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Efficacy and safety of GLP-1 receptor agonists as add-on to SGLT2 inhibitors in type 2 diabetes mellitus: A meta-analysis

Marco Castellana, Angelo Cignarelli, Francesco Brescia, Sebastio Perrini, Annalisa Natalicchio, Luigi Laviola & Francesco Giorgino*

GLP-1 receptor agonists (GLP-1RA) and SGLT2 inhibitors (SGLT2i) have been associated with improved glycemic control, body weight loss and favorable changes in cardiovascular risk factors and outcomes. We conducted a systematic review and meta-analysis to evaluate the effects of the addition of GLP-1RA to SGLT2i in patients with type 2 diabetes mellitus and inadequate glycemic control. Six databases were searched until March 2019. Randomized controlled trials (RCT) with a follow-up of at least 24 weeks reporting on HbA1c, body weight, systolic blood pressure, lipids, achievement of HbA1c < 7%, requirement of rescue therapy due to hyperglycemia and hypoglycemic events were selected. Four RCTs were included. Compared to SGLT2i, the GLP-1RA/SGLT2i combination was associated with greater reduction in HbA1c (−0.74%), body weight (−1.61 kg), and systolic blood pressure (−3.32 mmHg). A higher number of patients achieved HbA1c < 7% (RR = 2.15), with a lower requirement of rescue therapy (RR = 0.37) and similar incidence of hypoglycemia. Reductions in total and LDL cholesterol were found. The present review supports treatment intensification with GLP-1RA in uncontrolled type 2 diabetes on SGLT2i. This drug regimen could provide improved HbA1c control, together with enhanced weight loss and blood pressure and lipids control.

Diabetes mellitus is a chronic disease characterized by high prevalence, morbidity and excess mortality. It is a leading cause of cardiovascular disease, end-stage renal disease and blindness, causing a relevant economic impact on patients, their families and the health care system[1]. To reduce the incidence and progression of these complications, particularly microvascular, glycemic management aiming at blood glucose concentrations close to the normal range has been proved effective[2]. Management of hyperglycemia and other cardiovascular risk factors should be thus actively pursued, and combination therapies should be attentively considered in individuals with inadequate metabolic control[3].

In the last 10 years, two new drug classes have been available for type 2 diabetes therapy, GLP-1 receptor agonists (GLP-1RA) and SGLT-2 inhibitors (SGLT2i). GLP-1RA can be classified into short-acting (exenatide, lixisenatide) and long-acting (albiglutide, dulaglutide, exenatide long-acting release, liraglutide, semaglutide), based on their pharmacokinetic and pharmacodynamic profile. These agents stimulate insulin release in a glucose-dependent manner, promote reduction in glucagon secretion and hepatic glucose production, slow gastric emptying, and suppress appetite[4–7]. The most used SGLT2i include canagliflozin, dapagliflozin and empagliflozin. They inhibit glucose reabsorption by the kidney, thus increasing its excretion in the urine and ameliorating the effects of glucotoxicity on beta-cells; however, they increase glucagon levels. Both classes promote weight loss and blood pressure lowering, albeit with different and complementary mechanisms, and are characterized by a low risk of hypoglycemia[8]. Moreover, some of the agents in these drug classes have also been associated with reduction in cardiovascular events and mortality and nephroprotection[9–13].

Recently, a consensus report by the American Diabetes Association and the European Association for the Study of Diabetes on treatment of hyperglycemia in type 2 diabetes was released. In patients with established atherosclerotic cardiovascular disease or chronic kidney disease already taking SGLT2i, a combination of GLP-1RA and SGLT2i should be considered if further intensification of glycemic control is required[14]. The GLP-1RA/

Section of Internal Medicine, Endocrinology, Andrology and Metabolic Diseases, Department of Emergency and Organ Transplantation, University of Bari Aldo Moro, Bari, Italy. *email: francesco.giorgino@uniba.it

SGLT2i combination should be also preferentially used over other therapies in inadequately controlled patients in which promoting weight loss is a priority[14]. Considering their specific mechanistic synergy, tackling multiple pathophysiological defects of type 2 diabetes, the combination of GLP-1RA and SGLT-2i is expected to result in further decrease in HbA_{1c} with no further risk of hypoglycaemia, greater weight loss, and enhanced potential for cardiovascular and renal benefits, as compared with either drug class alone. Since studies evaluating the effects of the addition of GLP-1RA to SGLT2i in patients with inadequately controlled type 2 diabetes are now available, we performed a systematic review and meta-analysis focusing on traditional glycemic targets as well as on other major risk factors for cardiovascular disease, including hypertension, obesity, and dyslipidemia. Specifically, a comparison of the effects of the GLP-1RA/SGLT2i combination versus SGLT2i on HbA1c, body weight, systolic blood pressure (SBP), lipids, achievement of HbA1c < 7%, requirement of rescue therapy due to hyperglycemia, and incidence of hypoglycemic events was carried out.

Materials and Methods

The systematic review was registered in PROSPERO (CRD42018110532) and performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Supplementary Appendix)[15].

Search strategy. A four-step search strategy was planned. First, we identified keywords and MeSH terms in PubMed. Second, the terms "glucagon-like peptide-1 receptor agonist" and "sodium glucose cotransporter 2 inhibitor" (including exenatide, lixisenatide, albiglutide, dulaglutide, liraglutide, semaglutide, taspoglutide, canagliflozin, dapagliflozin, empagliflozin, ertugliflozin, ipragliflozin) were searched in PubMed, CENTRAL, ClinicalTrials.gov, EudraCT, Scopus and Web of Science. Third, randomized controlled trials (RCT) with a follow-up of at least 24 weeks analyzing GLP-1RA as add-on to SGLT2i in type 2 diabetes mellitus were selected. Fourth, references of included studies were searched for additional papers. The last search was performed on March 5th, 2019. No language restriction was adopted. Two investigators (MC, FG) independently searched papers, screened titles and abstracts of the retrieved articles, reviewed the full-texts, and selected articles for their inclusion.

Data extraction. The following information was extracted independently by the same investigators in a piloted form: 1) general information on the study (author, year of publication, study name, study type, follow-up period, number of patients, age, diabetes duration, ethnicity, sex, inclusion criteria of screened population, glucose-lowering medications at pre-screening, treatment of randomization, other anti-diabetes therapies allowed during the study); 2) end-points, including HbA1c, body weight, SBP, lipids, number of patients achieving an HbA1c target of less than 7%, number of patients requiring rescue therapy due to hyperglycemia, incidence of hypoglycemic events. The criteria for requirement of rescue therapy due to hyperglycemia and the definition of hypoglycemia for each study can be found in the Supplementary Appendix. The main paper and supplementary data were searched; if data was missing, the study protocol and pharmaceutical industry website were searched. Data were cross-checked, and any discrepancy was discussed.

Study quality assessment. The risk of bias of included studies was assessed independently by two reviewers (MC, FG) through the Cochrane Collaboration's tool for assessing risk of bias for the following aspects: random sequence generation; allocation concealment; blinding of participants and personnel; blinding of outcome assessment; incomplete outcome data; selecting reporting. For other bias, funding and authorship were assessed. Each domain was assigned low, unclear or high risk of bias[16].

Data analysis. The primary outcome was the change in HbA1c from baseline to the last available follow-up. Secondary outcomes included changes in body weight, SBP and lipids from baseline to the last available follow-up, achievement of an HbA1c target of less than 7%, requirement of rescue therapy due to hyperglycemia, incidence of hypoglycemic events. The first four endpoints were analyzed as continuous variables and summarized as weighted mean difference; the last three as dichotomous, and the risk ratios (RR) were estimated. If standard deviation was missing in a study for a specific outcome, it was calculated from standard error, 95% confidence interval or from interquartile range; if none of these were available, the largest among the other studies was reported. For studies with three arms, the shared one was used for comparison of the other two; this shared group was split into two groups with smaller sample size, and two comparisons were included. Heterogeneity between studies was assessed by using I^2, with 50% or higher regarded as high. Publication bias was assessed with Egger's test; the trim-and-fill method was used for estimating its effect. Sensitivity analyses by removing each study in turn were also performed. In particular, a specific sensitivity analysis was carried out to assess the impact of including patients on basal insulin as background therapy. All analyses were two-sided and were carried out using RevMan5.3 (The Cochrane Collaboration) and Prometa3.0 (Internovi) with a random-effect model; $p < 0.05$ was regarded as significant.

Ethics. These systematic review and meta-analysis were in accordance with the principles of the Declaration of Helsinki. Analyses were performed on data extracted from published papers.

Results

Study characteristics. A total of 1,489 papers were found, of which 390 on PubMed, 2 on ClinicalTrials.gov, 15 on EudraCT, 94 on CENTRAL, 762 on Scopus and 226 on Web of Science. After removal of 436 duplicates, 1,053 articles were analyzed for title and abstract; 964 records were excluded (systematic reviews, meta-analyses, non-randomized studies, comparison of therapy schemes other than the one reported above, cost-effectiveness studies, studies recruiting patients with type 1 diabetes mellitus, studies not in humans). The remaining 89 papers

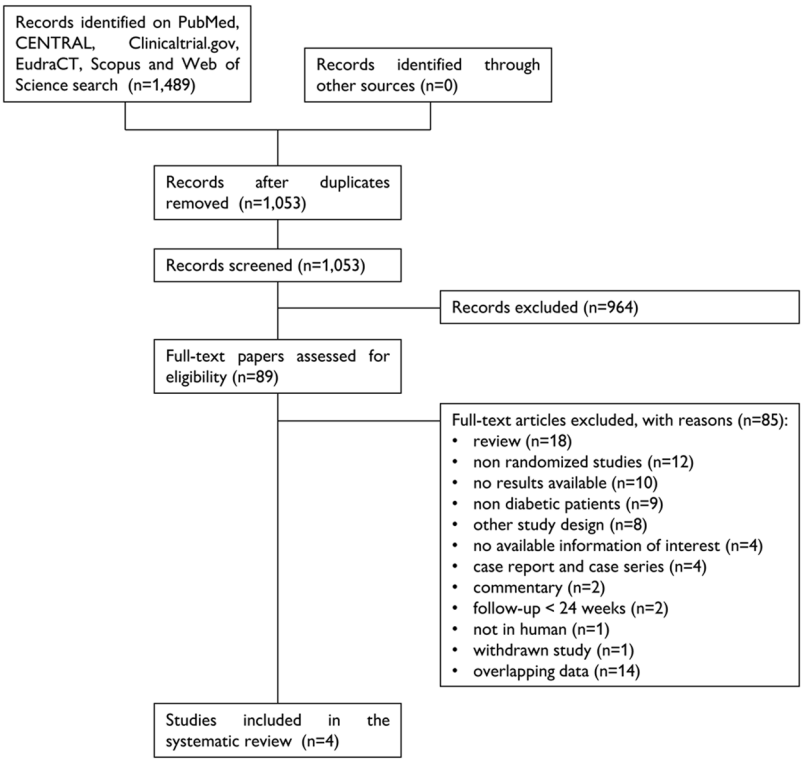

Figure 1. Flow-chart of the systematic review.

were retrieved in full-text and four articles were finally included in the systematic review (Fig. 1)[17–20]. No additional study was retrieved after screening the references of these papers.

Study quality assessment. The risk of bias of the included studies is shown in Supplementary Appendix. Random sequence generation was reported only in AWARD-10[18]. Allocation concealment and selective reporting bias were adequate in all. In DUAL IX, the open-label design led to the assignment of a high risk of bias for blinding of participants and personnel and blinding of outcome assessment: iDegLira and glargine share the route of administration and titration, thus a double-blind design could have been potentially considered[19]. In SUSTAIN 9, premature discontinuation was more frequent in the semaglutide arm compared to placebo, with possible attrition bias[20]. Finally, an industrial sponsor funded the study in all[17–20].

Qualitative analysis (systematic review). The characteristics of the included articles are summarized in Table 1. The studies were published between 2018 and 2019, had sample sizes ranging from 302 to 464 patients, and a follow-up from 24 to 52 weeks. All studies were randomized controlled, multinational and sponsored by industry (one by AstraZeneca, one by Eli Lilly, two by Novo Nordisk). One study examined dulaglutide, one exenatide QW, one iDegLira, and one semaglutide. Two studies were three-armed[17,18]. Participants were adult outpatients diagnosed with type 2 diabetes mellitus, with HbA1c 7–12% and BMI 20–45 kg/m^2. Regarding the glucose-lowering therapy at pre-screening, patients were on SGLT2i with or without metformin in three trials[18–20], metformin only in one[17]. 1,610 patients were included, 53% were males, and 85% were Caucasian. The weighted-mean age was 56.3 ± 9.7 years, and the weighted-mean duration of diabetes was 8.7 ± 6.1 years. 876 were randomized to GLP-1RA added to SGLT2i, while 734 to SGLT2i. Moreover, in DURATION-8, 231 patients were randomized to GLP-1RA added to placebo; they were not included in the present review.

Quantitative analysis (meta-analysis). The primary outcome was the change in HbA1c from baseline to the last available follow-up. The weighted-mean HbA1c at baseline was 8.5% with no difference between arms ($p = 0.30$). The GLP-1RA/SGLT2i combination was associated with an improved glycemic control, expressed as change in HbA1c, versus SGLT2i ($\Delta = -0.74\%$; 95% CI −1.15 to −0.33; $p < 0.001$; $I^2 = 95\%$) (Fig. 2, panel A). Moreover, the GLP-1RA/SGLT2i combination showed to be superior to SGLT2i in achieving an HbA1c value < 7% (RR = 2.15; 95% CI 1.20 to 3.86; $p = 0.01$; $I^2 = 96\%$), with fewer patients requiring rescue therapy due to hyperglycemia (RR = 0.37; 95% CI 0.15 to 0.89; $p = 0.03$; $I^2 = 53\%$) (Supplementary Appendix).

Body weight at baseline was 90.6 kg, with no differences between the study arms ($p = 0.48$). The GLP-1RA/SGLT2i combination caused a greater body weight loss versus SGLT2i ($\Delta = -1.61$ kg; 95% CI −2.83 to −0.38; $p = 0.01$; $I^2 = 88\%$) (Fig. 2, panel B).

SBP at baseline was 129 mmHg, with no differences between the two groups ($p = 0.91$). The GLP-1RA/SGLT2i combination was also associated with a lowering in SBP versus SGLT2i ($\Delta = -3.32$ mmHg; 95% CI −4.96 to −1.68; $p < 0.001$; $I^2 = 44\%$) (Fig. 2, panel C). In regard to incidence of hypoglycemic events, GLP-1RA added to SGLT2i showed a similar incidence as SGLT2i (RR = 1.43; 95% CI 0.46 to 4.52; $p = 0.54$; $I^2 = 94\%$). Finally,

Study name (identifier)	Author, year	GLP-1RA + SGLT2i arm	SGLT2i arm	Other therapies	Study type	Follow-up (weeks)	Number of patients	Population	Age (years)	Diabetes duration (years)	HbA1c	Body weight	Systolic blood pressure	Lipids	HbA1c < 7%	Rescue therapy	Hypoglycemic events
AWARD-10 (NCT02597049)	Ludvik, 2018	Dulaglutide QW + SGLT2i	Placebo + SGLT2i	Metformin	RCT	24	424	type 2 diabetes, HbA1c 7–9.5%, BMI ≤ 45 kg/m^2	57.3 (9.4)	9.4 (6.2)	x	x	x	x	x	x	x
DUAL IX (NCT02773368)	Philis-Tsimikas, 2019	iDegLira QD + SGLT2i	Glargine + SGLT2i	Metformin	RCT	26	420	type 2 diabetes, HbA1c 7–11%, BMI 20–40 kg/m^2	56.7 (10.3)	9.6 (6.3)	x	x	x	x	x		x
DURATION-8 (NCT02229396)	Jabbour, 2018	Exenatide QW + Dapagliflozin	Placebo + Dapagliflozin	Metformin	RCT	52	464	type 2 diabetes, HbA1c 8–12%	54.5 (9.5)	7.3 (5.7)	x	x	x	x	x	x	
SUSTAIN 9 (NCT03086330)	Zinman, 2019	Semaglutide QW + SGLT2i	Placebo + SGLT2i	Metformin, sulphonylurea	RCT	30	302	type 2 diabetes, HbA1c 7–10%	57 (9.5)	—	x	x	x	x	x	x	x

Table 1. Qualitative analysis of studies included in the systematic review. BMI, body mass index; GLP-1RA, glucagon-like peptide-1 receptor agonist; QD, once daily; QW, once weekly; RCT, randomized controlled trial; SGLT2i, sodium glucose cotransporter 2 inhibitor.

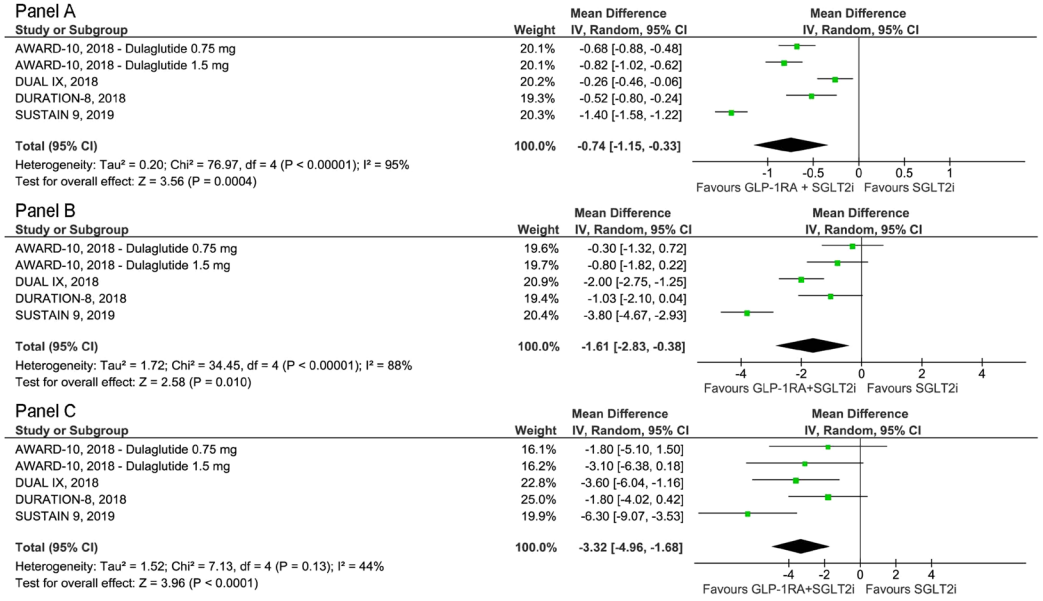

Figure 2. Forest plots of meta-analysis for change in HbA1c (panel A), body weight (panel B), and systolic blood pressure (panel C) from baseline to the last available follow-up.

reductions in total cholesterol ($\Delta = -0.17$ mmol/l; 95% CI −0.32 to −0.02; $p = 0.02$; $I^2 = 66\%$) and LDL cholesterol ($\Delta = -0.13$ mmol/l; 95% CI −0.24 to −0.03; $p = 0.01$; $I^2 = 50\%$) were observed, with no changes in either HDL cholesterol ($p = 0.61$) or triglycerides ($p = 0.09$) (Supplementary Appendix).

We found only one study with basal insulin as background therapy (DUAL IX)[19]. When this study was excluded from the analysis, improvements in HbA1c ($\Delta = -0.86\%$; 95% CI −1.25 to −0.47; $p < 0.001$; $I^2 = 93\%$), SBP ($\Delta = -3.25$ mmHg; 95% CI −5.43 to −1.06; $p = 0.004$; $I^2 = 57\%$), LDL cholesterol ($\Delta = -0.12$ mmol/l; 95% CI −0.25 to −0.00; $p = 0.05$; $I^2 = 61\%$) and chance of achieving an HbA1c value < 7% (RR = 2.52; 95% CI 1.78 to 3.58; $p < 0.001$; $I^2 = 75\%$) were confirmed. Similarly, there were neutral effects on the incidence of hypoglycemia ($p = 0.10$), HDL cholesterol ($p = 0.40$) and triglycerides ($p = 0.07$), as in the analysis of the full trial set. However, non-significant trends to greater effects on body weight loss ($\Delta = -1.50$ kg; 95% CI −3.16 to 0.17; $p = 0.08$) and total cholesterol ($\Delta = -0.18$ mmol/l; 95% CI −0.37 to 0.01; $p = 0.06$) were observed.

There was no evidence of publication bias, except for achievement of HbA1c < 7%, rescue therapy and hypoglycemic events; the statistical significance of these results was not changed following the application of the trim-and-fill method. In sensitivity analyses, the findings for changes in body weight, total and LDL cholesterol and rescue therapy were not always confirmed (Supplementary Appendix).

Discussion

This systematic review and meta-analysis was performed to identify a high level of evidence on the efficacy of the combination therapy with GLP-1RA and SGLT2i versus SGLT2i in patients with inadequately controlled type 2 diabetes. We found four RCTs, randomizing 1,610 adult patients with HbA1c between 7–12% and BMI between 20–45 kg/m^2 to either treatment. The GLP-1RA/SGLT2i combinations versus SGLT2i alone was associated

with a higher efficacy on HbA1c reduction, achievement of HbA1c < 7%, body weight loss, SBP reduction, and requirement of rescue therapy due to hyperglycemia, with a similar incidence of hypoglycemic events. A significant reduction in total and LDL cholesterol were also observed, with no changes in either HDL cholesterol or triglycerides.

Current guidelines recommend determining, assessing and pursuing regularly an HbA1c goal on an individual basis, given the strong predictive value for diabetes complications. HbA1c depends on the average glycemia in the previous 2–3 months, but it does not inform about postprandial hyperglycemia or hypoglycemia[3]. The use of some glucose-lowering drugs, specifically sulphonylureas and insulin, is largely limited by the risk of hypoglycemia, which is minimized when using GLP-1RA and SGLT2i[21]. Thus, the results of the present meta-analysis, showing that GLP-1RA/SGLT2i combinations are more effective than SGLT2i on HbA1c, while being characterized by a similar risk of hypoglycemia, are of interest.

Among GLP-1RA/SGLT2i combinations, it is noteworthy that a lower-magnitude reduction in HbA1c with a lower risk of hypoglycemia was found in DUAL IX compared to the other studies. Concerning HbA1c, one possible explanation is the trial design, since iDegLira was compared with glargine as add-on to SGLT2i, and both arms followed a treat to target approach; in regard to hypoglycemia incidence, it could be also due to the effects of liraglutide, differences in the insulin dose, as well as in the type of insulin, since lower rates of hypoglycemia have been reported for insulin degludec compared to insulin glargine U-100[19,22,23]. Moreover, in AWARD-10, an HbA1c reduction higher than reported could have been potentially achieved: a −0.54% HbA1c reduction was observed in the placebo arm, which could also represent a carry-over effect of the late introduction of SGLT2i in many patients (i.e., 3–6 months before study entry)[18].

Overweight and obesity represent common comorbidities in patients with type 2 diabetes mellitus. A weight loss of at least 5–10% is recommended in these patients, since this is usually associated with improvements in glycemic control and need for medications[3]. Both GLP-1RA and SGLT2i have been associated with weight loss, and one of them, received the approval for the treatment of obesity (i.e. liraglutide)[24,25]. Data from the SCALE Diabetes RCT showed a mean weight loss of 6.4 kg with liraglutide 3.0 mg once-daily and 5.0 kg with liraglutide 1.8 mg/die versus 2.2 kg in the placebo arm in overweight or obese patients with type 2 diabetes followed for 56 weeks; on the other hand, in SCALE Obesity and Prediabetes, a mean weight loss of 8.4 kg on liraglutide 3.0 mg once-daily versus 2.4 kg on placebo among obese patients without type 2 diabetes followed for 56 weeks was reported[26,27]. The above results suggest the difficulty to obtain weight loss with drugs recommended to treat obesity in patients with type 2 diabetes and at lower dosage[28]. This meta-analysis confirms that GLP-1RA induce a further −1.6 kg body weight reduction when added to SGLT2i in individuals with type 2 diabetes. It is worth noting that in SUSTAIN 9, baseline body weight was higher in the SGLT2i arm compared to GLP-1RA/SGLT2i arm; this could have led to an underestimation of treatment difference[20]. Also, in DURATION-8, a significantly higher proportion of participants lost at least 5% of body weight when treated with the combination of exenatide QW and dapagliflozin as compared to either exenatide or dapagliflozin (30.7%, 14.1%, and 21.3%, respectively)[17]. A proposed mechanism for the interaction is the suppression of appetite caused by GLP-1RA, limiting the increased food intake reported to occur with SGLT2i use, in addition to the SGLT2i-mediated glycosuria and consequent calorie loss[10]. Thus, when managing a type 2 diabetic patient with overweight or obesity and inadequately controlled HbA1c, the GLP-1RA/SGLT2i combination can be particularly useful in achieving both glycemic and body weight targets. Of note, in DUAL IX, the intervention and control groups differed in type and dose of basal insulin (e.g. degludec vs glargine), as already stated. Current evidence does not support any difference in change in body weight between these two insulins, therefore the overall results of our meta-analysis evaluating changes in body weight following the addition of GLP-1RA to SGLT2i should not be affected by the trial design and data of DUAL IX[29].

Hypertension and dyslipidemia represent frequent comorbidities in type 2 diabetes. Although not approved for, both GLP-1RA and SGLT2i have been shown to ameliorate hypertension, and GLP-1RA to improve dyslipidemia[25,30–32]. Therefore, an additive effect when they are used in combination is plausible and it is confirmed by this meta-analysis, in line with available data from other papers[25,33]. Noteworthy, the potential beneficial effect of GLP-1RA on LDL cholesterol may be nullified by the small increase observed with SGLT2i[25,34]. In regard to triglycerides and HDL cholesterol, a small impact has been reported for both classes. Indeed, since the combination of GLP-1RA/SGLT2i results in prominent reductions in HbA1c and body weight, a greater than observed effect on both triglycerides and HDL cholesterol was to be expected[35].

In DURATION-8, 695 patients were randomized to receive exenatide QW plus dapagliflozin (n = 231), exenatide QW (n = 231), or dapagliflozin (n = 233). This study allows to examine the interaction between the two drug classes on several endpoints. A less than additive effect was found on HbA1c, which could be explained by two hypotheses. As suggested by Polidori *et al*., a subadditive efficacy is expected in combination therapies because of different effective HbA1c levels at baseline on which each drug acts when given as component of a combination strategy, as compared with monotherapy[36]. According to other Authors, SGLT2i cause a rise in hepatic glucose production, which partially offsets the benefits of glycosuria; with increasing HbA1c levels, GLP-1RA may not be able to suppress gluconeogenesis and/or glycogenolysis induced by factors other than glucagon *per se*[37]. Whatever the mechanism, 38%, 30% and 16% of patients achieved an HbA1c < 7% in each arm, respectively. On the other hand, additive effects on body weight and SBP reductions were found, suggesting a mechanistic synergy between the two classes, as already noted[17].

In October 2019, a meta-analysis on SGLT2i and incretin-based agents combination therapy versus SGLT2i in patients with type 2 diabetes was published by Zhou *et al*.[38]. Three studies on the combination therapy with GLP-1RA and SGLT2i versus SGLT2i were included[17,18,20]. The Author concluded that the former was associated with a higher efficacy on HbA1c (Δ = −0.80%; 95% CI −1.14 to −0.45), body weight (Δ = −1.46 kg; 95% CI −2.38 to −0.54), and SBP (Δ = −2.88 mmHg; 95% CI −4.52 to −1.25); a higher risk of gastrointestinal disorders and

Study name (identifier)	GLP-1RA + SGLT2i arm	SGLT2i arm	Study type	Follow-up (weeks)	Number of patients	Population	Status
DECADE (2017-004709-42)	Exenatide QW + Dapagliflozin	Dapagliflozin	RCT	6	17	type 2 diabetes, HbA1c 7.5–10%	Ongoing
DECREASE (NCT03361098)	Exenatide BID + Dapagliflozin	Placebo + Dapagliflozin	RCT	16	64	type 2 diabetes, HbA1c 7–10%, BMI 30–40 kg/m^2	Recruiting
EXENDA (NCT03007329)	Exenatide QW + Dapagliflozin	Placebo + Dapagliflozin	RCT	24	90	type 2 diabetes, HbA1c 6.5–11%, BMI $\geq$ 25 kg/m^2	Recruiting
RESILIENT (2015-005242-60)	Exenatide QW + Dapagliflozin	Placebo + Dapagliflozin	RCT	32	120	type 2 diabetes, HbA1c 6.5–11%, BMI $\geq$ 30 kg/m^2	Ongoing

Table 2. Ongoing randomized controlled trials assessing GLP-1RA as add-on to SGLT2i. BMI, body mass index; BID, bis in die; GLP-1RA, glucagon-like peptide-1 receptor agonist; QW, once weekly; RCT, randomized controlled trial.

similar risk of genital infection, urinary tract infection and hypoglycemia were reported[38]. Despite the statement on the inclusion of the longest follow-up data to avoid duplicating results, data on both changes at week 28 at week 52, respectively, were reported for DURATION-8[38]. Also, no analysis was performed on other outcomes, including change in lipids from baseline to the last available follow-up, achievement of an HbA1c target of less than 7%, and requirement of rescue therapy due to hyperglycemia. Overall, the results of our meta-analysis are consistent with the data above, but evidence was gathered based on a greater number of patients and expanded on additional outcomes.

Several limitations of the present analysis should be discussed. The first limitation ascribes to its aims. Since data on adverse events other than hypoglycemia were not extracted, a full description of the benefits and limits of the addition of GLP-1RA to SGLT2i could not be performed. However, the proportion of patients experiencing treatment-emergent adverse events on GLP-1RA as add-on to SGL2i was similar to GLP-1RA only and in line with current literature, with most of them being of mild-to-moderate intensity[39,40]. Only four RCTs were found, and this is a second limitation. At least four ongoing studies were found, with a sample sizes ranging from 17 to 120 patients and a follow-up from 6 to 32 weeks (Table 2). Also, the results of a fifth study were recently published: in PIONEER-4, 183 patients on SGLT2i at baseline were randomized to oral semaglutide or liraglutide or placebo for 52 weeks. These patients could have potentially been included in our meta-analysis; however, data could not be retrieved even after contacting the corresponding Author of that study[41]. The results of the present review are thus meant to be exploratory; however, they will hardly change following the publication of the results of studies above. Thirdly, we found a high heterogeneity, so caution should be taken in generalizing the results to clinical practice. Specific properties of GLP-1RA and SGLT2i in each trial, study design, or patients' characteristics other than the extracted ones could explain the finding above. The duration of treatment with SGLT2i was different, since in AWARD-10 it was between 3 and 6 months for the majority of patients, compared to 11 months in SUSTAIN 9[18,20]. Also, we found only one study including patients on basal insulin as background therapy[19]. Fourthly, we were not able to assess the efficacy and safety of different doses of GLP-1RA, either as single drugs (e.g. liraglutide 1.2 mg versus liraglutide 1.8 mg once-daily) or according to the background therapy. Greater effects are to be expected when the highest available doses of GLP-1RA are prescribed to patients on SGLT2i. Lastly, this review included studies on type 2 diabetic patients with a baseline HbA1c ranging from 7 to 12% (in some cases with maximum HbA1c of 9.5%), and a follow-up to up to 52 weeks. Whether including subjects with higher HbA1c levels or a longer follow-up would have led to the same results is still to be assessed.

In conclusion, in patients with inadequately controlled type 2 diabetes mellitus, the addition of GLP-1RA to SGLT2i proved to be effective on HbA1c, body weight, SBP, and lipid profile. The chance of achieving HbA1c < 7% is increased, with no further risk of hypoglycemia. Current guidelines, trials results and findings of the present meta-analysis strongly support the GLP-1RA/SGLT2i combination as a strategic option in the management of patients with type 2 diabetes.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

M.C. and F.C. conceived the meta-analysis, developed the search strategy and provided statistical expertise. M.C. and A.C. drafted the manuscript. All Authors contributed to the development of the selection criteria, the risk of bias assessment strategy and data extraction criteria. All Authors read, provided feedback, and approved the final manuscript.

Competing interests

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Additional information

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Correspondence and requests for materials should be addressed to F.G.

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Association Between Glucagon-Like Peptide 1 Receptor Agonist and Sodium–Glucose Cotransporter 2 Inhibitor Use and COVID-19 Outcomes

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*Anna R. Kahkoska,[1] Trine Julie Abrahamsen,[2] G. Caleb Alexander,[3,4,5] Tellen D. Bennett,[6] Christopher G. Chute,[7] Melissa A. Haendel,[8] Klara R. Klein,[9] Hemalkumar Mehta,[3,4] Joshua D. Miller,[10] Richard A. Moffitt,[11] Til Stürmer,[12] Kajsa Kvist,[2] and John B. Buse,[9,13] N3C Consortium**

[1]Department of Nutrition, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC
[2]Novo Nordisk A/S, Copenhagen, Denmark
[3]Center for Drug Safety and Effectiveness, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
[4]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
[5]Division of General Internal Medicine, Johns Hopkins Medicine, Baltimore, MD
[6]Section of Informatics and Data Science, Department of Pediatrics, University of Colorado School of Medicine, Aurora, CO
[7]Schools of Medicine, Public Health, and Nursing, Johns Hopkins University, Baltimore, MD
[8]Center for Health AI, University of Colorado School of Medicine, Aurora, CO
[9]Division of Endocrinology and Metabolism, Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, NC
[10]Division of Endocrinology and Metabolism, Department of Medicine, Renaissance School of Medicine at Stony Brook University, Stony Brook, NY
[11]Department of Biomedical Informatics, Stony Brook University, Stony Brook, NY
[12]Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC
[13]NC Translational and Clinical Sciences Institute, University of North Carolina School of Medicine, Chapel Hill, NC

Corresponding author: John B. Buse, jbuse@med.unc.edu

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K.K. and J.B.B. made equal contributions as co-senior authors.

OBJECTIVE

To determine the respective associations of premorbid glucagon-like peptide-1 receptor agonist (GLP1-RA) and sodium–glucose cotransporter 2 inhibitor (SGLT2i) use, compared with premorbid dipeptidyl peptidase 4 inhibitor (DPP4i) use, with severity of outcomes in the setting of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

RESEARCH DESIGN AND METHODS

We analyzed observational data from SARS-CoV-2–positive adults in the National COVID Cohort Collaborative (N3C), a multicenter, longitudinal U.S. cohort (January 2018–February 2021), with a prescription for GLP1-RA, SGLT2i, or DPP4i within 24 months of positive SARS-CoV-2 PCR test. The primary outcome was 60-day mortality, measured from positive SARS-CoV-2 test date. Secondary outcomes were total mortality during the observation period and emergency room visits, hospitalization, and mechanical ventilation within 14 days. Associations were quantified with odds ratios (ORs) estimated with targeted maximum likelihood estimation using a super learner approach, accounting for baseline characteristics.

RESULTS

The study included 12,446 individuals (53.4% female, 62.5% White, mean ± SD age 58.6 ± 13.1 years). The 60-day mortality was 3.11% (387 of 12,446), with 2.06% (138 of 6,692) for GLP1-RA use, 2.32% (85 of 3,665) for SGLT2i use, and 5.67% (199 of 3,511) for DPP4i use. Both GLP1-RA and SGLT2i use were associated with lower 60-day mortality compared with DPP4i use (OR 0.54 [95% CI 0.37–0.80] and 0.66 [0.50–0.86], respectively). Use of both medications was also associated with decreased total mortality, emergency room visits, and hospitalizations.

CONCLUSIONS

Among SARS-CoV-2–positive adults, premorbid GLP1-RA and SGLT2i use, compared with DPP4i use, was associated with lower odds of mortality and other adverse outcomes, although DPP4i users were older and generally sicker.

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Diabetes is one of the comorbidities most strongly associated with severe coronavirus disease 2019 (COVID-19) in the U.S. (1). Data from early in the pandemic suggested approximately two times greater risk of death among individuals with type 2 diabetes compared with the risk for those without (2), as well as a greater risk of requiring hospitalization and intensive care (3,4).

Two classes of antihyperglycemic medications, glucagon-like peptide 1 receptor agonists (GLP1-RA) and sodium–glucose cotransporter 2 inhibitors (SGLT2i), have been associated with a reduction of cardiorenal events and mortality in large trials of cardiovascular outcomes (5–8), heart failure (9,10), and renal outcomes (11,12) in populations at high risk of cardiorenal events. Benefits associated with these medications appear most pronounced among individuals with type 2 diabetes and comorbid cardiovascular disease, heart failure, chronic kidney disease, and obesity (1,2,4,13,14), conditions that also incur the highest risk for severe COVID-19. Additionally, plausible mechanisms for the protective effects of GLP1-RA and SGLT2i in COVID-19, independent of their glycemic effects, have been speculated (15,16).

Yet, it is not known how the use of new antihyperglycemic medications is associated with severity of COVID-19. Therefore, our objective was to characterize the association of premorbid use of GLP1-RA and SGLT2i with COVID-19 outcomes. The study hypothesis was that use of both classes of medications would be associated with improved outcomes in the setting of COVID-19 infection. Characterizing these associations among individuals with type 2 diabetes may reveal interventional strategies to improve outcomes for a population at high risk for COVID-19–associated mortality. We selected individuals using dipeptidyl peptidase 4 inhibitors (DPP4i) as a comparator group because DPP4i, like the GLP1-RA and SGLT2i, are branded products that can be considered for second-line use after the initiation of metformin (17) and have been used in other real-world analyses to reduce the potential for confounding by clinical indication or socioeconomic status (18).

RESEARCH DESIGN AND METHODS

Study Design and Population

We analyzed data from a cohort study using COVID-19 data from health care systems across the U.S. contributing to the National COVID Cohort Collaborative (N3C) (19). The N3C cohort includes individuals with any encounter after 1 January 2020 and one or a combination of more than one of a set of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) laboratory tests, predefined based on diagnostic codes as defined by the N3C phenotype definition team (20,21).

For individuals included in N3C, the data set includes electronic health record (EHR) data from the same health system beginning 1 January 2018. In contrast to many COVID-19 data resources, the N3C data set encompasses individual-level data contributed by clinical sites across the U.S. The data set continues to grow as new individuals and institutions are added.

The University of North Carolina at Chapel Hill Office of Human Research Ethics determined that the research protocol did not constitute human subjects research (19). The study protocol was registered with the European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) on 5 October 2020 (no. 37860).

Individuals were included in the study if they were at least 18 years of age in 2020 with a positive SARS-CoV-2 PCR test and at least one ambulatory prescription of an antihyperglycemic medication of interest (GLP1-RA, SGLT2i, or DPP4i [according to the ATC codes listed in Supplementary Table 1]) in the 24 months preceding the SARS-CoV-2 PCR test. Information about drug prescriptions was based on information that captures prescriptions that were written or renewed during ambulatory visits and does not reflect dispensing. We excluded individuals with a history of both DPP4i and SGLT2i/GLP1-RA prescriptions within the previous 24 months of positive SARS-CoV-2 PCR test, i.e., concurrent use of DPP4i and either GLP1-RA or SGLT2i. Subjects on both GLP1-RA and SGLT2i contributed to both exposure arms. A total of 1,422 individuals had concurrent use of GLP-1RA and SGLT2i. We defined comorbidities based on the individual categories of diseases or diagnoses used to generate the updated Charlson Comorbidity Index (22). There were no inclusion criteria pertaining to diabetes diagnosis.

Measures, Definitions, and Outcomes

We defined the index date for each individual as the date of the first positive SARS-CoV-2 PCR test. The primary outcome was mortality within 60 days of any positive SARS-CoV-2 PCR test. Secondary outcomes were mortality during any time after index date (total mortality) and emergency room visits, hospitalization, and mechanical ventilation (i.e., intubation or ventilation) within 14 days of any positive SARS-CoV-2 PCR test. Compared with that for the other outcomes, the observation period for total mortality will vary between individuals, depending on the date of positive PCR test relative to the date of data release. All outcome assessments were consistent with a 2020 consensus statement on common outcome measures for COVID-19 clinical research (23).

Medical history and demographics were identified with use of all available data prior to the index date. Drug exposure were assessed with data from up to 24 months prior to the index date. Continuous variables, such as laboratory measurements and BMI, were also assessed with data from up to 24 months prior to the index date, with use of the most recent measurement.

Statistical Analysis

We conducted analyses in the order specified in the study protocol and as prespecified after the accrual of at least 150 deaths in the GLP1-RA and DPP4i populations pooled. Analyses were conducted on the N3C data with release dated 23 February 2021.

Baseline characteristics were summarized according to medication use with standardized mean differences (SMD) before and after propensity score weighting (PSW). Crude proportions for the primary and secondary outcomes were summarized.

To determine the association of GLP-1 RA and SGLT2i with outcomes, we used targeted maximum likelihood estimation (TMLE), using a super learner approach (24,25), as the primary statistical analysis method. TMLE is a semiparametric double robust method that improves the chance of correct model specification by allowing for flexible estimation using nonparametric machine learning methods for both the outcome and exposure model. The TMLE

uses the propensity scored exposure arms in the outcome model.

A sensitivity analysis examined inverse probability treatment weighted (IPTW) logistic regression using propensity scores. As prespecified in the protocol, stabilized weights were truncated at the 5% and 95% percentile, and covariates with SMD >0.1 after PSW were included in the outcome model. Both methods account for the baseline characteristics indicated in Table 1. In the case where the PSW exposure arms are not well-balanced, the TMLE produces larger SEs than the IPTW method. In addition to crude summaries, primary and secondary outcomes were also summarized after PSW.

For assessment of residual confounding relating to age and estimated glomerular filtration rate (eGFR), the primary and secondary analyses were repeated post hoc on an age-restricted cohort including only individuals aged 45–80 years and an eGFR-restricted cohort including only individuals with eGFR ≥45 mL/min/1.73 m^2. Both estimation procedures (i.e., TMLE and IPTW) were tested in the post hoc restricted cohorts.

As specified in the study protocol, missing values in continuous covariates were imputed based on an individual's medication arm, sex, and age with a linear regression model. Categorical covariates were imputed based on the majority category within the individual's medication arm. To evaluate the impact of imputing missing data in covariates, we performed a sensitivity analysis using only sex and age as covariates, which were, by definition, fully observed. Indicator variables for whether the individual has missing covariate information was also included in the TMLE model, which will induce wider confidence intervals if the information is missing not at random.

Significance testing was based on a 5% level. All analyses were done with Palantir Foundry hosted within the N3C Data Enclave, a cloud-based FedRAMP moderate-security enclave (19). Foundry is built on an Apache Spark back end, and analysis for this study was done with Python 3.6 and R 3.5.1. Statistical modeling was done with the tmle, ipw, and survey R packages.

RESULTS

As of 23 February 2021, there were 3,453,825 individuals across 42 contributing sites in the N3C database, including 629,242 with COVID-19. Of these, 12,446 individuals from 35 contributing sites were eligible for inclusion in the analyses (Supplementary Fig. 1). Crude and weighted baseline information is presented in Table 1 with SMD for weighted characteristics. Of the study population, 62.5% was White and 53.4% female, and mean ± SD age was 58.6 ± 13.1 years. The individuals in the DPP4i exposure arm were older and had a lower BMI than the individuals in the GLP1-RA and SGLT2i subgroups (age 64 years vs. 56 and 58 years, respectively, and BMI 33 kg/m^2 vs. 37 and 35 kg/m^2). For DPP4i there were also higher proportions of individuals with chronic kidney disease or end-stage renal disease, myocardial infarction, congestive heart failure, cancer, dementia, or stroke and there was a slightly lower use of insulin. After PSW, the exposure populations were comparable (Table 1). The distribution of the truncated propensity scores used in the model is shown in Supplementary Fig. 2.

The crude 60-day mortality rate in all individuals in the study was 3.11% (387 of 12,446) and differed according to class of premorbid medication use: 2.06% (138 of 6,692) and 2.32% (85 of 3,665) for individuals prescribed GLP1-RA and SGLT2i, respectively, and 5.67% (199 of 3,511) for individuals prescribed DPP4i (Table 2). Total mortality rate over the observation period was 2.29% (153 of 6,692) and 2.48% (91 of 3,665) for individuals prescribed GLP1-RAs and SGLT2i and 6.18% (217 of 3,511) for individuals prescribed DPP4i. The other crude secondary outcomes, including the proportion of emergency room visits, hospitalizations, and mechanical ventilation within 14 days of a positive SARS-CoV-2 test, are shown in Table 2. The primary and secondary outcomes after PSW are shown in Supplementary Table 2. After PSW, the 60-day mortality and total mortality in individuals prescribed GLP1-RA was 2.31% (149 of 6,475) and 2.58% (167 of 6,475), respectively, versus 4.86% (154 of 3,175) and 5.33% (169 of 3,175) in individuals prescribed DPP4i (Supplementary Table 2). The weighted 60-day mortality and total mortality rate was 2.70% (95 of 3,504) and 2.87% (100 of 3,504) for individuals prescribed SGLT2i vs. 4.74% (163 of 3,445) and 5.18% (178 of 3,445) for individuals prescribed DPP4i (Supplementary Table 2).

The results from the TMLE and IPTW analyses are presented as odds ratios (ORs) with 95% CIs in Fig. 1. Crude ORs for the same cohort are shown in Supplementary Table 3. The following results are reported from the TMLE analyses. Compared with DPP4i users, GLP1-RA users had lower odds of 60-day mortality (OR 0.54 [95% CI 0.37, 0.80]). The estimated risk difference in 60-day mortality between GLP1-RA and DPP4i use was −0.020 (95% CI −0.035, −0.0044), or 2.0 fewer deaths per 100 COVID-19 cases.

GLP1-RA use was also associated with lower odds relative to DPP4i use of total mortality (OR 0.56 [95% CI 0.39, 0.82]) and emergency room visits (OR 0.81 [95% CI 0.69, 0.96]), hospitalization (OR 0.73 [95% CI 0.62, 0.87]), and mechanical ventilation (OR 0.73 [95% CI 0.55, 0.97]) within 14 days of COVID-19 diagnosis.

Similar to GLP1-RA use, SGLT2i use showed lower odds of 60-day mortality relative to DPP4i use (OR 0.66 [95% CI 0.50, 0.86]). The estimated risk difference in 60-day mortality between SGLT2i and DPP4i use was −0.016 (95% CI −0.026, −0.0057), or 1.6 fewer deaths per 100 COVID-19 cases. SGLT2i use was associated with lower odds of total mortality (OR 0.63 [95% CI 0.49–0.82]), emergency room visits (OR 0.90 [95% CI 0.81, 0.998]) and hospitalization (OR 0.82 [95% CI 0.73, 0.91]) within 14 days of COVID-19 diagnosis. The odds of mechanical ventilation were not significantly different between SGLT2i and DPP4i use. Effect estimates generated from the IPTW analyses were consistent with TMLE estimates (Fig. 1).

The post hoc restricted cohort analyses (age 45–80 years and eGFR ≥45 mL/min/1.73 m^2)) yielded consistent effect estimates, with wider CIs reflecting smaller populations (Supplementary Table 4). Results from the sensitivity analysis in the full population, with adjustment only for age and sex, were also consistent with the main analysis (Supplementary Table 3).

CONCLUSIONS

Emerging evidence from the COVID-19 pandemic suggests that individuals with type 2 diabetes comprise a significant portion of the affected population and are at higher risk for severe outcomes including hospitalization and death (1,2). Due to the lack of a

Table 1—Demographics and clinical characteristics before and after PSW, according to premorbid medication use

	Crude characteristics				Weighted characteristics					
	All (N = 12,446)	GLP 1-RA users (N = 6,692)	SGLT2i users (N = 3,665)	DPP4i users (N = 3,511)	GLP 1-RA users (N = 6,475)	DPP4i users (N = 3,175)	SMD	SGLT2i users (N = 3,504)	DPP4i users (N = 3,445)	SMD
Age, years (N = 12,446)†	58.6 ± 13.1	55.7 ± 12.6	57.9 ± 11.7	64.1 ± 12.9	57.91 ± 12.64	59.88 ± 13.46	0.15	60.00 ± 11.66	61.00 ± 13.36	0.08
Sex, female (N = 12,446)†	6,641 (53.36)	3,953 (59.07)	1,642 (44.80)	1,759 (50.10)	3,640 (56.22)	1,696 (53.42)	0.06	1,629 (46.50)	1,635 (47.46)	0.02
Race, White (N = 11,146)†	7,781 (62.52)	4,286 (64.05)	2,422 (66.08)	2,014 (57.36)	4,705 (72.67)	2,273 (71.60)	0.02	2,564 (73.16)	2,483 (72.08)	0.02
Ethnicity, Hispanic or Latino (N = 11,347)†	1,472 (11.83)	778 (11.63)	433 (11.81)	427 (12.16)	765 (11.81)	377 (11.87)	<0.01	412 (11.77)	411 (11.94)	0.01
Current smoker†	680 (5.46)	361 (5.39)	215 (5.87)	185 (5.27)	347 (5.36)	170 (5.37)	<0.01	196 (5.61)	188 (5.46)	0.01
BMI, kg/m^2 (N = 7,044)†	35.4 ± 8.2	37.2 ± 8.1	35.2 ± 7.8	32.7 ± 8.0	35.96 ± 6.23	34.87 ± 6.98	0.16	34.30 ± 5.83	33.83 ± 6.67	0.08
Body weight , kg (N = 6,883)	101.7 ± 26.6	106.7 ± 26.3	102.9 ± 26.0	92.8 ± 25.9	103.80 ± 20.27	98.61 ± 22.18	0.24	99.86 ± 19.61	96.86 ± 21.43	0.15
Glycated hemoglobin, % (N = 9,928)†	8.0 ± 1.9	8.0 ± 2.0	8.2 ± 1.8	7.8 ± 1.9	7.98 ± 1.77	7.97 ± 1.71	<0.01	8.11 ± 1.59	8.04 ± 1.76	0.04
Glycated hemoglobin, mmol/mol	63.9 ± 20.8	63.9 ± 21.9	66.1 ± 19.7	61.7 ± 20.8	63.7 ± 19.3	63.6 ± 18.7	<0.01	65.1 ± 17.4	64.4 ± 19.2	0.04
Heart rate, bpm (N = 5,305)†	85.0 ± 15.7	86.4 ± 15.2	85.1 ± 15.5	83.3 ± 16.4	85.55 ± 10.30	84.72 ± 11.35	0.08	84.49 ± 10.59	84.19 ± 11.08	0.03
Systolic blood pressure, mmHg (N = 7,330)†	131.7 ± 19.1	131.2 ± 18.5	130.3 ± 17.9	133.5 ± 20.9	131.76 ± 14.62	132.21 ± 15.20	0.04	131.31 ± 14.53	132.01 ± 15.18	0.05
Diastolic blood pressure, mmHg (N = 7,328)†	75.8 ± 11.9	76.9 ± 11.6	76.2 ± 11.3	73.5 ± 12.2	75.99 ± 9.31	75.20 ± 9.56	0.08	75.27 ± 9.06	74.84 ± 9.47	0.05
eGFR, mL/min/1.73 m^2 (N = 10,098)†	77.5 ± 29.3	81.0 ± 28.3	81.8 ± 25.3	68.5 ± 31.7	78.41 ± 26.91	75.23 ± 29.99	0.11	78.22 ± 24.18	75.79 ± 29.05	0.9
Creatinine, mg/dL (N = 11,225)	1.2 ± 1.2	1.1 ± 1.0	1.0 ± 0.7	1.5 ± 1.6	1.16 ± 1.08	1.36 ± 1.45	0.15	1.08 ± 0.73	1.30 ± 1.30	0.21
ALT, units/L (N = 9,116)	31.0 ± 37.8	31.3 ± 41.7	32.2 ± 34.1	29.4 ± 28.2	30.99 ± 38.62	30.82 ± 24.72	0.01	31.48 ± 34.07	31.01 ± 25.07	0.02
AST, units/L (N = 9,593)	31.6 ± 68.9	30.8 ± 83.4	29.9 ± 26.8	33.6 ± 55.5	30.95 ± 71.73	32.93 ± 41.61	0.03	30.16 ± 25.59	33.00 ± 42.71	0.08
Medication										
Metformin†	7,667 (61.60)	4,020 (60.07)	2,556 (69.74)	2,128 (60.61)	3,941 (60.87)	1,957 (61.64)	0.02	2,359 (67.33)	2,253 (65.39)	0.04
Sulfonylurea†	3,381 (27.17)	1,487 (22.22)	1,096 (29.90)	1,217 (34.66)	1,689 (26.08)	945 (29.77)	0.08	1,137 (32.45)	1,146 (33.27)	0.02
Insulin†	6,587 (52.92)	3,713 (55.48)	1,928 (52.61)	1,848 (52.63)	3,540 (54.67)	1,733 (54.60)	<0.01	1,806 (51.53)	1,780 (51.68)	<0.01
Statin†	7,476 (60.07)	3,824 (57.14)	2,331 (63.60)	2,274 (64.77)	3,846 (59.40)	1,954 (61.56)	0.03	2,230 (63.64)	2,193 (63.66)	<0.01
ACEi/ARB†	7,321 (58.82)	3,797 (56.74)	2,302 (62.81)	2,150 (61.24)	3,759 (58.06)	1,891 (59.56)	0.03	2,182 (62.29)	2,130 (61.83)	0.01
Remdesivir	965 (7.75)	460 (6.87)	273 (7.45)	343 (9.77)	486 (7.51)	284 (8.93)	0.05	281 (8.02)	313 (9.09)	0.04
Medical history										
Myocardial infarction†*	1,141 (9.17)	502 (7.50)	363 (9.90)	393 (11.19)	548 (8.47)	300 (9.44)	0.03	354 (10.11)	355 (10.30)	0.01
Congestive heart failure†*	2,106 (16.92)	909 (13.58)	623 (17.00)	781 (22.24)	1,014 (15.65)	556 (17.51)	0.05	642 (18.32)	661 (19.17)	0.02
Cancer or metastatic cancer†*	1,228 (9.87)	566 (8.46)	308 (8.40)	457 (13.02)	633 (9.77)	348 (10.96)	0.04	358 (10.21)	377 (10.95)	0.02
Dementia or stroke†*	1,674 (13.45)	693 (10.36)	406 (11.08)	707 (20.14)	828 (12.79)	479 (15.08)	0.07	486 (13.87)	541 (15.71)	0.05
Chronic kidney disease or end-stage renal disease†	2,716 (21.82)	1,236 (18.47)	597 (16.29)	1,109 (31.59)	1,422 (21.96)	812 (25.57)	0.08	741 (21.14)	832 (24.14)	0.07
Peripheral vascular disease*	4,306 (34.60)	2,306 (34.46)	1,115 (30.42)	1,340 (38.17)	2,374 (36.66)	1,110 (34.96)	0.04	1,161 (33.14)	1,189 (34.51)	0.03
Mild liver disease*	1,620 (13.02)	913 (13.64)	493 (13.45)	411 (11.71)	866 (13.37)	397 (12.50)	0.03	463 (13.22)	408 (11.84)	0.04

Continued on p. 1568

Table 1—Continued

	Crude characteristics				Weighted characteristics				
	All (N = 12,446)	GLP 1-RA users (N = 6,692)	SGLT2i users (N = 3,665)	DPP4i users (N = 3,511)	GLP 1-RA users (N = 6,475)	DPP4i users (N = 3,175)	SMD	SGLT2i users (N = 3,504)	DPP4i users (N = 3,445)	SMD
Severe liver disease*	250 (2.01)	107 (1.60)	57 (1.56)	99 (2.82)	116 (1.79)	88 (2.76)	0.07	58 (1.65)	84 (2.45)	0.06
Pulmonary disease*	3,197 (25.69)	1,761 (26.32)	870 (23.74)	923 (26.29)	1,698 (26.22)	837 (26.38)	0.00	844 (24.08)	883 (25.63)	0.04
Coronary artery disease	2,497 (20.06)	1,139 (17.02)	792 (21.61)	841 (23.95)	1,269 (19.60)	625 (19.69)	0.00	816 (23.29)	723 (20.98)	0.06
Heart failure	1,986 (15.96)	850 (12.70)	583 (15.91)	743 (21.16)	950 (14.67)	532 (16.74)	0.06	604 (17.23)	624 (18.10)	0.02
Hypertension	9,456 (75.98)	5,012 (74.90)	2,832 (77.27)	2,764 (78.72)	4,972 (76.80)	2,426 (76.41)	0.01	2,757 (78.68)	2,636 (76.51)	0.05
Liver disease	766 (6.15)	394 (5.89)	231 (6.30)	224 (6.38)	392 (6.05)	207 (6.53)	0.02	222 (6.35)	206 (5.99)	0.02

For categorical parameters, data are n (%). For continuous parameters, data are means ± SDs. For weighted characteristics, data are shown after imputation of missing values. ACEi, ACE inhibitors; ARB, angiotensin receptor blockers. *Charlson Comorbidity Index category. †Characteristics included in model.

large cohort for evaluation, whether and how premorbid antihyperglycemic medication use may impact COVID-19–related outcomes have remained unclear. We examined these associations using N3C, a real-world U.S. database supporting the conduct of reproducible, transparent science investigating hypotheses in connection with COVID-19. Among adults with COVID-19, both GLP1-RA and SGLT2i use were associated with lower 60-day mortality compared with DPP4i use, as well as decreased total mortality, emergency room visits, and hospitalizations. GLP1-RA use was also associated with decreased odds of mechanical ventilation. Effect estimates were consistent across different statistical estimation strategies. In this analysis, DPP4i were selected as an active comparator because they are branded agents that have been well studied with minimal other clinical effects of concern, are among the five second-line therapies with prevalent use (17), and were recently suggested as an optimal comparator as a relatively newer agent among second-line therapies (18).

To date, investigations of potential COVID-19 risk factors have provided insights into individual characteristics that may increase the risk for poorer COVID-19–associated outcomes, such as age, race/ethnicity, socioeconomic status, obesity, and patterns of comorbidities (1,4,13,26). Meanwhile, the results from observational data on the impact of diabetes-related medications in the setting of COVID-19 have yielded mixed findings. For example, DPP4 inhibition garnered early interest as a target for the reduction of coronavirus infection severity via several potential mechanisms including decreased viral entry and immunomodulation (27). In the European Coronavirus SARS-CoV-2 and Diabetes Outcomes (CORONADO) study no associations were found between DPP4i and COVID-19 outcomes among individuals with diabetes in multivariate analyses (28). In an analysis of inpatient data from Wuhan, China, no association was found of glucose-lowering medications including metformin, insulin, secretagogues, or DPP4i with in-hospital mortality (29). Smaller observational COVID-19 studies from Europe and Asia suggested either benefits or no difference in mortality and other adverse outcomes with DPP4i therapy (28,30–32). For example, a series of studies from northern Italy suggested that premorbid use of DPP4i (30) and inpatient treatment with DPP4i in the setting of severe COVID-19 infection (31) were associated with reduced mortality, with similar trends reported from South Korea (33). Together, the potentially discrepant findings from observational studies underscore the need for confirmation via nonexperimental studies with a new user design as well as trial designs (34).

GLP1-RA have established anti-inflammatory effects, and preclinical studies indicate that GLP1-RA reduce cytokine production and lung inflammation (27). SGLT2i also may exert anti-inflammatory effects via increased fat utilization, shifts in energy metabolism, increases in hematocrit, selective reduction of interstitial volume with minimal changes in blood volume, and maintenance of cytosolic pH (16,35). In addition, both SGLT2i and GLP1-RA are associated with reduced risk of cardiovascular events and chronic kidney disease progression in patients at high risk (14), a population associated with higher mortality in individuals with COVID-19 (2). A recent population-based cohort study in Denmark compared the association of GLP1-RA and DPP4-i use and COVID-19 outcomes with that of SGLT2i use and COVID-19 outcomes and found that the use of incretin-based therapies was not associated with improved clinical outcomes (36), although statistical power was limited by a small sample size.

The research findings should be interpreted with the limitations of the study in mind. The main limitation of the study involves comparisons by prevalent drug prescribing rather than by drug initiation. This limits the causal interpretation of our findings as well as the validity of confounding control, e.g., by HbA_{1c}, since these measures are already affected by prior treatment (37).

We observed large differences in characteristics, including age and comorbidities, across treatment cohorts, with DPP4i users being older and generally sicker than the other two groups. Analytic methods to account for measured differences in these individual patient characteristics led to considerable attenuation of the ORs for both GLP1-RA and SLGT2i. Residual confounding due to, for example, severity of comorbidities or unmeasured confounding could still bias our results, with the true OR being even closer to the null. The post hoc analyses in age-restricted cohorts, in which the difference in mean age between the

Table 2—Crude primary and secondary outcomes according to premorbid medication use

	All (N = 12,446)	GLP1-RA users (N = 6,692)	SGLT2i users (N = 3,665)	DPP4i users (N = 3,511)
60-day mortality, E (%)	387 (3.11)	138 (2.06)	85 (2.32)	199 (5.67)
Total mortality, E (%)*	423 (3.40)	153 (2.29)	91 (2.48)	217 (6.18)
Emergency room visit, E (%)†	3,878 (31.16)	1,930 (28.84)	1,074 (29.30)	1,285 (36.60)
Hospitalization, E (%)†	3,163 (25.41)	1,465 (21.89)	851 (23.22)	1,172 (33.38)
Mechanical ventilation (intubation or ventilation), E (%)†	827 (6.64)	387 (5.78)	226 (6.17)	300 (8.54)

E, number of outcome events (first event only); *N*, total number of individuals; %, proportion of individuals with the outcome. *During the observation period. †Within 14 days after a positive SARS-CoV-2 test.

DPP4i group versus the GLP1-RA or SGLT2i group was <1 year after PSW (age 60.6 vs. 61.6 years for GLP1-RA vs. DPP4i, respectively, and 61.4 vs. 62.0 years for SGLT2i vs. DPP4i), suggest that associations with mortality were robust, particularly for GLP1-RA users compared with DPP4i users (Table 3). Finally, the results may be impacted by population differences reflecting the fact that DPP4i may be used in patients with chronic kidney disease and that the combination of major comorbidities was more frequent among DPP4i users than among the GLP-1RA or SGLT2i users. In the post hoc analyses in an eGFR-restricted cohort, with the aim of capturing a population with moderate-to-good renal function, point estimates were consistent with the main analyses, although the smaller cohorts were not adequately powered for statistical significance.

The results of this observational analysis may also be biased by factors that are difficult to measure and include in the analysis. In particular, GLP1-RA are more expensive than either of the other medications; unfortunately, data on socioeconomic status were not available for use in the current study, which represents a significant limitation of the data set. Other potential unmeasured confounders include differences in prescribing patterns across different care settings (i.e., primary care versus academic medical centers), variable delays in seeking treatment, heterogenous COVID-19 treatment protocols or therapies across different care settings and in different regions of the U.S., and differential clinical trajectories such as hyperglycemia or glycemic variability during infection, which may in turn influence outcomes (38).

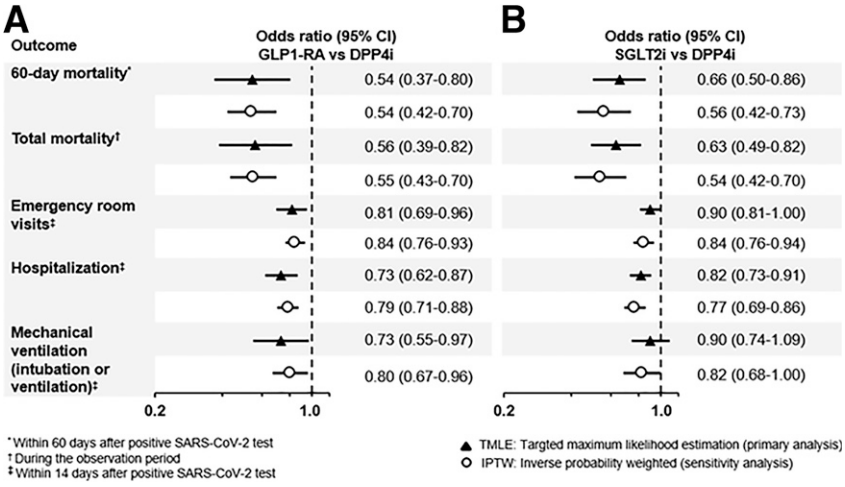

Figure 1—Forest plot depicting ORs for primary and secondary outcomes for patients with a COVID-19 diagnosis and prescription of a GLP1-RA, SGLT2i, or DPP4i, with TMLE (6) and IPTW (C). *A*: ORs for GLP1-RA vs. DPP4i. *B*: ORs for SGLT2i vs. DPP4i. *Within 60 days after positive SARS-CoV-2 test. †During the observation period. ‡Within 14 days after positive SARS-CoV-2 test.

There are several other limitations to the study. The COVID-19 diagnosis code does not represent a standardized time point in the clinical course, due to heterogeneity in timing of testing and assignment of COVID-19 diagnosis, which may contribute to heterogeneity in COVID-19 disease progression at the index date among individuals in the study. Individuals may be lost to follow-up; yet, underreporting of outcomes is expected to be independent of antihyperglycemic medication drug use with limited bias. The study population was defined by prescription of antihyperglycemic medication rather than diabetes ICD-10 code. Since EHR data, including diagnoses, prescriptions, and procedures, are only available when the individual is seen by a provider who contributes to the EHR system, any services conducted by providers external to the contributing EHR systems were not captured. This may limit data on outpatient diabetes regimens for new patient encounters. Although hospitalization rates were generally consistent with previously reported data (39), it is possible that some of the hospitalization events were not COVID-19 related; this limitation is partially addressed by the inclusion of other outcomes that are highly specific to COVID-19 such as mechanical ventilation. Per protocol, we did not consider comparisons with other antihyperglycemic medications, such as metformin monotherapy, and the study was not designed to assess interactions between different medications, such as how metformin may have enhanced associations of GLP1-RA or SGLT2i with improved outcomes. Finally, EHR data provide evidence of whether a drug was

Table 3—ORs in age-restricted cohort for 60-day mortality and secondary outcomes

	Model	GLP1-RA vs. DPP4i use	SGLT2i vs. DPP4i use
60-day mortality	Crude	0.44 (0.34–0.56)	0.45 (0.34–0.59)
	TMLE	0.59 (0.36–0.97)	0.69 (0.52–0.92)
	IPTW	0.52 (0.40–0.68)	0.60 (0.44–0.80)
Total mortality‡	Crude	0.44 (0.35–0.55)	0.44 (0.33–0.57)
	TMLE	0.60 (0.38–0.96)	0.67 (0.51–0.88)
	IPTW	0.53 (0.41–0.69)	0.58 (0.43–0.77)
Emergency room visit§	Crude	0.79 (0.72–0.87)	0.78 (0.70–0.87)
	TMLE	0.88 (0.74–1.04)	0.95 (0.85–1.07)
	IPTW	0.89 (0.80–1.00)	0.90 (0.80–1.00)
Hospitalization§	Crude	0.66 (0.60–0.73)	0.66 (0.59–0.74)
	TMLE	0.80 (0.67–0.95)	0.87 (0.77–0.98)
	IPTW	0.83 (0.74–0.93)	0.81 (0.72–0.91)
Mechanical ventilation (intubation or ventilation)§	Crude	0.75 (0.63–0.89)	0.79 (0.65–0.95)
	TMLE	0.77 (0.55–1.09)	0.95 (0.77–1.17)
	IPTW	0.79 (0.65–0.95)	0.87 (0.71–1.07)

Data are OR (95% CI). The age-restricted cohort included only individuals age 45–80 years (5,341 GLP1-RA users, 3,130 SGLT2i users, and 2,867 DPP4i users). TMLE: primary analysis. IPTW: sensitivity analysis. ‡During the observation period. §Within 14 days after a positive SARS-CoV-2 test.

prescribed—not whether the drug was reliably taken over time. The issue of unknown medication adherence may be particularly important in the setting of a pandemic, during which time economic or other disruptions may augment the challenges of daily adherence. ORs as a measure of association have limitations in the setting of IPTW. The N3C database is an evolving resource; the sample size is currently doubling every 4–6 weeks, and there are efforts to incorporate claims data and social determinants of heath. In the future, we hope to be able to address these limitations and potential residual confounding further. However, the overall effect sizes reported herein are large and robust to various analytic strategies and subgroup analyses, suggesting a potentially clinically relevant result.

There are several strengths of the study. The study population is geographically dispersed in the U.S. and demographically diverse, reflecting the impact of the pandemic on the nation. Currently, no diabetes-specific interventions are known to reduce the risk of a severe outcome of COVID-19, beyond the recommendations for the general population (3). This preliminary evidence for an association of antihyperglycemic medication use with COVID-19–related mortality and morbidity may be explored in the context of other infectious diseases or patient populations in the future. These data add to existing evidence for individual factors associated with risk for unfavorable outcomes.

A randomized global phase 3 trial of SGLT2i in the setting of diabetes and COVID-19 is currently ongoing and is expected to generate definitive data (Dapagliflozin in Respiratory Failure in Patients With COVID-19 [DARE-19], clinical trial reg. no. NCT04350593, ClinicalTrials.gov). A small prospective open-label blinded-evaluation study of semaglutide in COVID-19 is being conducted in Canada (Semaglutide to Reduce Myocardial Injury in PATIents With COVID-19 [SEMPATICO], NCT04615871). Given evidence from retrospective analyses, several randomized trials are planned or have been initiated to investigate the role for DPP4i, as well (NCT04542213, NCT04371978, NCT04341935, and NCT04365517).

In conclusion, this study provides evidence for antihyperglycemic medication class–based differences in COVID-19 outcomes, where premorbid GLP1-RA or SGLT2i prescribing is associated with lower mortality and other adverse clinical outcomes in the setting of a COVID-19 diagnosis as compared with DPP4i prescribing.

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BRIEF COMMUNICATION

Genetic Evidence for Repurposing of GLP1R (Glucagon-Like Peptide-1 Receptor) Agonists to Prevent Heart Failure

Iyas Daghlas, BS; Ville Karhunen, PhD; Devleena Ray, BSc; Verena Zuber, PhD; Stephen Burgess, PhD; Philip S. Tsao, PhD; Julie A. Lynch, PhD, RN; Kyung Min Lee, PhD; Benjamin F. Voight, PhD; Kyong-Mi Chang, MD; Emma H. Baker, PhD; Scott M. Damrauer, MD; Joanna M. M. Howson, PhD; Marijana Vujkovic, PhD, MSCE; Dipender Gill, BMBCh, PhD

BACKGROUND: This study was designed to investigate the genetic evidence for repurposing of GLP1R (glucagon-like peptide-1 receptor) agonists to prevent heart failure (HF) and whether the potential benefit exceeds the benefit conferred by more general glycemic control.

METHODS AND RESULTS: We applied 2-sample Mendelian randomization of genetically proxied GLP1R agonism on HF as the main outcome and left ventricular ejection fraction as the secondary outcome. The associations were compared with those of general glycemic control on the same outcomes. Genetic associations were obtained from genome-wide association study summary statistics of type 2 diabetes mellitus (228 499 cases and 1 178 783 controls), glycated hemoglobin (n=344 182), HF (47,309 cases and 930 014 controls), and left ventricular ejection fraction (n=16 923). Genetic proxies for GLP1R agonism associated with reduced risk of HF (odds ratio per 1 mmol/mol decrease in glycated hemoglobin 0.75; 95% CI, 0.64–0.87; P=1.69×10^{-4}), and higher left ventricular ejection fraction (SD change in left ventricular ejection fraction per 1 mmol/mol decrease in glycated hemoglobin 0.22%; 95% CI, 0.03–0.42; P=0.03). The magnitude of these benefits exceeded those expected from improved glycemic control more generally. The results were similar in sensitivity analyses, and we did not find evidence to suggest that these associations were mediated by reduced coronary artery disease risk.

CONCLUSIONS: This genetic evidence supports the repurposing of GLP1R agonists for preventing HF.

Key Words: diabetes mellitus ■ ejection fraction ■ GLP1R ■ heart failure ■ Mendelian randomization

Patients with type 2 diabetes mellitus are at increased risk of developing heart failure and evidence from randomized controlled trials supports that GLP1R (glucagon-like peptide-1 receptor) agonists reduce this risk.[1,2] The aim of this study was to leverage human genetic data within the Mendelian randomization paradigm to investigate whether effects of GLP1R agonists on heart failure risk and left ventricular ejection fraction (LVEF) exceed those of improved glycemic control more generally.

METHODS

All data used in this work are publicly available and anonymized. All contributing studies received appropriate ethical approval and patient consent.

Methodologic Overview

The Mendelian randomization (MR) approach uses genetic variants as proxies to investigate the causal effect of an exposure on an outcome.[3,4] This method

Correspondence to: Mr Iyas Daghlas, Harvard Medical School, 25 Shattuck St, Boston, MA 02115. E-mail: iyas_daghlas@hms.harvard.edu

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leverages the random allocation of genetic variants at conception to reduce any bias due to confounding and reverse causation that can limit causal inference in observational research. MR can be extended to investigate drug effects by leveraging genetic variation in genes (eg, *GLP1R*) encoding proteins corresponding to drug targets.[5]

Genetic Proxies for GLP1R Agonism and Glycemic Control

We identified genetic proxies for the effect of GLP1R agonism as genome-wide significant ($P<5\times10^{-8}$) and uncorrelated ($r^2<0.1$) variants in the *GLP1R* gene (genomic position on build GRCh37/hg19: chromosome 6:39 016 574–39 055 519) that associated with type 2 diabetes mellitus liability in the largest published genome-wide association study meta-analysis (228 499 cases and 1 178 783 controls; 79% European ancestry),[6] with directionally concordant and nominally significant ($P<0.05$) associations with glycated hemoglobin in the UK Biobank (n=344 182).[7] Unless otherwise stated, all downstream analyses were weighted by the variant association with glycated hemoglobin (mmol/mol). These variants were annotated for their sequence effects (eg, intron or missense), and we queried the Genotype-Tissue Expression v8 data set of 54 tissue types to determine whether the variants were associated with gene expression.[8] Variants were annotated as having directionally concordant associations with gene expression if they were associated with lower glycated hemoglobin and greater expression of *GLP1R* (or vice versa).

Genetic proxies for glycemic control more generally were identified through the same associations but considering genetic variants throughout the genome that were not located within 1megabase of *GLP1R*. Given the larger number of variants identified from throughout the genome, we used a stricter clumping threshold of $r^2<0.001$ to minimize bias due to linkage disequilibrium.

Heart Failure and Left Ventricular Ejection Fraction Genetic Association Estimates

Heart failure was the primary outcome for our analysis. We obtained genetic association estimates from the Heart Failure Molecular Epidemiology for Therapeutic Targets Consortium consisting of 47 309 cases and 930 014 controls of European ancestry.[9] Cases included patients with a clinical diagnosis of heart failure, irrespective of the ejection fraction. We further investigated LVEF as a secondary outcome using genetic association estimates from a study of cardiac magnetic resonance imaging derived LVEF in the UK Biobank (n=16 923, all of European ancestry).[10] LVEF was inverse normal-transformed, and the genetic association estimates are therefore presented in approximate SD units.

Statistical Analysis

For each of the variants used in MR analysis, we harmonized genetic associations with the exposure and outcome by aligning effect alleles, with no exclusion made for palindromic variants. We derived MR estimates considering genetically proxied GLP1R agonism and glycemic control more generally using the random-effects inverse-variance weighted method with intercept fixed at the origin,[3] orientating estimates to reduction in glycated hemoglobin (ie, the direction of drug effect). All MR analyses were performed using the TwoSampleMR package in R.[3] To assess for a GLP1R agonism drug class effect that exceeds the anticipated effect of glycemic control more generally, we tested for a significant difference between the respective MR estimates. The point estimate for this difference was obtained by taking the difference between the MR beta coefficients for the GLP1R and glycemic control estimates, and the SE for the difference was derived using the propagation of error method:

$$SE\left(\beta_{GLP1R}-\beta_{GLYCEMIA}\right)=\sqrt{SE\left(\beta_{GLP1R}\right)^2+SE\left(\beta_{GLYCEMIA}\right)^2},$$

where β_{GLP1R} and $\beta_{GLYCEMIA}$ are the MR estimates for the associations of genetically proxied GLP1R agonism and glycemic control with the outcomes.

Analyses investigating LVEF as a secondary outcome were considered exploratory, and so the *P* values were not corrected for multiple comparisons. All hypothesis tests were 2 sided.

Sensitivity Analyses

In sensitivity analyses considering GLP1R agonism we restricted the genetic proxies to coding variation in *GLP1R*, as these variants more plausibly relate to *GLP1R* function. Corresponding MR estimates that used a single proxy variant were derived using the Wald ratio with first-order SEs. We also performed analyses excluding any coding variants to ensure that they were not solely driving the MR estimates. To determine whether results were sensitive to our choice to weight the variants by their associations with glycated hemoglobin, we also performed analyses weighted by the log-odds of type 2 diabetes mellitus liability. MR estimates may be biased by horizontal pleiotropy if the genetic variants proxying GLP1R agonism influence heart failure risk or LVEF through a pathway independent of GLP1R agonism. We first tested for any such bias by calculating the Cochran Q test *P* value to assess for overdispersion in the MR estimates provided by each variant in the GLP1R agonism instrument. We then performed analyses using the weighted median

method, which provides consistent MR estimates if more than half of the weight from the genetic proxies comes from valid instrumental variables.[11]

To determine whether protective effects of GLP1R agonism on heart failure may be mediated by reduced coronary artery disease risk, we performed MR analyses investigating the effect of GLP1R agonism on coronary artery disease risk. We obtained genetic association estimates from a meta-analysis of data from the CARDIOGRAMplusC4D Consortium and UK Biobank consisting of 122 733 cases and 424 528 controls of European ancestry.[12]

RESULTS

Identification of Genetic Proxies for GLP1R Agonism and Glycemic Control More Generally

Three independent variants in *GLP1R* were identified as genetic proxies for GLP1R agonism, including 1 missense variant (rs10305420) and 2 intronic variants (rs2268647 and rs75151020; Tables S1–S2). Two of these variants were significantly associated with expression of *GLP1R* across several human tissues, and both variants had directionally concordant associations with *GLP1R* expression in pancreatic tissue (Table S3). A directionally discordant association with *GLP1R* expression in left ventricular and left atrial appendage myocardial tissue was identified for the intronic variant rs2268647 (Table S3). There were 350 variants available for use as proxies for glycemic control more generally in the heart failure data set (Table S4) and 334 variants available in the LVEF data set (Table S5).

Mendelian Randomization Analyses

Genetically proxied GLP1R agonism associated with a reduced risk of heart failure (odds ratio [OR] per 1 mmol/mol decrease in glycated hemoglobin, 0.75; 95% CI, 0.64–0.87; $P=1.69\times10^{-4}$). This estimate was similar in MR analysis only using the missense variant rs10305420 (OR, 0.62; 95% CI, 0.45–0.85; $P=2.59\times10^{-3}$). Analyses excluding this variant provided similar evidence of effect, suggesting that this variant did not solely drive the estimates (OR, 0.79; 95% CI, 0.67–0.92; $P=3.33\times10^{-3}$). Consistent with previous reports,[9] a genetically proxied improvement in overall glycemic control associated with reduced risk of heart failure (OR, 0.96; 95% CI, 0.94–0.97; $P=7.75\times10^{-11}$). This estimate was smaller in magnitude than the estimate obtained for genetically proxied GLP1R agonism ($P_{difference}=1.58\times10^{-3}$; Figure).

Genetically proxied GLP1R agonism associated with a higher LVEF (SD change in LVEF, 0.22; 95% CI, 0.03–0.42; $P=0.03$). There was no evidence of an association between genetically proxied glycemic control more generally and LVEF (SD change in LVEF, 0.00; 95% CI, −0.01 to 0.02; $P=0.67$). This estimate was smaller in magnitude than that obtained for genetically proxied GLP1R agonism ($P_{difference}=0.03$). Corresponding scatter plots for all analyses are provided in Figures S1–S5.

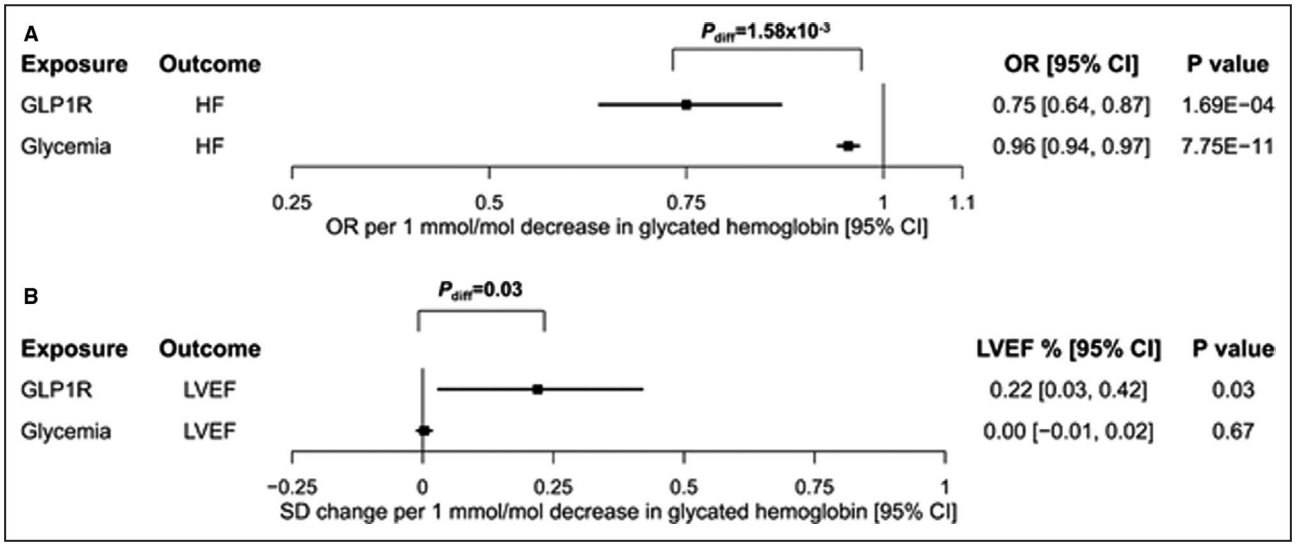

Figure. Forest plot depicting Mendelian randomization estimates for the association of genetically proxied GLP1R (glucagon-like peptide receptor) agonism and glycemic control more generally with (A) risk of heart failure (HF; 47 309 cases/930 014 controls) and (B) left ventricular ejection fraction (LVEF; n=16 923).

Estimates reflect the effect of a reduction in glycated hemoglobin on each of the respective outcomes (so as to orient estimates to GLP1R agonist drug effects). Squares correspond to point estimates, and the surrounding lines correspond to 95% CIs. diff indicates difference; and OR, odds ratio.

Sensitivity Analyses

Analyses weighting the genetic proxies for GLP1R agonism (OR per log-odds increase in type 2 diabetes mellitus liability, 0.48; 95% CI, 0.34–0.67; $P=2.87\times10^{-5}$) and overall glycemic control (OR, 0.90; 95% CI, 0.87–0.93; $P=1.81\times10^{-12}$) by type 2 diabetes mellitus liability showed similar evidence for a reduction in heart failure risk. There was no significant heterogeneity in the MR estimates generated by the different variants when considering either heart failure or LVEF as outcomes (Figures S1–S3). Results from analyses using the weighted median method showed significant protective associations of genetically proxied GLP1R agonism (OR, 0.77; 95% CI, 0.62–0.96; $P=0.02$) and improved glycemic control (OR, 0.98; 95% CI, 0.96–1.00; $P=0.04$) with heart failure risk, and directionally concordant but nonsignificant associations of genetically proxied GLP1R agonism with LVEF (SD change in LVEF, 0.18; 95% CI, −0.07 to 0.42; $P=0.16$; Table S6). We found no evidence for an association of genetically proxied GLP1R agonism with coronary artery disease risk (OR per 1 mmol/mol decrease in glycated hemoglobin, 1.02; 95% CI, 0.89–1.16; $P=0.80$).

DISCUSSION

In this MR study, we used human genetic data to identify proxies for GLP1R agonism and found evidence for their protective effect on risk of heart failure. In secondary analyses, we found associations of genetically proxied GLP1R agonism with increased LVEF. The magnitude of these estimates exceeded those generated using genetic proxies for glycemic control more generally, supporting a role for GLP1R signaling in preventing heart failure beyond an effect on glycemic control alone.[13] We did not find evidence of heterogeneity in the MR estimates generated by the genetic proxies for GLP1R agonism when considering either heart failure or LVEF as outcomes, and results were similar in sensitivity analyses using the weighted median method, with the null effect of GLP1R agonism on LVEF potentially attributable to low statistical power. The null effect of genetically proxied GLP1R agonism on coronary artery disease risk suggests that a reduced risk of heart failure is not attributable to chronic ischemic heart disease.

Our findings are consistent with meta-analyses of randomized controlled trials identifying a protective effect of GLP1R agonists on hospital admission with heart failure[1,2] and go further to provide genetic evidence supporting a drug effect on LVEF. Further clinical research is needed to determine contexts where GLP1R agonists may be repurposed for reducing risk of heart failure, particularly given the established effects of sodium glucose cotransporter 2 inhibitors for reducing progression of heart failure in patients with and without type 2 diabetes mellitus.[14]

A similar genetic approach was previously used to support a protective effect of GLP1R agonism on coronary artery disease risk[15]; however, our analyses did not replicate this finding. The previous investigation used a low-frequency missense variant, rs10305492, which is not in strong linkage disequilibrium with any of the variants included in our investigation (all pairwise $r^2<0.16$). We did not select this variant for inclusion in our analysis as its association with type 2 diabetes mellitus achieved only a nominal level of statistical significance ($P=0.001$), and not the more stringent genome-wide level of statistical significance achieved by the variants in our investigation. In contrast, meta-analyses of clinical trials have supported a nominally significant ($P=0.043$ before adjustment for multiple comparisons) beneficial effect of GLP1R agonism on myocardial infarction.[1] Given the small magnitude of this reported effect (hazard ratio, 0.91; 95% CI, 0.84–1.00[1]) and the span of the CIs from our MR estimates (95% CI, 0.89–1.16), it is plausible that the null MR estimate for coronary artery disease is attributable to low statistical power.

The key strength of our work is the use of randomly allocated genetic proxies to study the effects of GLP1R agonism. The genetic proxies used in these analyses were further validated by their associations with glycated hemoglobin, which reduces risk of bias due to winner's curse and permits the contextualization of the MR estimates on the glycated hemoglobin scale. The genetic associations with LVEF were adjusted for body mass index, which allowed standardization for body size. A study limitation is the absence of available large-scale genetic summary data for heart failure subtypes. Although we used a missense variant in *GLP1R* (rs10305420) and gene expression data to strengthen the validity of our findings, further experimental work is necessary to determine the mechanism by which these variants influence *GLP1R* expression or function. In particular, the directionally concordant association of the rs2268647 intronic variant on gene expression in the pancreas, but discordant effect on *GLP1R* expression in myocardial tissue warrants further exploration. The MR estimates reflect the consequence of a lifelong genetic perturbation of GLP1R signaling and cannot be extrapolated to predict the magnitude of effect from shorter, discrete pharmacological interventions. The limited number of genetic variants available to instrument GLP1R agonism precluded more extensive sensitivity analyses for horizontal pleiotropy. In particular, modeling an intercept term (as in the MR-Egger regression approach) can in some scenarios mitigate bias from unbalanced horizontal

pleiotropy but was not appropriate in our analysis of GLP1R agonism because of the availability of only 3 genetic proxies. We used summary-level genetic associations with heart failure and LVEF and therefore could not perform stratified analyses, such as by sex or diabetes mellitus status. Finally, these genetic data were predominantly gathered from individuals of European ancestry and these results may therefore not generalize to other ethnic groups.

CONCLUSIONS

In conclusion, we identified genetic proxies for the effects of GLP1R agonism, and applied these proxies in MR analyses to generate evidence supporting a protective effect on risk of heart failure. Further investigation of GLP1R agonist repurposing to prevent heart failure in the context of clinical trials is warranted.

ARTICLE INFORMATION

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Affiliations

Harvard Medical School, Boston, MA (I.D.); Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, UK (V.K., D.R., V.Z., D.G.); Medical Research Council Biostatistics Unit, Cambridge Institute of Public Health, Cambridge, UK (V.Z., S.B.); Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK (S.B.); VA Palo Alto Health Care System, Palo Alto, CA (P.S.T.); Department of Medicine, Stanford University School of Medicine, Stanford, CA (P.S.T.); Department of Veterans Affairs, VA Informatics and Computing Infrastructure, Salt Lake City Health Care System, Salt Lake City, UT (J.A.L., K.M.L.); Department of Internal Medicine, University of Utah School of Medicine, Salt Lake City, UT (J.A.L.); Corporal Michael J. Crescenz VA Medical Center, Philadelphia, PA (B.F.V., K.-M.C., S.M.D., M.V.); Department of Genetics (B.F.V.), Department of Systems Pharmacology and Translational Therapeutics (B.F.V.), Institute of Translational Medicine and Therapeutics (B.F.V.), Department of Medicine (K.-M.C., M.V.), University of Pennsylvania Perelman School of Medicine, Philadelphia, PA (S.M.D.); Clinical Pharmacology and Therapeutics Section, Institute of Medical and Biomedical Education and Institute for Infection and Immunity, St George's, University of London, London, UK (E.H.B., D.G.); Clinical Pharmacology Group, Pharmacy and Medicines Directorate, St George's University Hospitals NHS Foundation Trust, London, UK (E.H.B., D.G.); Novo Nordisk Research Centre Oxford, Oxford, UK (J.M.M.H., D.G.).

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Supplementary Material

Tables S1–S6
Figures S1–S5

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SUPPLEMENTAL MATERIAL

Table S1. Genetic proxies for GLP1R agonism, and estimates for their association with glycated hemoglobin, type 2 diabetes, heart failure, and left ventricular ejection fraction.

						Glycated hemoglobin			Type 2 diabetes			Heart failure			Left ventricular ejection fraction		
SNP	**Chr**	**Position**	**EA**	**OA**	**EAF**	**Beta**	**SE**	**P**	**Beta**	**SE**	**P**	**Beta**	**SE**	**P**	**Beta**	**SE**	**P**
rs10305420*	6	39016636	T	C	0.39	-0.051	0.016	1.30×10^{-3}	-0.032	0.004	5.11×10^{-14}	-0.024	0.008	2.63×10^{-3}	-0.009	0.011	4.1×10^{-1}
rs75151020	6	39031592	C	A	0.09	0.119	0.026	7.08×10^{-6}	0.041	0.007	1.37×10^{-9}	-0.022	0.013	1.04×10^{-1}	0.040	0.019	3.4×10^{-2}
rs2268647	6	39043178	T	C	0.52	0.066	0.015	1.51×10^{-5}	0.021	0.004	4.95×10^{-8}	0.019	0.007	1.12×10^{-2}	0.008	0.011	4.4×10^{-1}

Chr: chromosome; EA: effect allele; EAF: effect allele frequency; OA: other allele; SE: standard error; SNP: single nucleotide polymorphism. *: missense variant

Table S2. Linkage disequilibrium r^2 values for variants used as proxies for GLP1R agonism.

rsID	rs10305420	rs75151020	rs2268647
rs10305420	1.00	0.01	0.00
rs75151020	0.01	1.00	0.07
rs2268647	0.00	0.06	1.00

r^2 values were obtained using linkage disequlibrium data from the European subsample of the 1000 Genomes project (https://ldlink.nci.nih.gov/?tab=ldmatrix).

Table S3. Genetic proxies for GLP1R agonism, and estimates for their association with gene expression in the GTEx v8 database.

Gene Symbol	SNP	P-Value	NES	Directional concordance with glycated hemoglobin	Tissue
GLP1R	rs10305420	2.10E-09	0.23	Yes	Nerve - Tibial
GLP1R	rs10305420	3.80E-06	0.26	Yes	Adipose - Visceral (Omentum)
GLP1R	rs10305420	4.10E-06	0.25	Yes	Thyroid
GLP1R	rs10305420	2.30E-05	0.24	Yes	Pancreas
GLP1R	rs2268647	1.00E-09	0.31	No	Heart - Left Ventricle
GLP1R	rs2268647	2.10E-07	0.23	No	Heart - Atrial Appendage
GLP1R	rs2268647	3.40E-07	-0.33	Yes	Stomach
GLP1R	rs2268647	2.10E-06	-0.27	Yes	Pancreas
GLP1R	rs2268647	1.60E-05	-0.23	Yes	Thyroid
ANKRD18EP	rs2268647	2.20E-05	-0.12	Yes	Skin - Sun Exposed (Lower leg)

rs75151020 did not significantly influence gene expression in any of the tissues in the GTEx database. Variants were annotated as directionally concordant if they were associated with lower glycated hemoglobin and higher expression of *GLP1R* (or vice versa). NES: normalized effect size; SNP: single nucleotide polymorphism.

Table S4. Genetic proxies for glycemic control by any mechanism, and estimates for their association with glycated hemoglobin (for the outcome of heart failure).

SNP	Chromosome	Position	Effect allele	Other allele	EAF	Beta	SE
rs2482506	10	104563743	G	C	0.25	-0.053	0.018
rs79364741	10	114666651	T	C	0.01	-0.164	0.073
rs11196174	10	114734096	G	A	0.29	0.252	0.017
rs149692182	10	114752674	T	C	0.02	0.309	0.053
rs35676242	10	114757314	A	C	0.05	0.230	0.036
rs11257655	10	12307894	T	C	0.21	0.219	0.019
rs946859	10	13565429	A	G	0.47	-0.075	0.015
rs3122231	10	44027356	C	T	0.65	0.050	0.016
rs113899647	10	64850074	T	C	0.03	-0.189	0.044
rs949693	10	70354574	A	G	0.61	-0.050	0.016
rs11592899	10	71333783	A	G	0.34	-0.055	0.016
rs2812535	10	71456857	A	G	0.62	0.069	0.016
rs697239	10	80947438	C	T	0.46	-0.105	0.015
rs11201992	10	88117318	A	C	0.46	-0.038	0.015
rs1111875	10	94462882	T	C	0.41	-0.181	0.016
rs66536955	10	94737667	C	T	0.26	0.044	0.017
rs34041345	10	99174580	G	T	0.26	0.060	0.018
rs529623	11	117693255	C	T	0.52	-0.059	0.015
rs10893830	11	128044159	T	C	0.13	-0.058	0.023
rs10750397	11	128234144	G	A	0.72	-0.040	0.017
rs67232546	11	128398938	T	C	0.21	0.067	0.019
rs117316450	11	14518419	G	C	0.02	0.316	0.054
rs757110	11	17418477	A	C	0.64	-0.112	0.016
rs11042987	11	2201059	A	C	0.58	-0.034	0.016
rs10831668	11	2288412	T	C	0.02	0.234	0.060
rs231362	11	2691471	G	A	0.52	0.120	0.015
rs10767659	11	27686196	T	G	0.67	-0.041	0.016

rs60808706	11	2857233	A	G	0.05	-0.227	0.035
rs2289488	11	2892955	C	G	0.40	0.040	0.016
rs62618693	11	32956492	T	C	0.05	-0.144	0.037
rs523472	11	35031668	A	G	0.72	-0.056	0.017
rs3816605	11	47857253	C	T	0.45	-0.080	0.015
rs7483027	11	58128015	C	T	0.38	-0.061	0.016
rs174541	11	61565908	C	T	0.36	-0.098	0.016
rs1143756	11	65299595	G	A	0.29	0.100	0.017
rs3918296	11	69459036	G	C	0.03	-0.249	0.049
rs11602873	11	72460762	T	A	0.16	-0.187	0.021
rs11236524	11	75464344	C	T	0.09	0.069	0.027
rs4945090	11	76205018	A	T	0.60	0.036	0.016
rs12802861	11	8387806	T	C	0.28	-0.052	0.017
rs10830963	11	92708710	G	C	0.28	0.297	0.017
rs3020069	11	93057087	A	G	0.68	0.093	0.016
rs1426371	12	108629780	A	G	0.26	-0.074	0.018
rs79310463	12	118406696	T	C	0.13	0.104	0.023
rs56348580	12	121432117	C	G	0.31	-0.037	0.017
rs7975763	12	123604053	T	C	0.20	-0.057	0.019
rs11614914	12	133070294	T	C	0.33	0.078	0.016
rs12828318	12	133766122	G	A	0.18	-0.057	0.020
rs10841886	12	21864377	C	T	0.23	-0.082	0.018
rs1480029	12	26356032	A	G	0.46	0.042	0.015
rs3751239	12	27963676	G	C	0.20	-0.160	0.019
rs11063018	12	4288001	C	T	0.17	0.067	0.020
rs74862545	12	4365572	T	C	0.02	-0.279	0.052
rs76895963	12	4384844	G	T	0.02	-1.037	0.059
rs2732469	12	48712932	A	T	0.43	-0.258	0.015
rs61937817	12	57212823	G	T	0.11	0.060	0.024
rs11173646	12	61250814	T	A	0.82	-0.046	0.020
rs2257883	12	66216162	A	G	0.13	0.150	0.023
rs12371967	12	66346714	C	T	0.17	-0.043	0.020

rs10879261	12	71520761	G	T	0.41	0.068	0.016
rs11108094	12	95928113	A	C	0.07	0.099	0.030
rs6538805	12	97849120	C	T	0.39	-0.076	0.016
rs9587811	13	109946882	A	C	0.41	-0.056	0.016
rs314879	13	23309382	T	C	0.79	-0.069	0.019
rs34584161	13	26776999	G	A	0.24	-0.063	0.018
rs380854	13	33574631	A	G	0.58	-0.058	0.016
rs9316500	13	51094114	G	T	0.29	-0.067	0.017
rs7991679	13	58691107	A	T	0.16	-0.081	0.021
rs1215451	13	80715893	A	G	0.29	-0.131	0.017
rs112324411	14	101258584	T	C	0.07	-0.101	0.032
rs2295388	14	101309759	A	G	0.22	-0.073	0.019
rs4906272	14	103376031	T	C	0.16	0.046	0.021
rs12883788	14	33303540	T	C	0.46	0.060	0.015
rs7147483	14	38804675	C	T	0.25	-0.158	0.018
rs723355	14	47304091	A	G	0.50	-0.034	0.015
rs4902002	14	61229411	A	G	0.71	-0.034	0.017
rs242105	14	69459229	C	A	0.28	0.062	0.017
rs7156625	14	79942647	A	G	0.22	0.037	0.019
rs8010382	14	91963722	G	A	0.41	0.046	0.016
rs8043085	15	38828140	T	G	0.23	0.071	0.018
rs11856877	15	40620560	G	A	0.11	0.071	0.024
rs1473781	15	41818917	A	G	0.35	0.067	0.016
rs149336329	15	52587740	T	G	0.05	-0.272	0.037
rs7163757	15	62391608	T	C	0.43	-0.042	0.015
rs7178762	15	63871292	T	C	0.55	-0.057	0.015
rs9479	15	74328576	G	A	0.49	0.052	0.015
rs8033589	15	75596685	A	G	0.76	0.058	0.018
rs12910361	15	77782335	G	A	0.71	0.161	0.017
rs893617	15	90381278	T	C	0.72	-0.136	0.017
rs2290202	15	91512267	T	G	0.13	0.085	0.023
rs9927842	16	15153717	C	T	0.84	-0.056	0.021

rs8056890	16	28897452	A	G	0.36	0.105	0.016
rs8054556	16	29958216	A	G	0.47	0.077	0.015
rs55857387	16	300388	C	T	0.20	-0.142	0.019
rs8061528	16	3656482	T	C	0.21	0.092	0.019
rs2024449	16	53494617	C	T	0.44	-0.056	0.015
rs1421085	16	53800954	C	T	0.40	0.154	0.016
rs56125990	16	69742387	G	A	0.15	0.065	0.021
rs4788815	16	71634811	T	A	0.66	0.056	0.016
rs72802365	16	75246035	C	G	0.08	-0.163	0.029
rs2966117	16	81599271	T	G	0.48	0.059	0.015
rs11117364	16	88132199	G	A	0.68	0.066	0.017
rs9937296	16	88554480	C	T	0.86	0.069	0.023
rs66461358	16	89535257	C	T	0.15	0.060	0.021
rs12934854	16	950028	A	G	0.17	0.043	0.020
rs925095	17	17344653	T	C	0.39	-0.075	0.016
rs2297508	17	17715317	G	C	0.65	-0.150	0.016
rs117642733	17	21284910	T	C	0.05	0.107	0.039
rs9913225	17	27570622	A	G	0.58	-0.075	0.016
rs1109442	17	34862220	C	T	0.47	0.071	0.015
rs3110641	17	36047417	G	A	0.78	0.091	0.019
rs11651755	17	36099840	T	C	0.51	-0.124	0.015
rs3786017	17	3830340	C	T	0.11	0.054	0.025
rs8071043	17	3988451	C	T	0.33	0.066	0.016
rs1905339	17	40582296	C	T	0.34	0.097	0.016
rs35895680	17	47060322	A	C	0.33	-0.089	0.016
rs366577	17	4854480	T	C	0.60	-0.051	0.016
rs57767539	17	62203059	A	G	0.07	0.136	0.031
rs11658220	17	65646092	A	G	0.10	0.100	0.025
rs12603589	17	65825248	C	T	0.19	0.103	0.020
rs7224711	17	76772288	T	C	0.53	-0.069	0.015
rs303760	18	21083738	T	C	0.35	0.052	0.016
rs16965062	18	31581247	T	C	0.43	0.034	0.015

rs7227272	18	36746623	A	G	0.10	-0.062	0.026
rs410150	18	40066006	T	C	0.80	-0.047	0.019
rs17596995	18	53166594	A	G	0.20	-0.049	0.019
rs1517037	18	56878274	T	C	0.19	-0.093	0.020
rs6567160	18	57829135	C	T	0.23	0.097	0.018
rs74625348	18	60846430	C	G	0.23	-0.044	0.019
rs12963820	18	63426213	A	T	0.27	0.034	0.017
rs7240767	18	7070642	C	T	0.39	0.063	0.016
rs6565922	18	74558999	T	C	0.38	0.078	0.016
rs9384	19	13010643	T	G	0.38	-0.107	0.016
rs10404726	19	18834514	T	C	0.47	-0.035	0.015
rs58542926	19	19379549	T	C	0.08	0.139	0.029
rs924150	19	31829903	C	A	0.39	-0.087	0.016
rs4805881	19	33896432	C	A	0.67	-0.077	0.016
rs429358	19	45411941	C	T	0.16	-0.142	0.021
rs8107527	19	46158417	A	G	0.28	0.105	0.017
rs9304665	19	47602577	A	T	0.77	0.103	0.018
rs2115107	19	7968168	A	G	0.38	0.069	0.016
rs116843064	19	8429323	A	G	0.02	-0.150	0.055
rs7554251	1	11317932	C	T	0.73	0.036	0.017
rs1127215	1	117532790	T	C	0.42	-0.065	0.016
rs66464442	1	118171801	A	C	0.32	0.121	0.016
rs1493694	1	120526982	T	C	0.11	0.146	0.025
rs145904381	1	151017991	C	T	0.01	-0.266	0.071
rs2297607	1	154320942	G	A	0.24	0.051	0.018
rs6696888	1	155508882	A	G	0.68	-0.048	0.016
rs7546252	1	172368310	G	A	0.56	-0.092	0.015
rs539515	1	177889025	C	A	0.21	0.049	0.019
rs2816177	1	179248952	G	A	0.41	0.049	0.016
rs41304257	1	201849926	G	A	0.28	-0.042	0.017
rs61817176	1	206621028	C	A	0.52	-0.074	0.015
rs10916780	1	20707153	G	A	0.20	-0.045	0.019

rs340874	1	214159256	C	T	0.57	0.166	0.015
rs1337101	1	219726100	T	G	0.32	-0.095	0.016
rs348330	1	229672955	A	G	0.63	-0.119	0.016
rs10925635	1	235573486	C	A	0.64	0.046	0.016
rs17261915	1	26756856	C	T	0.25	0.074	0.018
rs3753693	1	29060898	T	C	0.41	-0.066	0.016
rs61779284	1	39855177	A	G	0.21	0.130	0.019
rs79090772	1	51209148	C	T	0.09	-0.219	0.027
rs2269247	1	64107284	T	C	0.18	-0.056	0.020
rs11583755	1	6672729	C	A	0.36	0.107	0.016
rs2613499	1	72751552	G	A	0.19	-0.052	0.019
rs10159026	1	96404462	T	C	0.25	-0.062	0.018
rs6137042	20	2100095	A	G	0.20	-0.050	0.019
rs7274134	20	22428284	T	C	0.25	-0.062	0.018
rs6059662	20	32675727	G	A	0.65	0.037	0.016
rs2038457	20	42239145	G	A	0.81	0.041	0.020
rs12625671	20	42994812	C	T	0.11	0.118	0.025
rs6066138	20	45594711	A	G	0.28	-0.135	0.017
rs6021276	20	50155386	C	T	0.64	-0.074	0.016
rs865034	20	51261615	C	T	0.66	0.040	0.016
rs4810145	20	57396495	C	T	0.52	0.068	0.015
rs6011155	20	62450664	C	T	0.37	-0.074	0.016
rs2240716	22	19969696	T	C	0.30	0.074	0.017
rs56392746	22	30451688	A	G	0.09	-0.138	0.026
rs75307421	22	32203334	A	G	0.02	0.151	0.061
rs138771	22	35705359	G	A	0.81	-0.055	0.020
rs1801645	22	50356850	T	C	0.74	-0.059	0.018
rs34506349	2	100598726	A	G	0.04	-0.099	0.038
rs79950062	2	111940612	C	T	0.13	-0.053	0.023
rs9308614	2	121337196	G	A	0.15	-0.090	0.022
rs6716394	2	146350724	A	G	0.54	-0.045	0.015
rs4668483	2	16231732	G	A	0.68	-0.040	0.016

rs10184004	2	165508389	T	C	0.41	-0.115	0.016
rs11680058	2	16574669	A	G	0.87	0.104	0.025
rs13406280	2	166610827	T	C	0.49	-0.047	0.015
rs72917531	2	175238176	A	C	0.19	-0.078	0.020
rs36051007	2	179545859	T	C	0.32	-0.035	0.017
rs67383253	2	181570394	C	T	0.37	-0.035	0.016
rs6712905	2	196952010	C	T	0.26	0.048	0.018
rs4482463	2	205375909	A	C	0.92	-0.063	0.029
rs34329895	2	208870017	G	A	0.60	-0.063	0.016
rs2943650	2	227105921	T	C	0.65	0.143	0.016
rs13415288	2	228971884	C	T	0.34	0.059	0.016
rs34339006	2	234271522	T	C	0.39	0.092	0.016
rs1260326	2	27730940	C	T	0.61	0.156	0.016
rs77165542	2	430975	T	C	0.04	-0.155	0.042
rs921069	2	43206922	G	A	0.58	-0.038	0.016
rs76675804	2	43611883	C	T	0.10	-0.311	0.026
rs10193538	2	58981064	T	G	0.61	0.072	0.016
rs243018	2	60586707	G	C	0.45	0.088	0.016
rs114213622	2	65243284	T	G	0.01	-0.303	0.080
rs10188334	2	653874	T	C	0.17	-0.087	0.020
rs12185610	2	65661468	C	A	0.41	-0.063	0.016
rs4671799	2	67622243	G	A	0.68	-0.036	0.016
rs4832290	2	86707504	C	T	0.77	-0.053	0.018
rs17036126	3	12287863	T	C	0.13	0.127	0.023
rs11708067	3	123065778	G	A	0.25	-0.262	0.018
rs17036160	3	12329783	T	C	0.12	-0.088	0.024
rs9873519	3	124921457	T	C	0.53	0.097	0.015
rs1224997	3	131631201	T	C	0.28	0.072	0.017
rs667920	3	136069472	T	G	0.77	0.038	0.018
rs9289556	3	138033181	T	C	0.73	-0.077	0.017
rs56243018	3	141101839	C	A	0.05	-0.214	0.036
rs28502438	3	149220109	C	T	0.43	-0.051	0.016

rs7633673	3	152084243	A	G	0.41	-0.086	0.016
rs11706810	3	160159921	C	T	0.48	-0.106	0.015
rs13099581	3	168226052	T	C	0.14	-0.060	0.022
rs8192675	3	170724883	C	T	0.29	-0.188	0.017
rs6444036	3	184901216	T	G	0.16	0.041	0.021
rs9859406	3	185534482	A	G	0.31	0.167	0.017
rs2041965	3	186648411	T	C	0.34	-0.083	0.016
rs6777684	3	187741842	G	A	0.61	0.134	0.016
rs13094957	3	23457080	C	T	0.20	-0.131	0.019
rs1470560	3	35670150	A	G	0.37	0.037	0.016
rs2624847	3	50174197	T	G	0.74	-0.084	0.017
rs13434089	3	63948566	C	T	0.12	-0.082	0.024
rs9870517	3	64708600	C	A	0.40	-0.096	0.016
rs1374915	3	71668037	C	T	0.42	-0.036	0.016
rs1523766	3	77670448	G	A	0.50	-0.031	0.015
rs978444	3	93981060	T	G	0.55	-0.057	0.015
rs3872707	3	9514016	A	G	0.12	0.049	0.023
rs7659468	4	103895317	G	T	0.49	-0.103	0.015
rs11728350	4	106078097	G	A	0.13	0.110	0.023
rs77141743	4	121774048	A	G	0.16	0.045	0.021
rs730831	4	1240299	G	T	0.04	-0.123	0.041
rs2604918	4	140879929	T	G	0.33	-0.063	0.016
rs2125799	4	156697784	C	T	0.33	0.060	0.016
rs28819812	4	157652753	A	C	0.32	-0.060	0.016
rs4865436	4	1788130	G	C	0.29	0.050	0.018
rs2169033	4	18044357	T	C	0.68	0.081	0.017
rs55691245	4	185716100	A	G	0.14	-0.160	0.022
rs7664347	4	20265535	C	T	0.64	-0.040	0.016
rs10938398	4	45186139	A	G	0.43	0.040	0.016
rs1996617	4	52798624	C	T	0.29	0.101	0.017
rs114447556	4	53207093	T	C	0.08	0.080	0.029
rs10937721	4	6306763	C	G	0.59	0.142	0.016

rs73222806	4	753840	G	C	0.05	0.098	0.035
rs6835992	4	76496817	G	A	0.69	0.066	0.017
rs993380	4	83584496	G	A	0.67	-0.059	0.016
rs28408270	4	95114572	T	G	0.47	-0.050	0.015
rs1961224	4	95999825	G	A	0.35	-0.065	0.016
rs141146025	5	101966291	A	C	0.02	0.129	0.058
rs75432112	5	102586407	A	G	0.05	0.195	0.036
rs329118	5	133861663	T	C	0.42	0.041	0.016
rs111686785	5	14738965	G	A	0.03	0.182	0.044
rs72734782	5	14789003	G	A	0.21	0.066	0.019
rs12514030	5	14810110	G	T	0.12	-0.103	0.023
rs1650505	5	158029734	A	G	0.21	0.060	0.019
rs4343858	5	176679407	A	G	0.23	-0.042	0.018
rs138373837	5	36219710	T	C	0.02	0.101	0.050
rs62366821	5	44875449	G	A	0.49	-0.055	0.015
rs10067659	5	52084365	C	G	0.79	-0.081	0.019
rs4865796	5	53272664	A	G	0.69	0.049	0.017
rs464605	5	55807370	T	C	0.75	0.080	0.019
rs34341	5	74934009	T	A	0.58	0.073	0.016
rs7732130	5	76435004	A	G	0.68	-0.132	0.016
rs6870983	5	87697533	T	C	0.21	-0.067	0.019
rs34483452	5	87986314	A	C	0.14	0.077	0.023
rs7752666	6	107445266	T	C	0.32	-0.035	0.017
rs80196932	6	117996631	C	T	0.16	-0.064	0.021
rs11759026	6	126792095	G	A	0.23	0.136	0.018
rs2876354	6	137295352	T	C	0.47	-0.083	0.016
rs7742292	6	138864489	C	T	0.40	0.041	0.016
rs2982521	6	139835329	T	A	0.63	-0.110	0.016
rs9390022	6	143056556	C	T	0.38	-0.042	0.016
rs1538247	6	153395344	C	T	0.30	0.093	0.017
rs2179168	6	15477030	A	G	0.80	0.046	0.019
rs501470	6	160770918	G	T	0.47	-0.089	0.015

rs4709746	6	164133001	T	C	0.13	-0.050	0.023
rs7774074	6	20517130	A	C	0.21	0.039	0.019
rs35261542	6	20675792	A	C	0.26	0.268	0.017
rs3117189	6	32033944	G	A	0.85	0.281	0.021
rs2780215	6	34236973	G	A	0.07	-0.110	0.033
rs7748962	6	43759927	A	G	0.77	0.113	0.018
rs9472139	6	43813711	C	G	0.29	0.065	0.017
rs3798519	6	50788778	C	A	0.18	0.107	0.020
rs9370243	6	53789830	T	G	0.08	0.079	0.028
rs9449295	6	64163807	C	T	0.54	0.036	0.015
rs9379084	6	7231843	A	G	0.12	-0.198	0.025
rs187653072	7	102976385	C	T	0.03	0.134	0.044
rs73184014	7	104516274	G	A	0.22	-0.053	0.019
rs6976111	7	117495667	A	C	0.30	0.074	0.017
rs13237518	7	12269593	A	C	0.41	0.048	0.016
rs3996350	7	130427057	C	G	0.50	-0.086	0.015
rs60251368	7	140522073	G	A	0.06	0.096	0.034
rs4252505	7	142607301	G	A	0.06	0.070	0.031
rs17168486	7	14898282	T	C	0.17	0.162	0.020
rs4725959	7	150534741	G	A	0.22	0.042	0.019
rs10228796	7	15064190	G	C	0.55	0.160	0.015
rs6946660	7	156948648	C	T	0.35	-0.107	0.016
rs11762413	7	2090387	G	C	0.25	-0.085	0.018
rs2188848	7	23884697	G	A	0.20	-0.055	0.019
rs860262	7	28194397	A	C	0.50	-0.158	0.015
rs917195	7	30728452	T	C	0.23	-0.073	0.018
rs730497	7	44223721	A	G	0.18	0.445	0.020
rs73121277	7	50577968	C	T	0.28	0.084	0.017
rs6975279	7	69649683	A	C	0.26	0.101	0.018
rs6956980	7	89803634	C	T	0.53	0.083	0.015
rs7834323	8	10671984	C	T	0.29	-0.074	0.017
rs727582	8	116650468	G	A	0.34	-0.093	0.016

rs13266634	8	118184783	T	C	0.31	-0.277	0.017
rs12056338	8	12643055	T	G	0.42	0.050	0.016
rs17772814	8	128711742	A	G	0.08	-0.099	0.029
rs1561927	8	129568078	T	C	0.73	-0.048	0.017
rs35753840	8	14148990	C	A	0.39	0.054	0.016
rs13268508	8	145525277	T	C	0.38	0.087	0.016
rs2953845	8	145972950	T	C	0.55	0.042	0.015
rs6558173	8	22492103	T	G	0.35	0.039	0.016
rs2725370	8	30852826	C	T	0.70	-0.049	0.017
rs57735787	8	34438332	G	A	0.25	-0.042	0.018
rs13262861	8	41508577	A	C	0.17	-0.121	0.021
rs7813865	8	57506937	C	T	0.29	0.041	0.017
rs10101067	8	72407374	C	G	0.08	0.092	0.029
rs28792187	8	74568099	G	A	0.07	0.123	0.030
rs1895874	8	95675372	A	G	0.50	0.048	0.015
rs10808671	8	95967372	G	A	0.53	-0.073	0.015
rs60384372	8	9974584	G	A	0.47	-0.056	0.015
rs1567353	9	1033773	G	C	0.31	0.035	0.017
rs10119430	9	111938268	A	G	0.79	-0.054	0.019
rs1431819	9	116943357	G	A	0.70	0.038	0.017
rs10818763	9	125689694	T	C	0.13	-0.108	0.023
rs10739629	9	126093422	T	C	0.51	-0.036	0.015
rs529565	9	136149500	C	T	0.32	0.164	0.017
rs28642213	9	139248082	G	A	0.75	0.169	0.018
rs12380322	9	19074538	G	A	0.39	0.051	0.016
rs10965247	9	22132729	G	A	0.18	-0.302	0.020
rs7018475	9	22137685	G	T	0.26	0.178	0.018
rs11788619	9	22258082	T	A	0.03	-0.134	0.048
rs2150854	9	28411949	T	G	0.33	0.072	0.016
rs4237150	9	4290085	C	G	0.40	0.091	0.016
rs67269808	9	81907986	G	A	0.06	-0.130	0.032
rs2796441	9	84308948	A	G	0.42	-0.096	0.016

rs7023781	9	96447178	T	C	0.27	0.058	0.017
rs10993072	9	96915002	T	C	0.32	0.083	0.016
rs28496034	9	98278332	G	C	0.33	-0.057	0.016

EAF: effect allele frequency; SE: standard error; SNP: single nucleotide polymorphism.

Table S5. Genetic proxies for glycemic control by any mechanism, and estimates for their association with glycated hemoglobin (for the outcome of left ventricular ejection fraction).

SNP	Chromosome	Position	Effect allele	Other allele	EAF	Beta	SE
rs2482506	10	104563743	G	C	0.25	-0.053	0.018
rs7090695	10	112801213	C	G	0.80	0.060	0.019
rs11196174	10	114734096	G	A	0.29	0.252	0.017
rs4918790	10	114830254	A	G	0.91	-0.117	0.028
rs4752351	10	121685016	C	T	0.20	0.087	0.019
rs11257655	10	12307894	T	C	0.21	0.219	0.019
rs946859	10	13565429	A	G	0.47	-0.075	0.015
rs3122231	10	44027356	C	T	0.65	0.050	0.016
rs949693	10	70354574	A	G	0.61	-0.050	0.016
rs11592899	10	71333783	A	G	0.34	-0.055	0.016
rs2812535	10	71456857	A	G	0.62	0.069	0.016
rs697239	10	80947438	C	T	0.46	-0.105	0.015
rs11201992	10	88117318	A	C	0.46	-0.038	0.015
rs1111875	10	94462882	T	C	0.41	-0.181	0.016
rs66536955	10	94737667	C	T	0.26	0.044	0.017
rs34041345	10	99174580	G	T	0.26	0.060	0.018
rs529623	11	117693255	C	T	0.52	-0.059	0.015
rs10893830	11	128044159	T	C	0.13	-0.058	0.023
rs10750397	11	128234144	G	A	0.72	-0.040	0.017
rs67232546	11	128398938	T	C	0.21	0.067	0.019
rs757110	11	17418477	A	C	0.64	-0.112	0.016
rs11042987	11	2201059	A	C	0.58	-0.034	0.016
rs2283167	11	2580063	A	G	0.14	-0.055	0.023
rs231362	11	2691471	G	A	0.52	0.120	0.015
rs10767659	11	27686196	T	G	0.67	-0.041	0.016
rs60808706	11	2857233	A	G	0.05	-0.227	0.035
rs2289488	11	2892955	C	G	0.40	0.040	0.016

rs74673753	11	32623621	T	A	0.06	-0.106	0.033
rs2956092	11	34908780	C	T	0.69	-0.059	0.017
rs3816605	11	47857253	C	T	0.45	-0.080	0.015
rs7483027	11	58128015	C	T	0.38	-0.061	0.016
rs174541	11	61565908	C	T	0.36	-0.098	0.016
rs12789028	11	65326154	A	G	0.20	0.084	0.019
rs11602873	11	72460762	T	A	0.16	-0.187	0.021
rs11236524	11	75464344	C	T	0.09	0.069	0.027
rs2513505	11	76230357	A	C	0.60	0.033	0.016
rs12802861	11	8387806	T	C	0.28	-0.052	0.017
rs10830963	11	92708710	G	C	0.28	0.297	0.017
rs3020069	11	93057087	A	G	0.68	0.093	0.016
rs1426371	12	108629780	A	G	0.26	-0.074	0.018
rs79310463	12	118406696	T	C	0.13	0.104	0.023
rs56348580	12	121432117	C	G	0.31	-0.037	0.017
rs7975763	12	123604053	T	C	0.20	-0.057	0.019
rs2066827	12	12871099	G	T	0.23	0.102	0.018
rs11614914	12	133070294	T	C	0.33	0.078	0.016
rs12828318	12	133766122	G	A	0.18	-0.057	0.020
rs10841886	12	21864377	C	T	0.23	-0.082	0.018
rs1480029	12	26356032	A	G	0.46	0.042	0.015
rs3751239	12	27963676	G	C	0.20	-0.160	0.019
rs7298690	12	4313438	C	T	0.21	0.060	0.019
rs3217893	12	4403876	T	C	0.09	-0.171	0.030
rs2732469	12	48712932	A	T	0.43	-0.258	0.015
rs61937817	12	57212823	G	T	0.11	0.060	0.024
rs11173646	12	61250814	T	A	0.82	-0.046	0.020
rs2257883	12	66216162	A	G	0.13	0.150	0.023
rs12371967	12	66346714	C	T	0.17	-0.043	0.020
rs10879261	12	71520761	G	T	0.41	0.068	0.016
rs11108094	12	95928113	A	C	0.07	0.099	0.030
rs6538805	12	97849120	C	T	0.39	-0.076	0.016

rs9587811	13	109946882	A	C	0.41	-0.056	0.016
rs314879	13	23309382	T	C	0.79	-0.069	0.019
rs34584161	13	26776999	G	A	0.24	-0.063	0.018
rs380854	13	33574631	A	G	0.58	-0.058	0.016
rs9316500	13	51094114	G	T	0.29	-0.067	0.017
rs7991679	13	58691107	A	T	0.16	-0.081	0.021
rs1215451	13	80715893	A	G	0.29	-0.131	0.017
rs112324411	14	101258584	T	C	0.07	-0.101	0.032
rs2295388	14	101309759	A	G	0.22	-0.073	0.019
rs4906272	14	103376031	T	C	0.16	0.046	0.021
rs12883788	14	33303540	T	C	0.46	0.060	0.015
rs7147483	14	38804675	C	T	0.25	-0.158	0.018
rs723355	14	47304091	A	G	0.50	-0.034	0.015
rs4902002	14	61229411	A	G	0.71	-0.034	0.017
rs242105	14	69459229	C	A	0.28	0.062	0.017
rs7156625	14	79942647	A	G	0.22	0.037	0.019
rs8010382	14	91963722	G	A	0.41	0.046	0.016
rs8043085	15	38828140	T	G	0.23	0.071	0.018
rs11856877	15	40620560	G	A	0.11	0.071	0.024
rs1473781	15	41818917	A	G	0.35	0.067	0.016
rs71472935	15	52565725	C	G	0.11	-0.145	0.025
rs7163757	15	62391608	T	C	0.43	-0.042	0.015
rs7178762	15	63871292	T	C	0.55	-0.057	0.015
rs9479	15	74328576	G	A	0.49	0.052	0.015
rs8033589	15	75596685	A	G	0.76	0.058	0.018
rs12910361	15	77782335	G	A	0.71	0.161	0.017
rs893617	15	90381278	T	C	0.72	-0.136	0.017
rs2290202	15	91512267	T	G	0.13	0.085	0.023
rs9927842	16	15153717	C	T	0.84	-0.056	0.021
rs8056890	16	28897452	A	G	0.36	0.105	0.016
rs8054556	16	29958216	A	G	0.47	0.077	0.015
rs55857387	16	300388	C	T	0.20	-0.142	0.019

rs8061528	16	3656482	T	C	0.21	0.092	0.019
rs2024449	16	53494617	C	T	0.44	-0.056	0.015
rs1421085	16	53800954	C	T	0.40	0.154	0.016
rs56125990	16	69742387	G	A	0.15	0.065	0.021
rs4788815	16	71634811	T	A	0.66	0.056	0.016
rs72802365	16	75246035	C	G	0.08	-0.163	0.029
rs2966117	16	81599271	T	G	0.48	0.059	0.015
rs11117364	16	88132199	G	A	0.68	0.066	0.017
rs9937296	16	88554480	C	T	0.86	0.069	0.023
rs66461358	16	89535257	C	T	0.15	0.060	0.021
rs12934854	16	950028	A	G	0.17	0.043	0.020
rs925095	17	17344653	T	C	0.39	-0.075	0.016
rs2297508	17	17715317	G	C	0.65	-0.150	0.016
rs9913225	17	27570622	A	G	0.58	-0.075	0.016
rs1109442	17	34862220	C	T	0.47	0.071	0.015
rs3110641	17	36047417	G	A	0.78	0.091	0.019
rs11651755	17	36099840	T	C	0.51	-0.124	0.015
rs3786017	17	3830340	C	T	0.11	0.054	0.025
rs8071043	17	3988451	C	T	0.33	0.066	0.016
rs1905339	17	40582296	C	T	0.34	0.097	0.016
rs35895680	17	47060322	A	C	0.33	-0.089	0.016
rs366577	17	4854480	T	C	0.60	-0.051	0.016
rs57767539	17	62203059	A	G	0.07	0.136	0.031
rs11658220	17	65646092	A	G	0.10	0.100	0.025
rs12603589	17	65825248	C	T	0.19	0.103	0.020
rs7224711	17	76772288	T	C	0.53	-0.069	0.015
rs303760	18	21083738	T	C	0.35	0.052	0.016
rs16965062	18	31581247	T	C	0.43	0.034	0.015
rs7227272	18	36746623	A	G	0.10	-0.062	0.026
rs410150	18	40066006	T	C	0.80	-0.047	0.019
rs17596995	18	53166594	A	G	0.20	-0.049	0.019
rs1517037	18	56878274	T	C	0.19	-0.093	0.020

rs6567160	18	57829135	C	T	0.23	0.097	0.018
rs74625348	18	60846430	C	G	0.23	-0.044	0.019
rs12963820	18	63426213	A	T	0.27	0.034	0.017
rs7240767	18	7070642	C	T	0.39	0.063	0.016
rs6565922	18	74558999	T	C	0.38	0.078	0.016
rs9384	19	13010643	T	G	0.38	-0.107	0.016
rs10404726	19	18834514	T	C	0.47	-0.035	0.015
rs58542926	19	19379549	T	C	0.08	0.139	0.029
rs924150	19	31829903	C	A	0.39	-0.087	0.016
rs4805881	19	33896432	C	A	0.67	-0.077	0.016
rs429358	19	45411941	C	T	0.16	-0.142	0.021
rs8107527	19	46158417	A	G	0.28	0.105	0.017
rs9304665	19	47602577	A	T	0.77	0.103	0.018
rs2115107	19	7968168	A	G	0.38	0.069	0.016
rs7554251	1	11317932	C	T	0.73	0.036	0.017
rs1127215	1	117532790	T	C	0.42	-0.065	0.016
rs66464442	1	118171801	A	C	0.32	0.121	0.016
rs1493694	1	120526982	T	C	0.11	0.146	0.025
rs115983556	1	149873582	C	A	0.08	-0.153	0.028
rs1194592	1	154324384	G	C	0.44	-0.044	0.015
rs3020781	1	155269776	G	A	0.27	0.086	0.017
rs7546252	1	172368310	G	A	0.56	-0.092	0.015
rs539515	1	177889025	C	A	0.21	0.049	0.019
rs2816177	1	179248952	G	A	0.41	0.049	0.016
rs41304257	1	201849926	G	A	0.28	-0.042	0.017
rs61817176	1	206621028	C	A	0.52	-0.074	0.015
rs10916780	1	20707153	G	A	0.20	-0.045	0.019
rs340874	1	214159256	C	T	0.57	0.166	0.015
rs1337101	1	219726100	T	G	0.32	-0.095	0.016
rs348330	1	229672955	A	G	0.63	-0.119	0.016
rs10925635	1	235573486	C	A	0.64	0.046	0.016
rs17261915	1	26756856	C	T	0.25	0.074	0.018

rs3753693	1	29060898	T	C	0.41	-0.066	0.016
rs61779284	1	39855177	A	G	0.21	0.130	0.019
rs79090772	1	51209148	C	T	0.09	-0.219	0.027
rs2269247	1	64107284	T	C	0.18	-0.056	0.020
rs11583755	1	6672729	C	A	0.36	0.107	0.016
rs2613499	1	72751552	G	A	0.19	-0.052	0.019
rs10159026	1	96404462	T	C	0.25	-0.062	0.018
rs6137042	20	2100095	A	G	0.20	-0.050	0.019
rs7274134	20	22428284	T	C	0.25	-0.062	0.018
rs6059662	20	32675727	G	A	0.65	0.037	0.016
rs2038457	20	42239145	G	A	0.81	0.041	0.020
rs12625671	20	42994812	C	T	0.11	0.118	0.025
rs6066138	20	45594711	A	G	0.28	-0.135	0.017
rs6021276	20	50155386	C	T	0.64	-0.074	0.016
rs865034	20	51261615	C	T	0.66	0.040	0.016
rs4810145	20	57396495	C	T	0.52	0.068	0.015
rs6011155	20	62450664	C	T	0.37	-0.074	0.016
rs2240716	22	19969696	T	C	0.30	0.074	0.017
rs56392746	22	30451688	A	G	0.09	-0.138	0.026
rs138771	22	35705359	G	A	0.81	-0.055	0.020
rs1801645	22	50356850	T	C	0.74	-0.059	0.018
rs79950062	2	111940612	C	T	0.13	-0.053	0.023
rs9308614	2	121337196	G	A	0.15	-0.090	0.022
rs6716394	2	146350724	A	G	0.54	-0.045	0.015
rs4668483	2	16231732	G	A	0.68	-0.040	0.016
rs10184004	2	165508389	T	C	0.41	-0.115	0.016
rs11680058	2	16574669	A	G	0.87	0.104	0.025
rs13406280	2	166610827	T	C	0.49	-0.047	0.015
rs72917531	2	175238176	A	C	0.19	-0.078	0.020
rs36051007	2	179545859	T	C	0.32	-0.035	0.017
rs67383253	2	181570394	C	T	0.37	-0.035	0.016
rs6712905	2	196952010	C	T	0.26	0.048	0.018

rs4482463	2	205375909	A	C	0.92	-0.063	0.029
rs34329895	2	208870017	G	A	0.60	-0.063	0.016
rs2943650	2	227105921	T	C	0.65	0.143	0.016
rs13415288	2	228971884	C	T	0.34	0.059	0.016
rs34339006	2	234271522	T	C	0.39	0.092	0.016
rs1260326	2	27730940	C	T	0.61	0.156	0.016
rs921069	2	43206922	G	A	0.58	-0.038	0.016
rs76675804	2	43611883	C	T	0.10	-0.311	0.026
rs10193538	2	58981064	T	G	0.61	0.072	0.016
rs243018	2	60586707	G	C	0.45	0.088	0.016
rs10188334	2	653874	T	C	0.17	-0.087	0.020
rs12185610	2	65661468	C	A	0.41	-0.063	0.016
rs4671799	2	67622243	G	A	0.68	-0.036	0.016
rs4832290	2	86707504	C	T	0.77	-0.053	0.018
rs17036126	3	12287863	T	C	0.13	0.127	0.023
rs11708067	3	123065778	G	A	0.25	-0.262	0.018
rs17036160	3	12329783	T	C	0.12	-0.088	0.024
rs9873519	3	124921457	T	C	0.53	0.097	0.015
rs1225004	3	131626991	C	T	0.28	0.071	0.017
rs667920	3	136069472	T	G	0.77	0.038	0.018
rs6766859	3	138055136	T	C	0.63	-0.091	0.016
rs34573045	3	149196752	G	C	0.43	0.049	0.015
rs7633673	3	152084243	A	G	0.41	-0.086	0.016
rs11706810	3	160159921	C	T	0.48	-0.106	0.015
rs13099581	3	168226052	T	C	0.14	-0.060	0.022
rs8192675	3	170724883	C	T	0.29	-0.188	0.017
rs6444036	3	184901216	T	G	0.16	0.041	0.021
rs9859406	3	185534482	A	G	0.31	0.167	0.017
rs2041965	3	186648411	T	C	0.34	-0.083	0.016
rs6777684	3	187741842	G	A	0.61	0.134	0.016
rs13094957	3	23457080	C	T	0.20	-0.131	0.019
rs1470560	3	35670150	A	G	0.37	0.037	0.016

rs2624847	3	50174197	T	G	0.74	-0.084	0.017
rs13434089	3	63948566	C	T	0.12	-0.082	0.024
rs9870517	3	64708600	C	A	0.40	-0.096	0.016
rs1374915	3	71668037	C	T	0.42	-0.036	0.016
rs1523766	3	77670448	G	A	0.50	-0.031	0.015
rs978444	3	93981060	T	G	0.55	-0.057	0.015
rs3872707	3	9514016	A	G	0.12	0.049	0.023
rs7659468	4	103895317	G	T	0.49	-0.103	0.015
rs11728350	4	106078097	G	A	0.13	0.110	0.023
rs77141743	4	121774048	A	G	0.16	0.045	0.021
rs2604918	4	140879929	T	G	0.33	-0.063	0.016
rs2125799	4	156697784	C	T	0.33	0.060	0.016
rs28819812	4	157652753	A	C	0.32	-0.060	0.016
rs4865436	4	1788130	G	C	0.29	0.050	0.018
rs2169033	4	18044357	T	C	0.68	0.081	0.017
rs55691245	4	185716100	A	G	0.14	-0.160	0.022
rs7664347	4	20265535	C	T	0.64	-0.040	0.016
rs10938398	4	45186139	A	G	0.43	0.040	0.016
rs1996617	4	52798624	C	T	0.29	0.101	0.017
rs114447556	4	53207093	T	C	0.08	0.080	0.029
rs10937721	4	6306763	C	G	0.59	0.142	0.016
rs75724417	4	757921	T	C	0.05	0.089	0.035
rs6835992	4	76496817	G	A	0.69	0.066	0.017
rs993380	4	83584496	G	A	0.67	-0.059	0.016
rs28408270	4	95114572	T	G	0.47	-0.050	0.015
rs1961224	4	95999825	G	A	0.35	-0.065	0.016
rs116782923	5	102331465	T	A	0.05	0.197	0.034
rs329118	5	133861663	T	C	0.42	0.041	0.016
rs9312873	5	14777799	G	A	0.10	-0.160	0.026
rs1650505	5	158029734	A	G	0.21	0.060	0.019
rs4343858	5	176679407	A	G	0.23	-0.042	0.018
rs62366821	5	44875449	G	A	0.49	-0.055	0.015

rs10067659	5	52084365	C	G	0.79	-0.081	0.019
rs4865796	5	53272664	A	G	0.69	0.049	0.017
rs464605	5	55807370	T	C	0.75	0.080	0.019
rs34341	5	74934009	T	A	0.58	0.073	0.016
rs7732130	5	76435004	A	G	0.68	-0.132	0.016
rs6870983	5	87697533	T	C	0.21	-0.067	0.019
rs34483452	5	87986314	A	C	0.14	0.077	0.023
rs7752666	6	107445266	T	C	0.32	-0.035	0.017
rs80196932	6	117996631	C	T	0.16	-0.064	0.021
rs11759026	6	126792095	G	A	0.23	0.136	0.018
rs2876354	6	137295352	T	C	0.47	-0.083	0.016
rs7742292	6	138864489	C	T	0.40	0.041	0.016
rs2982521	6	139835329	T	A	0.63	-0.110	0.016
rs9390022	6	143056556	C	T	0.38	-0.042	0.016
rs1538247	6	153395344	C	T	0.30	0.093	0.017
rs2179168	6	15477030	A	G	0.80	0.046	0.019
rs501470	6	160770918	G	T	0.47	-0.089	0.015
rs4709746	6	164133001	T	C	0.13	-0.050	0.023
rs7774074	6	20517130	A	C	0.21	0.039	0.019
rs35261542	6	20675792	A	C	0.26	0.268	0.017
rs3117189	6	32033944	G	A	0.85	0.281	0.021
rs2780215	6	34236973	G	A	0.07	-0.110	0.033
rs7748962	6	43759927	A	G	0.77	0.113	0.018
rs9472139	6	43813711	C	G	0.29	0.065	0.017
rs3798519	6	50788778	C	A	0.18	0.107	0.020
rs9370243	6	53789830	T	G	0.08	0.079	0.028
rs9449295	6	64163807	C	T	0.54	0.036	0.015
rs9379084	6	7231843	A	G	0.12	-0.198	0.025
rs62482399	7	102972707	T	C	0.09	0.087	0.027
rs73184014	7	104516274	G	A	0.22	-0.053	0.019
rs6976111	7	117495667	A	C	0.30	0.074	0.017
rs13237518	7	12269593	A	C	0.41	0.048	0.016

rs3996350	7	130427057	C	G	0.50	-0.086	0.015
rs60251368	7	140522073	G	A	0.06	0.096	0.034
rs4252505	7	142607301	G	A	0.06	0.070	0.031
rs17168486	7	14898282	T	C	0.17	0.162	0.020
rs4725959	7	150534741	G	A	0.22	0.042	0.019
rs10228796	7	15064190	G	C	0.55	0.160	0.015
rs6946660	7	156948648	C	T	0.35	-0.107	0.016
rs11762413	7	2090387	G	C	0.25	-0.085	0.018
rs2188848	7	23884697	G	A	0.20	-0.055	0.019
rs860262	7	28194397	A	C	0.50	-0.158	0.015
rs917195	7	30728452	T	C	0.23	-0.073	0.018
rs730497	7	44223721	A	G	0.18	0.445	0.020
rs73121277	7	50577968	C	T	0.28	0.084	0.017
rs6975279	7	69649683	A	C	0.26	0.101	0.018
rs6956980	7	89803634	C	T	0.53	0.083	0.015
rs7834323	8	10671984	C	T	0.29	-0.074	0.017
rs727582	8	116650468	G	A	0.34	-0.093	0.016
rs13266634	8	118184783	T	C	0.31	-0.277	0.017
rs12056338	8	12643055	T	G	0.42	0.050	0.016
rs17772814	8	128711742	A	G	0.08	-0.099	0.029
rs1561927	8	129568078	T	C	0.73	-0.048	0.017
rs35753840	8	14148990	C	A	0.39	0.054	0.016
rs13268508	8	145525277	T	C	0.38	0.087	0.016
rs2953845	8	145972950	T	C	0.55	0.042	0.015
rs6558173	8	22492103	T	G	0.35	0.039	0.016
rs2725370	8	30852826	C	T	0.70	-0.049	0.017
rs57735787	8	34438332	G	A	0.25	-0.042	0.018
rs13262861	8	41508577	A	C	0.17	-0.121	0.021
rs7813865	8	57506937	C	T	0.29	0.041	0.017
rs10101067	8	72407374	C	G	0.08	0.092	0.029
rs28792187	8	74568099	G	A	0.07	0.123	0.030
rs1895874	8	95675372	A	G	0.50	0.048	0.015

rs10808671	8	95967372	G	A	0.53	-0.073	0.015
rs60384372	8	9974584	G	A	0.47	-0.056	0.015
rs1567353	9	1033773	G	C	0.31	0.035	0.017
rs10119430	9	111938268	A	G	0.79	-0.054	0.019
rs1431819	9	116943357	G	A	0.70	0.038	0.017
rs10818763	9	125689694	T	C	0.13	-0.108	0.023
rs10739629	9	126093422	T	C	0.51	-0.036	0.015
rs529565	9	136149500	C	T	0.32	0.164	0.017
rs28642213	9	139248082	G	A	0.75	0.169	0.018
rs12380322	9	19074538	G	A	0.39	0.051	0.016
rs10965247	9	22132729	G	A	0.18	-0.302	0.020
rs7018475	9	22137685	G	T	0.26	0.178	0.018
rs2150854	9	28411949	T	G	0.33	0.072	0.016
rs4237150	9	4290085	C	G	0.40	0.091	0.016
rs67269808	9	81907986	G	A	0.06	-0.130	0.032
rs2796441	9	84308948	A	G	0.42	-0.096	0.016
rs7023781	9	96447178	T	C	0.27	0.058	0.017
rs10993072	9	96915002	T	C	0.32	0.083	0.016
rs28496034	9	98278332	G	C	0.33	-0.057	0.016

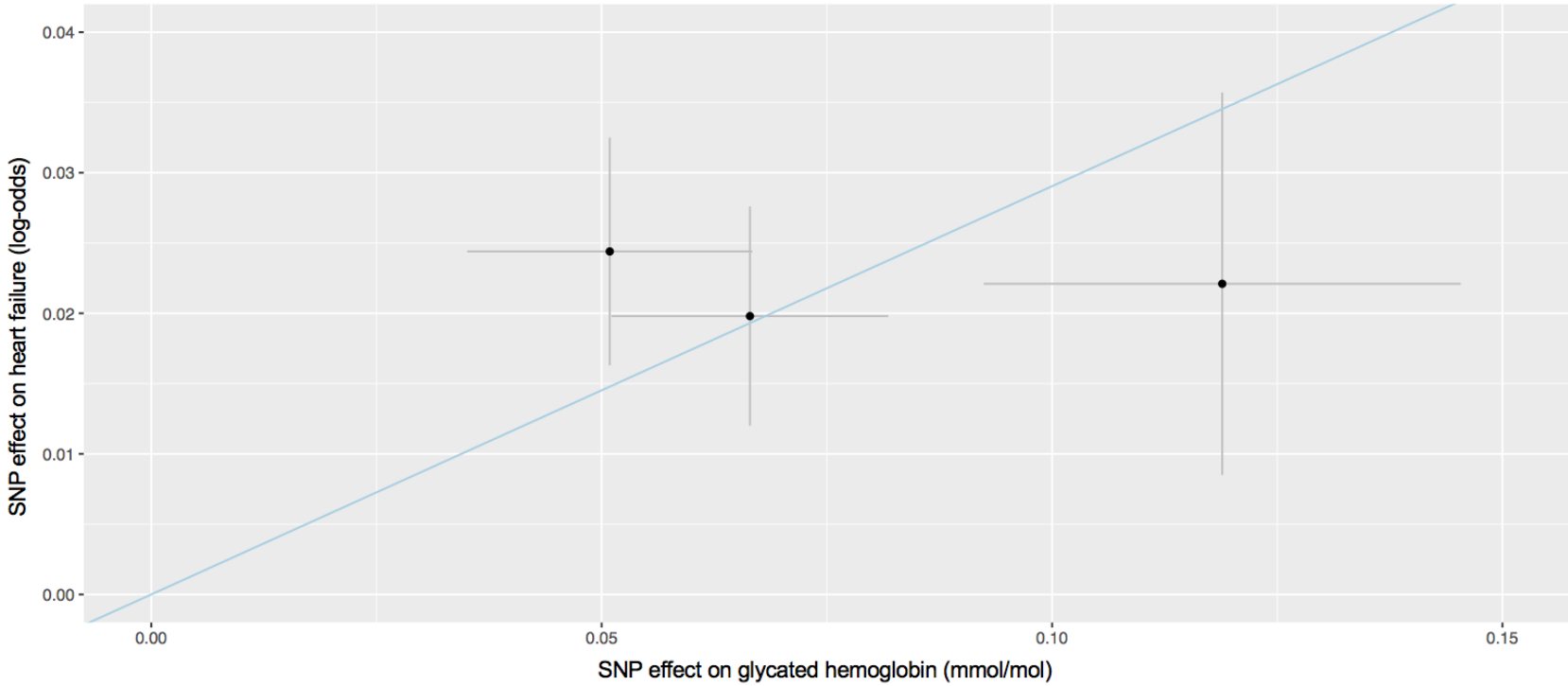
EAF: effect allele frequency; SE: standard error; SNP: single nucleotide polymorphism.

Table S6. **Weighted median sensitivity analyses for the association of genetically proxied glucagon-like peptide receptor (GLP1R) agonism and glycemic control more generally with heart failure (HF; 47,309 cases / 930,014 controls) and left ventricular ejection fraction (LVEF; *n*=16,923).**

Exposure	Outcome	N SNPs	Effect units	Effect	P value
GLP1R	HF	3	Odds ratio	0.77 [0.62, 0.96]	0.02
Glycemia	HF	350	Odds ratio	0.98 [0.96, 1.00]	0.04
GLP1R	LVEF	3	SD change in LVEF	0.18 [-0.07, 0.42]	0.16
Glycemia	LVEF	334	SD change in LVEF	0.00 [-0.02, 0.02]	0.89

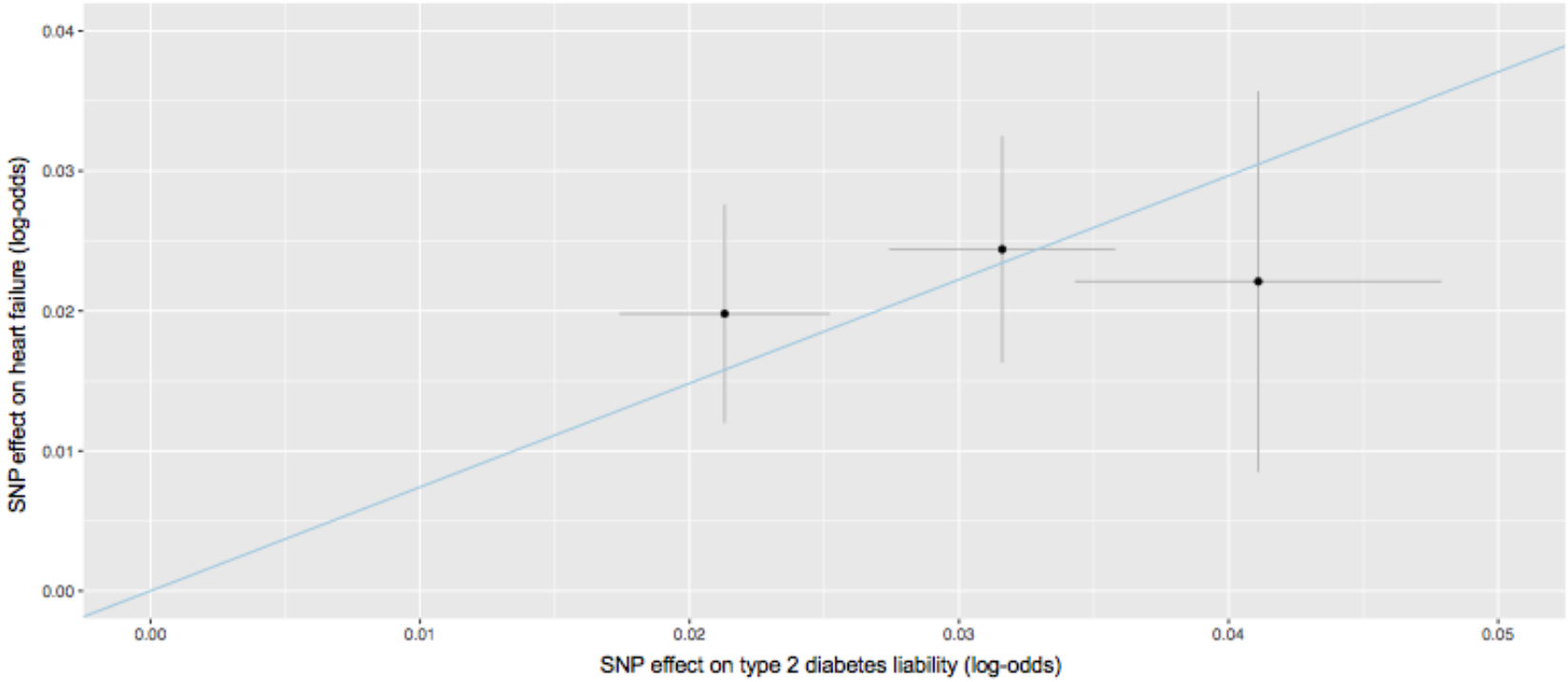
SD: standard deviation; SNP: single-nucleotide polymorphism.

Figure S1. Scatter plot displaying genetic associations of the GLP1R genetic proxies with glycated hemoglobin (mmol/mol, x-axis) and heart failure risk (log-odds, y-axis).



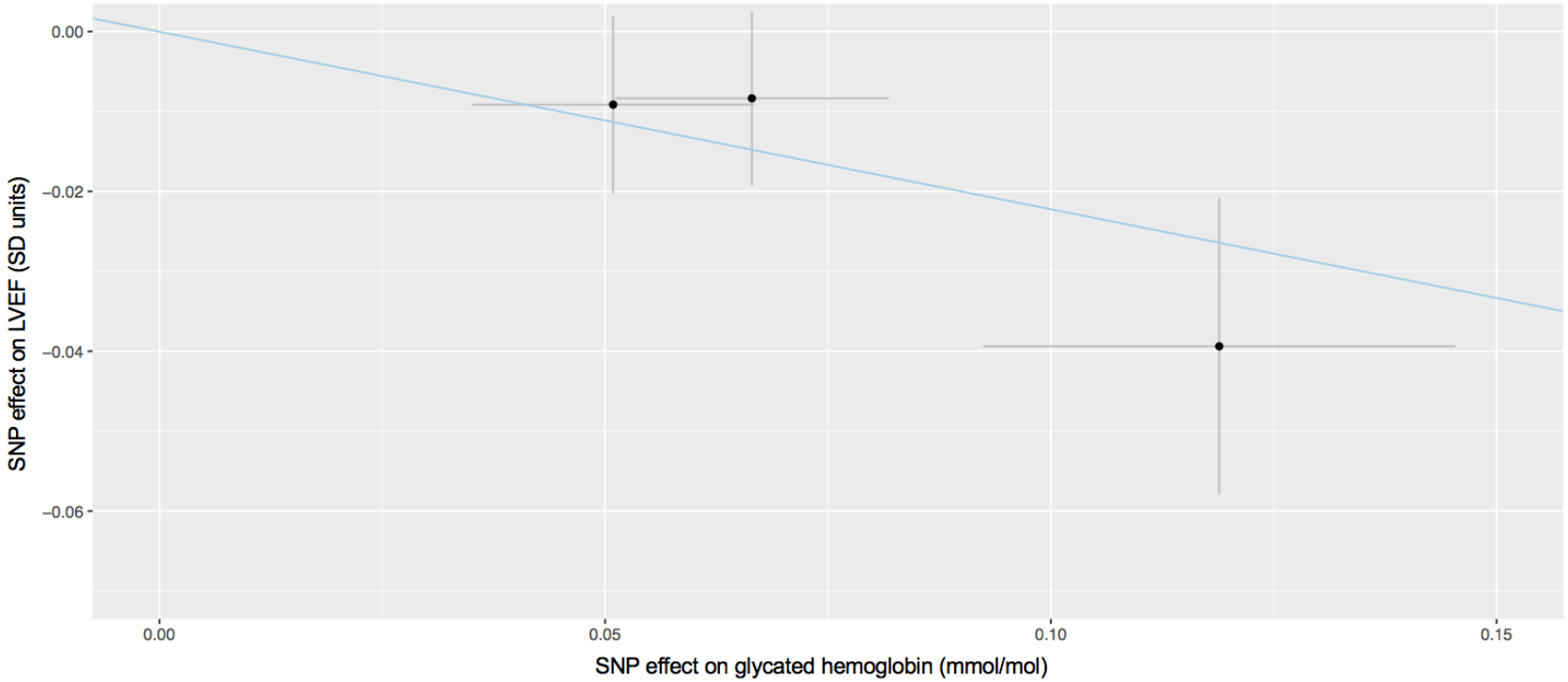
Each point represents a single genetic variant, with vertical and horizontal lines representing standard errors. The slope of the blue diagonal line represents the inverse-variance weighted Mendelian randomization estimate. The *P* value for the Cochran *Q* test for heterogeneity was 0.32. SNP: single-nucleotide polymorphism.

Figure S2. Scatter plot displaying genetic associations of the GLP1R genetic proxies with type 2 diabetes liability (log-odds, x-axis) and heart failure risk (log-odds, y-axis).



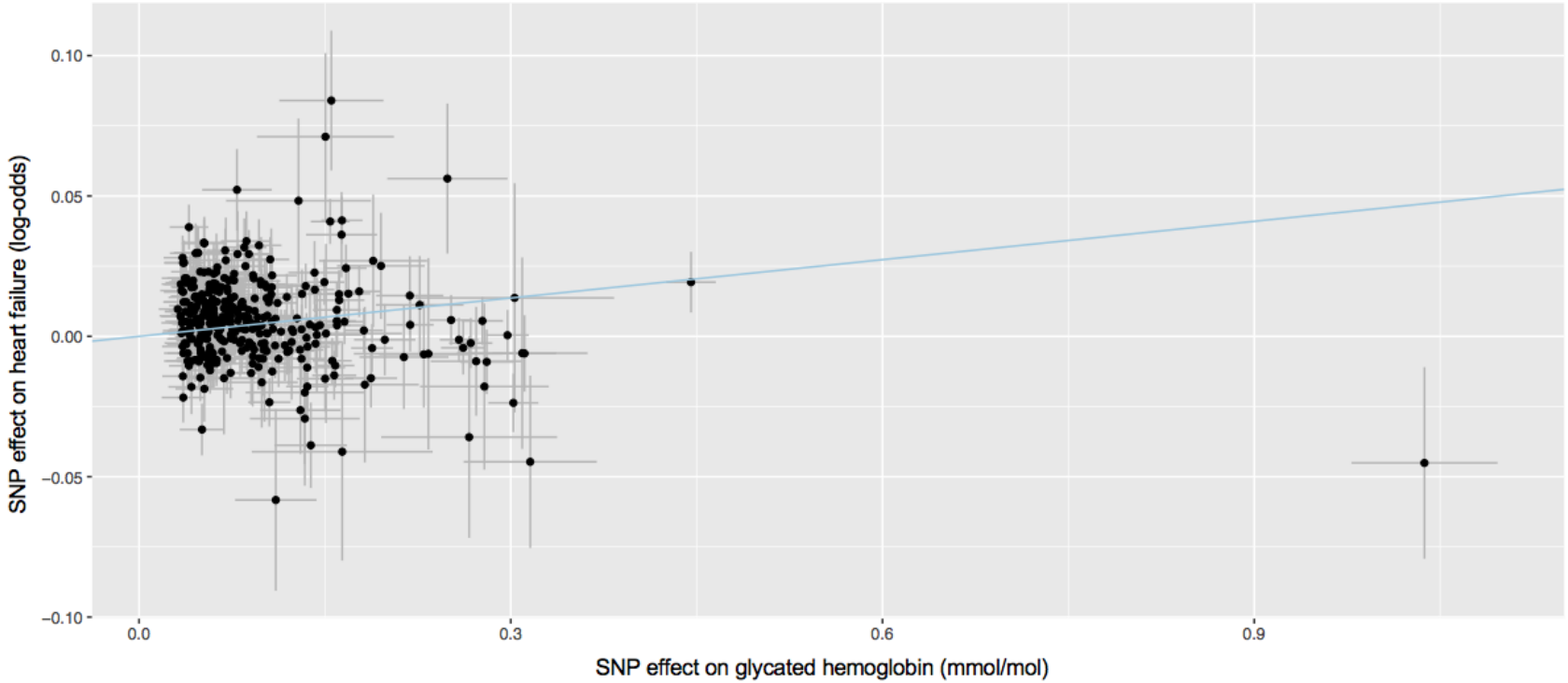
Each point represents a single genetic variant, with vertical and horizontal lines representing standard errors. The slope of the blue diagonal line represents the inverse-variance weighted Mendelian randomization estimate. The *P* value for the Cochran *Q* test for heterogeneity was 0.72. SNP: single-nucleotide polymorphism.

Figure S3. Scatter plot displaying genetic associations of the GLP1R genetic proxies with glycated hemoglobin (mmol/mol, x-axis) and left ventricular ejection fraction (standard deviation units, y-axis).



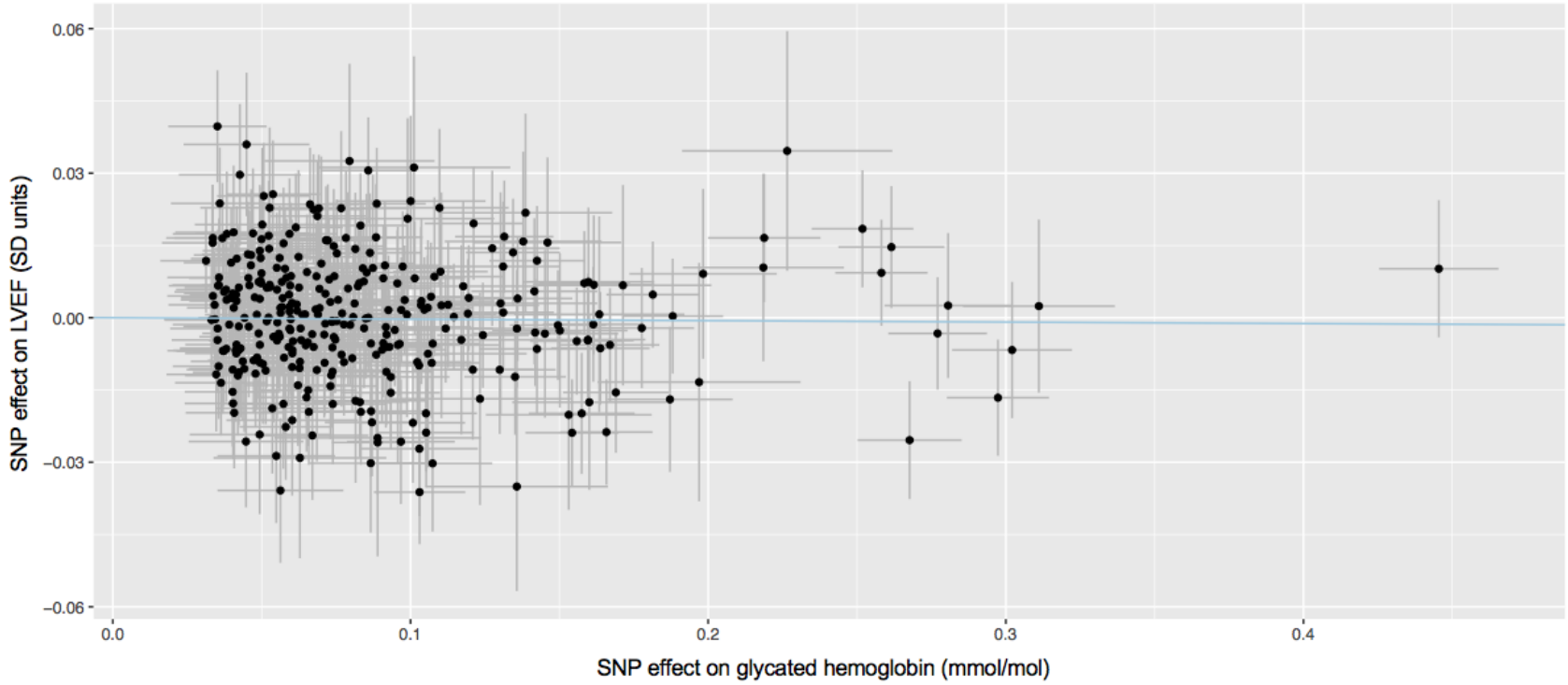
Each point represents a single genetic variant, with vertical and horizontal lines representing standard errors. The slope of the blue diagonal line represents the inverse-variance weighted Mendelian randomization estimate. The *P* value for the Cochran *Q* test for heterogeneity was 0.65. SNP: single-nucleotide polymorphism.

Figure S4. Scatter plot displaying genetic associations of the glycemia genetic proxies with glycated hemoglobin (mmol/mol, x-axis) and heart failure risk (log-odds, y-axis).



Each point represents a single genetic variant, with vertical and horizontal lines representing standard errors. The slope of the blue diagonal line represents the inverse-variance weighted Mendelian randomization estimate. SNP: single-nucleotide polymorphism.

Figure S5. Scatter plot displaying genetic associations of the glycemia genetic proxies with glycated hemoglobin (mmol/mol, x-axis) and left ventricular ejection fraction (standard deviation units, y-axis).



Each point represents a single genetic variant, with vertical and horizontal lines representing standard errors. The slope of the blue diagonal line represents the inverse-variance weighted Mendelian randomization estimate. SNP: single-nucleotide polymorphism.

ORIGINAL ARTICLE

Once-Weekly Semaglutide in Adults with Overweight or Obesity

John P.H. Wilding, D.M., Rachel L. Batterham, M.B., B.S., Ph.D., Salvatore Calanna, Ph.D., Melanie Davies, M.D., Luc F. Van Gaal, M.D., Ph.D., Ildiko Lingvay, M.D., M.P.H., M.S.C.S., Barbara M. McGowan, M.D., Ph.D., Julio Rosenstock, M.D., Marie T.D. Tran, M.D., Ph.D., Thomas A. Wadden, Ph.D., Sean Wharton, M.D., Pharm.D., Koutaro Yokote, M.D., Ph.D., Niels Zeuthen, M.Sc., and Robert F. Kushner, M.D., for the STEP 1 Study Group*

ABSTRACT

BACKGROUND

Obesity is a global health challenge with few pharmacologic options. Whether adults with obesity can achieve weight loss with once-weekly semaglutide at a dose of 2.4 mg as an adjunct to lifestyle intervention has not been confirmed.

METHODS

In this double-blind trial, we enrolled 1961 adults with a body-mass index (the weight in kilograms divided by the square of the height in meters) of 30 or greater (≥27 in persons with ≥1 weight-related coexisting condition), who did not have diabetes, and randomly assigned them, in a 2:1 ratio, to 68 weeks of treatment with once-weekly subcutaneous semaglutide (at a dose of 2.4 mg) or placebo, plus lifestyle intervention. The coprimary end points were the percentage change in body weight and weight reduction of at least 5%. The primary estimand (a precise description of the treatment effect reflecting the objective of the clinical trial) assessed effects regardless of treatment discontinuation or rescue interventions.

RESULTS

The mean change in body weight from baseline to week 68 was –14.9% in the semaglutide group as compared with –2.4% with placebo, for an estimated treatment difference of –12.4 percentage points (95% confidence interval [CI], –13.4 to –11.5; P<0.001). More participants in the semaglutide group than in the placebo group achieved weight reductions of 5% or more (1047 participants [86.4%] vs. 182 [31.5%]), 10% or more (838 [69.1%] vs. 69 [12.0%]), and 15% or more (612 [50.5%] vs. 28 [4.9%]) at week 68 (P<0.001 for all three comparisons of odds). The change in body weight from baseline to week 68 was –15.3 kg in the semaglutide group as compared with –2.6 kg in the placebo group (estimated treatment difference, –12.7 kg; 95% CI, –13.7 to –11.7). Participants who received semaglutide had a greater improvement with respect to cardiometabolic risk factors and a greater increase in participant-reported physical functioning from baseline than those who received placebo. Nausea and diarrhea were the most common adverse events with semaglutide; they were typically transient and mild-to-moderate in severity and subsided with time. More participants in the semaglutide group than in the placebo group discontinued treatment owing to gastrointestinal events (59 [4.5%] vs. 5 [0.8%]).

CONCLUSIONS

In participants with overweight or obesity, 2.4 mg of semaglutide once weekly plus lifestyle intervention was associated with sustained, clinically relevant reduction in body weight. (Funded by Novo Nordisk; STEP 1 ClinicalTrials.gov number, NCT03548935).

The authors' affiliations are listed in the Appendix. Address reprint requests to Dr. Kushner at Northwestern University Feinberg School of Medicine, 645 N. Michigan Ave., Suite 530, Chicago, IL 60611, or at rkushner@northwestern.edu.

*A complete list of investigators in the STEP 1 trial is provided in the Supplementary Appendix, available at NEJM.org.

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Obesity is a chronic disease and global public health challenge.[1-3] Obesity can lead to insulin resistance, hypertension, and dyslipidemia,[4] is associated with complications such as type 2 diabetes, cardiovascular disease, and nonalcoholic fatty liver disease,[2,5] and reduces life expectancy.[6] More recently, obesity has been linked to increased numbers of hospitalizations, the need for mechanical ventilation, and death in persons with coronavirus disease 2019 (Covid-19).[7,8]

Although lifestyle intervention (diet and exercise) represents the cornerstone of weight management,[1,2] sustaining weight loss over the long term is challenging.[9] Clinical guidelines suggest adjunctive pharmacotherapy, particularly for adults with a body-mass index (BMI, the weight in kilograms divided by the square of the height in meters) of 30 or greater, or 27 or greater in persons with coexisting conditions.[1,2,10] However, the use of available medications remains limited by modest efficacy, safety concerns, and cost.[3]

Semaglutide is a glucagon-like peptide-1 (GLP-1) analogue that is approved, at doses up to 1 mg administered subcutaneously once weekly, for the treatment of type 2 diabetes in adults and for reducing the risk of cardiovascular events in persons with type 2 diabetes and cardiovascular disease.[11] Semaglutide induced weight loss in persons with type 2 diabetes and in adults with obesity who were participants in a phase 2 trial,[12-14] findings that supported further investigation. The global phase 3 Semaglutide Treatment Effect in People with Obesity (STEP) program aims to evaluate the efficacy and safety of semaglutide administered subcutaneously at a dose of 2.4 mg once weekly in persons with overweight or obesity, with or without weight-related complications.[15]

This 68-week trial evaluated the efficacy and safety of semaglutide as compared with placebo as an adjunct to lifestyle intervention for reducing body weight and meeting other related end points in adults with overweight or obesity and without diabetes.

Methods

Trial Design and Oversight

We conducted a randomized, double-blind, placebo-controlled trial at 129 sites in 16 countries in Asia, Europe, North America, and South America. The sponsor (Novo Nordisk) designed the trial and oversaw its conduct. The design has been published previously.[15] The trial was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. The protocol (available with the full text of this article at NEJM.org) was approved by an independent ethics committee or institutional review board at each study site. Investigators were responsible for data collection, and the sponsor undertook site monitoring, data collation, and analysis. All authors had full access to study data, participated in drafting the manuscript (assisted by a sponsor-funded medical writer), approved its submission for publication, and vouch for the accuracy and completeness of the data and for the fidelity of the trial to the protocol.

Participants

We enrolled adults (18 years of age or older) with one or more self-reported unsuccessful dietary efforts to lose weight and either a BMI of 30 or greater or a BMI of 27 or greater with one or more treated or untreated weight-related coexisting conditions (i.e., hypertension, dyslipidemia, obstructive sleep apnea, or cardiovascular disease). A subgroup of participants with a BMI of 40 or less underwent dual-energy x-ray absorptiometry (DXA) to assess body composition. All participants provided written informed consent. Key exclusion criteria were diabetes, a glycated hemoglobin level of 48 mmol per mole (6.5%) or greater, a history of chronic pancreatitis, acute pancreatitis within 180 days before enrollment, previous surgical obesity treatment, and use of antiobesity medication within 90 days before enrollment. A full list of the eligibility criteria is provided in the Supplementary Appendix, available at NEJM.org.

Procedures

Participants were randomly assigned in a 2:1 ratio, through the use of an interactive Web-based response system, to receive semaglutide at a dose of 2.4 mg administered subcutaneously once a week for 68 weeks or matching placebo, in addition to lifestyle intervention; this 68-week period was followed by a 7-week period without receipt of semaglutide or placebo or lifestyle intervention. Semaglutide, administered with a prefilled pen injector, was initiated at a dose of 0.25 mg once weekly for the first 4 weeks, with the dose increased every 4 weeks to reach the maintenance

dose of 2.4 mg weekly by week 16 (lower maintenance doses were permitted if participants had unacceptable side effects with the 2.4-mg dose) (Fig. S1 in the Supplementary Appendix). Participants received individual counseling sessions every 4 weeks to help them adhere to a reduced-calorie diet (500-kcal deficit per day relative to the energy expenditure estimated at the time they underwent randomization) and increased physical activity (with 150 minutes per week of physical activity, such as walking, encouraged). Both diet and activity were recorded daily in a diary or by use of a smartphone application or other tools and were reviewed during counseling sessions. Participants discontinuing treatment prematurely remained in the trial.

END POINTS AND ASSESSMENTS

The coprimary end points were the percentage change in body weight from baseline to week 68 and achievement of a reduction in body weight of 5% or more from baseline to week 68. Confirmatory secondary end points (in hierarchical testing order) were achievement of a reduction in body weight of 10% or more and 15% or more by week 68 and the change from baseline to week 68 in waist circumference, systolic blood pressure, physical functioning score on the 36-item Short Form Health Survey (SF-36), version 2, and physical function score on the Impact of Weight on Quality of Life–Lite Clinical Trials Version (IWQOL-Lite-CT) questionnaire. (Assessments related to end points, along with supportive secondary and exploratory end points and safety assessments, are described in the Supplementary Appendix.) Body composition (total fat, total lean body mass, and regional [abdominal] visceral fat mass) was measured in the DXA subpopulation as a supportive secondary end point. Safety assessments included the number of adverse events occurring during the on-treatment period (the time during which participants received any dose of semaglutide or placebo within the previous 49 days, with any period of temporary interruption of the regimen excluded) and serious adverse events occurring between baseline and week 75. An independent external event adjudication committee reviewed selected adverse events (cardiovascular events and acute pancreatitis) and deaths. All standard assays were performed in a central laboratory.

STATISTICAL ANALYSIS

A sample size of 1950 participants provided an effective power of 99% for the coprimary and confirmatory secondary end points, tested in a prespecified hierarchical order. Efficacy end points were analyzed in the full analysis population (all randomly assigned participants according to the intention-to-treat principle); safety end points were analyzed in the safety analysis population (all randomly assigned participants exposed to at least one dose of semaglutide or placebo). Observation periods included the in-trial period (the time from random assignment to last contact with a trial site, regardless of treatment discontinuation or rescue intervention) and the on-treatment period. All results from statistical analyses were accompanied by a two-sided 95% confidence interval and corresponding P values (with significance defined as $P<0.05$). Supportive secondary end-point analyses were not controlled for multiple comparisons and should not be used to infer definitive treatment effects.

Two estimands — the treatment policy estimand (traditional intention-to-treat analysis, with effects assessed regardless of treatment discontinuation or rescue intervention) and the trial product estimand (effects assessed if the drug or placebo was taken as intended) — were used to assess treatment efficacy from different perspectives and accounted for intercurrent events and missing data differently, as described previously.[16] All analyses in the statistical hierarchy were based on the primary treatment policy estimand (details on analysis methods are provided in the Supplementary Appendix). All reported results are for the treatment policy estimand, unless stated otherwise.

RESULTS

STUDY PARTICIPANTS

From June through November 2018, a total of 1961 participants were randomly assigned to receive semaglutide (1306 participants) or placebo (655 participants). Overall, 94.3% of the participants completed the trial, 91.2% had a body-weight assessment at week 68, and 81.1% adhered to treatment (Fig. S2). Rescue interventions were received by 7 participants in the semaglutide group (2 had bariatric surgery and 5 received other antiobesity medication) and by 13 in the placebo

Table 1. Demographic and Clinical Characteristics of the Participants at Baseline.*

Characteristic	Semaglutide (N=1306)	Placebo (N=655)
Age — yr	46±13	47±12
Female sex — no. (%)	955 (73.1)	498 (76.0)
Race or ethnic group — no. (%)†		
White	973 (74.5)	499 (76.2)
Asian	181 (13.9)	80 (12.2)
Black or African American	72 (5.5)	39 (6.0)
Other	80 (6.1)	37 (5.6)
Hispanic or Latino ethnic group — no. (%)†	150 (11.5)	86 (13.1)
Body weight — kg	105.4±22.1	105.2±21.5
Body-mass index‡		
Mean	37.8±6.7	38.0±6.5
Distribution — no. (%)		
<30	81 (6.2)	36 (5.5)
≥30 to <35	436 (33.4)	207 (31.6)
≥35 to <40	406 (31.1)	208 (31.8)
≥40	383 (29.3)	204 (31.1)
Waist circumference — cm	114.6±14.8	114.8±14.4
Glycated hemoglobin — %	5.7±0.3	5.7±0.3
Prediabetes — no. (%)§	593 (45.4)	263 (40.2)
Blood pressure — mm Hg		
Systolic	126±14	127±14
Diastolic	80±10	80±10
Pulse — beats/min	72±10	72±10
Lipid levels — geometric mean mg/dl (coefficient of variation)¶		
Total cholesterol	189.6 (20.5)	192.1 (19.4)
HDL cholesterol	49.4 (25.6)	49.5 (25.0)
LDL cholesterol	110.3 (31.6)	112.5 (29.8)
VLDL cholesterol	24.5 (45.8)	24.9 (46.5)
Free fatty acids	12.3 (57.9)	12.7 (53.8)
Triglycerides	126.2 (47.4)	127.9 (49.0)
Estimated glomerular filtration rate — geometric mean ml/min/1.73 m^2 (coefficient of variation)‖	96.3 (18.7)	95.9 (18.3)
Coexisting conditions at the time of screening**		
Dyslipidemia — no. (%)	499 (38.2)	226 (34.5)
Hypertension — no. (%)	472 (36.1)	234 (35.7)
Knee osteoarthritis — no. (%)	173 (13.2)	102 (15.6)
Obstructive sleep apnea — no. (%)	159 (12.2)	71 (10.8)
Asthma or chronic obstructive pulmonary disease — no. (%)	147 (11.3)	80 (12.2)
Nonalcoholic fatty liver disease — no. (%)	101 (7.7)	62 (9.5)
Polycystic ovarian syndrome — no./total no. (%)††	62/955 (6.5)	34/498 (6.8)
Coronary artery disease — no. (%)	32 (2.5)	17 (2.6)

Table 1. (Continued.)

Characteristic	Semaglutide (N=1306)	Placebo (N=655)
No. of coexisting conditions at screening – no. (%)**		
None	328 (25.1)	163 (24.9)
1	337 (25.8)	187 (28.5)
2	298 (22.8)	135 (20.6)
3	183 (14.0)	96 (14.7)
4	96 (7.4)	43 (6.6)
≥5	64 (4.9)	31 (4.7)
SF-36‡‡		
Physical functioning score	51.0±6.9	50.8±7.9
Physical component summary score	51.1±7.3	51.1±7.9
Mental component summary score	55.4±5.7	55.5±5.9
IWQOL-Lite-CT§§		
Physical function score	65.4±24.0	64.0±24.4
Total score	63.6±21.2	63.3±20.9

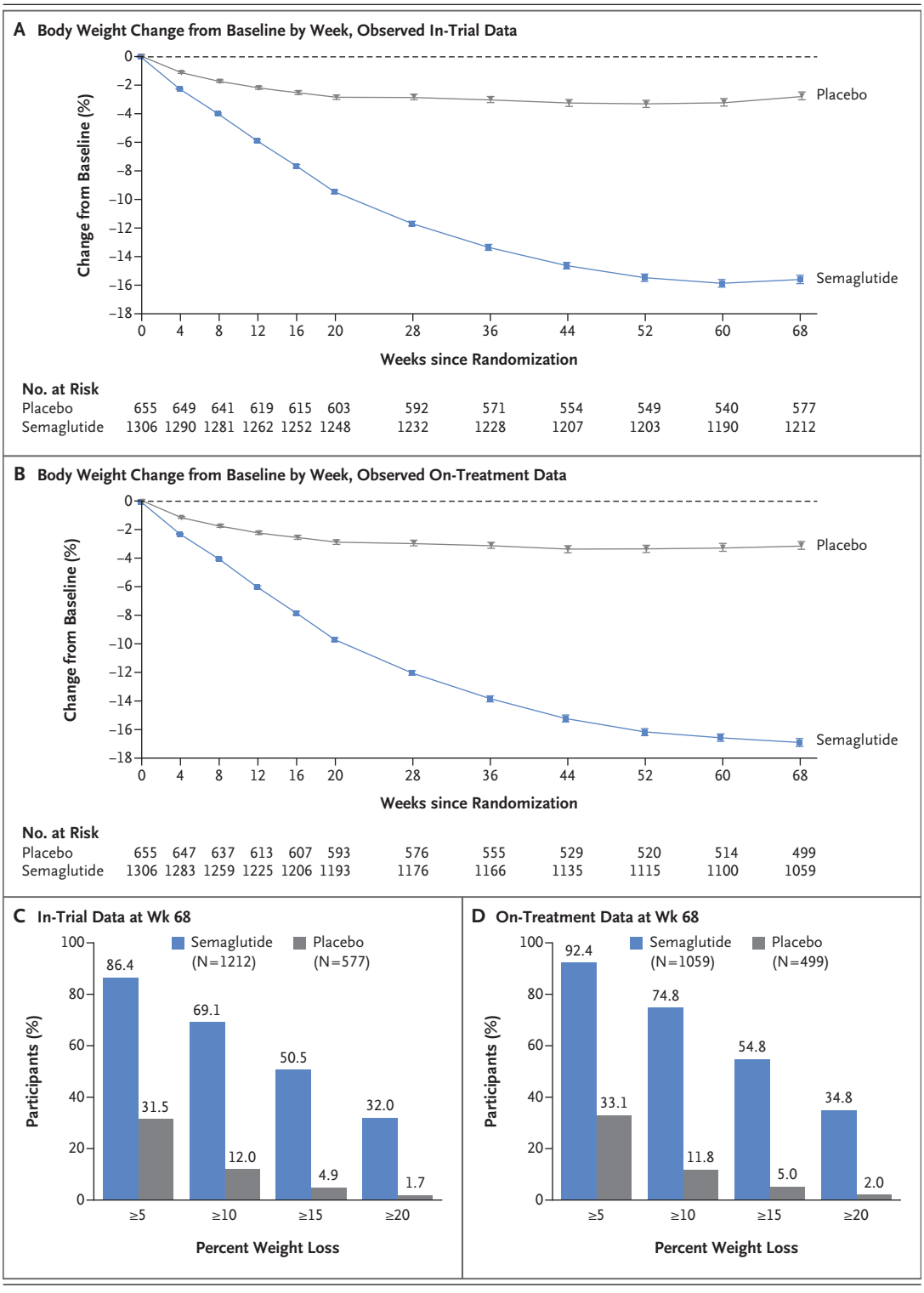
* Plus–minus values are means ±SD. HDL denotes high-density lipoprotein, LDL low-density lipoprotein, and VLDL very-low-density lipoprotein.
† Race and ethnic group were reported by the investigator. The category of "other" includes Native American, Hawaiian or other Pacific Islander, any other ethnic group, and "not applicable," the last of which is the way race or ethnic group was recorded in France.
‡ The body-mass index is the weight in kilograms divided by the square of the height in meters.
§ The presence of prediabetes was determined by investigators on the basis of available information (e.g., medical records, concomitant medication, and blood glucose variables) and in accordance with American Diabetes Association criteria.[17]
¶ Baseline lipid levels were reported for 1281 to 1301 participants per variable in the semaglutide group, and 645 to 649 participants per variable in the placebo group. The coefficient of variation is expressed as a percentage.
‖ The coefficient of variation is expressed as a percentage.
** A coexisting condition was a history of any of the following conditions, as reported at screening: dyslipidemia, hypertension, coronary artery disease, cerebrovascular disease, obstructive sleep apnea, impaired glucose metabolism, reproductive system disorders, liver disease, kidney disease, osteoarthritis, gout, or asthma or chronic obstructive pulmonary disease.
†† Data on polycystic ovarian syndrome include only female participants.
‡‡ Scores on the 36-Item Short-Form Health Survey (SF-36) are norm-based, transformed to a scale on which the 2009 general population of the United States has a mean score of 50 and a standard deviation of 10; higher scores indicate better quality of life. Baseline scores are reported for 1296 participants in the semaglutide group and 650 participants in the placebo group.
§§ Baseline scores on the Impact of Weight on Quality of Life–Lite Clinical Trials Version (IWQOL-Lite-CT; scores range from 0 to 100, with higher scores indicating better patient functioning) are reported for 1296 participants in the semaglutide group and 649 participants in the placebo group.

group (3 had bariatric surgery and 10 received other antiobesity medication).

Demographics and baseline characteristics were similar in the two treatment groups (Table 1). Most participants were female (74.1%) and White (75.1%), with a mean age of 46 years. The mean body weight was 105.3 kg, the mean BMI 37.9, and the mean waist circumference 114.7 cm; 43.7% had prediabetes. At screening, most participants (75.0%) had at least one coexisting condition. The baseline characteristics of the DXA subpopulation are provided in Table S1.

CHANGE IN BODY WEIGHT

In the semaglutide group, weight loss was observed from the first postrandomization assessment (week 4) onward, reaching a nadir at week 60 (Fig. 1A and 1B). For the treatment policy estimand (showing the effect regardless of treatment discontinuation or rescue intervention), the



estimated mean weight change at week 68 was –14.9% with 2.4-mg semaglutide, as compared with –2.4% with placebo (estimated treatment difference, –12.4 percentage points; 95% CI, –13.4 to –11.5; P<0.001). For the trial product estimand (showing the effect if the drug or placebo was taken as intended), the corresponding changes were –16.9% and –2.4% (estimated treatment

Figure 1 (facing page). Effect of Once-Weekly Semaglutide, as Compared with Placebo, on Body Weight.

Panels A and B show the observed mean percentage change from baseline in body weight over time among participants in the full analysis population during the in-trial observation period (the time from random assignment to last contact with a trial site, regardless of treatment discontinuation or rescue intervention) and during the on-treatment observation period (the time during which participants received semaglutide or placebo within the previous 2 weeks, with any period of temporary interruption of a regimen excluded). I bars indicate standard errors. The numbers at risk are the numbers of participants with available data contributing to the means at each visit. Panels C and D show the observed percentages of participants who had bodyweight reductions of at least 5%, 10%, 15%, and 20% from baseline to week 68 during the in-trial observation period and on-treatment observation period. Percentages were based on the number of participants for whom data were available at the week 68 visit — 1212 participants in the semaglutide group and 577 in the placebo group during the in-trial observation period and 1059 participants in the semaglutide group and 499 in the placebo group during the on-treatment observation period.

difference, –14.4 percentage points; 95% CI, –15.3 to –13.5).

Participants who received semaglutide were more likely to lose 5% or more, 10% or more, 15% or more, and 20% or more of baseline body weight at week 68 than those who received placebo (P<0.001 for the 5%, 10%, and 15% thresholds; the 20% threshold was not part of the statistical testing hierarchy) (Table 2, Fig. 1C and 1D, and Table S2). Among the participants for whom data were available at the week 68 visit (1212 participants in the semaglutide group and 577 in the placebo group), these thresholds were reached by 86.4% (1047 participants), 69.1% (838 participants), 50.5% (612 participants), and 32.0% (388 participants), respectively, in the semaglutide group, as compared with 31.5% (182 participants), 12.0% (69 participants), 4.9% (28 participants), and 1.7% (10 participants) in the placebo group (Fig. 1C, with on-treatment data shown in Fig. 1D and the cumulative distribution of change from baseline shown in Fig. S3). The change in body weight from baseline to week 68 was –15.3 kg in the semaglutide group as compared with –2.6 kg in the placebo group (estimated treatment difference, –12.7 kg; 95% CI, –13.7 to –11.7) (Fig. S4). Data on change in body weight and achieved reduction in body weight of 5% or more (coprimary end points) as well as confirmatory and selected supportive secondary end points for the trial product estimand are provided in Table S2.

OTHER CONFIRMATORY AND SUPPORTIVE SECONDARY END POINTS

Semaglutide was associated with greater reductions from baseline than placebo in waist circumference (–13.54 cm with semaglutide vs. –4.13 cm with placebo; estimated treatment difference, –9.42 cm; 95% CI, –10.30 to –8.53), BMI (–5.54 with semaglutide vs. –0.92 with placebo; estimated treatment difference, –4.61; 95% CI, –4.96 to –4.27), and systolic and diastolic blood pressure at week 68 (Table 2, Table S2, and Figs. S5 and S6). Benefits favoring semaglutide were also noted with respect to changes in glycated hemoglobin, fasting plasma glucose, C-reactive protein, and fasting lipid levels (Table 2).

EXPLORATORY END POINTS

Among participants with prediabetes at baseline, semaglutide was associated with improvements in glycated hemoglobin levels at week 68, and 84.1% of participants in the semaglutide group who had prediabetes at baseline, as compared with 47.8% of participants in the placebo group with prediabetes at baseline, reverted to normoglycemia. Results for these and other selected exploratory end points are presented in Table 2 and Table S3.

PHYSICAL FUNCTIONING AND OTHER PARTICIPANT-REPORTED OUTCOMES

SF-36 physical functioning scores (with possible norm-based scores ranging from 19.03 to 57.60) improved significantly more with semaglutide than with placebo at week 68 (P<0.001), and both SF-36 physical and mental component summary scores favored semaglutide (Table 2, Table S2, and Fig. S7). IWQOL-Lite-CT physical function scores improved significantly more with semaglutide than with placebo at week 68 (P<0.001) (Table 2 and Table S2), and there were favorable effects over placebo on IWQOL-Lite-CT total scores. The results of SF-36 and IWQOL-Lite-CT assessments showed that participants were more likely to have clinically meaningful within-person improvements in physical functioning with semaglutide than with placebo (Table S4).

Table 2. Coprimary, Confirmatory, and Selected Supportive Secondary and Exploratory End Points for the Treatment Policy Estimand.*

End Point	Semaglutide (N=1306)	Placebo (N=655)	Difference between Semaglutide and Placebo (95% CI)†	Odds Ratio	P Value
Coprimary end points assessed in the overall population					
Percent body-weight change from baseline to wk 68	−14.85	−2.41	−12.44 (−13.37 to −11.51)		<0.001
Participants with body-weight reduction ≥5% at wk 68 — %‡	86.4	31.5		11.2 (8.9 to 14.2)	<0.001
Confirmatory secondary end points assessed in the overall population					
Participants with body-weight reduction ≥10% at wk 68 — %‡	69.1	12.0		14.7 (11.1 to 19.4)	<0.001
Participants with body-weight reduction ≥15% at wk 68 — %‡	50.5	4.9		19.3 (12.9 to 28.8)	<0.001
Change from baseline to wk 68					
Waist circumference — cm	−13.54	−4.13	−9.42 (−10.30 to −8.53)		<0.001
Systolic blood pressure — mm Hg	−6.16	−1.06	−5.10 (−6.34 to −3.87)		<0.001
SF-36 physical functioning score	2.21	0.41	1.80 (1.18 to 2.42)		<0.001
IWQOL-Lite-CT physical function score	14.67	5.25	9.43 (7.50 to 11.35)		<0.001
Supportive secondary end points assessed in the overall population§					
Participants with body-weight reduction ≥20% at wk 68 — %‡	32.0	1.7		26.9 (14.2 to 51.0)	
Change from baseline to wk 68					
Body weight — kg	−15.3	−2.6	−12.7 (−13.7 to −11.7)		
Body-mass index	−5.54	−0.92	−4.61 (−4.96 to −4.27)		
Glycated hemoglobin — percentage points	−0.45	−0.15	−0.29 (−0.32 to −0.26)		
Fasting plasma glucose — mg/dl	−8.35	−0.48	−7.87 (−9.04 to −6.70)		
Diastolic blood pressure — mm Hg	−2.83	−0.42	−2.41 (−3.25 to −1.57)		
Lipid levels, ratio of wk 68 value to baseline¶					
Total cholesterol	0.97	1.00	0.97 (0.95 to 0.98)		
HDL cholesterol	1.05	1.01	1.04 (1.02 to 1.05)		
LDL cholesterol	0.97	1.01	0.96 (0.94 to 0.98)		
VLDL cholesterol	0.78	0.93	0.84 (0.81 to 0.87)		
Free fatty acids	0.83	0.93	0.89 (0.83 to 0.94)		
Triglycerides	0.78	0.93	0.84 (0.81 to 0.87)		
C-reactive protein, ratio of wk-68 value to baseline¶	0.47	0.85	0.56 (0.51 to 0.61)		

Exploratory end-point assessed in the prediabetes subpopulation¶				
Change in glycated hemoglobin level from baseline to wk 68 — percentage points**	−0.52	−0.17	−0.34 (−0.39 to −0.29)	
Participants with normoglycemia at wk 68 — (%)	84.1	47.8		

* The treatment policy estimand assesses treatment effect regardless of treatment discontinuation or rescue intervention; see Table S2 for corresponding data for the estimand (which assessed treatment effect assuming all participants adhered to treatment and did not receive rescue intervention). Continuous end-point analyses were conducted with the use of the analysis-of-covariance method, with randomized treatment as a factor and baseline end-point value as a covariate and a multiple imputation approach for missing data.[15] Analyses of categorical end points were conducted with the use of logistic regression, with the same factor and covariate.

† The difference is the estimated difference between the groups except in the case of lipid and C-reactive protein levels, for which the comparison is the ratio of values for semaglutide to those for placebo.

‡ Denominators for the percentages of participants observed to have body-weight reduction of ≥5%, ≥10%, ≥15%, and ≥20% at week 68 are the numbers of participants for whom data were available at the week 68 visit — 1212 participants in the semaglutide group and 577 participants in the placebo group.

§ Supportive secondary and exploratory end point analyses were not adjusted for multiplicity, and P values are therefore not reported for these end points.

¶ Ratios to baseline and corresponding baseline values were log-transformed before analysis.

‖ The exploratory end point in the prediabetes subpopulation was assessed in 593 participants in the semaglutide group and in 263 in the placebo group.

** The percentage-point change in glycated hemoglobin was not a prespecified end point.

CHANGE IN BODY COMPOSITION

In the DXA subpopulation (140 participants), total fat mass and regional visceral fat mass were reduced from baseline with semaglutide (Table S5). Although total lean body mass decreased in absolute terms (kg), the proportion of lean body mass relative to total body mass increased with semaglutide.

SAFETY AND SIDE-EFFECT PROFILE

Similar percentages of participants in the semaglutide and placebo groups reported adverse events (89.7% and 86.4%, respectively) (Table 3). Gastrointestinal disorders (typically nausea, diarrhea, vomiting, and constipation) were the most frequently reported events and occurred in more participants receiving semaglutide than those receiving placebo (74.2% vs. 47.9%). Most gastrointestinal events were mild-to-moderate in severity, were transient, and resolved without permanent discontinuation of the regimen (Fig. S8).

Serious adverse events were reported in 9.8% and 6.4% of semaglutide and placebo participants, respectively (Table 3), with the difference due primarily to a difference between the groups in the incidence of serious gastrointestinal disorders (1.4% of participants in the semaglutide group and 0% in the placebo group) and hepatobiliary disorders (1.3% with semaglutide and 0.2% with placebo). More participants in the semaglutide group than in the placebo group (7.0% vs. 3.1%) discontinued treatment owing to adverse events (mainly gastrointestinal events) (Table 3 and Fig. S9). One death was reported in each group, with neither considered by the independent external event adjudication committee to be related to receipt of semaglutide or placebo (Table 3).

Gallbladder-related disorders (mostly cholelithiasis) were reported in 2.6% and 1.2% of participants in the semaglutide and placebo groups, respectively. Mild acute pancreatitis (according to the Atlanta classification[18]) was reported in three participants in the semaglutide group (one participant had a history of acute pancreatitis, and the other two participants had both gallstones and pancreatitis); all recovered during the trial period. There was no difference between groups in the incidence of benign and malignant neoplasms. Additional safety variables are described in Table 3 and Table S6.

Table 3. Adverse Events.*

Adverse Event	Semaglutide (N=1306)			Placebo (N=655)		
	No. of participants (%)	No. of events	Events/100 person-yr	No. of participants (%)	No. of events	Events/100 person-yr
Any adverse event	1171 (89.7)	9658	566.1	566 (86.4)	3302	398.0
Serious adverse events	128 (9.8)	164	9.6	42 (6.4)	53	6.4
Adverse events leading to discontinuation of drug or placebo	92 (7.0)	123	7.2	20 (3.1)	23	2.8
Gastrointestinal disorders	59 (4.5)	78	4.6	5 (0.8)	5	0.6
Fatal events†‡	1 (0.1)	1	0.1	1 (0.2)	3	0.3
Adverse events reported in ≥10% of participants§						
Nausea	577 (44.2)	1068	62.6	114 (17.4)	146	17.6
Diarrhea	412 (31.5)	766	44.9	104 (15.9)	138	16.6
Vomiting	324 (24.8)	636	37.3	43 (6.6)	52	6.3
Constipation	306 (23.4)	390	22.9	62 (9.5)	73	8.8
Nasopharyngitis	281 (21.5)	480	28.1	133 (20.3)	216	26.0
Headache	198 (15.2)	387	22.7	80 (12.2)	104	12.5
Dyspepsia	135 (10.3)	179	10.5	23 (3.5)	30	3.6
Abdominal pain	130 (10.0)	175	10.3	36 (5.5)	41	4.9
Upper respiratory tract infection	114 (8.7)	158	9.3	80 (12.2)	116	14.0
Safety focus areas¶						
Gastrointestinal disorders‖	969 (74.2)	4309	252.6	314 (47.9)	739	89.1
Gallbladder-related disorders	34 (2.6)	42	2.5	8 (1.2)	8	1.0
Hepatobiliary disorders‖	33 (2.5)	40	2.3	5 (0.8)	5	0.6
Cholelithiasis	23 (1.8)	24	1.4	4 (0.6)	4	0.5
Hepatic disorders	31 (2.4)	37	2.2	20 (3.1)	24	2.9
Acute pancreatitis**	3 (0.2)	3	0.2	0	—	—
Cardiovascular disorders†	107 (8.2)	134	7.2	75 (11.5)	96	10.5
Allergic reactions	96 (7.4)	108	6.3	54 (8.2)	63	7.6
Injection-site reactions	65 (5.0)	99	5.8	44 (6.7)	82	9.9
Malignant neoplasms†	14 (1.1)	14	0.8	7 (1.1)	7	0.8
Psychiatric disorders‖	124 (9.5)	160	9.4	83 (12.7)	113	13.6
Acute renal failure	3 (0.2)	4	0.2	2 (0.3)	2	0.2
Hypoglycemia	8 (0.6)	15	0.9	5 (0.8)	7	0.8

* Adverse events are shown for the safety analysis population (all randomly assigned participants exposed to at least one dose of trial drug or placebo); since all participants received at least one dose of drug or placebo, the safety population is the same as the full-analysis population. Included are all adverse events that occurred during the on-treatment period (i.e., the period during which any dose of semaglutide or placebo was administered within the previous 49 days, with any period of temporary interruption of a regimen excluded), unless indicated otherwise. Adverse events were classified by severity as mild (causing minimal discomfort and not interfering with everyday activities), moderate (causing sufficient discomfort to interfere with normal everyday activities), or severe (preventing normal everyday activities).

† Included are events that were observed during the in-trial period (the time from random assignment to last contact with a trial site, regardless of treatment discontinuation or rescue intervention).

‡ In the semaglutide group, sudden cardiac death occurred in one participant with a medical history of hypertension and obstructive sleep apnea who had discontinued semaglutide. In the placebo group, death due to glioblastoma, aspiration pneumonia, and severe sepsis occurred in one participant each who had discontinued placebo.

§ Shown are the most common adverse events, according to the preferred term in the Medical Dictionary for Regulatory Activities (MedDRA), version 22.1, reported in 10% or more of participants in either treatment group.

¶ On the basis of therapeutic experience with glucagon-like peptide-1 receptor agonists and regulatory feedback and requirements, a number of safety focus areas were prespecified as being of special interest in the safety evaluation. Identified through searches of MedDRA, these preferred terms were judged to be relevant for each of the safety focus areas.

‖ This is a system organ class. (For gallbladder-related disorders, hepatobiliary disorders is the system organ class and cholelithiasis is the preferred term.)

** Acute pancreatitis was confirmed by the event adjudication committee.

DISCUSSION

In this trial, we found that adults with obesity (or overweight with one or more weight-related coexisting conditions) and without diabetes had a mean weight loss of 14.9% from baseline with semaglutide as an adjunct to lifestyle intervention. This loss exceeded that with placebo plus lifestyle intervention by 12.4 percentage points. The 14.9% mean weight loss that we observed in the semaglutide group is substantially greater than the weight loss of 4.0 to 10.9% from baseline with approved antiobesity medications.[3,19] Moreover, 86% of participants who received semaglutide, as compared with 32% of those who received placebo, lost 5% or more of baseline body weight, a widely used criterion of clinically meaningful response.[2,3,20,21] Weight loss with semaglutide stems from a reduction in energy intake owing to decreased appetite, which is thought to result from direct and indirect effects on the brain.[22-25] Weight loss with semaglutide was accompanied by greater improvements than placebo with respect to cardiometabolic risk factors, including reductions in waist circumference, blood pressure, glycated hemoglobin levels, and lipid levels; a greater decrease from baseline in C-reactive protein, a marker of inflammation; and a greater proportion of participants with normoglycemia. Semaglutide also improved physical functioning, as assessed by SF-36 and IWQOL-Lite-CT, a finding that is notable given that overweight and obesity significantly impair health-related quality of life.[26] Statistical superiority of semaglutide over placebo was achieved for all end points in the hierarchical testing procedure.

Weight loss of 10 to 15% (or more) is recommended in people with many complications of overweight and obesity (e.g., prediabetes, hypertension, and obstructive sleep apnea).[1,20,21,27] In the semaglutide group, approximately 70% of participants achieved a weight loss of at least 10%, and approximately 50% achieved a weight loss of at least 15%. Furthermore, one third of participants treated with semaglutide lost at least 20% of baseline weight, a reduction approaching that reported 1 to 3 years after bariatric surgery, particularly sleeve gastrectomy (approximately 20 to 30% weight loss).[28-31] The magnitude of reduction in cardiometabolic risk is assumed to be proportional to the amount of weight lost with both approaches (i.e., pharmacotherapy or surgery).[32]

Analyses from the DXA substudy suggested that semaglutide led to greater reduction in fat mass than lean body mass, a finding consistent with previous findings with semaglutide (at a dose of 1.0 mg) in persons with obesity[22] and in those with type 2 diabetes.[33] The weight loss and improvements with respect to cardiometabolic risk factors with semaglutide reported here will be complemented by an ongoing cardiovascular outcomes trial in participants with overweight or obesity and established cardiovascular disease (the SELECT trial; ClinicalTrials.gov number, NCT03574597).

Liraglutide administered subcutaneously once daily is the only GLP-1 receptor agonist approved for weight management.[3,19,34] Our trial showed greater mean placebo-corrected weight reductions with once-weekly 2.4-mg semaglutide plus lifestyle intervention (12.4%) than those reported with once-daily 3.0-mg liraglutide plus lifestyle intervention in the 56-week SCALE (Satiety and Clinical Adiposity — Liraglutide Evidence in Nondiabetic and Diabetic Individuals Obesity and Prediabetes) trial (4.5%).[34,35] In addition, the weight-loss phase with semaglutide persisted longer than that reported with liraglutide[35] and did not reach the nadir until week 60. However, these two studies differed in their participant population, which limits the robustness of between-study comparisons.

At week 68, 31% of participants who received placebo had lost at least 5% of baseline body weight, with 12% and 5% having achieved reductions of at least 10% and at least 15%, respectively, findings that show good adherence to lifestyle interventions. Similar results were observed at week 56 in the SCALE Obesity and Prediabetes trial.[35]

Currently, approved antiobesity drugs require administration once, twice, or three times daily,[3,19] and a once-weekly regimen may improve treatment adherence. The once-weekly 2.4-mg dose of semaglutide was chosen for the present study on the basis of pharmacokinetic modeling that suggested that the 2.4-mg weekly dose had a maximum steady-state concentration similar to a once-daily 0.4-mg dose investigated in a phase 2 dose-finding trial in participants with obesity.[14] The results of our study with once-weekly semaglutide at a 2.4-mg dose are consistent with the results of the phase 2 study, which showed an 11.6% greater reduction in body weight with

once-daily semaglutide at a dose of 0.4 mg than with placebo after 52 weeks of treatment.[14]

The safety of semaglutide was consistent with that reported in the phase 2 study with once-daily dosing in participants with obesity[14] and in the trials of once-weekly subcutaneous semaglutide in persons with type 2 diabetes (involving more than 8000 participants receiving doses up to 1 mg),[12] as well as with that reported for the GLP-1 receptor agonist class in general.[13,36] As is typical of this drug class,[13,37] transient, mild-to-moderate gastrointestinal disorders were the most frequently reported adverse events, and more participants in the semaglutide group than in the placebo group discontinued the assigned regimen after such events. Nausea was the most common gastrointestinal event, occurring primarily during the dose-escalation period, a finding similar to that reported with liraglutide at a dose of 3.0 mg.[35] Gallbladder-related disorders, principally cholelithiasis, were more common in the semaglutide group, a finding consistent with previous reports for GLP-1 receptor agonists[38,39] and with the known effects of rapid weight loss.[40,41] The incidence of cholelithiasis with semaglutide was in line with that of liraglutide at a dose of 3.0 mg.[35] No new safety concerns arose.

Strengths of this trial included the large sample size and high rates of adherence to the treatment regimen and completion of the trial. Limitations included the preponderance of women and White participants, the relatively short duration of the trial, the exclusion of persons with type 2 diabetes, and the potential that participants who were enrolled may represent a subgroup with greater commitment to weight-loss efforts than the general population. Although the DXA data we report provide greater insight into the weight-loss effects of semaglutide, such assessments were performed in only a subpopulation of participants.

Our trial showed that among adults with overweight or obesity (without diabetes), once-weekly subcutaneous semaglutide plus lifestyle intervention was associated with substantial, sustained, clinically relevant mean weight loss of 14.9%, with 86% of participants attaining at least 5% weight loss.

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A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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APPENDIX

The authors' affiliations are as follows: the Department of Cardiovascular and Metabolic Medicine, Institute of Life Course and Medical Sciences, University of Liverpool, Liverpool (J.P.H.W.), University College London Centre for Obesity Research, Division of Medicine, University College London (R.L.B.), the National Institute of Health Research, UCLH Biomedical Research Centre (R.L.B.), the Centre for Weight Management and Metabolic Surgery, University College London Hospital (R.L.B.), and the Department of Diabetes and Endocrinology, Guy's and St. Thomas' NHS Foundation Trust (B.M.M.), London, and the Diabetes Research Centre, University of Leicester (M.D.) and the NIHR Leicester Biomedical Research Centre (M.D.), Leicester — all in the United Kingdom; Novo Nordisk, Søborg, Denmark (S.C., M.T.D.T., N.Z.); the Department of Endocrinology, Diabetology, and Metabolism, Antwerp University Hospital, University of Antwerp, Antwerp, Belgium (L.F.V.G.); the Departments of Internal Medicine/Endocrinology and Population and Data Sciences, University of Texas Southwestern Medical Center (I.L.), and the Dallas Diabetes Research Center at Medical City (J.R.) — both in Dallas; the Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, Philadelphia (T.A.W.); York University, McMaster University and Wharton Weight Management Clinic, Toronto (S.W.); the Department of Endocrinology, Hematology, and Gerontology, Graduate School of Medicine, Chiba University and Department of Diabetes, Metabolism, and Endocrinology, Chiba University Hospital, Chiba, Japan (K.Y.); and the Division of Endocrinology, Feinberg School of Medicine, Northwestern University, Chicago (R.F.K.).

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ORIGINAL ARTICLE

Neuroprotection in Rats Following Ischaemia-Reperfusion Injury by GLP-1 Analogues—Liraglutide and Semaglutide

Maryna V. Basalay[1] · Sean M. Davidson[1] · Derek M. Yellon[1]

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Abstract

Purpose A substantial number of ischaemic stroke patients who receive reperfusion therapy in the acute phase do not ever fully recover. This reveals the urgent need to develop new adjunctive neuroprotective treatment strategies alongside reperfusion therapy. Previous experimental studies demonstrated the potential of glucagon-like peptide-1 (GLP-1) to reduce acute ischaemic damage in the brain. Here, we examined the neuroprotective effects of two GLP-1 analogues, liraglutide and semaglutide.

Methods A non-diabetic rat model of acute ischaemic stroke involved 90, 120 or 180 min of middle cerebral artery occlusion (MCAO). Liraglutide or semaglutide was administered either i.v. at the onset of reperfusion or s.c. 5 min before the onset of reperfusion. Infarct size and functional status were evaluated after 24 h or 72 h of reperfusion.

Results Liraglutide, administered as a bolus at the onset of reperfusion, reduced infarct size by up to 90% and improved neuroscore at 24 h in a dose-dependent manner, following 90-min, but not 120-min or 180-min ischaemia. Semaglutide and liraglutide administered s.c. reduced infarct size by 63% and 48%, respectively, and improved neuroscore at 72 h following 90-min MCAO. Neuroprotection by semaglutide was abolished by GLP1-R antagonist exendin(9-39).

Conclusion Infarct-limiting and functional neuroprotective effects of liraglutide are dose-dependent. Neuroprotection by semaglutide is at least as strong as by liraglutide and is mediated by GLP-1Rs.

Keywords Acute ischaemic stroke · Glucagon-like peptide-1 · Ischaemia-reperfusion injury · Middle cerebral artery occlusion · Neuroprotection

Introduction

At present, ischaemic stroke remains one of the most costly and devastating clinical syndromes in the world [1]. Recently, endovascular recanalization with mechanical thrombectomy has brought about a paradigm shift in the optimal management of this high-risk group of patients, demonstrating significant benefits in clinical trials [2]. Importantly, early reperfusion is currently the only therapy that has proven to limit infarct size in patients with acute ischaemic stroke [1, 3]. However, a substantial number of those patients who receive treatment with tPA and/or thrombectomy in the acute phase do not ever fully recover [4, 5]. This reveals the need to develop new adjunctive neuroprotective treatment strategies alongside reperfusion therapy [6].

Although glucagon-like peptide-1 (GLP-1) therapy has been associated with the treatment of type 2 diabetes [7, 8], the ability of GLP-1 to activate pro-survival pathways is well known [9]. Importantly, a number of preclinical studies have demonstrated neuroprotective effects of GLP-1 in non-diabetic and diabetic models of acute ischaemic stroke [8, 10]. In total, all these studies suggest that the administration of GLP-1 receptor (GLP-1R) agonists is one of the most promising treatments to pursue for patients immediately after stroke.

The purpose of the current study is to compare the effects of two different GLP-1 analogues—liraglutide [11] and semaglutide [12–14]. Liraglutide is a first-generation GLP-1 analogue designed to have a reduced susceptibility to enzymatic degradation and an extended plasma half-life in humans of 13 h following subcutaneous administration, in comparison to the ~ 2-min half-life for human native GLP-1 [11]. Semaglutide

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✉ Derek M. Yellon
d.yellon@ucl.ac.uk

[1] The Hatter Cardiovascular Institute, University College London, 67 Chenies Mews, London WC1E 6HX, UK

is a newer GLP-1 analogue with a greatly prolonged half-life in humans of 165 h (approximately 1 week) [13, 14]. For this reason, we hypothesised that semaglutide would confer prolonged neuroprotection during the reperfusion period compared with shorter-acting GLP-1 analogues.

While neuroprotection by liraglutide has previously been demonstrated in a number of preclinical studies using permanent or transient middle cerebral artery occlusion (MCAO) [10], semaglutide has never been examined in either stroke model.

Materials and Methods

Animals and Experimental Groups

Male non-diabetic Sprague-Dawley rats (220–250 g) were used. All animals were randomly allocated to groups with allocation being concealed.

Study 1 The rats were subjected to i.v. bolus of vehicle (saline) ($n = 6$) or liraglutide via the tail vein at the onset of reperfusion following a 90-min MCAO. Three doses of liraglutide were used (350 μg/kg ($n = 8$), 700 μg/kg ($n = 8$) and 1050 μg/kg ($n = 8$)). These doses were selected based on a previously published study which showed that 700 μg/kg was neuroprotective in rats [15]. Functional status was evaluated 24 h after MCAO. The rats were then immediately sacrificed, and infarct sizes measured.

Study 2 The functional and infarct-limiting effects of the maximal dose of liraglutide from study 1 were evaluated in rats subjected to 90-min ($n = 8$), 120-min ($n = 6$) or 180-min ($n = 5$) MCAO, followed by 24-h reperfusion, in comparison with the corresponding time-matched control groups ($n = 8$, $n = 8$ and $n = 5$ at each time point).

Study 3 The functional and infarct-limiting effects of s.c. administration of liraglutide 1050 μg/kg ($n = 9$) and semaglutide 12 μg/kg ($n = 10$) 5 min before the onset of reperfusion were evaluated in rats subjected to 90-min MCAO, followed by 72-h reperfusion and compared with the control group ($n = 8$). The chosen dose of semaglutide was based on a previous publication [16]. To assess the role of GLP-1Rs in these effects, in one group of animals, the GLP1-R antagonist exendin(9-39) (Ex(9-39), 50 μg/kg, i.v.) was administered 15 min before the injection of semaglutide ($n = 8$). The dose of Ex(9-39) was selected on the basis of our previous study [17]. Blood glucose was measured in three random rats in each group to make sure no significant decline of glucose concentration occurs in response to the administration of these drugs.

Transient Middle Cerebral Artery Occlusion

The intraluminal filament model of focal ischaemia was used [18, 19]. Briefly, under 2% isoflurane anaesthesia, a silicon-coated monofilament was advanced through the right common and internal carotid arteries towards the middle cerebral artery junction until resistance was felt (~ 2 cm). The animals were then allowed to recover in order to check for the presence of the functional signs of cortex brain ischaemia, such as walking towards the contralateral side, left forelimb flexion and body rotation to the left when held by the tail. Animals without any of these signs at this point were excluded from the study. The filament was withdrawn after 90, 120 or 180 min of occlusion.

Functional Status Evaluation

Behavioural neurological evaluation was performed using three previously reported scoring scales or neuroscores [20–23]. These scales consist of simple sensorimotor tasks (spontaneous activity, gait, postural signs, lateral resistance, limb placing and parachute reflex) for the assessment of the severity of neurological deficits. Higher neuroscores reflect stronger deficits, with the maximal possible score being 22. The merged 0–22-point scale is presented in supporting information on the website.

Infarct Size Measurement

The animals were euthanised with i.p. 100 mg/kg of sodium pentobarbital. The brains were immediately removed, sectioned at 1.25-mm intervals, stained with 1% triphenyl tetrazolium chloride (TTC) and fixed in formalin. The sections were photographed, and the resulting infarct areas were measured using ImageJ (the examples of the obtained images are presented on the website). Infarct sizes were presented as the hemispheric lesion volumes corrected for oedema (%HLVe) [24, 25].

Statistical Analysis

One-way ANOVA (Dunn's multiple comparison test) was used for statistical analysis of the data, following the Shapiro-Wilk normality test (GraphPad Prism 5, GraphPad Software, Inc., CA). Data are presented as median [25% percentile; 75% percentile]. Differences between groups were considered statistically significant when $P < 0.05$. Correlations were determined by Spearman's r analysis.

Results

Liraglutide Reduces Brain Damage in a Dose-Dependent Manner

Study 1 investigated the potential neuroprotective efficacy of single bolus of either 350, 700 or 1050 μg/kg liraglutide administered at the onset of reperfusion.

The %HLVe in the vehicle group was 40 [34; 46]. A total of 700 μg/kg or 1050 μg/kg liraglutide reduced infarct size by 74% ($P < 0.05$) and 90% ($P < 0.001$). No significant reduction in infarct size was observed in the animals treated with 350 μg/kg liraglutide (Fig. 1a). The median neuroscore after 24 h in the vehicle group was 10.0 (on the 0–22 scale) and was reduced with either 700 μg/kg ($P < 0.05$) or 1050 μg/kg ($P < 0.001$) liraglutide. No benefit was seen with 350 μg/kg liraglutide (median neuroscore of 10.0) (Fig. 1b). A significant correlation was found between infarct sizes and neuroscores on day 1 ($r = 0.86$; $P < 0.001$).

Reperfusion Delay is a Limiting Factor for Neuroprotection by Liraglutide

In study 2, we investigated whether the neuroprotective efficacy of 1050 μg/kg liraglutide was retained when the duration of MCAO was extended to 120 and 180 min.

The %HLVe in the control groups (MCAO duration of 90 min, 120 min and 180 min) were 42 [38; 46], 35 [31; 43] and 44 [39; 53] ($P > 0.05$ between all the control groups). Liraglutide reduced infarct size in the 90-min ischaemia groups ($P < 0.001$), but not in the 120- and 180-min groups (Fig. 2a). The median neuroscores in control groups subjected to 90-, 120- and 180-min ischaemia were 9.0, 9.0 and 10.0 respectively. In the corresponding liraglutide-treated groups, the medians were significantly reduced after 90-min ($P < 0.05$), but not 120-min and 180-min MCAO (Fig. 2b).

Neuroprotection by Semaglutide Is at Least as Powerful as by Liraglutide and Is Mediated by GLP-1Rs

Increasing the duration of reperfusion period to 72 h in study 3 was accompanied by reduced survival: 2 out of 10 animals died in the control group, 2 out of 11 in the liraglutide group, and 3 out of 11 in the group in which the GLP-1R antagonist Ex(9-39) was administered before semaglutide. The only mortality-free group (0 out of 10) was that in which the rats were treated with semaglutide alone. However, none of these differences in mortality was significant.

In this series of experiments, visible intracerebral haemorrhage was observed in some brains at slicing: 5 from the control group, 1 from the liraglutide group and 2 from the Ex(9-39) + semaglutide group. No large visible haemorrhages were revealed in the rats treated with semaglutide without the GLP-1R antagonist.

The %HLVe in surviving animals from the control group was 51 [43; 59]. Liraglutide and semaglutide each reduced infarct size at 72 h by 48% and 63% ($P < 0.01$ and $P < 0.001$ respectively). Ex(9-39) abolished the infarct-limiting effect of semaglutide ($P > 0.05$ vs. control) (Fig. 3a). The median neuroscore in the surviving animals from the control group was 12.0. In both liraglutide- and semaglutide-treated

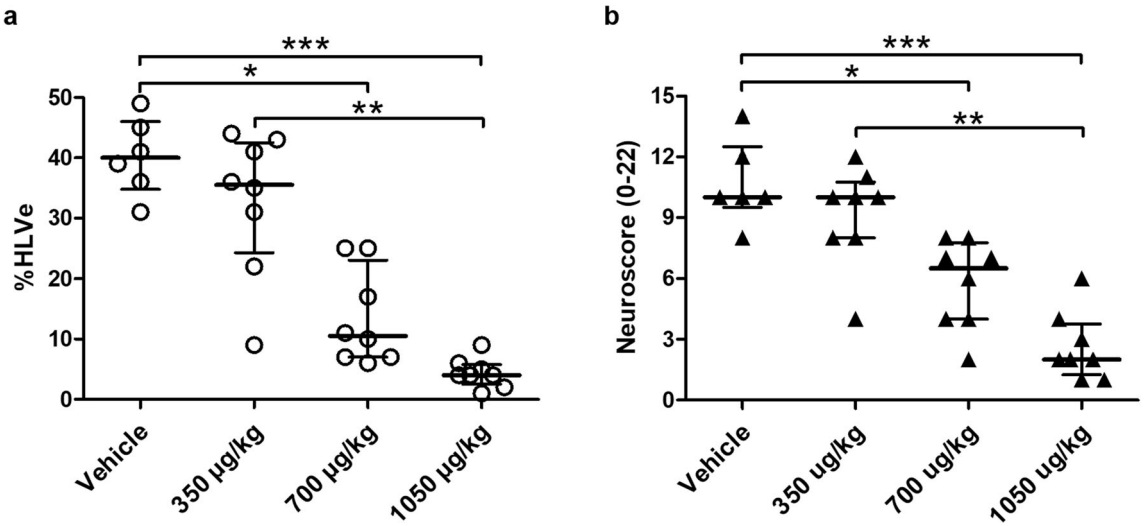

Fig. 1 Neuroprotection induced by liraglutide administration in the acute ischaemic stroke model is dose-dependent. Rats were subjected to 90-min MCAO, followed by 24-h reperfusion. Vehicle or liraglutide was administered as i.v. bolus at the onset of reperfusion. Hemispheric lesion volumes corrected for oedema (%HLVe) (**a**) and neuroscores (**b**) were evaluated at the end of reperfusion period. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

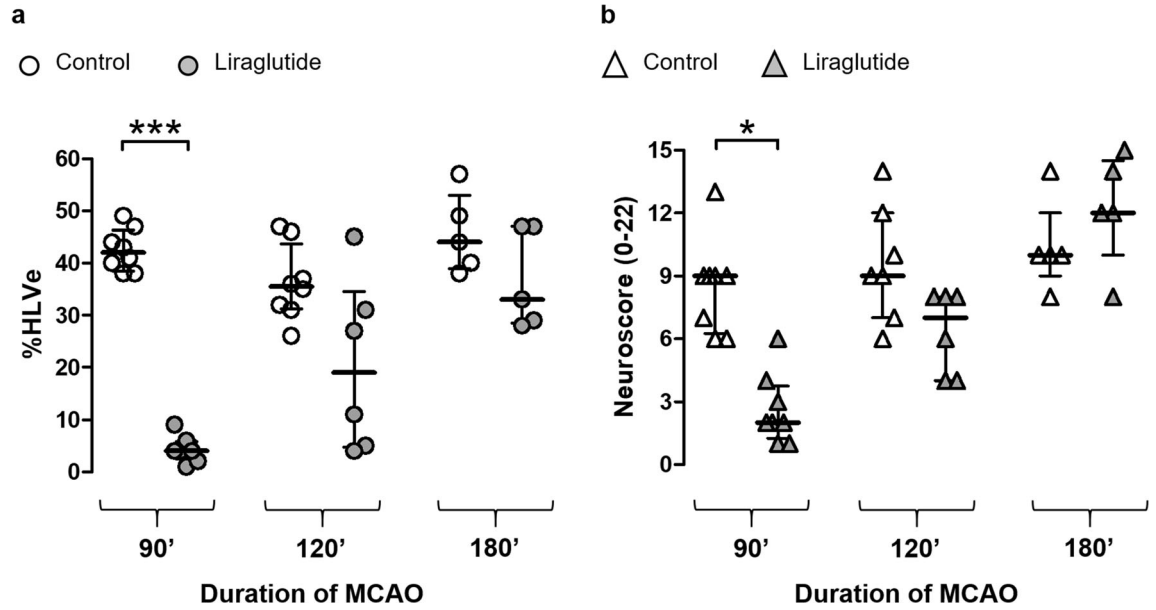

Fig. 2 Reperfusion delay is a limiting factor for neuroprotection by liraglutide. Rats were subjected to 90-, 120- or 180-min MCAO, followed by 24-h reperfusion. Liraglutide was administered as i.v. bolus at the onset of reperfusion. Hemispheric lesion volumes corrected for oedema (%HLVe) (**a**) and neuroscores (**b**) were evaluated at the end of reperfusion period. Individual data and median with interquartile range are shown for each group. $^{*}P < 0.05$; $^{***}P < 0.001$

groups, the neuroscore was significantly reduced to 6.0 ($P < 0.001$). In rats treated with semaglutide and GLP-1R antagonist, the neuroscore was unchanged at 11.0 (Fig. 3b). A significant correlation was found between infarct sizes and neuroscores on day 3 ($r = 0.9$; $P < 0.001$).

Discussion

Although the neuroprotective effects of liraglutide have been demonstrated previously in experimental studies [10], the current study is the first to show that the infarct-

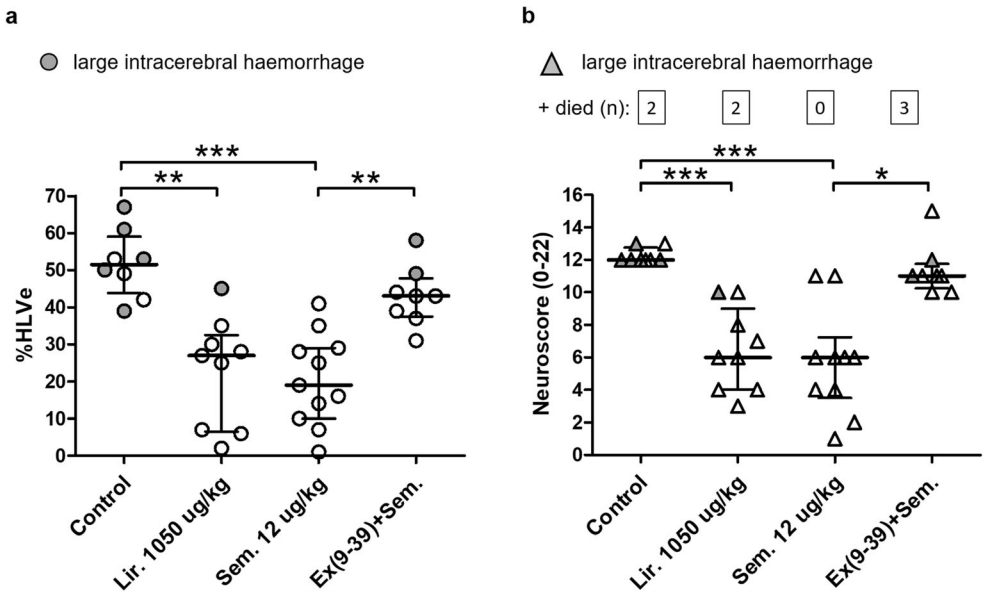

Fig. 3 Neuroprotection by semaglutide is at least as strong as by liraglutide and is mediated by GLP-1Rs. Rats were subjected to 90-min MCAO, followed by 72-h reperfusion. Liraglutide (Lir.) or semaglutide (Sem.) was administered s.c. 5 min before the onset of reperfusion. Hemispheric lesion volumes corrected for oedema (%HLVe) (**a**) and neuroscores (**b**) were evaluated at the end of reperfusion period. Individual data and median with interquartile range are shown for each group. The numbers of the animals who did not survive 72-h reperfusion or had to be euthanised due to having reached the humane endpoints are presented in the boxes. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

limiting effect of liraglutide is dose-dependent. Importantly, we used a clinically relevant model of transient focal brain ischaemia, as well as clinically applicable time of liraglutide administration, i.e., shortly before reperfusion. As the concentration and bioavailability of liraglutide can be variable following subcutaneous administration [14], we chose to administer it intravenously at the onset of reperfusion. According to data by Hunter and Hölscher, liraglutide is expected to cross the blood-brain barrier at the doses we tested, even in the normoxic state [26]. We regarded 1050 μg/kg as the maximal effective dose, as there were no significant differences in infarct size between 1050 and 700 μg/kg. We showed that the neuroprotective effects of liraglutide in an acute ischaemic stroke model are dose-dependent. The obtained data are in agreement with the study by Darsalia et al., demonstrating that the other synthetic form of GLP-1—exendin-4—reduces brain damage in a dose-dependent manner [27]. In addition, the infarct sizes in the current study correlated significantly with neuroscores, as in the study by Chauveau et al. [23].

It was shown previously that liraglutide administered 1 mg/kg per day does not reduce blood glucose level in non-diabetic rats [28]. Moreover, the neuroprotective effect of liraglutide in this study was independent of glycaemia normalisation [27]. For these reasons, we did not measure blood glucose dynamics in response to liraglutide treatment in all the animals, but only validated these previous data in three random rats of each group.

As the duration of brain ischaemia is a variable parameter in patients with an acute ischaemic stroke [29], in the second series of experiments, we examined the neuroprotective effects of 1050 μg/kg liraglutide bolus, administered at reperfusion, with more prolonged MCAO, specifically 120 and 180 min. Although there were no differences in infarct sizes between the control groups subjected to 90-, 120- and 180-min ischaemia, the infarct-limiting effect was not observed with 120-min and 180-min ischaemia. Similarly, the neuroscore improved significantly only in the 90-min treatment group. We were not able to find any direct evidence in the existing literature, as to the relationship between the duration of MCAO in rats and the corresponding ischaemic period in humans. However, it has been shown that rat brain infarcts increase in size progressively up to 120–180 min of MCAO [30], whereas reperfusion therapy in most patients with acute ischaemic stroke is effective within 6 h of focal brain ischaemia [3], suggesting ~ 2–3 times faster infarct progression in the brain of rats vs. humans.

In the third series of experiments, we compared the neuroprotective effects of liraglutide and the newer longer acting GLP-1 analogue, semaglutide. The principal clinic-related difference of semaglutide from liraglutide is the substantially longer half-life [11, 13, 14]. This means that the neuroprotection after single s.c. administration of semaglutide shortly before reperfusion could potentially cover a longer duration of the reperfusion process. For this reason, we extended the reperfusion period to 72 h to be able to observe the potential benefits of semaglutide due to its prolonged half-life. We showed that both these GLP-1 analogues reduce infarct size. Notably, the group treated with semaglutide was the only one in this series, where all the animals survived 72 h, and no large visible intracerebral haemorrhages were found. Although this latter observation still needs to be confirmed by further studies, this possible characteristic of semaglutide can be valuable for the patients with acute ischaemic stroke, especially those undergoing thrombolytic therapy [31–33]. Previously, it had been demonstrated that GLP-1R agonist exendin-4 ameliorates warfarin-associated haemorrhagic transformation after cerebral ischaemia [34]. Importantly, semaglutide has recently been shown in the SUSTAIN-6 trial to have clinical benefit in terms of reducing the rate of non-fatal stroke [34], although studies assessing functional outcome after stroke are still needed [35]. In this regard, our experimental study demonstrated improved functional recovery in rats treated with either liraglutide or semaglutide.

GLP1-R antagonist exendin(9-39) in our study abolished the neuroprotective effects of semaglutide, which indicates the key role of GLP-1Rs in these effects. This is in line with the previous study by Darsalia et al., where exendin-4 mediated neuroprotection in wild type, but not in *Glp-1r*-/- mice [36]. In addition, 2 animals in this group had large intracerebral haemorrhages. While GLP-1Rs are widely distributed in the brain [37, 38], the location of these receptors responsible for the neuroprotective effects is not known. However, regarding the possible protection from haemorrhage transformation of ischaemic stroke, the GLP-1Rs expressed in the endothelium [39] might be of more importance.

Conclusion

This study demonstrates that the GLP-1 analogues, liraglutide and semaglutide, reduce infarct size in a model of acute ischaemic stroke in non-diabetic rats. We believe this study indicates the potential for agents to be used in the clinical setting of ischaemic stroke.

Authors' Contribution Dr. Maryna Basalay contributed to conception and design, acquisition, analysis and interpretation of these experiments, drafted the manuscript and agreed to be accountable for all aspects of work ensuring integrity and accuracy. Dr. Sean Davidson contributed to the integration of the experiments and its design and critically revised the content of the manuscript. Professor Derek Yellon contributed to the conception and design, analysis and interpretation of the experiments and has critically revised the manuscript and approved its final version.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethics Approval All applicable international, national and/or institutional guidelines for the care and use of animals were followed. The experiments were performed in accordance with the European Commission Directive 2010/63/EU (European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes) and the UK Home Office (Scientific Procedures) Act (1986) with project approval from the respective Institutional Animal Care and Use Committees, in compliance with the ARRIVE guidelines.

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ORIGINAL RESEARCH

Once-Weekly Semaglutide Versus Once-Daily Liraglutide for the Treatment of Type 2 Diabetes: A Long-Term Cost-Effectiveness Analysis in Estonia

Samuel J. P. Malkin · Monika Russel-Szymczyk · Girtel Liidemann · Vallo Volke · Barnaby Hunt

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ABSTRACT

Introduction: Once-weekly semaglutide is a novel glucagon-like peptide-1 (GLP-1) analogue for the treatment of type 2 diabetes that was associated with greater reductions in glycated hemoglobin (HbA1c) and body mass index (BMI) versus once-daily GLP-1 analogue liraglutide in a recent network meta-analysis (NMA). The aim of the present study was to assess the long-term cost-effectiveness of once-weekly semaglutide 1 mg versus liraglutide 1.2 mg in Estonia.

Methods: Outcomes were projected over patient lifetimes using the IQVIA CORE Diabetes Model (version 9.0), with baseline cohort characteristics sourced from SUSTAIN 3 and changes in HbA1c, systolic blood pressure (SBP), and BMI associated with once-weekly semaglutide and liraglutide derived from the NMA. Patients were assumed to receive once-weekly semaglutide or liraglutide for 5 years before intensifying to basal insulin. Treatment effects were applied for the first 5 years, after which HbA1c increased to 7.0%, SBP followed a natural progression, and BMI reverted to baseline for the remainder of the analysis. Costs were expressed in euros (EUR) and estimated from a healthcare payer perspective. Utilities associated with diabetes and diabetes-related complications were taken from published sources.

Results: Once-weekly semaglutide 1 mg was associated with improvements in quality-adjusted life expectancy of 0.13 quality-adjusted life years (QALYs) versus liraglutide 1.2 mg. Direct costs were EUR 67 higher with once-weekly semaglutide, due to the increased acquisition cost, but this was mostly offset by cost savings due to avoidance of diabetes-related complications. Once-weekly semaglutide 1 mg was therefore associated with an incremental cost-effectiveness ratio of EUR 523 per QALY gained versus liraglutide 1.2 mg, which falls well below a willingness-to-pay threshold of EUR 52,390 per QALY gained (three times the Estonian GDP per capita).

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S. J. P. Malkin · B. Hunt (✉)
Ossian Health Economics and Communications, Basel, Switzerland
e-mail: hunt@ossianconsulting.com

M. Russel-Szymczyk
Novo Nordisk Pharma Sp. z o.o, Warsaw, Poland

G. Liidemann
Novo Nordisk A/S Eesti Filiaal, Tallinn, Estonia

V. Volke
Department of Physiology, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia

V. Volke
Tartu University Hospital, Tartu, Estonia

Conclusion: Once-weekly semaglutide was considered highly cost-effective versus liraglutide 1.2 mg for the treatment of patients with type 2 diabetes in Estonia.
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Keywords: Cost; Cost-effectiveness; Diabetes mellitus; Estonia; GLP-1 analogue; GLP-1 receptor agonist; Liraglutide; Semaglutide

PLAIN LANGUAGE SUMMARY

- Multifactorial treatments that target both reductions in blood sugar levels [measured via glycated hemoglobin (HbA1c)] and body weight are becoming increasingly important for the treatment of type 2 diabetes, with studies demonstrating that short-term improvements in these outcomes are associated with a reduction in the risk of long-term diabetes-related complications.
- In a recent network meta-analysis (NMA), once-weekly semaglutide was associated with greater efficacy versus once-daily liraglutide, with greater improvements in HbA1c and body weight in adult type 2 diabetes patients with inadequate glycemic control on multiple oral antidiabetic medications (OADs).
- With the prevalence and costs associated with diabetes spiraling, and healthcare payer budgets coming under increasing pressure, choosing cost-effective treatments is becoming increasingly important.
- The present analysis assessed the long-term cost-effectiveness of once-weekly semaglutide 1 mg versus liraglutide 1.2 mg for the treatment of adult type 2 diabetes patients with inadequate glycemic control on OADs from a healthcare payer perspective in Estonia.
- Once-weekly semaglutide 1 mg was associated with improved life expectancy and quality-adjusted life expectancy versus liraglutide 1.2 mg over patient lifetimes. Total costs were marginally higher with once-weekly semaglutide 1 mg, with the increased acquisition cost mostly offset by cost savings due to avoidance of diabetes-related complications.
- Once-weekly semaglutide 1 mg therefore offers a highly cost-effective alternative to liraglutide 1.2 mg for the treatment of adult type 2 diabetes patients with inadequate glycemic control on OADs in Estonia.

INTRODUCTION

Diabetes is associated with a significant clinical and economic burden in Estonia, with between 7% and 9% of the adult population affected, more than 2000 people per year hospitalized with the condition, and diabetes-related healthcare expenditure totaling USD 87 million in 2017 [1–5]. Improved glycemic control, measured via glycated hemoglobin (HbA1c), remains the key treatment target for patients with type 2 diabetes, with lowered HbA1c associated with a reduced incidence of long-term diabetes-related complications in landmark studies [6–10]. However, short-term improvements in systolic blood pressure (SBP) and body weight have also been shown to substantially reduce the risk of long-term complications [11–14]. Additionally, patients express a preference for treatments that do not increase body weight and require fewer injections [15, 16]. Therefore, treatments that target a variety of factors are becoming increasingly popular.

In Estonia, a high proportion of patients with type 2 diabetes fail to achieve glycemic control targets, with 50% found to have an HbA1c level greater than 7.0% and 61% not achieving an HbA1c value below 6.5% in 2009 [17, 18]. Similarly, patients with type 2 diabetes often struggle to maintain a normal weight, with only 6% of patients below a body mass index (BMI) of 25 kg/m^2 and more than 90% classified with BMIs greater than 27 kg/m^2 [17]. Additionally, only 37% of patients have an SBP of less than 140 mmHg [17]. Glycemic control and reductions in weight and SBP are particularly important for reducing the risk of cardiovascular complications, which is substantially higher in patients with type 2 diabetes

compared with the general population. The risk of death from cardiovascular complications is approximately two to three times higher in patients with type 2 diabetes versus people with no history of the disease, while cardiovascular disease is responsible for 52% of deaths in patients with type 2 diabetes [19, 20]. A 1% reduction in mean HbA1c has been associated with a 16% risk reduction for heart failure, a 4% risk reduction for myocardial infarction, and a 12% risk reduction for stroke, while modest weight losses of between 5% and 10% have been linked with significant improvements in cardiovascular disease risk factors [21, 22]. This exemplifies the need for treatments that target improvements in multiple clinical outcomes, not solely glycemic control.

Glucagon-like peptide-1 (GLP-1) receptor agonists are a class of diabetes treatments that have been associated with improved glycemic control and weight loss versus a variety of comparators [23–26]. In Estonia, the costs of GLP-1 receptor agonists are reimbursed for patients with type 2 diabetes with a BMI $\geq$ 35 kg/m^2, with once-daily injectable liraglutide 1.2 mg currently the most frequently used GLP-1 analogue [4].

Once-weekly semaglutide is a novel GLP-1 analogue that is approved for use in the European Union. Its safety and efficacy have been assessed versus a variety of comparators, and at different stages of the type 2 diabetes treatment algorithm, throughout the SUSTAIN clinical trial program [27, 29]. However, no head-to-head comparison data of once-weekly semaglutide versus liraglutide are available, with the recently completed SUSTAIN 10 trial yet to be published [30]. To fill this data gap, a network meta-analysis (NMA) conducted in adult patients with inadequate glycemic control on one or two oral antidiabetic medications (OADs) has been published [31]. The NMA was based on a systematic literature review and assessed the changes from baseline in HbA1c, SBP, and body weight in patients with inadequate glycemic control on one or two OADs, based on a Bayesian framework [31]. A total of 26, 15, and 25 studies were included in the HbA1c, SBP, and body weight networks, respectively. These showed that once-weekly semaglutide 1 mg was associated with statistically significant reductions in HbA1c and body weight and statistically nonsignificant reductions in SBP versus liraglutide 1.2 mg [31].

Healthcare in Estonia is almost wholly provided by a national health insurance service, known as the Estonian Health Insurance Fund, which is funded through taxation of the population and businesses. Approximately 95% of patients are covered through this mandatory insurance, which intends to cover at least 75% of the total healthcare expenditure for patients [32]. The aim of the present study was to assess the long-term cost-effectiveness of once-weekly semaglutide 1 mg versus liraglutide 1.2 mg for the treatment of adult patients with type 2 diabetes with inadequate glycemic control on OADs, based on data from the NMA, from an Estonian Health Insurance Fund perspective.

METHODS

Model Overview

The evaluation of cost-effectiveness was performed using the IQVIA CORE Diabetes Model (version 9.0), an internet-based, interactive computer model developed to project long-term health outcomes and economic consequences of implementing interventions for the treatment of type 1 and type 2 diabetes [33, 34]. Long-term outcomes projected by the model have been validated against real-life data, both at the time of initial publication in 2004 and in a more recent 2014 study [34, 35]. Outputs from the model include life expectancy (measured in life years), quality-adjusted life expectancy [measured in quality-adjusted life years (QALYs)], cumulative incidence and time to onset of diabetes-related complications, direct medical costs, and cost-effectiveness scatterplots and acceptability curves. Diabetes-related complications include cardiovascular events (angina, stroke, myocardial infarction, congestive heart failure, and peripheral vascular disease), renal complications (microalbuminuria, gross proteinuria, and end-stage renal disease), retinopathy diseases (macular edema, cataract, severe vision loss, and background and

proliferative retinopathy), and hypoglycemic events (severe and nonsevere), as well as ulcers, amputations, and neuropathy. Where an intervention is associated with clinical benefits and a cost increase, cost-effectiveness is assessed in the form of an incremental cost-effectiveness ratio (ICER), calculated as the incremental cost per unit of effect gained by using the novel intervention instead of the comparator.

Analyses were performed over a 50-year time horizon to capture all relevant long-term complications and associated costs and to assess their impact on life expectancy and quality of life, as recommended in guidelines for the assessment of cost-effectiveness of diabetes interventions [36]. In all base-case and sensitivity analyses, mortality was considered as a result of diabetes-related complications, with background mortality based on Estonia-specific life tables [37]. The UKPDS 68 risk equations were applied to predict the risk of cardiovascular complications [38]. A first-order Monte Carlo approach, capturing 1000 identical patients who are run through the model 1000 times, was used for base-case and sensitivity analyses, while a second-order Monte Carlo approach, with sampling applied to patient cohort characteristics, treatment effects, costs, utilities, and probabilities of events, was used for probabilistic sensitivity analysis (PSA). Clinical and cost outcomes were discounted at 5.0% per annum, in line with the guidelines for the assessment of medicinal products in the Baltic states [39].

Clinical Data

Baseline cohort characteristics were based on the subgroup of patients with a BMI $\geq$ 35 kg/m^2 in the SUSTAIN 3 clinical trial, with data extracted in a post hoc analysis (Table 1). This trial was chosen as it was used to inform the once-weekly semaglutide 1 mg arm of the NMA [31]. The proportion of patients using tobacco products (18.1%) was based on the trial data, but the number of cigarettes smoked per day was assumed to be the same as the general population in Estonia [4]. Similarly, mean weekly alcohol consumption was taken from Estonia-specific data for the general population [4].

Table 1 Baseline cohort characteristics of patients with a BMI $\geq$ 35 kg/m^2 in SUSTAIN 3

Characteristic	Mean (standard deviation)
Age at onset (years)	53.94 (10.52)
Duration of diabetes (years)	7.83 (5.19)[a]
Percentage male (%)	43.21
HbA1c (%)	8.37 (0.98)
Systolic blood pressure (mmHg)	134.63 (14.37)
Diastolic blood pressure (mmHg)	81.06 (8.42)
Total cholesterol (mg/dL)	188.63 (42.18)
HDL cholesterol (mg/dL)	47.10 (12.13)
LDL cholesterol (mg/dL)	105.08 (36.66)
Triglycerides (mg/dL)	196.23 (135.50)
BMI (kg/m^2)	41.04 (5.35)
Percentage smokers (%)	18.12
Cigarettes per day	13.00[b]
Alcohol consumption (oz/week)	4.66[b]

All data were taken from SUSTAIN 3, unless otherwise indicated

BMI body mass index, *HbA1c* glycated hemoglobin, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein

[a] Rounded to 8.00 in the analysis, as the model only accepts integer values for the duration of diabetes

[b] Based on a 2017 health technology assessment of GLP-1 receptor agonists [4]

Physiological parameter treatment effects applied in the first year of the analysis with once-weekly semaglutide 1 mg and liraglutide 1.2 mg were based on data from the NMA (Table 2). A random-effects model was used to assess changes in HbA1c, and fixed-effects models were used to assess changes in systolic blood pressure and body weight. These showed that once-weekly semaglutide 1 mg was associated with statistically significant reductions in

Table 2 Treatment effects included in the analysis

Parameter	Mean (standard error)	
	Once-weekly semaglutide 1 mg	Liraglutide 1.2 mg
HbA1c (%)	− 1.47 (0.12)*	− 0.87 (0.12)
Systolic blood pressure (mmHg)	− 6.28 (1.52)	− 4.45 (1.39)
BMI (kg/m^2)	− 1.35 (0.10)*	− 0.64 (0.10)

BMI body mass index, *HbA1c* glycated hemoglobin
*Statistically significant difference at 95% confidence level versus liraglutide 1.2 mg

HbA1c [− 1.5%, (95% confidence interval − 1.7 to − 1.2) versus − 0.9% (− 1.1 to − 0.6)] and body weight [− 3.8 kg (− 4.4 to − 3.3) versus − 1.8 kg (− 2.4 to − 1.2)] and statistically nonsignificant reductions in SBP [− 6.3 mmHg (− 9.3 to − 3.3) versus − 4.5 mmHg (− 7.2 to − 1.7)] versus liraglutide 1.2 mg [31]. Due to limitations in the published data, the NMA was based on all patients with diabetes receiving the study medications, so the present analysis assumes that the treatment effects are equivalent in patients with a BMI $\geq$ 35 kg/m^2. The outcomes included in the NMA that were applicable to an analysis using the IQVIA CORE Diabetes Model, encompassing changes from baseline in HbA1c, SBP, and body weight (converted to BMI) versus placebo, were applied in both treatment arms, with both statistically significant and nonstatistically significant differences included in line with modeling guidelines [40]. Where parameters were not included in the NMA, inputs were assumed to be 0 in both arms to ensure that these did not drive cost-effectiveness outcomes.

Treatment Duration, Switching, and Long-Term Parameter Progression

Patients were assumed to receive once-weekly semaglutide or liraglutide for the first 5 years of the analysis, in line with a 2017 health technology assessment of GLP-1 receptor agonists in Estonia [4]. After 5 years, treatment with once-weekly semaglutide or liraglutide was discontinued and patients were assumed to intensify to basal insulin therapy with insulin glargine U100 (Lantus®). This assumption recognizes that intensification from GLP-1 receptor agonists to basal insulin therapy will be required for patients to maintain glycemic control over the long term, due to the progressive nature of type 2 diabetes. Benefits in HbA1c and BMI associated with once-weekly semaglutide or liraglutide treatment were assumed to persist for the 5 years that patients received these treatments. On intensification to basal insulin therapy, HbA1c was brought to 7.0% in both treatment arms (based on guidelines released by the European Association for the Study of Diabetes) and BMI reverted to baseline for the remainder of the analysis [33, 41]. SBP was assumed to follow the UKPDS progression equation for the duration of the analysis. This resulted in a balanced cost-effectiveness analysis, with differences in HbA1c and BMI only maintained while there were differences in costs. Alternative treatment switching and parameter progression assumptions were explored in sensitivity analyses.

Costs, Resource Use, and Utilities

Costs were estimated from an Estonian healthcare payer perspective, specifically the Estonian National Health Insurance Fund, and expressed in euros (EUR). Unit costs of diabetes medications and consumables were based on retail prices, with calculations reflecting the acquisition cost reimbursed by the Estonian Health Insurance Fund [for needles, self-monitoring of blood glucose (SMBG) test strips, and SMBG lancets], and the maximum reimbursement quantities depending on the type of therapy patients received (for SMBG test strips and SMBG lancets).

Diabetes medication resource use was based on the trials from which the data were taken for the NMA in each arm of the analysis. Concomitant medication use (including metformin,

Table 3 Annual pharmacy costs in the base-case analysis

Item	Once-weekly semaglutide 1 mg	Liraglutide 1.2 mg	Basal insulin (intensification)
Annual medication costs	1367.86	1156.50	493.14
Annual metformin costs	50.40	50.40	50.40
Annual glimepiride costs	18.55	18.55	18.55
Annual pioglitazone costs	13.29	13.29	13.29
Annual needle costs	0.00	45.86	45.86
Annual SMBG testing costs	25.04	25.04	137.24
Total annual costs	1475.15	1309.65	758.48

All costs are expressed in euros (EUR)
SMBG self-monitoring of blood glucose

sulfonylurea, and thiazolidinedione) was based on the semaglutide 1 mg arm of the SUSTAIN 3 trial, and was assumed to be equal in both treatment arms. It was assumed that each patient received the defined daily dose (DDD) of each concomitant medication, with sulfonylurea treatment assumed to be glimepiride and thiazolidinedione treatment assumed to be pioglitazone. Patients receiving sulfonylurea were assumed to use three SMBG tests per week, but no SMBG use was directly associated with once-weekly semaglutide or liraglutide. Liraglutide required one needle per day for administration, but no needles were required in the once-weekly semaglutide arm, as these are included in the pack. Following intensification after 5 years, patients were assumed to receive the DDD (40 IU) of insulin glargine U100 (Lantus), with concomitant medication use equal in both treatment arms. Patients were assumed to use one needle and one SMBG test per day. Resource use was used to calculate annual treatment costs (Table 3).

The costs of diabetes-related complications in the year of the event and the annual follow-up costs were taken from a 2017 health technology assessment of GLP-1 receptor agonists, with the exception of the cost of severe hypoglycemia, which was taken from the insulin degludec assessment by the Estonian National Health Insurance Fund [4, 42].

Quality-of-life utilities associated with diabetes and diabetes-related complications were sourced from a 2014 review by Beaudet et al., while disutilities relating to hypoglycemia were taken from a 2013 publication by Evans et al. (published after the literature searches by Beaudet et al. had been conducted) [43, 44].

Sensitivity Analyses

As the long-term extrapolation of clinical and cost outcomes from short-term data is associated with uncertainty, sensitivity analyses were performed on key parameters to assess the robustness of the base-case findings. Analyses were performed with only statistically significant differences in treatment effects applied. The influence of the time horizon on projected outcomes was investigated via simulations with substantially shorter time horizons of 10 and 20 years applied, for which it should be noted that not all complications and costs were captured, as a 50-year time horizon was required for all modeled patients to have died. The effect of discounting on cost-effectiveness outcomes was assessed by applying discount rates of 0% and 10% in separate analyses. Simulations were prepared with only the HbA1c treatment difference between the treatment arms applied, to evaluate the impact of only this treatment effect on clinical and cost outcomes (i.e., changes in systolic blood pressure and BMI were the same in both arms).

Alternative parameter progressions were explored, with BMI differences between the

treatments maintained for patient lifetimes, and HbA1c in both treatment arms following the UKPDS progression equation from the start of the analysis. To assess variations in the treatment effects, the upper and lower 95% confidence interval limits of the estimated treatment differences of HbA1c and BMI were applied in four separate analyses. Alternative treatment switching patterns were explored by bringing treatment switching forward to the end of year 3 in both arms, and having it occur when HbA1c reached 7.5% following the application of the UKPDS progression equation from the first year of the analysis. The effect of overestimating or underestimating the costs of diabetes-related complications was assessed by increasing and decreasing these costs by 10%.

In 2014, an update to the IQVIA CORE Diabetes Model was released, incorporating data from the UKPDS 82 for several risk equations, and an analysis using this version of the model was performed. Although this version of the model has been validated, the model proprietors suggest that the update is used in a sensitivity analysis, with the previous version used for base-case analyses [35]. Further analyses tested the effect of using a larger BMI disutility, giving a greater impact to weight changes in the analysis, and alternative hypoglycemia disutilities, giving greater impact to nonsevere hypoglycemic events but smaller impact to severe events [45, 46]. Additionally, an analysis was performed with a diminishing hypoglycemia disutility model applied [47].

PSA was performed using the predefined function in the IQVIA CORE Diabetes Model to capture statistical uncertainty, with sampling applied to parameter inputs such as baseline characteristics, treatment effects, event risks, costs and utilities. These parameters were sampled from distributions, with the simulation run using a second-order Monte Carlo approach; 1000 unique cohorts, each containing 1000 patients, were run through the model to produce 1000 data points. The proportion of these points that fell under the willingness-to-pay threshold of EUR 52,390 per QALY gained was calculated, in addition to the mean outcomes.

Compliance with Ethics Guidelines

This article does not contain any studies with human participants or animals performed by any of the authors.

RESULTS

Base-Case Analysis

Long-term projections in patients with inadequate glycemic control on OADs indicated that once-weekly semaglutide 1 mg was associated with improvements in discounted life expectancy and discounted quality-adjusted life expectancy of 0.12 years and 0.13 QALYs, respectively, versus liraglutide 1.2 mg (Table 4). Improved clinical outcomes were a result of

Table 4 Long-term cost-effectiveness outcomes in the base-case analysis

Health outcomes	Once-weekly semaglutide 1 mg	Liraglutide 1.2 mg	Difference
Discounted life expectancy (years)	12.41 (0.13)	12.29 (0.13)	+ 0.12
Discounted quality-adjusted life expectancy (QALYs)	7.77 (0.08)	7.64 (0.08)	+ 0.13
Discounted direct costs (EUR)	25,183 (795)	25,116 (881)	+ 67
ICER based on life expectancy and direct costs	EUR 561 per life year gained		
ICER based on quality-adjusted life expectancy and direct costs	EUR 523 per QALY gained		

Values are means (standard deviations)
EUR euros, *ICER* incremental cost-effectiveness ratio, *QALYs* quality-adjusted life years

reduced cumulative incidence and delayed time to onset of diabetes-related complications with once-weekly semaglutide. Mean time to onset of any diabetes-related complication in the analysis was approximately 0.7 years longer with once-weekly semaglutide 1 mg compared with liraglutide 1.2 mg, with benefits observed across all micro- and macrovascular complications included in the analysis (Fig. 1).

Total direct costs were projected to be EUR 67 higher with once-weekly semaglutide 1 mg versus liraglutide 1.2 mg over patient lifetimes, driven by the higher acquisition costs over the first 5 years of the analysis and the increased survival and further treatment of patients in the long term (Fig. 2). Higher acquisition costs were mostly offset by cost savings due to the avoidance of diabetes-related complications with once-weekly semaglutide, most notably those relating to ulcers, amputation, and neuropathy (mean cost savings of EUR 449 per patient).

With improved clinical outcomes at an increased cost from a healthcare payer perspective, once-weekly semaglutide 1 mg was associated with an ICER of EUR 523 per QALY gained versus liraglutide 1.2 mg. This falls well below the suggested willingness-to-pay threshold of EUR 52,390 per QALY gained in Estonia (based on three times the Estonian GDP per capita [EUR 17,463], as recommended by the World

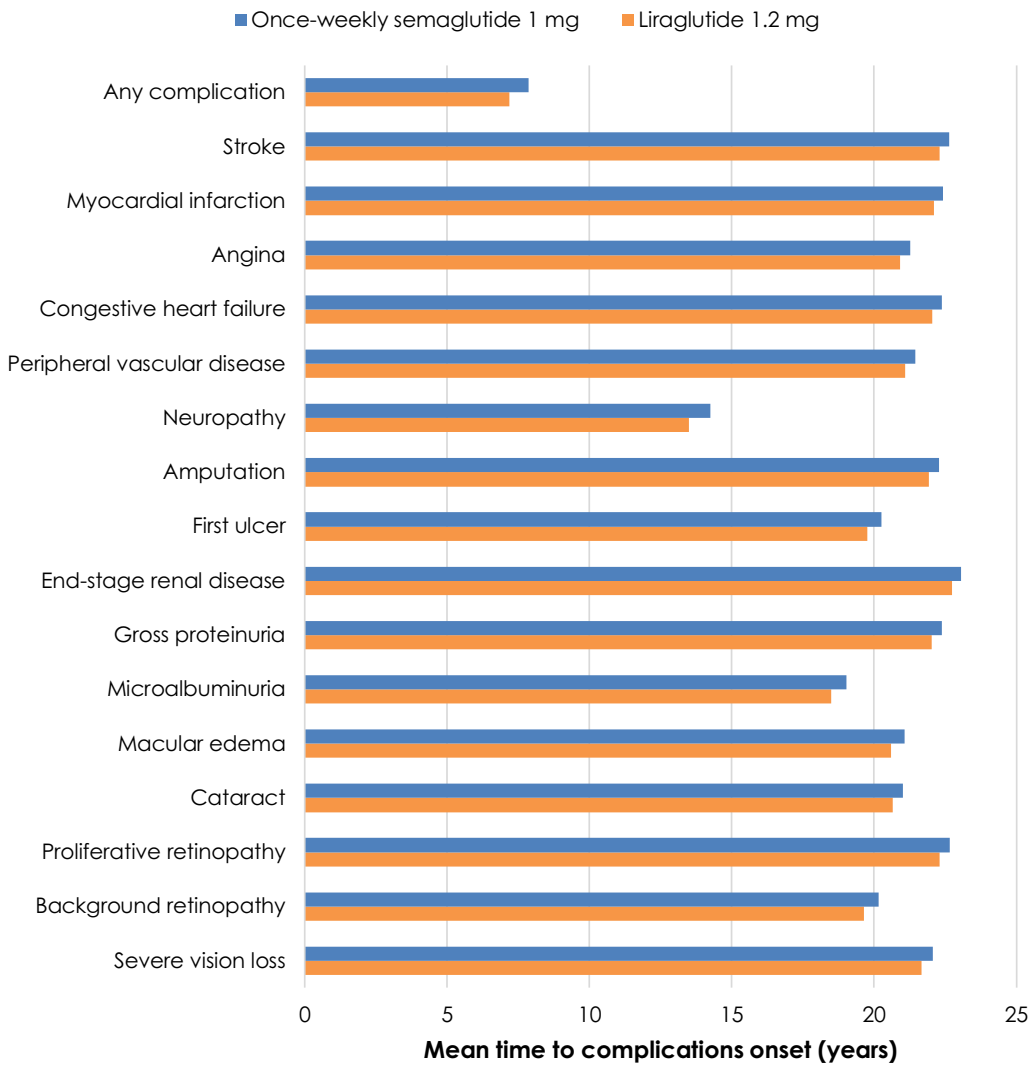
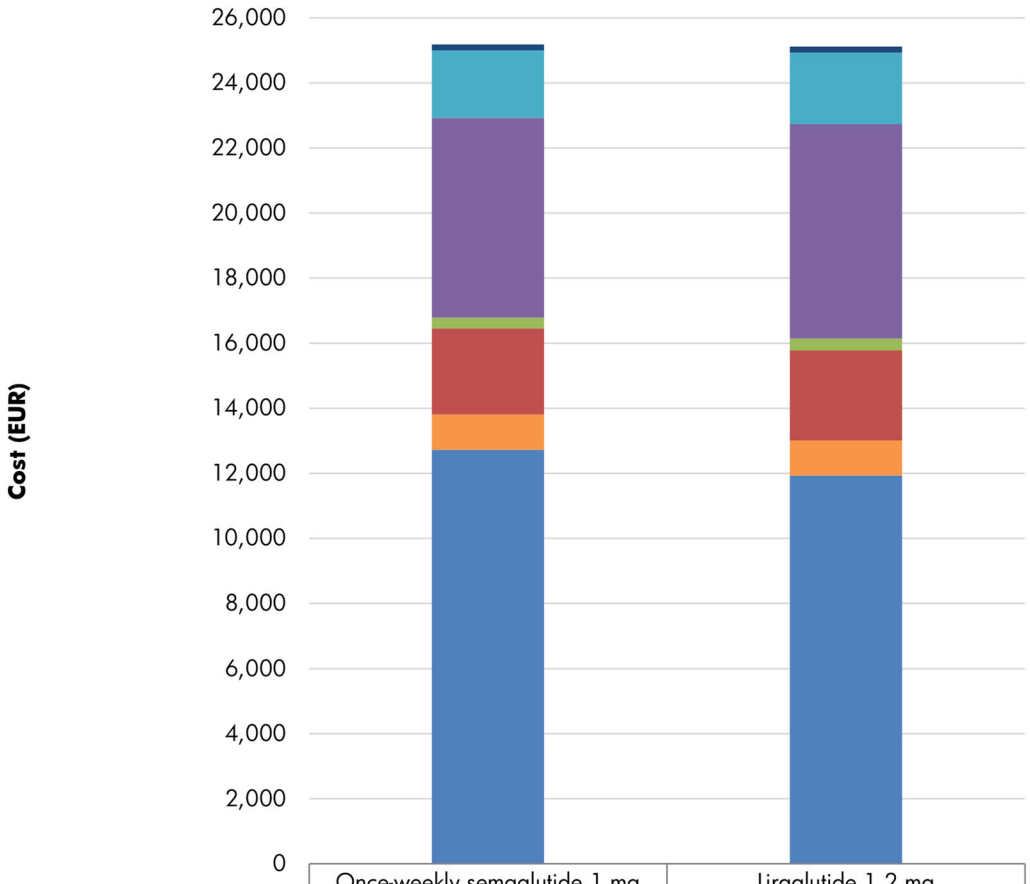


Fig. 1 Mean time to onset of diabetes-related complications



	Once-weekly semaglutide 1 mg	Liraglutide 1.2 mg
Total costs	25,183	25,116
Severe hypoglycemia	177	175
Non-severe hypoglycemia	0	0
Eye complications	2,084	2,206
Ulcer, amputation and neuropathy	6,139	6,588
Renal complications	325	375
Cardiovascular disease	2,652	2,764
Management	1,086	1,077
Treatment	12,720	11,931

Fig. 2 Direct costs over patient lifetimes. *EUR* euros

Health Organization), and once-weekly semaglutide 1 mg was therefore considered highly cost-effective versus liraglutide 1.2 mg [48, 49].

One-Way and Multi-Way Sensitivity Analyses

Sensitivity analyses showed that the base-case findings were robust to changes in the input parameters and assumptions used, with once-weekly semaglutide 1 mg remaining well below the suggested willingness-to-pay threshold of EUR 52,390 per QALY gained across all scenarios (Table 5). Including only the statistically significant differences between the treatment arms, specifically HbA1c and BMI, resulted in slightly decreased clinical benefits but also reduced incremental costs with once-weekly

Table 5 Sensitivity analysis results

Analysis	Discounted quality-adjusted life expectancy (QALYs)			Discounted direct costs (EUR)			ICER (EUR per QALY gained)
	Once-weekly semaglutide 1 mg	Liraglutide 1.2 mg	Difference	Once-weekly semaglutide 1 mg	Liraglutide 1.2 mg	Difference	
Base-case	7.77	7.64	+ 0.13	25,183	25,116	+ 67	523
Statistically significant differences only	7.76	7.64	+ 0.12	25,139	25,116	+ 23	195
20-year time horizon	6.79	6.69	+ 0.09	19,329	19,186	+ 143	1561
10-year time horizon	4.70	4.64	+ 0.06	12,356	11,934	+ 423	7354
0% discount rates	14.08	13.80	+ 0.28	54,245	54,661	− 416	Once-weekly semaglutide dominant
10% discount rates	5.11	5.04	+ 0.07	15,060	14,812	+ 248	3380
HbA1c difference only	7.73	7.64	+ 0.09	25,148	25,116	+ 32	356
BMI difference maintained for patient lifetimes	7.81	7.64	+ 0.17	25,205	25,116	+ 89	535
UKPDS HbA1c creep for duration of the analysis (no change upon treatment intensification)	7.31	7.21	+ 0.11	29,765	29,548	+ 217	2077
Upper 95% CI of HbA1c estimated treatment difference	7.80	7.64	+ 0.16	24,936	25,116	− 180	Once-weekly semaglutide dominant

Table 5 continued

Analysis	Discounted quality-adjusted life expectancy (QALYs)			Discounted direct costs (EUR)			ICER (EUR per QALY gained)
	Once-weekly semaglutide 1 mg	Liraglutide 1.2 mg	Difference	Once-weekly semaglutide 1 mg	Liraglutide 1.2 mg	Difference	
Lower 95% CI of HbA1c estimated treatment difference	7.74	7.64	+ 0.09	25,553	25,116	+ 437	4769
Upper 95% CI of BMI estimated treatment difference	7.78	7.64	+ 0.13	25,176	25,116	+ 60	453
Lower 95% CI of BMI estimated treatment difference	7.76	7.64	+ 0.12	25,233	25,116	+ 117	1019
Treatment switching at 3 years	7.72	7.62	+ 0.10	24,260	24,119	+ 141	1398
Treatment switching at 7.5% HbA1c threshold (using UKPDS progression)	7.26	7.14	+ 0.12	28,725	28,295	+ 430	3542
Cost of complications + 10%	7.77	7.64	+ 0.13	26,322	26,328	− 6	Once-weekly semaglutide dominant
Cost of complications −10%	7.77	7.64	+ 0.13	24,140	24,004	+ 136	1064
UKPDS 82 risk equations applied	7.93	7.87	+ 0.06	21,204	20,813	+ 391	6568
Lee et al.'s BMI disutility applied	7.00	6.86	+ 0.13	25,183	25,116	+ 67	504

Table 5 continued

Analysis	Discounted quality-adjusted life expectancy (QALYs)			Discounted direct costs (EUR)			ICER (EUR per QALY gained)
	Once-weekly semaglutide 1 mg	Liraglutide 1.2 mg	Difference	Once-weekly semaglutide 1 mg	Liraglutide 1.2 mg	Difference	
Diminishing hypoglycemia disutility applied	7.73	7.60	+ 0.13	25,183	25,116	+ 67	525
Currie et al.'s hypoglycemia disutility applied	7.83	7.70	+ 0.13	25,183	25,116	+ 67	520

BMI body mass index, *CI* confidence interval, *EUR* euros, *HbA1c* glycated hemoglobin, *ICER* incremental cost-effectiveness ratio, *QALY* quality-adjusted life year

semaglutide 1 mg, leading to an ICER of EUR 195 per QALY gained versus liraglutide 1.2 mg. Shortening the time horizon to 10 and 20 years (compared with the 50 years used in the base-case analysis) resulted in reduced clinical benefits and increased incremental costs, yielding ICERs of EUR 7354 and EUR 1561 per QALY gained, respectively, for once-weekly semaglutide 1 mg versus liraglutide 1.2 mg. These outcomes exemplify the fact that once-weekly semaglutide improves long-term outcomes, and that these benefits are not fully captured over shorter time horizons. Altering the discount rate also reflected these long-term benefits, with once-weekly semaglutide associated with greatly increased clinical benefits and cost savings when discount rates of 0% were applied, meaning it was considered dominant versus liraglutide 1.2 mg. Conversely, clinical benefits decreased and incremental costs increased when discount rates of 10% were applied, leading to an ICER of EUR 3380 per QALY gained for once-weekly semaglutide.

Applying only the difference in HbA1c versus liraglutide 1.2 mg in the once-weekly semaglutide 1 mg arm showed that greater reductions with once-weekly semaglutide were a substantial contributor to improved clinical outcomes versus liraglutide 1.2 mg, with only slightly reduced clinical benefits and incremental costs, resulting in an ICER of EUR 356 per QALY gained. Maintaining the BMI difference between the treatment arms after intensification increased the clinical benefit and incremental costs associated with once-weekly semaglutide, yielding an ICER of EUR 535 per QALY gained. Application of the UKPDS HbA1c progression equation resulted in reduced quality-adjusted life expectancy in both treatment arms, with increased incremental costs with once-weekly semaglutide compared with liraglutide 1.2 mg, leading to an ICER of EUR 2077 per QALY gained.

Use of the upper limit of the 95% confidence interval of the estimated treatment differences in HbA1c resulted in increased clinical benefits and cost savings with once-weekly semaglutide, meaning it was considered dominant versus liraglutide 1.2 mg. Application of the lower limit of the 95% confidence interval had the converse effect, with clinical benefits reduced and incremental costs increased. Use of the upper limit of the 95% confidence interval of the estimated treatment differences in BMI resulted in maintained clinical benefits from the base-case analysis and comparable

incremental costs, while application of the lower limit of the 95% confidence interval led to slightly reduced clinical benefits and increased incremental costs.

Treatment switching at 3 years, rather than the 5 years as in the base-case, resulted in smaller clinical benefits and increased incremental costs with once-weekly semaglutide, yielding an ICER of EUR 1398 per QALY gained versus liraglutide 1.2 mg. Application of the UKPDS HbA1c progression with treatment switching when HbA1c exceeded 7.5% led to slightly reduced clinical benefits and increased incremental costs for once-weekly semaglutide 1 mg versus liraglutide 1.2 mg.

Increasing the costs of treating diabetes-related complications resulted in small cost savings with once-weekly semaglutide, meaning it was considered dominant versus liraglutide 1.2 mg. Reducing the costs of complications had the converse effect, with incremental costs increased.

Using the UKPDS 82 risk equations to predict cardiovascular events resulted in smaller clinical benefits with once-weekly semaglutide 1 mg compared with the base-case analysis, with incremental costs increased. Application of alternative utilities relating to hypoglycemia and BMI resulted in only minor changes to clinical outcomes, and ICERs remained similar to the base-case analysis.

Probabilistic Sensitivity Analysis

PSA, performed to capture statistical uncertainty, showed similar mean results to the base-case but increased measures of variance around the mean outcomes. Once-weekly semaglutide 1 mg was associated with a mean incremental improvement in quality-adjusted life expectancy of 0.08 QALYs and higher mean costs of EUR 168 per patient versus liraglutide 1.2 mg. Therefore, once-weekly semaglutide 1 mg was associated with an ICER of EUR 2103 per QALY gained versus liraglutide 1.2 mg in the PSA. Based on the suggested willingness-to-pay threshold of EUR 52,390 per QALY gained, the modeling analysis indicated that the probability of once-weekly semaglutide 1 mg being cost-effective versus liraglutide 1.2 mg was 73.4% (Fig. 3).

DISCUSSION

The present analysis found once-weekly semaglutide 1 mg to be a highly cost-effective treatment option versus liraglutide 1.2 mg for the treatment of patients with type 2 diabetes with a BMI $\geq$ 35 kg/m^2 in Estonia. Life expectancy and quality-adjusted life expectancy were both improved with once-weekly semaglutide at a small cost increase over patient lifetimes from a healthcare payer perspective. Greater reductions in short-term clinical outcomes of HbA1c, body weight, and SBP resulted in a reduced cumulative incidence and delayed time to onset of long-term diabetes-related complications, leading to cost savings that mostly offset the higher acquisition costs associated with once-weekly semaglutide.

The positive impact of improvements in HbA1c and body weight on the risk of cardiovascular disease has been well documented [21, 22, 50]. Moreover, once-weekly semaglutide has been associated with additional cardiovascular benefits in the SUSTAIN 6 clinical trial, reducing the risk of a major cardiovascular event (a composite endpoint of cardiovascular death, nonfatal myocardial infarction, and nonfatal stroke) compared with placebo plus standard of care [51]. Liraglutide has also been associated with a reduced risk of cardiovascular disease versus placebo in the LEADER trial [52]. The present analysis did not capture the impacts on cardiovascular disease events identified in SUSTAIN 6 and LEADER, as risk equations based on these studies have not been incorporated into health economic models of diabetes.

Treatment guidelines for type 2 diabetes in Estonia recommend the introduction of a GLP-1 receptor agonist as either a second-line therapy in patients receiving oral monotherapy with an HbA1c $\geq$ 8.5%, or a fourth-line therapy in patients receiving oral triple therapy of metformin plus either sulfonylurea or thiazolidinedione and a dipeptidyl peptidase-4 (DPP-4) inhibitor or a sodium-glucose co-transporter

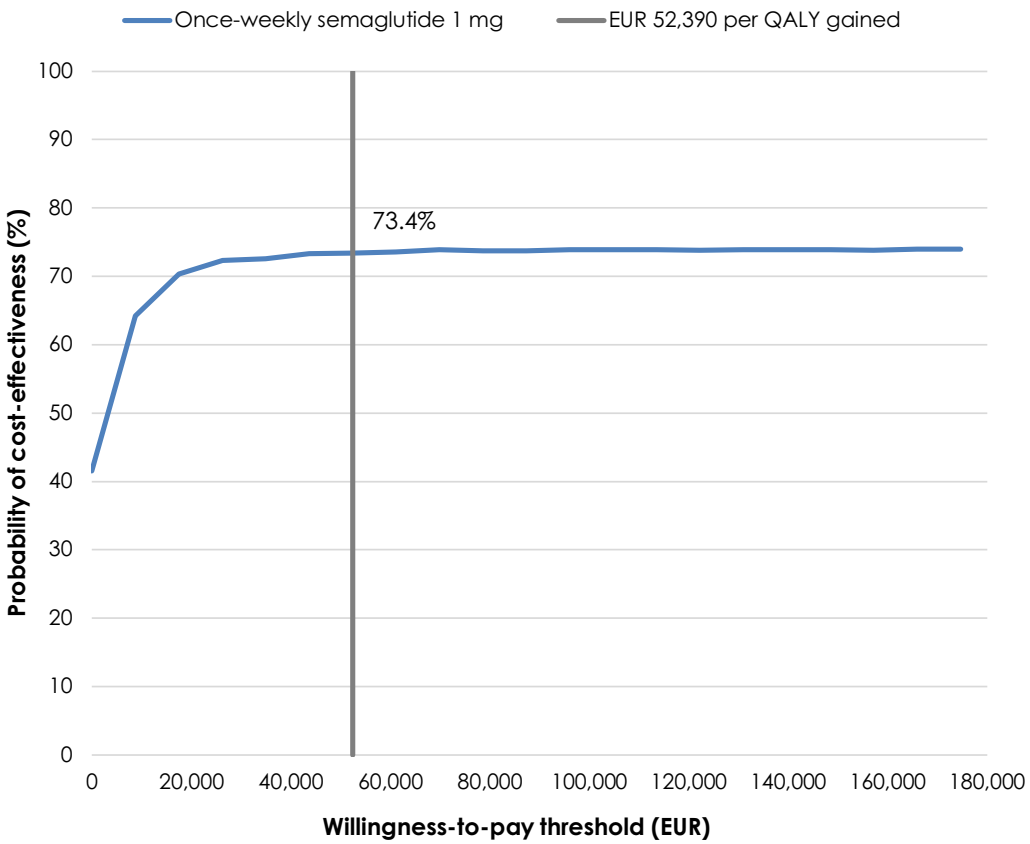


Fig. 3 Cost-effectiveness acceptability curve from the probabilistic sensitivity analysis. *EUR* euros, *QALY* quality-adjusted life year

2 (SGLT-2) inhibitor with an HbA1c > 7.0% [53]. The present analysis assessed the cost-effectiveness of once-weekly semaglutide in patients with inadequate glycemic control on one or two OADs, and found that once-weekly semaglutide 1 mg was a highly cost-effective option versus liraglutide 1.2 mg for those patients. Once-weekly semaglutide has also been shown to improve short-term outcomes versus both the DPP-4 inhibitor sitagliptin and the SGLT-2 inhibitor empagliflozin in SUSTAIN 2 and an NMA, respectively, and it could therefore be argued that once-weekly semaglutide is eligible to be used earlier in the treatment algorithm [54, 55]. Indeed, recent 2018 guidelines from the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) recommend GLP-1 receptor agonist therapy as the first-line injectable medication for treating type 2 diabetes [56]. Moreover, since once-weekly semaglutide requires fewer injections than once-daily liraglutide, and since patient preference is for simpler treatment regimens with fewer injections, treatment with once-weekly semaglutide could potentially improve patient adherence and alter preferences towards injectable GLP-1 receptor agonist therapy, given the benefits in HbA1c and body weight these treatments offer [15, 16, 23–26, 54, 55].

A limitation of the study was the reliance on relatively short-term clinical trial data to make long-term projections. However, this is common to a number of health economic analyses and, in the absence of long-term clinical trial data, extrapolation of short-term data remains one of the best available options to model chronic diseases. Indeed, projecting outcomes over patient lifetimes is recommended in the guidance for cost-effectiveness studies for patients with type 2 diabetes [36]. Additionally, the present analysis was conducted using a

published and extensively validated model, with numerous sensitivity analyses displaying the robustness of the base-case results [34, 35].

The use of data from an NMA, rather than a head-to-head clinical trial, could also be considered a potential shortcoming of the analysis. However, selection of the most appropriate comparator (in this case the most widely used GLP-1 receptor agonist in Estonia) was the first priority, and the use of evidence synthesis, using recommended methodologies, is becoming increasingly important and accepted for health technology assessment globally [57, 58].

A further limitation is that the NMA relied on published data that only reported outcomes for all patients, as the study publications identified by the reviewers did not report data for patients with BMI $\geq$ 35 kg/m^2 [31]. The present analysis applied the treatment effects for all patients in patients with BMI $\geq$ 35 kg/m^2, and this assumption of equivalent efficacy across these two populations represents a potential weakness. However, subgroup analyses have shown that once-weekly semaglutide is consistently efficacious across patient subgroups, with reductions in HbA1c and body weight observed in patients with higher BMIs similar to those seen in the full populations throughout the SUSTAIN clinical trials [59–61]. Therefore, while quantifying the impact of applying treatment effects from all patients in patients with BMI $\geq$ 35 kg/m^2 is difficult, it is unlikely to change the conclusion that once-weekly semaglutide 1 mg is cost-effective versus liraglutide 1.2 mg.

CONCLUSIONS

Once-weekly semaglutide 1 mg represents a highly cost-effective treatment option versus liraglutide 1.2 mg for the treatment of type 2 diabetes patients with inadequate glycemic control on one or two OADs in Estonia.

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Compliance with Ethics Guidelines. This article does not contain any studies with human participants or animals performed by any of the authors.

Data Availability. The datasets obtained and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Original Article
CLINICAL TRIALS AND INVESTIGATIONS

Obesity

Semaglutide 2.4 mg for the Treatment of Obesity: Key Elements of the STEP Trials 1 to 5

Robert F. Kushner [1], Salvatore Calanna[2], Melanie Davies[3,4,5], Dror Dicker[6,7], W. Timothy Garvey [8,9], Bryan Goldman[2], Ildiko Lingvay[10,11], Mette Thomsen[2], Thomas A. Wadden[12], Sean Wharton [13], John P.H. Wilding[14], and Domenica Rubino[15]

Objective: The obesity epidemic is a public health concern, warranting further research into pharmacological treatments for weight management (WM) as an adjunct to lifestyle interventions. The Semaglutide Treatment Effect in People with obesity (STEP) program aims to investigate the effect of semaglutide versus placebo on weight loss, safety, and tolerability in adults with obesity or overweight.

Methods: Across five phase 3 trials (NCT03548935, WM; NCT03552757, WM in type 2 diabetes; NCT03611582, WM with intensive behavioral therapy; NCT03548987, sustained WM; and NCT03693430, long-term WM), ~5,000 participants are being randomly assigned to receive semaglutide 2.4 mg once weekly subcutaneously versus placebo. Results will be available in 2020/2021. For all trials, the primary end point is change from baseline to end of treatment in body weight.

Results: Participants have a mean age of 46.2 to 55.3 years, are mostly female (mean: 74.1%-81.0%), and have a mean BMI of 35.7 to 38.5 kg/m^2 and a mean waist circumference of 113.0 to 115.7 cm.

Conclusions: The STEP program evaluates the efficacy and safety of semaglutide 2.4 mg subcutaneously once weekly in a broad population. The trials will provide insights on WM in people with obesity with and without type 2 diabetes and on long-term follow-up.

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Study Importance

What is already known?

- Lifestyle intervention can often be insufficient in treating obesity; however, when combined with pharmacological treatments, clinically relevant weight loss and amelioration of obesity complications can be achieved.
- The GLP-1 receptor agonist liraglutide is approved for the treatment of people with obesity; a phase 2 trial with semaglutide, a GLP-1 analogue, suggested greater efficacy.

What does this study add?

- The Semaglutide Treatment Effect in People with obesity (STEP trials 1-5) clinical development program is one of the largest clinical trial programs for the management of obesity and assessed the efficacy and safety of semaglutide 2.4 mg subcutaneously once weekly.
- The STEP program is designed to elucidate key aspects of the medical management of obesity across various races and ethnicities, including whether semaglutide 2.4-mg dosing once weekly is reliably effective (STEP trials 1-5) for patients with and without diabetes, as an adjunct to intensive behavioral therapy plus low-calorie diet, and with longer term administration for weight loss maintenance.

How might these results change the focus of clinical practice?

- These pivotal trials will provide data on the efficacy and safety of a new treatment, semaglutide, which is anticipated to provide clinically meaningful and durable weight loss beyond what is currently achievable with the available agents for obesity.

Introduction

Burden of obesity

Obesity is a chronic, relapsing, progressive disease (1) with a multifactorial origin, including genetic, metabolic, behavioral, sociocultural, and environmental factors (2,3). The clinical complications of obesity include cardiovascular diseases (CVD; e.g., ischemic heart disease, heart failure), metabolic diseases (type 2 diabetes [T2D]), mechanical dysfunction (musculoskeletal disorders [e.g., osteoarthritis]), sleep apnea, and malignancy (4-7). Around 13% to 19.5% of adults globally have obesity, and the prevalence of obesity is predicted to continue to rise (5,8). There is a recognition that much of the pathophysiology of obesity involves abnormal satiety and feeding signaling within the brain (9). The hypothalamus, mesolimbic system, and executive functioning are all implicated in the physiology of obesity (9). Thus, there is a necessity for developing more effective novel treatment approaches that address these central nervous system processes (2,9,10).

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Treatment of obesity

Lifestyle interventions are the cornerstone of weight management (WM) (11), but alone they are generally associated with moderate weight loss (WL) that is gradually regained (9,12,13). Maintaining WL is inherently difficult because of counter-regulatory neuroendocrine pathways that promote weight regain by influencing hunger and satiety, which are a component of appetite, and potentially by decreasing energy expenditure (14,15). Antiobesity medications (AOMs) may provide a valuable adjunct to lifestyle interventions, which typically have a limited effect on WL, to help people achieve and maintain healthy behaviors that are consistent with sustaining WL.

The US Food and Drug Administration and European Medicines Agency have approved AOMs that have been shown to achieve clinically significant WL when used as adjuncts to lifestyle interventions (2,16). However, most approved AOMs have moderate efficacy, quantified as a $<10\%$ reduction in mean WL over that achieved with lifestyle intervention alone, with significant limitations related to adverse effects, cost, or restrictions on use (2). There is a need for additional AOMs that can induce and sustain greater clinically meaningful WL and that have a convenient form of administration that improves associated complications, such as T2D and CVD. One potential new AOM is the glucagon-like peptide 1 (GLP-1) analogue semaglutide, which has been developed with these characteristic features in mind (11,17).

Semaglutide pharmacology

Semaglutide is a long-acting GLP-1 analogue that mimics the effects of native GLP-1, which promotes WL by reducing energy intake, increasing satiety and satiation, and reducing hunger, as well as enhancing glycemic control (17). Many GLP-1s have been approved for the treatment of T2D, but only liraglutide 3.0 mg daily has been approved for WM. Semaglutide is approved for treatment of diabetes at the dosage of ≤ 1.0 mg once weekly subcutaneously or in oral tablet form at a dosage of up to 14 mg (2,17-20).

Current phase 3 trials are investigating semaglutide as a new GLP-1 analogue for the treatment of obesity because greater WL was observed with semaglutide than liraglutide (21). In the phase 2 trial of semaglutide in adults with obesity, a 0.4-mg dose daily was well tolerated, and patients experienced a mean WL at week 52 from baseline of –13.8% compared with –7.8% for liraglutide 3.0 mg and –2.3% for placebo (21).

Semaglutide Treatment Effect in People with obesity program

The Semaglutide Treatment Effect in People with obesity (STEP) clinical trial development program is evaluating semaglutide 2.4 mg, administered subcutaneously once weekly, for WM in people with obesity or overweight. The purpose of the program is to demonstrate the effect, safety, and tolerability profile of semaglutide 2.4 mg on WL, to enable further clinical development, and to support regulatory approval of semaglutide for WM. The trial design, objectives, end points, and baseline characteristics of five of the STEP trials are presented in this article.

Methods

Trial designs

This article covers five of the ongoing phase 3, double-blinded, randomized, multicenter, and multinational trials that assess semaglutide (2.4 mg subcutaneously once weekly) versus placebo for WM in adults with obesity or overweight and with and without T2D (Table 1). All participants receive periodic counseling, and support for all trials is provided by a multidisciplinary team, including a dietitian or a similarly qualified health care professional. Nonmonetary incentives are provided throughout the program, such as kettle balls and jump ropes, to encourage exercise and a healthy lifestyle.

In the WM trial (STEP 1, NCT03548935), 1,961 adults with obesity or overweight, without T2D, are being randomly assigned in a 2:1 manner to receive semaglutide 2.4 mg or placebo to assess WL (Figure 1, Table 1). A subpopulation of participants will have their body composition assessed by dual-energy x-ray absorptiometry (DXA) to test the hypothesis that WL is primarily caused by reduction in fat mass, resulting from treatment, in accordance with the European Medicines Agency and US Food and Drug Administration guidelines (22,23).

In the WM trial in T2D (STEP 2, NCT03552757), 1,210 adults with obesity or overweight, and with T2D, are being randomly assigned 1:1:1 to receive either semaglutide 2.4 mg, semaglutide 1.0 mg once weekly, or placebo to assess WL (Figure 2, Table 1).

In the WM with intensive behavioral therapy (IBT) trial only conducted in the United States (STEP 3, NCT03611582), 611 adults with obesity or overweight, without T2D, are being randomly assigned in a 2:1 manner to receive semaglutide 2.4 mg or placebo to assess WL (Figure 3, Table 1). Treatment is administered as an adjunct to IBT, in addition to an initial 8-week, low-calorie diet, followed by 60 weeks of a hypocaloric diet and increased physical activity.

In the sustained WM trial (STEP 4, NCT03548987), 902 participants with obesity or overweight, without T2D, are being treated with semaglutide 2.4 mg once weekly. Those completing a 20-week run-in period are being randomly assigned in a 2:1 manner to receive continued semaglutide 2.4 mg or placebo for an additional 48 weeks to assess WL (Figure 4, Table 1). Approximately 750 eligible participants will be randomly assigned. In addition, it has a withdrawal trial design to assess the change in weight after switching from semaglutide to placebo.

[1] Division of Endocrinology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA. Correspondence: Robert F. Kushner (rkushner@northwestern.edu) [2] Novo Nordisk A/S, Søborg, Denmark [3] Diabetes Research Centre, University of Leicester, Leicester, UK [4] National Institute for Health Research Leicester Biomedical Research Centre, Leicester, UK [5] National Institute for Health Research Leicester Clinical Research Facility, Leicester, UK [6] Department of Internal Medicine, Hasharon Hospital Rabin Medical Center, Petah Tikva, Israel [7] Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel [8] Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, Alabama, USA [9] Birmingham Veterans Affairs Medical Center, Birmingham, Alabama, USA [10] Department of Internal Medicine/Endocrinology, UT Southwestern Medical Center, Dallas, Texas, USA [11] Department of Population and Data Sciences, UT Southwestern Medical Center, Dallas, Texas, USA [12] Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA [13] York University and Wharton Weight Management Clinic, Toronto, Ontario, Canada [14] Obesity and Endocrinology Research, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK [15] Washington Center for Weight Management and Research, Arlington, Virginia, USA.

TABLE 1 Enrollment, objectives, and end points for STEP trials

Trial	WM	WM in T2D	WM with IBT (US only)	Sustained WM	Long-term WM
Other trial names	STEP 1, NCT03548935	STEP 2, NCT03552757	STEP 3, NCT03611582	STEP 4, NCT03548987	STEP 5, NCT03693430
Participants enrolled, *n*	1,961	1,210	611	902	304
EOT, wk	68	68	68	68	104
Trial objectives	To show superiority of semaglutide 2.4 mg versus placebo[a] on WL and to compare safety and tolerability in adults with obesity or overweight, without T2D	To show superiority of semaglutide 2.4 mg versus placebo and semaglutide 1.0 mg OW[a] on WL and to compare safety and tolerability in adults with T2D and either obesity or overweight	To maximize the effect of semaglutide 2.4 mg versus placebo[b] on WL in adults with obesity or overweight, without T2D	To maintain the effect of semaglutide 2.4 mg versus placebo[a] on WL from randomization to EOT and baseline to EOT and to compare safety in adults with obesity or overweight who reached the target dose of semaglutide during run-in	To show superiority of semaglutide 2.4 mg versus placebo[a] on WL and to compare safety and tolerability in adults with obesity or overweight after 2 years of treatment
Primary end points					
Change from baseline to EOT in body weight, %	X	X	X	X[c]	X
≥ 5% WL from baseline after EOT, yes/no	X	X	X	NA	X
Confirmatory secondary end points					
≥ 10% WL from baseline after EOT, yes/no	X	X	X	NA	X
≥ 15% WL from baseline after EOT, yes/no	X	X	X	NA	X
Change from baseline to EOT					
Waist circumference, cm	X	X	X	X[c]	X
Systolic blood pressure, mmHg	X	X	X	X[c]	X
SF-36 physical functioning	X	X	X	X[c]	NA
IWQOL-Lite-CT physical function	X	X	NA	NA	NA
Other	NA	Body weight of semaglutide 2.4 mg OW versus semaglutide 1 mg, %; HbA_{1c}, % (mmol/mol)	NA	NA	NA

[a]As adjunct to lifestyle intervention (–500-kcal/d diet + 150 min/wk of physical activity).
[b]As adjunct to intensive behavioral therapy including initial 8-week low-calorie diet.
[c]Primary and confirmatory secondary efficacy end points in STEP 4 are changes between randomization (week 20) and EOT (68 weeks).
EOT, end of treatment; HbA_{1c}, hemoglobin A_{1c}; IBT, intensive behavioral therapy; IWQOL-Lite-CT, Impact of Weight on Quality of Life, Lite Clinical Trials Version; NA, not applicable; OW, once weekly; SF-36, Short Form36v2 Health Survey, Acute Version; STEP, Semaglutide Treatment Effect in People with obesity; T2D, type 2 diabetes; WL, weight loss; WM, weight management; X, included in trial.

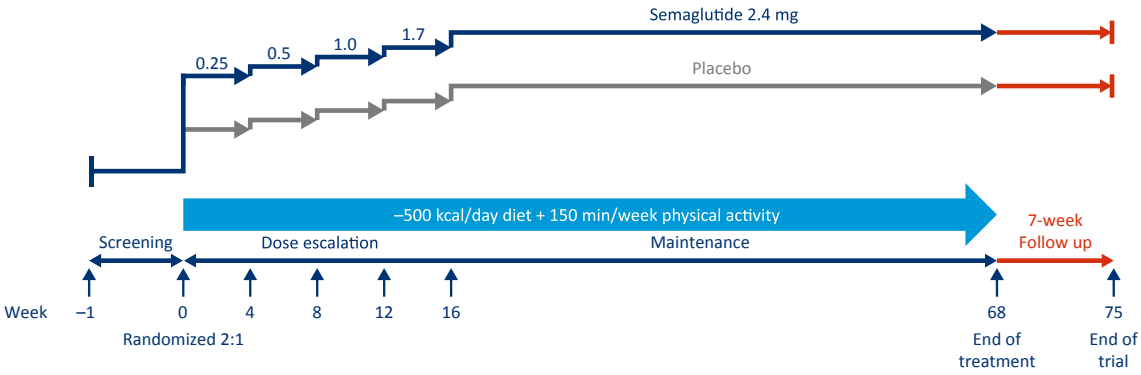

Figure 1 Weight management trial design (Semaglutide Treatment Effect in People with obesity 1). This is a 68-week, randomized, double-blind, placebo-controlled, two-armed, parallel-group, multicenter, multinational clinical trial, with 7 weeks of follow-up without treatment for safety assessments, comparing semaglutide 2.4 mg (subcutaneously, once weekly) with placebo, as an adjunct to lifestyle intervention, in people with obesity or overweight.

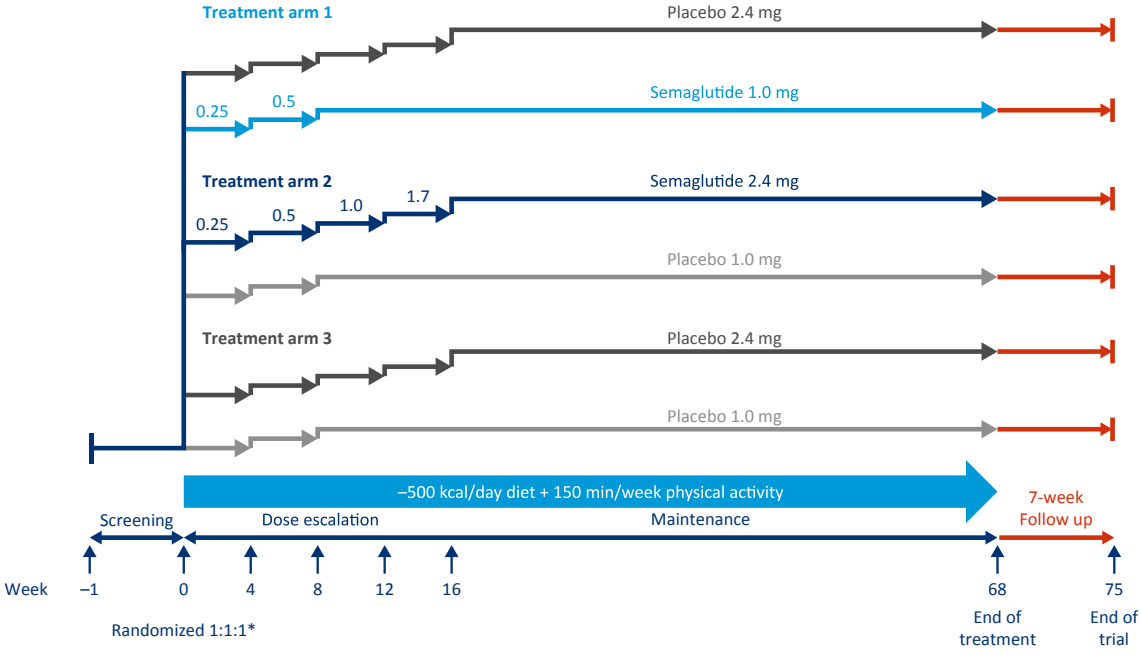

Figure 2 Weight management in type 2 diabetes (T2D) trial design (Semaglutide Treatment Effect in People with obesity 2). This is a 68-week, randomized, double-blind, double-dummy, placebo-controlled, three-armed, multicenter, multinational clinical trial, with 7 weeks of follow-up without treatment for safety assessments, comparing semaglutide 2.4 mg (subcutaneously, once weekly) with placebo, as an adjunct to lifestyle intervention, in people with obesity or overweight and T2D. *Randomization was stratified according to background diabetes treatment; diet and physical activity only or treatment with single-compound metformin or sodium-glucose cotransporter 2 inhibitor (SGLT2i) and single-compound agents for diabetes (sulphonylurea [SU] or glitazone) or combination treatment with up to three agents for diabetes (metformin, SU, SGLT2i, or glitazone).

In the long-term WM trial (STEP 5, NCT03693430), 304 participants with obesity or overweight, without T2D, are being randomly assigned in a 1:1 manner to receive semaglutide 2.4 mg or placebo to assess WL (Figure 5, Table 1) over a 2-year period.

Participants in all treatment groups, including placebo, are receiving the trial product as an adjunct to lifestyle intervention. In all trials except for the WM with IBT trial (STEP 3), this is defined as a 500-kcal/d deficit relative to the estimated total energy expenditure calculated at randomization together with a recommended 150 min/wk of physical activity.

Randomization and treatment

Randomization for all participants is being conducted by an interactive Web-based response system. In the WM in T2D trial (STEP 2), randomization is being stratified according to background diabetes

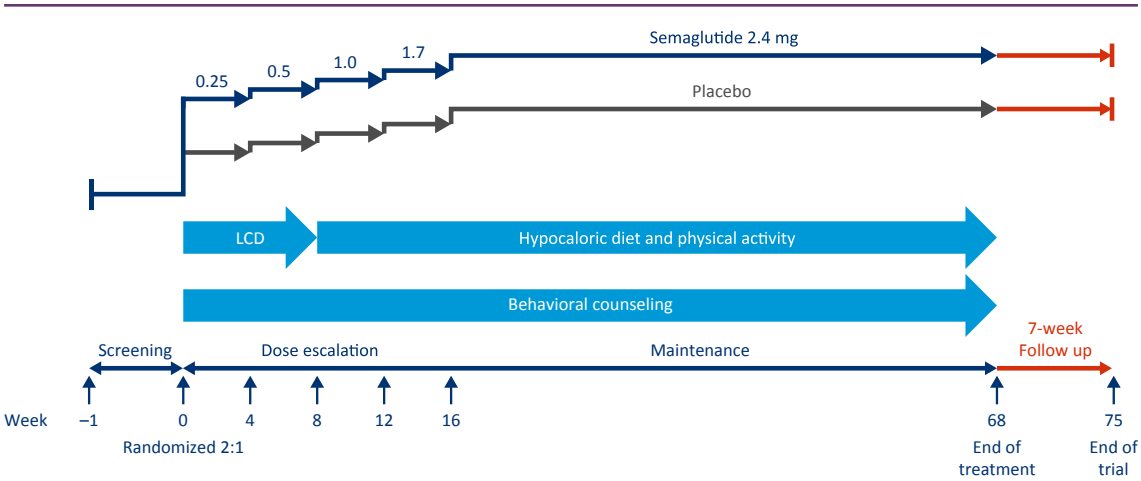

Figure 3 Weight management with intensive behavioral therapy trial design, only conducted in the United States (Semaglutide Treatment Effect in People with obesity 3). This is a 68-week, randomized, double-blind, placebo-controlled, two-armed, parallel-group, multicenter clinical trial, with 7 weeks of follow-up without treatment for safety assessments, comparing semaglutide 2.4 mg (subcutaneously, once weekly) with placebo, as an adjunct to intensive behavioral therapy and low-calorie diet (LCD), in people with obesity or overweight.

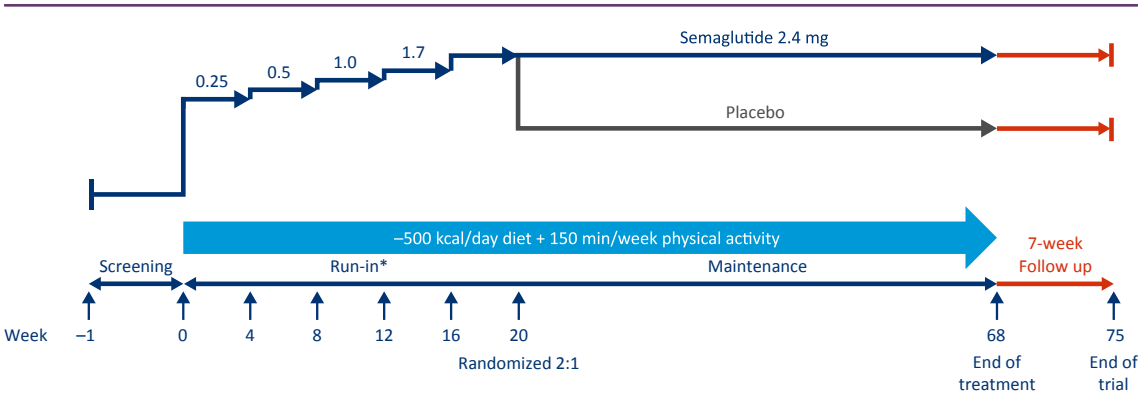

Figure 4 Sustained weight management trial design (Semaglutide Treatment Effect in People with obesity 4). This is a 68-week, randomized, double-blind, placebo-controlled, two-armed, multicenter, multinational withdrawal clinical trial, with 7 weeks of follow-up without treatment for safety assessments, comparing semaglutide 2.4 mg (subcutaneously, once weekly) with placebo, as an adjunct to lifestyle intervention, in people with obesity or overweight. *During the 20-week run-in period, participants start a dose escalation (visit 2 [week 0]) with semaglutide 2.4 mg (subcutaneously, once weekly) as an adjunct to a reduced-calorie diet and increased physical activity for 20 weeks. The run-in period includes 4 weeks at the target dose (semaglutide subcutaneously, 2.4 mg once weekly).

treatment: diet and physical activity only or treatment with single-compound metformin or sodium-glucose cotransporter 2 inhibitors and single-compound oral agents for diabetes (sulphonylurea or glitazone) versus combination treatment with up to three agents for diabetes (metformin, sulphonylurea, sodium-glucose cotransporter 2 inhibitor, or glitazone). Participants are being further stratified by screening value of hemoglobin A_{1c} (HbA_{1c}): < 8.5% versus ≥ 8.5%.

Trial populations

The key eligibility criteria are shown in Table 2. Male or female participants qualifying for eligibility are ≥ 18 years of age and have a history of at least one self-reported unsuccessful dietary effort to lose body weight. Adults are considered eligible for the DXA subtrial in STEP 1 if they have a BMI ≤ 40 kg/m^2 at screening and if the quality of the baseline DXA is found to be acceptable by the imaging laboratory before randomization into the subtrial. For the WM trials without T2D (STEP trials 1, 3, 4, and 5), eligible adults have a BMI ≥ 30 kg/m^2 or ≥ 27 kg/m^2 with the presence of weight-related complications (treated or untreated): dyslipidemia, obstructive sleep apnea, hypertension, or CVD. For the WM in T2D trial (STEP 2), eligible participants are required to have a BMI ≥ 27 kg/m^2 and a diagnosis of T2D (HbA_{1c}: 7%-10% [53-86 mmol/mol]) ≥ 180 days prior to the day of screening.

Adults are excluded from the trials if there is a self-reported change in body weight of > 5 kg within 90 days before screening. For trials that excluded patients with T2D (STEP trails 1, 3, 4, and 5), adults are excluded if they have a history of type 1 diabetes mellitus or T2D

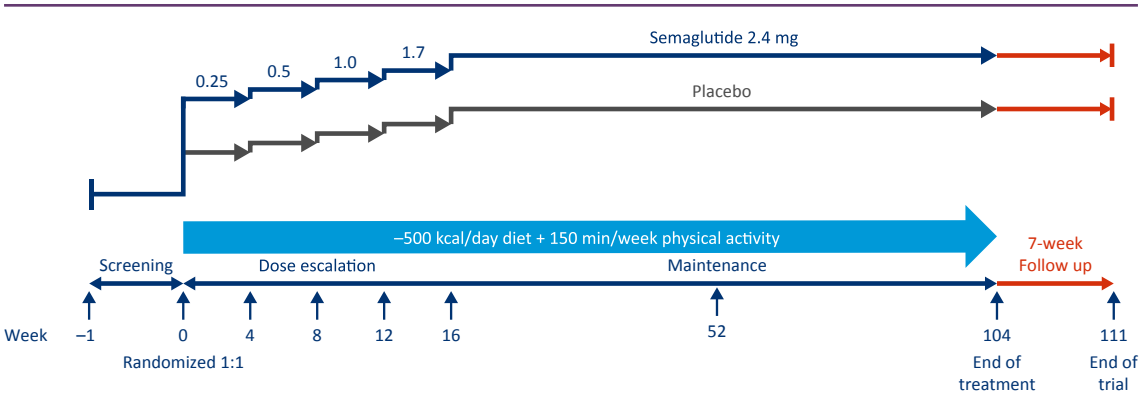

Figure 5 Long-term weight management trial design (Semaglutide Treatment Effect in People with obesity 5). This is a 104-week, randomized, double-blind, placebo-controlled, two-armed, parallel-group, multicenter, multinational clinical trial, with 7 weeks of follow-up without treatment for safety assessments, comparing semaglutide 2.4 mg (subcutaneously, once weekly) with placebo, as an adjunct to lifestyle intervention in people with obesity or overweight.

mellitus, $HbA_{1c} \geq 6.5\%$ (48 mmol/mol), or previous treatment with glucose-lowering agents or any AOM within the past 90 days before screening.

For all trials, treatment discontinuation can be decided by the investigator or participant. After discontinuation, participants are encouraged to continue to attend visits per the schedule and may be given the option to restart trial medication. Protocol-specified discontinuation criteria include safety concerns from the investigator, calcitonin level ≥ 100 ng/L, suspicion of pancreatitis, pregnancy or intention to become pregnant, and participation in another clinical trial. Participants can withdraw consent at any point and are considered lost to follow-up if they repeatedly fail to attend scheduled visits and cannot be contacted.

The protocols allow for dose reductions in case a participant does not tolerate the recommended target dose of 2.4 mg and may stay at the lower dose level of 1.7 mg once weekly, if needed. This is only allowed if the participant would otherwise discontinue trial treatment completely and if it is considered safe to continue trial treatment, per the investigator's discretion. It is recommended that the participant make at least one attempt to re-escalate to the recommended target dose of 2.4 mg once weekly. Dose is recorded at selected visits throughout the trials.

Outcome measures

The primary and confirmatory secondary end points are described in Table 1. For all trials, the primary endpoints are percentage change from baseline at randomization (note that this was from week 20 in STEP 4) to end of treatment (EOT) in body weight and ≥5% WL from baseline after EOT (not applicable for the sustained WM trial [STEP 4]). Confirmatory secondary trial endpoints include the proportion of participants achieving a body weight reduction ≥10% or ≥15% from baseline to EOT (not applicable for the sustained WM trial [STEP 4]). Other confirmatory secondary endpoints for all the trials are change from baseline to EOT (or change from randomization [week 20] to EOT for the sustained WM trial [STEP 4]), in waist circumference (centimeters), systolic blood pressure (millimeters of mercury), and clinical outcome assessments.

Assessments

Serial assessments of randomly assigned participants are conducted throughout all of the trials and include height, body weight, waist circumference, glucose metabolism (fasting plasma glucose, HbA_{1c}, fasting serum insulin), lipids (total cholesterol, free fatty acids, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, very-low-density lipoprotein cholesterol), biomarkers (high-sensitivity C-reactive protein, except in the sustained WM trial [STEP 4]), and vital signs (diastolic and systolic blood pressure [millimeters of mercury]). The WM in T2D trial (STEP 2) assessments also include self-measured fasting plasma glucose. If a BMI ≤ 22.5 kg/m^2 is reached, the recommended energy intake is recalculated, with no calorie deficit (maintenance diet) for the remainder of the trial.

Clinical outcome assessments are carried out throughout the duration of the STEP trials 1-4 and include the following measures for physical functioning: Short Form36v2 Health Survey, Acute Version (24); Impact of Weight on Quality of Life, Lite Clinical Trials Version (25,26); and Stanford Presenteeism Scale, version 2001. In addition, other measures included the Patient Global Impression of Status; Patient Global Impression of Change; International Consultation on Incontinence Questionnaire Urinary Incontinence Short Form; Work Productivity Activity Impairment–Specific Health Problem, version 2.0; Six-Minute Walk Test; and Weight-Related Sign and Symptom Measure.

For the WM with IBT trial (STEP 3), each IBT session consists of dietitian counseling and a participant handout based on the IBT protocol (27). Participants receive weekly intensive behavioral support in which they discuss progress, review their food diary/Web application (app), and address any adherence issues. For all other trials, participants receive diet and physical activity counseling provided by a dietitian or a similarly qualified health care professional. Counseling is provided every fourth week via visits/telephone contacts, and participants are instructed to record their food intake and physical activity daily via a paper diary, an app, or a similar tool.

For all trials, excluding the WM with IBT trial (STEP 3), the total energy expenditure is calculated by multiplying the estimated basal metabolic rate (as defined in the trial protocol) with a physical activity level value of 1.3.

Safety assessments of the randomly assigned participants for the trials include physical examinations, electrocardiograms, hematology

TABLE 2 Key eligibility criteria for STEP trials

Criteria	WM, STEP 1	WM in T2D, STEP 2	WM with IBT, STEP 3	Sustained WM, STEP 4	Long-term WM, STEP 5
Key inclusion					
Man or woman aged ≥ 18 years	X	X	X	X	X
BMI ≥ 27 kg/m^2	NA	X	NA	NA	NA
BMI ≥ 30 kg/m^2 or ≥ 27 kg/m^2 with ≥ 1 weight-related comorbidity (treated or untreated): hypertension, dyslipidemia, obstructive sleep apnea, or CVD	X	NA	X	X	X
History of at least 1 self-reported unsuccessful dietary effort to lose weight	X	X	X	X	X
Diagnosed with T2D ≥ 180 days prior to screening	NA	X	NA	NA	NA
Treated with diet and exercise alone or stable treatment with metformin, SU, SGLT2i, glitazone as single-agent therapy, or ≤ 3 agents for diabetes (metformin, SU, SGLT2i, or glitazone) according to local label	NA	X	NA	NA	NA
HbA_{1c} 7%-10% (53-86 mmol/mol)	NA	X	NA	NA	NA
Key exclusion					
HbA_{1c} ≥ 48 mmol/mol (6.5%) at screening[a]	X	NA	X	X	X
History of T1D or T2D	X	NA	X	X	X
Treatment with glucose-lowering agent(s) < 90 days before screening	X	NA	X	X	X
Treatment with GLP-1 RA < 180 days before screening	X	X	NA	NA	NA
Treatment with any medication for diabetes or obesity not stated in inclusion criteria < 90 days before screening	NA	X	NA	NA	NA
Treatment with any other investigational drugs for diabetes < 90 days before screening or any investigational drugs not affecting diabetes < 30 days before screening	NA	X	NA	NA	NA
Self-reported change in body weight > 5 kg (11 lb) < 90 days before screening	X	X	X	X	X
Uncontrolled thyroid disease: TSH > 6.0 mIU/L or < 0.4 mIU/L at screening[a]	X	X	X	X	X
Participants unable to adhere to low-calorie diet and physical activity	NA	NA	X	NA	NA
Acute pancreatitis < 180 days before screening	X	X	X	X	X
History or presence of chronic pancreatitis	X	X	X	X	X
Calcitonin ≥ 100 ng/L at screening[a]	X	X	X	X	X
Renal impairment eGFR 40 at screening[a]					
eGFR < 15 mL/min/1.73 m^2	X	NA	X	X	X
eGFR < 30 mL/min/1.73 m^2	NA	X	NA	NA	NA
eGFR < 60 mL/min/1.73 m^2	NA	X[b]	NA	NA	NA
MI, stroke, hospitalization for unstable angina, or TIA < 60 days before screening	X	X	X	X	X
Classified as New York Heart Association class 4	X	X	X	X	X

[a]Central laboratory measured.
[b]In participants treated with SGLT2i.
CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; GLP-1 RA, glucagon-like peptide 1 receptor agonist; HbA_{1c}, hemoglobin A_{1c}; IBT, intensive behavioral therapy; MI, myocardial infarction; NA, not applicable; SGLT2i, sodium-glucose cotransporter 2 inhibitor; STEP, Semaglutide Treatment Effect in People with obesity; SU, sulphonylurea; T1D, type 1 diabetes; T2D, type 2 diabetes; TIA, transient ischemic attack; TSH, thyroid-stimulating hormone; WM, weight management; X, included in trial.

and biochemistry assessments, detection of antibodies against semaglutide, and vital signs. Information on adverse events is collected throughout the trial periods, including the follow-up period off treatment.

Statistical analysis

Effect end points will be analyzed using the full analysis set, which includes all randomly assigned participants according to the intention-to-treat principle. Safety endpoints will be analyzed using the

safety analysis set, which includes all randomly assigned participants exposed to at least one dose of randomized treatment. Results from statistical analyses will generally be accompanied by two-sided 95% CIs and corresponding *P* values. Superiority will be claimed if *P* values are less than 5% ($P<0.05$) and the estimated treatment contrasts favor semaglutide 2.4 mg. The sample size for each trial gives an effective power (marginal powers multiplied) of 99% for STEP 1, 94% for STEP 2, 86% for STEP 3, 95% for STEP 4, and 43% for STEP 5. The sample sizes for the STEP trials 1 to 4 are primarily defined to support safety. The sample size for STEP 5 is primarily defined to support the co-primary end points. As there are two primary end points included in the statistical testing hierarchy for STEP trials 1 to 3 and STEP 5, significant superiority of semaglutide 2.4 mg versus placebo must be demonstrated for each primary end point.

The use of two estimands in the STEP program will address different scientific questions of interest, and both contribute to the full clinical picture. Including more than one estimand allows evaluation of the treatment effect from different perspectives. The treatment-policy estimand assesses the trial-population average treatment effect of semaglutide or placebo. All randomly assigned participants contribute to data analysis regardless of adherence to treatment or participants starting unplanned interventions such as other AOMs or bariatric surgery. For all trials, all analyses in the statistical testing hierarchy are addressing the treatment-policy estimand. The trial-product estimand will evaluate the treatment effect of semaglutide 2.4 mg versus placebo under the assumption that all participants remain on their randomized treatment for the entire planned trial duration. Trial-product estimand assessments include only participants who are taking the randomized treatment and have not initiated other AOMs or undergone bariatric surgery.

The treatment-policy and trial-product estimands correspond to the updated International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use regulatory guidelines on quantifying treatment effects of medications (28). For the treatment-policy estimand, continuous end points are analyzed using ANCOVA with randomized treatment as a factor and baseline end point value as a covariate. Missing data are imputed using a multiple imputation approach similar to that described by McEvoy (29). Estimates and standard deviations will be pooled across imputed data sets using the Rubin formula. All categorical end points will be assessed at EOT and analyzed by logistic regression using randomized treatment (and stratification groups for the WM in T2D trial [STEP 2]) as a factor and baseline end point value as a covariate. For analyses of end points, the estimated treatment difference and odds ratio (apart from the sustained WM trial [STEP 4]) between semaglutide 2.4 mg and placebo will be reported with the associated two-sided 95% CI and corresponding *P* value. For the trial-product estimand, continuous end points are analyzed using a mixed model for repeated measurements.

Ethics

The trials are being conducted in accordance with good clinical practice guidelines (30) and the principles of the Declaration of Helsinki (31). All 750 sites in the five studies received independent ethics-committee or institutional-review-board approval. The trials are designed and overseen by a steering group of clinical professionals, including representatives from the trial sponsor (Novo Nordisk, Søborg, Denmark).

Results

All the trials are ongoing, and results will be available in 2020, except for those from the long-term WM trial (STEP 5), which finishes in 2021. A total of 4,988 participants were enrolled across all five trials to receive either semaglutide or placebo (Supporting Information Table S1). The key baseline demographics and characteristics for the participants in each trial are shown in Table 3. The participants had a mean age of 46.2 to 55.3 years; were mostly female (74.1%-81.0%), excluding the WM in T2D trial (STEP 2); and had a mean BMI of 35.7 to 38.5 kg/m^2. Waist circumference, blood pressure, cholesterol levels, overall estimated glomerular filtration rate, high-sensitivity C-reactive protein, and glycemic status were generally well balanced across the trials.

The racial composition of each trial is primarily white (62.1%-93.1%), but overall there is broad variation in races/ethnicities across the trials. Both the WM with diabetes and the WM without diabetes trials (STEP 1 and STEP 2) have a higher proportion of Asians (13.7% and 26.2%, respectively) than the other trials. Compared with the WM with and without diabetes and long-term WM trials (STEP trials 1, 2, and 5), there are slightly more Hispanic or Latino participants in the WM with IBT trial (STEP 3, 19.8%) and slightly fewer in the sustained WM trial (STEP 4, 7.8%). As expected, the WM in T2D trial (STEP 2), versus the other trials, has numerically higher levels of fasting plasma glucose, HbA_{1c}, very-low-density lipoprotein, triglycerides, and free fatty acids.

Discussion

The STEP program represents the latest investigation to date of an AOM for chronic WM. These phase 3 trials aim to evaluate the effect of semaglutide 2.4 mg (administered subcutaneously once weekly) on WM in adults with obesity or overweight and provide a comprehensive overview of the efficacy, safety, and tolerability profile of semaglutide 2.4 mg.

Baseline characteristics are well balanced among randomized groups for many of the parameters. The baseline results presented from the sustained WM trial (STEP 4) are only available for the pre-randomized participants in the run-in phase. The variations in the race and ethnicity of participants across the trials are expected because of recruiting participants from various countries. The WM with or without diabetes trials (STEP 1 and STEP 2) are designed to have Asians as at least 10% of the population, which is the reason they had a higher proportion of Asians than the other trials. The WM in T2D trial (STEP 2) has a greater proportion of men (49.1%) than the other trials (19.0%-25.9%). There are slightly more Hispanic or Latino participants in the WM with IBT trial (STEP 3), and there are slightly fewer in the sustained WM trial (STEP 4) than in the other trials.

Baseline characteristics were generally comparable between the STEP trials presented in this article and the phase 2 trial of semaglutide (21). In comparison with the WM in T2D trial (STEP 2), the phase 2 trial had a numerically higher BMI (39.3 kg/m^2) and waist circumference (117.8 cm). This was due to STEP 2 having a lower threshold-for-BMI inclusion criterion (≥27 kg/m^2) than the phase 2 trial (≥30 kg/m^2). Overall, the phase 2 trial has a numerically lower HbA_{1c} level (5.5%) than the STEP trials, particularly for STEP 2 (21). However, cross-trial comparisons should be interpreted cautiously.

TABLE 3 Key baseline characteristics and demographics of participants

	WM, STEP 1, *N* = 1,961	WM in T2D, STEP 2, *N* = 1,210	WM with IBT, STEP 3, *N* = 611	Sustained WM, STEP 4, *N* = 902	Long-term WM, STEP 5, *N* = 304
Sex, *n* (%)					
Female	1,453 (74.1)	616 (50.9)	495 (81.0)	717 (79.5)	236 (77.6)
Male	508 (25.9)	594 (49.1)	116 (19.0)	185 (20.5)	68 (22.3)
Age, y	46.5 ± 12.7	55.3 ± 10.6	46.2 ± 12.7	46.4 ± 11.9	47.3 ± 11.0
Race, *n* (%)[a]					
White	1,472 (77.2)	751 (62.1)	465 (76.1)	751 (83.3)	283 (93.1)
Black or African American	111 (5.8)	100 (8.3)	116 (19.0)	123 (13.6)	12 (3.9)
Asian	261 (13.7)	317 (26.2)	11 (1.8)	19 (2.1)	2 (0.7)
American Indian or Alaskan Native	27 (1.4)	5 (0.4)	1 (0.2)	0	3 (1.0)
Native Hawaiian or other Pacific Islander	2 (0.1)	0	3 (0.5)	1 (0.1)	0
Other	33 (1.7)	37 (3.1)	15 (2.5)	8 (0.9)	4 (1.3)
Ethnic group, *n* (%)[a]					
Hispanic or Latino	236 (12.0)	155 (12.8)	121 (19.8)	70 (7.8)	39 (12.8)
Not reported	55 (2.8)	0	0	0	0
BMI, kg/m^2	37.9 ± 6.7	35.7 ± 6.3	38.0 ± 6.7	38.3 ± 7.0	38.5 ± 6.9
Waist circumference, cm	114.7 ± 14.7	114.6 ± 14.1	113.0 ± 15.5	115.1 ± 15.6	115.7 ± 14.8
FPG, mmol/L	5.3 ± 0.6	8.6 ± 2.2	5.2 ± 0.5	5.4 ± 0.6	5.3 ± 0.6
Blood pressure, mm Hg					
Systolic	126.5 ± 14.3	130.0 ± 13.5	124.4 ± 14.8	126.4 ± 14.3	125.5 ± 14.5
Diastolic	80.3 ± 9.6	79.8 ± 9.0	80.5 ± 9.7	80.9 ± 9.9	80.1 ± 9.4
HbA_{1c}, %	5.7 ± 0.32	8.1 ± 0.8	5.7 ± 0.3	5.7 ± 0.3	5.7 ± 0.3
Cholesterol, mmol/L					
Total	4.9 ± 20.0	4.4 ± 23.2	4.8 ± 19.7	5.0 ± 19.5	4.8 ± 19.1
HDL	1.3 ± 25.5	1.1 ± 24.5	1.3 ± 23.6	1.29 ± 24.6	1.23 ± 23.9
LDL	2.9 ± 28.7	2.3 ± 35.6	2.8 ± 28.5	3.0 ± 27.3	2.9 ± 26.1
VLDL	0.6 ± 51.1	0.8 ± 51.6	0.6 ± 48.1	0.6 ± 53.6	0.6 ± 48.1
Triglycerides, mg/dL	1.4 ± 70.0	1.8 ± 64.2	1.2 ± 49.8	1.4 ± 54.9	1.3 ± 48.6
Free fatty acids, mmol/L	0.4 ± 48.3	0.6 ± 44.6	0.4 ± 49.0	0.4 ± 51.5	0.4 ± 48.3
Overall eGFR, mL/min/1.73 m^2[b]	96.6 ± 17.2	93.7 ± 19.5	96.8 ± 19.5	97.6 ± 17.8	94.8 ± 16.6
hsCRP, mg/L	3.9 ± 117.5	3.4 ± 129.2	4.4 ± 99.3	NA	4.4 ± 125.2
Diabetes duration, y	NA	8.6 ± 6.2	NA	NA	NA
Glycemic status, *n* (%)					
Normoglycemia	1,106 (56.4)	NA	306 (50.1)	493 (54.7)	163 (53.6)
Prediabetes	855 (43.6)	NA	305 (49.9)	408 (45.3)	141 (46.4)

Baseline was defined as randomization for WM, WM in T2D, WM with IBT, and long-term WM trials and as start of run-in period for sustained WM trial. Plus-minus values are reported as means ± SD. For cholesterol, triglycerides, free fatty acids, overall eGFR, and hsCRP, plus-minus values are geometric means and coefficients of variations. For WM with or without T2D and maximizing and long-term WM trials (STEP trials 1-3 and 5), data are for all randomly assigned participants. For sustained WM trial (STEP 4), data are for all participants entering run-in period.

[a]Race and ethnic group were self-reported.

[b]eGFR is calculated according to chronic kidney disease–epidemiology collaboration (CKD-EPI) creatinine equation as defined by Kidney Disease: Improving Global Outcomes (KDIGO) (32).

eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HbA_{1c}, hemoglobin A_{1c}; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IBT, intensive behavioral therapy; LDL, low-density lipoprotein; NA, not applicable; STEP, Semaglutide Treatment Effect in People with obesity; T2D, type 2 diabetes; VLDL, very-low-density lipoprotein; WM, weight management.

At the time of initiation of the phase 2 trial, it was hypothesized that daily dosing of semaglutide would result in a more favorable tolerability profile compared with weekly administration, and, therefore, the once-daily administration of semaglutide was investigated. The phase 2 WM trial of semaglutide daily resulted in dose-dependent, clinically relevant WL over 52 weeks and associated with an acceptable tolerability profile with respect to gastrointestinal symptoms (21). However, based on comparisons with studies with weekly administration of semaglutide, it was reported that there was no difference in gastrointestinal adverse events with the daily versus weekly dosing regimen of semaglutide (33). Furthermore, using population pharmacokinetic modeling, it was estimated that a once-weekly maintenance dose of semaglutide 2.4 mg subcutaneously would not exceed the maximum concentration at steady state as obtained by the

once-daily semaglutide 0.4-mg subcutaneous dose. Hence, a decision was made to change dosing for semaglutide from daily to weekly to improve adherence and convenience for participants.

There are several trials that demonstrate the promising effects of GLP-1s in general and semaglutide in particular on WM in subpopulations of people with obesity. In the Satiety and Clinical Adiposity – Liraglutide Evidence in individuals with and without diabetes (SCALE) trial of 3,731 people without diabetes, WL was maintained at 56 weeks with liraglutide 3.0 mg versus placebo (−8.0% versus −2.6%, respectively; $P<0.001$) (34). The SCALE trial of 846 people with T2D and obesity or overweight demonstrated a 6.0% (6.4-kg) reduction of initial body weight with liraglutide 3.0 mg versus 1.8 mg and placebo (4.7% [5.0 kg] and 2.0% [2.2 kg], respectively) (35). The Semaglutide Unabated Sustainability in Treatment of Type 2 Diabetes (SUSTAIN) clinical development program, which included >8,000 people with T2D, demonstrated that semaglutide at doses of 0.5 mg and 1.0 mg weekly offered WM benefits of 2.5 to 5.7 kg and 2.0 to 7.9 kg, respectively (36). Liraglutide and semaglutide are administered at different frequencies and doses, and over a 24-hour period, they have distinct pharmacodynamic effects (37,38). However, they are equally associated with transient, mild, or moderate gastrointestinal symptoms, including nausea, vomiting, and diarrhea (17,39). Clinical experience shows that slowing the initial escalation of liraglutide and semaglutide helps mitigate these side effects; hence, we have designed the trials in the STEP program to have a slow titration of semaglutide over a 16-week period.

The primary goal of pharmacotherapies like semaglutide for chronic WM is to achieve a clinically meaningful WL when combined with lifestyle intervention and to provide long-term WM and to minimize weight regain (2). More importantly, semaglutide, through both direct and indirect actions, is hoped to meaningfully impact obesity-related comorbidities. Average WL of 10% to 15% has been shown to significantly alleviate many complications associated with obesity, including diabetes, hypertension, osteoarthritis, and gastroesophageal reflex disease (40-42). The benefits of WL have also been demonstrated in dyslipidemia, nonalcoholic fatty liver disease, sleep apnea, and stress incontinence (40-41,43,44).

Obesity can adversely affect physical and mental health and reduces health-related quality of life (45). The physical impairments appear to be more closely associated with severity of obesity than mental impairments (45). Despite a wide range of randomized controlled trials investigating these associations, a systematic review by Kolotkin et al. (45) found that these studies are inconclusive. Therefore, further investigations are necessary to explore the relationship between obesity and health-related quality of life besides the physical and health advantages of WL. The STEP trials have been designed to monitor patient-reported outcomes, including the Impact of Weight on Quality of Life, Lite Clinical Trials Version, and Short Form36v2 Health Survey, Acute Version.

One key difference among the STEP trials presented here is that, despite them all having the same primary end point of change in body weight, results from the primary end point in the sustained WM trial (STEP 4) will not reflect the full WL potential that is expected to be observed with semaglutide in the other STEP trials. This is due to the fact that in STEP 4, the randomization to semaglutide or placebo takes place after all participants have received a 20-week run-in treatment with semaglutide, and the primary end point for STEP 4 is the change in the percentage of body weight from randomization at week 20 to the EOT at week 68. There are currently four additional planned and ongoing trials in the STEP program. STEP 6 (NCT03811574) is investigating the efficacy and safety of semaglutide once weekly in East Asian adults with obesity or overweight. STEP TEENS (NCT04102189) is evaluating the efficacy and safety of semaglutide in adolescents. STEP 7 (NCT04251156) is planned to investigate the efficacy and safety of semaglutide also in the Chinese population. STEP 8 (NCT04074161) is assessing the effect and safety of semaglutide 2.4 mg once weekly compared with liraglutide 3.0 mg once daily on WM in people with obesity or overweight. Obesity is a strong risk factor for the development of diabetes, and both diabetes and obesity are associated with CVD; therefore, it is important to consider the effect of semaglutide on cardiovascular risk factors. Further research is ongoing in the Semaglutide Effects on Cardiovascular Outcomes in People With Overweight or Obesity (SELECT) trial (NCT03574597) to explore the impact of semaglutide 2.4 mg subcutaneously on reducing the risk of cardiovascular events in people with prior CVD and either obesity or overweight.

Conclusion

AOMs are an important treatment option for people living with obesity who are unable to lose weight and maintain WL or for those who do not meet the eligibility criteria for bariatric surgery or who failed to maintain WL following bariatric surgery. The STEP clinical development program with the GLP-1 analogue semaglutide provides rigorous assessment regarding the use of semaglutide 2.4 mg once weekly to treat people with obesity, with an effort to gain a greater understanding of WL, WL maintenance, safety, and tolerability in adults with obesity as an adjunct to lifestyle intervention. We anticipate that these trials will demonstrate that semaglutide represents a new and effective medication that can be used to improve the health and quality of life for patients with obesity.

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Deidentified participant data are available for this article on a specialized SAS data platform (SAS Institute, Cary, NC). Data sets from Novo Nordisk will be available permanently after research completion and approval of product and product use in both the European Union and United States. The study protocol and redacted clinical study report will be available according to Novo Nordisk data-sharing commitments. Access to data can be granted through a request proposal form, and the access criteria can be found online. Data will be shared with bona fide researchers submitting a research proposal requesting access to data. Data use is subject to approval by the independent review board according to the Institutional Review Board Charter (see novonordisk-trials.com).

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REVIEW

Weight Loss and Maintenance Related to the Mechanism of Action of Glucagon-Like Peptide 1 Receptor Agonists

Jamy Ard · Angela Fitch · Sharon Fruh · Lawrence Herman

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ABSTRACT

Obesity is a chronic disease associated with many complications. Weight loss of 5–15% can improve many obesity-related complications. Despite the benefits of weight reduction, there are many challenges in losing weight and maintaining long-term weight loss. Pharmacotherapy can help people with obesity achieve and maintain their target weight loss, thereby reducing the risk of obesity-related complications. The prevalence of obesity in the USA has been increasing over the past few decades, and despite the availability of approved anti-obesity medications (AOMs), people with obesity may not be accessing or receiving treatment at levels consistent with the disease prevalence. Reasons for low levels of initiation and long-term use of AOMs may include reluctance of public health and medical organizations to recognize obesity as a disease, lack of reimbursement, provider inexperience, and misperceptions about the efficacy and safety of available treatments. This article aims to inform primary care providers about the mechanism of action of one class of AOMs, glucagon-like peptide 1 receptor agonists (GLP-1RAs), in weight loss and longer-term maintenance of weight loss, and the efficacy and safety of this treatment class. GLP-1RA therapy was initially developed to treat type 2 diabetes. Owing to their effectiveness in reducing body weight, once-daily subcutaneous administration of liraglutide 3.0 mg has been approved, and once-weekly subcutaneous administration of semaglutide 2.4 mg is being investigated in phase III trials, for obesity management. Considerations regarding adverse effects and contraindications for different drug classes are provided to help guide treatment decision-

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J. Ard (✉)
Division of Public Health Sciences, Department of Epidemiology and Prevention, Wake Forest University School of Medicine, 1 Medical Center Blvd, Winston-Salem, NC 27157, USA
e-mail: jard@wakehealth.edu

A. Fitch
MGH Weight Center, Massachusetts General Hospital, Boston, MA, USA

A. Fitch
Harvard Medical School, Boston, MA, USA

A. Fitch
Endocrine Unit, Division of Endocrinology, Department of Medicine, Massachusetts General Hospital, Boston, MA, USA

S. Fruh
College of Nursing, University of South Alabama, Mobile, AL, USA

L. Herman
Doctor of Medical Science Program, University of Lynchburg, Lynchburg, VA, USA

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making when considering pharmacotherapy for weight management in patients with obesity.

PLAIN LANGUAGE SUMMARY

Obesity is a growing public health issue that increases the risk of developing heart disease, type 2 diabetes, and osteoarthritis. Weight loss can reduce the risk of developing these health problems but, despite this, levels of obesity remain high. Achieving and maintaining weight loss is challenging for many individuals. There is therefore a need for some patients to take medications to help them lose weight and prevent weight regain. Glucagon-like peptide 1 receptor agonists (GLP-1RAs) are a type of medication originally developed to treat type 2 diabetes, but are now being used for the treatment of obesity because they are effective at helping people to lose weight. One GLP-1RA, liraglutide, has been approved to treat obesity, and another, semaglutide, is in clinical trials. GLP-1RAs work by reducing the appetite and feelings of hunger, slowing the release of food from the stomach, and increasing feelings of fullness after eating. Most people can tolerate GLP-1RAs well. The most common side effects (nausea, vomiting, and diarrhea) are usually mild and occur in the first few weeks of treatment, reducing over time. Because of the difficulties many people face in maintaining weight loss, lifelong treatment may be needed. In clinical trials, GLP-1RAs were well tolerated and effective at helping people prevent weight regain, and may be a good option for long-term weight control and lowering patients' chances of serious health problems.

Keywords: Glucagon-like peptide 1 receptor agonist; Obesity; Pharmacotherapy; Antiobesity medication; Weight loss

Key Points
Many people with obesity have various health complications, but in spite of the benefits of weight loss, losing and maintaining weight is challenging.
Anti-obesity medication can help people with obesity achieve target weight loss and help to reduce the risk of regaining weight, thereby improving obesity-related health complications.
Glucagon-like peptide 1 receptor agonist therapy provides an effective and well-tolerated treatment option to help people with obesity achieve and maintain weight targets.

DIGITAL FEATURES

This article is published with digital features, including a plain language summary, and video animation, to facilitate understanding of the article. To view digital features for this article go to https://doi.org/10.6084/m9.figshare.14192567.

INTRODUCTION

Obesity (defined as a body mass index [BMI] $\geq 30\ kg/m^2$ in adults [1]) is a major health concern in the USA [2] and is associated with multiple complications, including cardiovascular disease, type 2 diabetes, and osteoarthritis [3–8]. Weight loss of 5–15% is recommended to improve many of the complications of overweight/obesity, with greater improvements observed with further weight reductions [5, 9]. Even a modest weight loss of 5% has been shown to improve cardiometabolic risk factors, including reduced systolic blood pressure and plasma triglyceride concentration, and increased multi-organ insulin sensitivity and β-cell function [6]. Despite evidence that

weight loss improves obesity-related complications, the age-adjusted prevalence of obesity in US adults in 2017–2018 was 42.4% [2], indicating an unmet need in the management of obesity.

Challenges for Achieving and Maintaining Weight Loss, and Pharmacotherapeutic Options for Obesity

Obesity is a chronic disease associated with high rates of relapse after achieving initial weight loss [10, 11]. As such, people with overweight or obesity may face many challenges losing weight and maintaining weight loss. These challenges can involve internal factors such as hormonal influence on the homeostatic regulation of body weight [11]. In addition, energy expenditure is decreased following diet-induced weight loss, with the implication that reduced food intake would need to be maintained in the long-term [12]. Other challenges include external factors due to an increasingly obesogenic environment. Such an environment encompasses the interrelated issues of exposure to high-density, highly caloric foods, the relatively low cost of these foods, and physical environments that limit the scope of physical activity [13]. Indeed, alterations in the levels of weight-regulating hormones along with the obesogenic environment explain why many individuals find maintaining a lower weight as challenging as the initial weight loss.

Given the risk that obesity represents to public health and the difficulty of achieving and maintaining weight loss via lifestyle changes alone, there is a need for pharmacological approaches to aid weight loss in some individuals. There are several anti-obesity medications (AOMs) currently available in the USA as an adjunct to lifestyle modification, each with differing mechanisms of action (Table 1). However, despite their availability, adoption of medications for the management of obesity remains low [14, 15]. Barriers to initiating treatment may include delayed recognition of obesity as a disease by public health and medical organizations, provider biases regarding patients with obesity, lack of reimbursement leading to out-of-pocket costs to patients, inadequate training of providers, historical safety issues with AOMs, and perceptions of patients and their caregivers regarding the efficacy of AOMs [14–17]. In the ACTION study, 3008 individuals with obesity and 606 healthcare providers were questioned on their obesity-related perceptions, attitudes, and behaviors. Only 27% of patients and 30% of healthcare providers believed prescription AOMs to be completely effective for weight management, and most survey respondents found other interventions to be more effective than AOMs [16]. Nearly all other interventions listed (including improved eating habits, exercise tracking, counseling or lifestyle modification, and visiting a dietitian) were perceived to be more effective than AOMs [16], indicating a lack of knowledge about the potential benefits of pharmacotherapy.

Development of Glucagon-Like Peptide 1 Receptor Agonists for the Management of Obesity

Glucagon-like peptide 1 receptor agonists (GLP-1RAs), including liraglutide and semaglutide, were initially developed for the treatment of type 2 diabetes but were found to be effective not only in reducing blood glucose levels but body weight as well [18–20]. Consequently, once-daily subcutaneous administration of liraglutide 3.0 mg was developed for the treatment of obesity [21] and once-weekly subcutaneous administration of semaglutide 2.4 mg is currently being investigated in phase III trials for this indication [22]. In contrast to other AOMs, which either suppress appetite or inhibit fat absorption [23], GLP-1RAs reduce body weight in a number of ways, decreasing appetite and hunger, and increasing satiety, resulting in reduced energy intake [24–26]. The purpose of this review is to further elucidate the mechanism of action of GLP-1RAs in helping individuals with overweight or obesity to achieve and maintain weight loss.

We searched PubMed and Embase databases using the terms glucagon-like peptide receptor agonists; obesity; anti-obesity; weight; overweight; bodyweight; overweight; anti-obesity

Table 1 Mechanism of action and efficacy of anti-obesity medications currently available in the USA [10, 21, 23, 37, 39, 43, 46, 47, 57, 62–65, 69–78]

Anti-obesity medication	Type of agent/ mechanism of action [23]	Trial information	Percentage of patients achieving categorial weight loss at 1 year		
			≥ 5% (or > 5%)	≥ 10% (or > 10%)	Percentage of patients achieving weight loss maintenance at 2 years
Liraglutide	· GLP-1RA · Reduces appetite and food cravings [21] · Increases satiety · Alters food preference and reward pathways [21]	Astrup et al., 2009; Astrup et al., 2012: placebo-controlled, randomized, 20-week trial for liraglutide (1.2, 1.8, 2.4, and 3.0 mg QD) with open-label comparator (orlistat 120 mg TID) + 84-week extension in patients with BMI 30–40 kg/m^2 Patients were on a 500-kcal/day energy-deficient diet and increased their physical activity	(Liraglutide 3.0 mg; orlistat; placebo) 73%; 44%; 28% (liraglutide vs. placebo or orlistat, $p \leq 0.0001$)	(Liraglutide 3.0 mg; orlistat; placebo) 37%; 14%; 10%	(Liraglutide 2.4/3.0 mg vs. orlistat) **≥ 5% weight loss**: 52% vs. 29% ($p < 0.001$) **≥ 10% weight loss**: 26% vs. 16% ($p = 0.04$)
		Pi-Sunyer et al., 2015: placebo-controlled, double-blind, randomized, 56-week trial of liraglutide 3.0 mg QD in patients with BMI ≥ 30 kg/m^2 (or ≥ 27 kg/m^2 with dyslipidemia or hypertension) Patients received counseling on lifestyle modification	(Liraglutide 3.0 mg; placebo) 63%; 27% ($p < 0.001$)	(Liraglutide 3.0 mg; placebo) 33%; 11% ($p < 0.001$)	NR
		Davies et al., 2015: placebo-controlled, randomized, double-blind, parallel-group 56-week trial of liraglutide 1.8 and 3.0 mg QD in patients with BMI ≥ 27 kg/m^2 with diabetes taking 0–3 OADs Patients were on a 500-kcal/day energy-deficient diet and increased their physical activity	(Liraglutide 3.0 mg; placebo) 54%; 21% ($p < 0.001$)	(Liraglutide 3.0 mg; placebo) 25%; 7% ($p < 0.001$)	NR
		Wadden et al., 2013: placebo-controlled, double-blind, randomized, 56-week trial of liraglutide 3.0 mg QD in patients with BMI ≥ 30 (or ≥ 27 with comorbidity) kg/m^2 after low-calorie-diet-induced weight loss Patients received diet and exercise counseling	(Liraglutide 3.0 mg; placebo)[a] 51%; 22% ($p < 0.0001$)	(Liraglutide 3.0 mg; placebo)[a] 26%; 6% ($p < 0.0001$)	NR
		Wadden et al., 2020b: placebo-controlled, double-blind, randomized, 56-week trial of liraglutide 3.0 mg QD plus IBT in patients with BMI ≥ 30 kg/m^2	(Liraglutide 3.0 mg; placebo) 62%; 34% ($p = 0.0003$)	(Liraglutide 3.0 mg; placebo) 31%; 20% ($p = 0.0469$)	NR
		Garvey et al., 2020: placebo-controlled, double-blind, randomized, 56-week trial of liraglutide 3.0 mg QD plus IBT in patients with BMI of ≥ 27 kg/m^2 and diabetes treated with basal insulin and ≤ 2 OADs	(Liraglutide 3.0 mg; placebo) 52%; 24.0% ($p < 0.0001$)	(Liraglutide 3.0 mg; placebo) 23%; 7% ($p < 0.0001$)	NR

Table 1 continued

Anti-obesity medication	Type of agent/ mechanism of action [23]	Trial information	Percentage of patients achieving categorial weight loss at 1 year		
			≥ 5%(or > 5%)	≥ 10%(or > 10%)	Percentage of patients achieving weight loss maintenance at 2 years
Naltrexone-bupropion	· Naltrexone: opioid antagonist · Bupropion: aminoketone antidepressant [62] · Suppresses appetite	Greenway et al., 2010: placebo-controlled, double-blind, randomized, 56-week trial of naltrexone-bupropion (NB16 and NB32[b]) BID in patients with BMI ≥ 30 (or ≥ 27 with comorbidity) to 45 kg/m^2 Patients were on a mild hypocaloric diet and exercise	(NB16; NB32; placebo) 39%; 48%; 16% (NB16/NB32 vs. placebo, both $p < 0.0001$)	(NB16; NB32; placebo) 20%; 25%; 7% (NB16/ NB32 vs. placebo, both $p < 0.0001$)	NR
		Apovian et al., 2013: placebo-controlled, double-blind, randomized, 56-week trial of naltrexone-bupropion (NB32) BID in patients with BMI ≥ 30 (or ≥ 27 with controlled hypertension and/or dyslipidemia) to 45 kg/m^2 Patients were on a 500-kcal/day energy-deficient diet, increased physical activity, and behavioral modification advice	(NB32; placebo) 51%; 17% ($p < 0.001$)	(NB32; placebo) 28%; 6% ($p < 0.001$)	NR
		Wadden et al., 2011: placebo-controlled, double-blind, randomized, 56-week trial of naltrexone-bupropion (NB32) QD and BMOD in patients with BMI ≥ 30 (or ≥ 27 with controlled hypertension and/or dyslipidemia) to 45 kg/m^2	(NB32; placebo) 66%; 43% ($p < 0.001$)	(NB32; placebo) 42%; 20% ($p < 0.001$)	NR
		Hollander et al., 2013: placebo-controlled, double-blind, randomized, 56-week trial of naltrexone-bupropion (NB32) QD in patients with BMI ≥ 27 and ≤ 45 kg/m^2 and type 2 diabetes treated with or without OADs Patients were on a 500-kcal/day energy-deficient diet, dietary counseling and advice on behavioral modification, including instructions to increase physical activity	(NB32; placebo) 45%; 19% ($p < 0.001$)	(NB32; placebo) 19%; 6% ($p < 0.001$)	NR

Table 1 continued

Anti-obesity medication	Type of agent/ mechanism of action [23]	Trial information	Percentage of patients achieving categorial weight loss at 1 year		
			≥ 5%(or > 5%)	≥ 10%(or > 10%)	Percentage of patients achieving weight loss maintenance at 2 years
Orlistat	· Reversible inhibitor of gastrointestinal lipases [65] · Inhibits fat absorption	Hauptman et al., 2000: placebo-controlled, double-blind, randomized, 2-year trial of orlistat (60 and 120 mg TID) in patients with BMI 30–44 kg/m^2 Patients were on an energy-deficient diet	(Orlistat 60 mg; orlistat 120 mg; placebo) 49%; 51%; 31% (Orlistat 60 mg/ 120 mg vs. placebo, both $p < 0.001$)	(Orlistat 60 mg; orlistat 120 mg; placebo) 24%; 29%; 11% (Orlistat 60 mg/ 120 mg vs. placebo, both $p < 0.001$)	(Orlistat 60 mg; orlistat 120 mg; placebo) **≥ 5% weight loss:** 34%; 34%; 24% (Orlistat 60 mg vs. placebo: $p = 0.03$; orlistat 120 mg vs. placebo: $p = 0.02$) **≥ 10% weight loss:** 15%; 19%; 7% (Orlistat 60 mg vs. placebo: $p = 0.008$; orlistat 120 mg vs. placebo: $p = 0.001$)
		Rössner et al., 2000: placebo-controlled, double-blind, randomized, 2-year trial of orlistat (60 and 120 mg) TID in patients with BMI 28–43 kg/m^2 Patients were on a 600-kcal/day energy-deficient diet	NR	(Orlistat 60 mg; orlistat 120 mg; placebo) 31%; 38%; 19% (Orlistat 60 mg vs. placebo: $p = 0.002$; orlistat 120 mg vs. placebo: $p = 0.001$)	(Orlistat 60 mg; orlistat 120 mg; placebo) **> 10% weight loss:** 29%; 28%; 19% (Orlistat 60 mg/ 120 mg vs. placebo: both $p < 0.05$)
Phentermine	· Phentermine: sympathomimetic amine anorectic [63] · Suppresses appetite	Kang et al., 2010: placebo-controlled, double-blind, randomized, 12-week trial of phentermine 30 mg QD in patients with obesity and controlled diabetes, hypertension, and dyslipidemia	(Phentermine; placebo) 96%; 21% ($p < 0.001$)	(Phentermine; placebo) 63%; 5% ($p < 0.001$)	NR

Table 1 continued

Anti-obesity medication	Type of agent/ mechanism of action [23]	Trial information	Percentage of patients achieving categorial weight loss at 1 year		
			≥ 5%(or > 5%)	≥ 10%(or > 10%)	Percentage of patients achieving weight loss maintenance at 2 years
Phentermine-topiramate	· Phentermine: sympathomimetic amine anorectic [64] · Topiramate: anti-epileptic drug · Suppresses appetite	Allison et al., 2012: placebo-controlled, randomized, 56-week trial of PT (3.75/23 mg or 15/92 mg) QD added to a reduced-energy diet in patients with BMI ≥ 35 kg/m^2 Patients were advised to follow a 500-kcal/day energy-deficient diet and received standardized diet and lifestyle-modification counseling	(PT 3.75/23 mg; PT 15/92 mg; placebo) 45%; 67%; 17% (PT 3.75/23 mg/15/92 mg vs. placebo, both $p < 0.0001$)	(PT 3.75/23 mg; PT 15/92 mg; placebo) 19%; 47%; 3% (PT 3.75/23 mg/15/92 mg vs. placebo, both $p < 0.0001$)	NR
		Gadde et al., 2011; Garvey et al., 2012: placebo-controlled, double-blind, randomized, 108-week trial of PT (7.5/46 mg or 15/92 mg) QD in patients with BMI 27–45 kg/m^2 and cardiometabolic disease Patients received standardized diet and lifestyle-modification counseling	(PT 7.5/46 mg; PT 15/92 mg; placebo) 62%; 70%; 21% (PT 5/46 mg/15/92 mg vs. placebo, both $p < 0.0001$)	(PT 7.5/46 mg; PT 15/92 mg; placebo) 37%; 48%; 7% (PT 5/46 mg/15/92 mg vs. placebo, both $p < 0.0001$)	(PT 7.5/46 mg; PT 15/92 mg; placebo) **≥ 5% weight loss:** 75%; 79%; 30% **≥ 10% weight loss:** 50%; 54%; 12% (PT 7.5/46 mg/15/92 mg vs. placeb, both $p < 0.0001$)

Percentages are rounded up to one decimal place

BID two times a day, *BMI* body mass index, *BMOD* intensive behavior modification, *GLP-1RA* glucagon-like peptide 1 receptor agonist, *IBT* intensive behavioral therapy, *NR* not reported, *OAD* oral antihyperglycemic drug, *PT* phentermine-topiramate, *QD* once-daily, *TID* three times per day

[a] Based on patients achieving ≥ 5% weight loss during the run-in period

[b] NB16: sustained-release naltrexone 16 mg per day plus sustained-release bupropion 360 mg per day combined in fixed-dose tablets; NB32: sustained-release naltrexone 32 mg per day plus sustained-release bupropion 360 mg per day combined in fixed-dose tablets

agents; appetite; food intake regulation; caloric; satiety; gastric emptying; energy intake; craving; cravings; eating control; safety; tolerability; tolerated; adverse; hypoglycemia; nausea; diarrhea; vomiting; gastrointestinal; long-term; durable; maintain*; sustain*. Records were limited to those in English language ($N = 247$). Records were excluded during screening if they were press releases, news reports, not relevant drug/indication/population, preclinical study, reviews, case reports, not a randomized trial, or not in humans. Searches last updated November 26, 2020 ($N = 16$). Supplementary searches were performed to identify overview of approved AOMs and background information. American Association of Clinical Endocrinologists and American College of Endocrinology,

and European guidelines for obesity were hand searched for relevant data.

This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

EFFECTS OF GLP-1RAS ON APPETITE, SATIETY AND HUNGER, AND GASTRIC EMPTYING

GLP-1RAs are attractive agents for the management of obesity owing to the actions of GLP-1 on appetite and energy intake.

GLP-1 is released from the L cells in the gut in response to energy intake, and facilitates a multitude of physiological actions, including a delay in gastric emptying [27]. In pharmacology trials, GLP-1RA treatment has been shown to delay gastric emptying within the first postprandial hour [25, 28], although overall gastric emptying did not appear to be affected [28], suggesting additional mechanisms of action in GLP-1RA-mediated weight loss.

In the central nervous system, GLP-1 receptors are located in the hypothalamus, which is involved in regulating food intake [24, 29, 30]. Coveleski et al. found that acute administration of the GLP-1RA exenatide resulted in reduced feelings of hunger in eight women with obesity. The reduced feelings of hunger were associated with an increase in functional connectivity of the nucleus tract solitaries with the hypothalamus and thalamus [31]. In addition, murine models show that liraglutide can access specific brain areas relevant for appetite regulation, binding GLP-1 receptors on proopiomelanocortin and cocaine- and amphetamine-regulated transcript (POMC/CART)-expressing arcuate nucleus neurons [32]. GLP-1 directly stimulates POMC/CART neurons and indirectly inhibits neuropeptide Y (NPY) and agouti-related peptide (AgRP) to increase measures of satiety and decrease hunger [32]. These effects of GLP-1 can lead to reduced energy intake [27], thereby facilitating weight loss (Fig. 1).

Studies investigating the mechanism of action of GLP-1RA therapy for causing weight loss provide evidence that GLP-1RA treatment is associated with reductions in appetite and hunger, lower preference for energy-dense foods, alteration in food reward pathways, decrease in food cravings, and improvement in eating control (Table 2) [25, 26, 33, 34].

Clinical Trials Demonstrating Reductions in Body Weight with GLP-1RAs

Several clinical trials have reported that the effects described above resulted in larger reductions in body weight with GLP-1RA therapy compared with placebo in participants with obesity. After 5 weeks of treatment with once-daily subcutaneous administration of liraglutide 1.8 mg and 3.0 mg, estimated reductions in body weight were − 2.1 kg and − 2.5 kg, respectively, vs. − 0.3 kg with placebo [25]. In another liraglutide trial, 16-week median (interquartile range) body weight reductions were − 5.8 kg (− 6.9, − 4.45) with liraglutide 3.0 mg and − 1 kg (− 3.5, 2.53) with placebo ($p < 0.003$) [35]. For once-weekly subcutaneous administration of semaglutide 1.0 mg, change from baseline in mean body weight after 12 weeks was − 5.0 kg vs. + 1.0 kg with placebo [26]. A recent 20-week, phase II trial investigated the effects of subcutaneous administration of semaglutide 2.4 mg on gastric emptying, appetite, and energy intake in patients with obesity. There was no significant difference between semaglutide and placebo in gastric emptying when corrected for week-20 body weight. However, patients receiving semaglutide 2.4 mg experienced reduced hunger, and increased fullness and satiety compared with placebo ($p < 0.02$). Ad libitum mean energy intake was also reduced by 35% for semaglutide 2.4 mg vs. placebo (1736 vs. 2676 kJ; estimated treatment difference [ETD], − 940 kJ; $p < 0.0001$). Patients receiving semaglutide 2.4 mg in this trial lost 9.9% of their body weight, compared with 0.4% in those receiving placebo [33]. In a phase II study of the long-acting GLP-1RA efpeglenatide (4 mg once weekly, 6 mg once weekly, 6 mg once every

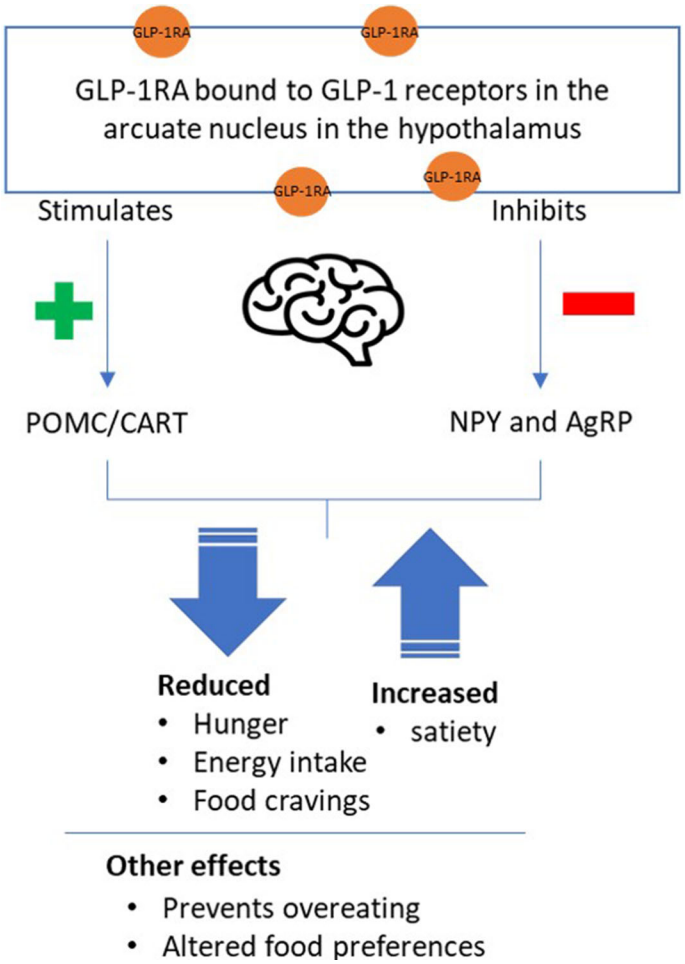

Fig. 1 Overview of the actions of GLP-1 in the central nervous system [27, 29, 32, 41]. *AgRP* agouti-related peptide, *CART* cocaine- and amphetamine-regulated transcript, *GLP-1RA* glucagon-like peptide 1 receptor agonist, *NPY* neuropeptide Y, *POMC* proopiomelanocortin

2 weeks, and 8 mg once every 2 weeks), patients with obesity and without diabetes had statistically significant reductions in body weight compared with placebo after 20 weeks of treatment (differences in least squares means were − 6.3 to − 7.2 kg; $p < 0.0001$) [36].

Longer-term data with GLP-1RAs include results from a phase III, 56-week study of liraglutide 3.0 mg vs. placebo in patients with obesity and without diabetes. After 56 weeks of treatment with liraglutide 3.0 mg vs. placebo, patients had mean body weight reductions of − 8.4 kg vs. − 2.8 kg (ETD − 5.6 kg; 95% confidence interval [CI] − 6.0 to − 5.1; $p < 0.001$) [37]. In a recently published landmark phase III study in patients with overweight or obesity (STEP 1), greater reductions in body weight were observed after 68 weeks of treatment with once-weekly semaglutide 2.4 mg vs. placebo (mean change from baseline − 14.9% vs. − 2.4%; ETD − 12.4%; 95 CI − 13.4 to − 11.5; $p < 0.001$) [38]. Similarly, in a 68-week phase III study comparing the effects of semaglutide 2.4 mg vs. placebo in adults with overweight or obesity without diabetes (STEP 3), mean body weight decreased 16.0% with semaglutide compared with 5.7% with placebo, both as adjunct to intensive behavioral therapy (ETD − 10.3%; 95% CI − 12.0 to − 8.6; $p < 0.0001$) [39]. Ongoing studies in the STEP program will

Table 2 Summary of clinical data on the effects of GLP-1RA therapy on energy intake, meal duration, appetite, satiety and hunger, food preferences, and gastric emptying [25, 26, 33]

Agent	Comparator(s)	Trial population	Trial duration	Energy intake[a]	Meal duration	Appetite	Satiety	Hunger	Food preference	1-h gastric emptying
Liraglutide 1.8 mg and 3.0 mg[b] [25]	Placebo	Individuals with obesity but without diabetes	Two treatment periods of 5 weeks with a 6–8-week washout period in between	Reduced by 16% vs. placebo($p = 0.003$)	NR	Reduced vs. placebo ($p = 0.0003$)	Reduced vs. placebo ($p = 0.002$)	Reduced vs. placebo ($p = 0.01$)	NR	23% lower vs. placebo ($p = 0.007$)
Semaglutide 1.0 mg [26]	Placebo	Individuals with obesity but without diabetes	12 weeks	Reduced by approx. 35% vs. placebo ($p < 0.0001$)	Shorter vs. placebo ($p = 0.0018$)	Reduced vs. placebo ($p = 0.0023$)	Increased (NS)	Reduced (NS)	Lower preference for high-fat and non-sweet foods vs. placebo ($p = 0.0016$)	27% lower vs. placebo ($p = 0.0012$) [27]
Semaglutide 2.4 mg [33]	Placebo	Individuals with obesity but without diabetes	20 weeks	35% lower vs. placebo ($p < 0.0001$)	NR	Reduced vs. placebo ($p = 0.001$)	Increased vs. placebo ($p < 0.02$)	Reduced vs. placebo ($p < 0.02$)	Reduced cravings for savory food ($p < 0.02$)	No clinically relevant effect vs. placebo

EE energy expenditure, *NR* not reported, *NS* non-significant

[a] Based on results for ad libitum lunch

[b] Results are for liraglutide 3.0 mg

provide further insights into the effects of once-weekly semaglutide 2.4 mg in a broader population [22].

In addition to the above findings, several studies have shown that GLP-1RA therapy results in larger proportions of patients achieving 5% and 10% body weight loss than with placebo (Table 1).

Safety and Tolerability of GLP-1RAs for the Treatment of Obesity

Since GLP-1RA therapy is used for the treatment of type 2 diabetes, there may be concerns about whether using an antidiabetic medication to treat obesity will increase the risk of hypoglycemia in people with obesity but without diabetes. However, the action of GLP-1 is glucose-dependent and blood glucose is only lowered by GLP-1 if concentrations are above fasting levels [40, 41]. This effect translates to a low risk of hypoglycemia in patients with type 2 diabetes treated with GLP-1RAs [42]. In individuals without diabetes, the potential for hypoglycemia with GLP-1RA treatment would therefore also be expected to be low. Indeed, Garvey et al. found that hypoglycemia was less common with liraglutide 3.0 mg than with placebo in individuals with overweight or obesity and insulin-treated type 2 diabetes [43]. Pi-Sunyer et al. also reported a low risk of hypoglycemia, with events occurring in similar proportions of patients with obesity but without diabetes who were treated with liraglutide 3.0 mg (1.3%) compared with placebo (1.0%) [37]. In a similar patient population of adults with obesity who did not have diabetes, no severe or blood glucose-confirmed symptomatic hypoglycemic events were reported with semaglutide 1.0 mg [26].

In studies of GLP-1RA therapy in individuals with type 2 diabetes, the most frequently reported adverse events tend to involve the gastrointestinal system, with nausea, vomiting, and diarrhea occurring in up to 51%, 19%, and 20% of patients, respectively [44]; however, in clinical trials for once-daily subcutaneous administration of liraglutide 3.0 mg in patients with obesity only, or with obesity and diabetes, these effects tended to be mild-to-moderate and transient. Nausea, vomiting, and diarrhea were typically the most common gastrointestinal adverse events, occurring in up to 48.4%, 23.2%, and 23.1% of participants, respectively, with events occurring predominantly during dose escalation and decreasing over time [10, 37, 43, 45–47]. Since gastrointestinal adverse effects are rarely severe and tend to diminish over time, they would therefore not be expected to cause a barrier to initiating and continuing treatment for most patients. Indeed, GLP-1RA therapy appears to be well tolerated overall, and proportions of clinical trial participants discontinuing treatment because of adverse events tend to be low (5.4–9.9%) [10, 37, 39, 43, 46, 47].

Management Strategies for Gastrointestinal Adverse Events with GLP-1RA Therapy

There are various strategies that can be employed to help manage or mitigate potential gastrointestinal adverse events when initiating a GLP-1RA for the treatment of overweight or obesity. A gradual dose-escalation is recommended for liraglutide 3.0 mg, starting with the initial dose of 0.6 mg per day for 1 week, then increasing the dose in weekly increments until the maximum therapeutic dose of 3.0 mg is reached [21]. If the patient experiences gastrointestinal adverse effects during dose escalation, uptitration can be delayed for an additional week [21]. This has been demonstrated by Gough et al. [48], who reported smaller proportions of patients experiencing gastrointestinal adverse events with a combination of insulin degludec and liraglutide (IDegLira) vs. liraglutide alone, attributing the findings to a more gradual dose escalation with IDegLira [48]. In the authors' anecdotal experience, when further uptitration is not tolerated, but treatment effects are noted, maintaining the patient at the lower tolerated dose may be preferable to discontinuation. This strategy was used for the STEP 1 trial of semaglutide 2.4 mg [38].

Other strategies include providing counseling regarding the potential gastrointestinal adverse events that could arise, such as nausea, vomiting, and diarrhea. Patients should be informed of dietary modifications that could help reduce symptoms such as smaller portion sizes and avoiding fatty foods [49].

ASSOCIATIONS BETWEEN WEIGHT LOSS AND GASTROINTESTINAL ADVERSE EVENTS ON GLP-1RA THERAPY

While gastrointestinal adverse events that occur with GLP-1RAs are mainly mild-to-moderate and transient, occurring particularly during dose escalation, there may be concerns that GLP-1RA-mediated weight loss could be due to these effects. Data collected from a randomized, placebo-controlled, double-blind trial of liraglutide 3.0 mg, in which nausea was the most frequent adverse event, showed that greater weight loss was associated with transient nausea and vomiting in participants with obesity but without diabetes [50]. In a phase II study, gastrointestinal side effects were most common during semaglutide dose escalation but weight loss persisted beyond these events and continued through the 52-week trial period [51]. The fact that patients in this trial continued to lose weight after gastrointestinal adverse effects had subsided suggests that these effects were not the cause of weight loss. A retrospective analysis of the DURATION trials found that overall, greater weight loss was associated with gastrointestinal adverse events with exenatide once weekly and exenatide twice daily. Conversely, the same analysis found no difference in weight loss for exenatide once weekly or liraglutide between patients experiencing gastrointestinal adverse events and those with none in DURATION-6 [52]. Furthermore, a study of patients with type 2 diabetes who were treated with once-weekly exenatide found significant reductions in weight regardless of whether patients experienced nausea or vomiting [53]. However, patients with type 2 diabetes were included in these trials [52, 53], which could have contributed to the observed differences.

GLP-1RA-MEDIATED WEIGHT LOSS MAINTENANCE

Obesity is a chronic condition, characterized by changes in weight-regulating hormones that drive weight regain following weight loss [11], therefore it is no surprise that maintaining weight loss can prove just as challenging as losing weight in the first place. Compensatory changes in the levels of weight-regulating hormones such as leptin, ghrelin, peptide YY, and gastric inhibitory peptide can counteract diet-induced weight loss, highlighting the difficulty in maintaining weight loss through diet alone [11]. Furthermore, when treated with an AOM, patients with obesity have experienced weight regain upon cessation of the AOM [10, 54]. This suggests that, to maintain weight loss, treatment for obesity should be considered chronic (as in the case of hypertension), rather than as a short course of treatment associated with acute illnesses [9, 55].

Treatment with an AOM often follows a pattern of initial weight loss that tends to level out, or "plateau", after a period of time on treatment [54, 56, 57]. Metabolic adaptation is most likely the reason for this plateau [11] rather than poor response or resistance to the medication. Given this pattern in weight loss following treatment with an AOM, anti-obesity therapy should be conceptualized as initiating weight loss followed by establishing a new weight plateau and assisting in maintaining this weight in the long term [9, 11].

One of the mechanisms thought to be responsible for weight regain or plateau is a reduction in circulating levels of leptin after initial weight loss [11, 58]. Indeed, it has been suggested that preservation of free leptin levels is involved in GLP-1RA-mediated maintenance of weight loss [58]. In a trial in which patients with obesity were treated with or without liraglutide 1.2 mg after diet-induced body weight loss of 12%, smaller decreases in free leptin and higher levels of PYY_{3-36} were observed with liraglutide vs. without liraglutide

[58]. In another trial, higher levels of PYY_{3-36} were also observed relative to baseline after 16 weeks of treatment with liraglutide 3.0 mg [34]. In addition, clinical evidence has shown that continued treatment with GLP-1RA therapy is associated with maintenance of weight loss [10, 47]. For example, after 2 years of treatment with liraglutide 2.4/3.0 mg in patients with obesity, 52% and 26% maintained at least 5% and at least 10% weight loss, respectively, compared with 29% and 16% of patients receiving orlistat [47] (Table 1). Furthermore, treatment with liraglutide 3.0 mg resulted in greater proportions of patients maintaining at least 5% weight loss over 56 weeks vs. placebo after an initial run-in period on a low-calorie diet (81.4% vs. 48.9%, respectively) [10]. These data suggest a potential for long-term benefit of liraglutide treatment in many patients, as weight loss of 5–15% has been shown to improve obesity-related complications including diabetes and cardiovascular disease risk factors [5, 9, 59]. In a cardiovascular outcomes trial of liraglutide 1.8 mg, weight loss was sustained over a median trial period of 3.5 years in patients with type 2 diabetes who had either cardiovascular disease or risk factors for cardiovascular disease [60]. Since obesity is associated with an increased risk of cardiovascular disease, the ongoing SELECT study is investigating whether once-weekly semaglutide 2.4 mg will reduce the risk of having cardiovascular events in patients with overweight or obesity and with prior cardiovascular disease [61].

PLACE OF GLP-1RAS IN THE TREATMENT OF OBESITY

Currently, pharmacotherapy is recommended as an adjunct to lifestyle modification for individuals with a BMI of at least 30 kg/m^2, or at least 27 kg/m^2 with comorbidity [5, 9]. Achieving body weight loss of 5–15% can improve cardiometabolic parameters including prediabetes, dyslipidemia, and hypertension [5, 9]; therefore, target weight loss should be defined on the basis of the individual patient's presentation. Adding an AOM to lifestyle modification can help patients to achieve these weight-loss targets, thereby reducing the risks of obesity-related complications [5].

Treatment Decision-Making

Treatment decision-making can be guided by the different mechanisms of action of AOMs to ascertain the suitability of the therapy for the individual patient. For instance, if patients present with symptoms such as early hunger or lack of satiety then a GLP-1RA may be appropriate compared with other available treatments that work solely by suppressing appetite or inhibiting fat absorption [23].

As well as taking into account the mechanism of action of each drug class, there are certain contraindications and adverse effects to consider, which could determine the suitability of the drug for individual patients. For example, naltrexone-bupropion is not suitable for patients with uncontrolled hypertension [62] and phentermine is contraindicated in patients with a history of cardiovascular disease [63]. Patients with cardiovascular risk factors may therefore find a GLP-1RA more appropriate owing to the improvements in cardiometabolic parameters that have been observed with this drug class [10, 37, 46, 47]. Naltrexone-bupropion has a black box warning for suicidal thoughts and behaviors and neuropsychiatric reactions [62]. In addition, phentermine-topiramate is contraindicated in those taking certain antidepressant drugs [64]. Therefore, liraglutide could be an alternative option to these drugs if the patient has ongoing mental health problems, although it should be avoided in patients with a history of suicidal attempts or active suicidal ideation [21]. Importantly, orlistat is contraindicated in patients with chronic malabsorption syndrome and cholestasis [65], conditions that require avoidance of fatty foods. Since GLP-1RA therapy has been shown to reduce preference for fatty foods [26], GLP-1RA therapy might be preferred for patients who would find it difficult to avoid fatty foods under normal circumstances. If other health problems such as prediabetes or polycystic ovary syndrome (PCOS) are present, GLP-1RA therapy

might be preferred over other treatment options. This is due to the reduced incidence of prediabetes in patients with obesity [37, 46, 47] and improved markers for ovarian function in patients with obesity and PCOS [66], compared with placebo.

Rapid weight loss has been associated with gallbladder-related disorders including cholelithiasis and cholecystitis [67, 68], and these adverse events, while rare, have been observed more with GLP-1RAs compared with placebo in clinical trials [21, 38]. There have been post-marketing reports of acute pancreatitis with liraglutide 3.0 mg; however, incidence of this adverse event in clinical trials was very low [21]. Although rare, healthcare providers should monitor their patients for symptoms of these adverse events, and treatment should be discontinued if gallbladder- or pancreatic-related disorders are suspected [21].

Although GLP-1RAs are generally well tolerated, there are some circumstances in which this drug class is not recommended. Liraglutide has a black box warning for thyroid C cell tumors [21], therefore this therapy is contraindicated for patients with a personal or family history of medullary thyroid carcinoma or multiple endocrine neoplasia syndrome type 2.

Suboptimal Treatment Response with AOMs

Once a treatment has been initiated, it is important to consider what to do in the event of a suboptimal treatment response. After treatment initiation with liraglutide 3.0 mg, it is recommended that, if a patient has not lost at least 4% of their baseline body weight after 16 weeks, the treatment should be discontinued because it is unlikely that significant weight loss will be achieved with the medication after this time point [21]. For other AOMs, it is suggested to stop treatment if weight loss of more than 5% has not been achieved after 12 weeks of treatment [9]. If a suboptimal treatment response occurs, another therapy could be selected taking into consideration the patient's presentation and the drug profile of the available options.

SUMMARY

GLP-1RA-mediated weight loss is achieved through multiple pathways including effects on the central nervous system such as reduced appetite, energy intake, and hunger, increased feelings of satiety, and altered food preferences. The risk of gastrointestinal adverse events is increased with GLP-1RA therapy but these effects tend to be mild-to-moderate and transient, and are not the reason for observed weight loss. Overall, GLP-1RAs provide a highly effective and well-tolerated treatment option to help individuals with obesity achieve and maintain body weight reductions of 5–10%, thereby improving weight-related complications in addition to weight loss.

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Compliance with Ethics Guidelines. This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

Data Availability. Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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Semaglutide seems to be more effective the other GLP-1Ras

Jens Juul Holst[1], Sten Madsbad[2]

[1]Department of Biomedical Sciences, [2]Department of Endocrinology, Copenhagen University Hospital Hvidovre, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Correspondence to: Jens Juul Holst, MD, DMedSci. Health Sciences Faculty, University of Copenhagen, The Panum Institute, Building 12.2.24, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark. Email: jjholst@sund.ku.dk.

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Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) were introduced as treatment options for type 2 diabetes (T2DM) in 2005 (1). They have become popular because of their efficacy and durability in relation to glycaemic control, and their low risk of hypoglycaemia in combination with weight loss in most patients (2,3).

GLP-1 RAs mimic the effects of native GLP-1, including potentiation of glucose-induced insulin secretion, inhibition of glucagon secretion, inhibition of gastric emptying and inhibition of appetite and food intake (2,3). Notably, the insulinotropic and glucagonostatic effects are glucose dependent, meaning that insulin secretion is only stimulated at euglycaemic or elevated glucose concentrations, while hypoglycaemia-induced glucagon secretion surprisingly is not inhibited. Therefore, the risk of hypoglycaemia is very low during treatment with a GLP-1 RA, unless it is combined with sulfonylureas or insulin (2-4).

The GLP-1 RAs fall into two categories, the short acting and the long acting agonists. Today the former only include agents identical to (Exenatide) or derived from (Lixisenatide) the Gila Monster salivary peptide, exendin 4 (5). With their subcutaneous half-lives of 2–3 hours, their effect wears off rapidly and mainly covers a single meal. It turns out that the effect on gastric emptying is primarily observed with the short acting GLP-1 RAs, since significant tachyphylaxis for this effect develops, within hours, upon continued exposure with a GLP-1 agonist, and the effect is nearly gone after few days' treatment with the long acting GLP-1 RAs (6,7). The explanation for this and for the absence of tachyphylaxis regarding the metabolic effects remains unknown. In addition, GLP-1 RAs reduce blood pressure during chronic treatment and increase pulse rate, both by still unknown mechanisms. The agonists also appear to reduce postprandial triglyceride concentrations (8-10) by an effect that appears to be independent of the effects on gastric emptying, but may reflect inhibition of chylomicron formation (11). Agonist treatment does not lead to fat malabsorption, though.

GLP-1 has repeatedly been reported to exert protective effects on the beta cells, originally by promoting beta-cell proliferation (which may only apply to young beta cells) and inhibition of cytokine- and FFA-induced apoptosis (12). This effect might be expected to reduce or halt the progression of type 2 diabetes, but the findings in this regard are unclear (13). In one study, beta cell function was evaluated after three years of treatment with high doses of a short acting GLP-1 RA (exenatide), and during this period there was no deterioration, but the same was true in the control group subjected to intensive insulin therapy (14), suggesting that both approaches may be protective. In the LEADER study of the cardiovascular safety of the long-acting GLP-1 RA, liraglutide, hemoglobin A1c levels remained almost unchanged over a period of up to 5 years without changes in the liraglutide dosing and in spite of significantly lesser increases in concomitant antidiabetic medications than in the placebo group. Since beta cell function would otherwise be expected to deteriorate significantly in a period of this duration, the finding is likely

to reflect some protective action on the beta cells, although the nature of this remains unclear (15).

Studies in rodent models of Parkinson's and Alzheimer's diseases and mouse models of ischaemic stroke have suggested that GLP-1 receptor agonist might have neuroprotective effects and prevent memory impairment (16-18). However, studies in humans have not supported the use of GLP-1 RA in cerebral diseases (19), except for one clinical trial of 48 weeks, which suggested that exenatide once weekly had positive effects in Parkinson's disease, which were sustained beyond the period of exposure (20). Whether the exenatide therapy affects the underlying disease pathophysiology or the result simply is secondary to long-lasting metabolic improvements is uncertain.

Apart from these actions, the GLP-1 RAs may also have protective cardiovascular effects and recently, three cardiovascular outcomes studies, showing beneficial effects of GLP-1 RAs on cardiovascular risk in patients with type 2 diabetes and heart problems have appeared (15,21,22). These results are likely to further support the enthusiasm for these agents.

The most common adverse events of The GLP-1 RAs are nausea and other gastrointestinal discomfort (2,3) which are usually mild to moderate and usually subside after a few weeks. A slow up-titration schedule often prevents most of the nausea. Other drawbacks of the GLP-1 RAs include the parenteral administration and the cost (2).

As a drug class, the GLP-1RAs have proven efficacy for lowering HbA1c and decreasing weight in T2D, with a reduced risk of hypoglycaemia compared with insulin or sulphonylureas (1,2,23). These characteristics underlie the inclusion of GLP-1RAs in various clinical practice guidelines. Their use as dual therapy with metformin after first-line metformin and as triple therapy (in combination with metformin and a sulphonylurea/thiazolidinedione/insulin) is part of the European Association for the Study of Diabetes/American Diabetes Association recommendations (1). GLP-1 RAs are recommended as monotherapy, dual therapy and triple therapy by the American Association of Clinical Endocrinologists/American College of Endocrinology guidelines (23).

Semaglutide once weekly

Liraglutide is a long-acting GLP-1 RA developed by NovoNordisk from the backbone of human (mammalian) GLP-1 (24). The prolonged action was obtained by addition of a palmitic acid moiety to residue no 26 via a glutamic acid linker (also the the Lys in position 34 was changed to Arg to prevent acylation at this residue), inspired by the experience gained by the company with acylated insulin (detemir). The acylation results in albumin binding, prolongation of the absorption phase from the injection site, reduced degradation by the enzyme dipeptidyl peptidase 4 (DPP-4) and prevention of renal elimination. The modification resulted in a s.c. half-life of 12–13 hours. On the basis of the experience with liraglutide, semaglutide was developed from liraglutide by changing 3 things: (I) Ala in position 8 was substituted to Aib (alpha-amino-iso-butyric acid; a change known to result in complete DPP-4 resistance); (II) substitution of the palmitic acid with a C-20 di-acid; and (III) introduction of a longer and more flexible linker. This increased its half-life in humans to 165 hours without significantly changing its ability to activate the GLP-1 receptor (25,26). This was interpreted to support a once weekly scheme of administration. Importantly, semaglutide was developed not only with respect to long duration of action, but also on the basis of its ability to stimulate both insulin secretion and inhibit food intake, and was selected among hundreds of acylated GLP-1 analogues, varied with respect to the fatty acid moiety, the linker and the peptide backbone.

The safety and efficacy of semaglutide has been evaluated in a series of phase 2 and 3 clinical studies among which the first 6 trials have been presented in public. In a 12-week phase 2 study, semaglutide reduced HbA1c by impressive 1.7% from a baseline of 8.1% and lowered body weight by up to 4.8 kg, which was greater than with liraglutide 1.8 mg QD (27). Semaglutide doses of 0.5 and 1.0 mg and a 4-week dose escalation scheme were then selected for the SUSTAIN phase 3 program (27). In SUSTAIN-1, semaglutide 0.5 and 1.0 mg in patients with type 2 diabetes reduced HbA1c from a baseline of 8.1% by 1.4% and 1.5% compared with placebo after 30 weeks, and about 73% reached a HbA1c below 7.0% and 60% below 6.5% (28). Weight loss was 2.8 and 3.6 kg greater than with placebo, respectively (28). In the 56 weeks SUSTAIN 2 trial, semaglutide 0.5 and 1.0 mg reduced HbA1c by 1.3% and 1.6% versus 0.5% with sitagliptin (baseline: 8.1%). Weight losses were 4.3, 6.1 and 1.9 kg, respectively (29). In the SUSTAIN-3 trial, semaglutide was compared with exenatide QW. After 56 weeks, semaglutide 1.0 mg reduced HbA1c by 1.5% from a baseline HbA1c of 8.3%, compared with 0.9% with exenatide QW, and 67% *vs.* 40% reached a HbA1c <7.0%, respectively. Weight losses were 5.6 and 1.9 kg, respectively. Gastrointestinal adverse events occurred in 42% and 33%, and injection site reactions were

reported by 1.2% and 22% respectively. In SUSTAIN-4, semaglutide was compared with insulin glargine in insulin naïve patients. After 30 weeks, the reduction in HbA1c was 1.2%, 1.6% and 0.8% from a baseline of 8.2% with 0.5 and 1.0 mg of semaglutide and insulin glargine, respectively (30). Weight loss was 3.5 and 5.2 kg versus a weight gain of 1.2 kg with insulin glargine (30). Risk of hypoglycaemia was also reduced with semaglutide. Efficacy and safety of semaglutide versus placebo as add-on to basal insulin were investigated in SUSTAIN-5. After 30 weeks (baseline HbA1c 8.4%) 61% and 79% versus 11% with 0.5 mg, 1.0 mg or placebo had achieved a HbA1c below 7.0%. Weight losses were 3.7, 6.4 and 1.4 kg, respectively.

In SUSTAIN-6, semaglutide given once weekly was evaluated in two doses (0.5 or 1.0 mg) versus placebo in 3,297 patients with type 2 diabetic (21). At baseline 83% had established cardiovascular disease, chronic kidney disease or both. After 104 weeks, the primary outcome: cardiovascular death, nonfatal myocardial infarction or nonfatal stroke was reduced by 26%, P<0.001, nonfatal myocardial infarction by 26%, P=0.12 and nonfatal stroke by 39%, P=0.04) (21). Rates of all-cause-mortality as well as cardiovascular mortality were similar in the two groups. In total 45 patients would need to be treated for 2 years to prevent one primary endpoint. Revascularization surgery rates were also greatly reduced by semaglutide compared with placebo and rates of new or worsening of nephropathy were significantly lover, but rates of retinopathy complications significantly higher with semaglutide (21). A similar worsening of diabetic retinopathy was observed in the DCCT studies of intensified insulin therapy in patients with type 1 diabetes, and this side effect is currently not considered specifically associated with semaglutide therapy.

The SUSTAIN-7 trial is a head-to-head comparison between semaglutide and dulaglutide as add-on to metformin during 40 weeks (press release Novo Nordisk 17. August 2017). Patients in the 0.5 mg semaglutide group had a reduction in HbA1c of 1.5% against a 1.1% reduction in the 0.75 mg dulaglutide group. Additionally, 1.0 mg of semaglutide reduced HbA1c by 1.8% compared with a decrease by 1.4% among patients treated with 1.5 mg dulaglutide. Those on 0.5 mg semaglutide lost on average 4.6 kg of body weight compared to 2.3 kg with 0.75 mg dulaglutide. The higher doses led to losses of 6.5 kg and 3.0 kg, respectively. The side effects including changes in retinopathy did not differ between the two GLP-1 RAs.

Overall, semaglutide seems at least as effective and possible more potent than the other GLP-1RAs. The safety profile of semaglutide did not differ from those reported with other GLP-1 RAs (21,28). Semaglutide has not yet been approved for treatment of type 2 diabetes, but the advisory committee of the FDA in October 2017 unanimously recommended approval of semaglutide diabetes therapy.

The unusual efficacy of semaglutide, not the least with respect to loss of appetite, has inspired the company to develop semaglutide further for obesity without diabetes. It has been suggested that higher doses of GLP-RAs are needed for the weight loss effects, but the use of higher doses of semaglutide was not supported by the phase 2 studies. Because it was felt that the limiting side effects were mainly caused by plasma concentration peaks reached early after the weekly injections, it was decided to investigate lower, but daily doses. In this way, with an agent with a half-life of 165 hours, it should be possible to almost completely eliminate troughs and peaks. This was tested in a 52-week double-blind phase 2 clinical trial with once-daily subcutaneous semaglutide in 957 people with obesity, randomised to 0.05 to 0.4 mg/day or placebo (n=100 per group). In this trial, weight losses up to 17.8 kg from 111 kg (BMI, 39) (13.8% *vs.* 2.3% placebo) were observed (press release 2017).

The effectiveness of dulaglutide has also led to attempts to deliver this GLP-1 RA by the oral route (31). For this, semaglutide was co-formulated with SNAC {Sodium N-[8-(2-hydroxybenzoyl) Amino] Caprylate (Eligen^{R})}, developed by the company Emisphere. This allows a very rapid absorption from the gastrointestinal tract (within minutes). But because of the long half-life of the compound, daily dosing is appropriate. The bioavailability is rather low (a few per cent) and variable, but, again because of the long half-life of the compound, all that is needed is a small dose to "top up" what is already present. This means that the plasma levels remain relatively constant in spite of the variable absorption. OraI semaglutide was evaluated in a phase 2 study of 600 patients with T2DM and a baseline HbA1c of 7.9%; their weight was 92 kg. Semaglutide was dosed as 2.5–40 mg orally for 26 weeks, and the results were compared to those obtained with 1 mg subcutaneous semaglutide dosed weekly. HbA1c decreased from –0.7% to –1.9% as compared to –0.3% with placebo and –1.9% with semaglutide 1 mg s.c. once weekly. Those treated with placebo experienced a weight loos of –1 kg whereas the maximal weight loss with both oral and s.c. semaglutide was –6.5 kg; the side effects were said to be similar in those receiving the high doses of oral semaglutide and those receiving the subcutaneous injections, and were reported to

diminish over time (32).

The question remains why semaglutide seems more effective that the other GLP-1RAs, including liraglutide. This question cannot currently be answered; obviously, the high, rather constant levels of the compound may contribute and also its efficacy with respect to receptor activation, possibly resulting from the full DPP-4 protection and the improved linker function. The weight effect of the GLP-1RAs is believed to be exerted via receptors in the central nervous system. These receptors are probably reached by the agonists in their free, non-protein bound form via leaks in the blood brain barrier, particularly the area postrema, the subfornical organ and the median eminence (33). But it is also possible that the acyl moiety of the acylated compounds facilitate entry into additional regions of the CNS, and that liraglutide and semaglutide may differ in this respect.

The recent demonstration of positive cardiovascular effects of the GLP-1 RAs is extremely encouraging in relation to the clinical use of these compounds. The best results so far have been obtained with semaglutide in the SUSTAIN 6 trial as mentioned above (21). The MACE effect in this trial was driven by a reduction in the incidences of cardiovascular events (nonfatal stroke, nonfatal myocardial infarction), and there were also significant, large beneficial effects on kidney function and a marked, highly significant reduction in revascularization procedures. Strikingly, however, there were no effects on cardiovascular mortality. In addition, there were pronounced effects on HbA1c and body weight. This might suggest that the therapy prevented these events from happening, and that the preventive effect was possibly due to the metabolic effects of the compound. Further studies are, however, required to settle this question, but the beneficial effect of the drug remains even though an explanation is currently unavailable.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Semaglutide is neuroprotective and reduces α-synuclein levels in the chronic MPTP mouse model of Parkinson's disease

Liping Zhang[1], LingYu Zhang[1], Lin Li[1], Christian Hölscher[2,3]

1: Key Laboratory of Cellular Physiology, Shanxi Medical University, Taiyuan, Shanxi, PR China

2: Second hospital Neurology Dept., Shanxi medical University, Taiyuan, Shanxi, PR China

3: Research and Experimental Center, Henan University of Chinese Medicine, Zhengzhou, Henan province, China

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Corresponding author:

Prof. Lin Li
Key Laboratory of Cellular Physiology,
Shanxi Medical University,
Taiyuan, PR China
Email: linlilin999@163.com

Abstract

Parkinson's disease (PD) is a progressive neurological motor control disorder. A key feature is the loss of midbrain dopaminergic neurons and the accumulation of aggregated alpha-synuclein (α-syn). No current treatment is on the market that slows or halts disease progression. Previous studies have shown that glucagon-like peptide-1 (GLP-1) receptor agonists have neuroprotective effects in animal models of PD. In addition, in a phase II clinical trial, the GLP-1 receptor agonist exendin-4 has shown good protective effects in PD patients. In the present study, we have investigated the neuroprotective effects of the GLP-1 analogues semaglutide (25nmol/kg ip. once every two days for 30 days) and liraglutide (25nmol/kg ip. once daily for 30 days) in the chronic MPTP mouse model of PD. Both drugs are currently on the market as a treatment for Type II diabetes. Our results show that both semaglutide and liraglutide improved MPTP-induced motor impairments. In addition, both drugs rescued the decrease of tyrosine hydroxylase (TH) levels, reduced the accumulation of α-syn, alleviated the chronic inflammation response in the brain, reduced lipid peroxidation, and inhibited the mitochondrial mitophagy signaling pathway, and furthermore increased expression of the key growth factor GDNF that protects dopaminergic neurons in the substantia nigra (SN) and striatum. Moreover, the long- acting GLP-1 analogue semaglutide was more potent compared with once daily liraglutide in most parameters measured in this study. Our results demonstrate that semaglutide may be a promising treatment for PD. A clinical trial testing semaglutide in PD patients will start shortly.

Key words: insulin; growth factors; oxidative stress; inflammation; GLP-1; incretin

1. Introduction

Parkinson disease (PD) is the second most common degenerative disease characterized by progressive loss of dopaminergic neurons in the substantia nigra pars compacta, motor impairments, and deposition of intraneuronal inclusions known as Lewy bodies [1]. The main symptoms are resting tremors, muscular rigidity, bradykinesia, and postural and gait abnormalities [2]. Recently, studies have shown a link between PD and type 2 diabetes (T2DM), another common chronic neurodegenerative disorder characterized by progressive hyperglycemia, pancreatic β-cell dysfunction and insulin resistance (IR) in peripheral tissues [3]. Both PD and T2DM are age-related chronic diseases, and these also share several genetic susceptibilities, such as single nucleotide polymorphisms in the growth factor signaling kinase gene Akt, which can increase individual's risk for developing PD and diabetes [4]. Insulin signaling was found to be impaired in the brains of PD patients, impairing energy utilization and cell repair [5-7]. Insulin is a key growth factor that protects neurons [8, 9].

Drugs that had been initially developed to treat type II diabetes have been re-purposed as treatments for Parkinson's disease [10, 11]. These drugs are long-lasting, protease resistant mimetics of the hormone and growth factor glucagon-like peptide -1 (GLP-1) [12-14]. GLP-1 is neuroprotective and can re-sensitize insulin signaling [15-17]. The GLP-1 mimetic exendin-4 (exenatide, Bydureon), which is on the market to treat T2DM, showed a therapeutic effect in different animal models of PD [18-21]. Exendin-4 was protective in a pilot clinical trial in PD patients (NCT01174810)[22, 23]. Importantly, a phase II clinical trial showed protective effects in PD patients even 3 months after treatment had been discontinued[24]. Liraglutide is a GLP-1 analogue [25, 26] that has an extended survival time in the blood stream and has a half-life of approximately 12 hours in humans [27, 28]. Therefore, it requires a once-daily dosing for treating diabetes [29]. Liraglutide also showed neuroprotective effects in animal models of PD and Alzheimer's disease [30, 31]. Liraglutide is currently being tested in a phase II trial in PD patients (clinical trial identifier NCT02953665).

Semaglutide is a modification of liraglutide that is protease-resistant by changing the amino

acid at position 8 and an extended spacer for the attached fatty acid [32], and is on the market as a new once- weekly drug to treat type II diabetes. It has been approved in the USA and Europe as a treatment for diabetes [33, 34]. A phase II clinical trial testing semaglutide in PD patients will start early 2019 (NCT03659682). Previously, we have investigated the neuroprotective effects of the once-weekly GLP-1 analogue semaglutide and compare it with liraglutide in the acute MPTP mouse model of PD [35]. However, the acute model is not considered to be a good representation of the pathology observed in PD, as the disease develops slowly over time. Therefore, we investigated these neuroprotective effective in the chronic MPTP treatment PD mouse model. Additionally, we analyzed the effects of the drugs on α-synuclein expression and on the impairments of autophagy that has been observed in the MPTP mouse model. If autophagy is impaired in the disease, proteins such as α-synuclein can accumulate and aggregate in the cell. As semaglutide will be tested in PD patients in a clinical trial, it is of vital importance to investigate the underlying molecular mechanisms of its actions.

2. Materials and methods

2.1. Chemicals and peptides

Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was purchased from Sigma-Aldrich (St Louis, MO, USA). Other chemicals used were of the highest quality commercially available. Semaglutide and liraglutide (peptide purity: 95.77%) was purchased from Synpeptide Co. (Shanghai, China). The quality was tested using HPLC and MALDI-TOF analysis.

The amino acid sequence of liraglutide:
HAEGTFTSDVSSYLEGQAAK[(γE)-(Pal)]EFIAWLVRGRG-OH
Pal = palmitoyl acid

The amino acid sequence of semaglutide [32]: HXEGTFTSDVSSYLEGQAAKN6-(N-(17-carboxy-1-oxoheptadecyl)-L-gamma-glutamyl-2-(2-(2-aminoethoxy)ethoxy)acetyl-2-(2-(2-aminoethoxy)ethoxy)acetyl)EFIAWLVRGRG-OH
X = aminoisobutyric acid;

2.2. Animals and drug treatments

Male C57BL/6 mice 8 weeks old (20-25g) were purchased from the Experimental Animal Center, Shanxi Medical University. The animals were maintained on 12 hour light/dark cycle and provided food and water ad libitum. Mice were randomly divided into six groups (N=12 animals per group). A: control group treated with saline alone; B: liraglutide group treated with liraglutide (25nmol/kg ip. once daily for 30 days); C: semaglutide group treated with semaglutide (25nmol/kg ip. once every two days for 30 days), D: MPTP group treated with MPTP alone (once daily 20mg/kg ip. for 30 days); E: MPTP (once daily 20mg/kg ip. for 30 days) + liraglutide treated group (25nmol/kg ip. once daily for 30 days). F: MPTP (20mg/kg ip. once daily for 30 days) + semaglutide- treated group (25nmol/kg ip. once every two days for 30 days). At the end of drug treatments, behavioral changes, neuronal damage, inflammatory markers, and other biomarkers were assessed.

All animal experiments were approved by the ethics committee of Shanxi Medical University.

2.3. Behavioral assessment

2.3.1. Open-field test

The open-field test is used to evaluate locomotor and exploratory activity of PD mice and was conducted on the 31th day after MPTP treatment. The open-field apparatus consisted of a circular arena (35cm diameter floor and 40cm high walls), and a computer tracking system (Etho Vision XT software, Noldus information technology, Wageningen, Netherlands). Each mouse was placed in the center of the apparatus, and tracking started immediately. After acclimatizing for 10 min, the distance travelled by the animal was recorded by the tracking system. The area was wiped with 75% alcohol and dried between each trial.

2.3.2. Rotarod performance

The rotarod test is to measure motor coordination in the mouse model of PD. The rotarod equipment (YLS-4C, Academy of medical sciences in Shandong, China) consisted of a rotating spindle and five individual compartments. All mice were pre-trained for 3 days prior to drug administration. The test consisted of three consecutive runs with a gradual increase in rpm up to a maximum 30 rpm for up to 180 seconds. The length of time was recorded as the latency period to fall. The experiment was repeated three times for each animal at 10 min rest

intervals.

2.3.3. Footprint gait test

The footprint test was described previously [35]. Briefly, the animal forelimbs were dipped in blue ink and the hind limbs in red ink to record footprints as they walked through a dark tunnel (10×10×50 cm). The footprints were recorded on a clean sheet of white paper placed on the floor of the tunnel. The two initial steps were excluded from the measurements, and only steps performed in a straight line were recorded. To avoid differences in the stride length as a result of velocity variations, the footprints were only recorded when the mice walked along the tunnel with a regular velocity, and excluding the mice that performed the test with perceptible velocity alterations. Stride lengths were determined by measuring the distance between each step on the same side of the body. The length of the shortest stride was subtracted from the length of the longest stride to determine the stride variability.

2.3.4. Grip Strength Test

Grip strength was measured by the digital grip strength meter 47200 (Ugo Basile, Italy). Grip strength testing is commonly used as an objective measure of muscle strength in the front legs. All mice were pre-trained for 3 days prior to test. Each mouse was placed in the platform apparatus, and to grasp a lever that can transmit the force value by its forelimbs, then they were pulled at the tail until release of grip to measure the muscle strength of their forelimbs. Each group was tested 3 times and measured in Newtons (N). If the third value was highest, the subject was tested until the value stopped increasing. The maximum muscle strength of each mouse was taken for statistical analysis.

2.4. Brain tissue preparation

All animals were killed on the 31th day of MPTP injection. After ethyl carbamate anesthetization, the brains of 6 mice per group were selected and the substantia nigra and striatum were dissected and immediately frozen at – 80℃ for immunoblot analysis. Another 6 mice per group were intracardially perfused with 20 ml saline and then fixed with 20 ml of cold 4% paraformaldehyde (PFA). Brains were immediately removed and post-fixed in 4%

PFA 24 hours for immunohistochemistry analysis.

2.5. Immunohistochemistry

The fixed brain tissue samples were embedded in paraffin, and sections were cut at 4μm with a semiautomatic microtome (Leica, Wetzlar, Germany). The sections encompassing the substantia nigra pars compacta (SNpc) and the striatum were placed on glass slides, then the paraffin was removed from the tissue sections with xylene, and the sections were rehydrated in descending concentrations of ethanol solutions. Then the sections were put into H2O2 (3%) for 10 min to block the activity of endogenous peroxidase. Antigen retrieval was performed by heating in 10 mmol/L citrate buffer for 10 min. After blocking with 5% BSA, sections were incubated with the primary antibody for tyrosine hydroxylase (TH) (rabbit anti-TH; 1:200; cat. No. ab75875, Abcam, Cambridge, UK), GFAP (rabbit anti-GFAP; 1:200; cat. No. PB0046, Boster Biotechnology Co., Ltd. Wuhan, China) and IBA1 (goat anti-IBA1; 1:200; cat. No. PB0517, Boster Biotechnology Co., Ltd. Wuhan, China), 4-HNE (rabbit anti-4-HNE; 1:400; cat. No. ab46545, Biosynthesis Biotechnology Co., Ltd. Beijing, China) at 37℃ for 1 hour. Then they were rinsed in PBS and incubated a secondary peroxidase-conjugated antibody kit (Boster, Wuhan, China) at 37℃ for 0.5 h. Stained sections were viewed under a Zeiss light microscope, and images were captured by a digital camera (Motic BA410; Motic, Xiamen, China). Quantitative analysis of DA neurons in SNpc was carried out in the region spanning from -2.92 mm to -3.40 mm relative to bregma. The region corresponding to the SNpc was clearly delineated, according to the mouse brain atlas of Paxinos and Franklin [36]. The magnification was kept the same for all measurements. Each mouse had one section analyzed with n=6 per group. Numbers of GFAP, IBA1, 4-HNE positive cells in SNpc were determined using Image-pro plus 6.0 software. All data were expressed as a percentage of control values. Abbreviations: 4-Hydroxynonenal (4-HNE); Glial fibrillary Acid Protein (GFAP); ionized calcium-binding adapter molecule 1 (IBA-1); B-cell lymphoma 2 (Bcl-2); Bcl-2 associated X protein (BAX); DA =dopamine

2.6. Western blots

Brain tissue with substantia nigra was stored at -80 °C for western blot analysis. The tissue

was weighed and cut into pieces in cold radio immune precipitation (RIPA) buffer (Beyotime Institute of Biotechnology, Shanghai, China). Two hours later, tissue lysates were added to phenylmethanesulfonyl fluoride (PMSF) and put on ice for 30 minutes. Tissue lysates were obtained by centrifugation at 12,000 rpm for 20 min at 4 C°. Protein concentration was measured by the bicinchoninic acid protein assay (Boster Biotechnology Co., Ltd. Wuhan, China). Samples mixed with loading buffer to the same concentration were boiled for 5 min. Samples with equivalent amounts of protein were run on 8%, 10% or 12% SDS-polyacrylamide gel and transferred protein band onto polyvinylidene difluoride (PVDF) membranes. Then, the membranes were blocked with 5% bovine serum albumin for two hours. The membranes were probed overnight at 4 °C with primary antibodies that specifically detect Bcl-2 (1:1000; #BS70205, Bioworld Technology, lnc. MN, USA), Bax (1:500; #BS2538, Bioworld Technology, lnc. MN, USA), and β-Actin (1:5000; #ab8227, Abcam, Cambridge, UK), α-Syn (1:1000; #2642, Cell Signaling Technology, lnc.), ATC7 (1:000; #BS6046, Bioworld Technology, lnc.MN, USA), LC3 (1:1000; #L7543, Sigma-Aldrich, lnc. USA)], Beclin 1 (1:000; #AP0768, Bioworld Technology, lnc. MN, USA), SQSTM1 (1:000; #AP6006, Bioworld Technology, lnc. MN, USA), or GDNF (1:500; #ab18956, Abcam, Cambridge, UK), followed by labeling with secondary antibodies (goat-anti-rabbit -IgG-horseradish peroxidase, HRP), 1:5000; (Abcam, Cambridge, UK) shake for 2 h. The relative immunoreactive bands were captured by a chemiluminescence imaging system (Sagecreation, Beijing, China), and visualized by using ECL-enhanced chemilluminescence (Boster Biotechnology Co., Ltd. Wuhan, China), and digitalized by the image analysis system of Quantity One 4.31 (Bio-Rad, Hercules, CA, USA). As an indicator for mitochondrial apoptosis, the ratio of BAX/Bcl-2 levels was computed and graphed. Abbreviations: autophagy chaperone mediator 7 (ATC7); sequestosome 1 (SQSTM1); 1A/1B-light chain 3 (LC3); LC3 binding protein 62 (p62)

2.7. Statistical analysis

All data were expressed as means ± S.E.M. All analysis was conducted using GraphPad Prism (Graph-Pad software Inc., San Diego, CA, USA). Statistical significance was performed by one-way ANOVA for multiple comparisons followed by Tukey's Multiple

Comparison Test. Statistical significance was set at $P < 0.05$.

3. Results

3.1 Semaglutide and liraglutide normalized motor impairments induced by MPTP

In the open field test, semaglutide alleviated the locomotor impairments induced by MPTP. A one-way ANOVA found an overall difference of distance travelled ($F=13.19$, $P<0.0001$) between all groups. There was no difference between control group and ns+liraglutide group and ns+semaglutide group. However a difference was found between the control groups and MPTP group ($p<0.001$). Furthermore, a difference was found between MPTP+liraglutide and MPTP group ($p<0.01$), and MPTP+semaglutide and the MPTP group ($p<0.001$). This shows that liraglutide and semaglutide were able to normalize the MPTP-induced impairments in locomotor and exploratory activity of mice. There was no significant difference between MPTP + semaglutide group and MPTP + liraglutide groups ($P>0.05$). N=12 per group, see Fig.1a.

In the rotarod test, a one-way ANOVA found an overall difference of the time spent on the rotating rod ($F=37.87$, $P<0.0001$) between all groups. There was no difference between control group and ns+liraglutide group and ns+semaglutide group. However a difference was found between the control group and MPTP group ($p<0.001$). Furthermore, a difference was found between MPTP+liraglutide and MPTP+semaglutide and the MPTP group ($p<0.001$). That is to say the two drugs were able to improve the bradykinesia and imbalance of mice induced by MPTP. Semaglutide was more effective than liraglutide($p<0.01$). N=12 per group, see Fig.1b.

In the Grip Strength Test, a one-way ANOVA found an overall difference of maximum muscle strength ($F=35.86$, $P<0.0001$) between all groups. There was no difference between control group and ns+liraglutide group and ns+semaglutide group, However a difference was found between the control groups and MPTP group ($p<0.001$). Furthermore, a difference was found between MPTP+liraglutide and MPTP+semaglutide and the MPTP group ($p<0.001$). That is to say the two drugs were able to improve the muscle strength of mice that was

impaired by MPTP. Semaglutide was more effective than liraglutide($p<0.05$). N=12 per group, see Fig.1c.

In the foot print test, a one-way ANOVA found an overall difference on the Step variation rate ($F=27.61$, $P<0.0001$) between all groups. There was no difference between control group and ns+liraglutide group and ns+semaglutide group. However a difference was found between the control groups and MPTP group ($p<0.001$). Furthermore, a difference was found between MPTP+liraglutide and MPTP+semaglutide and the MPTP group ($p<0.001$). That is to say the two drugs were able to improve the abnormal posture and gait of mice induced by MPTP. Semaglutide was more effective than liraglutide ($p<0.05$). N=12 per group, see Fig.1d.

3.2 Semaglutide and liraglutide attenuated dopaminergic neuronal loss in the SN induced by MPTP

In the histological analysis of the number of cells positive for the dopamine biomarker tyrosine hydroxylase (TH) in the substantia nigra, MPTP reduced the number of neurons significantly. In a one-way ANOVA with Tukey's multiple comparison test ($F=42.12$, $p < 0.0001$), MPTP groups showed fewer TH positive neurons in the SN than saline-treated mice ($p<0.001$). There was a difference between MPTP+liraglutide and MPTP group ($p<0.01$), and a difference between MPTP+semaglutide and MPTP group ($p<0.001$). Semaglutide was more effective than liraglutide ($p<0.05$), see Fig. 2.

3.3 Semaglutide and liraglutide alleviated astrocyte and microglia activation in the striatum

When analysing GFAP (astrogliosis) levels in the striatum: In a one-way ANOVA with Tukey's multiple comparison test ($F=432.2$, $p < 0.0001$). There was no difference between the control group and the ns+liraglutide group and the ns+semaglutide group. GFAP levels in the MPTP group were found to be far higher in the striatum compared to the control group ($P < 0.001$), a difference was found between the MPTP+liraglutide, MPTP+semaglutide and the MPTP group ($p < 0.001$), and semaglutide was more effective than liraglutide ($p<0.05$); N=6 per group, see Fig. 3a.

When assessing IBA-1 (microgliosis) levels in the striatum: In a one-way ANOVA with Tukey's multiple comparison tests ($F=187.2$, $p < 0.0001$), there was no difference between the control group and ns+liraglutide group and the ns+semaglutide group. In the MPTP group, IBA-1 levels were found to be higher than the control group ($p < 0.001$). A difference was found between the MPTP+liraglutide, MPTP+semaglutide and the MPTP group ($p < 0.001$), and the MPTP+semaglutide group differed from the MPTP+liraglutide group ($p < 0.001$), demonstrating that liraglutide and semaglutide can reduced microgliosis. Semaglutide was the more potent drug. N=6 per group. See Fig. 3b.

3.4 Semaglutide and liraglutide reduced lipid peroxidation in the striatum induced by MPTP

In the immunohistochemical analysis, 4-HNE was monitored as an indicator of lipid peroxidation. A one-way ANOVA found an overall difference of 4-HNE expression in the SN ($F=157.8$, $P<0.001$); There was no difference between the control group and ns+liraglutide group and the ns+semaglutide group. In the MPTP group, 4-HNE levels were found to be higher than in the control group ($p < 0.001$), but identical to the MPTP+liraglutide group and MPTP+semaglutide group. This shows that the liraglutide and semaglutide drugs reduced 4-HNE levels ($p < 0.001$). The MPTP+semaglutide group differed from the MPTP+liraglutide group ($p < 0.001$), demonstrating that both liraglutide and semaglutide reduced 4-HNE levels. Semaglutide was the more potent drug. N=6 per group. See Fig. 4.

3.5 Semaglutide and liraglutide reduced the levels of α-syn in the SN enhanced by MPTP treatment

In the western blot analysis, we investigated the levels of a-syn in the SN. A one-way ANOVA showed an overall difference ($F=139.7$, $p<0.0001$). In Tukey's multiple comparison tests, there was no difference between control group and ns+liraglutide group and ns+semaglutide group. In the MPTP group, α-syn levels were found to be higher than in the control group ($p < 0.001$), the MPTP+liraglutide group and MPTP+semaglutide group ($p < 0.001$). Tthe MPTP+liraglutide group was different from the MPTP+semaglutide group ($p <$

0.01). This shows that both liraglutide and semaglutide drug reduced α-syn levels, and that semaglutide was more effective. N=4 per group. See Fig. 5.

3.6 Semaglutide and liraglutide normalized the Bcl-2/BAX ratio in the SN impaired by MPTP

The increase of Bax/Bcl-2 levels in the substantia nigra of mice induced by MPTP was reversed by the two drugs. A one-way ANOVA showed an overall difference ($F=324.8$, $p<0.0001$). In Tukey's multiple comparison tests, there was no difference between control group and ns+liraglutide group and ns+semaglutide group. The overall levels of the anti-apoptotic signaling molecule Bcl-2 in SN was reduced by MPTP treatment, levels of the pro-apoptotic signaling molecule Bax in SNpc was increased by MPTP treatment, and the ratio of Bax/Bcl-2 was increased ($p< 0.001$), compared with control group. Liraglutide and semaglutide partly decreased the ratio of Bax/Bcl-2 by enhancing Bcl-2 levels and decrease Bax levels ($p< 0.001$). Semaglutide was the more potent drug ($p< 0.001$). N=4 per group, see Fig. 6.

3.7 Semaglutide and liraglutide increased autophagy-related proteins expression in the SN reduced by MPTP.

In the substantia nigra, we investigated the expression of a set of autophagy-related (Atg) proteins. Protein expression of Beclin1, Atg7, LC3 and P62 significantly differs among groups as evident by one-way ANOVA analysis ($p<0.0001$). Tukey's multiple comparison tests demonstrated that insurmountable ER stress induced by MPTP significantly suppresses Beclin1, Atg7, LC3, and P62 expression. Semaglutide and liraglutide treatment significantly enhanced levels of Beclin1, Atg7, L3 and P62. Additionally, semaglutide was more effective than liraglutide. N=4 per group, see Fig. 7.

3.8 Semaglutide and liraglutide increased GDNF expression in the SN reduced by MPTP.

In the western blot analysis, we investigated the expression of GDNF in the SN. A one-way ANOVA showed an overall difference ($F=67.88$, $p<0.0001$). In Tukey's multiple

comparison tests, there was no difference between the control group and ns+liraglutide group and ns+semaglutide group. In the MPTP group, GDNF levels were found to be lower than in the control group ($p < 0.001$), but identical to the MPTP+liraglutide group and MPTP+semaglutide group. This shows that the liraglutide ($p < 0.01$) and semaglutide ($p < 0.001$) partly increased GDNF levels. Semaglutide was the more potent drug ($p< 0.001$) compared to liraglutide. N=4 per group. See Fig. 8.

4. Discussion

PD is characterized by the progressive functional loss of dopaminergic neurons in the SN. One hypothesis is that the progressive deterioration of SN dopaminergic neurons may be caused by misfolding and aggregation of the protein alpha synuclein, disruption of the autophagy system, and mitochondrial dysfunction [37]. 1-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine (MPTP) is widely used to induce a Parkinson-like state in rodents [38, 39], which can cross the blood brain barrier. Then, MPTP is metabolized into the toxic cation1-methyl-4-phenylpyridinium (MPP+) by monoamine oxidase B [40]. MPP+ can kill primarily dopamine-producing neurons in SN [41, 42]. MPP+ interferes with complex1of the mitochondrial electron transport chain, which leads to the production of free radicals and ultimately to neuronal death in the SN [43, 44]. The MPTP animal model is a commonly used model of PD as this chemical can induce a PD-like phenotype in humans [45].

Our study demonstrates that both the GLP-1 analogue semaglutide and liraglutide effectively normalized locomotor and exploratory activity, improved bradykinesia, movement coordination and balance of mice, restored a weakening of muscle strength, and improved postural and gait abnormalities of MPTP-treated mice. These results are in agreement with our previous studies in the acute MPTP model [35]. Importantly, we have previously shown that GLP-1 mimetics do not affect food intake or insulin plasma levels in non-diabetic and non-obese animals [46]. We and others furthermore demonstrated that these drugs can enter the brain and are activate receptors on neurons [47-49].

Tyrosine hydroxylase (TH) is a key enzyme in the synthesis of the catecholamine

neurotransmitters [50] and is the principal regulator of dopamine synthesis in the CNS [51]. In order to investigate whether GLP-1could directly protect against MPTP-induced nigrostriatal degeneration, we measured the number of TH positive cells. Our results demonstrate that both drugs could increase the number in the SN, and show that the once-weekly semaglutide was more effective than the once-daily liraglutide. Chronic inflammation is playing a central role in PD pathogenesis because the release of cytokines promotes disease progression [52, 53]. Damaged dopaminergic neurons and activated microglial cells can stimulate astrocytes into immune-active status [54]. Moreover , the status of astrocyte activation and the release of pro-inflammatory cytokines is associated with impairment of the nigrostriatal system of MPTP treated mice [41, 55]. Recently a study reported that the presence of activated microglia in the SN and putamen of patients with a PD diagnosis [56]. Both central and peripheral inflammation responses are responsible for sustained progression of PD [57]. Our results demonstrate that both GLP-1 analogues can inhibit the inflammatory response. Importantly, semaglutide was more effective compared with liraglutide in our study where both drugs were tested at the same concentration.

Oxidative stress is a key feature of PD and of chronic inflammation that drives disease progression [58]. One study found that lipid peroxidation and the level of 4-Hydroxynonenal (4-HNE) in SNpc are increased in PD [59]. 4-HNE is one of the markers of membrane lipid peroxidation induced by the excessive generation of reactive oxygen species (ROS) [60, 61]. The generation of ROS by MPTP administration is partly due to the inhibition of the mitochondrial complex I activity [62]. 4-HNE furthermore activates BAD, a sensor for mitochondrial dysfunction, and accelerates mitochondrial mitophagy [63]. Our study demonstrates that both drugs can reduce 4-HNE levels in the midbrain of the mouse induced by MPTP treatment, protecting cells of oxidative stress. Again, semaglutide was more potent compared with liraglutide in this experiment.

The mechanisms underlying accumulation and aggregation of α-syn are considered to be based on over-expression and failure to clear α-syn by proteolysis and autophagy pathways [64, 65]. In addition, aberrant forms of α-syn, including oligomers and fibrils, are seen to interfere with normal cellular processes, promoting further aggregation of protein, leading to the spread of these toxic forms of α-syn from neuron to neuron, and ultimately to neuronal

death [66-68]. Oligomeric α-syn is proposed to play a central role in spreading protein aggregation in the brain with associated cellular toxicity, contributing to a progressive neurological decline. One study demonstrated that the cerebrospinal fluid (CSF) of patients with PD contained increased levels of α-syn oligomers when compared to controls [69]. We therefore decided to measure α-syn expression in the brain. We have previously shown that in the MPTP mouse model, α-syn expression is very much increased [63]. Our study demonstrates that the MPTP-induced increase of α-syn expression in the brain is reduced back to almost control levels by the drug, semaglutide again being more potent than liraglutide.

Apoptosis (the most common form of programmed cell death) is closely related to mitochondrial function, because the intrinsic apoptosis pathway is linked to mitochondrial depolarization[70]. B cell leukemia/lymphoma 2 (Bcl-2) -family proteins regulate the intrinsic apoptosis pathway by controlling mitochondrial outer membrane permeability [71]. The anti-apoptotic protein Bcl-2 can bind to the pro-apoptotic protein BAX (Bcl-2-associated X protein) to form heterodimers that modulate apoptosis [72]. Therefore, we measured the ratio of Bax/Bcl-2 levels. Our result show that MPTP treatment led to a decline of Bcl-2 levels and an increase of BAX in the SN, and GLP-1 analogues partly reversed this process. Our results show that the rate of mitophagy and eventually apoptosis is reduced in the brain after drug treatment. In addition, semaglutide showed an advantage compared with liraglutide.

Autophagy removes misfolded proteins and damaged mitochondria to prevent apoptosis caused by mitochondrial dysfunction [73-75]. Some studies show that the autophagy-lysosome system is impaired in PD animal models and evidence for this was also found in the analysis of postmortem PD brain tissue [76, 77]. In physiological conditions, apoptosis is blocked and autophagy maintains intracellular homeostasis; this balance is perturbed in PD [78, 79]. The activation of Beclin-1 leads to autophagosome formation and initiation of autophagy, and a reduced expression of this protein will impair this process. We also measured the conversion of microtubule-associated protein 1 light chain 3 beta-I/LC3B-I to LC3B-II which is important for the sequestration of the phagosome in autophagy. The conversion of LC3B-I to LC3B-II increased after GLP-1 treatment compare with the MPTP

group, and GLP-1 analogues upregulated beclin1 expression, indicating that GLP-1 signalling alleviates the inhibition of autophagy induced by MPTP. Autophagy dysfunction along with persistent ER stress can further trigger the excess accumulation of the autophagy adaptor protein p62, which contains a KEAP1 binding motif similar to the promotor of oxidative stress-reducing genes Nrf275 [80]. Accumulation of p62 leads to KEAP1 sequestration and inactivation, which, in turn, blocks nuclear Nrf2 localization and transcription of Nrf2 target genes [81]. This will lead to an impaired response to enhanced oxidative stress. These results are in line with our previous studies of liraglutide effects on autophagy [82].

Glial cell line-derived neurotrophic factor (GDNF) is one of the most potent trophic factors that have been identified for midbrain dopamine (DA) neurons, and plays an important role in the postnatal survival of mesencephalic dopamine neurons [83, 84]. In 1993 Glial cell line-derived neurotrophic factor (GDNF) was first been shown to protect embryonic dopaminergic neurons in vitro [85]. The therapeutic benefit of GDNF and NRTN has been demonstrated in phenotypic, toxin-induced (MPTP) rodent and nonhuman primate models of PD [86-89]. Our study demonstrates that the MPTP-induced loss of GDNF in the brain was reversed by both drugs, demonstrating that GLP-1 signalling can rescue the decrease of GDNF levels induced by MPTP. This is in line with previous studies [90, 91]. In addition, semaglutide showed an advantage compared with liraglutide

In summary, our result showed that semaglutide and liraglutide normalized impaired motor activity, increased the number of TH positive neurons in the SN, reduced the expression of α-syn, and decreased inflammation, oxidative damage and mitophagy while increasing autophagy, and furthermore increasing GDNF expression. These conclusion confirm our previous findings that GLP-1 receptor agonists have neuroprotective effects in PD mouse models[15]. In this mouse model of PD, semaglutide appears to be more effective than liraglutide under the conditions chosen in this study. As both liraglutide and semaglutide are in clinical trials in PD patients, we will be able to see if this outcome translates into the clinic.

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Conflict of interest

CH is a named inventor on patent applications that cover the use of GLP-1 receptor agonists as a treatment for Parkinson’s disease. The patent is owned by Ulster university, UK.

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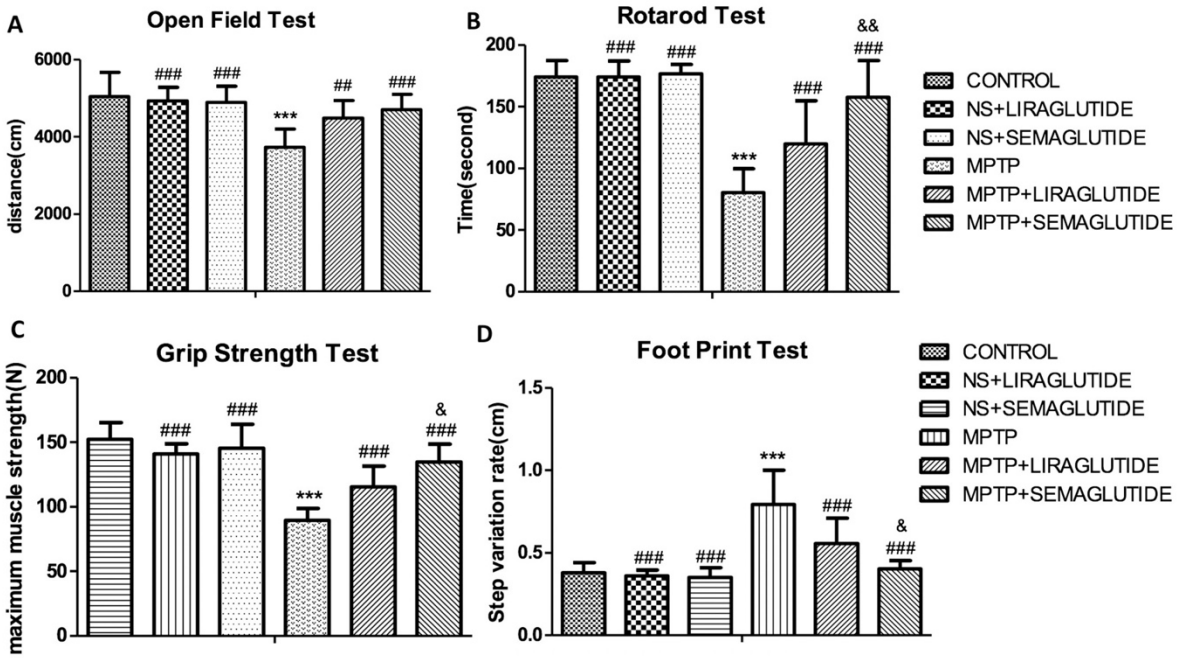

Fig. 1A. GLP-1 analogues protect from the MPTP-induced impairments in motor activity of mice. A difference was found between the control group and MPTP group. Furthermore, a difference was found between MPTP+ liraglutide and MPTP+semaglutide and the MPTP group. NS= normal saline. The values represent the means±S.E.M. ***=p<0.001 compared with the control group. ##=p<0.01, ###=p<0.001 compared with the MPTP group. n=12.

Fig. 1B. GLP-1 analogues improve the bradykinesia and imbalance of mice induced by MPTP. A difference was found between the control group and MPTP group. Furthermore, a difference was found between MPTP + liraglutide and MPTP+semaglutide and MPTP group. NS= normal saline. The values represent the means±S.E.M. ***=p<0.001 compared with the control group. ###=p<0.001 compared with the MPTP group. &&=p<0.01 compared with MPTP+liraglutide group. n=12.

Fig. 1C. GLP-1 analogues improve the muscle strength weakening of mice induced by MPTP. A difference was found between the control group and MPTP group. Furthermore, a difference was found between MPTP + liraglutide and MPTP+semaglutide and MPTP group. NS= normal saline. The values represent the means±S.E.M. ***=p<0.001 compared with the control group. ###=p<0.001 compared with the MPTP group. &=p<0.05 compared with MPTP+liraglutide group. n=12.

Fig. 1D. GLP-1 analogues improve the abnormal posture and gait of mice induced by MPTP. A difference was found between the control group and MPTP group. Furthermore, a difference was found between MPTP+ liraglutide and MPTP+semaglutide and MPTP group. NS= normal saline. The values represent the means±S.E.M. ***=p<0.001 compared with the control group. ###=p<0.001 compared with the MPTP group. &=p<0.05 compared with MPTP+liraglutide group. n=12.

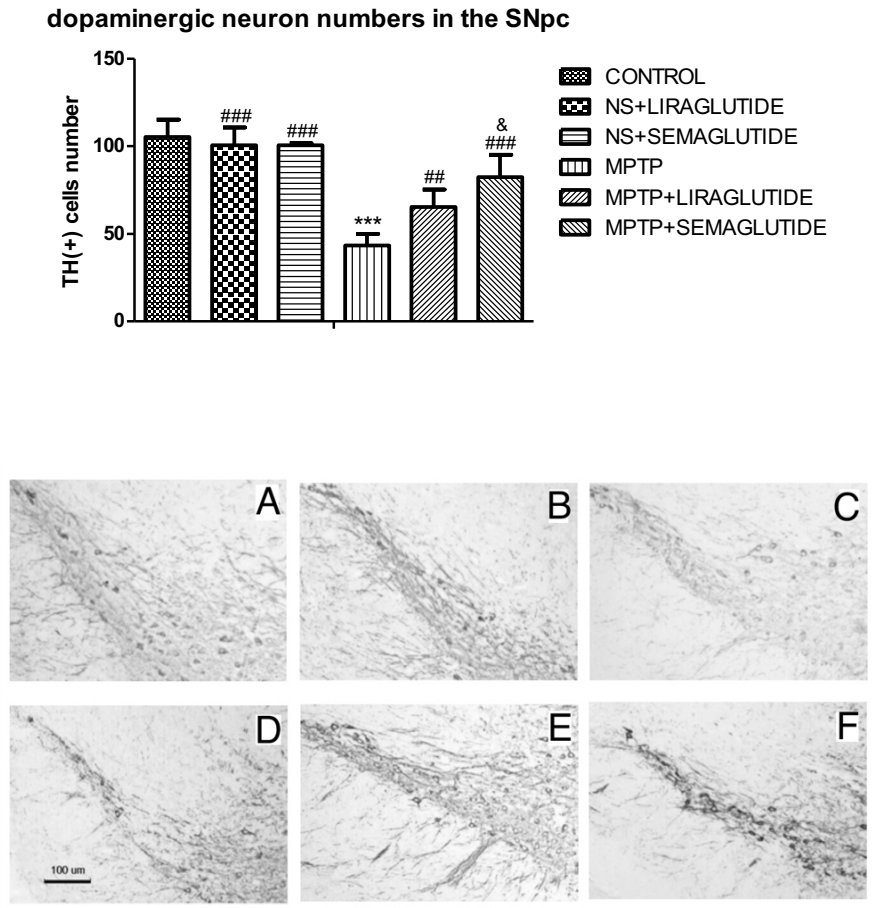

Fig. 2. GLP-1 analogues restored tyrosine hydroxylase (TH) positive dopaminergic neuron numbers in the substantia nigra. NS= normal saline. The values represent the means±S.E.M. ***=p<0.001 compared with the control group. ##=p<0.01, ###=p<0.001 compared with the MPTP group. &=p<0.05 compared with MPTP+liraglutide group. n=6. Examples of micrographs are given. A: CONTROL; B: NS+LIRAGLUTIDE; C: NS+SEMAGLUTIDE; D: MPTP; E: MPTP+LIRAGLUTIDE; F: MPTP+SEMAGLUTIDE. Scale bar in image D: 100 μm.

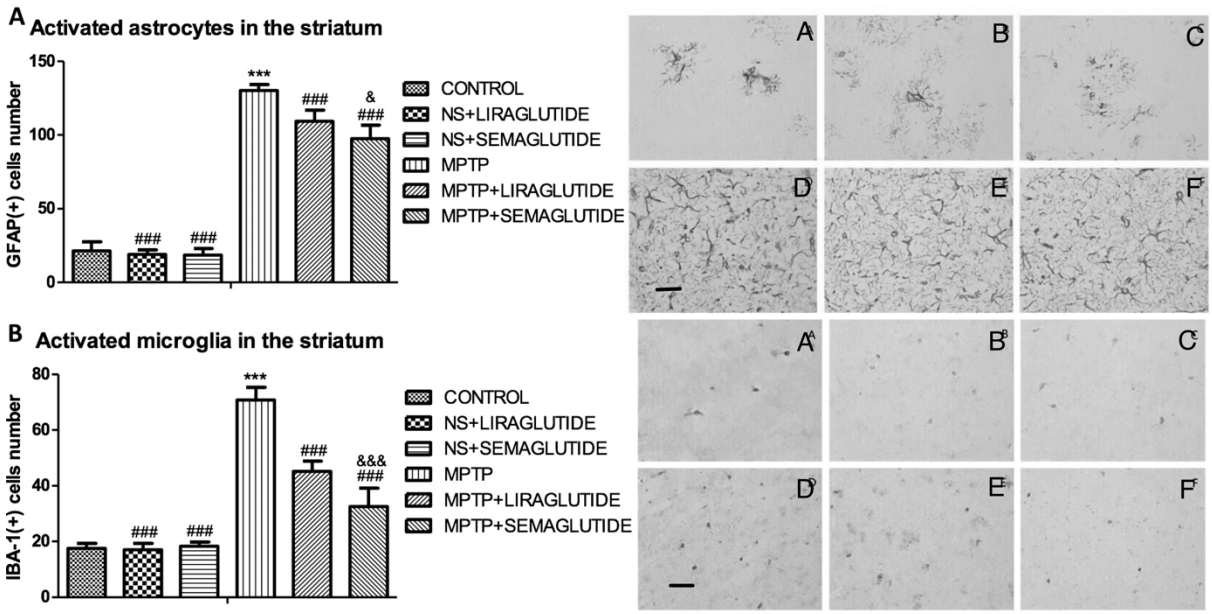

Fig. 3A: GLP-1 analogues reduced the astrocyte activation in the striatum of mice induced by MPTP. NS= normal saline. The values represent the means±S.E.M. ***=p<0.001 compared with the control group. ###=p<0.001 compared with the MPTP group. &&&=p<0.001 compared with MPTP+liraglutide group. n=6. Examples of micrographs are given. A: CONTROL; B: NS+LIRAGLUTIDE; C: NS+SEMAGLUTIDE; D: MPTP; E: MPTP+LIRAGLUTIDE; F:MPTP+SEMAGLUTIDE. Scale bar in image D: 25 µm.
Fig. 3B: GLP-1 analogues reduced the microglia activation in the striatum of mice induced by MPTP. NS= normal saline. The values represent the means±S.E.M. ***=p<0.001 compared with the control group. ###=p<0.001 compared with the MPTP group. &&&=p<0.001 compared with MPTP+liraglutide group. n=6. Examples of micrographs are given. A: CONTROL; B: NS+LIRAGLUTIDE; C: NS+SEMAGLUTIDE; D: MPTP; E: MPTP+LIRAGLUTIDE; F:MPTP+SEMAGLUTIDE. Scale bar in image D: 25 µm.

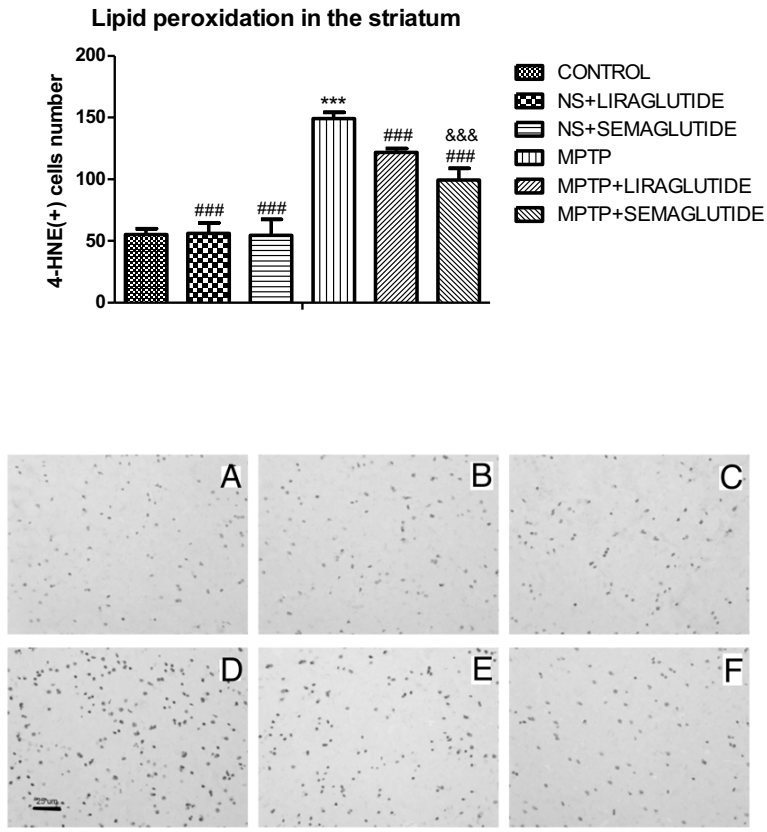

Fig.4. GLP-1 analogues reduced the oxidative stress 4-Hydroxynonenal expression in the striatum of mice induced by MPTP. NS= normal saline. The values represent the means±S.E.M. ***=p<0.001 compared with the control group. ###=p<0.001 compared with the MPTP group. &&&=p<0.001 compared with MPTP+liraglutide group. n=6. Examples of micrographs are given. A: CONTROL; B: NS+LIRAGLUTIDE; C: NS+SEMAGLUTIDE; D: MPTP; E: MPTP+LIRAGLUTIDE; F: MPTP+SEMAGLUTIDE. Scale bar in image D: 25 μm.

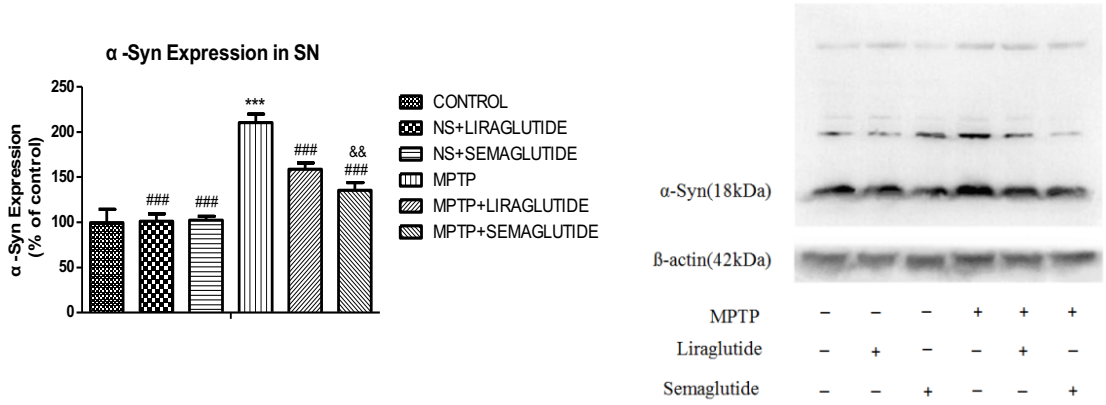

Fig. 5. GLP-1 analogues reduced the accumulation of α-Syn in the substantia nigra of mice induced by MPTP. NS= normal saline. The values represent the means ± S.E.M. ***=P < 0.001 compared with the control group. ###=P < 0.001compared with the MPTP group. &&=P<0.01 compared with the MPTP+LIRAGLUTIDE group; n=4 per group.

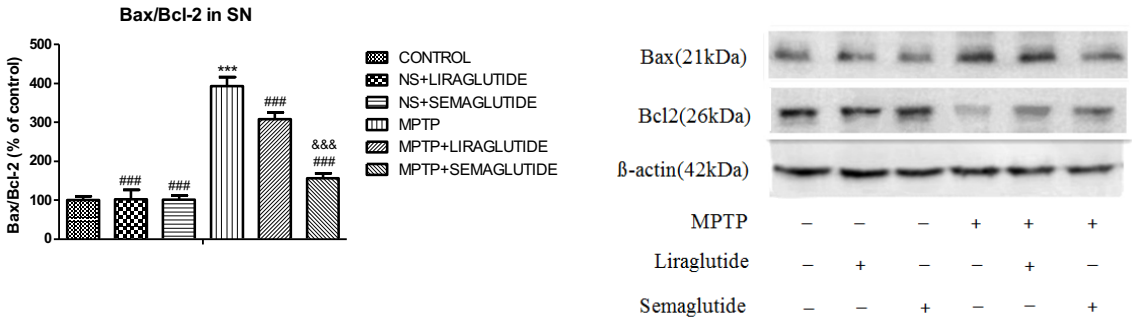

Fig. 6. GLP-1 analogues reversed the increase of ratio of mitophagy markers Bax/Bcl-2 in the substantia nigra of mice induced by MPTP. NS= normal saline. The values represent the means ± S.E.M. ***=P < 0.001 compared with the control group. ###=P < 0.001 compared with the MPTP group. &&&=P<0.001 compared with the MPTP+LIRAGLUTIDE group; n=4 per group.

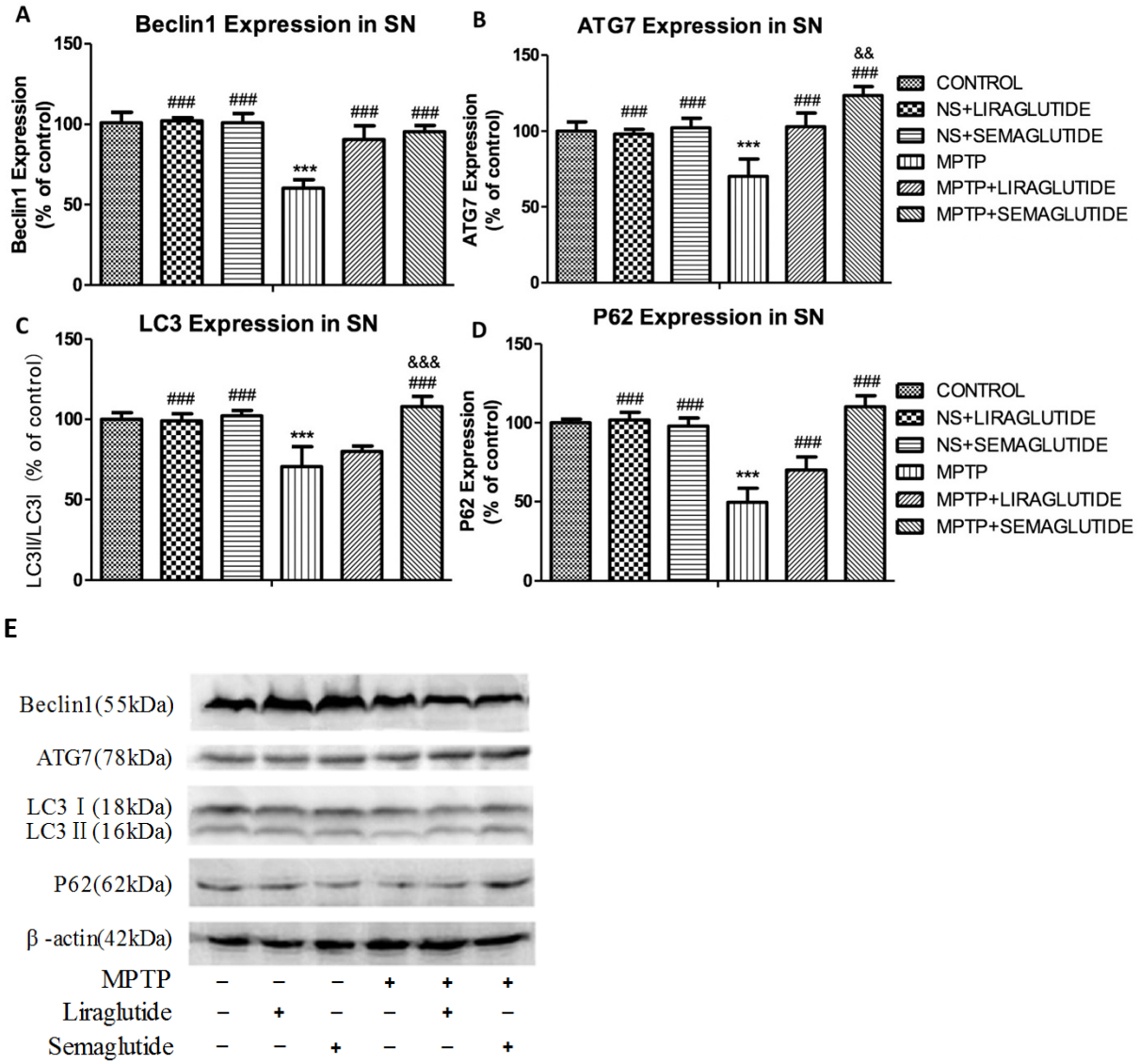

Fig. 7. GLP-1 analogues reverse the decrease of autophagy-associated markers. NS= normal saline. A: Beclin1, B: ATG7, C: LC3 and D: P62 expression and even upregulated autophagy in the substantia nigra of mice reduced by MPTP. E: sample western blot scans are shown. The values represent the means ± S.E.M. ***P < 0.001 compared with the control group. ###P < 0.001compared with the MPTP group. &&P<0.01 and &&&P<0.001 compared with the MPTP+LIRAGLUTIDE group; n=4 per group.

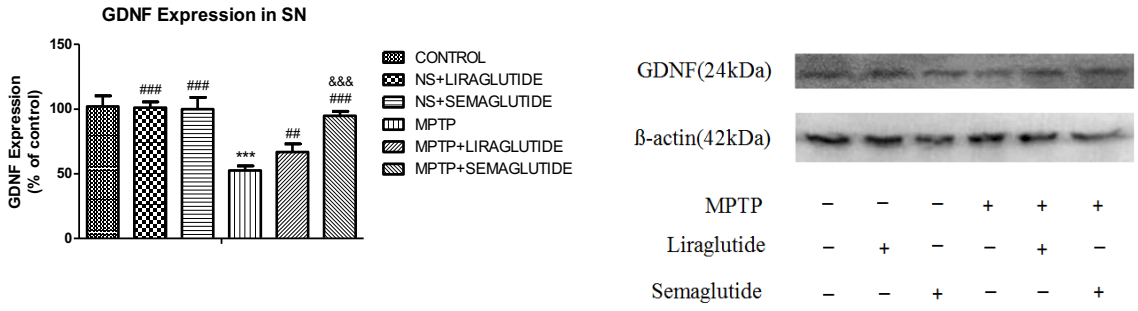

Fig. 8. GLP-1 analogues reverse the decrease of GDNF expression in the substantia nigra of mice reduced by MPTP. NS= normal saline. The values represent the means ± S.E.M. ***=P < 0.001 compared with the control group. ##=P < 0.01 and ###=P < 0.001compared with the MPTP group. &&&=P<0.001 compared with the MPTP+LIRAGLUTIDE group; n=4 per group.

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Superior weight loss with once-weekly semaglutide versus other glucagon-like peptide-1 receptor agonists is independent of gastrointestinal adverse events

Ildiko Lingvay ,[1] Thomas Hansen,[2] Stanislava Macura,[2] Michel Marre,[3,4] Michael A Nauck,[5] Raymond de la Rosa,[6] Vincent Woo,[7] Emre Yildirim,[2] John Wilding [8]

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For numbered affiliations see end of article.

Correspondence to
Dr Ildiko Lingvay; Ildiko.Lingvay@UTSouthwestern.edu

ABSTRACT

Introduction Gastrointestinal (GI) adverse events (AEs) are the most common AEs with glucagon-like peptide-1 receptor agonists (GLP-1RAs). Weight loss (WL) is slightly greater in people who experience GI AEs than those who do not. A previous mediation analysis of the SUSTAIN 1–5 trials indicated minor contribution of nausea/vomiting to the greater WL with once-weekly semaglutide versus comparators. Semaglutide demonstrated superior glycated hemoglobin and body weight (BW) reductions versus other GLP-1RAs in SUSTAIN 3 (versus exenatide extended release 2.0 mg), SUSTAIN 7 (versus dulaglutide) and SUSTAIN 10 (liraglutide 1.2 mg). The objective of this analysis was to assess if significantly greater WL with semaglutide versus other GLP-1RAs is mediated by nausea/vomiting and other GI AEs (diarrhea, constipation, dyspepsia) during dose escalation (baseline to week 12, when GI AEs are generally most prevalent) and from baseline to end of treatment (EOT: week 56 (SUSTAIN 3), 40 (SUSTAIN 7) or 30 (SUSTAIN 10)).

Research design and methods Subjects within trials were subdivided into those who reported (yes/no) nausea/vomiting or any other GI AE. Change from baseline in BW was assessed within each trial and subgroup. A mediation analysis separated WL into direct or indirect (mediated by GI AEs) effects.

Results From baseline to week 12 or EOT, the nausea/vomiting-mediated difference in WL was, respectively: 0.05 or 0.09 kg of 3.78 kg at EOT (SUSTAIN 3); 0.06 or 0.03 kg of 2.26 kg at EOT (low-dose comparison) and 0.08 or 0.04 kg of 3.55 kg at EOT (high-dose comparison) (SUSTAIN 7) and 0.05 or 0.09 kg of 3.82 kg at EOT (SUSTAIN 10).

Conclusions In SUSTAIN 3, 7 and 10, nausea/vomiting by week 12 (end of dose escalation) or throughout treatment contributed minimally (<0.1 kg) to the superior WL with semaglutide versus GLP-1RA comparators at EOT.

Significance of this study

What is already known about this subject?

- A previous mediation analysis, which evaluated the effect of nausea/vomiting on weight loss in the SUSTAIN 1–5 trials, showed that nausea/vomiting contributed only minimally to the superior weight loss with once-weekly semaglutide, a glucagon-like peptide-1 receptor agonist, versus mixed-class comparators.

What are the new findings?

- In this mediation analysis, we investigated the effect of nausea/vomiting within the glucagon-like peptide-1 receptor agonist (GLP-1RA) class (semaglutide versus other GLP-1RAs), which is known for its common but transient gastrointestinal (GI) adverse events (AEs).
- Nausea/vomiting contributed minimally to the significantly greater body weight (BW) reductions with semaglutide versus exenatide extended release (SUSTAIN 3; 56 weeks), dulaglutide (SUSTAIN 7; 40 weeks) or liraglutide (SUSTAIN 10; 30 weeks); these reductions were predominantly independent of the GI AEs of nausea, vomiting, diarrhea, dyspepsia and constipation.
- From baseline to week 12 (end of dose escalation) or to end of treatment (EOT), the nausea-/vomiting-mediated difference in BW loss was, respectively: 0.05 kg or 0.09 kg of 3.78 kg seen at EOT (SUSTAIN 3); 0.06 kg or 0.03 kg of 2.26 kg seen at EOT (low-dose comparison) and 0.08 kg or 0.04 kg of 3.55 kg seen at EOT high-dose comparison) (SUSTAIN 7) and 0.05 kg or 0.09 kg of 3.82 kg seen at EOT (SUSTAIN 10).
- Similarly, a minimal amount of the greater weight loss at EOT observed with semaglutide versus other GLP-1RAs was mediated by the individual GI AEs of nausea, vomiting, diarrhea, dyspepsia or constipation reported from baseline to week 12 and from baseline to EOT.

INTRODUCTION

The association between type 2 diabetes (T2D) and overweight/obesity is well established,[1 2] with more than 90% of people with T2D being overweight.[3] Individuals with T2D and overweight/obesity are at increased risk of developing T2D complications compared with people who are not overweight/obese.[4] Body weight (BW) reductions of ≥5% improve glycemic control, lipid levels and

Significance of this study

How might these results change the focus of research or clinical practice?

- The results of this analysis indicate that the superior weight loss observed with semaglutide versus GLP-1RA class comparators is mostly independent of GI AEs, the most common AEs in this class. These results are consistent with the previous findings in SUSTAIN 1–5 trials.

blood pressure.[5] BW control is an important component of an individualized, multifactorial approach to T2D management, as recommended in current treatment guidelines.[6 7]

Glucagon-like peptide-1 receptor agonists (GLP-1RAs) are recommended as second-line therapy (add-on to metformin) where minimizing weight gain, promoting weight loss or when hypoglycemia and cardiovascular risk reduction are considerations.[7–9] All available GLP-1RAs (dulaglutide, exenatide, liraglutide and lixisenatide) have demonstrated weight loss in people with T2D.[10–12] Semaglutide (Novo Nordisk, Denmark) is a GLP-1RA approved for the treatment of T2D as once-weekly (OW) subcutaneous[13] and once-daily oral formulations.[14] The efficacy and safety of OW semaglutide have been established in the global phase 3 SUSTAIN clinical trial program, encompassing subjects from across the continuum of T2D care.[15–24] In addition to significantly greater reductions in glycated hemoglobin (HbA_{1c}), semaglutide demonstrated superior reductions in BW versus all comparators across all SUSTAIN trials.[15–24]

The SUSTAIN 3, 7 and 10 trials compared semaglutide with the GLP-1RAs OW exenatide extended release (exenatide ER), OW dulaglutide and once-daily liraglutide, respectively. In these trials, mean BW loss was significantly greater with semaglutide versus comparators at end of treatment (EOT: weeks 56, 40 and 30 for SUSTAIN 3, 7 and 10, respectively): SUSTAIN 3: –5.6 kg vs –1.9 kg with semaglutide 1.0 mg vs exenatide ER 2.0 mg; SUSTAIN 7: –4.6 kg vs –2.3 kg with semaglutide 0.5 mg vs dulaglutide 0.75 mg and –6.5 kg vs –3.0 kg with semaglutide 1.0 mg vs dulaglutide 1.5 mg; SUSTAIN 10: –5.8 kg vs –1.9 kg with semaglutide 1.0 mg vs liraglutide 1.2 mg; all $p<0.0001$.[17 21 24]

Consistent with the GLP-1RA class,[25–27] gastrointestinal (GI) adverse events (AEs) were the most frequently reported AEs in the SUSTAIN 3, 7 and 10 trials: 42% with semaglutide 1.0 mg vs 33% with exenatide ER 2.0 mg in SUSTAIN 3; 43% with semaglutide 0.5 mg vs 33% with dulaglutide 0.75 mg and 44% with semaglutide 1.0 mg vs 48% with dulaglutide 1.5 mg in SUSTAIN 7; 44% with semaglutide 1.0 mg vs 38% with liraglutide 1.2 mg in SUSTAIN 10.[17 21 24] The five most commonly reported GI AEs in SUSTAIN 3, 7 and 10 were: nausea (23% with semaglutide 0.5 mg, 21%–22% with semaglutide 1.0 mg and 12%–20% with comparators); vomiting (10% with semaglutide 0.5 mg, 7%–10% with semaglutide 1.0 mg and 4%–10% with comparators); diarrhea (14% with semaglutide 0.5 mg, 11%–16% with semaglutide 1.0 mg and 8%–18% with comparators); dyspepsia (3% with semaglutide 0.5 mg, 4%–7% with semaglutide 1.0 mg and 3%–5% with comparators) and constipation (5% with semaglutide 0.5 mg, 5%–6% with semaglutide 1.0 mg and 3%–5% with comparators).[17 21 24 28]

Given the clinical significance of weight loss in T2D management, it is important to understand the mechanism by which semaglutide provides greater weight loss versus class comparators and, in particular, whether it is mediated by GI AEs. A previous mediation analysis examining superior weight loss with semaglutide versus mixed class comparators by GI AEs in the SUSTAIN 1–5 trials showed that only 0.07 kg of 2.3 kg (semaglutide 0.5 mg) and 0.5 kg of 6.3 kg (semaglutide 1.0 mg) of the treatment difference in weight loss was mediated by nausea/vomiting.[29]

To further determine if GI AEs of nausea/vomiting and others are associated with weight loss, we performed a *post hoc* mediation analysis to examine the extent to which the treatment difference with semaglutide versus the other GLP-1RAs in the SUSTAIN 3, 7 and 10 trials might be driven by a difference in GI AEs (indirect effects) or treatment (direct effect). Data on nausea and/or vomiting were pooled and data on nausea, vomiting, diarrhea, constipation and dyspepsia were analyzed individually.

MATERIALS AND METHODS

SUSTAIN 3, 7 and 10 trial designs

The designs of the SUSTAIN 3, 7 and 10 trials have been previously published.[17 21 24] Briefly, subjects with inadequately controlled T2D were randomized to receive: (1) in SUSTAIN 3, semaglutide 1.0 mg or exenatide ER 2.0 mg, in addition to existing oral antidiabetes drugs, over 56 weeks;[17] (2) in SUSTAIN 7, semaglutide 0.5 mg or 1.0 mg, or dulaglutide 0.75 mg or 1.5 mg in addition to metformin monotherapy, over 40 weeks;[21] (3) in SUSTAIN 10, semaglutide 1.0 mg or liraglutide 1.2 mg, in addition to 1–3 oral antidiabetes drugs, over 30 weeks.[24]

Semaglutide-treated subjects followed a fixed dose-escalation regimen:[17 21 24] the 0.5 mg maintenance dose was reached after 4 weeks of 0.25 mg OW and the 1.0 mg maintenance dose was reached after 4 weeks of 0.25 mg OW, followed by 4 weeks of 0.5 mg OW. Exenatide ER was administered in accordance with its prescribing information[30] (ie, no dose escalation) and dulaglutide was administered in accordance with its phase III clinical trial program (ie, no dose escalation).[31] The liraglutide 1.2 mg maintenance dose was reached after 1 week of 0.6 mg once daily.[24]

For all three trials, prior to trial initiation, the protocol, the consent form and the subject information sheet were reviewed and approved according to local regulations by appropriate health authorities and by an independent ethics committee/institutional review board. Written informed consent was obtained from all participants.

Post hoc analyses

Subjects in the SUSTAIN 3, 7 and 10 trials were subdivided according to whether or not they had reported either nausea or vomiting or both nausea and vomiting (nausea/vomiting), regardless of severity or duration. In addition, the subjects were subdivided according to whether or not they had reported any of the five most common GI AEs associated with semaglutide (nausea, vomiting, diarrhea, constipation or dyspepsia).

Change from baseline in BW by GI AEs

The change in BW from baseline to EOT (week 56 for SUSTAIN 3; week 40 for SUSTAIN 7; week 30 for SUSTAIN 10) in subjects who experienced GI AEs versus those who did not experience GI AEs was estimated from a mixed model for repeated measurements. The effect of GI AEs on the change from baseline in BW at EOT was compared in subjects with versus without GI AEs from baseline to week 12 (when GI AEs were found to peak and decline thereafter) and from baseline to EOT. The effect on the change in BW was analyzed by each of the five common GI AEs (nausea, vomiting, diarrhea, constipation or dyspepsia) individually and by nausea/vomiting. Analyses for BW change were performed on the full analysis set. Subjects who discontinued treatment/initiated rescue medication contributed to the analysis based on the data observed prior to their discontinuation of treatment or initiation of rescue medication.

Mediation analysis

As with the previous analysis,[29] a mediation analysis was performed to separate the overall effect of the GLP-1RAs on BW into direct or indirect (mediated by nausea or vomiting) effects, estimated using natural effect models with imputation-based estimation.[32] Missing BW data were imputed using observed data within the same treatment group assuming that data were missing at random. The question assessed by the direct effect was: what is the effect of changing the treatment from comparator to semaglutide while maintaining the mediator at a value observed in the comparator arm? Conversely, the question assessed by the indirect effect was: what is the effect of changing the level of mediator between semaglutide and comparator (exenatide ER, dulaglutide or liraglutide)? As some of these factors are counterfactual (ie, things that did not occur but were possible) and non-observable, a model was required to obtain estimates of the direct and indirect effects. The natural effect model for the estimation of direct and indirect effects included the interaction between treatment and GI AEs together with the baseline variables of BW and country as main effects, assuming no interaction between natural effects and baseline variables; standard errors of treatment differences were estimated by the bootstrap method. The model used to impute counterfactual values of BW also included the interaction between treatment and each baseline variable and the interaction between any GI AE and each baseline variable.

RESULTS

The presented results of the SUSTAIN 3, 7 and 10 trials focus on the category of subjects with/without nausea/vomiting, regardless of severity or duration (table 1; figures 1 and 2). The results, according to the common individual GI AEs associated with semaglutide (nausea, vomiting, diarrhea, constipation or dyspepsia), are provided in detail in the online supplemental material 1.

Subject disposition and baseline characteristics by nausea/vomiting

Overall baseline characteristics, which have been previously published, were broadly similar between the three trials, with the exception of a longer diabetes duration in subjects in SUSTAIN 3 and SUSTAIN 10 versus SUSTAIN 7 (minimum/maximum of the mean across treatment groups: 9.0–9.4 years and 8.9–9.6 years vs 7.0–7.7 years, respectively).[17 21 24] Greater proportions of subjects with nausea/vomiting (occurring from baseline to week 12 and from baseline to EOT) discontinued treatment than subjects without. Subjects with nausea/vomiting generally had lower baseline BW than subjects without. There were no other differences in baseline characteristics for subjects with or without nausea/vomiting (table 1).

Change from baseline in body weight in subjects with and without nausea/vomiting

BW reductions with all four GLP-1RAs were consistently greater in subjects who experienced nausea/vomiting than in those who did not, and reductions with semaglutide were consistently greater than those seen with exenatide ER, dulaglutide or liraglutide, regardless of nausea/vomiting (figure 1).

SUSTAIN 3 (semaglutide versus exenatide ER)

At EOT, a weight change of −7.0 kg was observed in subjects treated with semaglutide 1.0 mg experiencing nausea/vomiting from baseline to week 12 vs −5.3 kg in those who did not experience these events (p=0.0274). The corresponding values for exenatide ER were −2.5 vs −1.8 kg (p=0.4322; figure 1A).

In subjects treated with semaglutide 1.0 mg experiencing nausea/vomiting at any time from baseline to EOT, a weight change of −6.8 kg vs −5.3 kg at EOT was observed versus those who did not experience these events (p=0.0447). The corresponding values for exenatide ER were −3.3 vs −1.6 kg (p=0.0632).

Estimated treatment differences (ETDs) (95% CIs) favored semaglutide versus exenatide ER in all comparisons (figure 1A).

SUSTAIN 7 (semaglutide versus dulaglutide)

At EOT, the weight change in subjects experiencing nausea/vomiting from baseline to week 12 versus those who did not experience these events was −5.5

Table 1 Baseline characteristics and subject disposition of subjects with onset of nausea/vomiting from baseline to week 12 and at any time from baseline to end of treatment (yes/no) in the SUSTAIN 3, 7 and 10 trials

Treatment	Semaglutide 0.5 mg (SUSTAIN 7)		Semaglutide 1.0 mg (pooled)		Exenatide ER 2.0 mg (SUSTAIN 3)		Dulaglutide 0.75 mg (SUSTAIN 7)		Dulaglutide 1.5 mg (SUSTAIN 7)		Liraglutide 1.2 mg (SUSTAIN 10)	
N (total)	301		994		405		299		299		287	
Nausea/vomiting Yes/No	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N
Baseline to week 12												
N	71	230	213	781	50	355	37	262	63	236	54	233
Race, n (%)												
Asian	15 (21.1)	35 (15.2)	13 (6.1)	38 (4.9)	1 (2.0)	5 (1.4)	7 (18.9)	41 (15.6)	11 (17.5)	44 (18.6)	0 (0.0)	3 (1.3)
Black or African American	3 (4.2)	14 (6.1)	11 (5.2)	37 (4.7)	2 (4.0)	28 (7.9)	0 (0.0)	17 (6.5)	2 (3.2)	16 (6.8)	0 (0.0)	1 (0.4)
White	53 (74.6)	180 (78.3)	179 (84.0)	669 (85.7)	44 (88.0)	294 (82.8)	30 (81.1)	202 (77.1)	48 (76.2)	172 (72.9)	49 (90.7)	219 (94.0)
Other	0 (0.0)	1 (0.4)	10 (4.7)	37 (4.7)	3 (6.0)	28 (7.9)	0 (0.0)	2 (0.8)	2 (3.2)	4 (1.7)	5 (9.3)	10 (4.3)
Ethnic group, n (%)												
Hispanic or Latino	8 (11.3)	21 (9.1)	25 (11.7)	107 (13.7)	11 (22.0)	95 (26.8)	4 (10.8)	27 (10.3)	11 (17.5)	32 (13.6)	1 (1.9)	2 (0.9)
Not Hispanic or Latino	63 (88.7)	209 (90.9)	185 (86.9)	661 (84.6)	39 (78.0)	260 (73.2)	33 (89.2)	235 (89.7)	52 (82.5)	204 (86.4)	48 (88.9)	221 (94.8)
Other	0 (0.0)	0 (0.0)	3 (1.4)	13 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (9.3)	10 (4.3)
Baseline HbA_{1c}, %	8.3 (1.0)	8.3 (1.0)	8.2 (0.9)	8.3 (0.9)	8.4 (1.1)	8.3 (0.9)	8.2 (0.9)	8.2 (0.9)	8.2 (0.8)	8.2 (0.9)	8.2 (1.0)	8.3 (1.0)
Baseline BMI, kg/m^2	32.5 (7.2)	34.0 (7.1)	33.1 (6.3)	34.0 (7.0)	32.7 (6.2)	33.7 (6.2)	35.3 (6.5)	33.4 (6.9)	32.3 (6.9)	33.3 (6.5)	33.0 (7.8)	33.8 (6.8)
Baseline BW, kg	92.1 (28.4)	97.7 (22.9)	92.4 (19.4)	97.1 (22.0)	90.2 (16.7)	96.1 (20.9)	98.7 (22.9)	95.2 (23.0)	89.7 (21.9)	94.4 (21.7)	93.3 (25.2)	98.1 (20.8)
Exposure time, years	0.7 (0.3)	0.8 (0.2)	0.7 (0.3)	0.9 (0.3)	1.0 (0.4)	1.0 (0.3)	0.8 (0.1)	0.8 (0.1)	0.7 (0.3)	0.8 (0.2)	0.6 (0.2)	0.6 (0.1)
Duration of diabetes, years	7.4 (5.9)	7.8 (5.9)	8.8 (6.5)	8.7 (5.9)	9.6 (7.2)	9.4 (6.6)	7.0 (5.2)	7.0 (5.5)	7.1 (5.4)	7.8 (5.7)	8.2 (5.0)	9.1 (5.8)
Onset of rescue, n (%)	0 (0.0)	3 (1.3)	3 (1.4)	36 (4.6)	3 (6.0)	45 (12.7)	2 (5.4)	12 (4.6)	0 (0.0)	7 (3.0)	1 (1.9)	11 (4.7)
Discontinued treatment, n (%)	21 (29.6)	26 (11.3)	61 (28.6)	111 (14.2)	12 (24.0)	73 (20.6)	3 (8.1)	24 (9.2)	15 (23.8)	21 (8.9)	10 (18.5)	16 (6.9)
Withdrawal from trial, n (%)	5 (7.0)	17 (7.4)	17 (8.0)	37 (4.7)	6 (12.0)	30 (8.5)	1 (2.7)	12 (4.6)	3 (4.8)	12 (5.1)	2 (3.7)	3 (1.3)
Lost to follow-up, n (%)	3 (4.2)	6 (2.6)	9 (4.2)	13 (1.7)	2 (4.0)	8 (2.3)	0 (0.0)	8 (3.1)	1 (1.6)	7 (3.0)	1 (1.9)	2 (0.9)
At any time from baseline to EOT												
N	76	225	245	749	57	348	48	251	69	230	57	230
Race, n (%)												
Asian	16 (21.1)	34 (15.1)	14 (5.7)	37 (4.9)	1 (1.8)	5 (1.4)	8 (16.7)	40 (15.9)	12 (17.4)	43 (18.7)	0 (0.0)	3 (1.3)
Black or African American	4 (5.3)	13 (5.8)	13 (5.3)	35 (4.7)	2 (3.5)	28 (8.0)	1 (2.1)	16 (6.4)	4 (5.8)	14 (6.1)	0 (0.0)	1 (0.4)

Continued

Table 1 Continued

Treatment	Semaglutide 0.5 mg (SUSTAIN 7)		Semaglutide 1.0 mg (pooled)		Exenatide ER 2.0 mg (SUSTAIN 3)		Dulaglutide 0.75 mg (SUSTAIN 7)		Dulaglutide 1.5 mg (SUSTAIN 7)		Liraglutide 1.2 mg (SUSTAIN 10)	
White	56 (73.7)	177 (78.7)	207 (84.5)	641 (85.6)	51 (89.5)	287 (82.5)	39 (81.3)	193 (76.9)	51 (73.9)	169 (73.5)	51 (89.5)	217 (94.3)
Other	0 (0.0)	1 (0.4)	11 (4.5)	36 (4.8)	3 (5.3)	28 (8.0)	0 (0.0)	2 (0.8)	2 (2.9)	4 (1.7)	6 (10.5)	9 (3.9)
Ethnic group, n (%)												
Hispanic or Latino	8 (10.5)	21 (9.3)	26 (10.6)	106 (14.2)	11 (19.3)	95 (27.3)	4 (8.3)	27 (10.8)	11 (15.9)	32 (13.9)	1 (1.8)	2 (0.9)
Not Hispanic or Latino	68 (89.5)	204 (90.7)	216 (88.2)	630 (84.1)	46 (80.7)	253 (72.7)	44 (91.7)	224 (89.2)	58 (84.1)	198 (86.1)	50 (87.7)	219 (95.2)
Other	0 (0.0)	0 (0.0)	3 (1.2)	13 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (10.5)	9 (3.9)
Baseline HbA_{1c}, %	8.3 (1.0)	8.3 (1.0)	8.2 (0.9)	8.3 (0.9)	8.4 (1.1)	8.3 (0.9)	8.1 (0.9)	8.2 (0.9)	8.1 (0.8)	8.2 (0.9)	8.2 (1.0)	8.3 (1.0)
Baseline BMI, kg/m^2	32.9 (7.6)	33.9 (6.9)	33.4 (6.7)	33.9 (6.9)	33.0 (6.4)	33.7 (6.2)	34.6 (6.7)	33.5 (6.9)	32.3 (6.8)	33.3 (6.5)	33.4 (8.0)	33.7 (6.7)
Baseline BW, kg	92.8 (28.2)	97.6 (22.9)	93.4 (20.4)	97.0 (21.9)	91.0 (16.1)	96.1 (21.0)	97.3 (24.0)	95.3 (22.8)	89.8 (21.3)	94.5 (21.9)	94.0 (25.2)	98.0 (20.7)
Exposure time, years	0.7 (0.3)	0.8 (0.2)	0.8 (0.3)	0.9 (0.3)	1.0 (0.4)	1.0 (0.3)	0.8 (0.1)	0.8 (0.1)	0.7 (0.3)	0.8 (0.2)	0.6 (0.2)	0.6 (0.1)
Duration of diabetes, years	7.3 (5.8)	7.9 (6.0)	8.9 (6.5)	8.6 (5.8)	9.5 (7.1)	9.4 (6.7)	7.1 (5.4)	7.0 (5.5)	7.4 (5.8)	7.7 (5.6)	8.6 (5.2)	9.0 (5.8)
Onset of rescue, n (%)	0 (0.0)	3 (1.3)	3 (1.2)	36 (4.8)	3 (5.3)	45 (12.9)	2 (4.2)	12 (4.8)	0 (0.0)	7 (3.0)	1 (1.8)	11 (4.8)
Discontinued treatment, n (%)	21 (27.6)	26 (11.6)	67 (27.3)	105 (14.0)	13 (22.8)	72 (20.7)	5 (10.4)	22 (8.8)	15 (21.7)	21 (9.1)	10 (17.5)	16 (7.0)
Withdrawal from trial, n (%)	5 (6.6)	17 (7.6)	19 (7.8)	35 (4.7)	6 (10.5)	30 (8.6)	2 (4.2)	11 (4.4)	3 (4.3)	12 (5.2)	2 (3.5)	3 (1.3)
Lost to follow-up, n (%)	3 (3.9)	6 (2.7)	9 (3.7)	13 (1.7)	2 (3.5)	8 (2.3)	1 (2.1)	7 (2.8)	1 (1.4)	7 (3.0)	1 (1.8)	2 (0.9)

Data are mean (standard deviation) unless otherwise specified. Only subjects with non-missing subgroup information were selected.
AE, adverse event; BMI, body mass index; BW, body weight; EOT, end of treatment; exenatide ER, exenatide extended release; GI, gastrointestinal; HbA_{1c}, glycated hemoglobin.

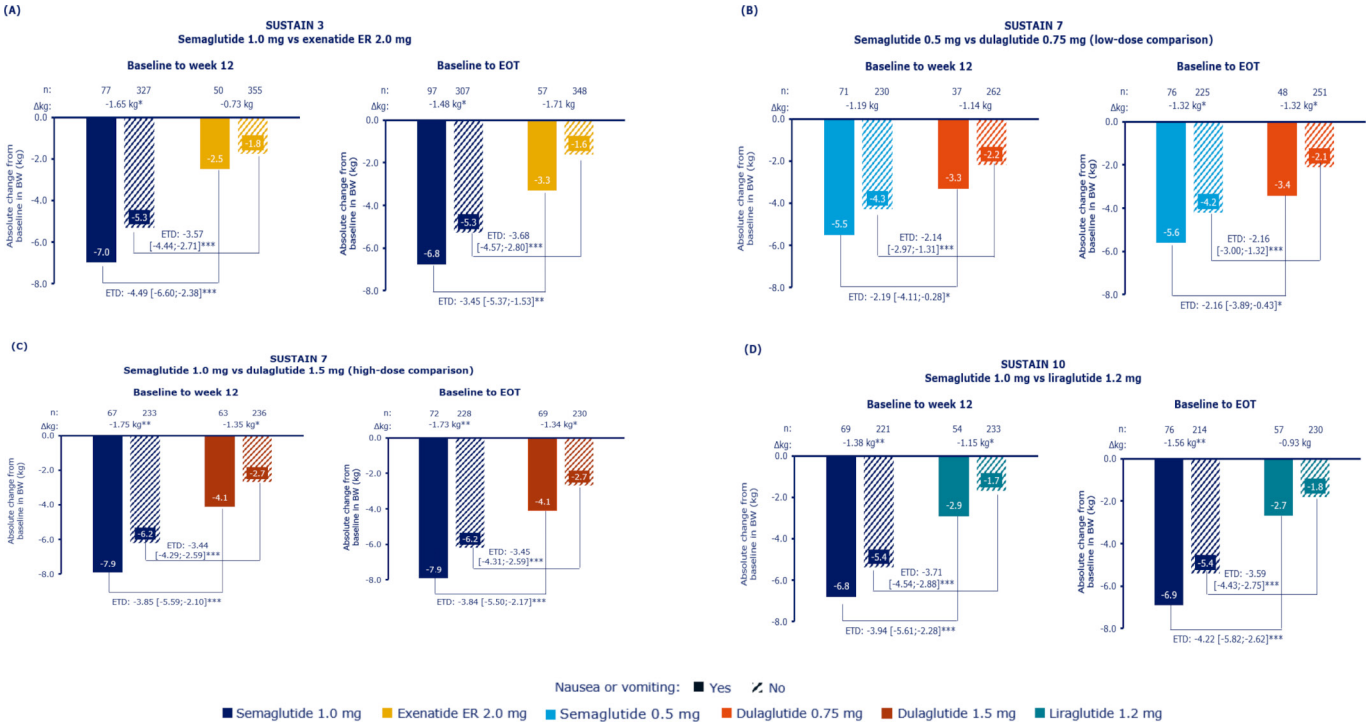

Figure 1 Absolute change from baseline in BW at EOT by nausea/vomiting occurring at any time from baseline to week 12 and at any time from baseline to EOT in SUSTAIN 3 (A), SUSTAIN 7 (B,C) and SUSTAIN 10 (D). *P<0.05; **p<0.01; ***p<0.0001. EOT was at week 56 for SUSTAIN 3, week 40 for SUSTAIN 7 and week 30 for SUSTAIN 10. Values are estimated means from a mixed model for repeated measurements analysis using 'on-treatment without rescue medication' data from subjects in the full analysis set. Values in square brackets indicate 95% CIs. BW, body weight; Δkg, differences in body weight within treatment arms; EOT, end of treatment; ETD, estimated treatment difference; exenatide ER, exenatide extended release.

kg vs –4.3 kg (p=0.0542) for semaglutide 0.5 mg and –7.9 kg vs –6.2 kg (p=0.0074) for semaglutide 1.0 mg (figure 1B,C). The corresponding values for dulaglutide 0.75 mg and dulaglutide 1.5 mg were –3.3 kg vs –2.2 kg (p=0.1153) and –4.1 kg vs –2.7 kg (p=0.0340), respectively.

In subjects experiencing nausea/vomiting at any time from baseline to EOT versus those who did not experience

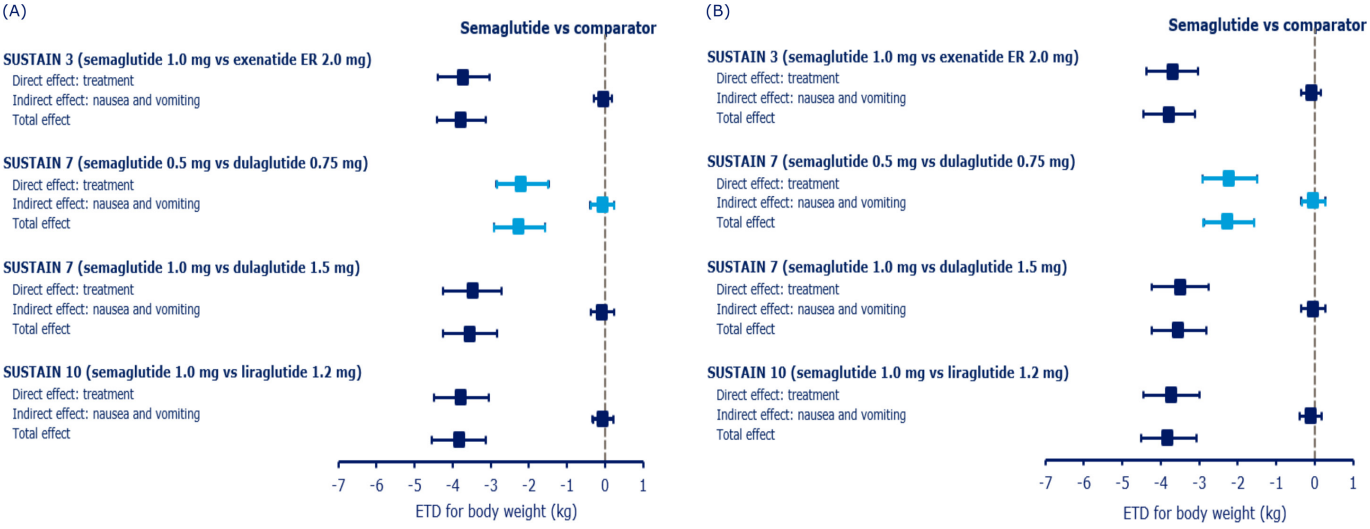

Figure 2 Mediation analysis of direct (due to treatment) and indirect (due to nausea or vomiting) effects on weight loss for subjects treated with semaglutide from baseline to week 12 (A) and from baseline to end of treatment (B) in the SUSTAIN 3, 7 and 10 trials. Data are 'on-treatment without rescue medication' ETDs (95% CIs) for the change from baseline at (A) at any time in the first 12 weeks and (B) week 56 (SUSTAIN 3), week 40 (SUSTAIN 7) or week 30 (SUSTAIN 10) from all randomized patients exposed to at least one dose of trial product (full analysis set). Post-baseline data were analyzed using a mixed model for repeated measurements that included the interaction of treatment and any nausea/vomiting. ETD, estimated treatment difference; exenatide ER, exenatide extended release.

these events, the weight change was –5.6 kg vs –4.2 kg at EOT (p=0.0236) for semaglutide 0.5 mg and –7.9 kg vs –6.2 kg for semaglutide 1.0 mg (p=0.0051; figure 1B,C). The corresponding values for dulaglutide 0.75 mg and dulaglutide 1.5 mg were –3.4 kg vs –2.1 kg (p=0.0375) and –4.0 kg vs –2.7 kg (p=0.226), respectively.

ETDs (95% CIs) favored semaglutide versus dulaglutide in all comparisons (figure 1B,C).

SUSTAIN 10 (semaglutide versus liraglutide)

At EOT, a weight change of –6.8 kg was observed in subjects treated with semaglutide 1.0 mg experiencing nausea/vomiting from randomization to week 12 vs –5.4 kg in those who did not experience these events (p=0.0071). The corresponding values for liraglutide were –2.9 vs –1.7 kg (p=0.0295; figure 1D).

In subjects treated with semaglutide 1.0 mg experiencing nausea/vomiting at any time from randomization to EOT, a weight change of –6.9 kg vs –5.4 kg at EOT was observed versus those who did not experience these events (p=0.0021). The corresponding values for liraglutide were –2.7 vs –1.8 kg (p=0.0528; figure 1D).

ETDs (95% CIs) favored semaglutide versus liraglutide in all comparisons (figure 1D).

Mediation analyses of BW reduction by nausea/vomiting

SUSTAIN 3 (semaglutide versus exenatide ER)

Mediation analyses showed that 0.05 kg of a total of 3.78 kg weight loss at EOT (week 56) observed with semaglutide versus exenatide ER in SUSTAIN 3 was mediated by nausea/vomiting from baseline to week 12 (p<0.0001; figure 2A). Similarly, only 0.09 kg of a total of 3.78 kg was mediated by nausea/vomiting at any time from baseline to EOT (p<0.0001; figure 2B).

SUSTAIN 7 (semaglutide versus dulaglutide)

In SUSTAIN 7, 0.06 kg of a total of 2.26 kg of the greater weight loss at EOT (week 40) observed with semaglutide 0.5 mg vs dulaglutide 0.75 mg and 0.08 kg of a total of 3.55 kg for semaglutide 1.0 mg vs dulaglutide 1.5 mg was mediated by nausea/vomiting from baseline to week 12 (both p<0.0001; figure 2A). In SUSTAIN 7, 0.03 kg of 2.26 kg of the greater weight loss at EOT observed with semaglutide 0.5 mg vs dulaglutide 0.75 mg and 0.04 kg of a total of 3.55 kg for semaglutide 1.0 mg vs dulaglutide 1.5 mg was mediated by nausea/vomiting at any time up to the EOT (both p<0.0001; figure 2B).

SUSTAIN 10 (semaglutide versus liraglutide)

Mediation analysis showed that 0.05 kg of a total of 3.82 kg weight loss at EOT (week 30) observed with semaglutide 1.0 mg vs liraglutide 1.2 mg in SUSTAIN 10 was mediated by nausea/vomiting from baseline to week 12 (p<0.0001; figure 2A). Similarly, only 0.09 kg of the total of 3.82 kg weight loss observed with semaglutide versus liraglutide at EOT was mediated by nausea/vomiting at any time up to the EOT (p<0.0001; figure 2B).

DISCUSSION

The rationale for conducting this posthoc analysis of SUSTAIN 3, 7 and 10 trials was to investigate whether GI AEs contributed to the superior weight loss observed with semaglutide versus the other GLP-1RAs, exenatide ER, dulaglutide or liraglutide. In this posthoc analysis, we found that in SUSTAIN 3, 7 and 10, subjects who experienced nausea/vomiting, or any of the five evaluated commonly reported GI AEs, generally had slightly greater weight loss compared with subjects who did not experience these symptoms (with some exceptions). In addition, treatment with semaglutide resulted in a significantly greater weight loss than with exenatide ER, dulaglutide or liraglutide, also in subjects who did not experience nausea/vomiting, suggesting that the superior weight loss observed with semaglutide was not related to the occurrence of these events. Mediation analyses support this observation and establish that the superior weight loss seen with semaglutide (2.26 to 3.82 kg) versus exenatide ER, dulaglutide or liraglutide was independent of GI AEs (only 0.03 to 0.09 kg due to nausea/vomiting). This is consistent with the previous analysis of the SUSTAIN 1–5 trials, which showed that a small amount (0.07 to 0.5 kg) of the total ETD (2.3 to 6.3 kg) in weight loss at EOT versus mixed-class comparators was due to nausea/vomiting[29]—thus, the majority of the weight-loss effect for semaglutide was not mediated by GI AEs such as nausea/vomiting.

Furthermore, in this analysis, there was no evidence of a temporal association between the incidence of GI AEs and weight loss at EOT. The prevalence of GI AEs with GLP-1RA treatment was previously found to peak within the initial 12 weeks of treatment and decline thereafter.[33] However, subjects in all treatment arms experienced weight loss between baseline and week 12, and from baseline to EOT (SUSTAIN 3, week 56; SUSTAIN 7, week 40; SUSTAIN 10, week 30).

Excess weight is an important contributing factor in the complex etiology of T2D,[4] and BW control is an important factor in the individualized management of T2D.[4 6 7] GLP-1RAs are established and effective therapies for T2D and can be prescribed at all stages of T2D.[6] In addition to managing glucose levels, GLP-1RAs also reduce BW,[7 34 35] and this potential for weight loss has been reflected by the GLP-1RA liraglutide (3.0 mg once daily) gaining approval as a treatment for obesity.[36 37] Because GI AEs including nausea, vomiting or diarrhea are the most common type of AE with GLP-1RAs,[25–27] it is important to establish whether the weight loss difference between treatment is mediated through the occurrence of GI AEs.

The previous mediation analysis of the SUSTAIN 1–5 trials showed that only a small component of the superior weight loss with semaglutide was associated with GI AEs.[29] Although GI AEs tend to be more common with semaglutide versus GLP-1RA comparators, they are usually reported during the dose-escalation phase of the trial[38] and, consistent with the GLP-1RA class, are generally mild to moderate in severity and transient in nature.[27]

In this analysis, the fact that greater weight loss with semaglutide versus class comparators was minimally affected by

GI AEs indicates involvement of alternative mechanisms. The unique physicochemical properties of semaglutide may contribute to the greater weight loss observed versus exenatide ER, dulaglutide or liraglutide. In a randomized controlled trial, semaglutide was associated with lower energy intake and higher BW loss versus placebo, the mechanisms likely being less appetite and food cravings, better control of eating and lower preference for fat-rich foods.[39] Other GLP-1RAs promote weight loss through a similar mechanism of action;[40] hence, the difference between semaglutide and other GLP-1RAs may just be quantitative. Although current evidence is limited to animal studies, the data suggest that semaglutide-associated weight loss is centrally mediated through the activation of areas of the brain involved in appetite control and reward, including the hypothalamus neural circuits, the arcuate nucleus, the pro-opiomelanocortin neurons and the nucleus of the tractus solitarius.[41–43]

Subjects experiencing nausea/vomiting had a lower baseline BW and were more likely to discontinue treatment compared with subjects not experiencing them. Of note, despite the lower baseline BW, these subjects still experienced greater weight loss with semaglutide; this could be because semaglutide produces weight loss, irrespective of baseline BW, across a range of exposures.[38]

The strengths of this study are: GI AEs were analyzed in week 12, which is the time-point when they peak, as well as any time from baseline to EOT; it is an intention-to-treat analysis; mediation analysis of BW reduction was used to calculate differences between groups (not only for nausea/vomiting but also for other GI AEs); semaglutide treatment resulted in significantly greater weight loss than comparators even in subjects who did not experience GI AEs which supports the hypothesis; similar results from SUSTAIN 1–5 trials also support the hypothesis.

Potential limitations of this *post hoc* analysis include its inherent retrospective nature and that it was not sufficiently powered to detect the effects assessed. For example, the small number of subjects per treatment arm in the groups that experienced GI AE; therefore, results should be interpreted in this context. Another possible limitation is the different durations of follow-up for subjects with GI AE in all three trials. In addition, the results should be viewed in the context that SUSTAIN 3, 7 and 10 were open-label trials and nausea is a subjective symptom. Furthermore, in the mediation analysis, the effect of 'one unit' mediator was assumed to be the same in the treatment arms being compared. Mediation analyses rely on strong, unverifiable assumptions, and the results of the analysis may be biased in case of potential unknown confounders that affect the risk of experiencing GI AEs as well as change in BW.

CONCLUSION

In this *post hoc* analysis of SUSTAIN 3, 7 and 10, nausea/vomiting contributed minimally to the significantly greater BW reductions with semaglutide versus exenatide ER, dulaglutide or liraglutide. These reductions were independent of the individual GI AEs of nausea, vomiting, diarrhea, dyspepsia and constipation in a subset of GLP-1RA class comparators with which GI AEs are the most commonly observed AEs.

Author affiliations
[1]Department of Internal Medicine/Endocrinology, University of Texas Southwestern Medical Center, Dallas, Texas, USA
[2]Novo Nordisk A/S, Søborg, Denmark
[3]Clinique Ambroise Paré, Neuilly sur Seine, France
[4]UMRS 1138, Metabolic Inflammation in Diabetes and its Complications Cordeliers Research Centre, Paris, France
[5]Diabetes Division, Katholisches Klinikum Bochum, St Josef Hospital, Bochum, Germany
[6]Endocrinology, Millennium Physician Group, Englewood, USA
[7]Endocrinology and Metabolism, University of Manitoba, Winnipeg, Manitoba, Canada
[8]Department of Cardiovascular and Metabolic Medicine, Institute of Life Course and Medical Sciences, University of Liverpool, Liverpool, UK

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ORCID iDs
Ildiko Lingvay http://orcid.org/0000-0001-7006-7401
John Wilding http://orcid.org/0000-0003-2839-8404

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ORIGINAL ARTICLE

WILEY

The effect of semaglutide 2.4 mg once weekly on energy intake, appetite, control of eating, and gastric emptying in adults with obesity

Martin Friedrichsen PhD[1] | Astrid Breitschaft MD[2] | Sayeh Tadayon MD[1] | Alicja Wizert PhD[1] | Dorthe Skovgaard MD[1]

[1]Novo Nordisk A/S, Søborg, Denmark

[2]Parexel International GmbH, Berlin, Germany

Correspondence
Martin Friedrichsen, PhD, Novo Nordisk A/S, Vandtårnsvej 108, 2860 Søborg, Denmark.
Email: mfrc@novonordisk.com

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Abstract

Aim: To investigate the effects of once-weekly subcutaneous (s.c.) semaglutide 2.4 mg on gastric emptying, appetite, and energy intake in adults with obesity.

Materials and Methods: A double-blind, parallel-group trial was conducted in 72 adults with obesity, randomized to once-weekly s.c. semaglutide (dose-escalated to 2.4 mg) or placebo for 20 weeks. Gastric emptying was assessed using paracetamol absorption following a standardized breakfast. Participant-reported appetite ratings and Control of Eating Questionnaire (CoEQ) responses were assessed, and energy intake was measured during ad libitum lunch.

Results: The area under the concentration–time curve (AUC) for paracetamol 0 to 5 hours after a standardized meal ($AUC_{0-5h,para}$; primary endpoint) was increased by 8% (P = 0.005) with semaglutide 2.4 mg versus placebo at week 20 (non-significant when corrected for week 20 body weight; P = 0.12). No effect was seen on $AUC_{0-1h,para}$, maximum observed paracetamol concentration, or time to maximum observed paracetamol concentration. Ad libitum energy intake was 35% lower with semaglutide versus placebo (1736 versus 2676 kJ; estimated treatment difference −940 kJ; P <0.0001). Semaglutide reduced hunger and prospective food consumption, and increased fullness and satiety when compared with placebo (all P <0.02). The CoEQ indicated better control of eating and fewer/weaker food cravings with semaglutide versus placebo (P <0.05). Body weight was reduced by 9.9% with semaglutide and 0.4% with placebo. Safety was consistent with the known profile of semaglutide.

Conclusions: In adults with obesity, once-weekly s.c. semaglutide 2.4 mg suppressed appetite, improved control of eating, and reduced food cravings, ad libitum energy intake and body weight versus placebo. There was no evidence of delayed gastric emptying at week 20, assessed indirectly via paracetamol absorption.

KEYWORDS

appetite, control of eating, energy intake, food craving, gastric emptying, GLP-1 analogue, glucagon-like peptide-1, obesity, randomized trial, semaglutide

1 | INTRODUCTION

Obesity is a growing global health crisis placing substantial burden on healthcare systems, with excess weight contributing to a range of detrimental effects, including increased risk of type 2 diabetes (T2D), cardiovascular disease, and mortality.[1,2] Despite the importance of weight loss in improving health outcomes for patients with overweight/obesity,[1,2] relatively few pharmacotherapies are approved for weight management.[3]

Glucagon-like peptide-1 receptor agonists (GLP-1RAs) were initially developed for improvement of glycaemic control in T2D.[4] Following the observation of weight reductions in T2D,[4] GLP-1RAs were studied in patients with overweight or obesity,[5,6] and a single agent (liraglutide 3 mg) is currently approved for weight management.[7,8] While liraglutide provided clinically relevant reductions in body weight of 5.4% relative to placebo in a pivotal study in overweight/obese patients,[6] there remains an unmet need for those patients for whom weight loss ≥10% is recommended.[1,2] Furthermore, other available antiobesity agents fail to achieve ≥10% weight loss and some are associated with safety concerns,[3,9] highlighting the need for effective, well-tolerated treatments.

Semaglutide is a GLP-1RA approved for the treatment of T2D as a once-weekly subcutaneous (s.c.) injection at doses up to 1.0 mg, and as a once-daily oral tablet (up to 14 mg), which is the first oral formulation of a GLP-1RA.[10–13] In phase 3 studies in patients with T2D, s.c. semaglutide 1.0 mg reduced body weight from baseline by up to 6.5 kg (at timepoints ranging from 30 to 104 weeks), with two to three times greater reductions than with other studied GLP-1RAs.[14–23] Semaglutide lowers body weight by reducing appetite and hunger, increasing satiety, reducing food cravings, altering food preferences and reducing energy intake.[24,25] An initial phase 2 dose-ranging study in patients with obesity demonstrated clinically relevant weight loss with s.c. semaglutide, when given as once-daily doses of up to 0.4 mg.[5] Once-weekly s.c. semaglutide is now in clinical development for weight management in patients with overweight/obesity, within the phase 3 Semaglutide Treatment Effect in People with obesity (STEP) trial programme, which is investigating the efficacy of once-weekly s.c. semaglutide 2.4 mg.[26]

In addition to their effects on regulation of energy intake and body weight, GLP-1RAs have been associated with delayed gastric emptying,[4,27,28] which has the potential to affect the absorption of concomitantly administered oral agents.[7,8,10–13] A 12-week study with semaglutide 1.0 mg in subjects with obesity indicated a delay in first hour gastric emptying.[29] We therefore conducted the present phase 1 trial in adults with obesity, with two main objectives: the primary objective was to investigate the effect of once-weekly s.c. semaglutide 2.4 mg on gastric emptying; the secondary objective was to investigate the effect of the 2.4 mg dose on appetite and energy intake, to provide further insight into the weight-reducing mechanism of action of semaglutide in obesity.

2 | MATERIALS AND METHODS

2.1 | Trial design

A single-centre, randomized, double-blind, placebo-controlled, parallel-group, phase 1 trial was conducted in Germany (NCT03842202). The trial consisted of a 20-week treatment period (including 21 doses of study drug) and a 7-week follow-up (Figure 1). The trial adhered to the Declaration of Helsinki and International Conference on Harmonisation Good Clinical Practice Guidelines, and was approved by the relevant institutional, ethical and regulatory bodies.

2.2 | Trial population

Participants were men and women, aged 18 to 65 years, with body mass index (BMI) of 30.0 to 45.0 kg/m^2. Informed consent was required before trial-related activities. Exclusion criteria included: clinically significant body weight change (≥5%) or dieting attempts in the prior 90 days; use of medications in the prior 14 days (other than contraceptives, occasional paracetamol or acetylsalicylic acid, or stable doses of antihypertensives or lipid-lowering drugs); use of weight-lowering drugs or drugs that may cause weight gain within the prior 12 months; presence of gastrointestinal disorders or symptoms of such disorders that may affect absorption of drugs or nutrients; prior obesity surgery or presence of gastrointestinal implant; and glycated haemoglobin (HbA1c) ≥48 mmol/mol or fasting glucose ≥7.0 mmol/L.

2.3 | Interventions

Participants were randomized equally to once-weekly s.c. semaglutide 2.4 mg (initially undergoing a 16-week dose-escalation consisting of 0.25, 0.5, 1.0 and 1.7 mg once weekly for 4 weeks each, followed by 2.4 mg for five doses; 21 doses in total over 20 weeks) or volume-matched placebo (with matching dose-escalation procedure; Figure 1). The randomization schedule was generated by the sponsor before the trial, and participants were assigned randomization numbers in ascending numerical order at the trial site. Participants were instructed to inject their allocated study drug on the same day each week (any time of day, irrespective of meals).

2.4 | Endpoints

The primary endpoint compared the effect of once-weekly s.c. semaglutide 2.4 mg and placebo on gastric emptying assessed by the paracetamol absorption method at week 20, using the area under the concentration–time curve (AUC) for paracetamol 0 to 5 hours after a standardized meal ($AUC_{0-5h,para}$). Paracetamol is commonly used as an indirect marker for gastric emptying,[30] and its use provided an approach consistent with a previous study of s.c. semaglutide 1.0 mg.[29] Secondary endpoints related to gastric emptying included

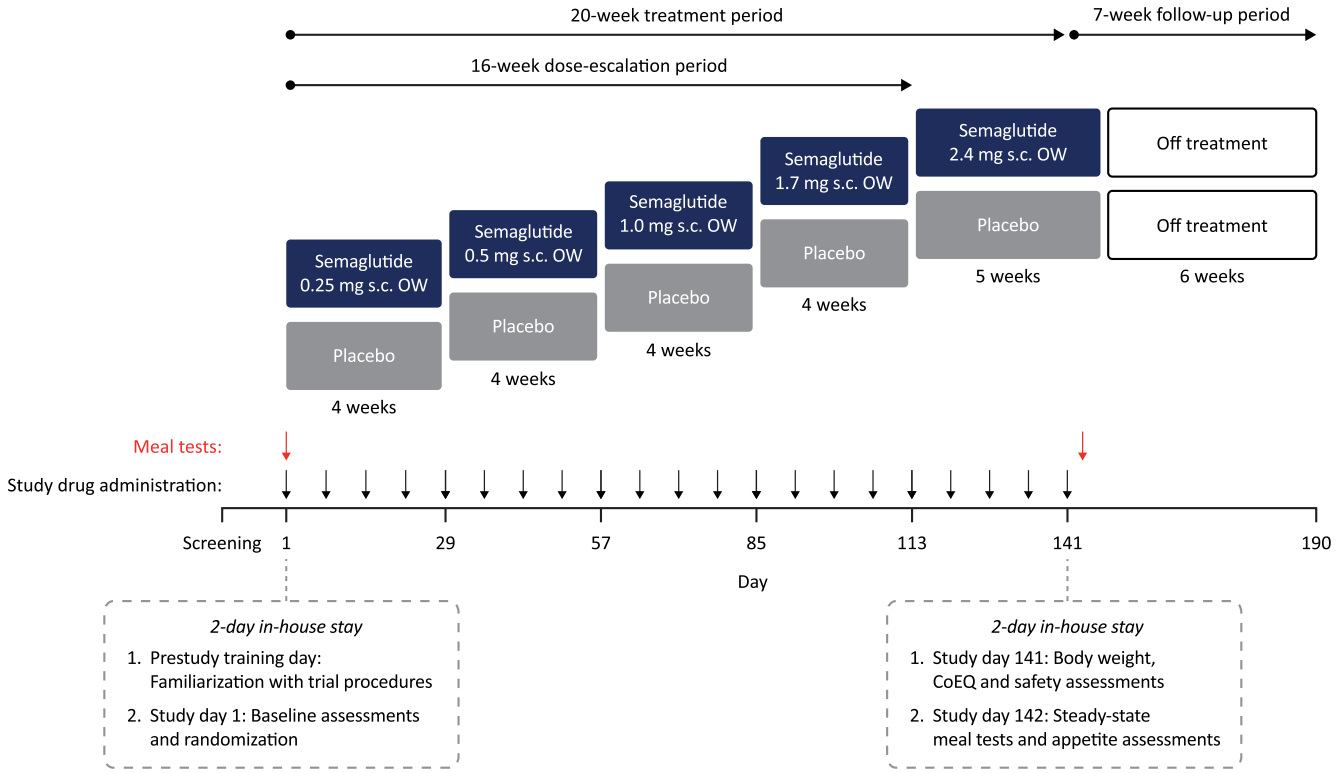

FIGURE 1 Trial design. OW, once weekly; s.c. subcutaneous

paracetamol AUC from 0 to 1 hour after a standardized meal ($AUC_{0-1h,para}$), maximum observed paracetamol concentration ($C_{max,para}$) and time to maximum observed paracetamol concentration ($t_{max,para}$).

Energy intake during the ad libitum lunch was compared between semaglutide and placebo at week 20 as a secondary endpoint.

The effect of semaglutide compared with placebo on appetite was assessed using mean postprandial participant-reported visual analogue scale (VAS) appetite ratings following a standardized breakfast meal at week 20, focusing on hunger, fullness, satiety, prospective food consumption and overall appetite suppression score (secondary endpoints). Additional exploratory endpoints included assessment of fasting and mean postprandial change from fasting ratings for VAS items measuring thirst, nausea and well-being following a standardized breakfast. Participant-reported control of eating was evaluated as an exploratory endpoint using the Control of Eating Questionnaire (CoEQ),[31] completed at week 20.

2.5 | Procedures and assessments

Following screening, eligible participants attended a 2-day in-house stay at the study centre. The first day of the in-house stay was a training day before the start of the treatment period, during which participants were familiarized with the study tasks and received an ad libitum meal (no data were collected). A 5-hour standardized meal test was performed on day 1 of the study (prior to initiating treatment [baseline timepoint]) and during a return visit on day 142 (the day after administration of the final dose of study drug, at the end of the 20-week treatment period; Figure 1). The meal test consisted of a breakfast meal of approximately 600 kcal (macronutrient composition of ~30 energy percentage [E%] fat, ~15 E% protein and ~55 E% carbohydrate), which participants were required to ingest within 15 minutes. A yoghurt containing paracetamol 1500 mg was included as part of the meal. Blood was sampled for paracetamol concentration using a venous catheter before the start of the meal (baseline), and at regular timepoints thereafter, for up to 5 hours postprandially, and participants completed several VAS 1 to 3 minutes prior to blood sampling. These VAS assessed appetite (hunger, satiety, fullness and prospective food consumption), thirst, well-being and nausea. A 100 mm scale was used, with the ends indicating the most extreme sensation the participants had ever experienced. The participants subsequently received an ad libitum lunch meal in excess, approximately 5 hours after the scheduled completion of the breakfast meal at baseline and after 20 weeks, and food consumption (kJ) was recorded. The participants were instructed to eat until they were pleasantly satiated.

The participants completed the CoEQ on day −1 (baseline for this analysis) and day 141, based on their experience over the prior 7 days, with ratings for each question recorded on a 100 mm VAS. The questionnaire included 19 questions relating to control of eating, intensity, frequency and type of food craving, appetite/hunger sensations and mood.

Body weight was recorded at baseline and after 20 weeks, and during the follow-up period. Safety assessments included adverse

event (AE) reporting, assessment of vital signs and laboratory tests (biochemistry, haematology and glucose metabolism).

2.6 | Statistical analysis

A sample size of 29 completers per treatment group was required to provide 90% power to detect a half-width of the 95% confidence interval (CI) for the log-transformed treatment ratio of 0.15 for the primary endpoint, assuming a standard deviation (SD) of 0.25 (based on a previous trial).[24] Assuming an estimated drop-out rate of 20%, 36 participants were planned to be randomized per group.

Statistical comparisons between groups were conducted using two-sided tests and at a 5% significance level. For the primary endpoint ($AUC_{0-5,para}$), data were log-transformed and analysed using an analysis of covariance (ANCOVA) model, with baseline as covariate and treatment as factor, and results presented as treatment ratio with 95% CI. Secondary endpoints were analysed in a similar way to the primary endpoint, but without log-transformation for those relating to ad libitum energy intake and appetite VAS. Mean postprandial values were calculated as the AUC for VAS ratings over 30 to 300 minutes after the standardized breakfast, divided by 270 minutes. For the appetite VAS, the overall appetite suppression score was calculated as the average of the four components: (satiety + fullness + [100 – hunger] + [100 – prospective food consumption]) / 4. Descriptive statistics were used for exploratory endpoints (except for CoEQ), changes in body weight and safety assessments.

The exploratory CoEQ endpoint was analysed using ANCOVA models for each CoEQ question, with change from baseline as response, baseline value of the respective question as covariate and treatment as factor. This approach differed from the prespecified methodology, which did not account for the baseline value. Additional post hoc analyses included analysis of the primary endpoint using an ANCOVA model, with log-transformed body weight at week 20 as an additional covariate, and analysis of percentage change in ad libitum energy intake from baseline to week 20 using an ANCOVA model with energy intake at baseline as covariate and treatment as factor.

3 | RESULTS

3.1 | Trial population

Seventy-two participants were enrolled between February and April 2019, and randomized to once-weekly s.c. semaglutide 2.4 mg (n = 36) or placebo (n = 36; Figure S1). Almost all participants (97.2%) completed the study; one participant in the semaglutide group withdrew consent before study end, and one participant in the placebo group was withdrawn following an AE (colonic abscess). Demographics and baseline characteristics were generally comparable between the groups; the majority of participants were men (61.1%), the mean age was 42.8 years, the mean body weight was 105.5 kg and the mean BMI was 34.4 kg/m^2 (Table 1).

3.2 | Gastric emptying

The $AUC_{0-5h,para}$ was 8% higher in the s.c. semaglutide 2.4 mg group compared with the placebo group at week 20 (estimated treatment ratio [ETR] 1.08; P = 0.0054). The difference in $AUC_{0-5h,para}$ between groups was no longer statistically significant when adjusted for body weight at week 20 in a post hoc analysis (ETR 1.05; P = 0.1218). No differences were found between semaglutide and placebo for other endpoints, including $AUC_{0-1h,para}$ (unadjusted ETR 0.99 [P = 0.8474]; body-weight-adjusted ETR 0.94 [P = 0.3069]), $C_{max,para}$ (unadjusted ETR 0.94 [P = 0.3299]; body-weight-adjusted ETR 0.90 [P = 0.1464]) and $t_{max,para}$ (unadjusted ETR 1.02 [P = 0.7540]; body-weight-adjusted ETR 1.02 [P = 0.7861]; Table S1; Figure S2). Median $t_{max,para}$ was 0.50 hours in both the semaglutide and placebo groups at week 20.

TABLE 1 Demographics and baseline characteristics

	Semaglutide s.c. 2.4 mg (N = 36)	Placebo (N = 36)	Total (N = 72)
Age, years	40.7 (12.2)	45.0 (9.5)	42.8 (11.1)
Sex, n (%)			
Male	24 (66.7)	20 (55.6)	44 (61.1)
Female	12 (33.3)	16 (44.4)	28 (38.9)
Race, n (%)			
Black or African American	1 (2.8)	0 (0.0)	1 (1.4)
White	35 (97.2)	36 (100.0)	71 (98.6)
Ethnicity, n (%)			
Not Hispanic or Latino	36 (100.0)	36 (100.0)	72 (100.0)
Body weight, kg	106.2 (16.2)	104.9 (14.0)	105.5 (15.0)
BMI, kg/m^2	34.2 (3.0)	34.6 (3.1)	34.4 (3.0)

Note: Data are mean (standard deviation), unless otherwise stated.
BMI, body mass index; s.c., subcutaneous

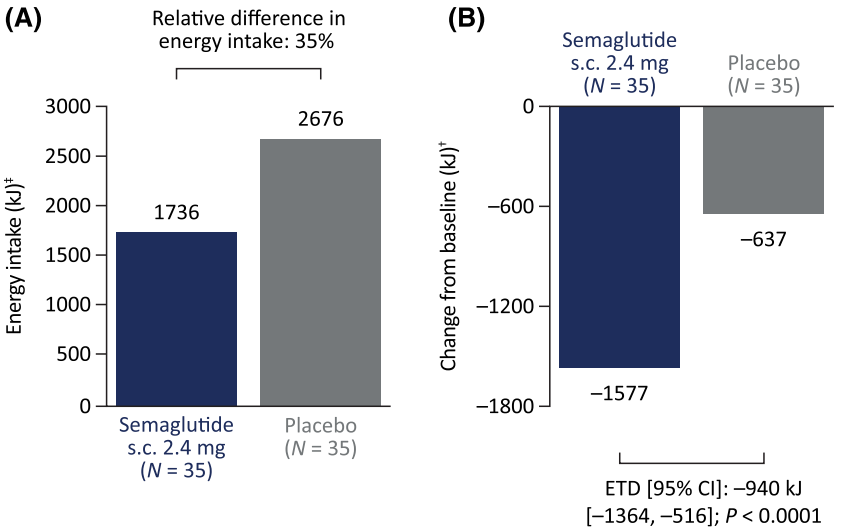

FIGURE 2 Ad libitum lunch energy intake at week 20 (A) and change from baseline in ad libitum lunch energy intake at week 20 (B). Estimates were calculated from analysis of covariance (ANCOVA) models using baseline energy intake of 3313 kJ, which corresponds to the average baseline value for all participants (semaglutide and placebo groups) who contributed to the analysis. ‡Obtained from an ANCOVA model with energy intake at baseline as a covariate and treatment as a factor. †Obtained from an ANCOVA model with change from baseline value to week 20 as response, energy intake at baseline as a covariate and treatment as a factor. CI, confidence interval; ETD, estimated treatment difference; s.c., subcutaneous

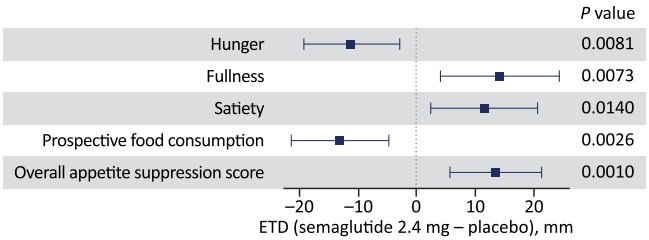

FIGURE 3 Postprandial appetite ratings after standardized breakfast at week 20. Overall appetite suppression score calculated as: (satiety + fullness + [100 – hunger] + [100 – prospective food consumption]) / 4. Each endpoint was analysed using the analysis of covariance model with baseline value of the respective endpoint as covariate and treatment as factor. The figure shows the estimated treatment difference for semaglutide versus placebo (boxes) and 95% confidence interval (whiskers). CI, confidence interval; ETD, estimated treatment difference; VAS, visual analogue score

3.3 | Ad libitum energy intake

The estimated mean ad libitum energy intake during lunch at week 20 was 35% lower in the s.c. semaglutide 2.4 mg group (mean 1736 kJ) compared with the placebo group (mean 2676 kJ; Figure 2A [see Table S2 for kcal values]). Relative to baseline, this represented a reduction of 1577 kJ at week 20 in the semaglutide 2.4 mg group compared with 637 kJ in the placebo group (estimated treatment difference [ETD] −940 kJ; P <0.0001; Figure 2B). When analysed in terms of the percentage change from baseline to week 20, estimated mean energy intake was reduced by 47.1% with semaglutide versus 18.6% with placebo (ETD 28.5%; P = 0.0001; post hoc analysis; Figure S3).

3.4 | Appetite

After a standardized breakfast, hunger and prospective food consumption VAS ratings were reduced, and fullness and satiety increased, with s.c. semaglutide 2.4 mg versus placebo (P <0.02 for all; Figure 3). The overall postprandial appetite suppression score after the standardized breakfast was higher with semaglutide versus placebo (ETD 13 mm; P = 0.001 [Figures 3 and S4]).

Ratings for thirst were similar in the s.c. semaglutide 2.4 mg group and placebo group at week 20. Overall, the mean ratings for nausea at week 20 were low in both groups and mean well-being ratings were high (exploratory endpoints; data not shown).

3.5 | Control of eating and food cravings

Participants' CoEQ scores at week 20 showed lower hunger with s.c. semaglutide 2.4 mg compared with placebo, better control of eating, and fewer and weaker food cravings, including reductions in both desire and craving for savoury foods, desire for sweet foods and craving for dairy foods (P <0.05 for all; Figure 4). In addition, fullness and contentment appeared increased with semaglutide compared with placebo (P <0.05).

3.6 | Body weight

By week 20, body weight was reduced from baseline by a mean (SD) of 10.4 kg (6.3) with s.c. semaglutide 2.4 mg and 0.4 kg (2.6) with placebo (descriptive statistics only), representing relative reductions from baseline of 9.9% and 0.4%, respectively.

3.7 | Safety

The number of participants reporting AEs was broadly similar in the s.c. semaglutide 2.4 mg group (29 participants [80.6%]) and placebo group (33 participants [91.7%]; Table S3). All AEs were mild or moderate in severity, with the exception of a single severe, serious AE (colonic abscess) in the placebo group, which led to trial withdrawal.

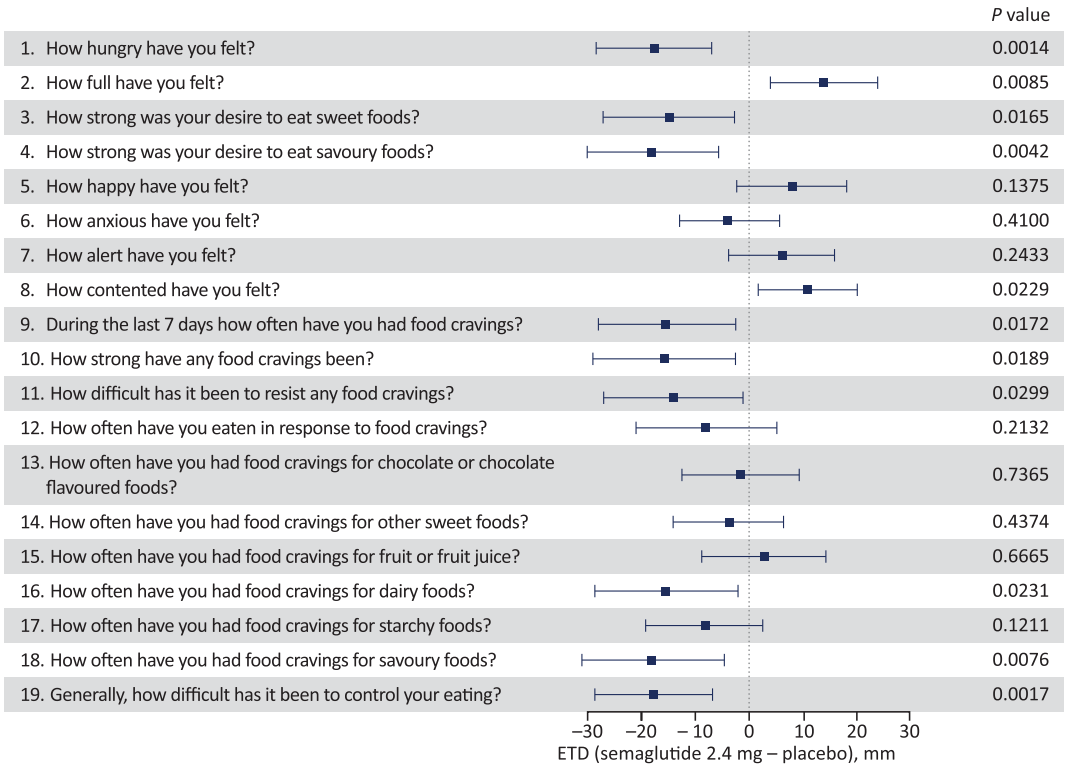

FIGURE 4 Control of eating and food cravings evaluated by the Control of Eating Questionnaire visual analogue scale at week 20. The Control of Eating Questionnaire was completed by participants at the end of the 20-week treatment period (day 141), based on their experience over the prior 7 days. Individual scores for each question were analysed using separate analysis of covariance models with change from baseline as response, baseline value of respective question as a covariate and treatment as factor (post hoc analysis methodology). The figure shows the estimated treatment difference (ETD) for semaglutide versus placebo (boxes) and 95% confidence interval (whiskers)

One serious AE was reported in the semaglutide group (injury-related after a motorcycle accident).

Decreased appetite was the AE reported by the greatest number of participants, and occurred in more participants with semaglutide than placebo (Table S3). Gastrointestinal AEs were reported more frequently in the semaglutide group (25 participants [69.4%]) compared with the placebo group (14 participants [38.9%]), with nausea and diarrhoea most commonly reported. Such events were all mild or moderate in severity and generally of short duration.

4 | DISCUSSION

This trial investigated the effect of once-weekly s.c. semaglutide 2.4 mg on gastric emptying, appetite and energy intake in participants with obesity. Using paracetamol absorption as an indirect measure for gastric emptying, we found no evidence of delayed gastric emptying with semaglutide 2.4 mg at week 20. Meal tests showed a reduction in appetite and energy intake with semaglutide relative to placebo, together with better control of eating, fewer and weaker food cravings, and clinically meaningful reductions in body weight.

Prior studies of GLP-1RAs on gastric emptying, energy intake and appetite have typically been of shorter durations and therefore often used crossover designs to reduce within-participant variability,[24,28,29] as used in a prior 12-week trial of s.c. semaglutide 1.0 mg in subjects with obesity.[24] In contrast, the present study used a parallel-group design, given that a 20-week treatment period was required to allow gradual dose-escalation to the 2.4 mg dose. In this study we instead accounted for within-participant variation by including a baseline evaluation and integrating this within the statistical analyses. In addition, baseline demographics and clinical characteristics were similar overall between the two groups.

Delayed gastric emptying would be anticipated to slow paracetamol absorption, and paracetamol absorption is therefore generally accepted as an indirect measure for gastric emptying. Semaglutide 2.4 mg was associated with a statistically significant 8% increase in paracetamol AUC_{0-5h} versus placebo, which might be partially explained by substantially lower body weight in the semaglutide group compared with placebo at week 20 (greater body weight is associated with reduced paracetamol absorption rates and increased clearance[32,33]). This was confirmed by a *post-hoc* analysis, which found that the difference in $AUC_{0-5h,para}$ between semaglutide and placebo was no longer statistically significant after adjusting for week 20 body weight. No differences were found between semaglutide and placebo for other paracetamol endpoints, with or without adjustment for week 20 body weight. We observed no reduction in paracetamol absorption (AUC_{0-5h}, AUC_{0-1h} or C_{max}) with once-weekly s.c. semaglutide 2.4 mg versus placebo at week 20, and no effect on $t_{max,para}$. In contrast, the 12-week crossover study of once-weekly s.c. semaglutide 1.0 mg reported a delay in paracetamol-assessed gastric emptying over the first

postprandial hour, but similarly found no significant difference in overall gastric emptying when assessed as paracetamol AUC 0 to 5 hours postprandially.[29] Similar effects were observed with oral semaglutide in another crossover study in participants with T2D, also using the paracetamol absorption test.[34] Our parallel-group study used a higher dose (2.4 mg once weekly) of semaglutide than that in the s.c. semaglutide 1.0 mg crossover study, and included a longer treatment period than both of these earlier studies (20 vs. 12 weeks). The absence of delayed paracetamol-assessed gastric emptying in our trial may therefore relate to more pronounced tachyphylaxis, arising from the longer treatment duration; such tachyphylaxis has previously been reported with long-acting GLP-1RAs.[4,28,35]

The present study provides further insights into the mechanisms through which semaglutide mediates body weight loss. Subcutaneous semaglutide 2.4 mg suppressed postprandial appetite, including a reduction in hunger and prospective food consumption, and an increase in satiety and fullness, with participants reporting that they were able to control their eating with less difficulty relative to placebo. CoEQ scores suggested an effect in terms of reduced intensity of desire for sweet and savoury foods, and reduced frequency of craving for dairy and savoury foods. These results are consistent with the prior study of once-weekly s.c. semaglutide 1.0 mg in participants with obesity, which similarly reported appetite suppression, improved control of eating and reduced food craving (particularly for savoury foods [craving for dairy and desire for sweet/savoury food were not assessed in that study]).[24] In animal studies, the anorexigenic actions of semaglutide have been shown to arise from effects on the central nervous system, mediated by GLP-1 receptors in the hypothalamus and hindbrain.[25,36] Such studies suggest that semaglutide directly activates brain areas that are accessible to the molecule and also causes indirect secondary modulation of neuronal activity in other brain areas, including those involved in appetite regulation, food intake, food preference, reward and meal termination, such as the lateral parabrachial nucleus.[25]

Once-weekly s.c. semaglutide 2.4 mg reduced body weight by 9.9% (10.4 kg) from baseline to week 20, in the absence of structured dietary and exercise intervention, compared with almost no change in the placebo group (0.4% [0.4 kg]). This reduction appears consistent with that seen in the phase 2 study with once-daily s.c. semaglutide 0.4 mg in obesity (when taking into account the additional contribution of structured dietary and physical activity counselling in the phase 2 study),[5] and is twice as great as the 5 kg reduction seen over 12 weeks in the study of once-weekly s.c. semaglutide 1.0 mg.[24] Our results demonstrate clinically relevant weight loss with once-weekly s.c. semaglutide 2.4 mg in participants with obesity during a relatively short 20-week treatment period (body weight assessment performed after only 4 weeks on the 2.4 mg dose). A greater weight loss may be achievable with longer-term treatment, which is being investigated in phase 3 studies with semaglutide 2.4 mg in adults with obesity.[26]

The body weight-lowering effects of once-weekly s.c. semaglutide 2.4 mg are likely to be related to reduced energy intake in response to effects on hedonic and homeostatic control of eating, manifesting as decreased appetite, increased satiety, reduced hunger, better control of eating and reduced food cravings. Mean ad libitum energy intake during lunch was 35% lower with s.c. semaglutide 2.4 mg versus placebo at week 20. Energy intake reductions were also found with once-weekly s.c. semaglutide 1.0 mg versus placebo in the crossover study in subjects with obesity, with reductions of 18% to 35% reported across ad libitum meals (lunch, evening meal and snack box).[24] A direct comparison of the energy intake results between these two studies is not possible due to key differences in study design (parallel vs. crossover; 20 vs. 12 weeks' treatment) and analysis methodology (adjustment for baseline in the present study, but not in the s.c. semaglutide 1.0 mg trial), and the potential for between-study differences in placebo effect.[24] It should be noted that the ad libitum lunch test used in the present study represents an isolated assessment of energy intake and may not capture the overall treatment difference in energy intake throughout the day, including other meals.

While it has been proposed that delayed gastric emptying could hypothetically contribute to reduced energy intake and weight loss with GLP-1RAs, the lack of notable effects of long-acting GLP-1RAs on gastric emptying renders this an unlikely mechanism for such agents.[37] We did not identify a role for gastric emptying in weight loss with semaglutide 2.4 mg, based on the paracetamol absorption test.

Overall, the incidence and nature of AEs was consistent with the known safety and tolerability profile of semaglutide,[5,14,24] with no new safety findings.

Key strengths of the present study include the fact that it was performed at a single centre, thereby reducing the potential for variations in study procedures, and the inclusion of a placebo group. While we did not use the "gold standard" scintigraphy-based approach for assessing gastric emptying, the paracetamol absorption test is widely used and is the methodology adopted in most prior studies of GLP-1RAs,[28] and enabled comparison with the results of the prior study of paracetamol-assessed gastric emptying with semaglutide 1.0 mg in obesity.[29] As an indirect measure, paracetamol absorption has been suggested to have limitations, particularly regarding its ability to reflect gastric emptying of solids, and the potential for short-term delays in gastric emptying to be missed if paracetamol absorption is only assessed several hours after administration.[28] In the present study, we attempted to mitigate these limitations by administering paracetamol as part of a semi-solid food (yoghurt; consistent with the semaglutide 1.0 mg study),[29] and by evaluating paracetamol concentration within the first hour post-dose and over a 5-hour period post-dose, as well as assessing the magnitude and timing of peak concentration ($C_{max,para}$ and $t_{max,para}$). At week 20, none of these assessments indicated a delay in paracetamol absorption with semaglutide 2.4 mg versus placebo, and there was no flattening of the paracetamol concentration–time curve, which, if present, would have suggested a delay in gastric emptying.

In conclusion, in adults with obesity, once-weekly s.c. semaglutide 2.4 mg suppressed appetite, improved control of eating, reduced the frequency and strength of food cravings, lowered ad libitum energy intake and was associated with clinically meaningful reductions in body weight versus placebo at week 20, with no evidence of

delayed gastric emptying as measured indirectly through paracetamol absorption.

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CONFLICT OF INTEREST

M.F., S.T. and D.S. are employees and shareholders of Novo Nordisk, the sponsor of this trial; A.W. is an employee of Novo Nordisk. A.B. is an employee of Parexel International GmbH; Parexel International GmbH was paid by Novo Nordisk for assistance in conducting the study.

AUTHOR CONTRIBUTIONS

Study design: A.B., M.F. and S.T. contributed to the study design and collection of data; A.W., D.S., M.F. and S.T. contributed to data analysis and interpretation. All authors contributed to the development of the manuscript and provided final approval for submission.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/dom.14280.

DATA AVAILABILITY STATEMENT

Data will be shared with bona fide researchers submitting a research proposal approved by the independent review board. Access request proposals can be found at novonordisk-trials.com. Data will be made available after research completion, and approval of the product and product use in the European Union and the USA. Individual participant data will be shared in data sets in a de-identified/anonymised format.

ORCID

Martin Friedrichsen https://orcid.org/0000-0003-3383-9593

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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