The novel dual glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 (GLP-1) receptor agonist tirzepatide transiently delays gastric emptying similarly to selective long-acting GLP-1 receptor agonists

Shweta Urva PhD1 | Tamer Coskun MD1 | Corina Loghin MD1 | Xuewei Cui PhD1 | Emily Beebe BS1 | Libbey O'Farrell BS1 | Daniel A. Briere MS1 | Charles Benson MD1 | Michael A. Nauck MD2 | Axel Haupt MD1

1Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana, USA
2Diabetes Division, Katholisches Klinikum Bochum, St. Josef Hospital, Ruhr-University Bochum, Bochum, Germany

Correspondence
Axel Haupt, MD, Lilly Corporate Center, Indianapolis, IN, USA.
Email: haupt_axel@lilly.com

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Abstract
The effect of dual glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptor agonist (RA) tirzepatide on gastric emptying (GE) was compared to that of GLP-1RA in non-clinical and clinical studies. GE was assessed following acute and chronic treatment with tirzepatide in diet-induced obese mice versus semaglutide or long-acting GIP analogue alone. Participants [with and without type 2 diabetes (T2DM)] from a phase 1, 4-week multiple dose study received tirzepatide, dulaglutide or placebo. GE was assessed by acetaminophen absorption. In mice, tirzepatide delayed GE to a similar degree to that achieved with semaglutide; however, these acute inhibitory effects were abolished after 2 weeks of treatment. GIP analogue alone had no effect on GE or on GLP-1’s effect on GE. In participants with and without T2DM, once-weekly tirzepatide (≥5 and ≥4.5 mg, respectively) delayed GE after a single dose. This effect diminished after multiple doses of tirzepatide or dulaglutide in healthy participants. In participants with T2DM treated with an escalation schedule of tirzepatide 5/5/10/10 or 5/5/10/15 mg, a residual GE delay was still observed after multiple doses. These data suggest that tirzepatide’s activity on GE is comparable to that of selective GLP-1RAs.

KEYWORDS
antidiabetic drug, GIP, GLP-1 analogue, incretin therapy, type 2 diabetes

1 | INTRODUCTION

Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are the two known incretins that enhance glucose-stimulated insulin secretion after nutrient ingestion.
GLP-1 receptor agonists (GLP-1RAs) affect glucose homeostasis by stimulating insulin secretion, suppressing glucagon, slowing gastric emptying (GE), and enhancing satiety to reduce food intake. The magnitude of effect on GE, unlike the effects on glycemic control or appetite, is attenuated with prolonged GLP-1 exposure. While GIP is the main incretin that stimulates glucose-dependent insulin secretion and affects postprandial fat metabolism, it has no known impact on GE.

The novel dual GIP and GLP-1RA tirzepatide has a mean half-life of ~5 days, supporting once-weekly dosing. In patients with type 2 diabetes (T2DM), tirzepatide demonstrated potent effects on both fasting and postprandial glycaemia and insulin response during an oral glucose tolerance test (OGTT). In patients with T2DM from a phase 2b study, tirzepatide showed superior efficacy in glycated haemoglobin (HbA1c) and body weight reduction compared with dulaglutide (up to −25.4 mmol/mol vs. −13.7 mmol/mol and −11.3 vs. −2.7 kg, respectively).

The GE rate is a major determinant of postprandial glycaemic excursions in both healthy participants and in participants with T2DM and has been linked to suppression of food intake and weight loss. Potentially, the robust glycaemic-lowering observed with tirzepatide could be partially attributable to its prolonged inhibitory effect on GE.

We investigated the GE effect of the long-acting dual GIP and GLP-1RA tirzepatide versus selective GLP-1RAs in non-clinical and clinical studies and the potential consequences for its glycaemic efficacy.

2 | MATERIALS AND METHODS

2.1 | Non-clinical methodology

Target engagement was evaluated in an intraperitoneal glucose tolerance test model. Male C57BL/6 mice, obtained from Taconic (Hudson, New York) at 16 to 22 weeks of age, were singly housed in microisolator cages on wood chip bedding, and standard food (5008 Teklad Global Diet; Envigo) and deionized water were available ad libitum. Lights were on a 12-h light/12-h dark cycle, and temperature and relative humidity were maintained between 21°C and 23°C and 45% and 65%, respectively. Tirzepatide, semaglutide or long-acting GIPRA were synthesized at Eli Lilly and Company (Indianapolis, Indiana) and formulated in 20 mM Tris HCl buffer pH 8. Mice (n = 5–10 per treatment) were subcutaneously dosed (10 mL/kg) with the vehicle or peptides and immediately fasted overnight, followed 17 hours later by an intraperitoneal bolus of 2 g glucose/kg (intraperitoneal glucose tolerance test). Blood glucose concentrations were measured up to 120 minutes after glucose administration using glucometers. Data were used to calculate the area under the curve (AUC). The AUC for each treatment was normalized to the average vehicle AUC such that vehicle represented 100%. The resulting dose–response curve was fit by a four-parameter logistic model in GraphPad Prism version 8 to estimate ED50. All in vitro binding and cAMP assays were performed as previously described.

Diet-induced obese (DIO) C57/B6 male mice (aged 24–26 weeks; Taconic) were randomized by body weight into seven groups of five mice. Before the 16-hour overnight fasting period, the mice were dosed subcutaneously with: vehicle (40 mM Tris-HCl buffer at pH 8.0), ascending doses of GLP-1RA (semaglutide 0.3, 1, 3, 10, 30 or 100 nmol/kg), dual GIP and GLP-1RA (tirzepatide 0.3, 1, 3, 10 or 30 nmol/kg), long-acting GIPRA (30, 100, 300, 1000 or 3000 nmol/kg; Eli Lilly and Company), or ascending doses of long-acting GIPRA (30, 100, 300 or 1000 nmol/kg), combined with a fixed efficacious dose of semaglutide (10 nmol/kg; Eli Lilly and Company). The next morning, mice were administered 0.5 mL (0.5 g) of freshly prepared semi-liquid diet by oral gavage (Table S2 in Appendix S1). Assessment of semi-liquid GE is described in the Appendix S1.

Gastric emptying delay was also assessed following chronic (ie, daily) treatment with semaglutide (1, 3 and 10 nmol/kg) or tirzepatide (1, 3 and 10 nmol/kg) in DIO mice for 14 days.

The mice were studied and maintained in accordance with the Institutional Animal Care and Use Committee of Eli Lilly and Company and the Guide for the Use and Care of Laboratory Animals by the National Institutes of Health.

2.2 | Clinical study design and participants

This phase 1, randomized, placebo-controlled, participant- and investigator-blinded study comprised three parts: a single-ascending dose and a 4-week multiple-ascending dose study in healthy participants, followed by a 4-week multiple-dose phase 1b proof-of-concept in participants with T2DM. Evaluation of GE was incorporated within the multiple-ascending dose and proof-of-concept parts of the study. Participants were randomly assigned to receive tirzepatide, dulaglutide 1.5 mg (healthy participants only) or placebo (Figure S1 in Appendix S1). The study design is described in the Appendix S1. The primary variables for analysis were maximum concentration (Cmax), area under the concentration versus time curve from time 0 to the last time point with a measurable concentration (AUC0–tlast), and time to Cmax (Tmax) of acetaminophen (APAP). A comparison of APAP pharmacokinetics on day −1 (prior to study drug) and following first and fourth dose of study drug was summarized. The trial (ClinicalTrials.gov: NCT02759107) was conducted in accordance with the Declaration of Helsinki, the Council for International Organizations of Medical Sciences International Ethical Guidelines, and the International Conference on Harmonisation Good Clinical Practices Guideline. Local institutional review boards approved the protocol.

2.3 | Study objectives

Gastric emptying was evaluated 2 hours post-meal as the percent of food retained in the stomach as a fraction of the amount of food given to DIO mice treated with long-acting GLP-1RA semaglutide, long-acting GIPRA, or dual GIP and GLP-1RA tirzepatide.

Gastric emptying was evaluated following treatment with tirzepatide versus dulaglutide and placebo in humans.
2.4 Statistical methods

In the non-clinical studies, statistical comparisons between groups were carried out using one-way ANOVA followed by Tukey’s multiple comparison test. Results are expressed as mean ± SE. In the clinical study, ratio of Cmax and AUC\(_{0-t\text{last}}\) of APAP were calculated on days 2 and 23 compared to day −1, and log-transformed to compare the GE effect of tirzepatide versus dulaglutide and placebo. A mixed-model repeated measure with treatment, day and treatment-by-day interaction as fixed effects, participant as random effect, and log-transformed baseline (day −1) as covariate was conducted. Geometric least squares mean (LSM), the ratio of geometric LSM and standard deviation are reported. The variable T\(_{\text{max}}\) of APAP was analysed using the Wilcoxon rank-sum test.

3 | RESULTS

3.1 Effect of tirzepatide on GE in mice

The affinity and potency of incretin ligands, namely, semaglutide, tirzepatide and long-acting GIPRA, used in the pre-clinical studies are presented in Table S1. Intraperitoneal glucose tolerance test results and effective doses (ED\(_{50}\)) are also reported in the same table. Semaglutide (Figure 1A) and tirzepatide (Figure 1B) inhibited GE, while long-acting GIPRA had no effect (Figure 1C). The combination of ascending doses of long-acting GIPRA to fixed dose of semaglutide had no further effect on GLP-1-induced GE delay (Figure 1D). The acute inhibitory effect on GE delay with semaglutide or tirzepatide was abolished after 2 weeks of treatment (Figure 1E).

3.2 Clinical baseline characteristics

A total of 86 participants (n = 33 healthy, n = 53 with T2DM) were included in this study. At baseline, healthy participants had a mean age of 40.3 years and 33 (100%) were male, with a mean body mass index (BMI) of 24.3 kg/m\(^2\) (Table S3). Participants with T2DM had a mean age of 56.8 years and 28 (52.8%) were male. The mean BMI was 31.2 kg/m\(^2\) and mean HbA1c concentration was 68.3 mmol/mol. Eight participants discontinued the study (healthy participants, n = 5; participants with T2DM, n = 3) due to scheduling conflicts, adverse events or discontinuation prior to receiving study drug.

3.3 Effects of tirzepatide on GE

No notable effects on GE were observed with tirzepatide 0.5 and 1.5 mg. At tirzepatide doses ≥4.5 mg in healthy participants and ≥5 mg in participants with T2DM, APAP Cmax decreased by 50% with a Tmax delay of 24 hours after a single dose. This effect diminished following multiple doses by day 23 (Figure 2A,B and Table S4). AUC was not

![Figure 1](image-url)
Impact on GE with tirzepatide 4.5 mg was comparable to that observed with dulaglutide (Figure S2A). The impact on GE in participants with T2DM was comparable to that of healthy participants (Figure 2C,D, Table S5 and Figure S2C,D). However, in participants with T2DM, tirzepatide 5 mg, 5/5/10/10 mg (data not shown) and 5/5/10/15 mg demonstrated dose-dependent glycaemic efficacy following an oral glucose tolerance test.5 In other words, the decrease in glucose exposure (AUC0–2h) from baseline was greater at day 23 versus day 2, despite the tachyphylaxis of GE delay observed over the same 4-week duration.5 Safety data are published5 and summarized in Appendix S1.

**DISCUSSION**

Gastric emptying is one of the critical factors affecting postprandial glycaemia and food intake, which is a complex interplay involving gastric, intestinal and central neuronal and humoral mechanisms.11 The role of GLP-1 in GE has been intensively studied.12 Research on GIP and its effect in GE is limited. Native GIP mimicking postprandial GIP blood levels and GIPRAs did not reveal any relevant effects on GE.3,13–15 In the present study, we investigated whether a dual GIP and GLP-1RA had any impact on GE and if the combination of GIP and GLP-1 could enhance the known GLP-1RA effects on GE in non-clinical and clinical settings.

In the absence of any available GIPRAs for clinical use, we first studied the effect of long-acting GIPRA versus GLP-1RAs in mice. Long-acting GIPRA had no effect on GE at pharmacological levels. As expected, long-acting GLP-1RA profoundly inhibited GE. Additionally, increasing concentrations of long-acting GIPRA on top of GLP-1RAs did not cause further GE delay, thereby clearly highlighting that GLP-1 was the main driver of decelerating GE. The extent of GE delay with acute tirzepatide treatment did not differ from that of long-acting GLP-1RA. Sustained exposure to GLP-1RA (via tirzepatide or using selective GLP-1RA, semaglutide) resulted in tachyphylaxis of the GE effect in mice, as demonstrated in previous studies.3,4

In our clinical studies, we found that tirzepatide's effect on GE was comparable to that of the selective GLP-1RA dulaglutide in healthy participants. In participants with T2DM, GE delay was observed following the first dose of tirzepatide 5 mg, but after
4 weeks of repeated dosing there was evidence of tachyphylaxis. However, after four weekly doses of 5/5/10/10 or 5/5/10/15 mg, GE delay, while diminished, was still apparent following the last dose.

Under similar experimental conditions, GE delay observed with tirzepatide and dulaglutide was comparable, thereby suggesting that the delay was driven by GLP-1RA and the GIP agonism did not seem to contribute to this effect.

Although the GE effect showed near-complete tachyphylaxis using the 5-mg dose, the effect on postprandial glucose (self-monitored blood glucose) was maintained following multiple 5-mg doses.5 It has also been shown that the impact of the 5-mg dose on weight loss continues beyond 4 weeks (−4.8 kg at 26 weeks vs. −2.5 kg at 4 weeks).6 This suggests that the attenuation of postprandial glycaemic rises, in the longer term, must be explained by other mechanisms over and above the waning effects on GE.1,2,13,14 These results are aligned with a prior report by Jelsing et al.,4 who demonstrated the importance of GLP-1-mediated GE delay in the immediate (ie, acute) regulation of food intake or glucose control. However, GLP-1’s effect on GE undergoes tachyphylaxis with chronic treatment but does not lead to a direct loss of glucose control or body weight effect, thereby demonstrating GLP-1’s differential regulation of metabolism.

The impact of long-acting incretin receptor agonists on GE was assessed as an exploratory measure within a phase 1 study of limited sample size. The study duration was 4 weeks, during which tirzepatide was rapidly escalated from 5 mg (the maximum tolerated dose as a single dose5), to 10 or 15 mg in the 5/5/10/10 and 5/5/10/15 mg cohorts. This limited duration did not permit evaluation of the steady-state effects of tirzepatide on GE delay, especially at the 10- and 15-mg doses. Nevertheless, although we were able to observe tachyphylaxis of GE delay at a fixed 5-mg dose, complete tachyphylaxis of GE delay within 4 weeks was not observed for the 5/5/10/10 or 5/5/10/15 mg cohorts. Longer-duration studies may be required to gain a better understanding on GE delay in relation to tachyphylaxis with tirzepatide. Studies with short-acting GLP-1RAs have shown residual effects on GE with long-term treatment.16

The treatment effect on GE delay may be influenced by the type of test used. In this study, we used an APAP test following an oral glucose tolerance test, as opposed to 'gold standard' methodology, such as scintigraphy or breath tests, to evaluate the impact of increasing dose levels and dose escalation schemes of dual GIP and GLP-1RA tirzepatide on GE within the context of a multiple-ascending dose study. This design allowed us to also compare the impact of a dual GIP and GLP-1RA versus a GLP-1RA. The technical complexities of scintigraphy and breath tests would limit such investigations within a phase 1 multiple-ascending dose clinical study and would require an additional stand-alone mechanism of action GE study. Future studies may be needed to assess the effect of tirzepatide on GE following a solid meal.

In conclusion, these data suggest that the novel dual GIP and GLP-1RA tirzepatide has similar effects on GE delay when compared with selective GLP-1RA. GE delay is not the key driver for tirzepatide-induced postprandial glycaemic efficacy and weight loss.

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CONFLICTS OF INTEREST

S.U., T.C., L.O., E.B., D.B., X.C., A.H., C.B. and C.L. are employees and shareholders of Eli Lilly and Company. M.A.N. has been a member of advisory boards or consulted for AstraZeneca, Boehringer Ingelheim, Eli Lilly and Company, Fractyl, GlaxoSmithKline, Intarcia, Menarini/Berlin Chemie, Merck, Sharp & Dohme and Novo Nordisk. His institution has received grant support from AstraZeneca, Boehringer Ingelheim, Eli Lilly and Company, GlaxoSmithKline, Intarcia, Menarini/Berlin Chemie, Merck, Sharp & Dohme, Novartis Pharma and Novo Nordisk A/S. He has served on the speakers’ bureau of AstraZeneca, Boehringer Ingelheim, Eli Lilly and Company, GlaxoSmithKline, Menarini/Berlin Chemie, Merck, Sharp & Dohme and Novo Nordisk A/S.

AUTHOR CONTRIBUTIONS

T.C., S.U., C.L., A.H. and C.B. contributed to the study design. A.H., C.L. and C.B. provided medical oversight during the trial. T.C. and X.C. were responsible for the statistical analyses. C.B. and A.H. are the guarantors of this work and, as such, take responsibility for the integrity of the data and the accuracy of the data analysis. All authors participated in interpretation of the data and critical review of the manuscript, had full access to all the data in the study and approved of this manuscript being submitted for publication.

ORCID

Shweta Urva https://orcid.org/0000-0002-3681-479X

Michael A. Nauck https://orcid.org/0000-0002-5749-6954

Axel Haupt https://orcid.org/0000-0003-4502-0470

REFERENCES

1. Imeryüz N, Yeğen BC, Bozkurt A, Coşkun T, Villanueva-Peañaacarrillo ML, Ulusoy NB. Glucagon-like peptide-1 inhibits gastric emptying via vagal afferent-mediated central mechanisms. Am J Physiol. 1997;273(4):G920-G927.

2. Unapathysivam MM, Lee MY, Jones KL, et al. Comparative effects of prolonged and intermittent stimulation of the glucagon-like peptide 1 receptor on gastric emptying and glycemia. Diabetes. 2014;63(2):785-790.

3. Nauck MA, Kemmeries G, Holst JJ, Meier JJ. Rapid tachyphylaxis of the glucagon-like peptide 1-induced deceleration of gastric emptying in humans. Diabetes. 2011;60(5):1561-1565.

4. Jelsing J, Vrang N, Hansen G, Raun K, Tang-Christensen M, Knudsen LB. Liraglutide: short-lived effect on gastric emptying—long lasting effects on body weight. Diabetes Obes Metab. 2012;14(6):531-538.

5. Coşkun T, Sloop KW, Loghin C, et al. LY3298176, a novel dual gip and glp-1 receptor agonist for the treatment of type 2 diabetes mellitus: from discovery to clinical proof of concept. Mol Metab. 2018;18:3-14.
6. Frías JP, Nauck MA, Van J, et al. Efficacy and safety of ly3298176, a novel dual gip and glp-1 receptor agonist, in patients with type 2 diabetes: a randomised, placebo-controlled and active comparator-controlled phase 2 trial. Lancet. 2018;392(10160):2180-2193.

7. Jones KL, Tonkin A, Horowitz M, et al. Rate of gastric emptying is a determinant of postprandial hypotension in non-insulin-dependent diabetes mellitus. Clin Sci (Lond). 1998;94(1):65-70.

8. Trahair LG, Horowitz M, Marathe CS, et al. Impact of gastric emptying to the glycemic and insulinemic responses to a 75-g oral glucose load in older subjects with normal and impaired glucose tolerance. Physiol Rep. 2014;2(11):e12204.

9. Acosta A, Camilleri M, Burton D, et al. Exenatide in obesity with accelerated gastric emptying: a randomized, pharmacodynamics study. Physiol Rep. 2015;3(11):e12610.

10. Jun LS, Showalter AD, Ali N, et al. A novel humanized GLP-1 receptor model enables both affinity purification and Cre-LoxP deletion of the receptor. PLoS One. 2014;9(4):e93746.

11. Goyal RK, Guo Y, Mashimo H. Advances in the physiology of gastric emptying. Neurogastroenterol Motil. 2019;31(4):e13546.

12. Camilleri M. Gastrointestinal hormones and regulation of gastric emptying. Curr Opin Endocrinol Diabetes Obes. 2019;26(1):3-10.

13. Nauck MA, Baller B, Meier JJ. Gastric inhibitory polypeptide and glucagon-like peptide-1 in the pathogenesis of type 2 diabetes. Diabetes. 2004;53(Suppl 3):S190-S196.

14. Edholm T, Degerblad M, Grybäck P, et al. Differential incretin effects of GIP and GLP-1 on gastric emptying, appetite, and insulin-glucose homeostasis. Neurogastroenterol Motil. 2010;22(11):1191-1200.

15. Meier JJ, Goetze O, Anstipp J, et al. Gastric inhibitory polypeptide does not inhibit gastric emptying in humans. Am J Physiol Endocrinol Metab. 2004;286(4):E621-E625.

16. Gentilella R, Pechtner V, Corcos A, Consoli A. Glucagon-like peptide-1 receptor agonists in type 2 diabetes treatment: are they all the same? Diabetes Metab Res Rev. 2018;35:e3070.

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

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