Kleiner fibrosis stage				
Change in score	−0·2 (0·8)	0·2 (1·0)	−0·4 (−0·8 to 0·1)	0·11
Patients with improvement	6 (26%)	3 (14%)	1·9 (0·5 to 6·7)	0·46†
Patients with worsening	2 (9%)	8 (36%)	0·2 (0·1 to 1·0)	0·04†

Data are n (%) or mean (SD). The mean of the two independent pathologists' scores for overall non-alcoholic fatty liver disease (NAFLD) activity score, steatosis, ballooning, inflammation, and fibrosis were used to compare the two treatment groups. The pathologists' agreement for overall NAFLD activity score using a weighted kappa was 0·854. *p values and mean changes from baseline were calculated by linear regression analysis using the baseline characteristic score and treatment as model covariates (equivalent to ANCOVA); for categorical comparisons, p values were determined by χ^2 analysis. †p value was determined by Fisher's exact test.

***Table 2*: Changes in liver histology after 48 weeks of treatment**

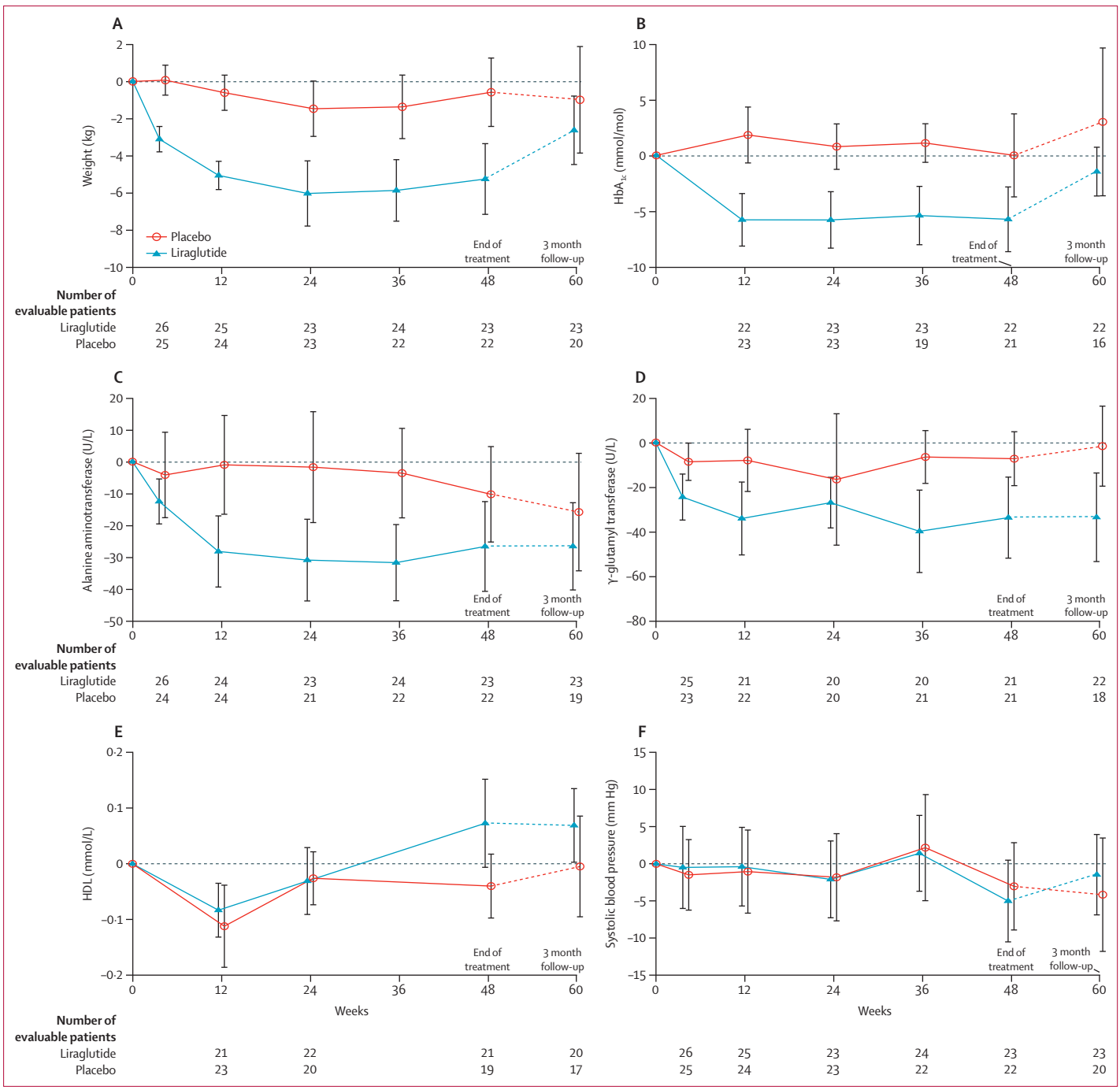

***Figure 2*: Changes from baseline in metabolic parameters and liver enzymes according to treatment group**
Mean change from baseline during treatment with liraglutide or placebo for up to 48 weeks followed by a 12 week post-treatment period are shown (dashed line) for (A) weight, (B) glycated haemoglobin A_{1c} (HbA_{1c}) concentration, (C) alanine aminotransferase concentration, (D) serum γ-glutamyl transpeptidase concentrations, (E) HDL cholesterol concentration, and (F) systolic blood pressure. Error bars show 95% CI.

Continuous secondary outcome measures were compared between treatment groups using linear regression, adjusting for parameter baseline values and allocated treatment (as model covariates, equivalent to ANCOVA), with multilevel modelling for key continuous outcome measures to account for repeated measures within each patient. Where appropriate we compared categorical secondary outcomes between treatment groups using χ^2 tests or Fisher's exact test. We used Stata Statistical Software version 12 for all statistical analysis. The trial is registered at ClinicalTrials.gov, number NCT01237119.

	Mean (SD) change from baseline to 48 weeks		Mean (95% CI) changes from baseline (liraglutide *vs* placebo)	p value*
	Liraglutide (n=23)	Placebo (n=22)		
Metabolic factors				
Glucose (mmol/L)	−1·0 (1·5)	0·72 (2·3)	−1·67 (−2·81 to −0·53)	0·005
Insulin (pmol/L)	−15·9 (54·7)	−34·7 (164·1)	−4·0 (−75·0 to 67·0)	0·91
HOMA-IR (glucose [mmol/L] × insulin [mmol × U/L])	−1·8 (3·7)	0·70 (9·49)	−2·74 (7·24 to 1·76)	0·23
Glycated haemoglobin A_{1c}				
Absolute concentration (mmol/mol)	−5·7 (6·9)	0·00 (8·7)	−5·18 (−9·91 to −0·44)	0·03
Percentage of total haemoglobin (%)	−0·53% (0·64%)	0·00% (0·80%)	−0·48% (−0·91% to −0·05%)	0·03
Non-esterified fatty acids (μmol/L)	−242 (374)	−121 (297)	−49 (−200 to 101)	0·51
ADIPO-IR (fasting non-essential fatty acid [mmol/L] × insulin [mmol × U/L])	−8·0 (10·1)	−7·6 (32·3)	−6·34 (−15·09 to 2·41)	0·15
Weight				
Absolute weight (kg)	−5·3 (4·7)	−0·6 (4·4)	−4·39 (−7·19 to −1·59)	0·003
Percentage (%)	−5·5 (4·9)	−0·7 (4·0)	−4·24 (−6·9 to −1·53)	0·003
Body-mass index (kg/m²)	−1·8 (1·67)	−0·3 (1·7)	−1·59 (−2·66 to −0·51)	0·005
Waist circumference (cm)	−3·52 (7·42)	−2·5 (7·6)	−2·54 (−7·32 to 2·25)	0·29
Systolic blood pressure (mm Hg)	−5·0 (13·4)	−3·0 (14·0)	−3·74 (−11·06 to 3·58)	0·31
Diastolic blood pressure (mm Hg)	0·6 (9·2)	2·4 (12·7)	−1·25 (−5·85 to 3·36)	0·59
Creatinine (mmol/L)	−2·2 (15·7)	0·5 (9·6)	2·36 (−5·06 to 9·79)	0·52
Lipids				
Total cholesterol (mmol/L)	0·01 (0·60)	−0·13 (0·91)	0·066 (−0·427 to 0·560)	0·79
HDL (mmol/L)	0·07 (0·19)	−0·04 (0·13)	0·134 (0·029 to 0·238)	0·01
LDL (mmol/L)	−0·1 (0·7)	−0·1 (0·9)	−0·126 (−0·622 to 0·371)	0·61
Triglycerides (mmol/L)	−0·02 (0·64)	0·18 (1·29)	−0·197 (−0·834 to 0·439)	0·53
Liver function				
Alanine aminotransferase (U/L)	−26·6 (34·4)	−10·2 (35·8)	−10·7 (−25·9 to 4·5)	0·16
Aspartate aminotransferase (U/L)	−15·8 (21·8)	−8·6 (28·3)	−6·7 (−19·3 to 5·9)	0·29
γ-glutamyl transferase (U/L)	−33·7 (42·5)	−7·2 (28·3)	−22·8 (−40·4 to −5·2)	0·01
Alkaline phosphatase (U/L)	−5·1 (11·7)	−1·2 (19·1)	−5·46 (−14·36 to 3·43)	0·22
Total bilirubin (μmol/L)	−1·7 (3·1)	−1·1 (3·1)	−0·63 (−2·52 to 1·26)	0·51
Albumin (g/L)	−0·2 (3·7)	−0·5 (3·2)	1·138 (−0·707 to 2·982)	0·22
Non-invasive biomarkers				
Caspase-cleaved cytokeratin-18 fragment M30 (U/L)	−185 (295)	−92 (327)	−86 (−188 to 16)	0·10
Enhanced liver fibrosis test	−0·3 (0·8)	0·1 (0·8)	−0·40 (−0·80 to 0·00)	0·05
Quality of life (SF-36v2)				
Physical component	1·9 (5·1)	−0·5 (8·0)	4·05 (0·20 to 7·90)	0·04
Mental component	−2·8 (6·9)	−3·3 (12·4)	1·50 (−4·64 to 7·65)	0·62
Daily dietary consumption (Block FFQ)				
Total calories (kcal)	−522 (708)	−291 (745)	−282 (−637 to 72)	0·12
Total protein (g)	−21 (28)	−7 (32)	−14 (−28 to 0)	0·06
Total fat (g)	−23 (29)	−19 (41)	−9 (−24 to 6)	0·22
Total carbohydrate (g)	−60 (101)	−23 (94)	−41 (−96 to 14)	0·14
Caffeine (mg)	3·4 (48·2)	−3·6 (67·2)	2·4 (−29·8 to 34·7)	0·88
Alcohol (g)	−1·8 (6·0)	−0·8 (2·9)	0·4 (−1·8 to 2·7)	0·69

HOMA-IR=homoeostasis model assessment-estimated insulin resistance. ADIPO-IR=adipose tissue insulin resistance. SF-36v2=Medical Outcomes Study 36-Item Short-Form Health Survey version 2. Block FFQ=Block Food Frequency Questionnaire. *p values and adjusted treatment changes determined by linear regression analysis regressing change on the baseline characteristic score and treatment.

***Table 3*: Changes in metabolic factors, liver function, non-invasive liver biomarkers, quality of life, and dietary consumption from baseline to 48 weeks**

Role of the funding source

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.

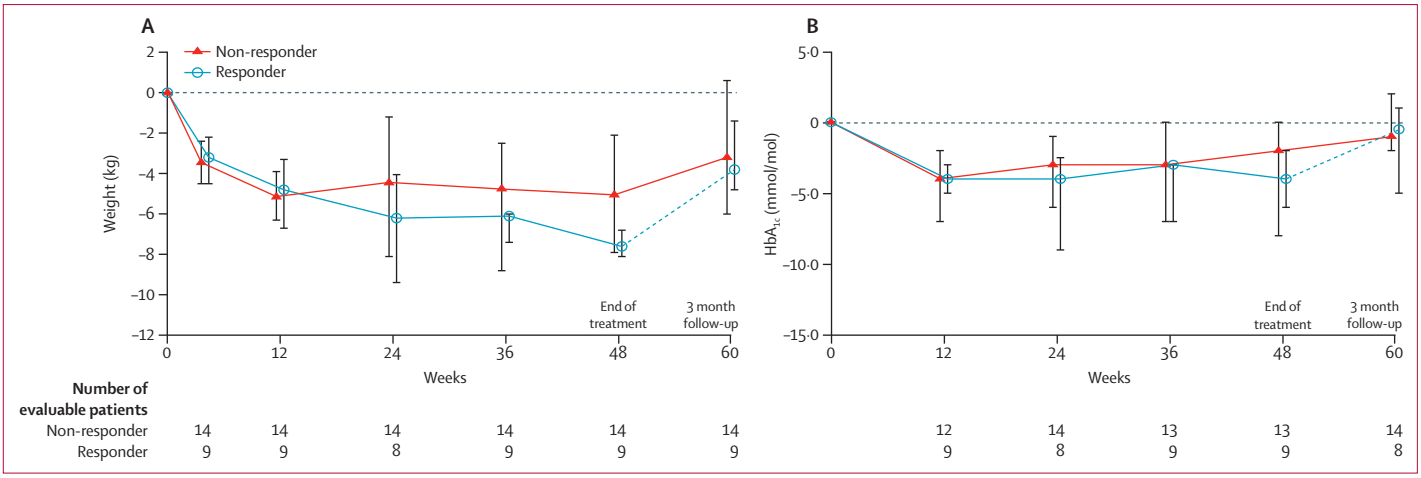

***Figure 3*: Changes from baseline in (A) weight and (B) glycated haemoglobin (HbA_{1c}) for patients with and without a histological response to liraglutide treatment**
Median changes from baseline in patients with histological improvement (responder) and no histological improvement (non-responders) receiving liraglutide treatment for 48 weeks and post-treatment follow-up (broken line) at 60 weeks. Error bars show IQR. Mean changes at 48 weeks and associated p values are reported in the appendix (p 23).

Results

Between Aug 1, 2010, and May 31, 2013, we randomly assigned 52 patients with histologically confirmed definite non-alcoholic steatohepatitis based on the central pathology review to receive liraglutide (n=26) or placebo (n=26; figure 1). With the exception of one patient in the placebo group, all patients received their assigned treatment. Three patients in each treatment group missed the end-of-treatment biopsies and withdrew from treatment. Baseline demographic, clinical, laboratory, and histological features were similar in the two groups (table 1). The mean total NAFLD activity score was 4·9 (SD 0·9; range 3·0–6·5) in the liraglutide group and 4·8 (0·9; 3·5–6·5) in the placebo group. On central review, of 52 patients, stage 3 fibrosis was present in ten (38%) patients in the liraglutide group and 11 (42%) patients in the placebo group, and cirrhosis was present in two (8%) patients in the liraglutide group and four (15%) patients in the placebo group.

23 (88%) patients in the liraglutide group and 22 (85%) patients in the placebo group had paired liver biopsies both at baseline and at 48 weeks, received treatment, and were included in the modified intention-to-treat analysis of the primary outcome measure (table 2). Nine (39%) of the 23 patients in the liraglutide group had resolution of definite non-alcoholic steatohepatitis with no worsening of fibrosis (table 2), thereby meeting the primary outcome (eight [38%] of 21 successes for the single-group analysis). A type I error of 0·027 and power of 89·5% were associated with this outcome under the same design conditions. In comparison, two (9%) of 22 patients in the placebo group had histological improvement (relative risk [RR] 4·3 [95% CI 1·0–17·7]; p=0·019; table 2).

A predefined sensitivity analysis of the primary outcome measure, in which patients with a missing end-of-treatment liver biopsy were defined as non-responders, showed that nine (35%) of 26 patients receiving liraglutide versus two (8%) of 26 patients receiving placebo achieved the primary outcome (appendix p 15). This equated to patients receiving liraglutide having a relative risk of 4·5 (95% CI 1·1–18·9; χ^2 test p=0·017) of achieving the primary outcome compared with patients in the placebo group. The odds ratio for the treatment effect resulting from a logistic regression analysis adjusting for the stratification factors of diabetes status and trial site was 7·8 (1·3–46·7, p=0·024; appendix p 17). No additional analyses were performed to account for missing data because low absolute numbers of dropout were recorded.

Three (38%) of eight patients with type 2 diabetes and six (40%) of 15 patients without type 2 diabetes achieved the primary outcome with liraglutide treatment (appendix p 16). Neither of the two patients assigned to placebo who achieved histological improvement had type 2 diabetes at baseline. The relative risk for non-diabetic patients achieving the primary endpoint in the liraglutide group was 3·4 (95% CI 0·8–14·4; p=0·11) compared with placebo. As none of the patients who responded to placebo had diabetes, a factor of 0·5 was added to all four values in the contingency table for diabetic patients. Using this adjustment, the relative risk for diabetic patients receiving liraglutide meeting the primary endpoint was 4·7 (0·3–75·0; p=0·20) relative to placebo. We found no evidence of heterogeneity (p=0·841). The relative risk of response when receiving liraglutide compared with placebo, adjusted for diabetes using the stratified Mantel–Haenszel test, was 3·7 (1·0–13·5; p=0·047).

Fewer patients in the liraglutide group had progression of fibrosis than in the placebo group, and a greater proportion of patients in the liraglutide group had improvements in steatosis and hepatocyte ballooning compared with the placebo group (table 2). However, no differences were seen in lobular inflammation and overall NAFLD activity score (table 2).

Serum γ-glutamyl transferase concentrations differed significantly between patients in the liraglutide and placebo groups after 48 weeks of treatment, whereas no difference was detected in the change in serum aminotransferase concentrations (figure 2, table 3). However, multilevel modelling (appendix p 20) of longitudinal parameters indicated significant differences in both alanine aminotransferase and γ-glutamyl transferase between the two treatment groups, thereby supporting the changes in concentration with time (figure 2). We detected a trend, albeit non-significant, towards a reduced concentration of serum biomarkers of hepatocyte injury (serum caspase-cleaved cytokeratin 18) in the liraglutide group; however, the serum enhanced liver fibrosis test results showed a reduction in fibrosis in patients receiving liraglutide compared with placebo (table 3).

48 weeks' treatment with liraglutide was associated with significant reductions in bodyweight and BMI (table 3) compared with placebo. Most of the beneficial effects of liraglutide related to weight were achieved by 12 weeks of treatment and were sustained throughout treatment (figure 2). Patients who received liraglutide also had significant improvements in HbA_{1c} concentrations compared with patients in the placebo group. Improvements in weight and HbA_{1c} concentration were confirmed by multilevel modelling (appendix p 20). Notably, weight increased and metabolic changes reverted towards baseline 12 weeks after liraglutide was discontinued (figure 2; appendix p 13). We found no significant difference in HDL concentration or systolic blood pressure between the two treatment groups when applying multilevel modelling.

We undertook post-hoc analysis of the clinical or laboratory test changes that occurred in patients who had resolution of non-alcoholic steatohepatitis with liraglutide treatment (nine responders) compared with patients who did not respond (14 non-responders; appendix p 24). Changes in weight and glycaemic control (ie, HbA_{1c} concentration) in patients receiving liraglutide were not significantly different in the comparison of responders and non-responders (figure 3).

Patients receiving liraglutide reported significant improvements in the physical component score of the SF-36vs questionnaire compared with patients in the placebo group (table 2).

Most adverse events 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). Another three patients in the liraglutide group withdrew from treatment because of needle phobia, work commitments, and loss to follow-up; these patients withdrew their consent from the study and did not undergo end-of-treatment liver biopsy.

We noted two (8%) serious adverse events in the liraglutide group (tuberculosis and migraines), both of which were deemed unrelated to treatment. No deaths or

	Liraglutide (n=26)	Placebo (n=26)
Overall treatment withdrawal rate	5 (19%)	5 (19%)
Treatment withdrawal due to adverse event	2 (8%)	1 (4%)
Participants with serious adverse event	2 (8%)	2 (8%)
Participants with grade 3 adverse event	3 (12%)*	4 (15%)
Participants with any grade of adverse event	23 (88%)	24 (92%)
Adverse event†		
Gastrointestinal disorders	21 (81%)	17 (65%)
Nausea	12 (46%)	10 (38%)
Diarrhoea	10 (38%)	5 (19%)
Abdominal pain	8 (31%)	7 (27%)
Vomiting	5 (19%)	3 (12%)
Constipation	7 (27%)	0
Dyspepsia	4 (15%)	1 (4%)
Flatulence	4 (15%)	0
Bloating	4 (15%)	0
Eye disorders	1 (4%)	5 (19%)
Cardiac disorders	3 (12%)	3 (12%)
General disorders and administration site conditions	13 (50%)	17 (65%)
Injection site reaction	9 (35%)	10 (38%)
Fatigue	4 (15%)	5 (19%)
Influenza-like symptoms	3 (12%)	6 (23%)
Peripheral oedema	2 (8%)	3 (12%)
Chills	4 (15%)	0
Non-specific pain	2 (8%)	4 (15%)
Infections and infestations	3 (12%)	9 (35%)
Chest infection	0	5 (19%)
Urinary tract infection	0	4 (15%)
Investigations	5 (19%)	11 (42%)
Increased γ-glutamyl transferase	0	3 (12%)
Increased aspartate aminotransferase	1 (4%)	3 (12%)
Metabolism and nutrition disorders	11 (42%)	6 (23%)
Anorexia (loss of appetite)	8 (31%)	2 (8%)
Musculoskeletal and connective disorders	8 (31%)	14 (54%)
Back pain	3 (12%)	7 (27%)
Arthralgia	1 (4%)	4 (15%)
Muscle cramps	0	3 (12%)
Nervous system disorders	14 (54%)	11 (42%)
Dizziness	6 (23%)	7 (27%)
Headaches or migraines	9 (35%)	7 (27%)
Psychiatric disorders	6 (23%)	7 (27%)
Depression	2 (8%)	6 (23%)
Renal and urinary disorders	2 (8%)	3 (12%)
Respiratory, thoracic, and mediastinal disorders	3 (12%)	6 (23%)
Cough	2 (8%)	3 (12%)
Skin and soft tissue disorders	7 (27%)	3 (12%)

* Grade 3 adverse events in the liraglutide group were headache, diarrhoea, syncope, and trapped nerve. †The listed adverse events (any severity) have an incidence greater than 10% in any treatment group, by system organ class and preferred term. No deaths were reported in the trial period (week 0–60). No cases of hepatitis or pancreatitis were reported. We monitored serum calcitonin concentrations and found no significant increase in either group, with the exception of one patient who received placebo. This calcitonin concentration was normal on further testing, which included review by an endocrinologist.

Table 4: **Adverse events**

cases of pancreatitis, hepatitis, or liver failure were reported during the trial. No patients developed antibodies against liraglutide when tested at week 60. Post-hoc analysis highlighted that the numbers of adverse events were similar between patients with and without advanced fibrosis (F3–F4; appendix p 29).

Discussion

In this double-blind, randomised, placebo-controlled phase 2 trial, the longacting GLP-1 analogue, liraglutide, met the predefined primary endpoint and led to resolution of non-alcoholic steatohepatitis in nine (39%) of 23 patients. Moreover, improvements in weight and glycaemic control with liraglutide might have a favourable effect on the future risk of cardiovascular disease and premature death in patients with non-alcoholic steatohepatitis, although longer-term outcome studies are needed to confirm this hypothesis. Study withdrawal (ie, no end-of-treatment biopsy) rates were the same in both treatment groups and had no effect on the primary endpoint. Liraglutide was safe and well tolerated, irrespective of the severity of underlying disease.

This study has a number of strengths. First, this is the first randomised, placebo-controlled trial to report the effect of a GLP-1 analogue on liver histology in patients with non-alcoholic steatohepatitis. Second, the study population included patients with and without type 2 diabetes and liver cirrhosis. Third, in light of the documented intra-variability and inter-variability in the assessment of liver biopsies,[27] we had two independent, blinded, central assessments of liver biopsies at baseline (same sections used to assess eligibility and effect of treatment) and at the end of treatment. This avoided inclusion of patients without definite non-alcoholic steatohepatitis, as was the case in the PIVENS trial[7] (21% of patients) and FLINT trial[8] (20% of patients). Fourth, we collated detailed recording of concomitant drug use (ie, lipid-lowering and anti-diabetic drugs) and dietary intake (ie, caffeine, vitamin E, alcohol) throughout 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 endpoint, and the level of histological resolution of non-alcoholic steatohepatitis with liraglutide (nine [39%] of 23 patients) was comparable to that previously reported with vitamin E (29 [36%] of 80 patients and pioglitazone (33 [47%] of 70 patients),[7] whereas histological resolution was achieved in 22 [22%] of 102 patients after treatment with obeticholic acid. The reported placebo rate (9%) was slightly lower than those previously described (13–21%),[7,8] but this difference is probably because clearance of non-alcoholic steatohepatitis in this study had to occur without any worsening of fibrosis (which has not been previously adopted).

Although patients in the liraglutide group met the primary endpoint, liraglutide did not result in significant mean changes in the composite NAFLD activity score, as reported with pioglitazone, vitamin E, and obeticholic acid.[7,8] Notably, a greater proportion of patients receiving 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 receiving liraglutide showed improved steatosis and hepatocyte ballooning, which would suggest that a larger study could identify significant mean changes in NAFLD activity score. Liraglutide also showed evidence of efficacy in a post-hoc analysis using the primary endpoints (which used the NAFLD activity score) in the FLINT and PIVENS trials (appendix p 23).

Resolution of non-alcoholic steatohepatitis was selected as the primary endpoint instead of changes in NAFLD activity score, in keeping with guidance from an expert consortium.[26] Notably, the NAFLD activity score does not predict liver-related morbidity or mortality, whereas the presence of non-alcoholic steatohepatitis (as opposed to simple NAFLD) is associated with a significant increase in liver-related outcomes and all-cause mortality.[3,4]

Recent data have revealed the importance of liver fibrosis as the key determinant of clinical outcomes in patients with non-alcoholic steatohepatitis.[29] Despite the relatively short duration of this trial, fewer patients receiving liraglutide had progression of fibrosis, and we also found a greater reduction in serum liver enzyme concentrations in patients within the liraglutide group than in the placebo group. The absence of a difference in mean change in fibrosis stage between the two groups probably reflects the duration of treatment, and a longer treatment course should be assessed. 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 (appendix p 18).

The clearance of non-alcoholic steatohepatitis by liraglutide is probably multifactorial 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, shows a possible continued, modest reduction in weight loss in responders. Post-hoc logistic regression analysis for additional covariates (appendix p 18) indicated that the effects of liraglutide are probably due to a combination of a direct hepatic effect (odds ratio for treatment effect adjusted for weight 4·12 [95% CI 0·66–25·8; p=0·131]) and an effect on weight loss. This synergistic effect would imply that the mechanism of action of GLP-1 analogues in non-alcoholic steatohepatitis is not solely explained by improvements in weight and metabolic phenotype, and indeed in-vitro studies have

shown that GLP-1 analogues improve the ability of hepatocytes to handle excess non-esterified fatty acids and lipid production by modulating lipid transport, beta-oxidation, and de-novo lipogenesis,[16,18,30] all of which have been implicated in the pathogenesis of non-alcoholic steatohepatitis. These observations have been confirmed in liraglutide-treated mice, in which reductions in hepatic steatosis, insulin resistance (via clamp technique), and endoplasmic reticulum oxidative stress happened in the absence of weight loss.[16,31] When this study was designed, liraglutide was only available at the 1·8 mg dose, and since then a higher dose (3·0 mg) has been approved for weight management.[12] A higher dose of liraglutide could possibly provide greater efficacy in the setting of non-alcoholic steatohepatitis, although the level of added benefit is unclear.

Safety data for the use of GLP-1 analogues in liver disease are limited to solitary case reports[20,21] and retrospective analysis of large cohorts of patients with type 2 diabetes and elevated transaminase concentrations.[23,24] Liraglutide was generally well tolerated in the study population and had a similar adverse event profile to placebo, with the exception of predictable gastrointestinal symptoms (mainly diarrhoea, constipation, and loss of appetite). These adverse events were, however, mainly transient and mild-to-moderate in severity.

At present, there is a significant unmet need of therapies for patients with non-alcoholic steatohepatitis cirrhosis. We therefore elected to include patients with cirrhosis in this study to pilot the efficacy, but importantly highlight the safety of liraglutide in this setting. Because cirrhosis is the final stage of the Kleiner scoring system (eg, 4/4), patients with cirrhosis might have been advantaged in achieving the primary endpoint since they could not have worsening of fibrosis. However, their inclusion did not inflate the histological response in the liraglutide group, as only one patient with cirrhosis met the primary endpoint, and this patient received placebo.

The unique combination of histological efficacy and improvement of the metabolic syndrome with liraglutide render it an attractive therapy for patients with non-alcoholic steatohepatitis and warrant further investigation in larger studies.

Contributors

MJA, SCG, JWT, and PNN (chief investigator) had the original concept of the LEAN trial. MJA, DB, PG, DS, SCG, JWT, RMB, SGH, and PNN designed the LEAN trial and wrote or reviewed all protocol versions. RMB and SGH did the central histopathology review of all pre-treatment and post-treatment liver biopsies. MJA and DB (senior trials coordinator) submitted all research ethics committee applications, MHRA clinical trial applications, and local research and development applications and coordinated the trial sites. PG (senior statistician) prepared the annual Data Management Committee reports and did all the statistical analysis. MJA, GPA, GA, MAA, and PNN recruited the participants, and MJA, GPA, DH, KG, DB, RP, JMH, GA, MAA, RMB, SGH, and PNN were responsible for data collection. MJA, PG, RMB, SGH, and PNN participated in data analysis and interpretation. MJA, PG, and PNN wrote the Article and all authors participated in the Article review. MJA, PG, and PNN were responsible for preparation of the tables and figures. MJA, PG, and PNN are guarantors.

Members of the LEAN trial team

All authors are members of the LEAN trial team. Additional members of the team are Manpreet Wilku, Christine Russell, Salma Iqbal, Christopher Corbett, Michelle Yun Kyong Lee, Jennifer Keely, and nursing staff at the Wellcome Trust Clinical Research Facility (Queen Elizabeth Hospital Birmingham/National Institute for Health Research Birmingham Liver Biomedical Research Unit/Cancer Research UK Clinical Trials Unit; Birmingham, UK); Maggie Nicholls and Susanne Henry (Nottingham University Hospitals NHS Trust/ Nottingham Digestive Diseases Biomedical Research Unity; Nottingham, UK); Martin Lewis, Erica Dixon, and Sally Myers (Hull Royal Infirmary; Hull, UK); and Samantha Sharman and Rebecca Bishop (St James's University Hospital; Leeds, UK).

Declaration of interests

PNN and MJA have received free trial drug supply from Novo Nordisk for use in the LEAN trial. 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 GlaxoSmithKline. PG, GPA, DB, RP, DS, DH, KG, JMH, GA, MAA, JWT, RMB, and SGH declare no competing interests.

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