Dual GLP-1 and glucagon receptor agonism reduces energy intake in type 2 diabetes with obesity

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Conflict of interest statement

RG, JK, MV, and AK have no conflicts of interest to declare. ME has served on advisory boards and/or received lecture fees and/or research support from Novo Nordisk, Eli Lilly, AstraZeneca, Medtronic, Dexcom, Ypsomed, Abbott Diabetes Care, Roche, NGM Pharma, Zucara, and Pila Pharma. VP, DR, DL, LH, LJ, PA, MR, MS, RCL are employees and stockholders of AstraZeneca. AP has received honoraria from Novo Nordisk.

ABSTRACT

Aims

Cotadutide is a GLP-1/glucagon receptor dual agonist that has demonstrated clinically significant weight loss in early phase studies. We aimed to establish which components of energy balance mediate this.

Materials and methods

We conducted a phase 2a, single-center, randomized, placebo-controlled trial in overweight and obese adults with type 2 diabetes. Following a 16-day single-blind placebo run-in, participants were randomized 2:1 to double-blind 42-day subcutaneous treatment with cotadutide (100–300 μg daily) or placebo. The primary outcome was percentage weight change. Secondary outcomes included change in energy intake (EI) and energy expenditure (EE).

Results

A total of 12 (63%) cotadutide and seven (78%) placebo participants completed the study. Mean (90%CI) weight change was –4.0% (–4.9, –3.1) and –1.4% (–2.7, –0.1) for cotadutide and placebo, respectively, $p = 0.011$. EI was lower with cotadutide vs placebo (–41.3% [–66.7, –15.9], $p = 0.011$). Difference in EE (per kJ/kg lean body mass) for cotadutide vs placebo was 1.0% (90%CI: –8.4, 10.4), $p = 0.784$, assessed by doubly labeled water, and –6.5% (–9.3, –3.7), $p < 0.001$, assessed by indirect calorimetry.

Conclusion

Weight loss with cotadutide is primarily driven by reduced EI, with relatively small compensatory changes in EE.

Trial registration

The study was registered at ClinicalTrials.gov (NCT03596177).

Funding

This study was funded by AstraZeneca.

Introduction

The rapidly increasing prevalence of type 2 diabetes (T2D), obesity, and associated metabolic disease, such as metabolic dysfunction associated steatohepatitis (MASH(1)), is a cause of considerable worldwide disease and economic burden (2-4). MASH and T2D can further contribute to the progression of kidney and cardiovascular disease (5). Weight loss has been associated with improvements in MASH (6) and glycemic control (7); therefore, weight loss is considered a key treatment target for patients with MASH and/or T2D (8, 9). Glucagon-like peptide-1 (GLP-1) receptor agonism has been shown to provide effective glycemic control in T2D. However, weight loss with GLP-1 receptor agonism is relatively modest at the doses at which maximum glucose reduction is achieved, ranging 1.5–6.0 kg in clinical trials (10) (11). This highlights the need for T2D treatments to complement glucose control with a greater reduction in body weight. A major challenge for achieving weight loss by reduced energy intake (whether through diet or pharmacotherapy) is the physiological compensatory reduction in energy expenditure (EE) that tends to counteract reduced energy intake (EI) (12, 13). Therapies that can simultaneously reduce appetite while preventing or limiting a reduction in EE might therefore allow greater weight loss and metabolic benefits.

One such approach is to combine the beneficial effects of GLP-1 receptor agonism with other therapies (glucose-dependent insulinotropic polypeptide [GIP] receptor agonism or antagonism, or glucagon receptor agonism). Tirzepatide, a novel dual GIP/GLP-1 receptor agonist, has shown promising findings with potent glucose-lowering effects and weight loss in patients with T2D (14).

Several trials in overweight and obese adults demonstrated an increase in EE following a short-term glucagon infusion (15-17). However, glucagon infusion longer than 72h was not associated with increased EE (18). In these studies, glucagon was used in low doses as high doses can induce nausea and vomiting (19).

Several GLP-1/glucagon receptor agonists are in development, including BI 456906, LY3305677, LY4347943, JNJ-54729518 (20, 21), HM15211 ,NNC9204-1706, Alt-801, and G3215 (22). Two further agents have been discontinued owing to excessive gastrointestinal adverse events during phase 2 studies (SAR425899) (22) and cardiovascular safety issues (NN117) (23). Although the magnitude of glucose lowering and weight loss varies across these agents, the optimal activity ratio of the two agonists from the perspective of health outcomes is yet to be determined.

Cotadutide is a synthetic linear peptide, which functions as a dual GLP-1/glucagon receptor agonist (24) and is under development for treating MASH and chronic kidney disease with T2D (25, 26). Glucagon signaling has potential metabolic benefits, such as decreased appetite (27) and food intake, reduced gastric emptying, increased EE, and beneficial renal and hepatic effects, which can act synergistically with GLP-1-RA (5, 15-17, 28-31). The beneficial renal effects of glucagon include natriuresis, increased renal vasodilation and an increase in estimated glomerular filtration rate(32), while favorable hepatic effects include increased lipid oxidation, reduced lipid synthesis, and increased bile acid production (33). With a 5:1 ratio of GLP-1 and glucagon signaling (26), cotadutide has been optimized so that the anti-glycemic properties of GLP-1 balance the glucose-elevating properties of glucagon action, such that the overall effect of cotadutide results in a glucose decrease while retaining appropriate glucagon signaling.

In rodent models, cotadutide promoted weight loss through both increased EE and reduced EI (34). Based on comparisons with liraglutide in the same study, increased EE might account for up to 25% of body weight loss while the remainder was attributable to reduced energy intake (34). Clinical studies with cotadutide in obese or overweight individuals with T2D demonstrated decreased body weight and improvements in glycemia (5, 25, 35), with the latter being mediated by increased insulin secretion and delayed gastric emptying (26). However, the interplay among EE, EI, and weight loss induced by cotadutide has not been studied in humans.

Here, we report a randomized, placebo-controlled, double-blind, phase 2a study performed in adults with T2D and obesity, which included a detailed examination of the mechanisms of cotadutide action. We show that cotadutide-associated weight loss is driven by markedly decreased EI, achieved without any major compensatory change in EE. We show that glycemic effects are likely mediated by increased insulin secretion and reduced gastric emptying.

Materials and methods

Study design and approval

This randomized, placebo-controlled, double-blind, phase 2a trial was conducted in a single center (Cambridge, UK). Study design and patient disposition are detailed in Figure S1 and Figure 1, respectively.

The study was approved by the East of England-Essex Research Ethics Committee (reference 18/EE/0204) and was performed according to 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 at ClinicalTrials.gov (NCT03596177). All patients provided a written informed consent before enrollment.

Participants

Participants were recruited via a radio advertisement and through local primary care practices between October 2018 and December 2019. The study was conducted in the NIHR/Wellcome Clinical Research Facility within the Cambridge Biomedical Campus. Overweight and obese (BMI > 28 and ≤ 40 kg/m^2) adults aged 30–75 years with well-controlled T2D (HbA1c ≤ 8.0%) were eligible. Participants receiving metformin monotherapy were eligible if there was no significant change (increase or decrease >50%) in the dose 3 months before screening. Individuals treated with adjuncts to metformin (dipeptidyl peptidase-4 inhibitors, sulfonylureas, glinides, or sodium-glucose co-transporter-2 inhibitors) were eligible after a 4-week washout period. The key exclusion criteria were using GLP-1 receptor agonists (GLP1-RA), insulin, or appetite-suppressing medications, acutely decompensated glycaemia, and regular high intensity exercise. Full list of eligibility criteria is shown in S1.

Randomization and follow-up

Eligible participants underwent a 16-day single-blind treatment with placebo to exclude non-compliers or placebo-responders thus increasing study power (36). Subsequently, participants were randomly assigned 2:1 to receive subcutaneous cotadutide or placebo for 42 days with a fixed-dose escalation of 100 µg daily for the first 4 days, 200 µg daily for the following 4 days and 300 µg daily for the remaining 34 days. Cotadutide and placebo were identically labeled and indistinguishable in appearance. Therefore, the pharmacists, participants, and study site personnel were masked to treatment allocation. Randomization was performed using an interactive web response system. All participants entered a 28-day follow-up period of safety monitoring with the final visit occurring 28 days post last dose.

Outcomes and procedures

The primary efficacy outcome was percentage change in body weight from the beginning to the end of treatment (day 17-59). Secondary efficacy outcomes included absolute and percentage change in the following variables: energy intake (kJ) from *ad libitum* lunch from day 16 to 32 and from day 16 to 59, total energy expenditure (TEE), and activity-related energy expenditure (AEE) from day 15 to 58, resting energy expenditure (REE) from day 16 to 32, body fat mass from day –1 to 59, fasting glucose, and $AUC_{glucose}$ during mixed meal tolerance test (MMTT) from day –1 to 59. All EE variables were expressed in kJ/kg of fat mass and additional analyses were performed using lean body mass instead of fat mass in separate models. Exploratory outcomes included: change in glucose metabolism parameters (insulin sensitivity, glucose effectiveness, first phase insulin response (FPIR), disposition index) derived from intravenous glucose tolerance test (IVGTT, detailed in S2) between days 1 to 14 and days 41 or 42, gastric emptying time from day 15 to 58, mean daily time (min) spent in activity, mean daily step count between day 1 to16 and day 44 to 59. EE was measured using indirect calorimetry and doubly labelled water technique. While the former is based on oxygen consumption and carbon dioxide production in a respiration chamber, the latter measures carbon dioxide production from isotope elimination in the urine. We measured and report both insulin and C-peptide responses during ivGTT and MMTT as latter are more closely linked to insulin secretion.Procedures are detailed in S3 and S4 (study protocol).

Statistical analysis

A sample size of 16 participants in the cotadutide group and eight participants in the placebo group was planned to provide >90% power to detect a 2.5% weight change from baseline difference between treatment groups at day 59 assuming SD=2.0% and two-sided α=0.1.

Descriptive statistics were used to summarize data at specified time points. The primary efficacy outcome of percentage change in body weight between days 17 and 59 was compared between cotadutide and placebo groups using an analysis of covariance (ANCOVA) adjusting for treatment group and baseline body weight. The analyses for EE variables was planned to be conducted using kJ/kg of fat mass. Additional sensitivity analyses were performed using kJ/kg of lean body mass and kJ/kg of total body mass. The aim of these analyses was to compare mean EE between treatment groups while controlling for the effect of covariates.

A modified intention-to-treat population was used for the analysis of primary and secondary efficacy outcomes, and analysis of covariance (ANCOVA) models adjusting for treatment group and baseline value of the outcome of interest were fitted. The LS-mean and the corresponding 90%CI are presented for ANCOVA models. Selected secondary and exploratory endpoints were analyzed using per-protocol population. Safety outcomes were analyzed in as-treated population. Last observation carried forward method was used to impute post-baseline missing values when applicable.

Statistical analyses were performed using SAS®, version 9.4 or higher. Analyses of IVGTT data were conducted using minimal model and parameter estimation (S2) in SAAM II software (version 2.3) and STATA (version 16). GraphPad Prism (version 9) was used for figure generation.

Results

Study population

From October 2018 to December 2019, 44 participants were screened for eligibility, of whom 28 were randomly assigned to receive cotadutide (n=19) or placebo (n=9). All 28 participants received placebo in the single-blind run-in period and 25 progressed to receive double-blind treatment (Figure 1) with cotadutide (n=18) or placebo (n=7). Of n=28 enrolled participants, 19 (68%) completed the study. Of the nine participants who discontinued, two participants withdrew and seven were discontinued for other reasons (Figure 1).

Demographic and baseline characteristics were balanced between treatment groups (Table 1). Participants were on average 60 years old and predominantly white men, with obesity and well-controlled T2D. Two participants in the cotadutide arm were overweight (BMI≥ 25 kg/m^2 and < 30 kg/m^2) while the rest of the participants in both groups were obese (BMI≥ 30 kg/m^2).

Body weight and composition

Cotadutide therapy resulted in 4% weight loss over 42 days of treatment, with significantly greater weight loss than placebo (between-group difference least squares [LS]-mean –2.6% [90%CI: –4.2, –1.0; p = 0.011]) (Table 2, Figure S2a). Data from home scales demonstrated a sequential weight reduction over time (Figure S2b). In keeping with the overall weight reduction, cotadutide resulted in greater reductions versus placebo in total fat mass (–3.3 [90%CI: –4.3, –2.3] kg vs –1.4 [90%CI: –2.8, –0.1] kg, p=0.085) and lean mass (–3.0 [90%CI: –3.6, –2.5] kg vs –1.4 [90%CI: –2.2, –0.6] kg, p=0.01, Table 2).

Self-reported hunger

There was no significant difference between treatment groups in any of the four items used to assess hunger measured by visual analogue scale (Table S1). However, there was a numerical increase in the sensation of feeling sick in the cotadutide group vs placebo with no statistical significance while a reduction in the desire to eat reduced in both groups (Table S1).

Energy intake and expenditure

We then examined in more detail how changes in EI and EE might underpin the cotadutide-induced changes in body weight and composition. The placebo group had an approximately 600 kJ higher EI at baseline which is a small difference in absolute terms. Strikingly, EI during *ad libitum* meal fell by 52% (relative change from day 16 to 59 vs placebo –41% [90%CI: –67, –16], p=0.011) (Table 3, Figure S2c).

Despite these changes in EI and weight loss, measured changes in EE were modest. TEE assessed by doubly labeled water technique showed no significant difference between treatment groups, with percentage change versus placebo of 1.0% (90%CI: –8.4, 10.4; p=0.784) expressed as kJ/kg of lean body mass and 5.0% (90%CI: –6.7, 16.7; p=0.710) expressed as kJ/kg of fat mass (Table S2). TEE measured by indirect calorimetry percentage change versus placebo was –6.5% (90%CI: –9.3, –3.7; p<0.001) expressed in kJ/kg of lean body mass and –3.2 (90%CI: –9.4, 3.0; p=0.384) expressed in kJ/kg of fat mass (Table 4). REE showed the same pattern as TEE, although with a small numerical increase in REE noted after 16 days of therapy in the cotadutide arm (+8.5% vs +1.2%; LS-mean difference 7.4% [90%CI: –1.7, 16.5; p=0.177]). There were no differences between placebo and cotadutide for activity-related energy expenditure (AEE). There was a small reduction in respiratory quotient in the cotadutide group between baseline and the end of treatment (LS-mean (90%CI) for % change: -0.73 (-2.28, 0.82) vs 2.30 (0.26, 4.33), cotadutide vs placebo, p=0.056) indicating a possible shift towards fat metabolism.

Physical activity

Greater weight loss in the cotadutide group was not explained by an increase in physical activity, with both daily step count (change from baseline [90%CI] –1286 [–2402, –170] steps vs 1021 [–442, 2484] steps, cotadutide vs placebo, p=0.044) and time spent in physical activity (change from baseline [90%CI] –11 [–20, –2] min/day vs 7 [–4, 19] min/day, cotadutide vs placebo, p=0.047) reduced in the cotadutide group (Figure S3, Table S3).

Glucose homeostasis

Fasting plasma glucose decreased in cotadutide group (change from baseline [90%CI] –2.1 [–2.5, –1.8] mmol/L and –0.7 [–1.2, –0.2] mmol/L, cotadutide vs placebo, p = 0.001). There was a significant reduction in glucose $AUC_{0\text{-}4h}$ during MMTT in cotadutide group (change from baseline –12.3% [90%CI: –19.7, –4.9], cotadutide vs placebo, p=0.010) (Table S4). Insulin responses to MMTT were increased by cotadutide ($AUC_{0\text{-}4h}$ difference in LS-mean percentage change from baseline 29.6% [90%CI: 1.5, 57.7], cotadutide vs placebo, p=0.085) (Table S4). C-peptide change followed the same pattern as that observed for insulin with increased responses during MMTT with cotadutide (Table S4).

During IVGTT-, there was a decrease in $AUC_{glucose}$ accompanied by marked increases in the $AUC_{insulin}$ and $AUC_{C\text{-}peptide}$ with cotadutide (Figures S4a-S4c). The median (IQR) FPIRin the cotadutide group increased from 117 (–193, 648) min*mU/L before treatment to 1967 (1303, 7474) min*mU/L. In the placebo group, FPIR was unchanged [489 (126, 1298) min*mU/L to 442 (64, 1048) min*mU/L; p 0.003 for the difference in change from baseline for cotadutide vs placebo] (Figure S4d).

The disposition index followed the same pattern as FPIR with median (IQR) increasing with cotadutide (9 [–14.3, 129.0] to 391 [218, 502]$*10^{-4}*min^{-1}*mU^{-1}*L^{-1}$ per $mU*L^{-1}$) but not in the placebo group (113 [22, 323] to 122 [19, 216]; p=0.003 for the difference in change from baseline for cotadutide vs placebo) (Figure 5e). Neither glucose effectiveness (change from baseline 0.4 [0.1, 1.7] vs 0 [–0.2, 0.1]*10^{-2}/min cotadutide vs placebo, *p*=0.504) nor insulin sensitivity (change from baseline 0.4 [–0.3, 0.6] vs -0.3 [–0.6, 0.2]*10^{-4} min^{-1} per mU/L cotadutide vs placebo, *p*=0.503) were altered by cotadutide (Figures S4f, S4g).

Gastric, small bowel, and colonic emptying time

There was a significant increase in gastric emptying time following treatment with cotadutide with change from baseline (90%CI) 6.7 hours (5.0, 8.4) vs 2.6 hours (0.4, 4.7) in the placebo group (*p*=0.019). There were no changes in the small bowel and colonic emptying time (Table S5).

Muscle biopsy

RNA sequencing showed eight genes that were differentially expressed in response to cotadutide treatment (Figure S5, Table S6). Among them, AC109583.4 (ENSG00000284672) was a novel uncharacterized long non-coding RNA. Targeted analysis indicated potential improvement in indices of insulin sensitivity (Figure S6, enrichment p=7*10^{-12}). Suggestive results were also obtained for lipid oxidation including increased expression of PGC1alpha in 3 out of 4 participants (Figure S7, enrichment p=0.005). No dysregulation was observed for the other selected marker sets.

Safety, pharmacokinetics, and immunogenicity

A higher proportion of participants receiving cotadutide experienced adverse events (AEs) and AEs that were considered related to treatment compared with the placebo group (all AEs: 94.4% vs 71.4%; treatment-related AEs: 88.9% vs 42.9%) (Table S7).

The most frequently reported AEs (>15% of participants in either group) were nausea, decreased appetite, ECG T-wave amplitude decrease, vomiting, constipation, contusion, diarrhea, eructation, and lethargy in the cotadutide group and diarrhea, nausea, and headache in the placebo group.

Most AEs were mild or moderate. Two participants in the cotadutide group (11.1%) had at least one event of grade≥3 severity.

Four participants receiving cotadutide (22.2%) had five AEs leading to permanent discontinuation of cotadutide (nausea in two participants and eructation, injection-site erythema, and a markedly decreased appetite in one participant each), and all were considered related to cotadutide. There was a low number of injection-site reactions (two participants in the cotadutide group [11.1%] reported three injection-site reactions), and an increase from baseline in heart rate was detected in both groups, which was in the order of 9 bpm. A trend toward a reduction in blood pressure was observed in cotadutide arm, and there were no clinically meaningful changes in laboratory values or ECGs.

Discussion

Our data show that dual GLP-1/glucagon receptor agonism with cotadutide leads to weight loss, primarily via a marked reduction in EI. We also provide evidence that the glucose-lowering actions of cotadutide are mediated by increased insulin secretion and prolonged gastric emptying. The metabolic benefits of GLP-1-RAare well established. In humans, weight loss with GLP-1-RAtherapy is predominantly driven by reduced EI, ranging from 15–20% (37-39) with associated weight loss of 1.5–6 kg over 30 weeks (10). Increased EE is seen with GLP-1-RA in rodent models and attributed to brown adipose tissue (BAT) activation (40), but this is unlikely to affect weight loss in humans (38, 39). Glucagon signaling plays a more dominant role in energy homeostasis through a variety of mechanisms, including upregulation of energy expensive catabolic processes, especially in the liver, and activation of BAT via FGF-21 in rodent models (41). Oxyntomodulin, a naturally occurring dual GLP-1 and glucagon receptor agonist, acts via the glucagon receptor to increase EE via BAT activation, an effect that is dependent on concurrent GLP-1 receptor signaling (41-43).

Clinical trials with oxyntomodulin (44, 45), showed 20–35% reductions in EI with single (46) or repeated doses (47). In this study, we found a reduction in EI with cotadutide of > 50% from baseline. Although we did not compare cotadutide therapy with GLP-1-RA directly, the reduction in EI was markedly greater than that previously reported with GLP-1-RA alone (48), suggesting that glucagon receptor agonism may act synergistically with GLP-1 signaling to reduce appetite. A recent phase 1b trial in healthy adults demonstrated that treatment with SAR425899 (GLP-1/glucagon co-agonist) over 19 days led to reduced selective metabolic adaptation (smaller decrease in sleeping metabolic rate than expected for weight loss) but there was no change in total EE measured by indirect calorimetry (49).

We performed detailed measures of EE. In preclinical studies with cotadutide, increases in EE were attributed to the preferential hepatic fat oxidation mediated via PGC1alpha and enhanced mitochondrial maintenance and function, with these effects being differentiated from a mono-GLP-1-RA(24). A study of oxyntomodulin in overweight and obese humans demonstrated that weight loss was achieved through both reduced EI and increased AEE, but not REE (50). In a double-blind, randomized, crossover trial using infusions of GLP-1, glucagon, GLP-1 and glucagon co-infusion, and placebo in obese adults without diabetes, Tan *et al.* demonstrated a significant increase in REE after glucagon and after GLP-1 and glucagon co-infusion, but not after GLP-1 alone (16). In contrast, Bagger *et al.* assessed both oxyntomodulin and separate and combined infusions of GLP-1 and glucagon in young healthy adults, reporting reduced EI for all compounds tested, but no significant changes in REE (51). A further study in humans infusing glucagon demonstrated increased REE, which was independent of BAT activation. These studies primarily evaluated acute glucagon infusions, while the effects of more sustained glucagon receptor agonism are less well characterized. Indeed, a study of a single patient with a glucagonoma observed minimal changes in EE (52). Furthermore, it has been demonstrated that insulin infusion offsets the effect of glucagon on REE, which may explain the inability to detect significant differences with combined GLP-1/glucagon receptor agonism (28). Changes in EE in this study were in keeping with the anticipated compensatory adaptations to weight loss, except for a small numerical increase in REE observed in early dosing (13, 53, 54). However, the observation of increased expression of markers of lipid oxidation (e.g., PGC1alpha) in skeletal muscle is intriguing, although the interpretation is limited by very small sample size in this sub-study. Despite the reduction in physical activity, there was no significant difference between treatment groups in TEE measured by doubly labeled water technique over 6 weeks.

We found a reduction in physical activity and daily step count in the cotadutide group while AEE during exercise was unchanged and TEE (kJ/kg lean mass) from indirect calorimetry decreased significantly in cotadutide group versus placebo. This suggests that weight loss was not explained by greater physical activity in the cotadutide group. It may be explained by the commonly reported AEs in the cotadutide group, which included lethargy, nausea and loss of appetite, but reduced physical activity has been observed in other studies (55) of glucose-lowering therapies, which do not share the same AE profile (sodium-glucose co-transporter-2 inhibitors and dipeptidyl peptidase-4 inhibitors). A structured exercise program could be considered in future studies with cotadutide to achieve greater weight loss and more metabolic benefits.

We observed lower fasting plasma glucose, reduced glucose and increased insulin and C-peptide excursions following a liquid meal in the cotadutide group. During IVGTT, we found a marked increase in FPIR in the cotadutide group. Therefore, glucose-lowering effects and improvements in postprandial glucose excursions associated with cotadutide are mediated by enhanced insulin secretion, rather than a change in responsiveness to insulin. Also likely contributing to improved glycemia, we found delayed gastric emptying consistent with previous studies with cotadutide (26) and short-acting GLP-1-RA (56).

A key strength of our study was comprehensive metabolic profiling of participants including 24-hour indirect calorimetry and doubly labeled water assessment to measure EE. Free-living physical activity was measured objectively and continuously throughout the study period, thus providing detailed data on time spent in moderate and vigorous intensity physical activity and step count with greater accuracy than self-report instruments, which are prone to bias. However, several limitations need to be considered. This was an exploratory study and power calculations were based on changes in body weight rather than EE. Therefore, it is possible that the statistical power to detect small changes in EE between the groups was not sufficient. Standardizing EE by fat mass and LBM assumes a direct proportional relationship between EE and fat mass or LBM which might not have been entirely appropriate if we fitted linear regression models and their intercepts were significant. We employed ANCOVA models including baseline measurement of EE to control individual differences at the start of the study allowing for a more accurate assessment of the treatment effect. We conducted a sensitivity analysis, adding a covariate of fat mass or LBM change from baseline to account for changes in body composition over time which can impact EE. This study lacks diversity in terms of sex and ethnicity and therefore generalizability of the findings to women and non-white individuals is limited.

Gastrointestinal AEs were self-reported in an unstructured way rather than using a standardized and validated questionnaire, which may have introduced bias. The duration of treatment was relatively short and long-term larger studies are required to assess the effects of cotadutide on weight regain and cardiovascular outcomes after stopping treatment. The lack of a GLP-1-RAgroup means that we are unable to compare directly dual versus single agonism.

In summary, cotadutide, a dual GLP-1/glucagon receptor agonist, achieved weight loss predominantly via a marked reduced EI rather than by changes in EE.

Data availability

Data generated or analyzed during the study are not publicly available but may be obtained from the corresponding author on reasonable request.

Author contributions

ME, VB, AP, LJ, and PA devised the study concept and design. RG, JK, ME and AP acquired the data. MS and MR analyzed the RNA-sequencing sub-study and wrote the corresponding sections of the manuscript. MV and AK provided analysis and modelling for the DLW sub-section of the study. DL, RH and RG performed the data analysis and all authors interpreted the data. RG and ME wrote the manuscript and all authors provided critical revision. All authors reviewed and approved the final draft for submission. ME is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the analysis.

Acknowledgments

The study was funded by AstraZeneca and supported by the NIHR Cambridge Biomedical Research Centre. The sponsor funded the study and provided overall oversight according to the Good Clinical Practice guidelines. The sponsor contributed to the design of the study together with the investigators. Data were collected by the investigators and research team according to the sponsor's instructions while the sponsor led data analysis. Data interpretation and writing of the study report were led by the investigators with input from the sponsor. Medical writing support was provided by Oxford PharmaGenesis Ltd, Oxford, UK, and was funded by AstraZeneca.

We are particularly grateful to nursing and research staff on the NIHR/Wellcome Clinical Research Facility for their expertise, Professor Roman Hovorka for mathematical modeling and to Daniel Chapman for study coordination. The University of Cambridge has received salary support for ME through the National Health Service in the East of England through the Clinical Academic Reserve. RG was supported by an NIHR Academic Clinical Fellowship. MV and AK were funded by the NIHR Cambridge Biomedical Research Centre (IS-BRC-1215-20014). The views expressed are those of the authors and not necessarily those of the

NIHR or the Department of Health and Social Care. We are grateful to Dan Griffiths and Elise Orford for the DLW sample analysis.

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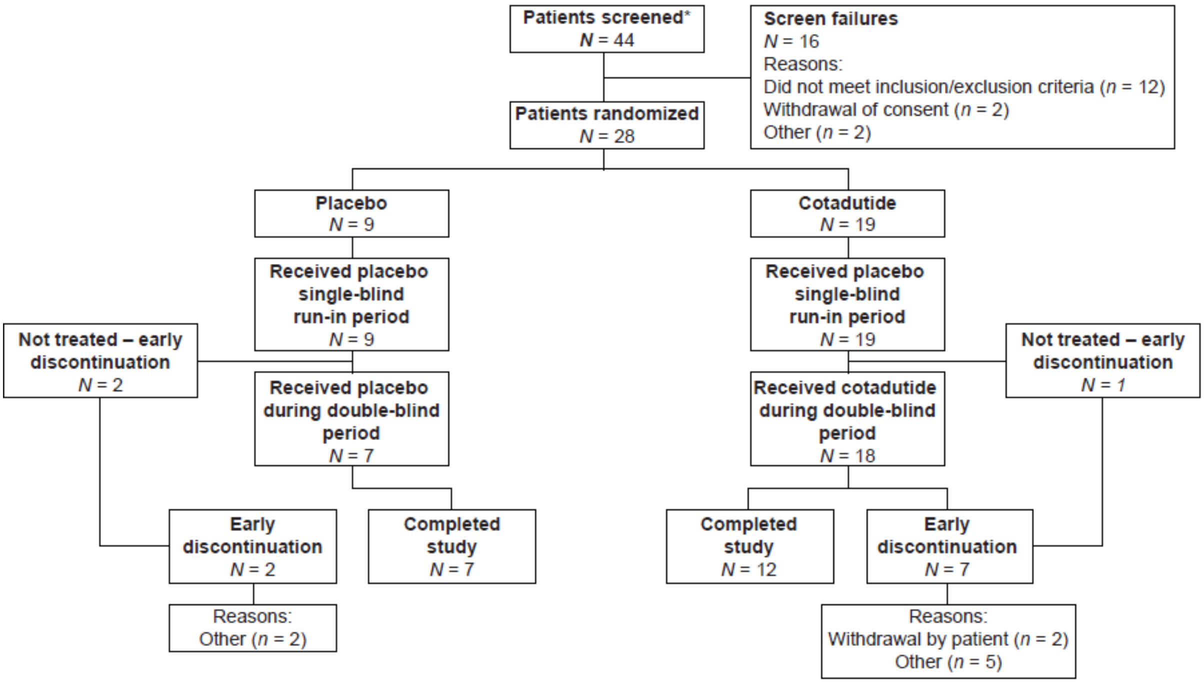
Figures

Figure 1. CONSORT diagram showing participant disposition



*Informed consent was signed.

Tables

Table 1. Participant demographics and baseline characteristics, as-treated population

Parameter	Cotadutide N = 19	Placebo N = 9
Age (years)		
Mean (SD)	59.5 (8.4)	62.2 (7.2)
Sex		
Female	1 (5.3%)	2 (22.2%)
Male	18 (94.7%)	7 (77.8%)
Race		
Black or African American	0	1 (11.1%)
Native Hawaiian or Other Pacific Islander	1 (5.3%)	0
White	18 (94.7%)	8 (88.9%)
Weight (kg)		
Mean (SD)	99.3 (10.8)	101.4 (16.0)
Height (cm)		
Mean (SD)	175.3 (6.2)	170.8 (8.8)
Body mass index (kg/m^2)		
Mean (SD)	32.2 (2.2)	34.5 (2.8)
HbA_{1c} (%)		
Mean (SD)	7.0 (0.5)	6.7 (0.4)
Duration of T2D (years)		
Mean (SD)	7.3 (5.5)	8.7 (8.5)
Diabetes complications, n (%)		
Any	2 (10.5%)	0
Retinopathy	1 (5.3%)	0
Autonomic neuropathy	0	0
Peripheral neuropathy	1 (5.3%)	0
Nephropathy	0	0
Angiopathy	0	0
Other	0	0

HbA_{1c}, glycated hemoglobin A1c; SD, standard deviation; T2D, type 2 diabetes.

Table 2. Body composition assessed by dual energy absorptiometry, mITT population

	Cotadutide (N=12)	Placebo (N=7)	Difference in LS-mean vs placebo (90%CI)	p-value
Total body weight (kg), mean (SD)				
Baseline (day 17)	96.5 (10.8)	103.5 (10.5)		
Day 59	93.2 (10.4)	102.6 (11.3)		
Absolute change from baseline to day 59, LS-mean (90%CI)	-3.8 (-4.7, -3.0)	-1.3 (-2.5, -0.1)	-2.5 (-4.1, -1.0)	0.009
Percentage change from baseline to day 59, LS-mean (90%CI)	-4.0 (-4.9, -3.1)	-1.4 (-2.7, -0.1)	-2.6 (-4.2, -1.0)	0.011
Total fat mass (kg), mean (SD)				
Baseline (day –1)	34.7 (5.9)	40.2 (9.9)		
Day 59	30.7 (4.8)	38.6 (9.7)		
Absolute change from baseline to day 59, LS-mean (90%CI)	–3.3 (–4.3, –2.3)	–1.5 (–2.8, –0.1)	–1.8 (–3.6, –0.1)	0.085
Percentage change from baseline to day 59, LS-mean (90%CI)	–9.3 (–12.3, –6.4)	–4.2 (–8.2, –0.2)	–5.1 (–10.4, 0.1)	0.107
Lean body mass (kg), mean (SD)				
Baseline (day –1)	61.1 (7.2)	62.8 (3.3)		
Day 59	57.5 (6.7)	61.4 (3.4)		
Absolute change from baseline to day 59, LS-mean (90%CI)	–3.0 (–3.6, –2.5)	–1.4 (–2.2, –0.6)	–1.6 (–2.6, –0.7)	0.010
Percentage change from baseline to day 59, LS-mean (90%CI)	–5.0 (–5.9, –4.0)	–2.3 (–3.6, –1.0)	–2.7 (–4.3, –1.0)	0.012
Total fat:lean mass ratio, mean (SD)				
Baseline (day –1)	0.57 (0.1)	0.64 (0.2)		
Day 59	0.54 (0.1)	0.63 (0.2)		
Absolute change from baseline to day 59, LS-mean (90%CI)	–0.03 (–0.04, –0.02)	–0.01 (–0.03, 0.01)	–0.02 (–0.04, 0.00)	0.145
Percentage change from baseline to day 59, LS-mean (90%CI)	–4.8 (–7.3, –2.4)	–1.7 (–4.9, 1.6)	–3.2 (–7.3, 1.0)	0.204
Visceral adipose tissue mass (kg), mean (SD)				
Baseline (day –1)	3.0 (1.0)	3.6 (1.1)		
Day 59	2.3 (0.8)	3.1 (1.1)		
Absolute change from baseline to day 59, LS-mean (90%CI)	–0.5 (–0.7, –0.3)	–0.4 (–0.7, –0.1)	–0.1 (–0.5, 0.2)	0.533
Percentage change from baseline to day 59, LS-mean (90%CI)	–17.1 (–23.8, –10.4)	–15.0 (–24.0, –6.0)	–2.2 (–13.8, 9.4)	0.748

CI, confidence interval; LS, least squares; mITT, modified intention to treat; SD, standard deviation. p-value is for the difference in LS-mean vs placebo from ANCOVA model adjusted for treatment arm and baseline value of the outcome. In the cotadutide group, complete data were available for N=12 participants on day 59. In the placebo group, the number of participants with complete data stayed N=7 throughout the study. In the total body weight analysis, day 17 was considered baseline while for the body composition analysis day -1 (start of placebo run-in period) was considered baseline.

Table 3. Energy intake from *ad libitum* lunch, mITT population

	Cotadutide (N=14)	Placebo (N=7)	Difference in LS-mean vs placebo (90%CI)	p-value
Total energy intake (kJ), mean (SD)				
Day 16	3004.6 (1472.2)	3615.3 (1544.8)		
Day 32	1672 (1104)	3412.7 (1403.2)		
Day 59	1622.4 (1195.5)	3094.9 (1325.1)		
Absolute change from baseline to day 32, LS-mean (90%CI)	–1677.5 (–2045.3, –1309.6)	–126.3 (–647.4, 394.8)	–1551.2 (–2190.5, –911.9)	<0.001
Percentage change from baseline to day 32, LS-mean (90%CI)	–50.7 (–63.5, –37.8)	–5.2 (–23.3, 13.0)	–45.5 (–67.8, –23.2)	0.002
Absolute change from baseline to day 59, LS-mean (90%CI)	–1743.3 (–2235.4, –1251.3)	–410.8 (–1107.9, 286.3)	–1332.5 (–2187.7, –477.3)	0.015
Percentage change from baseline to day 59, LS-mean (90%CI)	–51.9 (–66.5, –37.3)	–10.6 (–31.3, 10.1)	–41.3 (–66.7, –15.9)	0.011

CI, confidence interval; LS, least squares; mITT, modified intention to treat; SD, standard deviation.
p-value is for the difference in LS-mean vs placebo from ANCOVA model adjusted for treatment arm and baseline value of the outcome. In the cotadutide group, complete data were available for N=14 participants on day 32 and for N=14 participants on day 59. In the placebo group, the number of participants with complete data stayed N=7 throughout the study. In this analysis, day 16 was considered the baseline.

Table 4. Energy expenditure from indirect calorimetry, mITT population

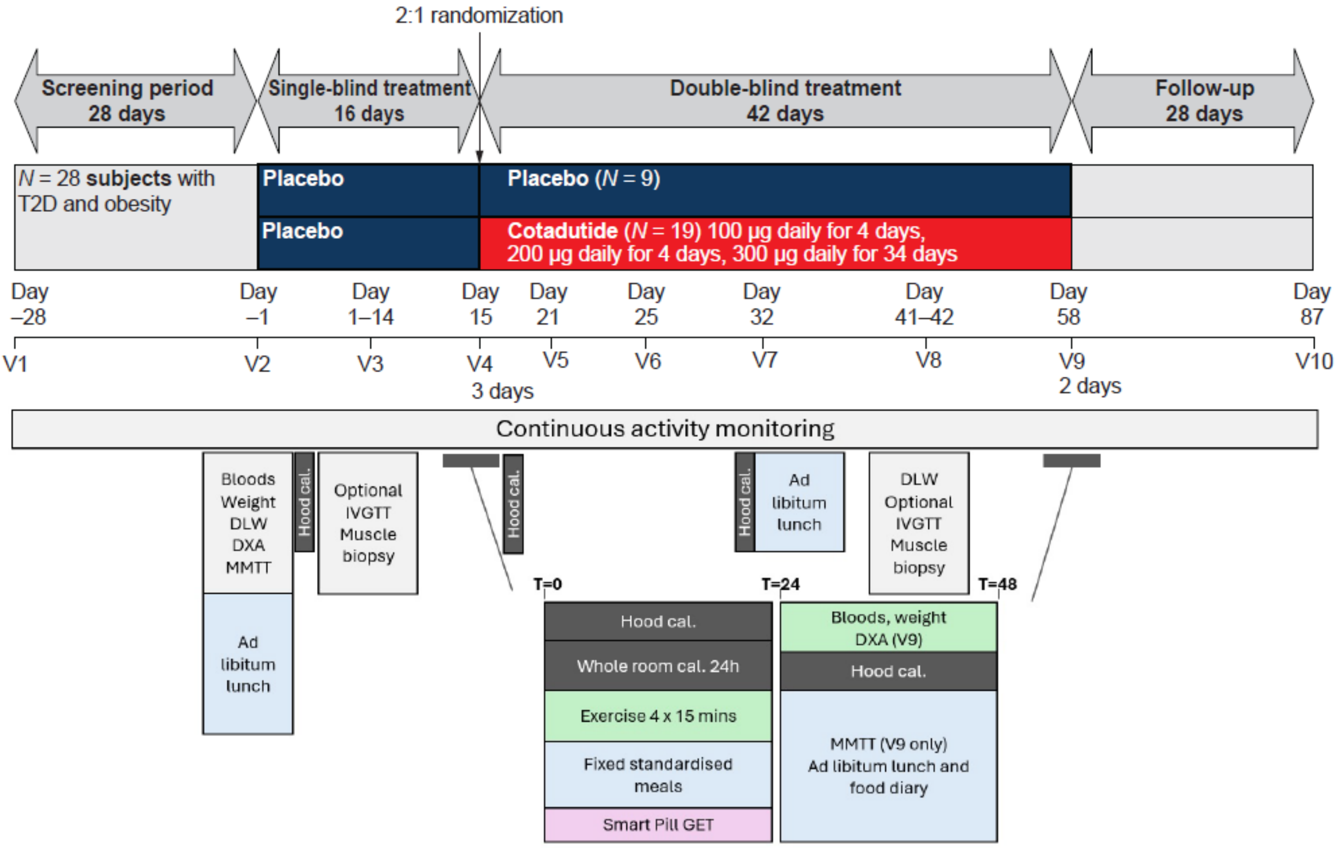
	Cotadutide (N=12)	Placebo (N=7)	Difference in LS-mean vs placebo (90%CI)	p-value
Energy expenditure per kg of lean body mass				
Total EE (kJ/kg LBM)				
Day 15, mean (SD)	150.4 (15.0)	153.2 (6.8)		
Day 58, mean (SD)	142.1 (9.8)	153.3 (6.1)		
Absolute change from baseline to day 58, LS-mean (90%CI)	–10.3 (–13.1, –7.5)	0.43 (–3.2, 4.1)	–10.7 (–15.3, –6.2)	< 0.001
Percentage change from baseline to day 58, LS-mean (90%CI)	–6.2 (–7.9, –4.5)	0.32 (–1.9, 2.5)	–6.5 (–9.3, –3.7)	<0.001
Activity EE (kJ/kg LBM)				
Day 15, mean (SD)	17.3 (2.6)	19.9 (3.8)		
Day 58, mean (SD)	17.0 (1.7)	19.0 (2.1)		
Absolute change from baseline to day 58, LS-mean (90%CI)	–1.4 (–2.1, –0.8)	–0.2 (–1.1, 0.6)	–1.2 (–2.3, –0.1)	0.072
Percentage change from baseline to day 58, LS-mean (90%CI)	–6.7 (–10.2, –3.3)	0.24 (–4.3, 4.8)	–6.9 (–12.8, 1.1)	0.054
Resting EE (kJ/kg LBM)				
Day 15, mean (SD)	98.7 (7.6)	94.6 (7.3)		
Day 16, mean (SD)	126.3 (12.8)	127.3 (14.4)		
Day 17 pre-dose, mean (SD)	124.3 (11.1)	126.9 (14.5)		
Day 17 post-dose, mean (SD)	120.4 (13.2)	124.5 (14.6)		
Day 32, mean (SD)	129.7 (11.5)	125.6 (9.7)		
Day 58, mean (SD)	98.1 (6.6)	100.2 (8.4)		
Absolute change from baseline to day 58, LS-mean (90%CI)	–0.6 (–2.6, 1.4)	5.1 (2.5, 7.8)	–5.7 (–0.1, 2.3)	0.010
Percentage change from baseline to day 58, LS-mean (90%CI)	–0.4 (–2.5, 1.6)	5.4 (2.8, 8.1)	–5.9 (–9.3, –2.5)	0.008

CI, confidence interval; EE, energy expenditure; LBM, lean body mass; LS, least squares; mITT, modified intention to treat; SD, standard deviation. p-value is for the difference in LS-mean vs placebo from ANCOVA model adjusted for treatment arm and baseline value of the outcome. In the cotadutide group, complete data were available for N=12 participants on day 58. In the placebo group, the number of participants with complete data stayed N=7 throughout the study. In this analysis, day 15 was considered the baseline and day 58 the final time point.

NB Equilibration was not achieved in 4 participants in the cotadutide group and 4 participants in the placebo group.

Supplementary appendix

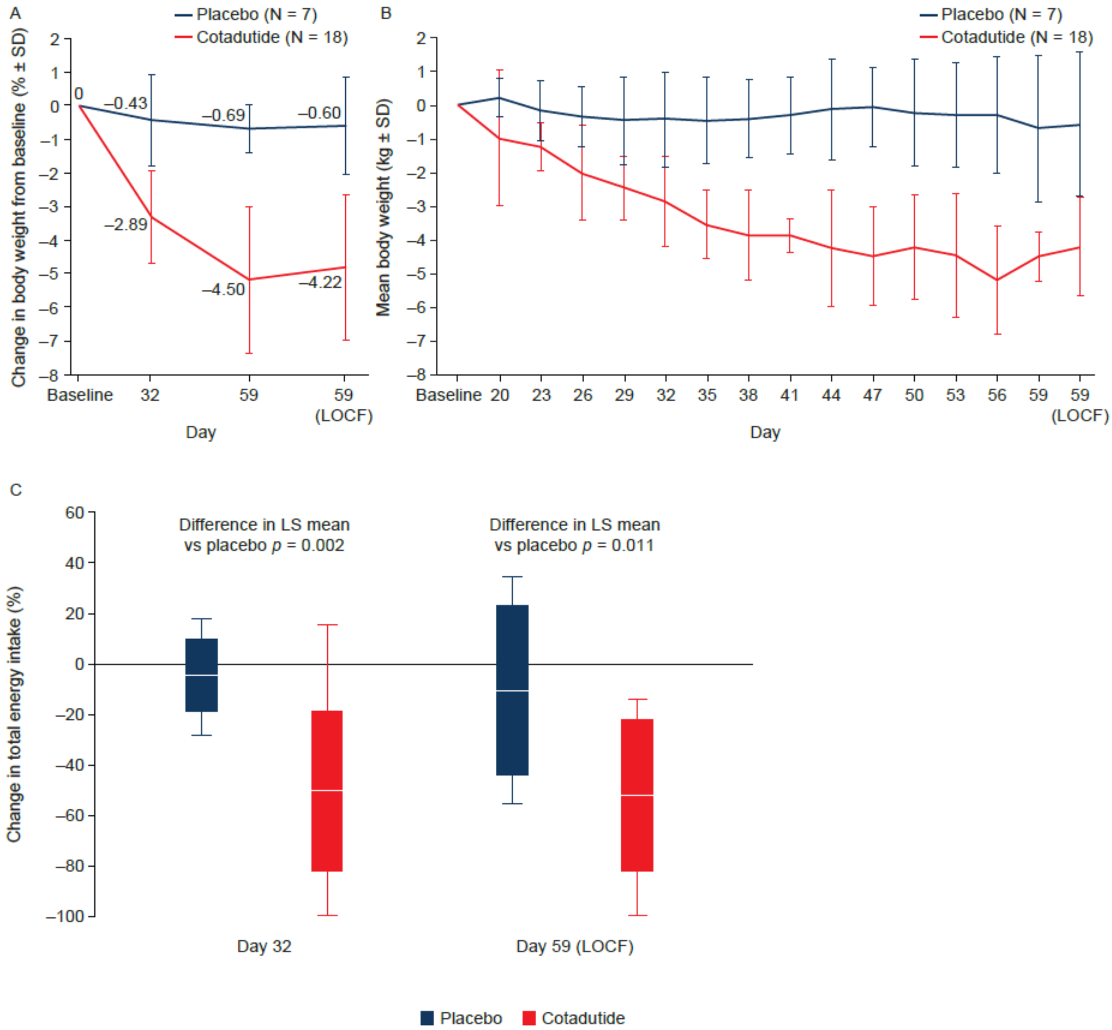
Figure S1. Study design



Visit 3 could occur any time from day 1 to day 14. Visit 8 could occur on day 41 or day 42.

Cal. calorimetry; DLW, doubly-labelled water; DXA, dual X-ray absorptiometry; GET, gastric emptying time; h, hours; IVGTT, intravenous glucose tolerance test; mins, minutes; MMTT, mixed-meal tolerance test; T, time; T2D, type 2 diabetes; V, visit.

Figure S2. Changes from baseline in (A) body weight, (B) body weight recorded by subjects at home by visit (mITT population), (C) energy intake from ad libitum meal



Panels A and B data are mean (± SEM). Fig C boxes show mean and SD, whiskers are maximum and minimum values.

LOCF, last observation carried forward, mITT, modified intention to treat; SD, standard deviation; SEM, standard error of the mean.

Figure S3. (A) Mean daily step count, and (B) mean time spent in physical activity

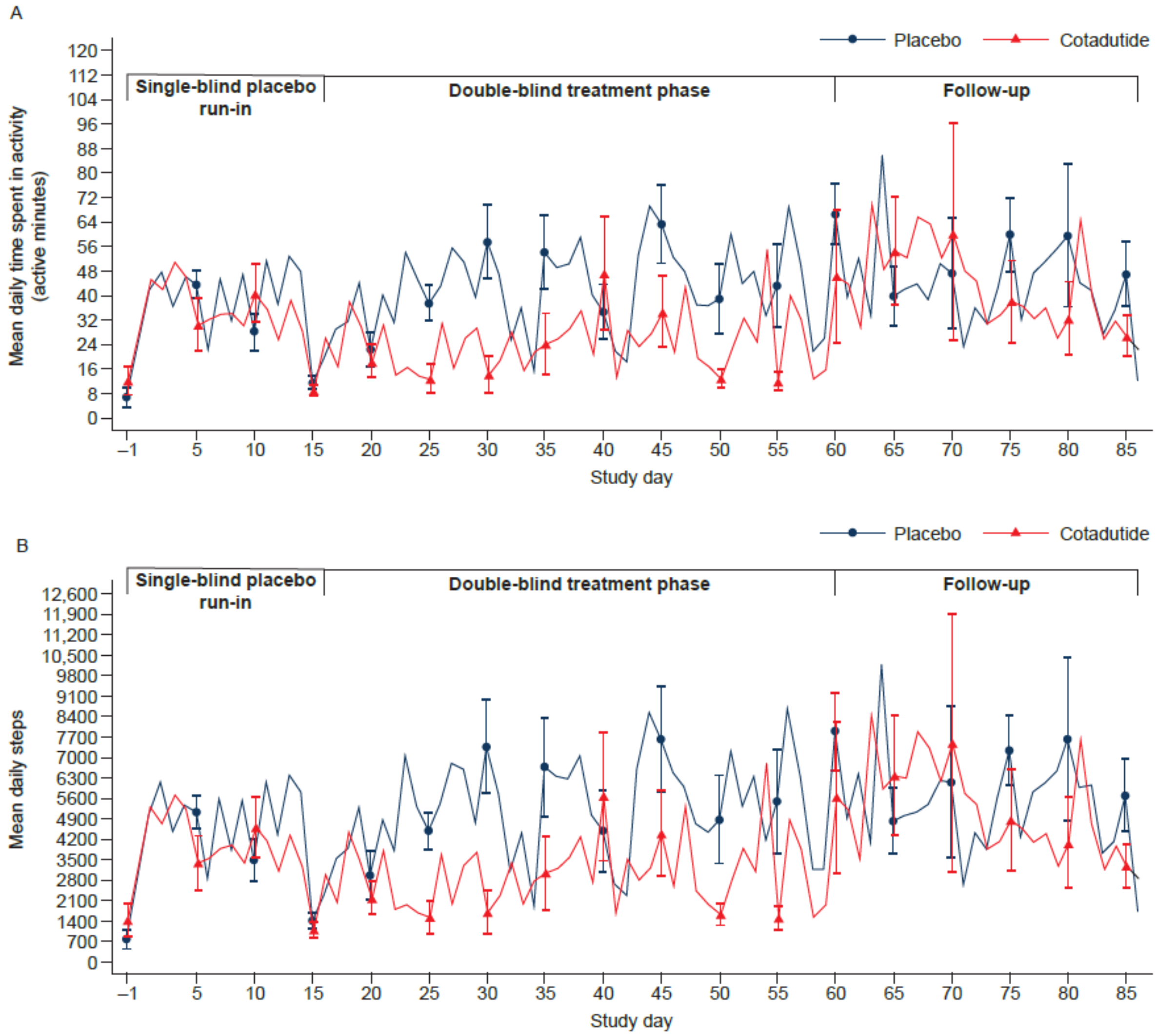
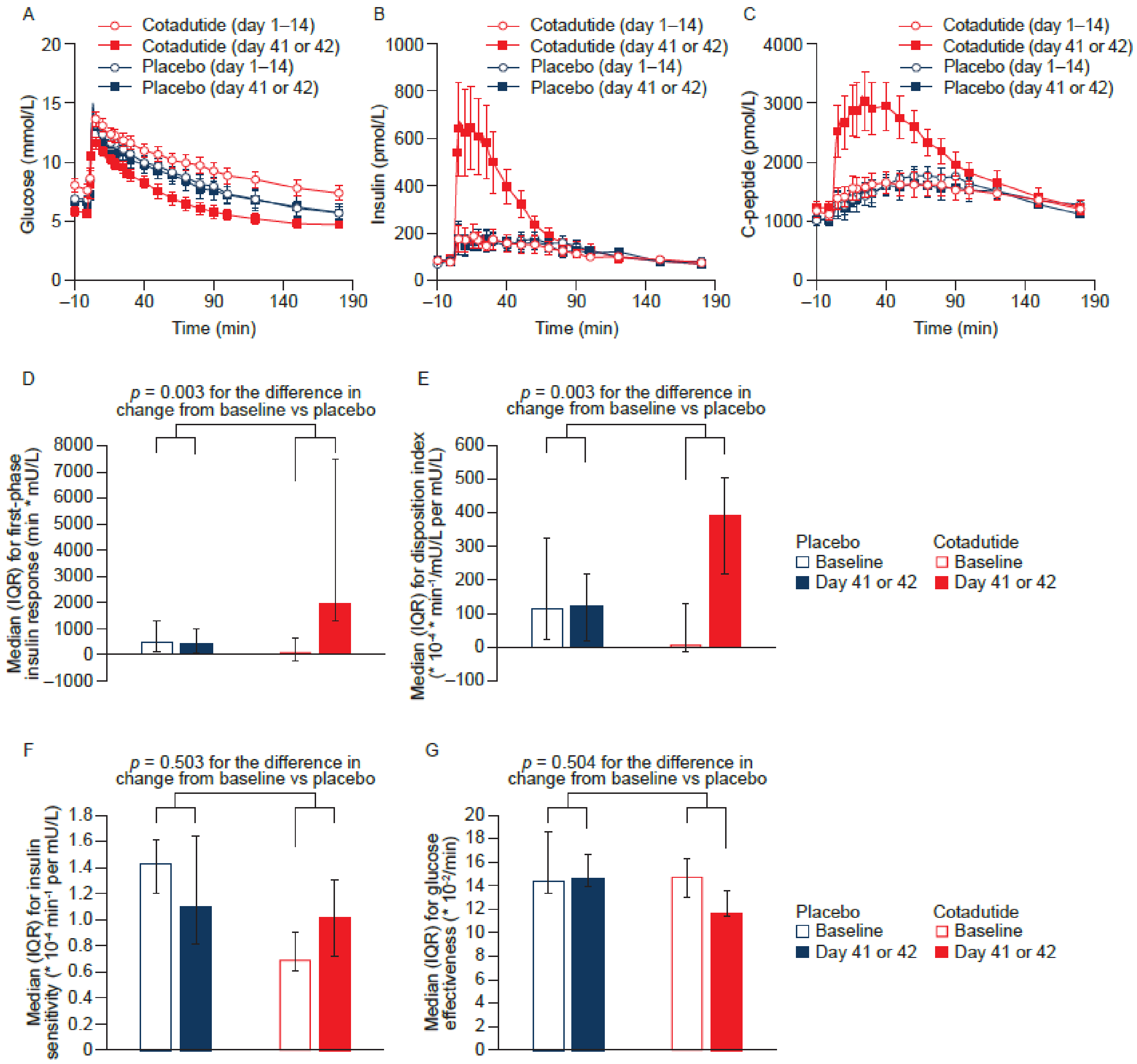


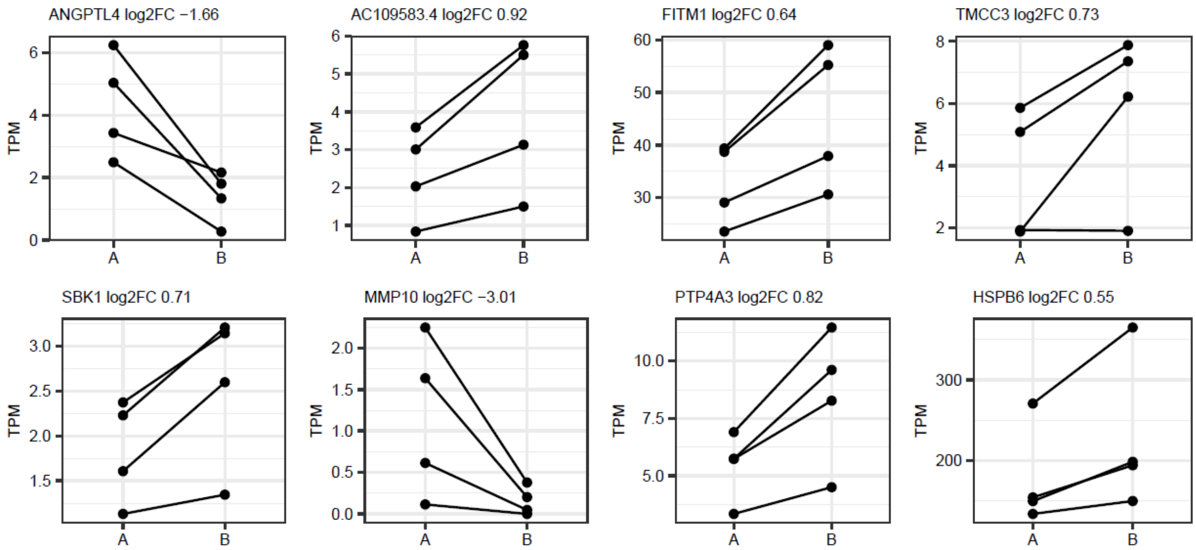
Figure S4. Glucose homeostasis assessed by IVGTT. (A) Glucose from IVGTT, (B) Insulin from IVGTT, (C) C-peptide from IVGTT, (D) first-phase insulin response, (E) disposition index, (F) insulin sensitivity, and (G) glucose effectiveness.



Data are mean (± SEM) for panels A-C; and median (IQR) for panels D-G.

IQR, interquartile range; IVGTT, intravenous glucose tolerance test.

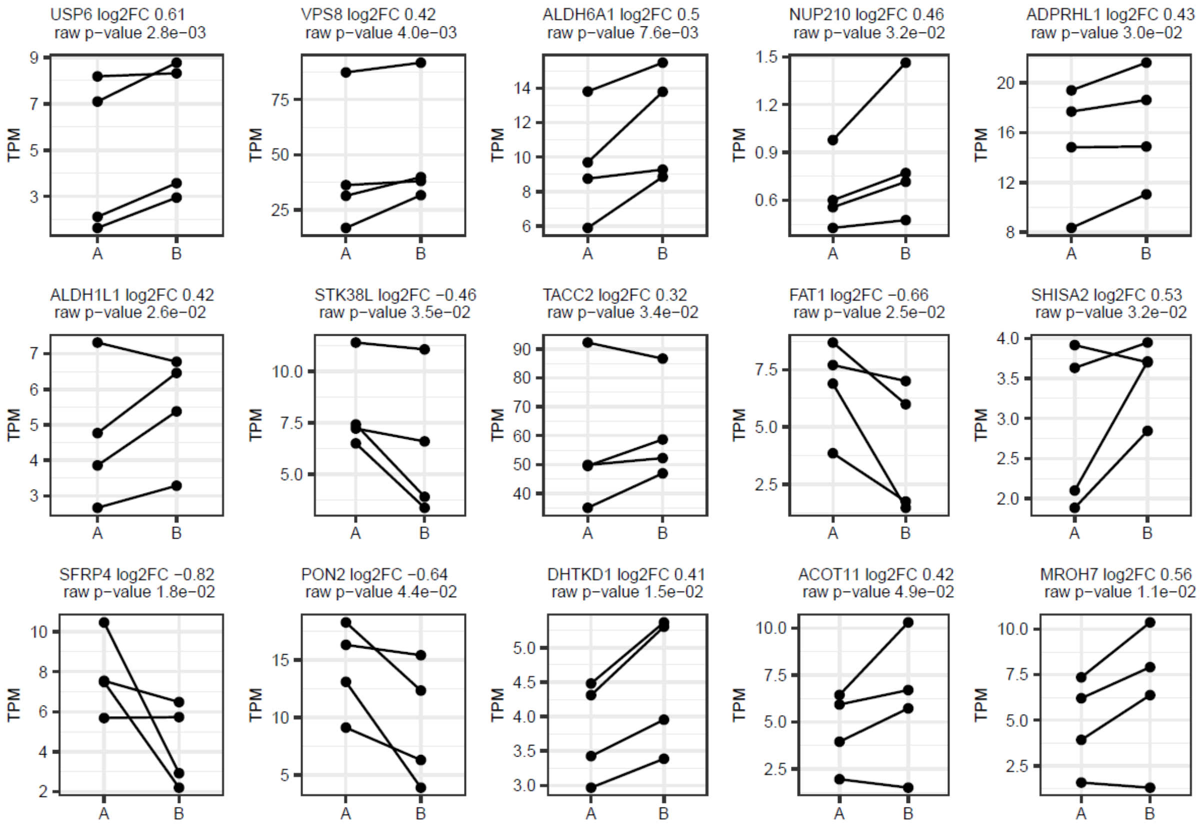
Figure S5. Differentially expressed genes from Supplementary Table 5 plotted as post- vs pre-treatment change in each study participant



A denotes pre-treatment biopsies. B denotes post-treatment biopsies. All genes satisfy FDR < 0.05.

FDR, false discovery rate; TPM, transcripts per million.

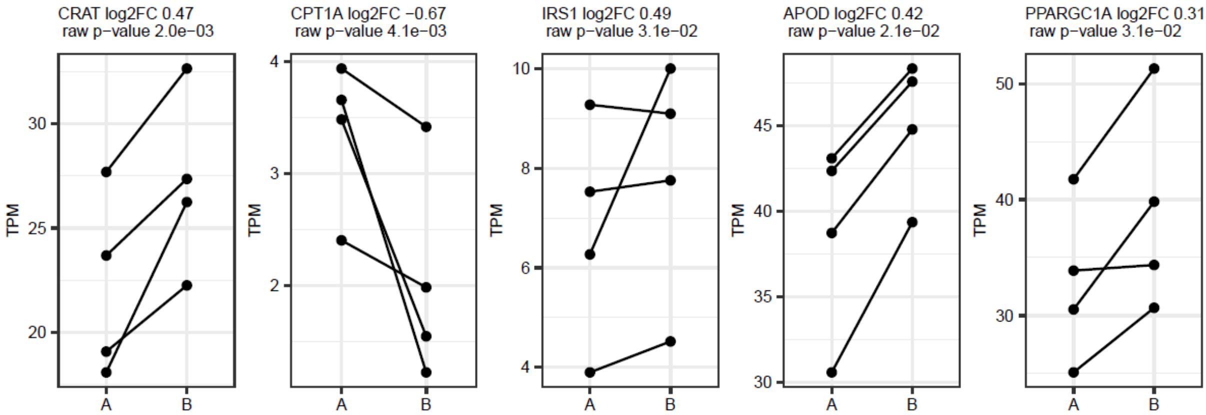
Figure S6. Markers indicating improvement in insulin sensitivity



A are pre-treatment biopsies. B are post-treatment biopsies.

TPM, transcripts per million.

Figure S7. Markers indicating improvement in lipid oxidation



A are pre-treatment biopsies. B are post-treatment biopsies.

TPM, transcripts per million.

Table S1. Hunger scores assessed by visual analogue scale (VAS)

	Cotadutide (N = 12)	**Placebo (N = 7)**	**Difference in LS-mean vs placebo (90%CI)**	**p-value**
How hungry do you feel right now?				
VAS score (cm)				
Day 15, mean (SD)	2.66 (1.30)	2.72 (1.34)		
Day 58, mean (SD)	2.04 (1.15)	2.31 (1.48)		
Absolute change from day 15 to day 58, LS-mean (90%CI)	-0.60 (-0.96, -0.24)	-0.40 (-0.88, 0.08)	-0.20 (-0.80, 0.40)	0.568
How sick do you feel right now?				
VAS score (cm)				
Day 15, mean (SD)	0.84 (0.78)	0.51 (0.24)		
Day 58, mean (SD)	1.44 (2.26)	0.47 (0.31)		
Absolute change from day 15 to day 58, LS-mean (90%CI)	0.66 (-0.25, 1.56)	0.01 (-1.19, 1.21)	0.64 (-0.88, 2.17)	0.472
How pleasant would it be to eat right now?				
VAS score (cm)				
Day 15, mean (SD)	3.11 (1.43)	3.21 (1.85)		
Day 58, mean (SD)	2.34 (1.27)	2.68 (1.67)		
Absolute change from day 15 to day 58, LS-mean (90%CI)	-0.75 (-1.12, -0.38)	-0.51 (-1.00, -0.03)	-0.24 (-0.85, 0.38)	0.514
How much could you eat right now?				
VAS score (cm)				
Day 15, mean (SD)	3.21 (1.38)	3.12 (1.68)		
Day 58, mean (SD)	2.39 (1.28)	2.63 (1.55)		
Absolute change from day 15 to day 58, LS-mean (90%CI)	-0.85 (-1.23, -0.48)	-0.51 (-1.0, -0.03)	-0.34 (-0.96, 0.27)	0.344

CI, confidence interval; VAS, visual analogue scale; LS, least squares; SD, standard deviation. Day 15 was considered baseline.

Table S2. Total energy expenditure assessed by doubly labeled water technique

	Cotadutide (N = 12)	**Placebo (N = 7)**	**Difference in LS-mean vs placebo (90%CI)**	**p-value**
Energy expenditure per kg of fat mass				
Total EE (kJ/kg FM)				
Day 17, mean (SD)	376.5 (106.0)	343.1 (63.4)		
Day 58 or 59, mean (SD)	354.0 (111.7)	309.2 (66.6)		
Absolute change from baseline to day 58 or 59, LS-mean (90%CI)	–21.0 (–49.9, 8.0)	–36.6 (–74.7, 1.5)	15.6 (–32.6, 63.8)	0.634
Percentage change from baseline to day 58 or 59, LS-mean (90%CI)	–5.2 (–12.2, 1.8)	–10.2 (–19.5, –1.0)	5.0 (–6.7, 16.7)	0.710
Energy expenditure per kg of lean body mass				
Total EE (kJ/kg LBM)				
Day 17, mean (SD)	205.5 (34.4)	211.8 (22.6)		
Day 58 or 59, mean (SD)	183.7 (37.1)	187.5 (26.4)		
Absolute change from baseline to day 58 or 59, LS-mean (90%CI)	–22.4 (–35.2, –9.7)	–23.3 (–40.0, –6.6)	0.9 (–20.2, 21.9)	0.479
Percentage change from baseline to day 58 or 59, LS-mean (90%CI)	–10.3 (–16.0, –4.6)	–11.3 (–18.8, –3.8)	1.0 (–8.4, 10.4)	0.784

CI, confidence interval; EE, energy expenditure; FM, fat mass; LBM, lean body mass; LS, least squares; SD, standard deviation.

Table S3. Accelerometer-measured physical activity parameters throughout the study, mITT population

	Cotadutide (N = 18)	Placebo (N = 7)	Difference in LS-mean vs placebo (90%CI)	p-value
Time spent in activity (min/day), mean (SD)				
Baseline (day 1 to 16)	43 (29)	39 (5)		
End (day 44 to 59)	33 (23)	49 (13)		
Absolute change from baseline to day 59, LS-mean (90%CI)	–11 (–20, –2)	7 (–4, 19)	–18 (–33, –4)	0.047
Percentage change from baseline to day 59, LS-mean (90%CI)	–11 (–25, 3)	22 (4, 41)	–33 (–56, –10)	0.023
Daily step count, mean (SD)				
Baseline (day 1 to 16)	5025 (3340)	4728 (714)		
End (day 44 to 59)	3927 (2629)	6007 (1968)		
Absolute change from baseline to day 59, LS-mean (90%CI)	–1286 (2402, –170)	1021 (–442, 2484)	–2307 (–4152, –462)	0.044
Percentage change from baseline to day 59, LS-mean (90%CI)	–11 (–26, 3)	24 (5, 43)	–35 (–59, –11)	0.022

ANCOVA, analysis of covariance; CI, confidence interval; LS, least squares; mITT, modified intention to treat; SD, standard deviation. p-value is for the difference in LS-mean vs placebo from ANCOVA model adjusted for treatment arm and baseline value of the outcome. In the cotadutide group, complete data were available for N = 12 participants on day 59. In the placebo group, the number of participants with complete data remained at N = 7 throughout the study.

Table S4. Glucose homeostasis parameters from mixed meal tolerance test ($AUC_{0–4h}$), mITT population

	Cotadutide (N = 18)	Placebo (N = 7)	Difference in LS-mean vs placebo (90%CI)	p-value
Glucose $AUC_{0–4h}$ (h * mmol/L), mean (SD)				
Baseline (day –1)	35.5 (10.2)	30.5 (3.7)		
Day 59	26.9 (3.1)	30.3 (4.8)		
Absolute change from baseline to day 58 (h*mmol/L), LS-mean (90%CI)	–8.3 (–10.0, –6.5)	–3.5 (–5.8, –1.2)	–4.8 (–7.7, –1.8)	0.012
Percentage change from baseline to day 58, LS-mean (90%CI)	–19.1 (–23.4, –14.8)	–6.8 (–12.5, –1.0)	–12.3 (–19.7, –4.9)	0.010
Insulin $AUC_{0–4h}$ (h * mU/L), mean (SD)				
Baseline (day –1)	128.1 (64.7)	134.3 (74.1)		
Day 59	141.9 (59.0)	127.9 (65.9)		
Absolute change from baseline to day 58 (h*mU/L), LS-mean (90%CI)	11.8 (–15.7, 39.3)	–4.4 (–40.4, 31.6)	16.2 (–29.1, 61.5)	0.542
Percentage change from baseline to day 58, LS-mean (90%CI)	27.3 (10.3, 44.4)	–2.2 (–24.6, 20.1)	29.6 (1.5, 57.7)	0.085
C-peptide $AUC_{0–4h}$ (h * µg/L), mean (SD)				
Baseline (day –1)	24.8 (7.5)	24.3 (4.9)		
Day 59	27.1 (6.9)	24.2 (5.6)		
Absolute change from baseline to day 58 (h * µg/L), LS-mean (90%CI)	3.1 (0.1, 6.2)	0.0 (–4.0, 4.0)	3.1 (–1.9, 8.1)	0.299
Percentage change from baseline to day 59, LS-mean (90%CI)	21.5 (12.0, 31.1)	0.6 (–12.0, 13.1)	21.0 (5.2, 36.8)	0.034

ANCOVA, analysis of covariance; $AUC_{0–4h}$, area under the curve from 0 to 4 hours; CI, confidence interval; LS, least squares; mITT, modified intention to treat; SD, standard deviation. p-value is for the difference in LS-mean vs placebo from ANCOVA model adjusted for treatment arm and baseline value of the outcome. In the cotadutide group, complete data were available for N = 12 participants on day 59. In the placebo group, the number of participants with complete data remained at N = 7 throughout the study.

Table S5. Gastric, small bowel, colonic emptying time and whole gut transit time as measured by SmartPill, mITT population

	Cotadutide (N = 18)	Placebo (N = 7)	Difference in LS-mean vs placebo (90%CI)	p-value
Gastric emptying time (hours), mean (SD)				
Baseline (day 15)	14.0 (4.8)	14.4 (5.6)		
Day 58	19.9 (3.1)	16.3 (4.3)		
Absolute change from baseline to day 58 (hours), LS-mean (90%CI)	6.7 (5.0, 8.4)	2.6 (0.4, 4.7)	4.1 (1.4, 6.9)	0.02
Percentage change from baseline to day 58, LS-mean (90%CI)	82.1 (58.8, 105.4)	34.5 (5.2, 63.7)	47.7 (10.0, 85.3)	0.04
Small bowel emptying time (hours), mean (SD)				
Baseline (day 15)	9.0 (11.1)	6.2 (4.1)		
Day 58	6.9 (3.2)	5.5 (2.0)		
Absolute change from baseline to day 58 (hours), LS-mean (90%CI)	–0.1 (–1.7, 1.6)	–1.4 (–3.8, 1.0)	1.4 (–1.6, 4.3)	0.43
Percentage change from baseline to day 58, LS-mean (90%CI)	47.4 (14.5, 80.3)	–1.7 (–50.7, 47.3)	49.1 (–10.1, 108.3)	0.2
Colonic emptying time (hours), mean (SD)				
Baseline (day 15)	16.6 (10.5)	14.0 (8.4)		
Day 58	16.4 (9.9)	10.3 (8.7)		
Absolute change from baseline to day 58 (hours), LS-mean (90%CI)	–0.3 (–5.6, 5.0)	–5.8 (–13.8, 2.2)	5.5 (–4.3, 15.3)	0.3
Percentage change from baseline to day 58, LS-mean (90%CI)	43.8 (–8.6, 96.2)	–21.0 (–99.9, 58.0)	64.75 (–31.7, 161.2)	0.3
Whole-gut transit time (hours), mean (SD)				
Baseline (day 15)	39.7 (16.1)	34.6 (11.6)		
Day 58	43.2 (12.5)	32.1 (8.9)		
Absolute change from baseline to day 58 (hours), LS-mean (90%CI)	6.7 (0.6, 12.9)	–4.4 (–12.2, 3.3)	11.15 (1.2, 21.1)	0.07
Percentage change from baseline to day 58, LS-mean (90%CI)	32.6 (13.4, 51.8)	–1.3 (–25.4, 22.8)	33.9 (2.9, 64.8)	0.07

ANCOVA, analysis of covariance; CI, confidence interval; LS, least squares; mITT, modified intention to treat; SD, standard deviation. p-value is for the difference in LS-mean vs placebo from ANCOVA model adjusted for treatment arm and baseline value of the outcome. For gastric emptying parameters, in the cotadutide group, complete data were available for N = 15 participants on day 15 for small-bowel emptying time, colonic emptying time, and whole gut transit time (N = 16 for gastric emptying time), and N = 11 participants on day 58 for all four parameters. In the placebo group, the number of participants with complete data remained at N = 7 throughout the study for gastric emptying time and whole-gut transit time; complete data were available for N = 5 participants on day 58 for small-bowel emptying time and colonic emptying time.

Table S6. The eight differentially expressed genes after vs before cotadutide treatment in skeletal muscle biopsies

Ensembl ID	Symbol	log2FoldChange	lfcSE	stat	p-value	FDR
ENSG00000167772	ANGPTL4	-1.66	0.35	-4.80	1.6E-06	0.02
ENSG00000284672	AC109583.4	0.92	0.19	4.73	2.28E-06	0.02
ENSG00000139914	FITM1	0.64	0.15	4.35	1.39E-05	0.04
ENSG00000057704	TMCC3	0.73	0.17	4.32	1.53E-05	0.04
ENSG00000188322	SBK1	0.71	0.17	4.30	1.72E-05	0.04
ENSG00000166670	MMP10	-3.01	0.68	-4.43	9.52E-06	0.04
ENSG00000184489	PTP4A3	0.82	0.19	4.31	1.62E-05	0.04
ENSG00000004776	HSPB6	0.55	0.13	4.25	2.13E-05	0.05

Table shows output from the DESeq2 analysis. Ensembl ID is a stable database identifier corresponding to specific genes and does not change over time (in contrast to gene symbol). Log2FoldChange indicates the average difference in expression after versus before treatment with cotadutide lfcSE indicates uncertainty around the log2FoldChange estimate. Stat is the value of the test statistic for the gene. FDR is the Benjamini-Hochberg false discovery rate (p-value adjusted for multiple testing).

Table S7 Overall Summary of Treatment-emergent Adverse Events during the Double-blind Treatment Period - As-treated Population[a]

Participants with	Placebo N = 7	Cotadutide N = 18	Total N = 25
At least one event	5 (71.4%)	17 (94.4%)	22 (88.0%)
At least one investigational product related event	3 (42.9%)	16 (88.9%)	19 (76.0%)
At least one event of ≥ Grade 3 severity [b]	0	2 (11.1%)	2 (8.0%)
Death (Grade 5 severity [b])	0	1 (5.6%)	1 (4.0%)
At least one serious [c] event	0	2 (11.1%)	2 (8.0%)
At least one serious [c] and/or ≥ Grade 3 severity [b] event	0	2 (11.1%)	2 (8.0%)
At least one investigational product related serious [c] event	0	0	0
At least one event leading to discontinuation of investigational product	0	4 (22.2%)	4 (16.0%)

[a]Participants were counted once for each category regardless of the number of events. Population and participants were analysed according to their randomised treatment group.

[b]Grade 3: Severe, Grade 4: Life-threatening, Grade 5: Fatal.

[c]Serious adverse event criteria: death, life-threatening, required inpatient hospitalisation, prolongation of existing hospitalisation, persistent or significant disability/incapacity, important medical event, congenital anomaly/birth defect (in the offspring of the participant).

Note on serious adverse events

Two participants in the cotadutide group (11.1%) had at least one event of grade≥3 severity. One was a death (coronary artery thrombosis in a 72-year-old male, cotadutide group). The participant had a long-standing history (>30 years) of coronary artery disease and received a single dose of cotadutide; the death was considered unrelated to cotadutide. The other serious adverse event (grade 3 muscle hematoma) followed a muscle biopsy but was considered unrelated to cotadutide.

Note on pharmacokinetics and immunogenicity

Geometric mean pre-dose concentrations of cotadutide on day 32 and 59 were 5.43 ng/mL and 6.33 ng/mL, respectively, following 300 µg daily subcutaneous injections of cotadutide starting on day 25. Between-participant variability of cotadutide pre-dose concentrations, as measured by geometric coefficient of variation, was moderate, with estimates of 32.6% and 24.9% for days 32 and 59, respectively.

At baseline, no participants tested positive for cotadutide antibodies in either treatment group. In total, three participants receiving cotadutide (21.4%) developed cotadutide-specific anti-drug antibodies post-baseline and, for all three participants, the titer was low and remained low at the follow-up visit (median titer:5).

Supplement 1 (S1): Full list of eligibility criteria

Inclusion criteria

1. Subjects aged ≥ 30 and ≤ 75 years at screening.
2. Provision of signed and dated written informed consent (except for consent for genetic and non-genetic research and additional optional assessments) before any protocol-related procedures.
3. Body mass index > 28 and ≤ 40 kg/m^2 at screening.
4. Glycated hemoglobin (HbA1c) ≤ 8.0% at screening.
5. Diagnosed with type 2 diabetes mellitus with glucose control managed with metformin, with or without a dipeptidyl peptidase IV (DPPIV) inhibitor, sodium–glucose co-transporter-2 inhibitor (SGLT2i), sulfonylurea, or glitinide, where no significant dose change (increase or decrease > 50%) has occurred in the 3 months before screening; if the subject is on dual therapy, a 4-week washout of the non-metformin therapy (DPPIV inhibitor, SGLT2i, sulfonylurea, or glitinide) will be required before visit 4.
6. Female subjects of childbearing potential must have a negative pregnancy test at screening and randomization, and must not be lactating.
7. Female subjects of childbearing potential who are sexually active with a non-sterilized male partner must be using at least one highly effective method of contraception (see section 10.2 of the study protocol for definition of females of childbearing potential and for a description of highly effective methods of contraception) from screening and must agree to continue using such precautions up until 4 weeks after the last dose of investigational product.

Exclusion criteria

1. History of, or any existing, condition(s) that, in the opinion of the investigator, would interfere with evaluation of the investigational product, put the subject at risk, influence the subject's ability to participate, or affect the interpretation of the results of the study and/or any subject unable or unwilling to follow study procedures.
2. Any subject with a cardiac pacemaker or implanted/portable electronic device.
3. Any subject who has received another investigational product as part of a clinical study or a glucagon-like peptide-1 (GLP-1) analog-containing preparation within the last 30 days or 5 half-lives of the drug (whichever is longer) at the time of screening (visit 1).
4. Any subject who has received any of the following medications within the specified time frame before visit 2: herbal preparations or drugs licensed for control of body weight or appetite (e.g., orlistat, bupropion, naltrexone, phentermine-topiramate,

phentermine, lorcaserin, opiates, domperidone, metoclopramide, or other drugs known to alter gastric emptying).

5. Concurrent participation in another study with an investigational product and prior randomization in this study is prohibited.
6. Severe allergy/hypersensitivity to any of the proposed study treatments, excipients, or standardized meals.
7. Symptoms of acutely decompensated blood glucose control (e.g., thirst, polyuria, weight loss), a history of type 1 diabetes mellitus, or diabetes being treated with daily subcutaneous insulin in the 90 days before screening.
8. Abnormal thyroid stimulating hormone level of < 0.03 mIU/L or > 10 mIU/L confirmed in two consecutive tests.
9. Regularly engage in high-intensity exercise at least 3 times per week or have done so in the previous 3 months.
10. Clinically significant inflammatory bowel disease, gastroparesis or other severe disease, or surgery affecting the upper gastrointestinal tract (including weight-reducing surgery and procedures) which may affect gastric emptying or could affect the interpretation of safety and tolerability data.
11. Acute or chronic pancreatitis with or without amylase > 1000 IU/L and/or lipase > 600 IU/L at screening.
12. Significant hepatic disease (except for non-alcoholic steatohepatitis or non-alcoholic fatty liver disease without portal hypertension or cirrhosis) and/or subjects with any of the following results at screening:
    (a) Aspartate transaminase (AST) ≥ 3 × upper limit of normal (ULN)
    (b) Alanine transaminase (ALT) ≥ 3 × ULN
    (c) Total bilirubin ≥ 2 × ULN.
13. Impaired renal function defined as estimated glomerular filtration rate (eGFR) < 45 mL/min/1.73 m^2 at screening (GFR estimated according to the Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] or the Modification of Diet in Renal Disease [MDRD] using MDRD Study Equation isotope dilution mass spectrometry-traceable SI units).
14. Poorly controlled hypertension defined as:
    (a) systolic blood pressure (BP) > 180 mmHg
    (b) diastolic BP or > 100 mmHg

    after 10 minutes of supine rest and confirmed by repeated measurement at screening. Subjects who fail BP screening criteria may be considered for 24-hour ambulatory BP monitoring at the discretion of the investigator. Subjects who maintain a mean 24-hour

BP ≤ 180/100 mmHg with a preserved nocturnal dip of > 15% will be considered eligible.

15. Unstable angina pectoris, myocardial infarction, transient ischemic attack, or stroke in the 3-month period before screening, or subjects who have undergone percutaneous coronary intervention or a coronary artery bypass graft within the past 6 months, or who are due to undergo these procedures at the time of screening.
16. Severe congestive heart failure (New York Heart Association Class III or IV).
17. Basal calcitonin level > 50 ng/L at screening or history/family history of medullary thyroid carcinoma or multiple endocrine neoplasia.
18. History of neoplastic disease in the 5-year period before screening, except for adequately treated basal cell, squamous cell skin cancer, or in situ cervical cancer.
19. Any positive results for serum hepatitis B surface antigen, hepatitis C antibody, and human immunodeficiency virus antibody.
20. Substance dependence or history of alcohol abuse and/or excess alcohol intake.
21. Involvement of any AstraZeneca, MedImmune, contract research organization, or National Institute for Health Research/Wellcome Trust Cambridge Clinical Research Facility employee or their close relatives.

Supplement 2 (S2): Intravenous glucose tolerance test (IVGTT) procedure, data processing and analysis

Procedure

Participants were fasted for 12 hours overnight before IVGTT. An intravenous cannula was inserted into a vein in the antecubital fossa. A bolus of 300 mg/kg (lean mass) of 20% glucose solution was administered intravenously. Blood samples were drawn before and for up to 180 minutes after the glucose infusion at T = -10, -1 (just before glucose infusion) and 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150, and 180 minutes.

Minimal model and parameter estimation

Insulin sensitivity (S_I) and glucose effectiveness (S_G) were calculated using the minimal model of glucose tolerance. The minimal model defines S_I (the ability of insulin to enhance the net glucose disappearance from plasma) and S_G (the ability of glucose to promote its own disposal) and is described by two differential equations:

$$dg/dt = -[p1 + x(t)]g(t) + p1gb \qquad g(0) = gb + D/V \qquad (1)$$

$$dx/dt = -p2x(t) + p3[i(t) - ib] \qquad x(0) = 0 \qquad (2)$$

where g(t) is the plasma glucose concentration, i(t) is the plasma insulin concentration, x(t) is insulin action above basal, gb and ib are end-test glucose and insulin concentrations, D is the amount of exogenous glucose injected at time 0, and p1, p2, p3, and V are model parameters. The parameter V represents the distribution volume of glucose.

Insulin sensitivity is defined as the ratio $S_I = p3/p2$ and glucose effectiveness as the ratio $S_G = p1$.

Glucose measurements before 8 minutes were excluded from the parameter estimation to allow early mixing by the one-compartment model.

Parameters from the minimal model were estimated for each subject by the weighted non-linear regression analysis. The package SAAM II v 1.2.1 (SAAM Institute, Seattle, WA, USA) was used for individual parameter estimation.

First-phase insulin response (FPIR) was computed as an incremental area under the curve in the first 10 minutes of the test. Disposition index (DI) was calculated as the product of S_I and FPIR. Differences in the changes in S_I, S_G, FPIR, and DI from visit 3 to visit 8 between the treatment groups were assessed using the Mann-Whitney U test due to departure from normality. STATA v16 (StataCorp LLC, College Station, TX, USA) and GraphPad Prism8

(GraphPad Software, Boston, MA, USA) were used for the calculation of FPIR and DI, descriptive statistics, and to generate graphical outputs.

Supplement 3 (S3): Study procedures

Energy intake was assessed during *ad libitum* lunch using single-blind measure of food consumption using a Universal Eating Monitor, where weight of the food and rate of consumption was recorded. Food of known macronutrient content was served. Participants were advised to eat freely until they felt comfortably full. There was no time limit on the duration of the meal. During the meal, the quantity of food ingested was recorded by study site personnel while the participants were not aware that food consumption was being recorded. Participants were not instructed to follow a specific diet between the study visits.

A visual analogue scale with 4 items was used to assess self-reported hunger ranging from "not at all" to "extremely". The scale is presented in the study protocol.

24hr Energy Expenditure indirect room calorimetry.

On day -1, participants arrived at the NIHR Cambridge Clinical research Facility at 0700am, fasted for at least 12 hours. After laying down for a one hour rest period, resting energy expenditure (REE) was assessed using a ventilated hood attached to a portable indirect calorimeter (GemNutrition, Daresbury, UK). The REE procedure and calibrations have been previously described (1). In brief, participants were asked to remain still, awake and not interact with others for 40 min during the measurements. Gas exchange measurements of the room were collected for the first 10 min, followed by ventilated hood measurement of the participant for 20 min with a further 10 min of room gas analysis at the end of the measurement.

On day 15, participants arrived at 0700am, fasted for 12 hours. After a repeat REE measurement, participants entered the room calorimeter at 0900 for 24hr EE and macronutrient oxidation assessment. A 24h urine collection was started for protein estimation. A standardised breakfast was served at 0915am, cycling at 25W for 15min began at 1130am, a standardised lunch was served at 1230pm, further cycling at 25W for 15min at 1430 and 1730. At 1800 a standardised evening meal was served followed by a final cycling bout at 2030pm. The calorimeter lights were switched off at 2300pm. Upon waking, the participant passed urine, completing the 24hr urine collection, and the door of the calorimeter was opened, indicating the end of the room calorimeter analysis. The participant remained in bed and a final REE assessment was performed using the ventilated hood.

Standardised meals were calculated using Schofield *et al*.(2) for energy requirements plus a physical activity score of 1.35. The composition of meals were 50-55% carbohydrates, 30-35% fat and 12-15% protein.

On day 32 the REE assessment was repeated and on day 58 the room calorimeter procedure was repeated. The calorimeter rooms have 10.4 m^2 floor area and 24 m^3 volume. They are constructed as comfortable bed-sitting rooms within the NIHR Cambridge CRF. They have outside windows and washbasins, comfortable beds and chairs, TV and internet computers. Instrumentation and calibration procedures have been previously described (3).

O_2 consumption and CO_2 production were calculated by the method described by Brown *et al.(4).* Macronutrient oxidation rates were calculated to better than 20 g/d for carbohydrate (CHO) and 9.5 g/d for fat, using the expressions of Murgatroyd *et al*.(5), with macronutrient respiratory quotients and energy equivalents of oxygen from Elia and Livesey (6).

Body composition was measured using dual energy x-ray absorptiometry [DXA] GE Lunar iDXA.

Doubly labeled water assessment of energy expenditure was performed at baseline (day -1) and at the end of treatment (day 58 or 59). Following a baseline sample collection of urine, each participant was given a dose of doubly labeled water consisting of 105 mg/kg $^{2}H_2O$ and 225 mg/kg $H_2{}^{18}O$. Further urine samples were collected at 2, 3, 4 hours and 18 days post dose. Samples were analyzed in singlicate for ^{2}H and ^{18}O enrichment using isotope ratio mass spectrometers. All samples were measured alongside secondary reference standards previously calibrated against the primary international standards Vienna-Standard Mean Ocean Water (vSMOW) and Vienna-Standard Light Antarctic Precipitate (vSLAP; International Atomic Energy Agency, Vienna, Austria). The rate of carbon dioxide production was calculated from pool sizes and rate constants determined from the intercepts and slopes of the log transformed data and converted to TEE assuming a fixed respiratory quotient of 0.85.

Free-living physical activity was assessed using a non-waterproof wrist-worn accelerometer Striiv (Striiv, Inc. Redwood City, CA,US) during the study period. Time spent in activity and step count were derived from the accelerometer data.

Mixed meal tolerance test (MMTT) was performed following a minimum 12-hour fast. After consumption of a standardized solid meal within 5 minutes, timed serial blood samples were obtained for measurements of glucose, gut, and pancreatic hormone levels through 240 minutes, with no additional food intake during this time.

Gastric emptying was measured using SmartPill™ Motility Testing System. Participants were required to swallow a SmartPill™ motility capsule with the first standardized meal following a 12-hour overnight fast. The SmartPill™ measures pressure, pH, transit time, and temperature as it passes through the entire gastrointestinal tract, sending a signal to the receiver once it has been expelled from the body (through the feces).

The key safety assessment included measurements of vital signs, electrocardiogram, safety laboratory analyses (serum biochemistry and blood count), and measurement of capillary blood glucose every 72 hours using a standardized glucometer. Treatment-emergent AEs were defined using the Medical Dictionary for Regulatory Activities and elicited via participant self-report. The investigator determined whether the event was related to the study drug.

Pharmacokinetic evaluation of cotadutide was conducted using a validated liquid chromatography-tandem mass spectrometry method. Immunogenicity was assessed by measures of anti-drug antibody positive responses.

A full list of secondary, exploratory, and safety outcomes and all procedures used in this study is provided in the study protocol (Supplement 4).

Muscle biopsies

Optional muscle biopsies were performed before and after treatment to evaluate changes in mitochondria, muscle protein, and RNA expression following treatment with cotadutide. Quadriceps muscle was used for the site of the biopsy. The selected site was the same for both biopsies. The area was shaved (if necessary) and cleaned and a muscle biopsy was performed using a muscle biopsy needle following instillation of local anaesthetic and using aseptic technique. The biopsy site was closed using wound closure strips or absorbable sutures, and a suitable dressing. Participants were given wound-care advice.

RNA sequencing

Pre-processing was performed with an internal pipeline. Short sequencing reads were trimmed with NGmerge 0.3 (7). Alignment to human genome GRCh38 was performed with STAR 2.7.2b (8). Expression was quantified with Salmon 1.1.0 (9) and bcbioRNASeq 0.3.28 (10). Quality control readouts were aggregated and reviewed in a multiQC 1.9 report (11).

Small pieces of skin and adipose tissue may be co-sampled in muscle biopsies. We evaluated potential contamination based on adipose and skin tissue markers. The biopsy pair from one patient displayed levels of contamination that might affect downstream analysis and was excluded. The final sample size constituted four paired biopsies.

Paired-sample differential expression analysis was performed with DESeq2 0.2.10 (12). Genes with Benjamini-Hochberg False Discovery Rate < 0.05 were considered significant.

In addition, targeted analysis was performed for glucose transporters, glycogen biosynthesis (GO:0005978), pentose phosphate pathway (GO:0006098) and glycolysis (GO:0061621), markers of lipid oxidation (GO:0034440) and lipogenesis (GO:0008610), mitochondrial enzymes, and markers of insulin sensitivity in skeletal muscle (13). These marker sets were selected as potentially relevant based on prior publications for GLP-1 mimetics and oxyntomodulin (14-16). If at least 4% of a reference marker set was dysregulated with raw $p < 0.05$, enrichment of the marker set was evaluated with Chi-squared test.

Supplement 4 (S4): Study protocol

The study protocol is available via the following link: https://storage.googleapis.com/ctgov2-large-docs/77/NCT03596177/Prot_002.pdf

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