Semaglutide improves postprandial glucose and lipid metabolism, and delays first-hour gastric emptying in subjects with obesity

Authors: Hjerped JB¹, Flint A¹, Brooks A², Axelsen MB¹, Kvist T¹, Blundell J³

Author affiliations: ¹Novo Nordisk A/S, Søborg, Denmark; ²Covance Clinical Research Unit Ltd, Leeds, UK; ³University of Leeds, Leeds, UK

Contact details for corresponding author:
Address: Julie B. Hjerped, Vandtaarnsvej 108-110, 2860 Søborg, Denmark
E-mail: jlhj@novonordisk.com
Phone: +45 30751915

Short running head: Semaglutide effects on postprandial glucose and lipid metabolism, and gastric emptying

Structured abstract

Aim: To investigate the effects of semaglutide on fasting and postprandial glucose and lipid responses, and on gastric emptying.

Materials and Methods: This was a randomised, double-blind, placebo-controlled, two-period, crossover trial. Subjects with obesity (N=30) received once-weekly subcutaneous semaglutide, dose-escalated to 1.0 mg, or placebo. After each 12-week treatment period, glucose and lipid metabolism were assessed before and after standardised meals. Gastric emptying (paracetamol absorption test) and peptide YY (PYY) response were also assessed.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/dom.13120

This article is protected by copyright. All rights reserved.
Results: Semaglutide treatment significantly lowered fasting concentrations of glucose and glucagon, and increased insulin versus placebo (estimated treatment ratio: 0.95 [95% confidence interval: 0.91, 0.98]; 0.86 [0.75, 0.98]; 1.45 [1.20, 1.75], respectively).

Postprandial glucose metabolism significantly improved with semaglutide versus placebo (incremental area under the curve 0–5 hours [iAUC\textsubscript{0-5h}]; estimated treatment difference: glucose –1.34 mmol\textperiodcentered h/L [–2.42, –0.27]; insulin –921 pmol\textperiodcentered h/L [–1461, –381]; C-peptide –1.42 nmol\textperiodcentered h/L [–2.33, –0.51]). Fasting and postprandial lipid metabolism improved with semaglutide versus placebo. First-hour gastric emptying after the meal was delayed versus placebo (AUC\textsubscript{0-1h}; estimated treatment ratio: 0.73 [0.61, 0.87]); this may have contributed to the lower postprandial glucose increase in semaglutide-treated subjects. Overall gastric emptying (AUC\textsubscript{0-5h}) was not statistically different between treatments. Fasting and postprandial PYY responses were significantly lower with semaglutide versus placebo ($p=0.0397$ and $p=0.0097$, respectively).

Conclusion: Semaglutide improved fasting and postprandial glucose and lipid metabolism. Overall gastric emptying was similar to placebo; however, the observed first-hour delay with semaglutide may contribute to a slower entry of glucose into the circulation.
Introduction

Type 2 diabetes (T2D) is a progressive metabolic disease with increasing prevalence.[1] T2D is characterised by chronic hyperglycaemia caused by insulin resistance or reduced insulin secretion.[1, 2] Despite the availability of several anti-diabetic drugs, there remains an unmet need for better therapies because a significant proportion of individuals with T2D do not achieve recommended treatment targets for glycaemic control.[3] Inadequately controlled T2D can result in various complications, including an increased risk of cardiovascular diseases.[1] Hyperlipidaemia often co-exists with T2D and is a clear risk factor for atherosclerotic cardiovascular diseases.[4] Controlling hyperlipidaemia is one of the central recommendations of the American Heart Association to reduce the risk of cardiovascular disease.[5]

Glucagon-like peptide 1 (GLP-1) and peptide PYY (PYY) are gut hormones, colocalised in intestinal L cells, that are released in response to nutrient intake.[6, 7] GLP-1 stimulates insulin secretion and inhibits glucagon secretion in a glucose-dependent manner.[8] At physiological levels, GLP-1 is also associated with an inhibitory effect on gastric emptying and lowering of body weight, due to reduced appetite and decreased energy intake.[8-11] These properties have led to the development of GLP-1 receptor agonists (GLP-1RAs) as a treatment option for individuals with T2D, with one GLP-1RA also developed for the treatment of obesity.[12]

GLP-1RAs have been associated with improved beta-cell function,[13, 14] and have been shown to lower postprandial glucose levels and reduce lipid responses.[15-18] In addition, GLP-1RAs have the potential to affect gastric emptying,[19] with an apparently diminished response over time with long acting GLP-1RAs.[20]

Semaglutide is a human GLP-1 analogue currently in development for once-weekly treatment of T2D. Semaglutide has 94% structural homology with native human GLP-1 [21,
22] with three important modifications: an amino acid substitution at position 8 that makes it less susceptible to degradation by dipeptidyl peptidase-4; lysine acylation of the peptide backbone, with a spacer and C-18 fatty di-acid chain at position 26 that provides strong, specific binding to albumin; and another amino acid substitution at position 34, which prevents C-18 fatty di-acid binding at the wrong site.[21] These modifications give semaglutide an extended half-life of around 1 week,[21] making it suitable for once-weekly administration,[23, 24] which has the potential for improving patient compliance and quality of life,[22] compared with first-generation GLP-1RAs that require once- or twice-daily dosing.[25] Semaglutide is associated with dose-dependent reductions in haemoglobin A1c (HbA1c) levels and body weight in individuals with diabetes.[26].

Previously, we reported that semaglutide reduced body weight and ad libitum energy intake after 12 weeks’ treatment, compared with placebo.[27] This finding was supported by different aspects of homeostatic and hedonic appetite parameters.[27] Here, we report data from the same study on the effects of semaglutide compared with placebo on fasting and postprandial glucose and lipid responses, as well as its effects on gastric emptying.

**Materials and Methods**

*Study design*

Details of the study design have been described elsewhere.[27] Briefly, this was a single-centre, randomised, double-blind, two-period, placebo-controlled, crossover study (NCT02079870, EudraCT no. 2013-000012-24). It was conducted in compliance with the International Conference on Harmonisation Good Clinical Practice guidelines[28] and the Declaration of Helsinki.[29]

Subjects were randomised 1:1 to one of two treatment sequences: semaglutide-placebo or placebo-semaglutide, and received either semaglutide or volume-matched placebo
administered subcutaneously (s.c.) once-weekly. The starting dose was 0.25 mg (4 weeks), escalating to 0.5 mg (4 weeks) and thereafter 1.0 mg (4 weeks). Subjects received a 5th dose of 1.0 mg at the last visit (an in-house stay) of each treatment period, when assessments were conducted. The two treatment periods were separated by a washout period of 5–7 weeks, to allow for elimination of semaglutide, before starting the second treatment period.

**Trial population**

Eligible subjects were 18 years of age or older, with obesity defined as a body mass index (BMI) of 30–45 kg/m², HbA₁c <6.5%, and a stable body weight (<3 kg body weight change during the past 3 months prior to screening). Key exclusion criteria included: diagnosis of type 1 or 2 diabetes; anticipated change in lifestyle (e.g. eating, exercise or sleeping pattern, including excessive participation in strenuous exercise, as judged by the investigator) during the trial period; history of chronic or idiopathic acute pancreatitis; personal/family history of medullary thyroid carcinoma or multiple endocrine neoplasia syndrome type 2; previous surgical treatment for obesity; or use of any medication that could interfere with the trial results. Written informed consent was obtained from all participants before any study-related activities commenced.

**Assessments and endpoints**

At the end of each 12-week treatment period, on Day 1 of the in-house stay, subjects were standardised in regard to meals, physical activity and sleep. The last dose of trial drug was administered in the evening. On Day 2, a standardised carbohydrate-rich breakfast was served at ~8:00 a.m. and assessments were performed over a 5-hour postprandial period. The total energy content of the standardised breakfast was 600 kcal (2.51 MJ, with an approximate macronutrient composition, energy percentage [E%] 55% carbohydrate, 30 E% fat and 15 E% protein). The breakfast included a yoghurt that contained 1500 mg paracetamol (Zentiva, Surrey, UK), to allow measurement of gastric emptying.[30] Before
the start of the breakfast (fasting) and up to 5 hours afterwards (postprandial), blood was sampled for measurement of glucose, insulin, C-peptide, glucagon, paracetamol and PYY₃₋₃₆ (referred to as PYY). Gastric emptying was assessed by calculating the endpoints derived from paracetamol concentration profiles. Additionally, endpoints were derived from first-hour glucose, insulin, C-peptide, glucagon, paracetamol and PYY concentration profiles.

On Day 4, a standardised fat-rich breakfast was served at ~8:00 a.m. and an 8-hour standardised fat-rich meal test performed. The standardised fat-rich breakfast had a total energy content of 1000 kcal (4.18 MJ, approximate macronutrient composition: 66 E% fat, 19 E% carbohydrate and 15 E% protein). Before (fasting) and up to 8 hours after (postprandial) the start of the breakfast, blood was sampled for measurement of parameters of lipid and glucose metabolism. Low-density lipoprotein (LDL), high-density lipoprotein (HDL) and total cholesterol were assessed in the fasted state only, while glucose, insulin, C-peptide, glucagon, triglyceride (TG), free fatty acid (FFA), very-low-density-lipoprotein (VLDL) cholesterol and apolipoprotein B48 (Apo-B48) were assessed both fasting and postprandially.

Analytical and statistical methods

Blood samples were taken from subjects by venepuncture or cannulation, and serum and plasma were prepared using standard procedures. Plasma concentrations of glucagon and PYY₃₋₃₆ were measured using validated competitive radioimmunoassays (RIAs; GL-32K glucagon RIA and PYY-67HK human PYY₃₋₃₆-specific RIA; both Millipore, UK). Paracetamol was measured in plasma by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The following parameters were measured in serum using standard validated methods; Apo-B48 (ApoB-48 Human ELISA; Biovendor Research and Diagnostic Products, UK), glucose, insulin and C-peptide (ADVIA Centaur CP Immunoassay System; Siemens Healthineers, UK), FFA (IL ILab 650 Chemistry Analyser; Diamond Diagnostics, UK), HDL, LDL, total...
cholesterol, TG and VLDL by ELISA (automated methods: Pacific Biomarkers Inc., USA, for further details see: https://pacbio.com).

Fasting values and paracetamol endpoints were analysed using a linear mixed model on log-transformed data, including treatment and treatment period as fixed effects and subject as random effect. Incremental area under the concentration–time curves (iAUC) of glycaemic and lipid parameters were analysed using a linear mixed model, including treatment and treatment period as fixed effects, fasting value as covariate, and subject as random effect.

Four subjects did not complete the standardised carbohydrate-rich breakfast during the second treatment period (17–40% of meals not eaten) and were excluded from the statistical analyses of the endpoints related to this meal test, with the exception of the data points relating to gastric emptying, since all subjects consumed the paracetamol-containing yoghurt. Two subjects did not complete the standardised fat-rich breakfast and were excluded from the statistical analyses of endpoints relating to this meal test. A sensitivity analysis of gastric emptying was done for the four subjects not completing the standardised breakfast but who consumed the paracetamol-containing yoghurt, as well as for one subject who on one occasion received placebo instead of semaglutide, and one subject who had a measurable plasma paracetamol concentration at baseline. Furthermore, sensitivity analyses were performed for glucose and TG endpoints for the one subject who received placebo instead of semaglutide on one occasion. The sensitivity analyses findings supported the overall results.

The effects of gastric emptying on first-hour postprandial glucose absorption was assessed in an exploratory analysis of glucose results, from the same meal as the paracetamol sampling was performed. Glucose measured before and up to 1 hour after the standardised breakfast ($\text{AUC}_{0-1\text{h}}$ for glucose) was further analysed with or without adjustment for the subject-mean-centred log-transformed $\text{AUC}_{0-1\text{h}}$ for paracetamol concentration as covariate.
Results

Subject characteristics

A total of 30 subjects were randomised to once-weekly semaglutide or placebo. At baseline, mean (standard deviation [SD]) age, body weight, height and BMI were 42 (11) years, 101.3 (10.5) kg, 1.73 (0.08) m and 33.8 (2.5) kg/m², respectively. Two-thirds of the study subjects were male and 90% were Caucasian. Two subjects withdrew due to gastrointestinal (GI) adverse events (AEs) during the first treatment period while receiving semaglutide, resulting in 28 subjects completing both treatment periods.

Glucose metabolism

Glucose

At the end of the 12-week treatment period, subjects receiving semaglutide had lower mean fasting concentrations of glucose, compared with placebo (estimated treatment ratio [ETR]: 0.95 [95% CI: 0.91, 0.98]; \( p = 0.0079 \)), prior to the standardised carbohydrate-rich breakfast (Table 1). Following the standardised carbohydrate-rich breakfast, postprandial increments (iAUC0-5h) for glucose were 38.5% lower with semaglutide, compared with placebo (estimated treatment difference [ETD]: –1.34 mmol*h/L [95% CI: –2.42, –0.27]; \( p = 0.0163 \)) (Figure 1, Table 1). Before the fat-rich breakfast, fasting concentrations of glucose were significantly lower for subjects treated with semaglutide versus placebo (ETR: 0.95 [95% CI: 0.92, 0.98]; \( p = 0.0036 \)) (Table 1). Following the standardised fat-rich breakfast, postprandial values (iAUC0-8h) of glucose were 32.0% lower with semaglutide, compared with placebo (ETD: –1.41 mmol*h/L; \( p = 0.0087 \)) (Supplementary Figure 1, Table 1).

Insulin and C-peptide

At the end of the 12-week treatment period, subjects receiving semaglutide had
significantly higher mean fasting concentrations of insulin and C-peptide, compared with placebo (ETR: 1.45 [95% CI: 1.20, 1.75]; \( p = 0.0005 \); and ETR: 1.35 [95% CI: 1.20, 1.52]; \( p < 0.0001 \), respectively), prior to the standardised carbohydrate-rich breakfast (Table 1).

After the standardised carbohydrate-rich breakfast, postprandial increments (iAUC\(_{0-5h}\)) were 43.4% and 28.7% lower for insulin and C-peptide, respectively, with semaglutide compared with placebo (ETD: \(-921\) pmol*h/L [95% CI: \(-1461, -381\)]; \( p = 0.0018 \); and ETD: \(-1.42\) nmol*h/L [95% CI: \(-2.33, -0.51\)]; \( p = 0.0033 \), respectively) (Supplementary Figure 1, Table 1).

Before the fat-rich breakfast, subjects treated with semaglutide also had higher fasting concentration of insulin and C-peptide, although only borderline significant for insulin (ETR: 1.18 [95% CI: 0.99, 1.41]; \( p = 0.0569 \); and ETR: 1.23 [95% CI: 1.09, 1.38]; \( p = 0.0012 \), respectively, Table 1). After the standardised fat-rich breakfast, postprandial values (iAUC\(_{0-8h}\)) of insulin and C-peptide were 35.7% and 30.6% lower for insulin and C-peptide, respectively, with semaglutide compared with placebo (ETD: \(-1105\) pmol*h/L, \( p = 0.0028 \); and ETD: \(-2.25\) nmol*h/L; \( p = 0.0005 \), respectively) (Supplementary Figure 1, Table 1).

Glucagon

At the end of the 12-week treatment period, subjects receiving semaglutide had lower mean fasting concentrations of glucagon, compared with placebo (ETR: 0.86 [95% CI: 0.75, 0.98]; \( p = 0.0224 \)), prior to the standardised carbohydrate-rich breakfast (Table 1).

Following the standardised carbohydrate-rich breakfast, there was no significant difference in postprandial increments (iAUC\(_{0-5h}\)) for glucagon between semaglutide and placebo (Figure 1, Table 1).

Before and following the standardised fat-rich breakfast, fasting and postprandial values (iAUC\(_{0-8h}\)) for glucagon tended to be lower with semaglutide than with placebo (\(-13\%\) and \(-27.3\%\), respectively) (Supplementary Figure 1, Table 1).
**Lipid metabolism**

Fasting total cholesterol and HDL cholesterol were lower with semaglutide, compared with placebo (ETR: 0.89 [95% CI: 0.86, 0.92]; \(p<0.0001\); and ETR: 0.92 [95% CI: 0.88, 0.96]; \(p=0.0002\); respectively), whereas no difference was observed for fasting LDL cholesterol (ETR: 0.95 [95% CI: 0.86, 1.06]; \(p=0.3906\)). Fasting concentrations of TG and VLDL were significantly lower (12% and 21%, respectively) with semaglutide, compared with placebo (\(p<0.02\)) (Table 2). No difference in FFA and ApoB-48 was observed.

Following the fat-rich breakfast, postprandial values (iAUC\(_{0-8h}\)) were significantly lower for TG (–40.7%), VLDL (–42.8%) and ApoB-48 (–49.6%) with semaglutide, compared with placebo (ETD: –4.51 mmol*h/L, –1.17 mmol*h/L and –0.046 g*h/L, respectively; \(p<0.01\) for all) (Figure 2; Table 2). No difference in FFA was observed.

**Gastric emptying**

As assessed by paracetamol concentrations, gastric emptying during the first hour, following the standardised carbohydrate-rich breakfast, was 27% lower with semaglutide, compared with placebo (AUC\(_{0-1h}\) ETR: 0.73 [95% CI: 0.61, 0.87]; \(p=0.0012\)). There was no significant difference between treatments for the overall postprandial gastric emptying (AUC\(_{0-5h}\): ETR: 0.94 [95% CI: 0.88; 1.01]) (Figure 3a).

**Effect of gastric emptying on glucose response**

Following the standardised carbohydrate-rich breakfast, the postprandial increment for glucose within the first hour (iAUC\(_{0-1h}\)) was 37.8% lower with semaglutide than with placebo (ETD: –0.56 mmol*h/L [95% CI: –0.88, –0.23]; \(p=0.0018\)). When gastric emptying during the first hour after the meal was included as a covariate, treatment difference was less pronounced (ETD: –0.33 mmol*h/L [95% CI: –0.70, 0.05]; \(p=0.0829\)), indicating that approximately 40% of the early glucose response may be explained by the rate of gastric emptying.
**PYY response**

At the end of the 12-week treatment period, subjects receiving semaglutide 1.0 mg had lower mean fasting PYY concentrations, compared with those receiving placebo (ETR: 0.72 [95% CI: 0.53; 0.98], \( p = 0.0397 \)). Postprandial values (iAUC\(_{0-5h}\)) for PYY concentrations, following the standardised carbohydrate-rich breakfast, were 46.3% lower with semaglutide, compared with placebo (ETD: \(-46.10 \text{ pg}\cdot\text{h/mL}; \ p = 0.0097\) ) (**Figure 3b**).

**Discussion**

In this study we report that fasting and postprandial glucose and lipid metabolism were improved with semaglutide treatment, compared with placebo.

Postprandial glucose and lipid metabolism are important aspects when considering overall glycaemic and lipid control because most individuals spend a significant amount of time during the day in a non-fasting state.[19, 31]

Following 12 weeks’ treatment with semaglutide, there were generally lower postprandial increments of glucose-related parameters, including glucose, glucagon, insulin and C-peptide. However, the differences in glucose parameters were less pronounced between treatments following the fat-rich breakfast, possibly due to the expected reduction in glucose absorption associated with a high fat intake,[32] and/or the lower absolute carbohydrate amount in the fat-rich breakfast versus the carbohydrate-rich breakfast (50 g vs 83 g).[33]

Our findings in a population with obesity, but not T2D, are in alignment with results from previous studies with GLP-1RAs.[15-17, 34, 35] Liraglutide has been shown to significantly reduce mean postprandial glucose and glucagon (AUC\(_{0-5h}\)), compared with placebo, both in subjects with T2D,[15] and obese, non-diabetic adults.[35] Similarly, albiglutide and
dulaglutide, both of which are once-weekly GLP-1RAs, have been shown to lower fasting and postprandial glucose concentrations. [17, 34] In relation to glycaemic control, GLP-1RAs increase insulin secretion in a glucose-dependent manner, suppress glucagon, and slow gastric emptying, which affects the postprandial glucose response [13, 14, 19]. The observed reduction in postprandial insulin and C-peptide in this trial may, in part, be due to the reduction in glucose concentrations observed after 12 weeks' treatment. It is conceivable that semaglutide may produce a greater response in subjects with T2D with higher glycaemia. [15, 35, 36] In another 12-week study with semaglutide in subjects with T2D, a pronounced improvement of beta-cell function was shown, and the impact on 24-hour glucose, insulin and C-peptide responses during a test day with three standardised meals was similar to the observations in the current study. [36] The effects of semaglutide on insulin and glucose are interdependent. In the fasting state, the concentrations of insulin and C-peptide are significantly increased. However, after 12-weeks' treatment, in the
postprandial state the lower insulin increments should be interpreted in light of the concurrent lower glucose increments.

Previously we reported an observed body weight reduction of 5.0 kg following 12 weeks of semaglutide treatment.[27] Body weight loss is known to improve insulin sensitivity.[37] Improved insulin sensitivity would result in a decrease in glucose concentrations, and, thus, less insulin demand. Therefore, weight loss reported in this study may have affected the glucose and lipid responses. Similar findings have been reported with liraglutide, which improved both postprandial glycaemia and induced weight loss.[35] The substantial reduction in body weight reported in this study may, therefore, be an indirect route by which semaglutide influences postprandial glycaemic and lipid parameters. The long-term effects of continuous subcutaneous infusion of GLP-1 also showed decreases in both fasting and postprandial glucose concentrations as well as decreases in HbA₁c and body weight.[38]

Our study is the first to investigate the effect of semaglutide treatment on postprandial lipid absorption and metabolism. Following a standardised fat-rich breakfast, subjects treated with semaglutide had lower postprandial TG, VLDL and ApoB-48, over 8 hours (iAUC₀⁻₈ₘ). Postprandial ApoB-48, a marker of TG uptake from the gut, is known to be involved in the assembly of chylomicron particles required for the absorption of TGs.[39] Chylomicron production has been shown to be increased in subjects with T2D and insulin resistance.[39] In this study the lower postprandial TG concentrations corresponded well with lower postprandial ApoB-48 concentration profiles for semaglutide versus placebo. It is plausible that by reducing the serum concentration of ApoB-48, semaglutide in turn reduces the postprandial absorption of serum TGs. In a series of studies in hamsters and mice, Hsieh and colleagues showed that the GLP-1 receptor is essential for regulation of the intestinal lipid and lipoprotein metabolism, through control of intestinal lipoprotein synthesis and secretion.[40] This was confirmed in humans by Vergès and colleagues, who recently reported on the effects of liraglutide on the metabolism of ApoB-48.[41] Treatment with
liraglutide significantly decreased postprandial hyperlipidaemia in subjects with T2D via a mechanism of reduced ApoB-48 production and increased ApoB-48 catabolism.[41]

The frequency of obesity is reported to be greater in high-fat than in low-fat consumers.[42] Therefore, the demonstrable effect of semaglutide on blood lipids during a high-fat meal is highly relevant for subjects with obesity, who often consume energy-dense, high-fat foods that contribute to hyperlipidaemia.

In our study, fasting concentrations of TG and VLDL were also significantly lower with semaglutide than with placebo. In addition, total cholesterol and HDL cholesterol were lower with semaglutide than with placebo, whereas LDL cholesterol appeared comparable between treatments. In longer-term studies with semaglutide, 1–2 years treatment modestly improved various lipid parameters in subjects with T2D, compared with placebo or sitagliptin.[43, 44] These findings are consistent with those for other GLP-1RAs, including liraglutide and exenatide.[18, 45, 46]

Hyperlipidaemia is a well-known risk factor for cardiovascular diseases.[47, 48] Thus, lowering serum TGs may reduce the risk of cardiovascular diseases such as atherosclerosis.[5] In particular, lowering of non-fasting TG could be of clinical importance since higher concentrations have been shown to be associated with an increased risk of myocardial infarction, ischaemic heart disease and death.[48] The effect of semaglutide on cardiovascular outcomes in subjects with T2D has been reported in the 2-year SUSTAIN 6 study.[44] Results from this study showed that among subjects with T2D at high cardiovascular risk, the rate of first occurrence of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke was significantly lower in those receiving semaglutide than in those receiving placebo.[44] The effect of semaglutide on postprandial glucose and lipid metabolism may have contributed to these findings.
GLP-1RAs have the capacity to slow gastric emptying in a variable but marked manner, when administered acutely, which may represent a key mechanism contributing to their glucose-lowering effect.[19] However, some investigations indicate that this phenomenon is transient and the effect on gastric emptying diminishes over time.[49, 50] The differing durations of action of GLP-1RAs seem to influence gastric emptying with continued dosing. The slowing of gastric emptying induced by several long-acting GLP-1 agonists,[49, 51] (though not by exenatide twice-daily or lixisenatide) lessens with time, perhaps indicative of adaptation over time.[19] In our study, after 12 weeks’ treatment, there was no statistically significant difference between semaglutide and placebo for the overall rate of postprandial gastric emptying. A delayed gastric emptying response was detected during the first hour after the standardised breakfast, compared with placebo. This finding is consistent with previous findings for liraglutide.[18, 19] Gastric emptying during the first hour was a significant covariate in the statistical analysis of postprandial glucose response following the standardised breakfast, indicating that approximately 40% of the initial glucose response could be explained by the rate of gastric emptying, and that gastric emptying contributed to the lower postprandial increase in glucose response observed when subjects were treated with semaglutide.

We used the paracetamol absorption test to measure the rate of gastric emptying. Although it provides an indirect estimation of gastric emptying, this technique is reported to correlate generally well with scintigraphy.[52] A limitation with all gastric emptying tests is the considerable inter-individual variation;[52] however, this was counteracted by intra-subject comparisons in this study. Despite claimed limitations in drawing conclusions about solids from paracetamol studies,[52] it should be noted that paracetamol in this study was added to the semi-solid part of the meal (yoghurt). In a previous study investigating the effect of liraglutide on gastric emptying,[18] a comparable set-up was used and similar results were observed with the paracetamol test and octanoate breath test (labelling the solid part of the
meal) during the same meal. Furthermore, the results on gastric emptying obtained in the current study agree with observations in other long-acting GLP-1RAs studies.[53, 54]

The lower PYY response observed with semaglutide is in alignment with a previous study in which GLP-1 infusions appeared to have an inhibitory effect on PYY release, suggesting possible feedback (suppression) of GLP-1 on L-cell function in the acute setting.[55] GLP-1RAs have also been reported to reduce postprandial endogenous GLP-1 and PYY concentrations.[56] Like GLP-1, PYY responses correlate with nutrient exposure in the gut.

For the standardised breakfast, the amount consumed was the same for those receiving semaglutide or placebo, but a lower postprandial PYY response was expected with semaglutide treatment, due to the lower initial gastric emptying. The lower fasting level of PYY may further be explained by a semaglutide-induced lower food intake during the treatment period, reported from the same study.[27] Therefore, it is not clear if the reduced PYY response in this study is due to semaglutide-induced direct suppression of L-cell secretion, or indirectly by lower postprandial stimulation of L-cells caused by delayed gastric emptying or by longer-term lower food intake due to the documented impact of semaglutide on appetite regulation.[27]

The strengths of this study include the crossover design, as subjects served as their own control, the high degree of standardisation and the frequent sampling used, of up to 5 and 8 hours after the standardised meals. A potential weakness is that, of those subjects receiving semaglutide, four did not ingest all of their standardised breakfast meal (consuming approximately 60–83%) and, likewise, two subjects consumed only part of their standardised fat-rich breakfast. Therefore, related data derived from these subjects were excluded from the glucose and TG results. Nevertheless, sensitivity analyses confirmed the results from the primary analyses.
To conclude, fasting and postprandial glucose and lipid metabolism were improved after 12 weeks of treatment with semaglutide, compared with placebo. Overall, 5 hours postprandial gastric emptying was similar for semaglutide and placebo; with semaglutide, however, a delay was observed during the first hour after a meal, possibly contributing to a slower entry of glucose into the circulation.

Acknowledgements
We thank all the participants, investigators and trial-site staff who were involved in the conduct of the trial. We also thank Haydn Liang PhD, and Ian Seymour PhD (both from AXON Communications) for medical writing and editorial assistance.

Author contributions
JB, AF and JBH designed the study. AB and JB conducted the study. TK participated in the data analysis. MBA, AB, JB, AF and JBH participated in the collection, analysis and interpretation of data. All authors were involved in the writing and revision, and gave final approval, of the manuscript.

Funding
This study was funded by an unrestricted grant from Novo Nordisk A/S.

Conflict of interests
MBA, AF, JH and TK are full-time employees of, and hold shares in, Novo Nordisk A/S. AB has received research grants from Novo Nordisk. JB has received research, travel and accommodation grants within the submitted work from Novo Nordisk A/S, and advisory and speaker fees outside the submitted work from Novo Nordisk A/S.
**Figures**

**Figure 1.** Mean glucose metabolism profiles after standardised, carbohydrate-rich breakfast. Error bars represent standard error.
**Figure 2.** Mean lipid metabolism profiles after standardised, fat-rich breakfast. Error bars represent standard error. VLDL, very-low-density lipoprotein.
**Figure 3a.** Gastric emptying: mean paracetamol profiles following standardised carbohydrate-rich breakfast. Error bars represent standard error.

![Paracetamol](image1)

**Figure 3b.** Mean PYY profile following standardised, carbohydrate-rich breakfast. Error bars represent standard error.

![PYY](image2)
### Table 1. Glucose metabolism parameters and PYY after 12 weeks’ treatment with semaglutide versus placebo

<table>
<thead>
<tr>
<th></th>
<th>Fasting values</th>
<th>Postprandial values (iAUC₀⁻⁵h) following carbohydrate-rich breakfast</th>
<th></th>
<th></th>
<th></th>
<th>Relative difference (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Semaglutide</td>
<td>Placebo</td>
<td>ETR</td>
<td>95% CI</td>
<td>p</td>
<td>Semaglutide</td>
</tr>
<tr>
<td></td>
<td>1.0 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.0 mg</td>
</tr>
<tr>
<td>Glucose metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>5.34 mmol/L</td>
<td>5.63 mmol/L</td>
<td>0.95</td>
<td>0.91, 0.98</td>
<td>0.0079</td>
<td>2.14 mmol*h/L</td>
</tr>
<tr>
<td>Insulin</td>
<td>166 pmol/L</td>
<td>115 pmol/L</td>
<td>1.45</td>
<td>1.20, 1.75</td>
<td>0.0005</td>
<td>1203 pmol*h/L</td>
</tr>
<tr>
<td>Glucagon</td>
<td>89.39 pg/mL</td>
<td>104.22 pg/mL</td>
<td>0.86</td>
<td>0.75, 0.98</td>
<td>0.0224</td>
<td>31.29 pg*h/mL</td>
</tr>
<tr>
<td>C-peptide</td>
<td>0.886 nmol/L</td>
<td>0.656 nmol/L</td>
<td>1.35</td>
<td>1.20, 1.52</td>
<td>0.0001</td>
<td>3.541 nmol*h/L</td>
</tr>
<tr>
<td>PYY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PYY</td>
<td>33.89 pg/mL</td>
<td>47.11 pg/mL</td>
<td>0.72</td>
<td>0.53; 0.98</td>
<td>0.0397</td>
<td>53.40 pg*h/mL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Fasting values</th>
<th>Postprandial values (iAUC₀⁻⁸h) following fat-rich breakfast</th>
<th></th>
<th></th>
<th></th>
<th>Relative difference (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Semaglutide</td>
<td>Placebo</td>
<td>ETR</td>
<td>95% CI</td>
<td>p</td>
<td>Semaglutide</td>
</tr>
<tr>
<td></td>
<td>1.0 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.0 mg</td>
</tr>
<tr>
<td>Glucose metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>5.14 mmol/L</td>
<td>5.40 mmol/L</td>
<td>0.95</td>
<td>0.92, 0.98</td>
<td>0.0036</td>
<td>2.99 mmol*h/L</td>
</tr>
<tr>
<td></td>
<td>Insulin</td>
<td>Glucagon</td>
<td>C-peptide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------</td>
<td>----------</td>
<td>-----------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>135 pmol/L</td>
<td>114 pmol/L</td>
<td>1105 pmol*h/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.18</td>
<td>0.87</td>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.99, 1.41</td>
<td>0.74, 1.02</td>
<td>0.74, 1.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0569</td>
<td>0.0905</td>
<td>0.0905</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1993 pmol*h/L</td>
<td>165.65 pg*h/mL</td>
<td>165.65 pg*h/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0028</td>
<td>0.0582</td>
<td>0.0582</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-35.7%</td>
<td>-27.3%</td>
<td>-27.3%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CI, confidence interval; ETD, estimated treatment difference (semaglutide – placebo); ETR, estimated treatment ratio (semaglutide/placebo); iAUC_{0-5h}, incremental area under the 0–5-hour curve; iAUC_{0-8h}, incremental area under the 0–8-hour curve; PYY, peptide YY. *Estimated treatment difference/estimated mean for placebo x 100%.
Table 2. Lipid metabolism parameters after 12 weeks’ treatment with semaglutide versus placebo

<table>
<thead>
<tr>
<th>Lipid metabolism parameters</th>
<th>Fasting values</th>
<th>Postprandial values (iAUC&lt;sub&gt;0-8h&lt;/sub&gt;) following fat-rich breakfast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ETR</td>
<td>95% CI</td>
</tr>
<tr>
<td>TGs</td>
<td>0.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.80, 0.98</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.66, 0.95</td>
</tr>
<tr>
<td>Apo B48</td>
<td>1.02</td>
<td>0.86, 1.21</td>
</tr>
<tr>
<td>FFA</td>
<td>0.99</td>
<td>0.88, 1.11</td>
</tr>
</tbody>
</table>

ApoB-48, apolipoprotein B48; CI, confidence interval; ETD, estimated treatment difference (semaglutide – placebo); ETR, estimated treatment ratio (semaglutide/placebo); FFA, free fatty acids; iAUC<sub>0-8h</sub>, incremental area under the 0–8-hour curve; TG, triglyceride; VLDL, very-low-density lipoprotein.

<sup>a</sup>p<0.05. <sup>b</sup>Estimated treatment difference/estimated mean for placebo x 100%.
References

13 Hare KJ, Vilsbøll T, Asmar M, Deacon CF, Knop FK, Holst JJ. The glucagonostatic and insulinotropic effects of glucagon-like peptide 1 contribute equally to its glucose-lowering action. Diabetes 2010; 59: 1765-1770


26 Nauck MA, Petrie JR, Sesti G et al. A Phase 2, Randomized, Dose-Finding Study of the Novel Once-Weekly Human GLP-1 Analog, Semaglutide, Compared With Placebo and Open-Label Liraglutide in Patients With Type 2 Diabetes. Diabetes Care 2016; 39: 231-241


34 Umpierrez GE, Blevins T, Rosenstock J et al. The effects of LY2189265, a long-acting glucagon-like peptide-1 analogue, in a randomized, placebo-controlled, double-blind


37 Schenk S, Harber MP, Shrivastava CR, Burant CF, Horowitz JF. Improved insulin sensitivity after weight loss and exercise training is mediated by a reduction in plasma fatty acid mobilization, not enhanced oxidative capacity. J Physiol 2009; 587: 4949-4961


41 Vergès B, Duvillard L, Bouillet B et al. Liraglutide reduces postprandial hyperlipidaemia by increasing apoB48 catabolism and by reducing apoB48 production. 52nd EASD Annual Meeting 2016; Abstract #635


