Liraglutide suppresses postprandial triglyceride and apolipoprotein B48 elevations after a fat-rich meal in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled, cross-over trial

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Aims: Postprandial triglyceridaemia is a risk factor for cardiovascular disease (CVD). This study investigated the effects of steady-state liraglutide 1.8mg versus placebo on postprandial plasma lipid concentrations after 3 weeks of treatment in patients with type 2 diabetes mellitus (T2DM).

Methods: In a cross-over trial, patients with T2DM (n = 20, 18–75 years, BMI 18.5–40 kg/m²) were randomized to once-daily subcutaneous liraglutide (weekly dose escalation from 0.6 to 1.8 mg) and placebo. After each 3-week period, a standardized fat-rich meal was provided, and the effects of liraglutide on triglyceride (primary endpoint AUC₀–₈ h), apolipoprotein B48, non-esterified fatty acids, glycaemic responses and gastric emptying were assessed. ClinicalTrials.gov ID: NCT00993304. Funding: Novo Nordisk A/S.

Results: After 3 weeks, mean postprandial triglyceride (AUC₀–₈ h liraglutide/placebo treatment-ratio 0.72, 95% CI [0.62–0.83], p = 0.0004) and apolipoprotein B48 (AUC₀–₈ h ratio 0.65 [0.58–0.73], p < 0.0001) significantly decreased with liraglutide 1.8 mg versus placebo, as did iAUC₀–₈ h and C_{max} (p < 0.001). No significant treatment differences were observed for non-esterified fatty acids. Mean postprandial glucose and glucagon AUC₀–₈ h and C_{max} were significantly reduced with liraglutide versus placebo. Postprandial gastric emptying rate [assessed by paracetamol absorption (liquid phase) and the ¹³C-octanoate breath test (solid phase)] displayed no treatment differences. Mean low-density lipoprotein and total cholesterol decreased significantly with liraglutide versus placebo.

Conclusions: Liraglutide treatment in patients with T2DM significantly reduced postprandial excursions of triglyceride and apolipoprotein B48 after a fat-rich meal, independently of gastric emptying. Results indicate liraglutide’s potential to reduce CVD risk via improvement of postprandial lipaemia.

Keywords: antidiabetic drug, GLP-1 analogue, lipid-lowering therapy, phase I-II study, randomized trial, type II diabetes

Introduction

The prevalence of type 2 diabetes mellitus (T2DM) continues to increase [1]. Most individuals with T2DM have insulin resistance and are at increased risk of developing cardiovascular disease (CVD) [2]. Dyslipidaemia accompanying T2DM plays an important role in the pathogenesis of atherosclerosis, and postprandial triglyceridaemia is a distinct component of diabetic dyslipidaemia [3]. Postprandial triglyceridaemia is an independent risk factor for CVD in individuals with and without T2DM [4–6]. The increased CVD risk may be attributed to the formation of lipids and lipoproteins generated through chylomicron metabolism during the postprandial triglyceridaemia [7,8]. These lipid particles can penetrate the endothelial cell layer and have atherogenic or proinflammatory actions [7]. Therefore, there is a need for treatments that can reduce postprandial lipid concentrations, and reduce the risk of CVD.

Acute intravenous administration of the incretin hormone glucagon-like peptide-1 (GLP-1) has been shown to lower postprandial triglyceride levels in healthy volunteers [9]. However, GLP-1 is rapidly inactivated by the enzyme dipeptidyl peptidase-4 (DPP-4) [8,10]. Short-term treatment with vildagliptin and sitagliptin, selective DPP-4 inhibitors, has been shown to reduce postprandial lipaemia in T2DM [10,11], presumably by inhibiting the inactivation of endogenous GLP-1 by DPP-4. Also, treatment with the GLP-1 receptor agonist exenatide reduces postprandial triglyceride and lipoprotein concentrations in T2DM, both in the short term [12,13] and after one year [14].

Liraglutide, a human GLP-1 analogue with 97% homology to endogenous GLP-1 and a half-life of about 13 h, is approved for the treatment of T2DM, administered by once-daily
subcutaneous injection at doses up to 1.8 mg. Long-term clinical trials with liraglutide have shown its ability to improve glycaemic control and reduce body weight [15], but its effects on postprandial triglyceridaemia have not been investigated. The aim of this study was to investigate the effect of steady-state treatment with liraglutide 1.8 mg versus placebo on postprandial triglyceride and other lipid parameters, including the apolipoprotein B48 (apoB48; the intestinally derived structural lipoprotein in chylomicrons, which are secreted following ingestion of lipids), following a standardized fat-rich meal in patients with T2DM. As changes in postprandial lipaemia may be correlated to a delay in gastric emptying [9,16], this was also assessed. A reduction of postprandial triglyceridaemia would add to the therapeutic value of liraglutide with respect to its potential to reduce the risk of CVD in T2DM.

Methods
Participants
Participants were recruited between October 2009 and December 2010 at one research site in Denmark and one in Germany, and included men and women with T2DM (diet and exercise-treated, or treated with oral anti-diabetic drugs (OADs; metformin, sulphfonylureas and glinides) in mono- or combination therapy) diagnosed ≥6 months previously, aged 18–75 years, with body mass index (BMI) 18.5–40 kg/m², fasting serum triglycerides 0.5–5.0 mmol/l and HbA1c 6.5–10.0%. Main exclusion criteria: pulse ≥91 or <100 beats/min, systolic blood pressure (BP) <91 or >160 mmHg, diastolic BP <51 or >100 mmHg, impaired liver function, impaired renal function (creatinine clearance <60 ml/min [17]), previous insulin treatment, known clinically significant active CVD, and alcoholism/drug abuse or positive alcohol/drug tests at screening. Use of statins was allowed if treatment was stable for ≥3 months before screening. Participants provided written informed consent. The protocol was approved by local ethics committees, and the trial was performed in accordance with the Declaration of Helsinki and ICH Good Clinical Practice. The study is registered at ClinicalTrials.gov, number NCT00993304.

Study Design and Procedures
This was a randomized, double-blind, placebo-controlled, cross-over trial in 20 participants (figure 1). Treatment order of liraglutide 1.8 mg or placebo was determined through random assignment (1:1). Randomization was stratified by gender and country. Treatment allocation was blinded to participants, investigators and sponsor.

Individuals randomized to liraglutide underwent weekly dose escalation, starting at 0.6 mg/day (with 0.6 mg increments to 1.8 mg). Liraglutide vehicle was used as placebo to ensure blinding. Liraglutide (6.0 mg/ml) and placebo were provided in identical FlexPen® devices (Novo Nordisk A/S, Bagsværd, Denmark) and were administered subcutaneously in the abdomen. Participants were instructed to administer liraglutide or placebo before bedtime on days 1–16 and between 21:00 hours and 22:00 hours on days 17–21 (to standardize timing of the pharmacokinetic (PK) and meal-test evaluations). The total planned treatment period was 42 days (21 days on each treatment). A wash-out period of 3–9 weeks was included to ensure that the lipid response in the second period was not influenced by the first period, and to ensure elimination of liraglutide in liraglutide-treated individuals. There were a total of 10 visits: screening, randomization immediately before treatment period 1 (comprising 3 weekly visits), a visit at the start of treatment period 2 (also comprising 3 weekly visits), and a follow-up visit.

Gastric emptying was assessed by the paracetamol absorption technique [18] and the 13C-octanoate breath test [19] during a test meal. On day 22 (at steady-state) in both treatment periods, participants received 1.5 g paracetamol [3 × 500 mg tablets of Panodil® Brus, GlaxoSmithKline, Copenhagen, Denmark (Danish site), or Paracetamol-ratiopharm®, Ratiopharm GmbH, Ulm, Germany (German site)] and 100 mg 13C-octanoate (Cambridge Isotope Laboratories Inc., Andover, MA, USA) as markers of gastric emptying. The paracetamol was dosed orally at start of the breakfast test meal, which was served at the approximate time of peak liraglutide concentration (tmax) [20], 10–11 h post-dosing. The test meal consisted of white bread, rye bread, butter, cheese (45%), sausage, bacon, scrambled egg, milk (1.5% fat) and decaffeinated coffee or tea. 13C-octanoate was added to the egg yolk prior to cooking. The energy content of the test meal was 969 kcal (4055 kJ) at the Danish site (macronutrient composition 65 energy% [E%] fat, 16 E% protein and 19 E% carbohydrate) and 961 kcal (4021 kJ) at the German site (macronutrient composition 61 E% fat, 19 E% protein and 20 E% carbohydrate). The minimal differences between sites were not considered important. The test meal was to be consumed within 15 min.

Clinical Outcomes
The primary objective of the trial was to investigate the effect of treatment with steady-state liraglutide 1.8 mg versus placebo on postprandial plasma triglyceride concentrations following a standardized high-fat (>60 E%) meal. The primary endpoint was the total area under the postprandial triglycerides concentration–time curve from meal-time until 8 h (AUC0–8h), measured after 3 weeks. Other secondary efficacy endpoints included apoB48, NEFA, glucose, insulin,
C-peptide and glucagon, and gastric emptying measures, assessed after 3 weeks. Changes from baseline in body weight and lipids and steady-state PK of liraglutide 1.8 mg, all after 3 weeks, were also assessed. Safety endpoints included vital signs, adverse events, standard laboratory tests, and concentrations of pancreatic enzymes lipase and amylase.

Measurement of apoB48 was performed using an enzyme-linked immunosorbsent assay (BioVendor GmbH, Heidelberg, Germany) [21]. The average within-assay coefficient of variation was 3.5%. Triglyceride and glucose concentrations in plasma were measured using enzymatic assays (Roche Diagnostics GmbH, Mannheim, Germany). NEFA were analysed using an enzymatic colorimetric assay (Roche, Penzberg, Germany).

Other laboratory analyses were performed using standard methods. Data for 13C-octanoate were analysed by Aarhus University, Foulum, Tjele, Denmark [22]. Liraglutide plasma concentration was measured by Huntingdon Life Science Ltd., Alconbury, UK [20]. All other analyses were performed by Nuvisan Pharma Services, Neu-Ulma, Germany.

**Statistical Analysis**

The AUC for postprandial triglyceride was compared using a two-sided t-test of equal means with 5% significance and at least 80% power. The trial was powered to show a difference in the baseline-corrected triglyceride AUC0–8h ratio of 30% for liraglutide versus placebo. The intra-subject standard deviation of the difference in log-transformed AUC0–8h for triglyceride was assumed to be 0.30 [23]. Hence, it was determined that at least 13 completing individuals with two usable triglyceride profiles were required, and 20 to be randomized (dropouts could be replaced).

Data analysis was performed on the full analysis set, comprising all randomized individuals. Analyses were 2-sided and performed at 5% significance. We used a **ANOVA** for prespecified analyses of iAUC0–8h, log-transformed AUC0–8h and Cmax, and for post hoc analyses of fasting values for lipids and glycaemic parameters. The model included treatment, treatment period, country, gender and use of statins as fixed factors, and subject as a random effect. The primary null hypothesis was no difference between treatments. For iAUC0–8h and log-transformed AUC0–8h and Cmax for glucose, insulin, C-peptide and glucagon, the same **ANOVA** model was used, excluding use of statins. This model was also used for analysis of log-transformed postprandial paracetamol endpoints, and for a post hoc analysis of the insulin/glucose iAUC0–8h ratio. For paracetamol endpoints, liraglutide and placebo treatments were declared equivalent if the 90% CI of the ratio for AUC0–60min, AUC0–8h, and Cmax were fully contained within the interval 0.80–1.25. Change in body weight was analysed using an **ANOVA** model with treatment, treatment period, country, and gender as fixed factors, subject as a fixed effect, and baseline value as covariate. For non-transformed endpoints, least-square means and treatment differences were estimated in the **ANOVA** model, and presented together with 95% CI. For log-transformed endpoints, the estimated treatment differences and the corresponding CI were back-transformed to the original scale and presented as the ratio between liraglutide and placebo, with corresponding 95% CI.

We analysed changes in total, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol from baseline **post hoc**, using an **ANOVA** model with treatment, treatment period, country, gender and use of statins as fixed factors, subject as random effect and baseline value as covariate. A **post hoc** analysis of the ratio of HDL/LDL cholesterol used an **ANOVA** model with treatment, treatment period and subject as fixed effect, and baseline value as covariate. SAS (version 9.1; SAS Institute, Cary, NC, USA) was used for analyses.

AUC0–8h was derived using standard model-free, non-compartmental methods and calculated using the trapezoidal method with observed values and actual time points. iAUC0–8h was defined as the AUC0–8h above the fasting value (15 min prior to meal). Decremental AUC0–8h (dAUC0–8h) for NEFA was defined as the AUC0–8h above the curve but below the fasting value.

**Results**

**Subject Demographics**

Twenty white individuals (11 male and 9 female, aged 53–73 years, BMI 24–39 kg/m²) were randomized and exposed in the trial (Table 1), and included in the full analysis set; 8 were from Denmark and 12 were from Germany. Of those randomized, 18 completed the trial. Postprandial meal-test endpoints for six individuals were excluded from analyses due to non-compliance with protocol requirements.

**Lipids**

After 3 weeks of treatment, no significant treatment differences for mean fasting values of triglyceride or NEFA were observed, whilst fasting concentrations of apoB48 were statistically significantly lower with liraglutide 1.8 mg than placebo (Table 2).

Mean postprandial responses for triglyceride and apoB48 were markedly lower with liraglutide compared with placebo (figure 2A, B). Both triglyceride and apoB48 rose substantially after 3 weeks, were also assessed. Safety endpoints included vital signs, adverse events, standard laboratory tests, and concentrations of pancreatic enzymes lipase and amylase.

**Table 1.** Baseline characteristics of all 20 randomized and exposed participants.

| Sex, n (%) | Male | 11 (55%) |
| Age (years) | 62.7 (53.0–73.0) |
| BMI (kg/m²) | 30.8 (24.2–39.4) |
| Duration of diabetes (years) | 8.3 (2.3–17.7) |
| Triglyceride (mmol/l) | 1.9 (0.5–3.8) |
| Use of statins, n (%) | Yes | 9 (45%) |
| No | 11 (55%) |
| Co-treatment with OADs, n (%) | Metformin monotherapy | 11 (55%) |
| Sulphonylurea monotherapy | 2 (10%) |
| Metformin + sulphonylurea | 5 (25%) |
| No OADs | 2 (10%) |

Data are mean (range) unless specified. BMI, body mass index; OAD, oral anti-diabetic drug.
Table 2. Comparison of changes in plasma concentrations of triglycerides, apolipoprotein B48, non-esterified fatty acids, glucose, insulin, C-peptide and glucagon after 3 weeks of treatment.

| Parameter          | Liraglutide, 1.8 mg | Placebo | Treatment-ratio (R) or difference (D) for liraglutide vs. placebo | p value* |
|--------------------|---------------------|---------|------------------------------------------------------------------|---------|
| Triglycerides      | n = 16              | n = 14  |                                                                 |         |
| Fasting value (mmol/l) | 1.4                | 1.7     | R: 0.84 (0.68–1.05)                                              | 0.11    |
| AUC_{0–8h} (h*mmol/l) | 13.7               | 19.0    | R: 0.72 (0.62–0.83)                                              | 0.0004  |
| iAUC_{0–8h} (h*mmol/l) | 2.9                | 6.7     | D: -3.9 (–5.8; -2.0)                                             | 0.0008  |
| iAUC per h (mmol/l)   | 0.4 (0.3)          | 0.8 (0.5) | –                                                                  |         |
| C_{max} (mmol/l)     | 2.1                 | 3.2     | R: 0.66 (0.56–0.78)                                              | 0.0002  |
| t_{max} (h)          | 4.2 (2.5)          | 4.2 (0.7) | –                                                                  |         |
| Apolipoprotein B48   | n = 15              | n = 14  |                                                                 |         |
| Fasting value (g/l)   | 0.006              | 0.008   | R: 0.71 (0.57–0.88)                                              | 0.006   |
| AUC_{0–8h} (h*g/l)   | 0.08               | 0.12    | R: 0.85 (0.58–0.73)                                              | <0.0001 |
| iAUC_{0–8h} (h*g/l)   | 0.03               | 0.07    | D: -0.03 (–0.05; -0.02)                                          | 0.0003  |
| C_{max} (g/l)        | 0.01               | 0.02    | R: 0.59 (0.46–0.75)                                              | 0.0009  |
| t_{max} (h)          | 3.7 (1.8)          | 4.4 (0.8) | –                                                                  |         |
| NEFA                | n = 16              | n = 14  |                                                                 |         |
| Fasting value (mmol/l) | 0.65               | 0.60    | R: 1.10 (0.92–1.30)                                              | 0.26    |
| AUC_{0–8h} (h*mmol/l) | 3.8                | 3.9     | R: 0.98 (0.86–1.1)                                               | 0.78    |
| dAUC_{0–8h} (h*mmol/l) | 1.9            | 1.5     | D: 0.31 (–0.38; 0.99)                                            | 0.34    |
| C_{min} (mmol/l)     | 0.25               | 0.21    | R: 1.2 (0.93–1.5)                                                | 0.14    |
| iAUC per h (mmol/l)   | 3.8 (1.4)          | 2.3 (1.2) | –                                                                  |         |
| Glucose              | n = 15              | n = 12  |                                                                 |         |
| Fasting value (mmol/l) | 6.0                | 7.9     | R: 0.76 (0.68–0.83)                                              | 0.0001  |
| AUC_{0–8h} (h*mmol/l) | 55.9               | 68.5    | R: 0.82 (0.73–0.92)                                              | 0.004   |
| iAUC_{0–8h} (h*mmol/l) | 10.5              | 11.2    | D: -0.66 (–4.0; 2.7)                                             | 0.67    |
| iAUC per h (mmol/l)   | 1.2 (0.8)          | 1.6 (0.8) | –                                                                  |         |
| C_{max} (mmol/l)     | 10.0               | 11.9    | R: 0.84 (0.72–0.98)                                              | 0.03    |
| t_{max} (h)          | 1.6 (0.67)         | 1.6 (0.72) | –                                                                  |         |
| Insulin              | n = 15              | n = 14  |                                                                 |         |
| Fasting value (pmol/l) | 83.1               | 61.8    | R: 1.4 (1.1–1.7)                                                 | 0.02    |
| AUC_{0–8h} (h*pmol/l) | 1554              | 1256    | R: 1.2 (1.1–1.4)                                                 | 0.01    |
| iAUC_{0–8h} (h*pmol/l) | 909              | 796     | D: 113 (–142; 368)                                               | 0.36    |
| iAUC per h (pmol/l)   | 113 (46.4)         | 100 (39.1) | –                                                                  |         |
| C_{max} (pmol/l)     | 407                | 320     | R: 1.3 (1.0–1.6)                                                 | 0.04    |
| t_{max} (h)          | 2.0 (0.77)         | 1.5 (0.88) | –                                                                  |         |
| C-peptide            | n = 15              | n = 14  |                                                                 |         |
| Fasting value (nmol/l) | 1.01              | 0.77    | R: 1.3 (1.2–1.5)                                                 | 0.0004  |
| AUC_{0–8h} (h*nmol/l) | 15.1              | 13.3    | R: 1.1 (1.0–1.3)                                                 | 0.03    |
| iAUC_{0–8h} (h*nmol/l) | 7.1              | 7.3     | D: -0.22 (–1.5; 1.0)                                             | 0.70    |
| C_{max} (nmol/l)     | 2.9                | 2.5     | R: 1.2 (1.0–1.4)                                                 | 0.03    |
| t_{max} (h)          | 2.6 (0.74)         | 2.6 (0.87) | –                                                                  |         |
| Glucagon             | n = 15              | n = 14  |                                                                 |         |
| Fasting value (ng/l)   | 93.1               | 99.5    | R: 0.94 (0.77–1.14)                                              | 0.47    |
| AUC_{0–8h} (h*ng/l)   | 864               | 961     | R: 0.90 (0.82–0.98)                                              | 0.02    |
| iAUC_{0–8h} (h*ng/l)   | 131              | 201     | D: -70 (–167; 267)                                               | 0.15    |
| C_{max} (ng/l)       | 145               | 164     | R: 0.89 (0.81–0.98)                                              | 0.02    |
| t_{max} (h)          | 1.4 (1.4)          | 1.3 (1.6) | –                                                                  |         |

Data for liraglutide and placebo are estimated means, except for t_{max} and iAUC per h [mean (s.d.)]. N numbers show the number of participants included in the analysis, which differs according to parameter. Fasting values were measured 15 min prior to meal start.

*Obtained from an ANCOVA model. For incremental (i) and decremental (d; NEFA) AUC_{0–8h}, estimated treatment differences (and 95% CI) are shown.

The remaining endpoints were log-transformed, and for these, the estimated treatment differences and the corresponding CI were back-transformed to the original scale and presented as the ratio between liraglutide and placebo, with corresponding 95% CI. For triglycerides, apolipoprotein B48 and NEFA endpoints, the model included treatment, treatment period, country, gender and statin use as fixed factors, and subject as a random effect. For glucose, insulin, C-peptide and glucagon endpoints, the same ANCOVA model was used, excluding statin use.

from fasting values with placebo (Table 2), peaking 4–5 h after the meal, an effect that was abolished with liraglutide treatment. For triglyceride, the endpoints AUC_{0–8h} and iAUC_{0–8h} were 28% and 57% lower, respectively, with liraglutide compared with placebo, and C_{max} was also reduced. The mean iAUC/h for triglyceride was reduced by 50% with liraglutide versus placebo. Similarly, AUC_{0–8h} and iAUC_{0–8h} for apoB48 were 33% and 57% lower, respectively, with liraglutide versus placebo, and C_{max} occurred earlier with liraglutide. Mean postprandial NEFA declined from fasting values in the first 2 h after the meal, an effect that was abolished with liraglutide treatment.
Effect of liraglutide and placebo on postprandial triglyceride (A), apolipoprotein B48 (B) and NEFA (C) concentrations. Data are presented as mean ± s.e.m.

Figure 2. Effect of liraglutide and placebo on postprandial triglyceride (A), apolipoprotein B48 (B) and NEFA (C) concentrations. Data are presented as mean ± s.e.m.

meal (figure 2C). The decline appeared to be less pronounced but persisted longer with liraglutide compared to placebo. However, no statistically significant differences between treatments were observed for AUC₀–₈h, dAUC₀–₈h or Cₘ₉₉₉₉.

Glycaemic Parameters

After 3 weeks of treatment, mean fasting glucose concentrations were significantly lower with liraglutide 1.8 mg than placebo, and mean fasting concentrations of insulin and C-peptide were significantly higher with liraglutide than placebo (Table 2).

Mean postprandial responses for glucose, insulin, C-peptide and glucagon increased from fasting levels in the first 1–2 h after the meal (figure 3). For glucose and glucagon, both AUC₀–₈h and Cₘ₉₉₉₉ were statistically significantly reduced with liraglutide versus placebo (Table 2). In contrast, insulin and C-peptide AUC₀–₈h and Cₘ₉₉₉₉ were significantly increased with liraglutide versus placebo. No statistically significant differences between treatments were observed for iAUC₀–₈h for any glycaemic parameter. In a post hoc analysis of the insulin/glucose ratio for iAUC, no significant difference between liraglutide and placebo was found.

Gastric Emptying

After 3 weeks of treatment, the mean postprandial paracetamol concentration profiles were similar for liraglutide 1.8 mg and placebo (figure 4A). Gastric emptying assessed by paracetamol concentration was found to be equivalent for liraglutide and placebo, as the 90% CIs of the ratios for AUC₀–₆₀₉₉₉₉ [treatment-ratio 1.02, 95% CI (0.86–1.21)], AUC₀–₈h [treatment-ratio 1.06 (1.02–1.11)] and Cₘ₉₉₉₉ [treatment-ratio 1.00 (0.83–1.19)] were all fully contained within the interval 0.80–1.25. These results were supported by ¹³C-octanoate breath test, where no apparent difference between treatments was observed (figure 4B).

Body Weight

Mean body weight at baseline was 88.2 kg (Table 1). After 3 weeks, participants on liraglutide 1.8 mg lost an estimated 1.1 kg mean body weight, and those on placebo gained an estimated mean 0.7 kg (treatment-difference −1.8 kg [−2.5; −1.0], p < 0.0001).

Changes in Other Lipid Parameters

Changes from baseline in total, LDL and HDL cholesterol were analysed post hoc. Mean total cholesterol at baseline was 5.27 mmol/l (s.d. 1.20) for liraglutide 1.8 mg and 5.13 (1.22) mmol/l for placebo. Total cholesterol decreased with liraglutide and placebo by estimated means 0.55 and 0.13 mmol/l, respectively [treatment-difference −0.43 (−0.82; −0.03) mmol/l, p = 0.04]. Mean LDL-cholesterol concentrations at baseline were 2.93 (0.96) mmol/l for liraglutide and 2.83 (0.99) mmol/l for placebo. LDL-cholesterol decreased with liraglutide by an estimated mean 0.14 mmol/l and increased with placebo by 0.15 mmol/l (treatment-difference −0.30 [−0.59; −0.01] mmol/l, p = 0.04). Mean HDL cholesterol concentrations at baseline were 1.40 (0.33) mmol/l for liraglutide and 1.34 (0.32) mmol/l for placebo. HDL cholesterol also decreased with both treatments (0.16 and 0.09 mmol/l); no effect of liraglutide on HDL cholesterol was observed (treatment-difference −0.07 [−0.18; 0.03] mmol/l, p = 0.18). Similarly, no effect of liraglutide 1.8 mg on HDL/LDL ratio was observed by post hoc analysis (treatment-difference 0.04 [−0.02; 0.10], p = 0.20). The effect of liraglutide on lipids was unaffected by co-treatment with statins (data not shown).

PK Endpoints

Steady-state PK was assessed after dosing on day 21 after 3 weeks of liraglutide treatment. The geometric mean AUC₀–₂₄₉₉₉₉ for liraglutide was 561,404 h × pmol/l (CV 36); Cₘ₉₉₉₉ was
A

Figure 3. Effect of liraglutide and placebo on postprandial glucose (A), insulin (B), C-peptide (C) and glucagon (D) concentrations. Data are presented as mean ± s.e.m.

29,820 pmol/l (CV 39) ; and terminal half-life ($t_{1/2}$) was 14.1 h (CV 15.2).

Safety

Liraglutide was generally well tolerated. The frequency of adverse events, predominantly gastrointestinal disorders, was greater in the liraglutide group (Table 3). Two participants withdrew from the trial: one because of an adverse event of vomiting (moderate severity) after 18 days on liraglutide (1.8 mg dose), and one because of difficulties in blood sample drawing. Three participants on liraglutide experienced a total of 10 hypoglycaemic episodes (none severe), including eight in one participant who was also on a sulphonylurea. No hypoglycaemic episodes occurred with placebo recipients. Mean systolic BP decreased slightly from 137 (s.d. 11) to 136 (12) mmHg with liraglutide treatment, and increased from 135 (15) to 142 (15) mmHg with placebo. Mean diastolic BP increased slightly with both treatments (by 2.0 and 0.5 mmHg, respectively). Finally, an increase in mean (s.d.) pulse from 71 (10) to 82 (11) beats/min was observed with liraglutide. A minimal increase was observed with placebo (74 [11] to 75 [8] beats/min).

There appeared to be a treatment-related asymptomatic increase in median serum lipase activity, remaining below the upper limit of normal (UNL) range. One clinically significant laboratory abnormality of increased lipase was reported in the liraglutide group.

Discussion

In T2DM, steady-state liraglutide treatment significantly reduced postprandial triglyceride and apoB48 concentrations compared with placebo after 3 weeks of treatment, whereas no treatment differences were observed with respect to gastric emptying, either for the liquid or solid phase. Reductions in postprandial glucose and glucagon responses, as well as in LDL and total cholesterol concentrations from baseline, were also observed with liraglutide. Body weight decreased, as expected.

This is the first trial to demonstrate that liraglutide has beneficial effects on the postprandial lipid response, in agreement with results from other trials with native GLP-1 [9] and the GLP-1 receptor agonist exenatide [12–14]. A single subcutaneous injection of exenatide markedly reduced postprandial triglyceride and apoB48 responses to a fat-rich meal in 35 individuals with impaired glucose tolerance or recent-onset T2DM [12]. Moreover, 2 weeks of treatment with exenatide compared to the DPP-4 inhibitor sitagliptin inhibited 2-h postprandial triglyceride and delayed gastric emptying in T2DM [13]. Interestingly, DPP-4 inhibitors concomitantly with an increase in circulating GLP-1 cause a reduction in postprandial triglyceride and apoB48 responses [24]. Postprandial lipaemia is also ameliorated by several non-incretin therapies, including insulin and insulin analogues, acarbose, metformin, sulphonylureas, statins and fibrates [25,26].
The effect of exenatide on postprandial lipaemia may be influenced by several mechanisms mediated by GLP-1 receptor signalling, as well as delayed gastric emptying [14]. The apolipoprotein apoB48 is present in chylomicrons that transport ingested lipids from the intestine to the liver for storage; in the current trial, apoB48 decreased in parallel with triglyceride. The mechanism responsible for the lowering of postprandial triglyceride with liraglutide may therefore be due to a decreased secretion of apoB48-containing chylomicron particles in the intestinal mucosa and subsequent reduced absorption of triglyceride [8]. This hypothesis is supported by the fact that fasting apoB48 concentrations were lower with liraglutide compared with placebo after 3 weeks of treatment. Increased catabolism of triglyceride by adipose tissue may also have occurred [8]. Moreover, GLP-1 has been shown to attenuate postprandial triglyceride secretion [9]. The precise mechanism is yet to be determined, as many factors can influence circulating triglyceride concentrations [5,23].

For example, postprandial triglyceridaemia is known to be modulated by meal composition, with differential effects of fat type and protein quality [23,27,28]. However, this trial had a standardized meal with high-fat content to maximize the response, and a cross-over design to emphasize the treatment effect.

The attenuated postprandial decline in NEFA with liraglutide compared to placebo has also been reported with exenatide treatment [12], and may be explained by a suppressing effect of insulin on adipocyte lipolysis. Nevertheless, no statistically significant differences between treatments in any of the postprandial NEFA parameters were observed.

The protein content in the tested meal would be expected to induce a postprandial glucagon response peaking shortly after the meal, as observed [29]. As reported previously, and consistent with the mechanism of action of liraglutide [30], this study confirmed a reduction in glucagon AUC0–8h and Cmax with liraglutide compared to placebo, whereas fasting glucagon concentrations and iAUC0–8h were not significantly different. The observed reductions in fasting glucose and AUC0–8h and Cmax with liraglutide versus placebo, and the corresponding improvements in insulin, are consistent with previous findings [15,31]. In T2DM, chronically elevated glucose (and lipid) concentrations may cause beta-cell damage in the presence of insulin resistance, due to an impaired metabolism [32]. Since the current study compares liraglutide treatment with that of placebo, the improvement in postprandial lipaemia with liraglutide could, at least partly, be attributable to suppression of postprandial glycaemia.

The effects of liraglutide on postprandial triglyceride and apoB48 excursions appeared to be independent of gastric emptying. No differences in gastric emptying between the two treatments were observed after 3 weeks with either the paracetamol absorption technique, which assesses the gastric emptying rate of the liquid phase, or the 13C-octanoate breath test, which assesses solid phase gastric emptying. The presence of fat in the small intestine can delay gastric emptying [33]; hence ingestion of the fat-rich test meal could have slowed gastric emptying and masked any additional effect of liraglutide. Results may also partly reflect tachyphylaxis/desensitisation of the gastric emptying response to liraglutide, as has been showed after chronic liraglutide treatment in preclinical experiments in rats [34]. Tachyphylaxis of gastric emptying has also been reported with native GLP-1 in humans [35]. However, clinical results with liraglutide to date are equivocal: equivalence in gastric emptying between liraglutide and placebo over the full postprandial period after short-term treatment [36], but slower gastric emptying with liraglutide during the initial hour [37]. This study suggests that gastric emptying is not of great importance for the effect of liraglutide on triglyceride levels.

Mean total cholesterol concentrations were reduced to a greater extent with liraglutide than placebo. Mean LDL-cholesterol concentrations decreased with liraglutide and increased with placebo, but remained above recommended

### Table 3. Adverse events by system organ class and preferred term.

| Adverse events                                      | Liraglutide 1.8 mg (n = 20) | Placebo (n = 20) |
|-----------------------------------------------------|-----------------------------|------------------|
|                                                     | N (%)                       | E                | N (%)                       | E                |
| Total adverse events                                | 12 (60)                     | 27               | 6 (30)                      | 8                |
| Gastrointestinal disorders                          | 8 (40)                      | 16               | 1 (5)                       | 1                |
| Nausea                                              | 5 (25)                      | 5                | 1 (5)                       | 1                |
| Dyspepsia                                           | 3 (15)                      | 4                | 0                           | 0                |
| Constipation                                        | 2 (10)                      | 2                | 0                           | 0                |
| Vomiting                                            | 2 (10)                      | 2                | 0                           | 0                |
| Other                                               | 3 (15)                      | 3                | 0                           | 0                |
| Metabolism and nutrition disorders                  | 6 (30)                      | 6                | 0                           | 0                |
| Investigations                                     | 2 (10)                      | 2                | 0                           | 0                |
| Ear and labyrinth disorders                         | 1 (5)                       | 1                | 1 (5)                       | 1                |
| General disorders & administration site conditions  | 1 (5)                       | 1                | 1 (5)                       | 1                |
| Infections and infestations                         | 1 (5)                       | 1                | 2 (10)                      | 2                |
| Nasopharyngitis                                     | 1 (5)                       | 1                | 1 (5)                       | 1                |
| Pneumonia                                           | 0                           | 1                | 1 (5)                       | 1                |
| Musculoskeletal and connective tissue disorders      | 0                           | 1                | 1 (5)                       | 1                |
| Nervous system disorders                            | 0                           | 2                | 2 (10)                      | 2                |

N (%), number and proportion of individuals with an event; E, number of events.
Figure 4. Effect of liraglutide and placebo on gastric emptying. Mean postprandial paracetamol concentration–time profiles (A) and 13C-octanoate excretion–time profiles (B).

LDL-cholesterol goals with both treatments [38]. Mean HDL cholesterol levels at baseline were below the recommended levels of above 1.5 mmol/l [39], and remained so after 3 weeks as decreases were observed with both treatments.

Liraglutide was generally well tolerated. As observed previously with liraglutide, the most frequently reported side-effects were gastrointestinal, but dose escalation helps to mitigate these events [40]. The clinical significance of the pulse increase and slight increase in median lipase (remaining below UNL) with liraglutide is currently unknown, but is consistent with previous trials with liraglutide and other GLP-1 receptor agonists [41,42]. Liraglutide has also been associated with low (<1%) major adverse cardiovascular event rates that were similar to or lower than those estimated for comparators [43].

Limitations of the study include its short duration and the high-fat content of the test meal, which may not be representative of a normal meal but was nevertheless in line with recommendations for studying postprandial lipaemia [44]. Furthermore, an impact of the weight loss observed with liraglutide on some of the endpoints cannot be ruled out.

In conclusion, treatment with liraglutide in patients with T2DM significantly reduced postprandial increases of triglyceride and apoB48 after a fat-rich meal, an effect that appeared to be independent of gastric emptying. The overall positive effects on lipids suggest further benefits of liraglutide in the treatment of T2DM, in addition to its positive effects on glycaemic control and body weight, and provide more evidence of the potential of liraglutide to reduce CVD risk.

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Conflict of Interest

K. H. has received speaker fees from Novo Nordisk, Merck, Sanofi-Aventis, Eli Lilly, Bristol-Myers Squibb, and AstraZeneca; fees for advisory board meetings from Novo Nordisk, Merck and Bristol-Myers Squibb; as well as research grants from Novo Nordisk and Sanofi-Aventis. T. A. B, M. D. and A. F. are employees of Novo Nordisk and own stock in the company. A. P., L. S. M. and H. J. have no conflicts of interest to disclose. K. H., T. A. B. and A. F. designed the study. K. H., T. A. B., M. D., A. P., L. S. M., H. J. and A. F. contributed to the acquisition and interpretation of data. M. D. performed the statistical analyses. The article was prepared with assistance from a Novo Nordisk medical writer, with interpretation and contributions from all authors, who approved the final draft. The corresponding author had full access to data and final responsibility for the decision to submit for publication.

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