JGH Open An open access journal of gastroenterology and hepatology
JGHF
doi:10.1002/jgh3.12464

ORIGINAL ARTICLE

Liraglutide reduces attenuation coefficient as a measure of hepatic steatosis during 16 weeks' treatment in nondiabetic obese patients: A pilot trial

Xiao Jing Wang,* Ping Gong,† Chenyun Zhou,† Chengwu Huang,† U-Wai Lok,† Shanshan Tang,† Ann Taylor,* Deborah Eckert,* Shigao Chen† and Michael Camilleri*

*Clinical Enteric Neuroscience Translational and Epidemiological Research (C.E.N.T.E.R.), and Division of Gastroenterology and Hepatology, Department of Medicine and †Division of Ultrasound Research, Department of Radiology, Mayo Clinic, Rochester, Minnesota, USA

Key words
body mass index, GLP-1 analog, hepatic steatosis, ultrasound imaging.

Accepted for publication 13 November 2020.

Correspondence
Michael Camilleri, Mayo Clinic, 200 First Street S.W., Charlton Building, Rm. 8-110, Rochester, MN 55905, USA.
Email: camilleri.michael@mayo.edu

Declaration of conflict of interest: None
Author contribution: Xiao Jing Wang and Ping Gong drafted the initial manuscript, which was revised by Michael Camilleri. Chenyun Zhou, Chengwu Huang, U-Wai Lok, Shanshan Tang, and Shigao Chen obtained ultrasound measurements on patients and reviewed the manuscript. Ann Taylor and Deborah Eckert recruited all patients and coordinated appointments, as well as reviewed manuscript.

Funding support: National Institutes of HealthR21-DK121943RO1-DK67071

Abstract

Background and Aim: Liraglutide, a long-acting GLP-1 analog, is approved for the treatment of obesity with improvements in fasting blood glucose, hemoglobin A1c, and cardiovascular health. Our aim was to measure the impact of liraglutide dose for obesity on hepatic steatosis measured by ultrasound.
Methods: A single-center, randomized, double-blind, placebo-controlled pilot trial was undertaken in nondiabetic obese, otherwise healthy patients aged 18–65 years. Participants were randomly assigned to receive subcutaneous liraglutide (3.0 mg) or placebo over 16 weeks with dose escalation following US Food and Drug Administration guidelines. Both groups received standardized nutritional and behavioral counseling during the 16 weeks. Hepatic fat content was measured by ultrasound at baseline, 8 weeks, and 16 weeks as an attenuation coefficient (ACE). Effects of treatment were assessed using t-test for the entire groups and for patient subgroup with baseline ACE >0.66 (indicating significant steatosis).
Results: Among 30 patients (93% female) enrolled, 16 were randomized to placebo and 14 to liraglutide. Baseline body mass indices (BMIs) and average age were similar in the two groups. After 16 weeks, the liraglutide group had a significant improvement in steatosis ACE scores (-0.068 ± 0.02 *vs* -0.0077 ± 0.02 placebo, $P = 0.05$). Change in steatosis was positively correlated with change in BMI ($R^2 = 0.402$, $P = 0.0007$). Within the liraglutide group, patients with baseline ACE >0.66 had improvement in ACE (-0.134 ± 0.03) compared to those without significant steatosis (-0.041 ± 0.02, $P = 0.05$).
Conclusions: In this pilot trial, liraglutide, 3.0 mg over 16 weeks, reduced hepatic steatosis; a reduction in hepatic steatosis is correlated with BMI reduction, and effects are particularly evident in those with a significant degree of steatosis by ultrasound imaging.

Introduction

Liraglutide, a long-acting GLP-1 analog, is a GLP-1 receptor agonist currently approved for the treatment of obesity, as well as diabetes. Liraglutide has shown improvements in fasting blood glucose, hemoglobin A1c, waist circumference, and cardiovascular health including blood pressure.

Non-alcoholic fatty liver disease (NAFLD) is increasingly recognized as a complication of obesity. Weight loss is the most reliable treatment for improvement of hepatic steatosis, and loss of 10% bodyweight may reverse steatosis and steatohepatitis.[1] Hypoglycemic agents such as metformin,[2] piaglitazone,[3] and the GLP-1 agonists liraglutide and exenatide have inconsistent effects on hepatic steatosis in diabetes. In a meta-analysis of different doses of liraglutide in diabetic patients with abnormal liver enzymes who were uncontrolled on oral medications or lifestyle alone, only liraglutide 1.8 mg showed a weight-dependent improvement in hepatic steatosis (LEAD-2).[4] A subsequent study of NASH patients performed in the United Kingdom showed that liraglutide 1.8 mg led to the resolution of NASH and improvement of the fibrosis stage compared to placebo (LEAN).[5] In a study of patients with diabetes, liraglutide 1.8 mg administered for 26 weeks reduced subcutaneous, but not visceral, fat or hepatic steatosis as evaluated by magnetic resonance imaging.[6] The American Association for the Study of Liver Disease recommended pioglitazone for biopsy-proven NASH in patients

with and without diabetes but concluded that evidence was not strong enough to recommend liraglutide to treat NAFLD.[1]

The effect of liraglutide, dosed for obesity management (that is escalated to 3.0 mg), on hepatic steatosis has been evaluated predominantly in patients with diabetes.

Our hypothesis was that weight loss associated with liraglutide 3.0 mg daily results in a reduction in hepatic steatosis. Our aim was to measure the effect of liraglutide 3.0 mg daily on hepatic steatosis measured ultrasonographically in obese patients without diabetes.

Methods

Study design and participants. A single-center, randomized, double-blind, placebo-controlled pilot study was undertaken to study the impact of liraglutide 3.0 mg compared to placebo (both daily dosed by subcutaneous injection for 16 weeks) on weight loss and hepatic steatosis. The study was conducted exclusively at the Mayo Clinic (Rochester, MN, USA) and was approved by its Institutional Review Board (IRB #15-001783); all patients provided informed consent.

Nondiabetic patients, aged 18–65 years, with body mass index (BMI) ≥30 kg/m^2 who were otherwise healthy and residing within 125 miles of Rochester, MN, USA, were recruited. Patients with unstable medical or psychiatric disease or any condition that would interfere with study conduct or interpretation were excluded.

Standard US Food and Drug Administration recommendations on the use of liraglutide were followed for eligibility. Screening questionnaires appraised psychiatric symptoms,[7] alcohol use disorders,[8] eating disorders,[9] and intake of medications—whether prescribed or over the counter (except multivitamins)—within 7 days of the study.

Individuals with delayed gastric emptying of solids (>90th percentile of normal values according to gender[10]) were excluded as it was considered potentially dangerous to significantly increase the delay in gastric emptying with a GLP-1 receptor agonist. Delayed gastric emptying was defined as less than 36% emptied at 2 h and less than 87% emptied at 4 h in men and less than 31% emptied at 2 h and less than 81% at 4 h in women.[10]

Randomization and masking. A randomization schedule, which was computer generated by the study statistician's office, was submitted to the Mayo Clinic Research Pharmacy. Participants were randomly assigned (1:1) to liraglutide or placebo, with no stratification factors. Allocations were concealed by the study pharmacists, who assigned patients to treatment groups and were physically separated from the Clinical Research Trials Unit at Mayo Clinic, where the patients were enrolled by the study coordinators.

The study personnel (coordinators and technicians performing measurements, and the physicians involved in the study) did not have knowledge of the next assignment in the sequence, which could not be revealed by the research pharmacists or guessed by the study staff.

Group assignments were blinded to the participants, study staff, and care providers until the data were transmitted to the statistician for data lock. No formal evaluation of the success of study masking was conducted.

Procedures. All participants underwent screening visits and baseline measurements of gastrointestinal, behavioral, and psychological factors and received the same doses and dose escalation (Fig. 1). Liraglutide was purchased from Novo Nordisk (Plainsboro, NJ, USA) and stored in the Mayo Clinic Research Pharmacy; all liraglutide and saline placebo supplies were dispensed from the research pharmacy.

Liraglutide was administered as recommended by the US Food and Drug Administration: initiated at 0.6 mg daily for 1 week, with instructions to increase by 0.6 mg weekly until 3.0 mg was reached (over 4 weeks). Once the maintenance dose of 3.0 mg was reached (typically) by week 5, participants returned every 4 weeks to obtain a new supply of the study drug. Similar weekly volume increments were used for placebo injections during the dose escalation phase.

Participants received education and a pamphlet with directions for use provided by nurses in the Clinical Research Trials Unit who were not associated with the research study. All participants also received standardized dietetic and behavioral advice for weight reduction therapy.

Concomitant medications. Permitted concomitant medications during the study were birth control pill, estrogen, and any "as needed" medication as long as they did not alter gastric emptying or accommodation or satiation.

We assessed safety and tolerability throughout the study by evaluation of adverse events, vital signs, fasting blood glucose, and physical examination. These assessments were conducted at baseline and at visits for dose escalations at weeks 2, 3, 4, and 5, as well as follow-up visits at weeks 8 and 16.

Assessments

Hepatic steatosis and fibrosis assessments. Hepatic steatosis and fibrosis were assessed noninvasively at baseline, 8 weeks, and 16 weeks[11] utilizing ultrasound technology.

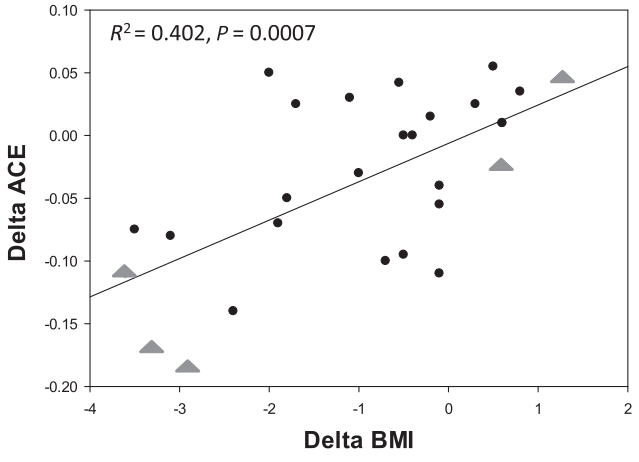

Figure 1 Linear regression of change in body mass index (BMI) compared to change in hepatic steatosis (attenuation coefficient [ACE]). ▲, Steatosis at baseline; ●, non steatosis at baseline.

Method to measure steatosis. Steatosis was assessed by ultrasonic attenuation, which was measured using a new ultrasound attenuation estimation method named Reference Frequency Method utilizing the General Electric LE9 system.[11] This ultrasonic attenuation coefficient (ACE) is an estimate of the total ultrasonic attenuation (go-and-return path) in liver and is expressed in dB/cm/MHz. This shear wave technology has been validated against magnetic resonance (MR) elastography in the evaluation of hepatic fibrosis.[12] In addition, utilization of the same technology for assessment of hepatic steatosis in patients at baseline and postintervention (8 and 16 weeks) provides some degree of internal validity of the method. Prior to the current study, there had not been any demonstration of responsiveness to treatment in the measured parameters of steatosis.

Procedure. Ultrasound data used for ACE were acquired from the most uniform liver parenchyma section, avoiding major vessels, cysts, or the gallbladder. For each patient, 10 repeated ACE measurements were performed, with the patient breathing freely.

Method to measure fibrosis. Measurements were obtained by using the General Electric LE9 system with an abdominal two-dimensional (2D) shear wave elastography application. LOGIQ E9 2D shear wave elastography uses comb-push excitation to produce shear waves and a time-aligned sequential tracking method to detect shear wave signals.[13] The comb-push technique simultaneously produces multiple shear waves inside the tissue to improve the shear wave signal-to-noise ratio, followed by directional filtering to remove the shear wave interferences, which allows robust reconstruction of 2D shear elasticity maps.[14,15]

Procedure. This is included in the Appendix I. The acquisition region of interest (ROI) was placed at least 1.5–2.0 cm from the Glisson capsule to avoid shear wave measurement bias.[16]

Outcomes. The prespecified primary end-point for the clinical trial as registered under ClinicalTrials.gov (NCT03523273) was to evaluate the relationship of weight loss and gastric emptying and accommodation in patients undergoing liraglutide treatment for obesity. The objective of this subgroup pilot study, conducted as part of the larger study, was to understand changes in liver function and hepatic steatosis in patients who were otherwise without significant comorbidities and who were undergoing treatment for obesity with liraglutide. Therefore, this pilot trial was not conducted to study established NAFLD or steatohepatitis. The primary end-points of this pilot substudy were changes in ACE and fibrosis scores at weeks 8 and 16 of treatment compared to baseline. This evaluation was then stratified to evaluate patients with baseline steatosis as measured by ACE >0.66, indicative of significant baseline steatosis, and those without baseline steatosis, that is, ACE <0.66.[17] This cutoff was determined *a priori* based on prior work correlating ultrasound testing with MR elastography findings.[17]

Bodyweight changes at weeks 8 and 16 compared to baseline were also analyzed, although this was not the primary focus of this study, and the same participants were enrolled in a study of the relationship of effect on gastric emptying and efficacy of liraglutide on weight loss (ClinicalTrials.gov Identifier: NCT03523273).

Adverse events, including nausea, abdominal pain, diarrhea, light headedness, and injection site reactions, were assessed and recorded by the study coordinators and physicians at each study visit.

Statistical power. Given that our hypothesis was that liraglutide's effect on steatosis was associated with its effect on weight, the study power was anchored on the effects of liraglutide on bodyweight. Utilizing prior studies on the effect of liraglutide on weight loss and gastric emptying conducted at our center,[18] we determined that 15 patients in each treatment group would provide 80% power (at $\alpha = 0.05$) to detect a difference in weight loss. Statistical analysis was conducted to address the hypothesis that there was a treatment effect with liraglutide compared with placebo on the study end-points and that there was a correlation between weight loss and reduction in steatosis. Statistical significance was prespecified at a *P*-value less than or equal to 0.05.

Statistical analysis and handling of missing data. Analysis was conducted using intention-to-treat principles. All available data from randomized patients who participated in ultrasound evaluation were used in the statistical analyses. In patients who did not complete all three ultrasound evaluations, all available data points were used for assessment of the analysis of change, for example, in ACE. In addition, for each missing data value, we imputed the average value for all patients in the study (i.e. the mean for all patients randomized in the study to either liraglutide or placebo) and reduced the degrees of freedom by one for each data value imputed for that end-point. This method has been used extensively in the literature, including our prior clinical trial of liraglutide in obesity.[18]

We analyzed the effects of liraglutide and placebo on change in hepatic steatosis and fibrosis at weeks 8 and 16 of the treatment period compared to baseline values, using t-tests of mean change. We then performed subgroup analysis on patients who had baseline ACE >0.66, indicating significant steatosis. Spearman correlations were used to assess the relationship between change in steatosis and weight loss at weeks 8 and 16. All analyses were performed with JMP version 14.

Results

Participant disposition and demographics. A total of 30 patients were enrolled between July 2018 and April 2019. Sixteen participants were assigned to the placebo arm and 14 to the treatment arm. One participant in the placebo arm and one participant in the liraglutide arm withdrew before week 8. Two additional participants in the liraglutide group withdrew between weeks 8 and 16. Overall, six data points were missing of a total of 90 observations in 30 patients—two from the placebo and four from the liraglutide arms. Thus, there was imputation of <7% of data, and the missing data were replaced by the average at the respective time points for the entire cohort of patients, with a reduction in the degrees of freedom for the number of points imputed at each time of assessment.

Table 1 Baseline demographics and measurements

	Placebo (n = 16)	Liraglutide (n = 14)	P-value
Female, % (n)	94 (15)	93 (13)	0.92
Age, years, mean (SD)	35.7 (11.0)	36.4 (8.9)	0.84
Baseline BMI, kg/m^2,mean (SD)	38.4 (5.7)	37.0 (4.3)	0.48
Baseline ACE, mean (SD)	0.63 (0.03)	0.64 (0.04)	0.80
Baseline fibrosis, mean (SD)	6.59 (0.59)	5.48 (0.63)	0.21
% with baseline steatosis, % (n)	31.3 (5)	28.6 (4)	0.87

ACE, attenuation coefficient; BMI, body mass index; SD, standard deviation.

Demographics in the two groups did not differ significantly (Table 1). The mean BMI was 38.4 ± 5.7 kg/m^2 in the placebo group compared to 37.0 ± 4.3 kg/m^2 in the liraglutide group (P = 0.48). Baseline steatosis measurements (ACE) and fibrosis scores did not differ between groups; similar numbers of patients in each group had steatosis at baseline (25% [n = 4] in placebo *vs* 35.8% [n = 5] in liraglutide group).

Hepatic steatosis. At week 16 of intervention, patients receiving liraglutide had an improvement in steatosis scores as measured by ACE scores (−0.068 ± 0.02 *vs* −0.0077 ± 0.02 placebo, P = 0.0506). Patients in the liraglutide arm had statistically significant improvement in BMI (−1.91 ± 0.37 kg/m^2) compared to placebo (−0.21 + 0.32 kg/m^2, P = 0.002). The change in steatosis was positively correlated with a change in BMI (R^2 = 0.402, P = 0.0007; Fig. 1), with a linear regression model showing that a 0.3 kg/m^2 change in BMI was positively associated with a 0.1-point change in ACE.

Among patients with baseline ACE >0.66, there was significant improvement in mean ACE scores with liraglutide (−0.134 ± 0.053) compared to placebo (0.0025 ± 0.041, P = 0.012) (Fig. 2). Within the liraglutide group, patients with baseline ACE >0.66 had improvement in ACE (mean −0.134 ± 0.053) compared to those with ACE <0.66 (−0.041 ± 0.056, P = 0.054) (Fig. 2).

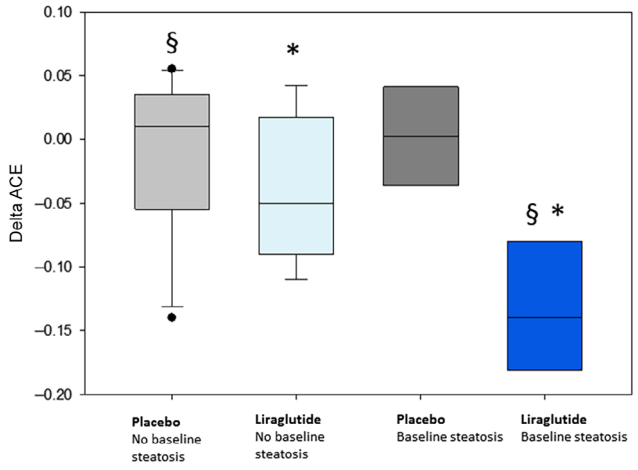

Figure 2 Change in hepatic steatosis (attenuation coefficient [ACE]) by group. *§Statistically significance difference between groups.

Hepatic fibrosis. None of the participants had evidence of advanced fibrosis at baseline on imaging. Changes in fibrosis were not significantly different between the groups after 16 weeks of treatment.

Discussion

Our study has shown that, in nondiabetic obese patients, liraglutide 3.0 mg daily was superior to placebo in reducing hepatic steatosis measured ultrasonagraphically with a validated method that appraised attenuation of ultrasound waves within the liver. In addition, the degree of change of hepatic steatosis was significantly and positively correlated with the effects on BMI. We were unable to observe changes in hepatic fibrosis, in part because of the relatively short (16 weeks) duration of the trial in the context of a prolonged process like fibrosis and also because there was no significant fibrosis in the cohort recruited for this study. Among our study patients with obesity, 30% had a degree of hepatic steatosis at baseline high enough to be considered NAFLD, which is similar to the global prevalence of NAFLD of 25%.[19] Liraglutide 3.0 mg daily decreased the degree of hepatic steatosis in patients with NAFLD at baseline. This effect was significant when compared to the impact of placebo on patients with baseline NAFLD and to the effect of liraglutide on patients without baseline NAFLD. The correlation of improvement in steatosis with weight loss observed in the current study was consistent with prior studies of hepatic steatosis.[20]

GLP-1 agonists, including liraglutide, impact the development of hepatic steatosis in multiple points of the proposed pathogenetic "two-hit" pathways of insulin resistance and inflammation[21] and decreased autophagy,[22] leading to lipid deposition. Mouse models suggest that liraglutide promotes lipolysis, increased free fatty acid uptake, and oxidation, leading to decreased triglycerides.[23] Liraglutide also promotes autophagy in *in vitro* cell lines and rat models, decreasing lipid overaccumulation and ameliorating high fat diet-induced NAFLD by activating autophagy and thereby maintaining normal hepatocyte function by promoting turnover of long-lived proteins.[24] Liraglutide also actively promotes lipolysis[23] and decreases inflammatory markers, including TNF-a and NK-kB, in the liver of mice fed a high fat diet.[25] Liraglutide also inhibits inflammasome activation, decreases reactive oxygen species generation, and attenuates mitochondrial dysfunction.[26]

Strengths/limitations. The strengths of this study include the double-blind and randomized design. Patients in both arms were provided behavioral counseling and check-in visits at the

same interval. A majority of the participants completed dose escalation of liraglutide, and 87% completed the study.

Ultrasound measurements were obtained by the same team of trained researchers to minimize operator error. The ultrasound operators were blinded to the treatment assigned to the study participants. The degree of steatosis was measured utilizing ultrasound shear wave technology throughout the course of the study. Utilization of ACE as a measure of hepatic steatosis is a technique that has not undergone large trial external validation against existing techniques such as MR elastography or transient elastography. However, utilization of the same technology for patient assessment at baseline and postintervention at 8 and 16 weeks allowed for internal validity as we evaluated delta-ACE as our end-point. In addition, this pilot trial has demonstrated responsiveness of this end-point in a randomized, placebo-controlled study. Shear wave technology has been validated against MR elastography in the evaluation of hepatic fibrosis.[12]

The study is limited by the small number of patients with significant NAFLD with no significant fibrosis identified at baseline. We were unable to assess the impact of liraglutide on hepatic fibrosis given that none of our patients had fibrosis at baseline. Our statistical analysis is limited by our study size, as well as the use of imputation in 4.3% and 10.5% of data points missing in placebo and liraglutide arms, respectively. Despite these limitations, there were statistically significant differences in the improvement of steatosis based on *a priori* determined power calculations based on an effect size on BMI that could be achieved with a total of 30 patients assigned to two treatment groups. This is an additional strength as the patient cohort was likely representative of obese individuals rather than a potentially skewed population with already established or clinically evident NAFLD.

Given these pilot study results, a further trial of 3.0 mg liraglutide with larger samples of obese, nondiabetic patients with known NAFLD and even hepatic fibrosis should be undertaken. The 3.0-mg dose can also be compared to the effects of a 1.8-mg dose given the more widespread use of the latter dose in patients with type 2 diabetes mellitus who also manifest hepatic steatosis.

Conclusion

Liraglutide 3 mg dose is superior to placebo in numerically (P = 0.0506) reducing hepatic steatosis in nondiabetic obesity. Patients with significant steatosis at baseline had the greatest degree of improvement in ACE with liraglutide; this was statistically significant over placebo treatment. Reduction in BMI with liraglutide 3.0 mg over 16 weeks was positively correlated with improvement in hepatic steatosis as measured using an ultrasound-based ACE.

Acknowledgments

The authors thank Mrs. Cindy Stanislav for excellent secretarial assistance. Michael Camilleri takes full responsibility for the conduct of the study. He had access to the data and control of the decision to publish. Michael Camilleri's research on obesity is supported by RO1-DK67071 grant from National Institutes of Health. Dr. Shigao Chen's work is supported by R21-DK121943 grant from National Institutes of Health.

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Appendix I

The patient was placed in the left posterior oblique position and with the right arm across the chest. The sonographers used the ninth, eighth, or seventh intercostal spaces in the right anterior axillary line, depending on the image qualities, to image the liver and obtain measurements. The size of the acquisition ROI was always the same by system default. The depth of the ROI depended on the thickness of the body wall but should have been the same for each patient across different intercostal spaces. Ten repeated shear wave elastography (SWE) measurements were acquired with end-expiration breath holds for each patient. Each SWE acquisition takes about 1–2 s (acquisition time plus cooling time). A total of 10 2D SWE images were acquired for each patient.