Received: 14 September 2022 | Revised: 25 November 2022 | Accepted: 8 December 2022

DOI: 10.1111/dom.14944

ORIGINAL ARTICLE

WILEY

Semaglutide delays 4-hour gastric emptying in women with polycystic ovary syndrome and obesity

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Funding information
Slovenian Research Agency, Grant/Award Numbers: P3-0343, P3-0298

Abstract

Aim: To evaluate the effect of once-weekly subcutaneous semaglutide 1.0 mg on the late digestive period of gastric emptying (GE) after ingestion of a standardized solid test meal by using technetium scintigraphy, the reference method for this purpose.

Methods: We conducted a single-blind, placebo-controlled trial in 20 obese women with polycystic ovary syndrome (PCOS; mean [range] age 35 [32.3-40.8] years, body mass index 37 [30.7-39.8] kg/m^2) randomized to subcutaneous semaglutide 1.0 mg once weekly or placebo for 12 weeks. GE was assessed after ingestion of [^{99m}Tc] colloid in a pancake labelled with radiopharmaceutical by scintigraphy using sequential static imaging and dynamic acquisition at baseline and at Week 13. Estimation of GE was obtained by repeated imaging of remaining [^{99m}Tc] activity at fixed time intervals over the course of 4 hours after ingestion.

Results: From baseline to the study end, semaglutide increased the estimated retention of gastric contents by 3.5% at 1 hour, 25.5% at 2 hours, 38.0% at 3 hours and 30.0% at 4 hours after ingestion of the radioactively labelled solid meal. Four hours after ingestion, semaglutide retained 37% of solid meal in the stomach compared to no gastric retention in the placebo group ($P = 0.002$). Time taken for half the radiolabelled meal to empty from the stomach was significantly longer in the semaglutide group than the placebo group (171 vs. 118 min; $P < 0.001$).

Conclusion: Semaglutide markedly delayed 4-hour GE in women with PCOS and obesity.

KEYWORDS
gastric emptying, obesity, PCOS, scintigraphy, semaglutide

1 | INTRODUCTION

Subcutaneous (s.c) semaglutide, a long-acting glucagon-like peptide-1 receptor agonist (GLP-1RA), is the first second-generation drug for the treatment of obesity.[1] Human studies confirmed that it reduces appetite, increases satiety, prolongs feeling of abdominal fullness and limits caloric intake.[2-4] The main mechanism involves its uptake into specific brain regions and direct interactions with homeostatic and hedonic centres for regulation of energy.[2]

It has also been proposed that semaglutide could hypothetically contribute to reduced energy intake and weight loss by delaying gastric emptying (GE) due to prolonged feeling of fullness,[5] although such an

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assumption has not been supported by observations with the short-acting GLP-1RA lixisenatide.[6] Physiologically, glucagon-like peptide-1 (GLP-1) decelerates GE by relaxation of stomach muscle, inhibition of antral and duodenal motility, and stimulation of pyloric pressure.[7,8] However, it is also noted that, in general, there is no significant correlation of intrinsic rate of GE with postprandial GLP-1 response. Rather, in a given individual, the GLP-1 response to the small intestinal nutrient load is predictive of GE.[9] The evidence for notable effects of semaglutide on GE are inconclusive.[10-16] To date, it has been reported that s.c. semaglutide had either no effect on GE or delayed GE only within the first hour, without any effect on the late digestive period of GE.[15-17]

Measurement of GE requires a precise technique. Regrettably, all conclusions that semaglutide has no notable effect on the late digestive period of GE were made based on an indirect method of evaluation through ingestion, absorption and determination of plasma level of paracetamol.[16] There are a few key limitations of such an assessment. First, paracetamol is typically administered as a liquid, more closely following the exponential pattern of GE, which is governed by a distinct GE profile and mechanism characterized by initial retention. By contrast, solids are triturated to a small particle size before emptying at a relatively constant rate. Second, GE has been frequently calculated by paracetamol absorption at the end of a 4-, 5- or 6-hour appraisal, at which time much of the paracetamol will have had the opportunity to be absorbed, consequently missing the potential impact of GE on the plasma paracetamol profile. Such studies that express the paracetamol area under the curve (AUC) over 4 to 6 hours underestimate the impact of GE in the late digestive period. Some studies have avoided this potential limitation by assessing the paracetamol AUC from 0 to 1 hour, which provides a more precise assessment of the early phase of GE[18] Altogether, the paracetamol test is accurate for evaluation of the kinetics of liquids, whereas it is inaccurate when testing GE over the first hour, within the late digestive period of GE. The implicit assumption that paracetamol is absorbed from the stomach concordant with a solid meal is incorrect.[19]

Scintigraphy is considered a reference method for this purpose.[20,21] Our study is the first to evaluate the effect of s.c. semaglutide on GE of a solid meal in obese women with polycystic ovary syndrome (PCOS) using scintigraphy. The primary outcome was gastric retention, expressed as the percentage of tracer retained at specific times up to 4 hours after meal ingestion and the time taken for half the radiolabelled meal to empty from the stomach ($T_{1/2}$).

2 | MATERIALS AND METHODS

2.1 | Trial design

We conducted a single-centre, randomized, single-blind, placebo-controlled trial, comparing the effect of semaglutide versus placebo on the GE of a solid meal in obese women with PCOS, without other comorbidities, using scintigraphy.

The study was conducted in accordance with the Declaration of Helsinki and approved by the National Medical Ethics Committee (approval number 0120-258/2019/12). It is registered at www.Clinical Trials gov as NCT04263415. All subjects were informed of the study aims and signed the written consent form before entering the study.

2.2 | Trial population

Twenty White women, aged 35 (32.3-40.8) years with a body mass index (BMI) of 37 (30.7-39.8) kg/m^2, diagnosed with PCOS and obesity were recruited from outpatients at the Department of Endocrinology, Diabetes and Metabolic Diseases, University Medical Centre, Ljubljana. The diagnosis of PCOS was established using the Rotterdam criteria, specifically, phenotype A, characterized as concomitant presence of irregular menstrual cycles, hyperandrogenism, and polycystic ovarian morphology.[22] Exclusion criteria were any known serious chronic illness, including diabetes mellitus, angina pectoris, coronary heart disease, congestive heart failure, severe renal and hepatic impairment, inflammatory bowel disease, gastroparesis, cancer, chronic obstructive lung disease, psychiatric and neurological disease. Additional exclusion criteria were other clinical features, including GE disorders such as gastroesophageal reflux, hypothyroidism, gastric resection, or medication that may alter GE (eg, metoclopramide, domperidone, cimetidine, parasympatholytics and sympathomimetics). Given that metformin slows GE in patients with type 2 diabetes,[23] 10 subjects who had been treated with metformin before the enrolment were instructed to discontinue metformin 1 month before the start of the study. Further exclusion criteria were: failure of the subject to ingest the entire meal; the use of medications that cause clinically significant weight gain or loss; previous bariatric surgery; history of idiopathic acute pancreatitis; family or personal history of multiple endocrine neoplasia type 2 or familial medullary thyroid carcinoma; current smoking; pregnancy, expecting pregnancy or breast feeding; and allergy to any of the ingredients in the study medication.

2.3 | Study protocol

Participants were randomized in a 1:1 ratio to either semaglutide or placebo. As a method of randomization, the RAND program in Excel was used. The RAND function embedded in Excel generates random numbers using the Mersenne Twister algorithm, which is one of standard algorithms used in the majority of statistics, database and mathematics programs, such as Mathematica, Stata, SPSS, GAUSS, as well as being a standard source of randomly generated numbers in various programming languages, such as Python and C++. The algorithm generates integers in the range between 0 and (2^{19937} – 1) and then calculates a value on the interval between 0 and 1.

Subjects in the semaglutide group received semaglutide (Ozempyc; Novo Nordisk A/S, Bagsvaerd, Denmark) once weekly as s.c. injections in the abdomen. The placebo group administered saline with prefilled saline syringes in the placebo pen using the same method and volume as the semaglutide group. Semaglutide was

initiated at a dose of 0.25 mg once weekly for the first 2 weeks, was escalated by rapid titration to 0.5 mg/week for 2 weeks and then was increased to 1.0 mg once weekly for the remaining treatment period for 8 weeks.

Gastric emptying was assessed at baseline and at Week 13 by scintigraphy, after ingestion of a standardized solid meal, using sequential static imaging and dynamic acquisition. Additionally, at baseline and at the end of the study, all participants underwent standard anthropometric measurements: height, weight, waist and neck circumference. A fasting blood sample was drawn for determination of glucose, luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione, dehydroepiandrosterone sulphate (DHEAS), total and free testosterone and lipids. This was followed by a 2-hour 75-g oral glucose tolerance test (OGTT) conducted in the morning, between 7:00 AM and 9:00 AM, after a 12-hour overnight fast. Blood samples for glucose were drawn at 0 and 120 minutes into the OGTT. The homeostatic model assessment of insulin resistance (HOMA-IR) index was calculated as a measure of insulin resistance. Clinical safety assessment was performed at the beginning and at the end of the treatment period.

2.4 | Assessment of biochemical variables

Glucose levels were determined using a standard glucose oxidase method (Beckman Coulter Glucose Analyzer; Beckman Coulter, Inc.). LH and FSH were determined using an immunometric assay (Diagnostic Products Corporation). Androstenedione and DHEAS were measured by specific double-antibody radioimmunoassay (RIA) using 125I-labelled hormones (Diagnostic Systems Laboratories). Total and free testosterone levels were measured by coated-tube RIA (DiaSorin, S.p.A. [Salluggia, Italy] and Diagnostic Products Corporation, respectively). Intraassay variations ranged from 1.6% to 6.3%, and interassay variations ranged from 5.8% to 9.6% for the applied methods. Pre- and posttreatment samples from each subject were assayed in the same assay run.

The OGTT was performed as recommended.[24] Blood samples for glucose were drawn at 0, and 120 minutes into the OGTT.

2.5 | Assessment of GE of solid meal

Gastric emptying was assessed by scintigraphy using dynamic and sequential static imaging after ingestion of a pancake labelled with a ^{99m}Tc-radiopharmaceutical. Estimation of GE was obtained by repeated imaging of remaining ^{99m}Tc activity at fixed time intervals over 4 hours and the $T_{1/2}$ of GE was calculated.

2.6 | Subject preparation

Participants were oligoamenorehoeic. If they had a menstrual period during the course of the study, they were tested in the first week of their menstrual cycle, before oestrogen and progesterone peak. They had not ingested anything for at least 8 hours before the investigation. Abstinence of alcohol and caffeine intake were required 24 hours prior to the investigation.

2.7 | Essentials for the procedure

A commercial pancake mixture was used for preparation of pancakes (Dr Oetker, produced in business division Jánossomorja, Hungary, 2020). In addition to the radiopharmaceutical, the following ingredients were used for 10 pancakes: 0.45 L water and one pre-prepared powdered pancake mix 215 g (ingredients: wheat flour, glucose syrup, whey eggs, sugar, salt, thickener [guar gum]).The contents of 100 g of the mixture comprised: fat 3.70 g, of which saturates 1.20 g; carbohydrate 26.00 g, of which sugars 5.80 g; protein 6.10 g; salt 0.41 g. A pancake was labelled with 30 MBq of ^{99m}Tc-labelled macroaggregated albumin (MAA). Two pancakes were ingested by each subject. Subjects were seated during ingestion. ^{99m}Tc-MAA labelling was performed through an internally validated standardized procedure for which the ^{99m}Tc-MAA provides the highest labelling efficiency and stability (estimated at 99 ± 0.5% at 90 minutes post labelling).[25]

2.8 | Image acquisition processing

All investigations were scheduled in the morning between 8:00 AM and 10:00 AM. Dynamic imaging was performed within the first hour after ingestion, with subsequent static imaging at hourly intervals up to 4 hours after ingestion. Imaging was performed in the supine position; in the intervals between imaging the subjects were seated or standing.

A region of interest (ROI) was drawn around the stomach (gastric ROI), with an appropriately placed adjacent ROI for background correction on the image obtained by the conjugate view (geometric mean) method. The detected counts in the gastric ROI represented the activity remaining in the stomach at specific timepoints, from which the time-activity curve was reconstructed. All data were corrected for radioactive decay. The $T_{1/2}$ of GE was calculated from the time-activity curves. An example of GE in one subject before and after intervention with semaglutide is shown in Figure 1A,B.

2.9 | Statistical analysis

Continuous variables were described using median (25%-75% range). Normality of the distribution was evaluated using the Shapiro-Wilk test. As multiple variables were not normally distributed, nonparametric statistical tests were used for analysis. The nonparametric Mann-Whitney test was used to compare the distribution of continuous variables between different treatment groups. The nonparametric Wilcoxon signed-rank test was used for comparison of continuous variables for related samples (values before and after intervention). Spearman's rho correlation coefficient was used to assess the correlation between continuous variables. *P* values below 0.05 were considered statistically

significant. All statistical analyses were performed using IBM SPSS Statistics, version 27.0 (IBM Corporation).

In previous studies on healthy volunteers, the standard deviation for GE $T_{1/2}$ in female participants was approximately 28.7 min.[20] By including 10 subjects in each treatment group, we could detect differences in absolute GE $T_{1/2}$ in the range of ±38 min, with 80% study power (calculation performed using Power and Sample Size Calculation version 3.0.43).

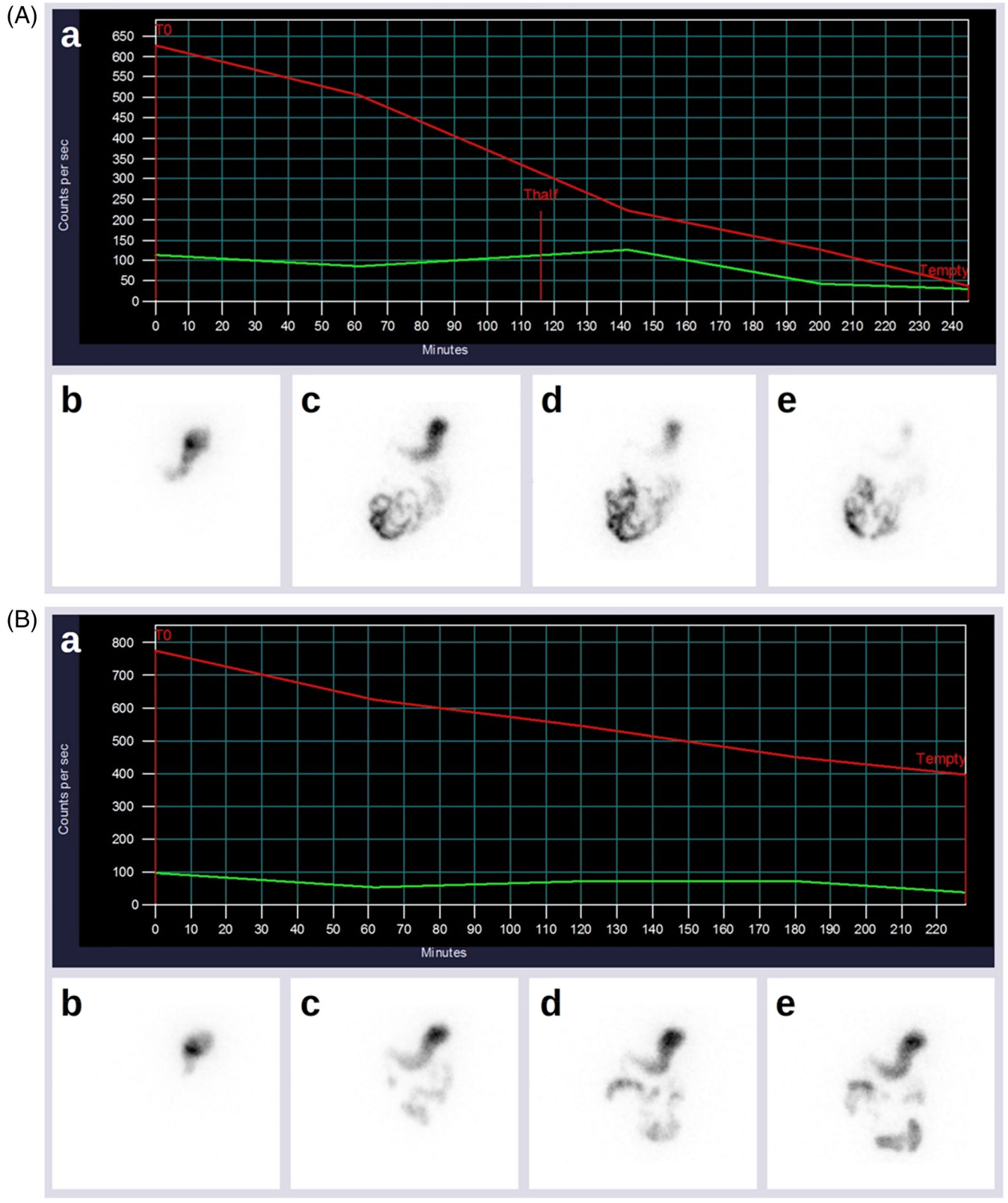

FIGURE 1 Legend on next page.

3 | RESULTS

One subject was excluded from the placebo group because she could not perform the follow-up visit due to COVID-19 infection in the 10th week of study. Nineteen subjects completed the study: 10 in the semaglutide group and 9 in the placebo group. There were no significant differences at baseline in any of the variables between the treatment groups. Baseline characteristics of the study outcomes are provided in Table 1.

3.1 | Gastric emptying

Comparing pre- and posttreatment values, semaglutide significantly delayed GE. From baseline to Week 13, the estimated retention of gastric contents in the semaglutide group increased by 3.5% at 1 hour after ingestion of the radioactively labelled meal, 25.5% at 2 hours, 28.0% at 3 hours and 30.0% at the 4 hours after ingestion. In comparison with placebo, a statistically significant difference in retention of gastric contents between groups was observed 30 minutes after ingestion and persisted throughout the observation time up to 4 hours. Four hours after ingestion, semaglutide retained 37% of solid meal in the stomach compared to no gastric retention in the placebo group (Table 2, Figure 2). $T_{1/2}$ was significantly longer in the semaglutide group as compared to the placebo group (171 min vs. 118 min; $P < 0.001$). Pre-post intervention difference in $T_{1/2}$ in the placebo group was 13 minutes, which again could be attributed to variability of GE. The difference in pre-post treatment value in semaglutide group was 43 minutes. Therefore, an increase in $T_{1/2}$ of 30 minutes could be attributed to the intervention. There were no significant correlations between baseline $T_{1/2}$ and change in $T_{1/2}$ in subjects treated with placebo (Spearman's rho = −0.283, $P = 0.460$) or semaglutide, even though subjects with higher baseline $T_{1/2}$ tended to have a smaller change in $T_{1/2}$ in the semaglutide group (Spearman's rho = −0.647, $P = 0.083$).

3.2 | Changes in anthropometric measurements

There was a significant decrease in body weight, abdominal circumference and neck circumference in the semaglutide group ($P = 0.005$, $P = 0.018$ and $P = 0.017$, respectively) at Week 13 compared to baseline (Table 1). The differences did not reach statistical significance when compared between groups (Table 1).

3.3 | Metabolic changes

In the semaglutide group, treatment intervention resulted in a statistically significant reduction of glucose value at 120 minutes into the OGTT ($P = 0.013$) and a significant decrease in glycated haemoglobin (HbA1c) value ($P = 0.007$) at Week 13 compared to baseline. LDL increased in the placebo group ($P = 0.0043$). The between-group differences were not statistically significant. There were no statistically significant differences in other metabolic variables at this early timepoint of the intervention. There were no significant correlations between change in HbA1c and change in $T_{1/2}$ in subjects treated with placebo (Spearman's $\rho = 0.197$, $P = 0.612$) or semaglutide (Spearman's $\rho = 0.446$, $P = 0.268$). The mean pre- and posttreatment values of the metabolic variables are presented in Table 1.

3.4 | Endocrine changes

At 12 weeks, a statistically significant reduction in androstenedione and free testosterone and an increase in SHBG were noted in the semaglutide group. The total testosterone significantly increased in the placebo group. However, the between-group differences in endocrine variables were not statistically significant (Table 1).

3.5 | Adverse events

Nausea and dyspepsia were the most common adverse events experienced by four out of 10 participants in the semaglutide group and by one out of nine subjects in the placebo group. Adverse events were transient, mild to moderate in severity and subsided with time. None of the participants discontinued treatment due to gastrointestinal events or other reasons.

4 | DISCUSSION

This is the first study that investigated the effect of s.c. semaglutide on GE by using scintigraphy, the reference method for this purpose. We demonstrated that s.c. semaglutide significantly delayed GE in obese women with PCOS. Furthermore, 4 hours after ingestion, semaglutide retained 37% of solid meal in the stomach, as compared with

FIGURE 1 A, Example of gastric emptying (GE) in a subject with polycystic ovary syndrome (PCOS) at baseline. Emptying of gastric contents is shown with the reconstructed curve (a) from the obtained data estimating $T_{½}$ at 116 minutes and retention of gastric contents at 1, 2, 3 and 4 hours after ingestion of the radioactively labelled standard meal estimated at 81%, 35%, 20% and 6%, respectively (b to e). Rapid appearance of radioactive contents is demonstrated in the small bowel with minimal retention in the stomach at 4 hours. The red curve (in panel a) represents emptying of gastric contents; the green curve (in panel a) represents the background activity. B, Example of delayed GE in a subject with PCOS at 13 weeks of treatment with semaglutide. Delayed emptying of gastric contents is shown in the same subject as in (A) with the reconstructed curve (a), with $T_{½}$ not reached and retention of gastric contents at 1, 2, 3 and 4 hours after ingestion of the radioactively labelled standard meal estimated at 81%, 70%, 56% and 51%, respectively (b to e). In comparison to (A), there is significant retention of radioactive contents in the stomach at 4 hours and delayed appearance of the labelled meal in the small bowel. The red curve (in panel a) represents emptying of gastric contents; the green curve (in panel a) represents the background activity

TABLE 1 Comparison of clinical characteristics of subjects treated with semaglutide (N = 10) or placebo (N = 9)

Group Characteristic	Placebo group			Semaglutide group			Between-group comparison pre-treatment	Between-group comparison post-treatment
	Pretreatment Median value (25%-75%)	Posttreatment Median value (25%-75%)	p^a	Pre-treatment Median value (25%-75%)	Post-treatment Median value (25%-75%)	p^a	p^b	p^b
Weight, kg	93 (83-105.5)	91 (88-5-107)	.079	104.5 (89.8-111.8)	99.5 (82.8-106)	.005	.278	1.000
BMI, kg/m^2	31.0 (29.3-39.5)	32.5 (29.6-40.0)	.092	37.9 (33.2-41.4)	35.9 (30.6-39.7)	.005	.182	.780
Neck circumference, cm	36 (34-39.5)	36 (34-40.8)	.673	36.8 (36-38.6)	35.5 (33.8-38)	.018	.842	.720
Waist circumference, cm	94 (86.5-112)	95 (86.5-112.5)	.944	104 (98-113.5)	104 (92.5-108)	.017	.182	.842
Systolic BP, mmHg	123 (119-129)	117 (111.5-124)	.123	122 (116.8-129.3)	112 (106.8-123.3)	.284	.780	.211
Diastolic BP, mmHg	80 (73.5-84.5)	79 (66-81.5)	.286	80 (77.3-88.8)	77.5 (71-81.3)	.090	.661	.905
Pulse, beats/min	78 (68.5-92)	85 (78-89.5)	.138	72 (71.3-80.5)	89.5 (75.8-96.5)	.014	.447	.549
Glucose 0 min OGTT, mmol/L	5.7 (5.4-6)	5.8 (5.4-6.1)	.496	5.6 (5.2-5.9)	5.4 (5.1-5.5)	.260	.905	.065
Glucose 120 min OGTT, mmol/L	6.5 (5.3-7.5)	5.8 (5.2-9.6)	.765	6.3 (5.7-6.7)	5.4 (4.6-5.6)	.013	.661	.053
Insulin 0 min OGTT, mU/L	10.3 (8.2-25)	14.1 (10.1-23.5)	.515	12.3 (10.8-15)	12.9 (7.1-19.7)	.646	.905	.661
Insulin 120 OGTT min, mU/L	112 (50.7-183.5)	110 (59.4-201)	.859	75.8 (49.5-94.1)	55.6 (39.9-92)	.575	.182	.095
HOMA-IR	2.4 (2.1-6.5)	3.3 (2.6-6.2)	.378	3.2 (2.7-3.5)	3.1 (2.6-6.2)	.595	.842	.549
Glucose, mmol/L	4.9 (4.7-5.2)	5 (4.7-5.4)	.674	4.8 (4.5-5.1)	4.8 (4.6-4.8)	.952	.549	.133
HbA1c, mmol/mol	33 (33-37)	34 (31-37)	1.000	36(32-39)	32(31-36)	.007	.604	.447
Cholesterol, mmol/L	5.3 (4.5-6.1)	5.1 (4.9-7.1)	.083	4.9 (4.5-5.4)	4.8 (4.6-5.7)	.552	.497	.095
HDL, mmol/L	1.3 (1.1-1.7)	1.4 (1.2-1.7)	.862	1.2 (1.1-1.5)	1.1 (1-1.3)	.055	.720	.079
LDL, mmol/L	3 (2.5-3.5)	3.2 (2.8-4.5)	.043	3 (2.7-3.6)	3.1 (3-3.6)	.959	.661	.780
TG, mmol/L	1.2 (0.8-2.5)	2.1 (1-2.4)	.953	1.3 (1.1-1.7)	1.2 (1.1-1.6)	.878	.842	.278
FSH, E/L	6.5 (4.8-7.7)	4.9 (4.1-6.1)	.051	5.5 (4.8-6.5)	5.9 (5.2-7.5)	.413	.315	.243
LH, E/L	7.3 (6.1-13.3)	10.2 (8.1-16.4)	.051	5.5 (2.9-9.7)	6.1 (3.8-9.4)	.475	.278	.043
DHEAS, µmol/L	6.49 (4.73-8.09)	5.54 (4.2-8.67)	.214	5.91 (4.31-11.45)	5.5 (4.09-8.68)	.214	.842	.968
SHBG, nmol/L	34 (17.7-53)	27.3 (18-46.4)	.678	23.3 (14.4-29)	27.8 (18-35.2)	.012	.156	.604
FAI	4 (1.5-5.5)	3 (1.5-4)	.059	3.5 (2.8-9.3)	3 (2-6.3)	.120	.447	.604
Total testosterone, nmol/L	0.78 (0.7-1.17)	1.24 (0.7-1.56)	.028	1.01 (0.7-1.57)	0.7 (0.7-1.2)	.063	.400	.095
Free testosterone, pmol/L	5.27 (4.04-7.01)	5.55 (3.82-7.6)	1.000	8.27 (5.25-12.58)	5.41 (3.48-8.81)	.013	.065	1.000
Androstenedione, nmol/L	6.28 (4.93-9.79)	7.7 (4.87-9.42)	.594	7.35 (4.98-8.91)	4.94 (3.65-7.26)	.005	.720	.156

Abbreviations: BP, blood pressure; DHEAS, dehydroepiandrosterone sulphate; FAI, free androgen index; FSH, follicle-stimulating hormone; HbA1c, glycated haemoglobin; HDL high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein cholesterol; LH, luteinizing hormone; OGTT, oral glucose tolerance test; SHBG, sex hormone-binding globulin; TG, triglycerides.

[a]Calculated using Wilcoxon signed-rank test.

[b]Calculated using Mann-Whitney test.

TABLE 2 Comparison of gastric emptying evaluated by scintigraphy using static and dynamic imaging between the semaglutide and placebo group

Characteristic, % of estimated retention of gastric contents	Placebo group, N = 9			Semaglutide group, N = 10			Between-group comparison posttreatment
	Pretreatment Median value (25%-75%)	Posttreatment Median value (25%-75%)	*P*[a]	Pretreatment Median value (25%-75%)	Posttreatment Median value (25%-75%)	*P*[a]	*P*[b]
Dynamic imaging							
Td 15 min	94 (92.5-96.5)	94 (91.5-95.5)	0.726	95 (92.8-97)	96.5 (93.8-99)	0.311	0.113
Td 30 min	89 (87-91.5)	89 (84.5-90)	0.310	91 (89-93.3)	92.5 (87.8-95)	0.283	0.050
Td 45 min	83 (77.5-85)	82 (78.5-86)	0.953	87.5 (85.5-88.5)	90 (83.8-93.3)	0.256	0.013
Td 60 min	79 (69.5-81.5)	78 (73-82.5)	0.678	83 (80.8-87.3)	85.5 (82.3-90.5)	0.332	0.017
Static imaging							
Ts 1 h	73 (69.5-78)	79 (74.5-81)	0.327	83.5 (79.8-87.3)	87 (84.8-89.3)	0.092	<0.001
Ts 2 h	31 (29-36.5)	43 (36.5-53)	0.109	46.5 (25-55.5)	72 (60-78)	0.009	0.001
Ts 3 h	5 (2-9)	18 (13-32.5)	0.061	22 (6.5-34.5)	50 (35.5-58.8)	0.046	0.008
Ts 4 h	0 (0-1.5)	0 (0-10.5)	0.262	7 (0.8-14.5)	37 (17-47.5)	0.028	0.002
$T_{1/2}$, min	105 (90.5-117)	118 (108-132)	0.139	128 (108.5-141.8)	171 (154-187.5)	0.036	<0.001

Abbreviations: $T_{1/2}$, time taken for half the radiolabelled meal to empty from the stomach; *Td*, duration of dynamic imaging; *Ts*, duration of static imaging.
[a]Calculated using Wilcoxon signed-rank test.
[b]Calculated using Mann-Whitney test.

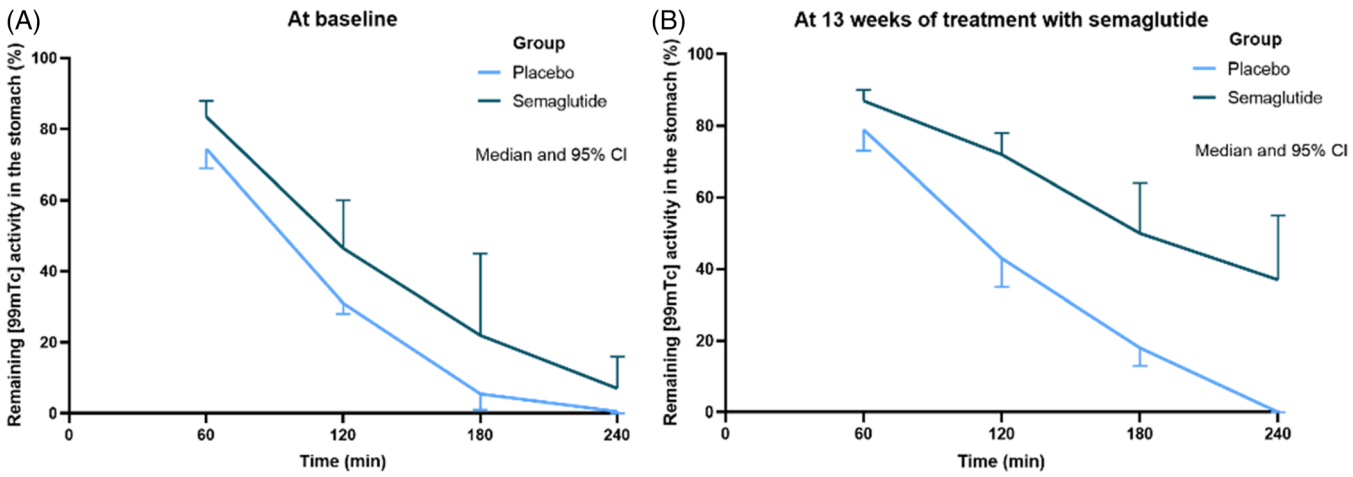

FIGURE 2 Median values of gastric emptying during sequential static imaging. CI, confidence interval

no gastric retention in the placebo group. We therefore confirmed the significant effect of s.c. semaglutide on GE throughout 4 hours, including the late digestive period of GE.

Currently, the evidence for notable effects of GLP-1RAs on GE is inconclusive. While it has been consistently demonstrated that short-acting GLP-1RAs delayed GE, in that this is the main mechanism through which they act on post-meal glycaemic rises,[26-30] notable effects of long-acting GLP-1RAs have not been confirmed.[3,15,16,29,31-36] Regrettably, most of the conclusions for long-acting GLP-1RAs were based on a paracetamol absorption test because of its advantages of simplicity, availability and absence of ionizing radiation.

As assessed by paracetamol test, the long-acting GLP-1RA liraglutide delayed GE only within the first hour, whereas there was no impact in the late digestive period of GE.[31-33] The effectiveness of s.c. semaglutide on GE was assessed by paracetamol test in two previous studies.[15,16] Hjerpsted et al. primarily aimed to investigate the role of s.c. semaglutide 1.0 mg on body weight and ad libitum energy intake after 12 weeks of treatment, and GE was characterized as a secondary outcome.[16] GE during the first hour after the standardized carbohydrate-rich breakfast was 27% lower with semaglutide as compared with placebo. However, there was no significant difference between the groups in the first to the fifth hour or for overall postprandial GE.[16] Friedrichsen et al. reported that in adults with obesity, s.c. semaglutide 2.4 mg did not cause any delay in GE at Week 20.[15] No effect was seen on paracetamol area under the concentration-time curve (AUC) for paracetamol 0 to 1 hour or for 1 to 5 hours after a

standardized meal including yoghurt that contained 1500 mg paracetamol.[15]

These observations should be interpreted in line with the limitations of this indirect method because paracetamol empties with the liquid phase of the meal and does not accurately measure the late digestive period of GE. While the plasma paracetamol AUC 0 to 1 hour may provide an assessment of liquid emptying, the absence of difference after that time (1 to 4 hours) does not establish that an effect on emptying is only sustained for 1 hour.[19] Thus, any conclusions regarding the late digestive phase of GE based on the paracetamol test should perhaps be considered as misleading.[19]

A second alternative method frequently used to evaluate GE is the ^{13}C-isotope breath test.[29,34-36] The isotope breath test, with the rate of GE calculated using a mathematical model, is simple, not expensive and not associated with a radiation burden.[19] Appropriate mathematical modelling results in reasonable correlation with scintigraphic results.[37] The few studies that employed the 13-C octanoate breath test did not find any effects of liraglutide on the GE of either solids or liquids.[29,34-36] However, caution is required with the use of the breath test for GLP-1RAs because of the potential confounding effect of changes in small intestinal motility induced by GLP-1RAs.[38]

Scintigraphy is the most physiological method for quantitative study of GE. It is considered as a reference method for the GE of solids.[20,21,39] It directly measures the activity remaining in the stomach.[19] The obtained results are reproducible over time.[39] Serial testing can accurately determine the effectiveness of therapy.[39] Scintigraphy has been previously used only in one study with the long-acting GLP-1RA, liraglutide.[36] Compared with placebo, liraglutide 3.0 mg delayed $T_{1/2}$ at 5 and 16 weeks. Over time, there was a reduced effect on GE, most likely related to tachyphylaxis due to continuous substantial stimulation of GLP-1 receptors associated with the long-acting GLP-1RA.[26,28,36]

Until now, the potential impact of s.c. semaglutide on GE has not yet been evaluated using scintigraphy. Our study clearly demonstrated that s.c. semaglutide delayed the early and late digestive period of GE. At 4 hours after meal ingestion, semaglutide led to 37% gastric retention of solid meal. The upper limit of normal for gastric retention in healthy subjects at 4 hours has been determined to be a maximum of 10%[39] and retention of more than 30% of solid meal at this time period has already been categorized as non-physiologically "slow" GE.[17,21]

Furthermore, we observed a significant reduction of androstenedione and free testosterone in the semaglutide group. The results are in line with observations that exenatide and liraglutide reduce androgens in preclinical and clinical studies on PCOS.[40] Moreover, we noticed an increase in sex hormone-binding globulin (SHBG) in the semaglutide group. An increase in SHBG has been linked with improvement of hyperandrogenism via reduced bioavailability of testosterone.[41] Similar to our finding, treatment with liraglutide increased SHBG and reduced free androgen index.[42] Currently, there are no other data about the impact of semaglutide on androgen profile in PCOS.

The main limitation of the present study was the small sample size, which was conditioned by the demanding methodology. The role of scintigraphy as a "gold standard" for the measurement of GE has been disputed due to its shortcomings, including complexity and radiation exposure.[43] However, the statistical power was sufficient to detect differences. In addition, there was a 7% to 17% difference at different timepoints of GE between the placebo and semaglutide groups before the treatment intervention. The differences could be attributed to variability of GE.[19] Therefore, some of the posttreatment difference might also be attributed to this variability in GE. Furthermore, other underlying conditions, such as hyperresponsiveness to semaglutide or predisposition to greater placebo effect, may have biased the posttreatment results. Lastly, the semaglutide group appears to be heavier than the placebo group, although the difference was not statistically significant. Previous studies reported that BMI was not significantly associated with GE results,[20,21] implying that the between-group differences in body weight and BMI probably had no significant impact on the main outcome.

The main strength of our study was the assessment of GE of a solid meal with the rarely used reference method for this purpose.[20,21,39] Furthermore, the included study cohort was a group of obese women with PCOS without diabetes and with no other concomitant clinical feature or therapy as a confounding factor. It has been reported previously that gender is significantly associated with GE results.[44,45] The GE of solids in premenopausal females was slower than that in males, irrespective of the phase of the menstrual cycle.[20,21] Sex hormones alone were unlikely to explain the slower GE observed in females.[46] It has also been shown that high glucose levels have an adverse effect on GE[21] and that potential asymptomatic gastroparesis in subjects with type 2 diabetes may confer a relatively low risk of further slowing GE.[47] On the other hand, BMI was not significantly associated with GE results.[20,21] By selecting a female obese PCOS cohort without diabetes, other concomitant disease or medications, we excluded many variables that might potentially have affected the results of our study.

In conclusion, we demonstrated that s.c. semaglutide 1.0 mg led to substantial gastric retention over a period of 4 hours after ingestion of a solid meal, whereas previous reports have not provided evidence for a notable effect of s.c. semaglutide on the late digestive period of GE.[3,15,16,29,31-36] The discordant observations expose the limitations of the paracetamol absorption test for the assessment of GE and demonstrate how interpretation of the results is mitigated by testing prolonged absorption over the first hour.

While it has been previously proposed that long-acting GLP-1RAs could hypothetically contribute to reduced energy intake and weight loss by delaying GE, the lack of evidence for notable effects renders this an unlikely mechanism. Results from our study imply further exploration of peripheral mechanisms through which s.c. semaglutide, particularly at a dose of 2.4. mg/week, could potentially contribute to reduced food and energy intake.

AUTHOR CONTRIBUTIONS

Mojca Jensterle and Andrej Janez: Conceptualization, methodology, data curation, validation, formal analysis, writing–original draft, writing–review and editing. Mojca Jensterle, Simona Ferjan: Data curation,

writing–original draft, writing review and editing. Katja Zaletel, Luka Ležaič and Aljaž Sočan: Investigation, data curation, writing–original draft. Katja Goričar: statistical calculations.

FUNDING INFORMATION

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The study was supported by Slovenian Research Agency grants no. P3-0298, no. P3-0343. The planning and conduction of the study, the interpretation of data, and the writing of the present manuscript were completely independent of the funder.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/dom.14944.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Jensterle M, Ferjan S, Ležaič L, et al. Semaglutide delays 4-hour gastric emptying in women with polycystic ovary syndrome and obesity. *Diabetes Obes Metab*. 2023;25(4):975-984. doi:10.1111/dom.14944