Efficacy, safety, and mechanistic insights of cotadutide a dual receptor glucagon-like peptide-1 and glucagon agonist

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Trial Registration: ClinicalTrials.gov, NCT03244800.
Funding: AstraZeneca

DISCLOSURES

VERP, DR, TW, DCH, **BH, and ATC** are employees and shareholders of AstraZeneca. **MP** is an employee and shareholder for AstraZeneca and is included in a patent application (nb 16/0803,064). **MGP, BK,** and **HS** have nothing to disclose. **TH** has received grants from

MedImmune, AstraZeneca, Adocia, Boehringer Ingelheim, Dance Pharmaceuticals, Johnson & Johnson, MSD, Nordic Bioscience, Poxel, Novo Nordisk, Zealand Pharma, Eli Lilly, Mylan, Roche Diagnostics, Sanofi, Saniona, and Senseonics. He has received personal fees from Eli Lilly, Mylan, Novo Nordisk, Sanofi, and Dexcom. He has also received nonfinancial support from Eli Lilly, Mylan, Zealand Pharma, and Dexcom. **LP-M** has received travel grants and speaker honoraria from Eli Lilly and Novo Nordisk. **PA** is an employee and shareholder of AstraZeneca and has a pending dosing patent for cotadutide (WO2017153575 A1). **JJM** has received grants from MedImmune, AstraZeneca, Novo Nordisk, Sanofi, MSD, and Boehringer Ingelheim. He has received personal fees from Novo Nordisk, Sanofi, MSD, Servier, Boehringer Ingelheim, and Eli Lilly.

ABSTRACT

Context: Cotadutide is a dual receptor agonist with balanced glucagon-like peptide-1 (GLP-1) and glucagon activity.

Objective: To evaluate different doses of cotadutide and investigate underlying mechanisms for its glucose-lowering effects.

Design/setting: Randomized, double-blind, phase 2a study conducted in two cohorts at five clinical trial sites

Patients: 65 adult overweight/obese patients with type 2 diabetes mellitus; 63 completed the study; 2 were withdrawn due to AEs.

Intervention: Once-daily subcutaneous cotadutide or placebo for 49 days. Doses (50-300 µg) were uptitrated weekly (cohort 1) or biweekly (cohort 2).

Main outcome measures: Coprimary end points (cohort 1) were percentage changes from baseline to end of treatment in glucose $AUC_{0–4h}$ post–mixed-meal tolerance test (MMTT) and weight. Exploratory measures included postprandial insulin and gastric emptying time (GET; cohort 2).

Results: Patients received cotadutide (cohort 1, n=26; cohort 2, n=20) or placebo (cohort 1, n=13; cohort 2, n=6). Significant reductions were observed with cotadutide vs placebo in glucose $AUC_{0–4h}$ post MMTT (LS mean [90% CI]: -21.52% [-25.68, -17.37] vs 6.32% [0.45, 12.20]; *P*<0.001) and body weight (-3.41% [-4.37, -2.44] vs -0.08% [-1.45, 1.28]; *P*=0.002). A significant increase in insulin $AUC_{0–4h}$ post MMTT was observed with cotadutide (19.3 mU.h/L [5.9, 32.6]; *P*=0.008) and GET was prolonged on day 43 with cotadutide vs placebo ($t_{½}$: 117.2 minutes vs -42.9 minutes; *P*=0.0392).

Conclusion: These results suggest that the glucose-lowering effects of cotadutide are mediated by enhanced insulin secretion and delayed gastric emptying.

PRECIS

Cotadutide in overweight or obese patients with type 2 diabetes mellitus significantly reduced glucose levels via insulinotropic effects and delayed gastric emptying time versus placebo.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a heterogenous disorder characterized by hyperglycemia, insulin resistance and beta cell insufficiency (1). Pathological features of T2DM include increased endogenous glucose production, predominantly in the liver, and reduced glucose uptake into peripheral tissues, leading to impaired fasting and postprandial glycemic control (1,2). Poor diet and lack of exercise increase metabolic burden and drive weight gain, insulin resistance, dyslipidemia, and other features of the metabolic syndrome (3,4). Despite the development of new glucose-lowering therapies in the last decade, morbidity and mortality rates associated with T2DM have increased (5,6) and the proportions of patients reaching desired glycemic targets have not changed (7). In addition to stabilizing blood glucose levels, new therapies for T2DM should, therefore, aim to promote weight loss.

Glucagon-like peptide-1 (GLP-1) receptor agonists are established therapies for T2DM and provide effective glycemic control and modest weight loss (8). Interestingly, pharmacodynamic effects of this drug class differ according to pharmacokinetic profiles. Short-acting GLP-1 receptor agonists (eg, lixisenatide) tend to promote more effective postprandial glucose reduction through delayed gastric emptying, whereas longer-acting agonists (eg, liraglutide or once-weekly preparations) have a more pronounced effect on fasting glucose, which is achieved via glucose-dependent insulin secretion and presumed glucagonostatic effects (8-10). It is believed that tachyphylaxis to the effects of long-acting GLP-1 receptor agonists on gastric emptying may, in part, explain some of these observed differences (8,11). Some studies in obese subjects, however, report that liraglutide 3.0 mg resulted in a delay in gastric emptying at 5 weeks that diminished by 16 weeks (12).

Glucagon receptor agonism may appear counterintuitive as a treatment modality for T2DM given the known effect of glucagon in increasing hepatic glucose output (13). However,

glucagon can promote weight loss via suppression of appetite and enhanced energy expenditure (14,15), which could occur via upregulation of energy-expensive metabolic processes such as amino acid catabolism, ureagenesis and fatty acid oxidation. Glucagon can also promote a delay in gastric emptying time (GET) (16), which may have favorable effects on postprandial glucose levels, and in certain conditions, glucagon is also an insulin secretagogue, potentiating insulin secretion (17,18).

In support of this, oxyntomodulin, an endogenous dual agonist for GLP-1 and glucagon receptors, is increased along with GLP-1 following bariatric surgery (19), and is believed to contribute to weight loss and improved glucose control seen after bariatric surgery (20). In addition, short-term treatment with oxyntomodulin in overweight or obese patients with or without T2DM significantly decreased body weight and elicited favorable gluco-regulatory effects (21,22). Peptides that combine an optimal ratio of GLP-1 and glucagon receptor agonism could, therefore, promote glucose-lowering activity and harness the beneficial effects of dual agonism on weight loss.

Cotadutide is a dual receptor agonist with balanced GLP-1 and glucagon activity that is under development for T2DM, obesity, and nonalcoholic steatohepatitis. Clinical and preclinical studies in T2DM have shown that cotadutide improves glycemic control and promotes body weight loss (23,24). In a randomized, controlled, phase 2a study, treatment with cotadutide resulted in a significant reduction in postprandial and fasting glucose levels, and body weight, compared with placebo in obese or overweight patients with T2DM (24).

This randomized, placebo-controlled, double-blind, phase 2a study evaluated the efficacy and safety of different doses of cotadutide in overweight or obese patients with T2DM. Continuous glucose monitoring (CGM) was used to evaluate glycemic control in this study population. As an

exploratory analysis, GET and insulin levels were measured to investigate the underlying mechanism for postprandial glucose-lowering activity with cotadutide.

MATERIALS AND METHODS

Study design and procedures

In this randomized, placebo-controlled, double-blind, phase 2a study, 2 cohorts of overweight or obese patients with T2DM in 5 study sites in Germany received once-daily subcutaneous cotadutide (AstraZeneca, Gaithersburg, MD, USA) or placebo for 49 days on different titration schedules (**Figures 1a** and **1b**). In cohort 1, CGM sensors (Freestyle Libre® Pro, Abbott Diabetes Care, Inc., Alameda, CA, USA) were applied 2 days prior to study-drug treatment and worn continuously through the end of the study (day 49). In cohort 2, serial measures of GET were undertaken throughout the study and at follow-up.

Patients

Eligible patients (aged ≥18 years) had T2DM and were all taking metformin monotherapy, with a glycated hemoglobin A1c (HbA1c) measurement of 48–69 mmol/mol (6.5–8.5%), and a body mass index of 27–40 kg/m^2 (inclusive). Patients receiving metformin monotherapy were eligible if no significant dose changes (increase or decrease ≥500 mg/day) occurred in the 3 months prior to screening. Patients receiving adjuncts to metformin (dipeptidyl peptidase-4 inhibitors, sulfonylureas, glinides, or sodium-glucose co-transporter-2 inhibitors) were allowed following a 4-week washout period. Patients were excluded if they had a medical condition that could interfere with the study, had acutely decompensated blood glucose control, or a history of type 1 diabetes mellitus or diabetic ketoacidosis. Patients were also excluded if they received once-daily subcutaneous insulin within 90 days of screening, received an agent containing a GLP-1 analogue within the 30 days of screening or before 5 half-lives of the drug, or had received any investigational product as part of a clinical study.

Randomization and masking

In cohort 1, patients were randomly assigned 2:1 to receive subcutaneous cotadutide or placebo equivalent at 50 µg for 7 days, followed by 100 µg for 7 days, then 200 µg for 7 days, and finally 300 µg for 28 days. In cohort 2, patients were randomly assigned 3:1 to receive subcutaneous cotadutide or placebo equivalent at 50 µg for 14 days, followed by 100 µg for 14 days, then 200 µg for 14 days, and finally 300 µg for 7 days. Randomization was performed using an interactive voice/web response system.

In both cohorts, patients were not given any dietary advice throughout the course of the study but were required to fast overnight for at least 10 hours prior to mixed-meal tolerance tests (MMTT). MMTTs were performed by administering 237 mL of Ensure Plus milkshake (350 calories) that comprised 51 g carbohydrates, 22 g sugars, 13 g protein, and 11 g fat (Abbott Laboratories, Columbus, OH). During the MMTT, plasma samples were taken at -15, 0, 15, 30, 60, 90, 120, 150, 180, and 240 minutes. All patients were followed-up for 28 days post-last treatment with the study drug. CGM sensor readings were double blinded during the study, and sensors were changed every 14 days. A 1-hour activation period and 12-hour warm-up period were factored into subsequent data analyses. All patients, investigators, and study-site personnel were blinded to study-drug treatment allocation.

Outcome Measures

The coprimary end points (assessed in cohort 1 only) were the percentage change from baseline to day 49 in glucose area under the concentration–time curve 0–4 hours ($AUC_{0–4h}$ post–MMTT and to day 50 in body weight. Secondary efficacy end points assessed in cohort 1 included the change from baseline to day 49 in HbA1c (EDTA tube, Beckton Dickson [BD], Franklin Lakes, NJ) levels, fasting plasma glucose (fluoride tube, BD, Franklin Lakes, NJ; enzymatic assay, Roche Cobas 8000, Indianapolis, IN) levels at study drug doses of 50 µg and

300 µg, and the proportion of patients with a weight loss ≥5%. Pooled analysis (cohorts 1 and 2) of glucose total AUC during MMTT on day 7 of treatment was assessed as a secondary end point. Venous samples were collected via venipuncture or drawn through a catheter during serial sampling. Covance Central Laboratories, Conshohocken, PA, performed assays for the study, unless otherwise specified.

Exploratory end points assessed in cohort 1 included the change from baseline to day 7 and day 49 in fasting plasma insulin (EDTA tube, BD, Franklin Lakes, NJ; chemiluminescence, Beckman-Coulter DxI 800, Brea, CA), insulin $AUC_{0–4h}$ post MMTT, and serum C-peptide levels (plain separation tube, BD, Franklin Lakes, NJ; chemiluminescence, Siemens Advia Centaur XP, Tarrytown, NY). Changes from baseline to day 49 in levels of serum free fatty acid (serum separation tube [SST], BD, Franklin Lakes, NJ; enzymatic assay, Roche Cobas 8000, Indianapolis, IN), serum beta-hydroxybutyrate (plain separation tube, BD, Franklin Lakes, NJ; enzymatic assay, Roche Cobas 8000, Indianapolis, IN), point-of-care capillary plasma ketones (Abbott Laboratories, Columbus, OH), plasma amino acids (lithium-heparin tube; BD Franklin Lakes, NJ; mass spectrometry, Burlington, NC), low-density lipoproteins (LDL), triglycerides and total cholesterol to high-density lipoproteins (HDL) ratios (SST, BD, Franklin Lakes, NJ) and serum urea (SST, BD, Franklin Lakes, NJ) were also assessed in cohort 1.

Exploratory end points assessed in cohort 2 included change from baseline to day 49 in glucose $AUC_{0–4h}$ post-MMTT, body weight, and HbA1c levels. GET was also assessed as an exploratory end point in cohort 2. GET was measured (as described previously (25)) following at least a 10-hour fast using a ^{13}C-octanoate breath test in conjunction with a standardized egg meal with crackers at baseline, days 15, 29, 43, 50, and at follow-up (28 days post-last treatment). The nutritional content of the cracker and egg meal was 280 kcal: 11.1 g (16%) protein, 18.9 g (60%)

fat, 16.1 g (23%) carbohydrates, and 0.9 g (1%) fiber. The meal contained 60 mL of water to dissolve the egg powder and was administered with another 250 mL of water.

Breath samples were centrally analyzed for $^{13}CO_2$ by y isotope-selective nondispersive infrared spectrometry (Analysen Technik, Bremen, Germany). GET was assessed 2.5 hours after administration of cotadutide or placebo on days 15, 29, and 43. No drug was administered prior to assessments at baseline, day 50, or follow-up. Glucose and insulin samples were drawn concurrently at baseline, day 50, and follow-up. GET was estimated using time for retention of ^{13}C to decline to 50% (GET $t_{½}$) and time at which the percentage of ^{13}C dose excreted per unit time reached its peak (GET t_{lag}). Metformin therapy was not washed out prior to GET assessments; other agents known to delay gastric emptying such as opiates, domperidone, and metoclopramide were excluded during the study.

CGM was used to measure the change from baseline in the percentage time spent above target glycemic range (>140 mg/dL [>7.8 mmol/L]), within target glycemic range (70 mg/dL to 140 mg/dL [3.9 mmol/L to 7.8 mmol/L]; inclusive), and clinically significant hypoglycemia (<54 mg/dL [<3.0 mmol/L]) over 7 days at each dose level. The average time spent within target glycemic range in the 49-day treatment period was also measured. Ambulatory glucose profiles across the entire treatment period and within the first few days of dosing were qualitatively assessed. Post hoc analyses on the comparability of interstitial glucose measurements to plasma glucose measurements during MMTT were assessed.

The pharmacokinetics of cotadutide at 50 µg and 300 µg were assessed in cohorts 1 and 2. Plasma concentrations of cotadutide were measured with liquid chromatography-mass spectrometry and used stable-isotope–labelled cotadutide for the internal standard (LGC group, Middlesex, UK). In cohort 1, samples for pharmacokinetic analyses were collected predose on

days 1, 7, 15, 23, 29, 36, 43, and 50. On days 22 and 49 (300 µg dose level), samples were collected predose, at 1 and 2 hours (± 15 min); and at 4, 6, 8, and 12 hours (± 30 min) postdose. In cohort 2, samples were collected predose on days 2, 8, 15, 29, 43, 49, and 50. On days 1, 7, and 14 (50 µg dose level) samples were collected predose, at 1 and 2 hours (± 15 min); and at 4, 6, 8, and 12 hours (± 30 min) postdose.

Pharmacokinetic parameters measured included maximum observed plasma concentration (C_{max}), time to maximum observed plasma concentration (t_{max}), half-life ($t_{1/2}$), area under the concentration-time curve to the end of dosing (AUC_t), and area under the concentration-time curve from time 0 to infinity ($AUC_{0\text{-}inf}$). To characterize the pharmacokinetic profile of cotadutide at 300 µg, full pharmacokinetic profiles were collected from patients in cohort 1 during the first (day 22) and last day of dosing (day 49). Similarly, to characterize the pharmacokinetic profile of cotadutide at 50 µg, full pharmacokinetic profiles were collected from patients in cohort 2 on days 1, 7, and 14.

Safety was assessed as a secondary end point in both cohorts. Treatment-emergent adverse events (TEAEs) were defined using the Medical Dictionary for Regulatory Activities (version 20.0 or higher) and elicited via subject self-report. The investigator determined whether the event was related to the study drug. Change from baseline over 7 days in the percentage of patients with nausea and vomiting was assessed as exploratory end points in both cohorts. In cohort 1, ambulatory blood pressure monitoring (ABPM) assessed changes from baseline in pulse rate, systolic blood pressure, and diastolic blood pressure on day 49 and at 28 days after last treatment (follow-up).

Statistical analysis

A planned sample size of 39 patients for cohort 1 (cotadutide, n=26; placebo, n=13) provided 97% power to detect a change from baseline in glucose AUC of 28% (assuming standard deviation [SD]=20.0%) and 85% power to detect a change from baseline in body weight of 2.3% (assuming SD=2.2%) between treatment groups. For the prespecified analysis to detect a 12% change from baseline to day 7 in glucose AUC at the cotadutide 50 µg dose level with 80% power, it was determined that by pooling cohorts 1 and 2, the combined sample size for cotadutide (n=44) plus placebo (n=19) would enable benchmarking to prior studies (24). All statistical tests were 2-sided with a significance level set at 0.1. No adjustments for multiplicity were applied.

Efficacy analyses were performed in the intent-to-treat population (all patients who received ≥1 dose of the study drug and analyzed according to randomized treatment group). Safety analyses were performed in the as-treated population (all patients who received ≥1 dose of the study drug and analyzed according to study drug received). Pharmacokinetic analyses were performed in the pharmacokinetic population (defined as all participants who received ≥1 dose of cotadutide and had ≥1 pharmacokinetic sample taken that was above the lower limit of quantification).

For the coprimary end points and continuous secondary or exploratory efficacy end points, analysis of covariance (adjusted for treatment and measurement at baseline) was used. For proportion-related secondary or exploratory efficacy end points, a logistic regression with fixed effects of treatment and baseline measurement was used. Descriptive statistics were used to summarize safety data. ABPM measures were analyzed as summary measures (mean values) collected across a 24-hour period. Statistical analyses were performed using SAS® System version 9.3 or higher (SAS Institute Inc., Cary, NC, USA).

Study approval

The protocol was approved by an independent ethics committee (Arztekammer Nordrhein, Dusseldorf, Germany). The study was performed in accordance with the International Council for Harmonization Guidance for Good Clinical Practice, the ethical principles outlined in the Declaration of Helsinki, and applicable regulatory requirements. The study was registered under ClinicalTrials.gov (NCT03244800). All patients provided written and informed consent prior to study enrollment.

RESULTS

Between August 10, 2017 and February 8, 2018, 65 patients were randomized to receive either cotadutide (cohort 1, n=26; cohort 2, n=20) or placebo (cohort 1, n=13; cohort 2, n=6; **Figure 2**). Patient demographics and baseline characteristics were generally well balanced between treatment groups in cohort 1; however, in cohort 2, patients receiving cotadutide had a numerically longer duration of T2DM compared with those receiving placebo (**Table 1**).

The coprimary end points were met. In cohort 1, significant reductions from baseline to day 49 were observed with cotadutide vs placebo in glucose $AUC_{0–4h}$ post-MMTT (least squares [LS] mean [90% confidence interval]: -21.52% [-25.68, -17.37] vs 6.32% [0.45, 12.20]; $P < 0.001$; **Figure 3a** and **3c**). In cohort 1, significant reductions from baseline to day 50 were observed with cotadutide vs placebo in percent body weight (-3.41% [-4.37, -2.44] vs -0.08% [-1.45, 1.28]; $P = 0.002$; **Figure 3b**). Similarly, in cohort 2, significant reductions from baseline were observed with cotadutide vs placebo to day 49 in percentage glucose $AUC_{0–4h}$ post-MMTT ($P < 0.001$; **Figure 3d**) and to day 50 in body weight ($P < 0.001$; **Figure 3e**).

In cohorts 1 and 2, significant reductions from baseline to day 49 were observed with cotadutide vs placebo in fasting plasma glucose (both $P < 0.001$) and HbA1c levels ($P < 0.001$ and $P = 0.037$, respectively; **Table 2**). Pooled data from both cohorts revealed greater reductions in glucose AUC during MMTT with cotadutide 50 µg on day 7 vs placebo or baseline (**Table 2**). In both cohorts, a progressive and dose-dependent decrease in body weight over time was observed with cotadutide, with the greatest magnitude of weight loss observed in cohort 1 at 300 µg for 28 days. A greater proportion of patients achieved ≥5% decrease in body weight from baseline to day 50 with cotadutide vs placebo in cohort 1 (42% [11/26] vs 8% [1/13]; $P = 0.040$) and in cohort 2 (20% [4/20] vs 0%; P = not estimable) (**Table 2**).

Over the 52 days of CGM in cohort 1, 95% (37/39) of patients had >70% data completeness, 77% [30/39] had >80% data completeness, and 62% [24/39] had >90% data completeness (**Figure 4**). Consistent reductions in mean daily glucose levels over the treatment period were observed with cotadutide (**Figure 5a**). The mean percentage of time spent within the target glycemic range during the study was significantly greater with cotadutide vs placebo (67.9% vs 43.2%; *P* = 0.002; **Figure 5b**). An improvement in glycemic variability, as measured by coefficient of variation in CGM over 7 days, was evident at all tested doses of cotadutide vs placebo (minimum–maximum; 0.19–0.22 vs 0.25–0.26; all $P \leq 0.030$; **Figure 5c**). CGM profiles revealed consistent reductions in glucose during the day and overnight (**Figure 6**).

In cohort 2, a dose-dependent delay in GET was observed with cotadutide vs placebo over time, with a lesser, but discernable, difference at day 50 and at follow-up (**Figure 7**). A significant delay in GET $t_{½}$ was observed with cotadutide vs placebo on day 43 (LS mean; 117.2 minutes vs -42.9 minutes; *P* = 0.0392; **Table 3**). A significantly prolonged GET t_{lag} was observed with cotadutide vs placebo on day 43 (LS mean; 46.5 minutes vs -27.3 minutes; *P* = 0.0479]; **Table 3**). A numerical increase in GET $t_{1/2}$ and GET t_{lag} was seen at all dose levels; and GET t_{lag} increased with exposure up to 200 µg. There was less of a delay in GET on day 50 at 300 µg with similar exposure; however, this was observed alongside a significant reduction in glucose AUC (-25.3%; 90% CI: -28.2, -22.4; $P < 0.0001$). Despite a marked reduction in glucose levels in cohort 2, insulin levels on day 50 were not statistically different with cotadutide vs placebo (**Figure 8**). A delay in peak insulin level was also evident (**Figure 8b**).

In cohort 1, a significant reduction from baseline to day 49 with cotadutide vs placebo was observed in alanine levels (LS mean [90% CI]: -60.5 µmol/L [-102.1, -18.9] vs 48.7 µmol/L [-11.5, 108.9]; *P* = 0.017; **Table 4**). Numerical reductions were observed in the levels of other amino acids, including cystine, glutamate, isoleucine, tryptophan, and tyrosine (**Table 4**). No

clinically or statistically significant changes from baseline to day 49 were observed with cotadutide vs placebo in levels of beta hydroxybutyrate, point-of-care measured ketones, urea or free fatty acids (**Table 4**). Additionally, in cohort 1, numerical reductions from baseline to day 49 in LDL cholesterol level and total cholesterol:HDL ratios, and significant reductions in triglyceride levels were observed with cotadutide vs placebo (**Table 4**).

In cohort 1, significant changes from baseline to day 49 were observed with cotadutide vs placebo in insulin $AUC_{0–4h}$ post MMTT (LS mean [90% CI]: 19.3 mU.h/L [5.9, 32.6] vs -20.6 mU.h/L [-40.1, -1.2; *P* = 0.008]; **Figure 9**). Nonsignificant changes from baseline to day 49 were observed with cotadutide vs placebo in fasting insulin, but a numerical increase in C-peptide levels was observed (LS mean [90% CI]: 0.20 µg/L [-2.22, 0.62] vs 0.71 µg/L [0.41, 1.01; *P* = 0.101]).

In the cotadutide treatment group, TEAEs were reported in 85% (22/26) and 75% (15/20) of patients in cohorts 1 and 2, respectively (**Table 5**). No deaths or serious adverse events occurred during this study period. A TEAE ≥grade 3 of vomiting was reported in 1 subject treated with cotadutide in cohort 1 (**Table 5**). Decreased appetite was the most common TEAE in cohort 1 with cotadutide (50% [13/26]; **Table 5**). The incidence of nausea and vomiting observed with cotadutide were 19% (5/26) and 12% (3/26), respectively, in cohort 1, and 35% (7/20) and 20% (4/20), respectively, in cohort 2 (**Table 5**). No TEAEs of clinically significant hypoglycemia were reported in either cohort 1 or 2. ABPM revealed a significant increase from baseline to day 49 in pulse rate with cotadutide vs placebo (*P*<0.001; **Figure 10a**). No significant differences between treatment groups were observed for systolic or diastolic blood pressure (**Figures 10b** and **10c**).

Repeat daily treatment with cotadutide at a dose range of 50 µg to 300 µg suggested linear pharmacokinetics for C_{max} and AUC (**Table 6**). Cotadutide was associated with a t_{max} of 4–6 hours, and a $t_{½}$ of approximately 8–9 hours at the tested doses (**Table 6**).

DISCUSSION

This study demonstrates that treatment of patients with cotadutide up to a dose of 300 µg for 49 days significantly decreased glucose levels and body weight compared with placebo in overweight or obese patients with T2DM. Taking into account placebo-corrected differences and all plasma- and CGM-based assessments of glycemic control, comparable glucose-lowering efficacy was observed at the lower dose level of 50 µg and efficacy showed no signs of plateau over the 49-day treatment period. In comparison to results from a previous randomized, controlled, ascending dose and phase 2a cotadutide trial (24), use of a starting dose of 50 µg resulted in a lower incidence of gastrointestinal adverse events. A longer duration of dosing, however, may be required to determine dose-dependent differences in efficacy. The safety profile of cotadutide is comparable to approved GLP-1 analogues at this stage in development (26,27). We have previously published a summary comparing the efficacy of cotadutide to that of GLP-1 analogues, accepting the limitations of benchmarking of studies of differing durations, sizes, and study populations (24).

This study demonstrates that cotadutide promotes glucose lowering via a combination of delayed GET and an insulinotropic effect. Signs of partial tachyphylaxis in the effects on GET were seen after prolonged dosing. This was, however, observed alongside a persistent insulinotropic effect, as evidenced by the lack of change in postprandial insulin despite reduced glucose levels recorded during the egg meal on day 50, with the limitation that C-peptide was not measured. The findings in this study are consistent with previously published results which included concurrent serial measurements of C-peptide and demonstrated concordance between insulin and C-peptide levels (24), suggesting enhanced secretion of insulin, rather than altered clearance. Prior studies evaluating insulin excursion and GET following administration of a GLP-1 analogue, show a concurrent reduction in postprandial insulin or C-peptide levels in the presence of a delay in GET (28). Moreover, increased or unchanged insulin levels in the

absence of a delay in GET have been reported (8). The profile for cotadutide demonstrates either a significant increase or unchanged postprandial insulin AUC and a delay in achieving peak insulin levels. This signature is presumably reflective of the combined effects of augmented insulin release and a delay in GET.

Given the known impact of glucagon on GET and the differential effects of short- and long-acting GLP-1 analogues (9,10), it is unclear whether such effects are mediated via GLP-1 or glucagon receptor agonism. It is established that GLP-1 promotes a delay in GET via transmission of cholinergic and peptidergic inhibitory signals to the vagus nerve and tachyphylaxis is thought to arise through adaptation of the parasympathetic nervous system (11,29). The mechanisms of glucagon-mediated delay in GET are less well characterized; however, inhibition of gastrointestinal contractions have been recorded in response to glucagon (16). Moreover, a direct effect on smooth muscle or sympathetic control have been proposed to mediate tonic effects in the gut (30). Tachyphylaxis to the effects of glucagon has also been reported (30). In addition to the observed tachyphylaxis, data from CGM demonstrate that postprandial glucose lowering occurs rapidly with cotadutide. This may be suggestive of an effect on the autonomic nervous system which could be due to either GLP1 or glucagon receptor agonism.

The magnitude of delay in GET observed with cotadutide was within the range observed for short-acting GLP-1 analogues (25). This suggests that there is no additive or synergistic effect in the setting of dual agonism of glucagon and GLP-1 receptors. Interestingly, the timing of onset of tachyphylaxis was not consistent with that previously observed in the GLP-1 analogue class (8) although, to the best of our knowledge, there are no prior studies evaluating serial measures of GET during dose escalation. This observation and the pharmacokinetic profile of cotadutide (which is closer to that of a long-acting GLP-1 analogue) raises the possibility that

glucagon receptor agonism may contribute to the effect of cotadutide on delaying GET. Future studies evaluating gastric motility and contrasting to short-acting and long-acting GLP-1 analogues may help to decipher the contribution of GLP-1 vs glucagon receptor engagement in this setting.

A limitation to this study was that analysis of GET included missing data due to unrecordable delays in GET in several patients during the dosing phase. In addition, although ^{13}C octanoate is an accepted method for determining gastric emptying delay, criticisms of this methodology have been raised (31). Scintigraphy is the gold standard for assessing gastric emptying (32) but is not widely available due to limits on subject exposure to local radiation. The lack of washout of metformin may influence GET (33) and differences in the absorption and kinetics of glucose and octanoate could influence the interpretation of the results. Moreover, GLP-1 agonists have been found to affect small intestinal function (34), suggesting that cotadutide could also influence small intestinal absorption.

Cotadutide resulted in a significant reduction in the levels of the glucogenic amino acid alanine in comparison to placebo. This may provide evidence for glucagon receptor engagement given the known effects of glucagon in modulating amino acid levels. Although flux measurements were not undertaken in this study, this observation may imply that alanine is being consumed by gluconeogenesis, which is a predictable effect of glucagon receptor agonism associated with increased energy expenditure (35). However, cotadutide had pronounced effects in reducing fasting and overnight glucose levels. These observations are not indicative of an increase in hepatic glucose output under fasted conditions which would be expected if cotadutide promoted a net increase in gluconeogenesis and glycogenolysis. Moreover, there were no significant changes detected in fasting free-fatty acid levels or ketogenesis with cotadutide. The reason for this is unclear. It is, however, possible that concurrent increases in insulin masked the ability to

detect any changes in these substrates. Although glucagon facilitates ureagenesis, it also promotes urinary excretion of urea (36); therefore, point measures of urea are unlikely to provide reliable evidence of glucagon receptor engagement. As such, dynamic testing of urea synthesis could provide better insights into the effects of cotadutide on the metabolism of urea compared to point estimates. Further studies evaluating endogenous glucose production and glucose uptake, and effects on free-fatty acid oxidation eg, using indirect calorimetry, would be required to better understand pharmacodynamics of cotadutide.

Cotadutide was well tolerated at all dose levels. Extending the titration schedule to two weeks did not appear to improve tolerability. TEAEs in cohort 2 coincided with egg meals used for GET assessments, which may have led to the higher incidence of TEAEs in this cohort. A limitation of this study for evaluating tolerability was a lack of statistical power to draw inferences between the two cohorts and the absence of validated questionnaires. Larger studies are needed to further evaluate the tolerability of cotadutide at different titration schedules.

In summary, cotadutide stabilized glucose levels rapidly and delivered consistent reductions in fasting and postprandial glucose for 49 days. CGM demonstrated a rapid and comprehensive effect on glucose. Cotadutide promoted sustained and clinically relevant weight loss in a substantial proportion of patients. Longer term studies are warranted to further assess the clinical utility of cotadutide in obese or overweight patients with T2DM.

AUTHOR CONTRIBUTIONS

All authors contributed to the writing of this manuscript, approved the final version for submission, and are accountable for all aspects of the work. **VERP** contributed to the trial design, data interpretation, data analysis, and conceptualization of this study. **DR** contributed to the study design and data interpretation. **TW** contributed to the study design and data analysis. **DH** contributed to data analysis. **MP** contributed to the study design, data analysis and interpretation. **ATC** contributed to analysis and interpretation of data. **MGP**, **TH**, **LP-M**, **HS**, **BK,** and **JJM** contributed to the conduct of this study. **PDA** and **BH** contributed to study design, supervising of this study, and interpretation of data.

ACKNOWLEDGMENTS

This study was funded by AstraZeneca. V Ruvini Jayasinghe, PhD, of Oxford PharmaGenesis Inc., Newtown, PA, USA, provided editorial assistance, which was funded by AstraZeneca.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

REFERENCES

1. American Diabetes Association. Standards of medical care in diabetes—2017. *Diabetes Care.* 2017;**40(suppl 1)**:S1-S129.
2. Petersen MC, Vatner DF, Shulman GI. Regulation of hepatic glucose metabolism in health and disease. *Nat Rev Endocrinol.* 2017;**13**(10):572-587.
3. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: pathogenesis and treatment. *Lancet.* 2008;**371**(9631):2153-2156.
4. Pitsavos C, Panagiotakos D, Weinem M, Stefanadis C. Diet, exercise and the metabolic syndrome. *Rev Diabet Stud.* 2006;**3**(3):118-126.
5. International Diabetes Federation. IDF Diabetes Atlas, 8th edition. 2017; http://www.diabetesatlas.org/. Accessed September 20, 2018.
6. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol.* 2018;**14**(2):88-98.
7. Carls G, Huynh J, Tuttle E, Yee J, Edelman SV. Achievement of glycated hemoglobin goals in the US remains unchanged through 2014. *Diabetes Ther.* 2017;**8**(4):863-873.
8. Meier JJ. GLP-1 receptor agonists for individualized treatment of type 2 diabetes mellitus. *Nat Rev Endocrinol.* 2012;**8**(12):728-742.
9. Trujillo JM, Nuffer W, Ellis SL. GLP-1 receptor agonists: a review of head-to-head clinical studies. *Ther Adv Endocrinol Metab.* 2015;**6**(1):19-28.
10. Hare KJ, Vilsbøll T, Asmar M, Deacon CF, Knop FK, Holst JJ. The glucagonostatic and insulinotropic effects of glucagon-like peptide 1 contribute equally to its glucose-lowering action. *Diabetes.* 2010;**59**(7):1765-1770.
11. 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.

12. Halawi H, Khemani D, Eckert D, O'Neill J, Kadouh H, Grothe K, Clark MM, Burton DD, Vella A, Acosta A, Zinsmeister AR, Camilleri M. Effects of liraglutide on weight, satiation, and gastric functions in obesity: a randomised, placebo-controlled pilot trial. *Lancet Gastroenterol Hepatol.* 2017;**2**(12):890-899.

13. Jiang G, Zhang BB. Glucagon and regulation of glucose metabolism. *Am J Physiol Endocrinol Metab.* 2003;**284**(4):E671-E678.

14. Holst JJ, Wewer Albrechtsen NJ, Pedersen J, Knop FK. Glucagon and amino acids are linked in a mutual feedback cycle: the liver-α-cell axis. *Diabetes.* 2017;**66**(2):235-240.

15. Habegger KM, Heppner KM, Geary N, Bartness TJ, DiMarchi R, Tschöp MH. The metabolic actions of glucagon revisited. *Nat Rev Endocrinol.* 2010;**6**(12):689-697.

16. Mochiki E, Suzuki H, Takenoshita S, Nagamachi Y, Kuwano H, Mizumoto A, Itoh Z. Mechanism of inhibitory effect of glucagon on gastrointestinal motility and cause of side effects of glucagon. *J Gastroenterol.* 1998;**33**(6):835-841.

17. Curry DL. Glucagon potentiation of insulin secretion by the perfused rat pancreas. *Diabetes.* 1970;**19**(6):420-428.

18. Song G, Pacini G, Ahrén B, D'Argenio DZ. Glucagon increases insulin levels by stimulating insulin secretion without effect on insulin clearance in mice. *Peptides.* 2017;**88**:74-79.

19. Laferrère B, Swerdlow N, Bawa B, Arias S, Bose M, Olivan B, Teixeira J, McGinty J, Rother KI. Rise of oxyntomodulin in response to oral glucose after gastric bypass surgery in patients with type 2 diabetes. *J Clin Endocrinol Metab.* 2010;**95**(8):4072-4076.

20. Dimitriadis GK, Randeva MS, Miras AD. Potential hormone mechanisms of bariatric surgery. *Curr Obes Rep.* 2017;**6**(3):253-265.

21. Wynne K, Park AJ, Small CJ, Patterson M, Ellis SM, Murphy KG, Wren AM, Frost GS, Meeran K, Ghatei MA, Bloom SR. Subcutaneous oxyntomodulin reduces body weight in

overweight and obese subjects: a double-blind, randomized, controlled trial. *Diabetes.* 2005;**54**(8):2390-2395.

22. Shankar SS, Shankar RR, Mixson LA, Miller DL, Pramanik B, O'Dowd AK, Williams DM, Frederick CB, Beals CR, Stoch SA, Steinberg HO, Kelley DE. Native oxyntomodulin has significant glucoregulatory effects independent of weight loss in obese humans with and without type 2 diabetes. *Diabetes.* 2018;**67**(6):1105-1112.

23. Henderson SJ, Konkar A, Hornigold DC, Trevaskis JL, Jackson R, Fritsch Fredin M, Jansson-Löfmark R, Naylor J, Rossi A, Bednarek MA, Bhagroo N, Salari H, Will S, Oldham S, Hansen G, Feigh M, Klein T, Grimsby J, Maguire S, Jermutus L, Rondinone CM, Coghlan MP. Robust anti-obesity and metabolic effects of a dual GLP-1/glucagon receptor peptide agonist in rodents and non-human primates. *Diabetes Obes Metab.* 2016;**18**(12):1176-1190.

24. Ambery P, Parker VE, Stumvoll M, Posch MG, Heise T, Plum-Moerschel L, Tsai LF, Robertson D, Jain M, Petrone M, Rondinone C, Hirshberg B, Jermutus L. MEDI0382, a GLP-1 and glucagon receptor dual agonist, in obese or overweight patients with type 2 diabetes: a randomised, controlled, double-blind, ascending dose and phase 2a study. *Lancet.* 2018;**391**(10140):2607-2618.

25. Meier JJ, Rosenstock J, Hincelin-Méry A, Roy-Duval C, Delfolie A, Coester HV, Menge BA, Forst T, Kapitza C. Contrasting effects of lixisenatide and liraglutide on postprandial glycemic control, gastric emptying, and safety parameters in patients with type 2 diabetes on optimized insulin glargine with or without metformin: a randomized, open-label trial. *Diabetes Care.* 2015;**38**(7):1263-1273.

26. Prasad-Reddy L, Isaacs D. A clinical review of GLP-1 receptor agonists: efficacy and safety in diabetes and beyond. *Drugs Context.* 2015;**4**:212283.

27. Kumarathurai P, Anholm C, Larsen BS, Olsen RH, Madsbad S, Kristiansen O, Nielsen OW, Haugaard SB, Sajadieh A. Effects of liraglutide on heart rate and heart rate

variability: a randomized, double-blind, placebo-controlled crossover study. *Diabetes Care.* 2017;**40**(1):117-124.

28. Yamada Y, Senda M, Naito Y, Tamura M, Watanabe D, Shuto Y, Urita Y. Reduction of postprandial glucose by lixisenatide vs sitagliptin treatment in Japanese patients with type 2 diabetes on background insulin glargine: A randomized phase IV study (NEXTAGE Study). *Diabetes Obes Metab.* 2017;**19**(9):1252-1259.
29. Tong J, D'Alessio D. Give the receptor a brake: slowing gastric emptying by GLP-1. *Diabetes.* 2014;**63**(2):407-409.
30. Taylor I, Duthie HL, Cumberland DC, Smallwood R. Glucagon and the colon. *Gut.* 1975;**16**(12):973-978.
31. Parker HL, Liu D, Curcic J, Ebert MO, Schwizer W, Fried M, Steingoetter A. Gastric and postgastric processing of ^{13}C markers renders the ^{13}C breath test an inappropriate measurement method for the gastric emptying of lipid emulsions in healthy adults. *J Nutr.* 2017;**147**(7):1258-1266.
32. Abell TL, Camilleri M, Donohoe K, Hasler WL, Lin HC, Maurer AH, McCallum RW, Nowak T, Nusynowitz ML, Parkman HP, Shreve P, Szarka LA, Snape WJ, Jr., Ziessman HA, American Neurogastroenterology and Motility Society and the Society of Nuclear Medicine. Consensus recommendations for gastric emptying scintigraphy: a joint report of the American Neurogastroenterology and Motility Society and the Society of Nuclear Medicine. *J Nucl Med Technol.* 2008;**36**(1):44-54.
33. Borg MJ, Bound M, Grivell J, Sun Z, Jones KL, Horowitz M, Rayner CK, Wu T. Comparative effects of proximal and distal small intestinal administration of metformin on plasma glucose and glucagon-like peptide-1, and gastric emptying after oral glucose, in type 2 diabetes. *Diabetes Obes Metab.* 2019;**21**(3):640-647.
34. Thazhath SS, Marathe CS, Wu T, Chang J, Khoo J, Kuo P, Checklin HL, Bound MJ, Rigda RS, Crouch B, Jones KL, Horowitz M, Rayner CK. The glucagon-like peptide 1

receptor agonist exenatide inhibits small intestinal motility, flow, transit, and absorption of glucose in healthy subjects and patients with type 2 diabetes: a randomized controlled trial. *Diabetes.* 2016;**65**(1):269-275.

35. Heppner KM, Habegger KM, Day J, Pfluger PT, Perez-Tilve D, Ward B, Gelfanov V, Woods SC, DiMarchi R, Tschöp M. Glucagon regulation of energy metabolism. *Physiol Behav.* 2010;**100**(5):545-548.

36. Tamura S, Kawata S, Fukuda K, Inui Y, Kakimoto H, Ishiguro H, Namba M, Kono N, Matsuzawa Y. Effects of glucagon on urinary excretion of urea and on plasma ammonia level in argininosuccinate synthetase deficiency. *J Gastroenterol.* 1994;**29**(1):31-34.

LEGENDS FOR FIGURES AND TABLES

Figures:

Figure 1: Study design. Study design for cohort 1 (**a**) and cohort 2 (**b**).

Figure 2: Patient disposition. CONSORT diagram showing patient disposition for the study.

Figure 3: **Coprimary and secondary efficacy end points.** Percentage change from baseline to day 49 in glucose $AUC_{0–4h}$ post-MMTT (**a**) and to day 50 in body weight (**b**; coprimary end points) and the glucose excursion profile over time (**c**) in cohort 1. Percent change from baseline to day 49 in glucose $AUC_{0–4h}$ post MMTT (**d**) and to day 50 in body weight (**e**) in cohort 2. Data are LS means (90% CI) analyzed by ANCOVA.

Figure 4: Individual data completeness for CGM in cohort 1. Data completeness for CGM for each patient in cohort 1 over 52 days.

Figure 5: CGM data in cohort 1. CGM data showing daily glucose levels (**a**) (data are mean ± SEM); the proportion of time spent in target glycemic levels (**b**) (ie, 70–140 mg/dL or 3.9–7.8 mmol/L [inclusive]; data are mean [SD]); and the coefficient of variation of glucose levels over 7 days at each dosing level (**c**). Data analyzed using ANCOVA or a logistic regression model for proportion related end points.

Figure 6: Glucose levels measured using CGM in cohort 1. Mean glucose levels every 4 hours over the initial treatment period. Data are mean ± SEM.

Figure 7: GET in cohort 2. GET estimations based on Wagner-Nelson method at baseline (**a**); day 15 (**b**); day 29 (**d**); day 43 (**d**); day 50 (**e**); and after a 28-day follow-up period (**F**). Data are mean ± SEM.

Figure 8: Glucose and insulin levels during GET assessments in cohort 2. Glucose (**a**) and insulin levels (**b**) during GET assessments in cohort 2. Data are mean ± SEM. Note delay in peak insulin in panel (**b**) on day 50 in the cotadutide-treated group.

Figure 9: Insulin excursion profile in cohort 1. Insulin AUC during MMTT over time in cohort 1. Data are mean ± SD.

Figure 10: Effects on BP in cohort 1. Change from baseline in pulse rate (**a**), systolic BP (**b**), and diastolic BP (**c**), at the end of the study and at follow-up in cohort 1. Data are LS mean (90% CI) and analyzed with ANCOVA.

Tables:

Table 1. Patient demographics and baseline characteristics. . Data are mean (SD), unless otherwise specified.

Table 2. Secondary efficacy end points. Data are LS mean change from baseline to the end of treatment, unless otherwise specified.

Table 3. Change from baseline over time in GET $t_{½}$ and GET t_{lag}, and change from baseline to day 50 in GET plasma glucose $AUC_{0–4\ h}$ and to day 49 in GET plasma insulin $AUC_{0–4\ h}$.

Table 4. Changes from baseline to day 49 in amino acids ketones, fatty acids, urea, and the lipid profile in cohort 1. Data for baseline values are mean (SD); data for change from baseline values are LS mean (90% CI), unless otherwise specified.

Table 5. Summary of safety and TEAEs by SOC and PT (≥15%).

Table 6. Pharmacokinetics of cotadutide.

Table 1. Patient demographics and baseline characteristics. Data are mean (SD), unless otherwise specified.

Parameter	Cohort 1		Cohort 2	
	Cotadutide (n = 26)	Placebo (n = 13)	Cotadutide (n = 20)	Placebo (n = 6)
Age, years	58.7 (8.5)	60.2 (5.6)	61.9 (6.0)	60.3 (9.5)
Sex, n (%)				
Female	7 (27)	4 (31)	10 (50)	1 (17)
Male	19 (73)	9 (69)	10 (50)	5 (83)
Race, n (%)				
White	25 (96)	13 (100)	20 (100)	6 (100)
Pacific Islander	1 (4)	0	0	0
Weight, kg	95.6 (17.2)	93.8 (21.0)	92.4 (8.7)	93.7 (9.6)
BMI, kg/m^2	31.5 (3.5)	31.6 (3.8)	31.1 (3.5)	31.2 (3.5)
Blood pressure, mmHg				
Systolic	133.5 (13.9)	133.9 (11.7)	139.8 (17.7)	128.0 (8.8)
Diastolic	82.2 (8.6)	84.1 (8.1)	88.2 (11.2)	82.2 (10.2)
Duration of T2DM, years	9.4 (7.2)	8.1 (8.7)	12.3 (5.8)	8.9 (4.2)
Fasting plasma glucose, mmol/L	8.7 (2.1)	8.6 (1.6)	9.2 (1.9)	9.4 (1.7)
HbA1c				
%	7.26 (0.58)	7.19 (0.52)	7.47 (0.58)	7.48 (0.72)
mmol/mol	55.8	55.1	58.1	58.3
Glucose AUC$_{0–4h}$ post MMTT, mmol.h/L	41.0 (9.5)	40.1(6.3)	46.4 (8.6)	45.8 (8.6)
Fasting insulin, mU/L	9.80 (5.62)	10.47 (11.32)	8.83 (5.62)	9.09 (4.26)
C-peptide, µg/L	2.54 (0.84)	2.64 (1.47)	1.43 (0.91)	2.52 (0.67)

AUC, area under the plasma concentration–time curve; BMI, body mass index; HbA1c, glycated hemoglobin A1c; MMTT, mixed-meal tolerance test; SD, standard deviation; T2DM, type 2 diabetes mellitus.

Table 2. Secondary efficacy and exploratory end points. Data are LS mean change from baseline to the end of treatment, unless otherwise specified.

Parameter	Cohort 1		Cohort 2		Day 7 Pooled[a]	
	Cotadutide (n = 26)	Placebo (n = 13)	Cotadutide (n = 20)	Placebo (n = 6)	Cotadutide (n = 46)	Placebo (n = 19)
Glucose $AUC_{0–4h}$, %	-21.52	6.32	-34.24	1.68	-28.84	-1.49
90% CI	-25.68, -17.37	0.45, 12.20	-36.69, 29.80	-6.44, 9.80	-31.28, -26.40	-5.25, 2.27
vs placebo	*P* < 0.001		*P* < 0.001		*P* < 0.001	
Body Weight, %	-3.41	-0.08	-2.90	0.40		
90% CI	-4.37, -2.44	-1.45, 1.28	-3.58, 2.22	-0.84, 1.64	–	
vs placebo	*P* = 0.002		*P* < 0.001			
FPG, mmol/L	-1.96	-0.13	-2.97	-0.63		
90% CI	-2.37, -1.55	-0.71, 0.45	-3.27, -2.66	-1.20, -0.07	–	
vs placebo	*P* < 0.001		*P* < 0.001			
HbA1c, %	-0.67	-0.07	-0.83	-0.41		
90% CI	-0.82, -0.53	-0.27, 0.14	-0.99, -0.68	-0.70, -0.13	–	
vs placebo	*P* < 0.001		*P* = 0.037			
Insulin $AUC_{0–4h}$, mU.h/L	19.29	-20.63	2.18	16.83		
90% CI	5.94, 32.64	-40.07, -1.19	-12.62, 16.99	-8.33, 41.99	–	
vs placebo	*P* = 0.008		*P* = 0.401			
≥ 5% weight loss, n (%)	11 (42)	1 (8)	4 (20)	0		
Odds ratio	10.76		NE		–	
90% CI	1.61, 72.03		NE			
vs placebo	*P* = 0.040		NE			

[a]Pooled data are from cohorts 1 and 2, showing LS mean change from baseline to 7 days of treatment at the cotadutide 50 µg dose.
$AUC_{0–4h}$, area under the curve, 0 to 4 hours; CI, confidence interval; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin A1c; LS, least squares; NE, not estimable.

Table 3. Change from baseline over time in GET $t_{½}$ and GET t_{lag}, and change from baseline to day 50 in GET plasma glucose $AUC_{0–4\ h}$ and to day 49 in GET plasma insulin $AUC_{0–4\ h}$.

Parameter	Cotadutide (n = 20)	Placebo (n = 6)
GET $t_{½}$, min		
Day 15		
N	16	6
Mean change from BL (SD)	42.9 (109.4)	-19.7 (61.8)
Day 29		
N	15	6
Mean change from BL (SD)	118.1 (246.8)	-23.3 (13.4)
LS mean change from BL (90% CI)[a]	98.9 (23.1, 174.6)	-37.4 (-172.7, 98.0)
vs placebo	*P* = 0.1469	
Day 43		
N	13	6
Mean change from BL (SD)	126.8 (218.1)	-21.7 (49.8)
LS mean change from BL (90% CI)[a]	117.2 (56.2, 178.3)	-42.9 (-152.0, 66.2)
vs placebo	*P* = 0.0392	
Day 50		
N	16	6
Mean change from BL (SD)	15.9 (55.4)	-30.8 (33.2)
LS mean change from BL (90% CI)[a]	8.8 (-12.6, 30.2)	-34.9 (-73.2, 3.3)
vs placebo	*P* = 0.1020	
Follow-up[b]		
N	17	6
Mean change from BL (SD)	-34.1 (49.1)	-38.2 (33.0)
LS mean change from BL (90% CI)[a]	-27.6 (-41.0, -14.2)	-45.4 (-69.5, -21.4)
vs placebo	*P* = 0.2795	
GET t_{lag}, minutes		
Day 15		
N	16	6
Mean change from BL (SD)	23.2 (63.4)	-14.5 (38.6)
Day 29		
N	15	6
Mean change from BL (SD)	32.6 (93.0)	-18.8 (8.6)
LS mean change from BL (90% CI)[a]	28.3 (0.9, 55.7)	-23.3 (-72.2, 25.6)
vs placebo	*P* = 0.1285	
Day 43		
N	13	6
Mean change from BL (SD)	54.8 (118.1)	-19.3 (33.9)
LS mean change from BL (90% CI)[a]	46.5 (16.9, 76.0)	-27.3 (-80.0, 25.4)
vs placebo	*P* = 0.0479	
Day 50		
N	16	6
Mean change from BL (SD)	7.6 (35.5)	-24.0 (23.9)
LS mean change from BL (90% CI)[a]	4.8 (-9.0, 18.6)	-24.4 (-49.0, 0.2)
vs placebo	*P* = 0.0898	
Follow-up[c]		
N	17	6
Mean change from BL (SD)	-21.7 (27.7)	-29.3 (21.9)
LS mean change from BL (90% CI)[a]	-17.2 (-26.9, -7.4)	-31.4 (-48.8, -14.0)
vs placebo	*P* = 0.2338	
GET plasma glucose $AUC_{0–4h}$, mg.h/dL		
Mean at BL (SD)	700.9 (146.5)	703.3 (135.4)
LS mean % change from BL (90% CI)	-25.3 (-28.2, -22.4)	-4.4 (-9.4, 0.6)

vs placebo	P < 0.001	
GET plasma insulin $AUC_{0–4h}$, pmol.h/L		
Mean at BL (SD)	339.4 (179.8)	334.7 (155.2)
LS mean change from BL (90% CI)	30.3 (-88.7, 149.3)	101.33 (38.6, 164.0)
vs placebo	*P* = 0.373	

[a]Last observation carried forward. [b]Follow-up period was 28 days.

AUC, area under the concentration–time curve; BL, baseline; CI, confidence interval; GET, gastric emptying time; GET $t_{½}$, time for retention of ^{13}C to decline to 50%; GET t_{lag}, time at which the percentage of ^{13}C dose excreted per unit time reached its peak; LS, least squares; MMTT, mixed-meal tolerance test; SD, standard deviation.

Table 4. Changes from baseline to day 49 in amino acids ketones, fatty acids, urea, and the lipid profile in cohort 1. Data for baseline values are mean (SD); data for change from baseline values are LS mean (90% CI), unless otherwise specified.

Parameter	Cotadutide (n = 26)	Placebo (n = 13)	*P* value vs placebo
Glucogenic amino acids			
Alanine, µmol/L			
Baseline	438.24 (105.95)	417.84 (68.59)	–
Change from baseline	-60.52 (-102.14, -18.91)	48.69 (-11.49, 108.88)	0.017
Arginine, µmol/L			
Baseline	60.58 (17.84)	63.50 (19.92)	–
Change from baseline	-3.27 (-9.91, 3.36)	-0.06 (-9.65, 9.52)	0.645
Asparagine, µmol/L			
Baseline	54.98 (9.52)	50.97 (8.59)	–
Change from baseline	1.04 (-3.35, 5.43)	2.55 (-3.85, 8.96)	0.747
Aspartic acid, µmol/L			
Baseline	3.13 (1.05)	3.06 (1.13)	–
Change from baseline	0.53 (-0.74, 1.80)	-0.03 (-1.86, 1.10)	0.673
Cystine, µmol/L			
Baseline	34.32 (8.76)	36.26 (9.85)	–
Change from baseline	1.21 (-3.25, 5.68)	-5.12 (-8.21, -2.03)	0.057
Glutamate, µmol/L			
Baseline	100.58 (36.73)	103.85 (82.26)	–
Change from baseline	-5.78 (-19.87, 8.31)	11.41 (-8.93, 31.76)	0.248
Glutamine, µmol/L			
Baseline	515.03 (75.31)	516.12 (131.84)	–
Change from baseline	24.31 (-10.62, 59.24)	10.26 (-40.16, 60.68)	0.701
Glycine, µmol/L			
Baseline	199.54 (59.59)	182.93 (57.45)	–
Change from baseline	-14.95 (-29.19, -0.72)	-28.07 (-48.72, -7.43)	0.385
Histidine, µmol/L			
Baseline	85.74 (15.44)	78.96 (11.72)	–
Change from baseline	-8.57 (-13.51, -3.63)	-9.35 (-16.55, -2.15)	0.882
Methionine, µmol/L			
Baseline	24.98 (4.96)	24.17 (5.61)	–
Change from baseline	-2.18 (-4.37, 0.01)	-1.09 (-4.26, 2.07)	0.637
Proline, µmol/L			
Baseline	286.16 (100.31)	264.88 (93.11)	–
Change from baseline	9.88 (-23.77, 43.53)	46.47 (-2.23, 95.17)	0.305
Serine, µmol/L			
Baseline	123.41 (23.44)	110.81 (18.18)	–
Change from baseline	14.13 (3.64, 24.62)	5.47 (-9.95, 20.89)	0.448
Valine, µmol/L			
Baseline	297.95 (57.45)	285.20 (68.32)	–
Change from baseline	-40.75 (-61.44, -20.06)	-6.76 (-36.66, -23.15)	0.124
Ketogenic amino acids			
Leucine, µmol/L			
Baseline	172.95 (36.93)	162.23 (29.04)	–
Change from baseline	24.34 (-37.03, -11.64)	-5.15 (-23.53, 13.23)	0.157
Lysine, µmol/L			
Baseline	213.45 (39.91)	203.47 (39.43)	–
Change from baseline	-42.83 (-57.61, -28.05)	-15.42 (-36.80, 5.95)	0.084

Ketogenic and glucogenic amino acids			
Isoleucine, µmol/L			
Baseline	89.23 (23.81)	85.46 (16.40)	–
Change from baseline	-2.87 (-9.71, 3.98)	2.16 (-7.73, 12.05)	0.485
Phenylalanine, µmol/L			
Baseline	66.62 (13.75)	62.53 (9.06)	–
Change from baseline	-4.32 (-7.87, -0.77)	-3.66 (-8.80, 1.48)	0.861
Threonine, µmol/L			
Baseline	105.07 (27.24)	90.79 (18.81)	–
Change from baseline	-11.28 (-21.54, 1.03)	-16.37 (-31.39, -1.34)	0.646
Tryptophan, mg/dL			
Baseline	1.27 (0.24)	1.25 (0.29)	–
Change from baseline	-0.09 (-0.17, -0.02)	0.01 (-0.10, 0.11)	0.204
Tyrosine, µmol/L			
Baseline	68.87 (14.55)	66.23 (18.76)	–
Change from baseline	-9.94 (-15.54, -4.34)	1.08 (-7.02, 9.18)	0.068
Free fatty acids, mmol/L			
Baseline	0.64 (0.21)	0.55 (0.17)	–
Change from baseline	-0.04 (-0.09, 0.02)	0.03 (-0.05, 0.10)	0.293
Plasma beta-hydroxybutyrate, µmol/L			
Baseline	174.0 (155.0)	163.3 (79.7)	–
Fasted state	41.03 (-3.87, 85.92)	16.23 (-47.28, 79.73)	0.594
4 hours postdose	-50.08 (-73.45, -26.71)	-37.28 (-70.33, -4.22)	0.597
Point-of-care capillary ketones, mg/dL			
Baseline	27.8 (51.7)	36.6 (62.7)	–
Fasted state	-25.67 (-26.36, -24.98)	-26.60 (-27.58, -25.62)	0.201
4 hours postdose	-27.41 (-27.90, -26.92)	-26.71 (-27.41, -26.02)	0.174
Blood urea nitrogen			
Baseline	5.56 (1.25)	5.60 (1.22)	–
% Change from baseline	-11.25 (22.74)	-5.96 (14.52)	–
Lipid Profile			
LDL cholesterol, mmol/L			
Baseline	2.74 (0.84)	2.82 (0.91)	–
Change from baseline	-0.27 (-0.43, -0.11)	0.05 (-0.18, 0.29)	0.066
Triglycerides, mmol/L			
Baseline	2.02 (0.94)	2.37 (1.36)	–
Change from baseline	-0.40 (-0.62, -0.18)	0.11 (-0.20, 0.42)	0.031
Total cholesterol: HDL			
Baseline	3.47 (0.99)	3.55 (1.02)	–
Change from baseline	-0.03 (-0.21, 0.16)	0 (-0.27, 0.26)	0.905

CI, confidence interval; LDL, low-density lipoprotein; LS, least square; HDL, high-density lipoprotein; SD, standard deviation.

Table 5. Summary of safety and TEAEs by SOC and PT (≥15%).

Parameter, n (%)	Cohort 1		Cohort 2	
	Cotadutide (n = 26)	Placebo (n = 13)	Cotadutide (n = 20)	Placebo (n = 6)
TEAEs	22 (85)	6 (46)	15 (75)	3 (50)
Treatment-related AEs	17 (65)	2 (15)	14 (70)	0
Grade ≥ 3 TEAE	1 (4)	0	0	0
Serious AE	0	0	0	0
Deaths	0	0	0	0
TEAEs by SOC and PT				
Gastrointestinal disorders	11 (42)	2 (15)	9 (45)	0
Constipation	4 (15)	0	1 (5)	0
Dyspepsia	4 (15)	0	2 (10)	0
Nausea	5 (19)	0	7 (35)	0
Vomiting	3 (12)	0	4 (20)	0
General disorders and administration-site reactions	5 (19)	1 (8)	7 (35)	0
Injection-site erythema	0	0	5 (25)	0
Infections and infestations	4 (15)	3 (23)	5 (25)	0
Viral upper respiratory tract	3 (12)	3 (23)	3 (15)	0
Metabolism and nutrition disorders	13 (50)	2 (15)	1 (5)	0
Decreased appetite	13 (50)	2 (15)	0	0
Musculoskeletal and connective tissue disorders	2 (8)	1 (8)	0	1 (17)
Back pain	0	1 (8)	0	1 (17)
Nervous system disorders	3 (12)	0	5 (25)	2 (33)
Headache	2 (8)	0	4 (20)	2 (33)
Reproductive system and breast disorders	0	0	0	1 (17)
Vaginal hemorrhage	0	0	0	1 (17)
Respiratory, thoracic, and mediastinal disorders	3 (12)	0	1 (5)	1 (17)
Cough	1 (4)	0	0	1 (17)
Nasal obstruction	0	0	0	1 (17)

AE, adverse event; PT, preferred term; SOC, systems organ class; TEAE, treatment-emergent adverse event.

Table 6. Pharmacokinetics of cotadutide.

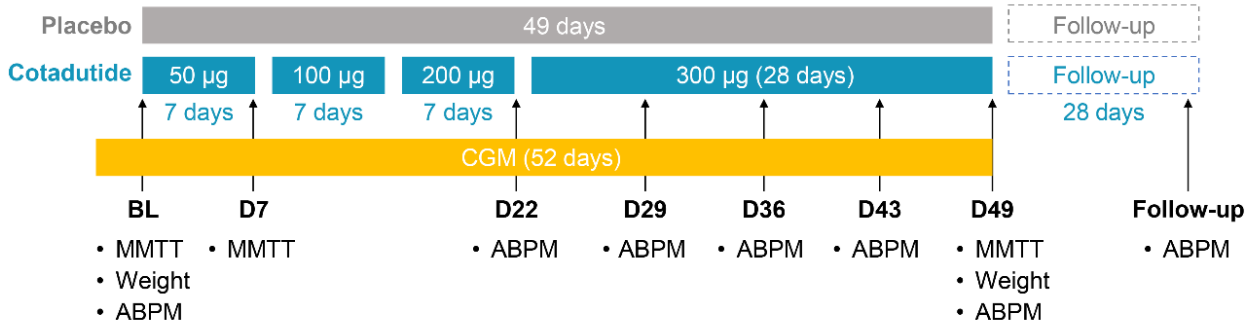
Pharmacokinetic parameter[a]	Cohort 1: 300 µg, day 49 (n = 25)	Cohort 2: 50 µg, day 14 (n = 20)
C_{max}, ng/mL	14.8 (5.76, 33.3)	2.7 (1.5, 3.8)
t_{max}, h[b]	4 (2, 8)	6 (4, 12)
$t_{½}$, h	8.4 (7.8, 9.4)	9.4 (8.7, 10.5)
AUC_t, ng.h/mL	248.8 (86.6, 558.6)	46.8 (26.4, 65.6)
$AUC_{0–inf}$, ng.h/mL	318.8 (250.9, 395.8)	62.8 (52.8, 72.7)
Accumulation ratio	1.5 (1.1, 3.0)	1.5 (1.2, 1.9)

[a]Data are geometric means (range), unless otherwise specified.
[b]Median (range).
$AUC_{0–inf}$, area under the concentration–time curve from time 0 to infinity; AUC_t, area under the concentration–time curve from time 0 to infinity; CI, confidence interval; C_{max}, maximum observed concentration; $t_{1/2}$, half-life; t_{max}, time to maximum observed concentration.

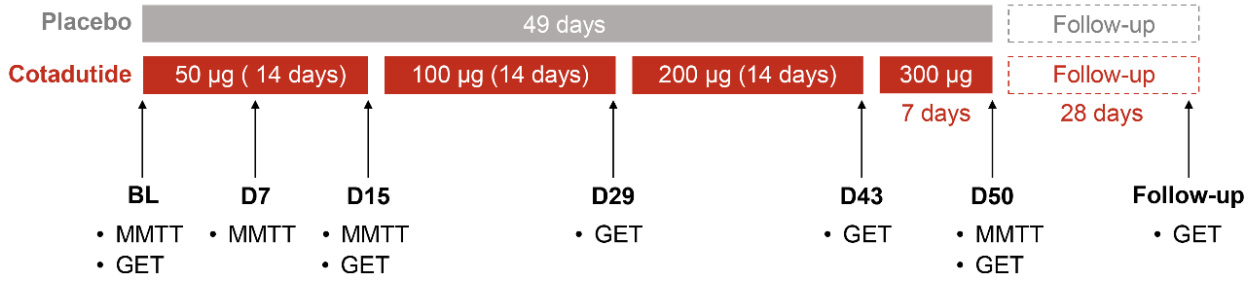
FIGURES

Figure 1: Study design. Study design for cohort 1 (**a**) and cohort 2 (**b**).

(a)

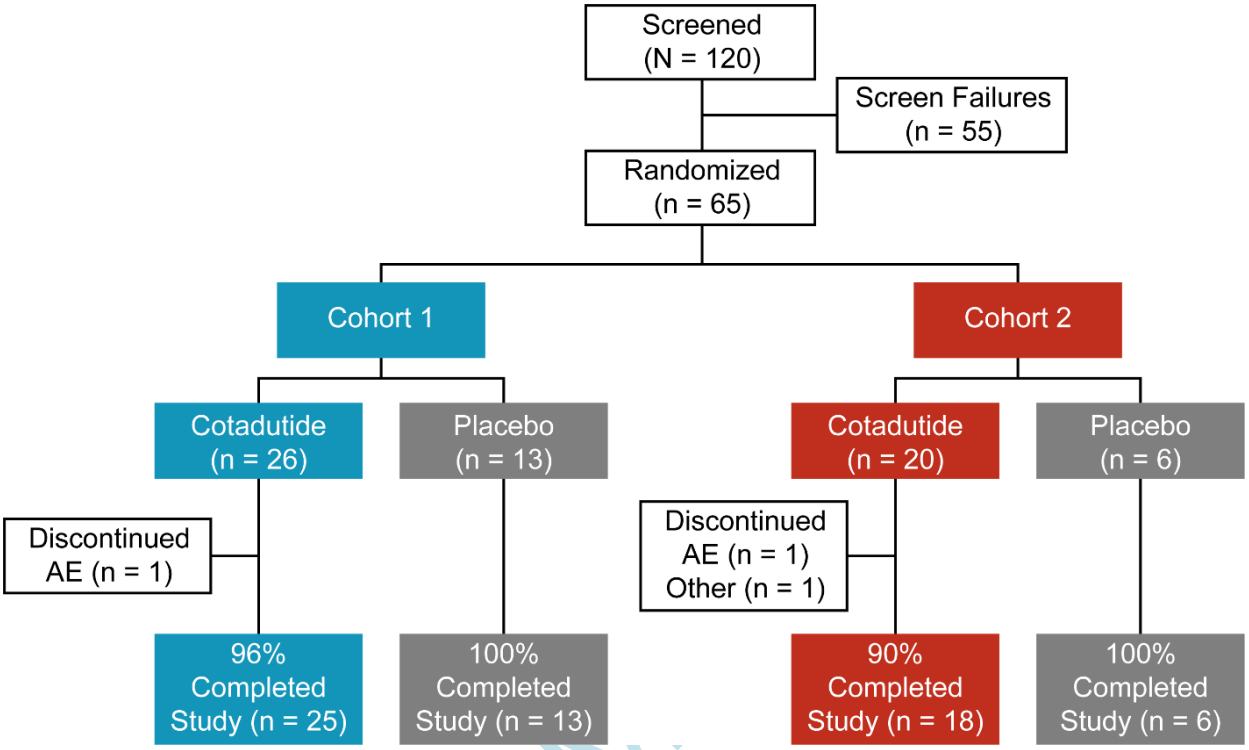


(b)



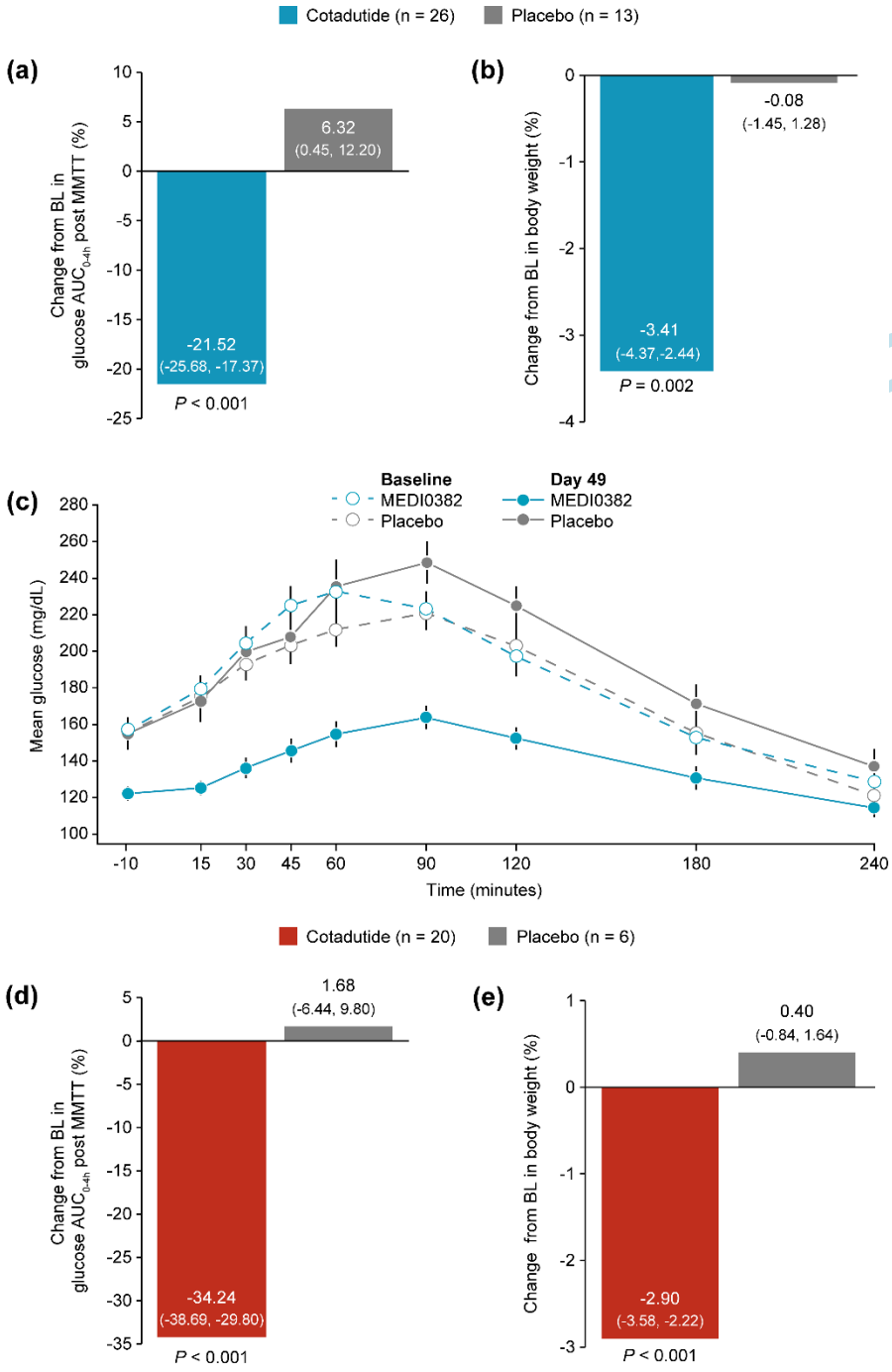
ABPM, ambulatory blood pressure monitoring; BL, baseline; D, day; GET, gastric emptying time; MMTT, mixed-meal tolerance test.

Figure 2: Patient disposition. CONSORT diagram showing patient disposition for the study.



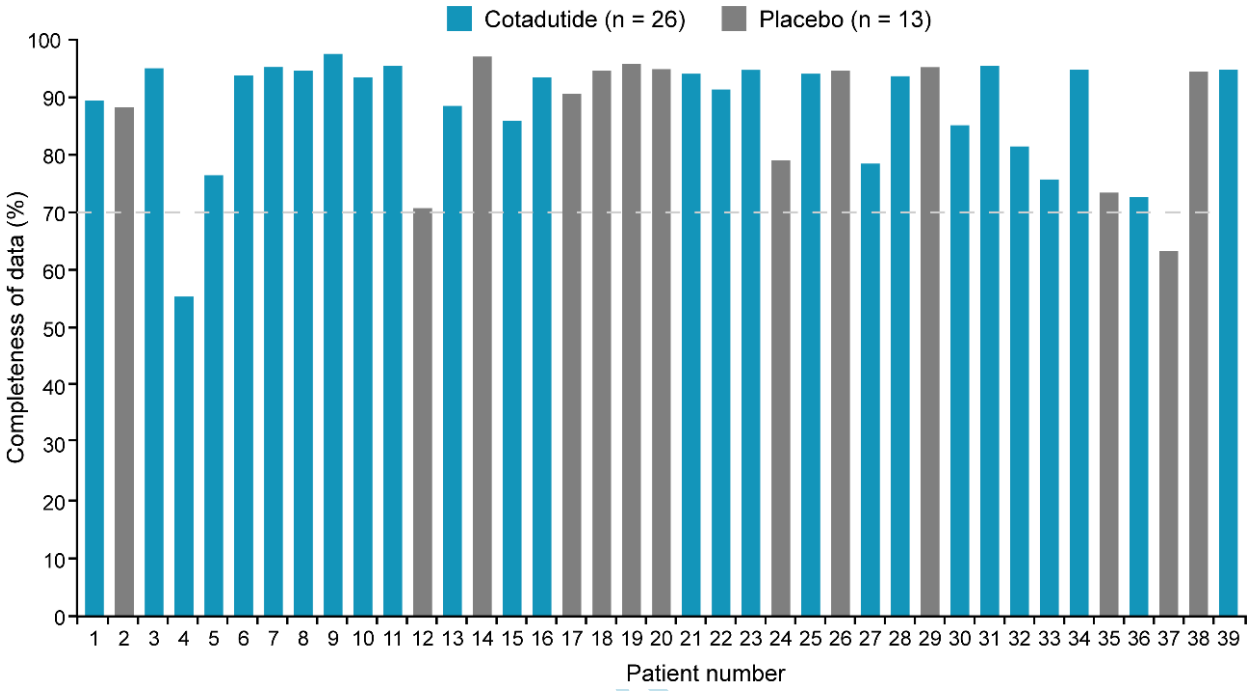
AE, adverse event.

Figure 3: **Coprimary and secondary efficacy end points.** Percentage change from baseline to day 49 in glucose $AUC_{0–4h}$ post-MMTT (**a**) and to day 50 in body weight (**b**; coprimary end points) and the glucose excursion profile over time (**c**) in cohort 1. Percent change from baseline to day 49 in glucose $AUC_{0–4h}$ post MMTT (**d**) and to day 50 in body weight (**e**) in cohort 2. Data are LS means (90% CI) analyzed by ANCOVA.



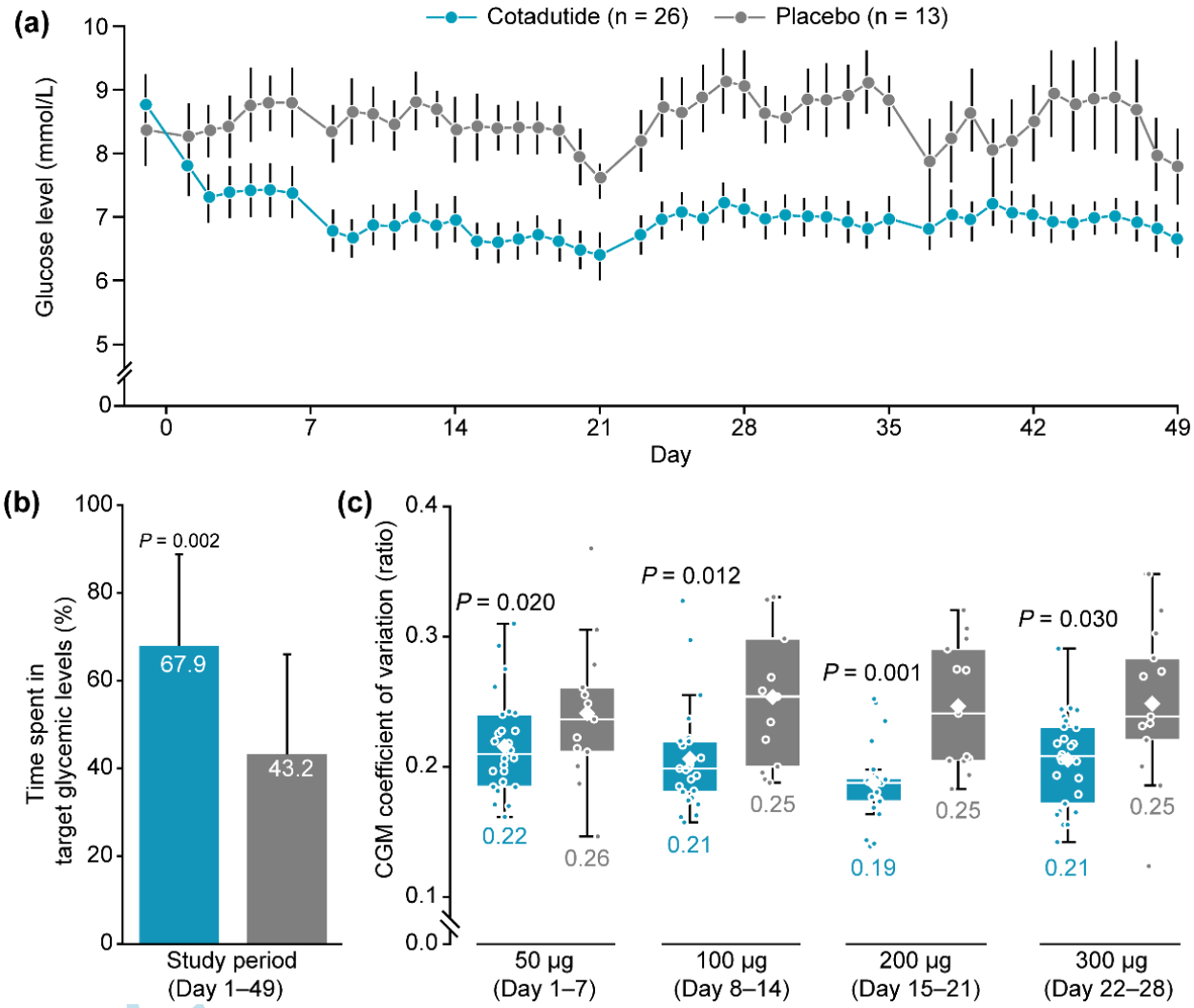
ANCOVA, analysis of covariance; AUC, area under the plasma concentration–time curve; BL, baseline; CI, confidence interval; LS, least squares; MMTT, mixed-meal tolerance test.

Figure 4: Individual data completeness for CGM in cohort 1. Data completeness for CGM for each patient in cohort 1 over 52 days.



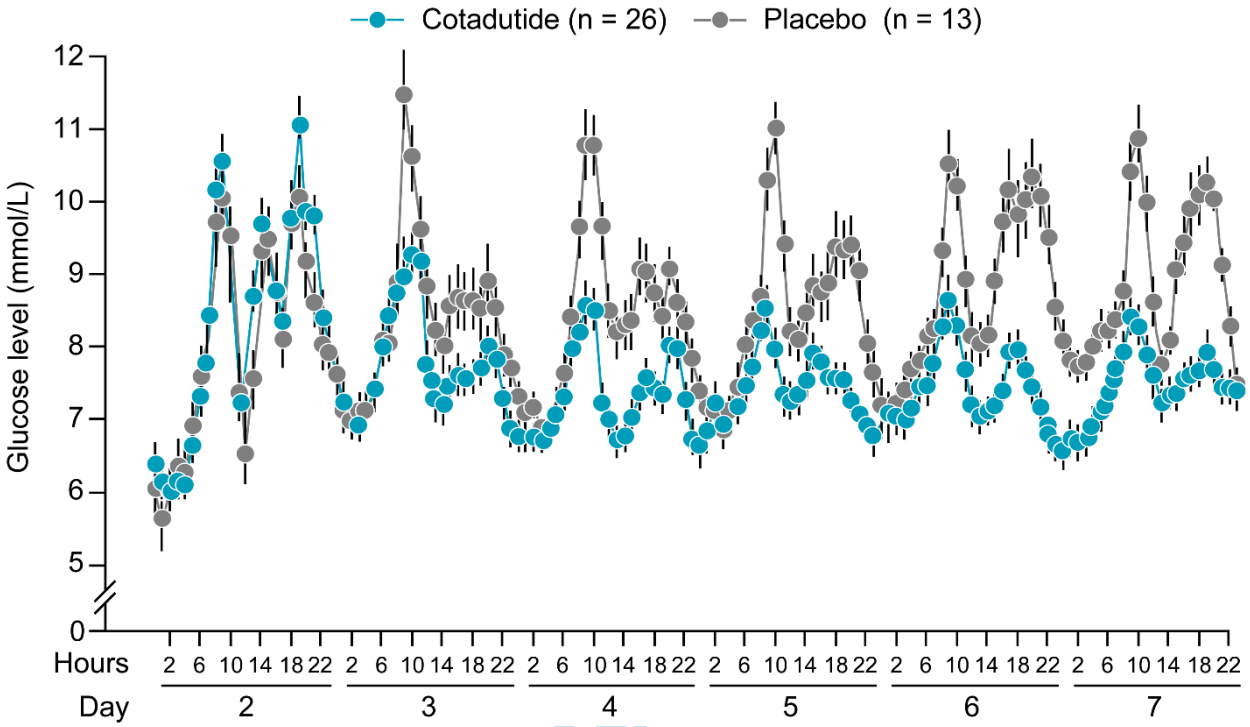
CGM, continuous glucose monitoring.

Figure 5: CGM data in cohort 1. CGM data showing daily glucose levels (**a**) (data are mean ± SEM); the proportion of time spent in target glycemic levels (**b**) (ie, 70–140 mg/dL or 3.9–7.8 mmol/L [inclusive]; data are mean [SD]); and the coefficient of variation of glucose levels over 7 days at each dosing level (**b**). Data analyzed using ANCOVA or a logistic regression model for proportion related end points.



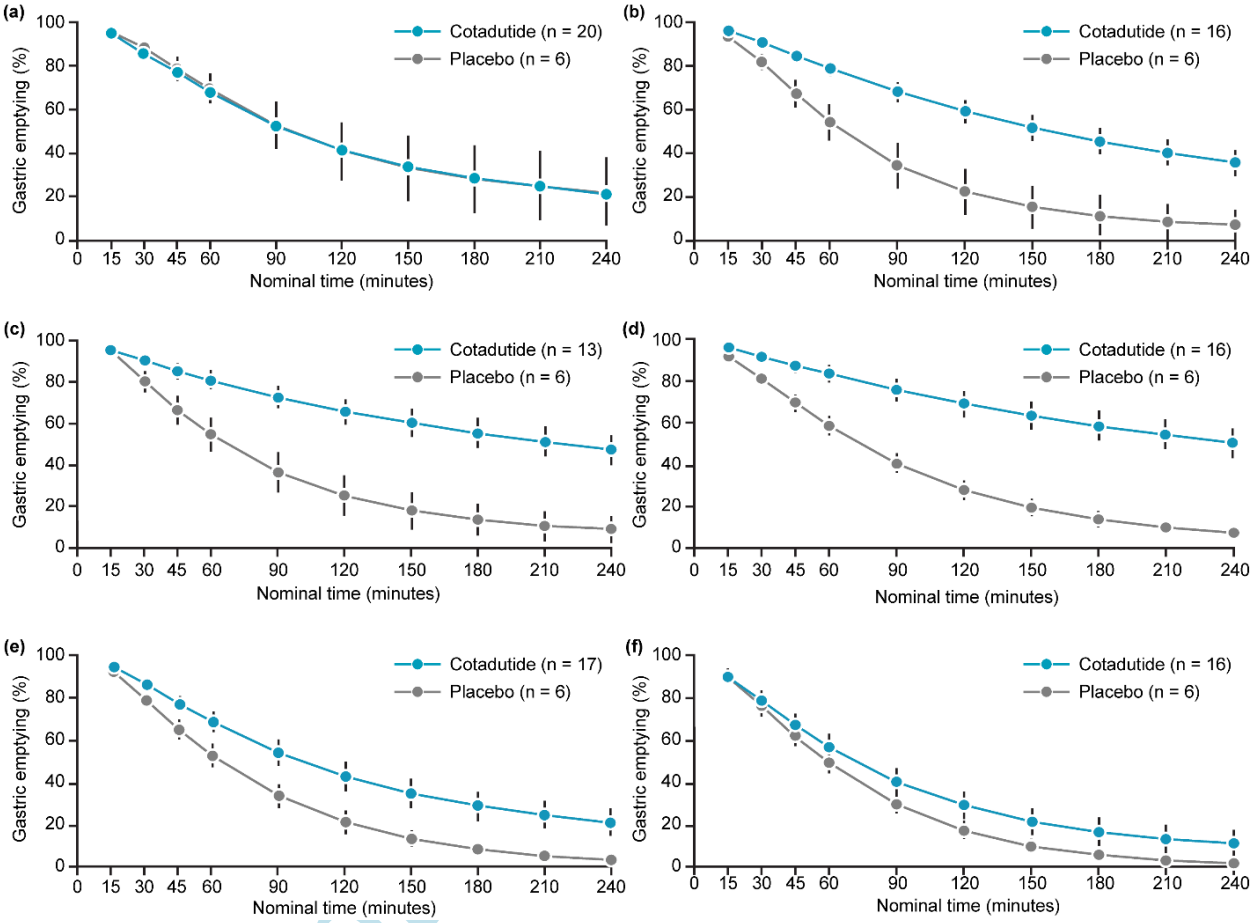
ANCOVA, analysis of covariance; CGM, continuous glucose monitoring; SEM, standard error of the mean.

Figure 6: Glucose levels measured using CGM in cohort 1. Mean glucose levels every 4 hours over the initial treatment period. Data are mean ± SEM.



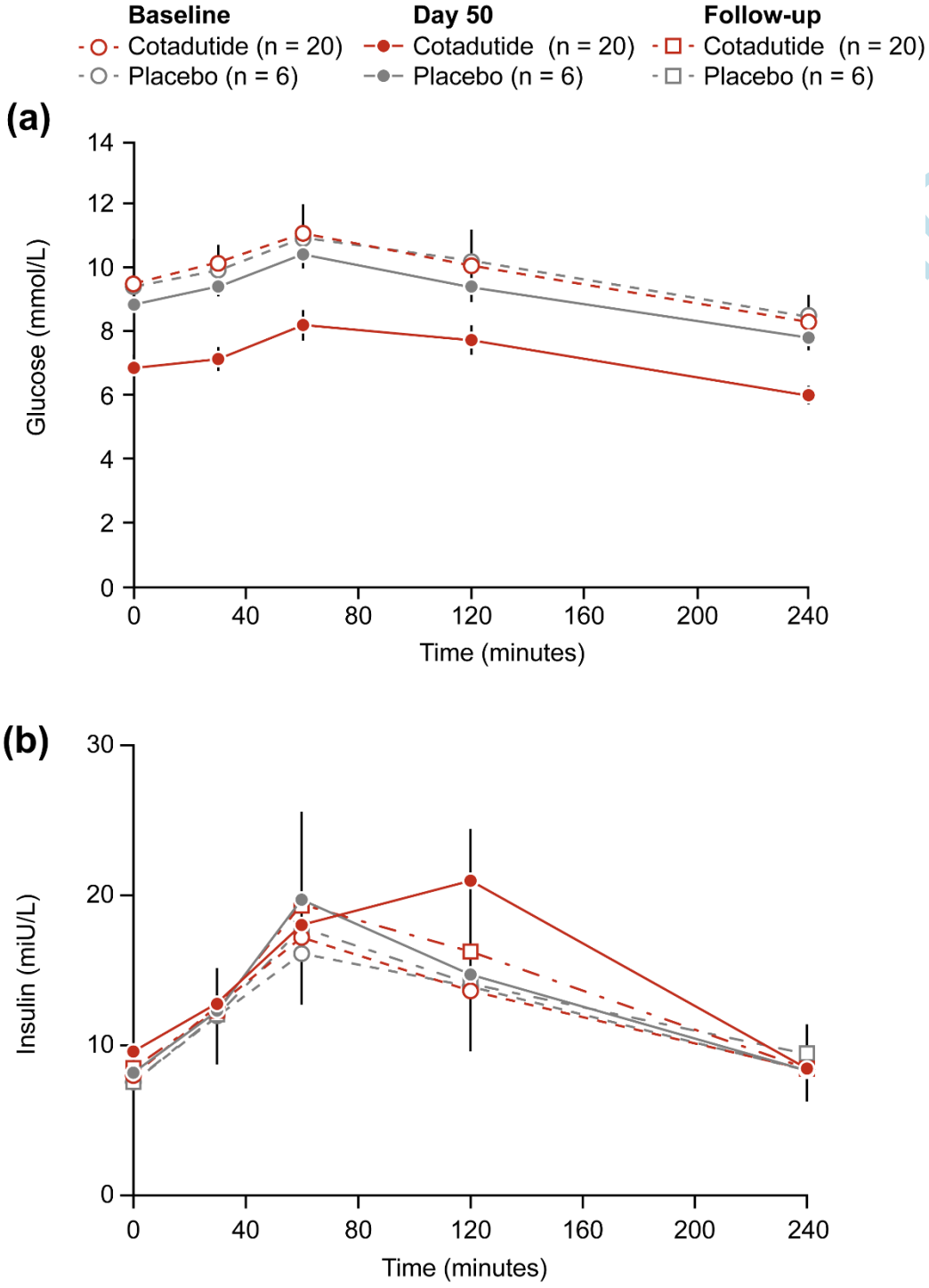
CGM, continuous glucose monitoring; SEM, standard error of the mean.

Figure 7: GET in cohort 2. GET estimations based on Wagner-Nelson method at baseline (**a**); day 15 (**b**); day 29 (**c**); day 43 (**d**); day 50 (**e**); and after a 28-day follow-up period (**f**). Data are mean ± SEM.



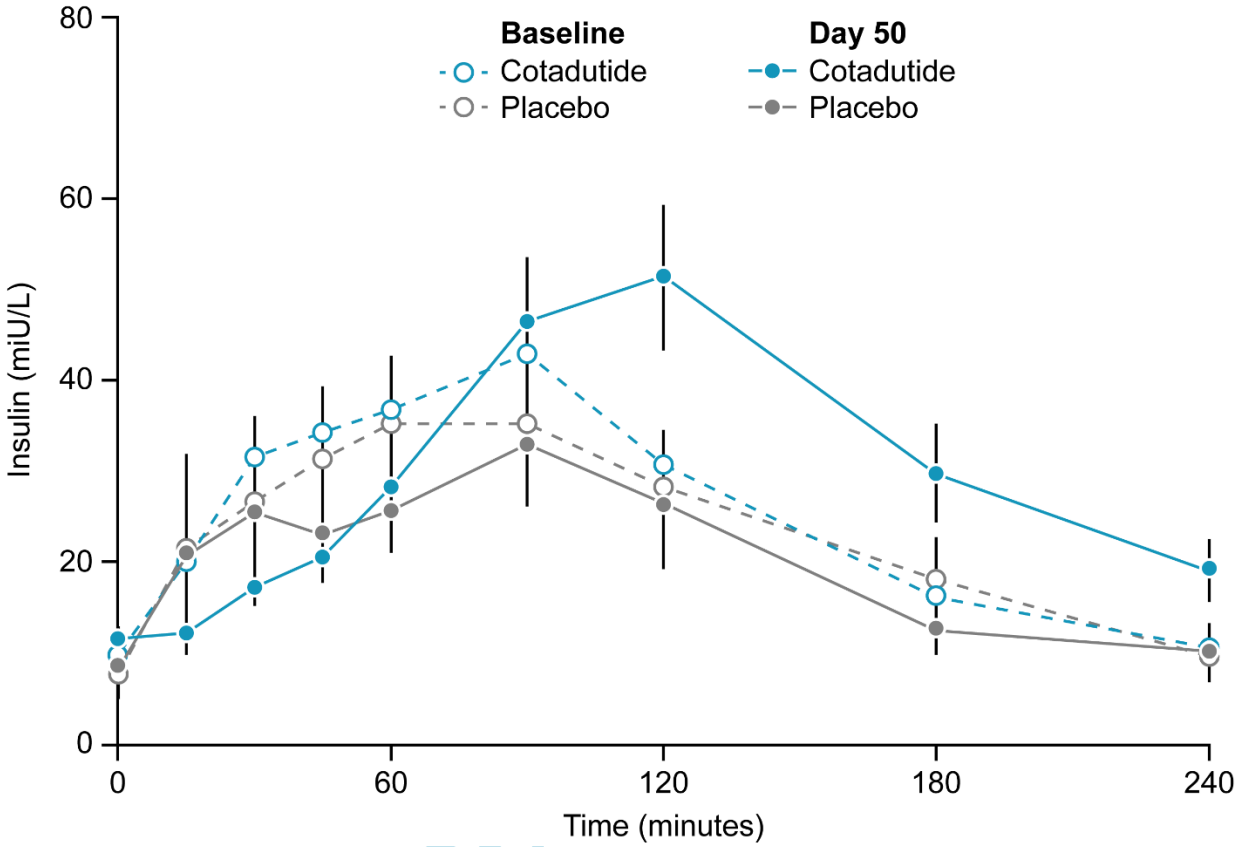
GET, gastric emptying time; SEM, standard error of the mean.

Figure 8: Glucose and insulin levels during GET assessments in cohort 2. Glucose (**a**) and insulin levels (**b**) during GET assessments in cohort 2. Data are mean ± SEM. Note delay in peak insulin in panel (**b**) on day 50 in the cotadutide-treated group.



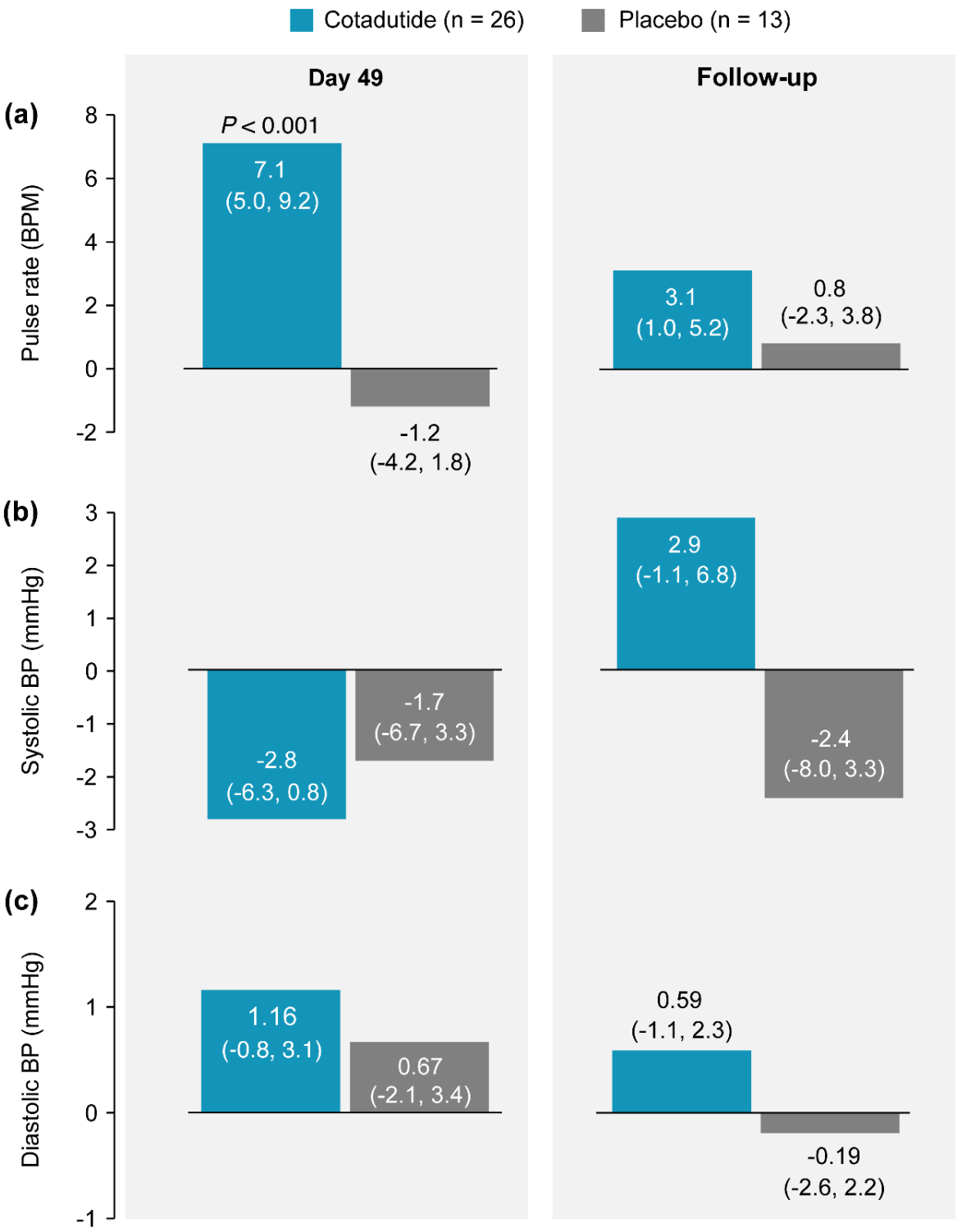
AUC, area under the concentration–time curve; GET, gastric emptying time; SEM, standard error of the mean.

Figure 9: Insulin excursion profile in cohort 1. Insulin AUC during MMTT over time in cohort 1. Data are mean ± SD



MMTT, mixed-meal tolerance test; SD, standard deviation.

Figure 10: Effects on BP in cohort 1. Change from baseline in pulse rate (**a**), systolic BP (**b**), and diastolic BP (**c**), at the end of the study (day 49) and at follow-up in cohort 1. Data are LS mean (90% CI) and analyzed with ANCOVA.



ANCOVA, analysis of covariance; BP, blood pressure; BPM, beats per minute; CI, confidence interval; LS, least squares.