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Acute concomitant GIP receptor antagonism during GLP-1 receptor agonism does not affect appetite, resting energy expenditure or food intake in patients with type 2 diabetes and overweight/obesity

Short running title: Combining GIPR antagonism and GLP-1R agonism in type 2 diabetes

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Abstract

Aims: When combined with glucagon-like peptide 1 (GLP-1) receptor (GLP-1R) agonism, antagonising the glucose-dependent insulinotropic polypeptide (GIP) receptor (GIPR) reduces body weight in rodent models of obesity. Here, we investigated the acute effects of GIPR antagonism combined with a GLP-1 infusion on determinants of body weight in patients with type 2 diabetes and overweight/obesity.

Materials and methods: In a randomised, double-blind, placebo-controlled, crossover design, human synthetic GLP-1(7-36)NH₂ (0.75 pmol/kg/min) was infused together with the selective GIPR antagonist GIP(3-30)NH₂ (1,200 pmol/kg/min) or placebo for 320 minutes on two separate days covering an initial oral liquid mixed meal test and a terminal ad libitum meal. Appetite sensations, resting energy expenditure (REE) and food intake were evaluated, and subcutaneous adipose tissue (SAT) biopsies were analysed for triglyceride content.

Results: Ten patients with type 2 diabetes and overweight/obesity (mean±SD; HbA₁c 52±9 mmol/mol (7±1%); BMI 32.5±4.8 kg/m²) were included. Compared to placebo, infusion of the GIPR antagonist GIP(3-30)NH₂ added to a GLP-1 infusion had no effect on appetite sensations, REE, food intake, or SAT triglyceride content during an ad libitum meal. Compared to placebo, GIP(3-30)NH₂ lowered plasma glucagon by -12.4±5.9% (p=0.037), and reduced serum insulin by -32.5±8.0% (p=0.027).

Conclusions: During short term infusion, we found no effect of GIPR antagonism added to GLP-1R agonism on appetite sensations, REE, SAT triglyceride content or food intake in patients with type 2 diabetes and overweight/obesity.

Keywords: GLP-1, GIP; GIP receptor antagonist; incretin hormones; incretins; type 2 diabetes; obesity.

Abbreviations

bsAUC, baseline-subtracted AUC
GIP, glucose-dependent insulinotropic polypeptide
GIPR, glucose-dependent insulinotropic polypeptide receptor
GLP-1, glucagon-like peptide 1
GLP-1R, glucagon-like peptide 1 receptor
GLP-1RA, glucagon-like peptide 1 receptor agonist
ISR, insulin secretion rate
IQR, inter quartile range
MMT, mixed meal test
REE, resting energy expenditure
RQ, respiratory quotient
SAT, subcutaneous adipose tissue
VAS, visual analogue scale
Background

In type 2 diabetes (as well as other forms of diabetes), the insulinotropic effect of exogenous glucose-dependent insulinotropic polypeptide (GIP) is markedly reduced or even lost in contrast to that of exogenous glucagon-like peptide 1 (GLP-1), which retains its insulinotropic effect to a greater degree (1,2). Optimised glycaemic control improves the insulinotropic effect of exogenous GIP in patients with type 2 diabetes suggesting that diabetic GIP resistance is a reversible phenomenon (2,3). Furthermore, we recently showed that endogenous GIP has insulinotropic properties in type 2 diabetes (4). Several GLP-1 receptor (GLP-1R) agonists (GLP-1RAs) are now approved for the treatment of type 2 diabetes and obesity (5). Previously, GIP was described as an obesity hormone (7,8). In recent years, paradoxically, both GIPR agonism and GIPR antagonism have shown anti-obesity effects when combined with GLP-1RA in preclinical models (8–12). Potentially, the beneficial effects of GIPR agonists arise from functional GIPR antagonism elicited by GIPR internalisation (13,14). Several new treatment modalities for type 2 diabetes and obesity combining GLP-1RA with GIPR agonism or GIPR antagonism are emerging (14). However, no human data are currently available describing the combined effects of GLP-1RA and GIPR antagonism.

We investigated the effect of GIPR antagonism combined with GLP-1RA on determinants of body weight regulation in patients with type 2 diabetes and overweight/obesity.

Methods

Study protocol

The Scientific-Ethical Committee of the Capital Region of Denmark (H-17006969) and The Danish Data Protection Agency (HGH-2019-001; I-suite no.6801) approved the protocol. The study was registered at www.clinicaltrials.gov (NCT03702660). Data from two separate experimental days involving GIP(3-30)NH2 and saline infusions have previously been published (4).

Study design and participants

We included 10 men with type 2 diabetes and overweight/obesity in this randomised, double-blind, placebo-controlled, crossover study, including 210-minute liquid mixed meal tests (MMTs) on two separate occasions with a concomitant infusion of GLP-1(7-36)NH2 (0.75 pmol/kg/min) combined with the GIPR antagonist GIP(3-30)NH2 (1,200 pmol/kg/min) or placebo (for inclusion and exclusion criteria and description of the peptide infusions, please refer to Supplementary materials).

Experimental procedures and analyses

Study days were preceded by an overnight fast. Infusions were initiated 20 minutes before the MMTs. The standardised MMTs consisted of 200 ml Nutridrink Compact® (energy content per 100 ml: 240 kcal from 9.6 g protein, 9.3 g fat, and 29.7 g carbohydrate (15.0 g glucose/100g))
mixed with 100 ml of water with 1,500 mg paracetamol for evaluation of gastric emptying (15). After 210 minutes, an ad libitum meal test was served (pasta Bolognese; energy content per 100 g: 135 kcal from 5.3 g protein, 5.6 g fat, and 15.0 g carbohydrate) (16), followed by termination of the infusions and an abdominal SAT biopsy, taken during local anaesthesia (time point 230 min). We measured RQ and REE by indirect calorimetry (baseline, time points 30, 90, 150 min) (CCM Express, MedGraphics, Medical Graphics Corp, St Paul, MN, USA). REE and RQ were calculated based on 15-minute measurements during which a steady-state period of at least 4 minutes was identified. The estimates were derived by calculating a mean of seven out of 10 breaths, excluding the three most outlying measures. Patients were instructed not to do strenuous physical activity 60 hours prior to the study day. Confounding factors were minimised by a stable room temperature and a strictly calm surrounding eliminating disturbances. Visual analogue scales (VAS) were used to evaluate appetite, satiety, thirst, fullness, prospective eating, wellbeing, and nausea (time points 0, 60, 120, 180, 230 min). Blood samples for analysis of plasma/serum glucose, glucagon, GIP, GLP-1, GIP(3-30)NH₂, C-peptide, insulin, paracetamol, and lipids, and the SAT biopsies were handled as previously described (4). For a description of the hormonal analyses, please refer to the supplementary materials.

Statistical analyses and calculations

Results are presented as mean±SD unless otherwise stated. Comparisons were performed using two-tailed Student’s paired t tests or Wilcoxon non-parametric test. p values <0.05 were considered statistically significant. Please refer to Supplementary materials for details.

Results

Baseline characteristics have been described previously (4). In brief, the 10 men with type 2 diabetes had a mean (±SD) HbA₁c of 52±11 mmol/mol (7±3.2%) and a mean BMI of 32.5±4.8 kg/m². Please refer to Supplementary table 1 for additional baseline characteristics.

Peptide infusions

Plasma concentrations of GLP-1 reached a steady state of 55.1±6.0 pmol/l during GLP-1+GIP(3-30)NH₂ and 57.0±6.5 pmol/l during GLP-1+placebo (Figure 1A). GIP(3-30)NH₂ reached a steady state of 75.9±3.9 nmol/l during GLP-1+GIP(3-30)NH₂ (Figure 1B). The antagonist/agonist ratio was >1900, which significantly inhibits GIP(1-42)-induced actions (17). We observed no adverse reactions to the infusions.

Appetite measures and energy expenditure

VAS scores for satiety, fullness, appetite, prospective eating, thirst, wellbeing, and nausea, and Ad libitum meal consumption were similar during both interventions (Figure 2). Energy expenditure did not change during co-infusion of GLP-1+GIP(3-30)NH₂ compared to infusion
of GLP-1+placebo, whether measured at baseline, or at time point 30 min or 90 min, but at time point 150 min energy expenditure was lower during co-infusion of GLP-1+GIP(3-30)NH₂ compared to infusion of GLP-1+placebo ($p=0.055$). The RQ was similar during both interventions (Supplementary table 2, Figure 1J-K).

Paracetamol absorption test and plasma glucose

Paracetamol absorption was similar during both interventions (Figure 1C). Due to GLP-1-induced deceleration of gastric emptying (18), postprandial plasma glucose concentrations declined slightly and similarly on both study days (Figure 1D).

Insulin, C-peptide, ISR, and glucagon

We observed no difference in baseline levels of insulin, C-peptide or glucagon (Figure 1E-F,H). Compared with GLP-1+placebo, infusion of GLP-1+GIP(3-30)NH₂ reduced C-peptide by 27.7±36.4% ($p=0.027$) (mean±SEM) and insulin levels by −32.5±8.0% ($p=0.014$). The ISR and the ISR/glucose ratio were lower during infusion of GLP-1+GIP(3-30)NH₂ compared to GLP-1+placebo (Figure 1G). We found no difference in the insulinoenic index (Δinsulin$_{0-30}$min/Δglucose$_{0-30}$min) (data not shown). GLP-1+GIP(3-30)NH₂ significantly reduced glucagon levels by −12.4±5.9%, compared with GLP-1+placebo.

GIP

Baseline concentrations, peak concentrations, and time-to-peak of total plasma GIP were similar on both study days (Figure 1I).

Triglyceride content of the SAT biopsy and plasma lipids

SAT triglyceride content was unchanged during infusion of GLP-1+GIP(3-30)NH₂ compared to GLP-1+placebo (Figure 1L). Baseline concentrations and bsAUC of plasma triglyceride, NEFA, glycerol, and cholesterol (total, HDL, LDL, VLDL) were similar during the two interventions (Supplementary table 2).

Discussion

We found no effect of co-infusion of the GIPR antagonist GIP(3-30)NH₂ on appetite sensations, REE, RQ, food intake, or SAT triglyceride content during infusion of GLP-1 in 10 patients with type 2 diabetes and overweight/obesity.

In preclinical models, both GIPR agonism and GIPR antagonism combined with GLP-1RA result in body weight reductions by lowering food intake and fat mass (8,9,20), but effects of GIPR antagonism combined with GLP-1RA have not previously been studied in humans. In persons with overweight/obesity without diabetes, exogenous GIP added to exogenous GLP-1 increased ad libitum food intake (21), whereas in persons with overweight/obesity and GLP-1RA-treated type 2 diabetes, exogenous GIP did not alter ad libitum food intake (21). In
contrast, long-term treatment with the GIPR/GLP-1R co-agonist tirzepatide causes greater body weight reductions than GLP-1RA mono-therapy (22). The present results do not exclude that chronic pharmacological GIPR stimulation may reduce appetite and food intake. Supraphysiological GIPR agonism might cause functional GIPR antagonism due to GIPR internalisation, which combined with GLP-1R agonism may reduce body weight (19). A hypothetical advantage of GIPR antagonism for the treatment of obesity is inhibition of GIP-mediated fat deposition in the SAT. However, we found no difference in SAT triglyceride content, nor in the present data or in our previous comparison of GIPR antagonism alone compared to placebo in patients with type 2 diabetes (4). Effects of long-term GLP-1RA and GIPR antagonism have not been described in humans.

GIP(3-30)NH2 reduced insulin secretion during GLP-1 infusion demonstrating that endogenous GIP exerts insulinotropic action even in the face of supraphysiological GLP-1 concentrations. The finding that the beta cell responsiveness to endogenous GIP seems to differ from that of exogenous GIP should be further explored in future studies. During infusion of GLP-1, we observed strong inhibition of gastric emptying and an increase in insulin secretion before ingestion of the liquid mixed meal, explaining the lack of a postprandial rise in plasma glucose, making this study a functional fasting study. The effect of GLP-1 on the postprandial glucose levels have likely overshadowed any effects of GIPR antagonism on plasma glucose and represents a limitation of the study. Other limitations include the number of patients included and the short-term duration of the study.

In conclusion, the body weight-lowering effect of GIPR antagonism when added to GLP-1RA treatment observed in preclinical models could not be substantiated in these 10 patients with type 2 diabetes and overweight/obesity.

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Data availability

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

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Declaration of interest

SS, LLK, and FD have nothing to disclose. AHS is the CEO and co-founder of Antag Therapeutics ApS. BH is co-founder of Bainan Biotech ApS. TV has served on scientific advisory panels and/or speakers’ bureaus or has served as a consultant to and/or received research support from Amgen, AstraZeneca, BMS, Boehringer Ingelheim, Eli Lilly, GSK, MSD/Merck, Mundipharma, Novo Nordisk, Sanofi, and SunPharma. JJH has served on scientific advisory panels for and/or has received speaker honoraria from Novo Nordisk and MSD/Merck. JJH is one of the founders of Antag Therapeutics ApS. MMR is one of the founders of Antag Therapeutics ApS, Bainan Biotech ApS, and Synklinio ApS. MBC is co-founder of and minority shareholder in Antag Therapeutics ApS. LSG has been a speaker for Eli Lilly and is co-founder of Antag Therapeutics ApS. FKK has served on scientific advisory panels and/or been part of speaker’s bureaus for, served as a consultant to, and/or received research support from Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, Carmot Therapeutics, Eli Lilly, Gubra, MedImmune, MSD/Merck, Mundipharma, Norgine, Novo Nordisk, Sanofi, and Zealand Pharma; and is a co-founder of and minority shareholder in Antag Therapeutics ApS.

Author contribution statement

SS, LSG, AHS, JJH, MMR, MBC, and FKK designed the clinical study, and SS and LSK performed the clinical study. BH and JJH performed RIA and ELISA analyses. FD was responsible for the NEFA and glycerol measurements. SS, LSG, and FKK performed and were responsible for the data analysis and wrote the manuscript. All authors contributed to the interpretation of data, reviewed and edited the manuscript, and approved the final version.
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Figure legends

Figure 1. Total plasma GLP-1 (A), plasma GIP(3-30)NH₂ (B), serum paracetamol (C), plasma glucose (D), serum insulin (E), serum C-peptide (F), beta cell function assessed as ISR / plasma glucose (G), plasma glucagon (H), total plasma GIP (I), REE (J), RQ (K), and SAT triglyceride content (L) during a liquid MMT initiated at time point 0 (indicated by dotted line) and concomitant infusion of GLP-1+placebo (dark blue squares) or GLP-1+GIP(3-30)NH₂ (light blue squares) in 10 patients with type 2 diabetes. Infusions were initiated 20 minutes before MMT ingestion (indicated by dashed line). Data are presented as mean±SEM (n = 10). MMT, mixed meal test; bsAUC, baseline-subtracted AUC; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; ISR, insulin secretion rate; REE, resting energy expenditure; RQ, respiratory quotient, SAT, subcutaneous adipose tissue.

Figure 2. VAS measures of patient-evaluated sensations of appetite, satiety, thirst, fullness, prospective eating, wellbeing, and nausea, and composite appetite scores, and food consumption during the ad libitum meal at time point 210 min (indicated by the vertical line). Study days included a liquid MMT initiated at time point 0 min (indicated by dotted line) and concomitant infusion of GLP-1+placebo (dark blue squares) or GLP-1+GIP(3-30)NH₂ (light blue squares). Infusions were initiated 20 minutes before MMT ingestion (indicated by dashed line). CAS was calculated from the VAS assessment of appetite-related measures (hunger+prospective food consumption (100–satiety)+(100–fullness)/4). Data are presented as mean±SEM (n=10). MMT, mixed meal test; CAS, composite appetite score; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; VAS, visual analogue score.
Infusion of GLP-1 (0.75 pmol/kg/min) ± GIP(3-30)NH₂ (1,200 pmol/kg/min)

A) GLP-1 (pmol/l)

B) GIP(3-30)NH₂ (pmol/l)

C) Paracetamol (mmol/l)

D) Glucose (mmol/l)

E) Insulin (pmol/l)

F) C-peptide (pmol/l)

G) Glucagon (pmol/l)

H) AUC

I) GIP (pmol/l)

J) REE (kcal/day)

K) RQ (V CO₂/V O₂)

L) SAT triglyceride content (mg/g adipose tissue)

- GLP-1 + placebo
- GLP-1 + GIP(3-30)NH₂