Endogenous Glucose-Dependent Insulinotropic Polypeptide Contributes to Sitagliptin-Mediated Improvement in β-Cell Function in Patients With Type 2 Diabetes

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Dipeptidyl peptidase 4 (DPP-4) degrades the incretin hormones glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide (GIP). DPP-4 inhibitors improve glycemic control in type 2 diabetes, but the importance of protecting GIP from degradation for their clinical effects is unknown. We included 12 patients with type 2 diabetes (mean ± SD BMI 27 ± 2.6 kg/m^2, HbA_{1c} 7.1 ± 1.4% [54 ± 15 mmol/mol]) in this double-blind, placebo-controlled, crossover study to investigate the contribution of endogenous GIP to the effects of the DPP-4 inhibitor sitagliptin. Participants underwent two randomized, 13-day treatment courses of sitagliptin (100 mg/day) and placebo, respectively. At the end of each treatment period, we performed two mixed-meal tests with infusion of the GIP receptor antagonist GIP(3-30)NH_2 (1,200 pmol/kg/min) or saline placebo. Sitagliptin lowered mean fasting plasma glucose by 1.1 mmol/L compared with placebo treatment. During placebo treatment, postprandial glucose excursions were increased during GIP(3-30)NH_2 compared with saline (difference in area under the curve ± SEM 7.3 ± 2.8%) but were unchanged during sitagliptin treatment. Endogenous GIP improved β-cell function by 37 ± 12% during DPP-4 inhibition by sitagliptin. This was determined by the insulin secretion rate/plasma glucose ratio. We calculated an estimate of the absolute sitagliptin-mediated impact of GIP on β-cell function as the insulinogenic index during sitagliptin treatment plus saline infusion minus the insulinogenic index during sitagliptin plus GIP (3-30)NH_2. This estimate was expressed relative to the maximal potential contribution of GIP to the effect of sitagliptin (100%), defined as the difference between the full sitagliptin treatment effect, including actions mediated by GIP (sitagliptin + saline), and the physiological response minus any contribution by GIP [placebo treatment + GIP (3-30)NH_2]. We demonstrate insulinotropic and glucose-lowering effects of endogenous GIP in patients with type 2 diabetes and that endogenous GIP contributes to the improved β-cell function observed during DPP-4 inhibition.

Dipeptidyl peptidase 4 (DPP-4) is a widely expressed enzyme that degrades the incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (1). DPP-4 inhibitors are efficient, safe, and frequently used as second-line glucose-lowering agents in the management of type 2 diabetes (2). Because the insulinotropic effect

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of exogenous GIP is severely reduced or even absent in patients with type 2 diabetes (3,4), DPP-4 inhibition is generally believed to act mainly through preventing GLP-1 degradation. However, reduced GIP-induced insulin secretion can, to some extent, be restored following optimization of glycemic control in patients with type 2 diabetes (5,6). Recently, using the highly selective GIP receptor (GIPR) antagonist GIP(3-30)NH_2 (7), we demonstrated that endogenous GIP *1*) exerts greater potentiation of glucose-stimulated insulin secretion compared with endogenous GLP-1 in healthy individuals (8,9) and *2*) contributes significantly to postprandial insulin secretion in patients with type 2 diabetes (10). Furthermore, in preclinical studies, inhibition of the GLP-1 receptor (GLP-1R) by antagonism or receptor knockout does not eliminate the glucose-lowering effect of DPP-4 inhibition (11,12), whereas the effect is lost in double–incretin receptor knockout mice and GLP-1–negative mice treated with GIPR-antagonizing antibodies (13–15). Clinical studies using the GLP-1R antagonist exendin(9-39)NH_2 also have shown that endogenous GLP-1 accounts for only approximately one-half of the glucose-lowering and insulinotropic effects of DPP-4 inhibition in patients with type 2 diabetes; thus, it was speculated that the remaining effect of DPP-4 inhibition is mediated by GIP (16,17). In the current study, we infused the GIPR antagonist GIP(3-30)NH_2 or saline during treatment with the DPP-4 inhibitor sitagliptin in patients with type 2 diabetes to test the hypothesis that endogenous GIP contributes to the insulinotropic and glucose-lowering effects of DPP-4 inhibitor treatment.

RESEARCH DESIGN AND METHODS

Study Design and Approvals

In this randomized, double-blind, placebo-controlled, crossover study, patients were randomly assigned to receive treatment with sitagliptin (Januvia, Merck, Branchburg, NJ) 100 mg once daily or placebo in two separate 13- or 14-day treatment periods with an interposed washout period of 1–3 weeks. Study medication was add-on therapy to a continuing stable dose of metformin. Study medication was purchased through the Capital Region Pharmacy (Herlev, Denmark), which also performed the blinding of active treatment and placebo treatment by encapsulating sitagliptin and placebo tablets to obtain identical appearance. Randomization was performed by otherwise uninvolved personnel using an online random sequence generator (https://www.random.org). All participants and study personnel were blinded to the type of treatment. The study was approved by the health research ethics committees of the Capital Region of Denmark (identification no. H-18040916) and by the Danish Data Protection Agency (journal no. VD-2019-193) and was performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients before inclusion.

Study Participants

Participants were recruited through local advertisement and from local general practitioners. Inclusion was based on a screening visit, which included a clinical examination and blood and urinary tests. Inclusion criteria were age ≥18 years, type 2 diabetes for >3 months, ongoing stable treatment with lifestyle interventions and metformin, HbA_{1c} <9% (75 mmol/mol), and BMI >27 kg/m^2. The exclusion criteria were any medication that could not be paused for 12 h, plasma ALT >210 units/L, renal impairment (estimated glomerular filtration rate <60 mL/min/1.73 m^2), complicating heart disease, New York Heart Association heart failure functional classification group III or IV, anemia (hemoglobin <8.3 mmol/L for men and <7.3 mmol/L for women), special dietary preferences or planned weight loss, and pregnancy or lactation.

GIPR Antagonist and Placebo Infusions

Synthetic human GIP(3-30)NH_2 (custom synthesized by CASLO ApS, Lyngby, Denmark), with a purity of 98.55% confirmed by mass spectrometry, was dissolved in sodium hydrogen carbonate with 0.2% human albumin (CSL Behring, Marburg, Germany), tested for sterility and endotoxins by the Capital Region Pharmacy, and stored at −20°C. Under sterile conditions, the peptide was diluted to a volume of 1,000 mL in sodium chloride (9 mg/mL; Fresenius Kabi, Uppsala, Sweden) with 0.2% human albumin. Placebo infusions were 1,000 mL sodium chloride with 0.2% human albumin with an appearance identical to the GIP(3-30)NH_2 infusions.

Experimental Procedures

At the end of each treatment period, two randomized study days, each including a standardized liquid mixed-meal test with a concomitant infusion of either GIP(3-30)NH_2 (1,200 pmol/kg/min) or saline (placebo) were performed. All study days were preceded by a 10-h overnight fast. The study drug sitagliptin or placebo was administered 2 h before ingestion of the liquid mixed-meal test, and all other medications (including metformin) were discontinued 12 h before each study day. Two cannulas were inserted in a cubital vein bilaterally, one for blood sampling and one for peptide/placebo infusion. The meal was ingested evenly over 5 min from time 0 min. The meal consisted of 480 kcal/200 mL Nutridrink Compact (energy content: 240 kcal/100 mL, 9.6 g protein, 9.3 g fat, and 29.7 g carbohydrate [15.0 g glucose]) to which was added a solution of 1.5 g acetaminophen in 100 mL water (for assessment of acetaminophen absorption rate as a proxy for gastric emptying) (18). The infusion was initiated 20 min before meal ingestion and was continued for 5 h. At time −25, 15, 75, 150, and 245 min, duplicate blood pressure and heart rate measures were obtained, and a mean value was calculated. Participants evaluated sensations of appetite, satiety, thirst, fullness, prospective eating, well-being, and nausea on a visual analog scale at time −30, 0, 15, 30, 60, 90, 120, 180, 240, and 300 min. Blood was drawn at fixed intervals (time −25, −20, 0, 15, 30, 45,

60, 90, 120, 180, 240, and 300 min). For bedside analysis of plasma glucose, blood was distributed into sodium fluoride–coated tubes and centrifuged immediately. For GIP, GLP-1, GIP(3-30)NH_2, proinsulin, and glucagon, blood was distributed into chilled tubes containing EDTA and a specific DPP-4 inhibitor (valine pyrrolidide 0.01 mmol/L, a gift from Novo Nordisk, Måløv, Denmark) and immediately cooled on ice. For insulin, C-peptide, and acetaminophen analysis, blood was collected in dry tubes containing separator gel and serum clot activator (silica particles) and left to coagulate at room temperature for 20 min. Samples in EDTA and dry tubes were centrifuged for 20 min at 2,000*g* and 4°C and then kept on ice while plasma/serum was transferred to chilled storage tubes also kept on ice and then frozen. Plasma and serum samples were stored at −20°C until batch analysis.

Analytical Procedures

Plasma glucose was measured at bedside using the glucose oxidase method (YSI model 2900 STAT Plus Analyzer; Yellow Springs Instruments, Yellow Springs, OH). Intact biologically active plasma GIP was analyzed by an in-house radioimmunoassay using an N-terminally directed antiserum as previously described (19). Intact biologically active plasma GLP-1 was analyzed by a previously described in-house ELISA (20). Plasma levels of total GIP (C terminus) (9), total GLP-1 (C terminus) (21), and GIP(3-30)NH_2 (22) were measured by in-house radioimmunoassays as previously described. According to the manufacturer's instructions, plasma glucagon and proinsulin were analyzed by ELISAs (Mercodia, Uppsala, Sweden). Serum insulin and C-peptide were analyzed by sandwich immunoassays using direct chemiluminescence (ADVIA Centaur XP; Siemens, Munich, Germany).

Calculations and Statistical Analyses

Data are presented as mean ± SEM unless otherwise stated. Data from blood sample analyses are presented as baseline, peak, time to peak, area under the curve (AUC), and baseline-subtracted AUC (bsAUC) values as prespecified in the protocol. When available, baseline values were calculated as a mean of time −30-, −15-, and 0-min values. According to our sample size calculation, 12 participants were needed to detect a 30% difference in bsAUC for C-peptide with a power of at least 80% and a two-sided significance level of 5% based on values reported for a similar population (mean ± SD C-peptide levels 9.1 ± 2.8 ng/mL × h) (16). The primary end point was differences in AUC for plasma glucose concentrations during GIP(3-30)NH_2 infusion and saline infusion during sitagliptin treatment. Key secondary end points were the contribution of endogenous GIP to the insulinotropic effect of sitagliptin, postprandial changes in serum insulin and C-peptide, serum C-peptide/plasma glucose ratio, insulin secretion rate (ISR), plasma glucagon, and blood pressure. AUC and bsAUC were calculated by the trapezoidal method. We evaluated differences using a two-sample Student *t* test (two-tailed). Repeated-measures one-way ANOVA was applied to analyze variations among more than two estimates. Using the deconvolution method, we calculated ISR based on detected serum C-peptide concentrations and population-based variables for C-peptide kinetics in combination with weight, height, sex, and age (23). A two-sided $P < 0.05$ was chosen to indicate statistically significant differences.

Statistical analyses were performed using GraphPad Prism 9.0.0 software (GraphPad Software, San Diego, CA). The contribution of endogenous GIP to the insulinotropic effect of sitagliptin was assessed as the observed reduction in insulinogenic indices (*II*) (assessed as ISR/plasma glucose and serum C-peptide/plasma glucose) relative to the maximal potential contribution made by GIP to sitagliptin as previously done for GLP-1 and the DPP-4 inhibitor vildagliptin (16):

$$= \frac{II(sitagliptin + saline) - II(sitagliptin + GIP(3-30)NH_2)}{II(sitagliptin + saline) - II(placebo + GIP(3-30)NH_2)}.$$

As expressed in this equation, we calculated an estimate of the absolute sitagliptin-mediated impact of GIP on β-cell function as the insulinogenic index during sitagliptin treatment plus saline infusion minus the insulinogenic index during sitagliptin plus GIP(3-30)NH_2. This estimate was expressed relative to the maximal potential contribution of GIP to the effect of sitagliptin (100%), defined as the difference between the full sitagliptin treatment effect, including actions mediated by GIP (sitagliptin + saline), and the physiological response minus any contribution by GIP [placebo treatment + GIP(3-30)NH_2]. Mean arterial pressure (MAP) was calculated as (systolic blood pressure + [2 × diastolic blood pressure])/3.

Data and Resource Availability

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

RESULTS

Study Participants

We included four women and eight men with metformin-treated type 2 diabetes (duration of diabetes 11 ± 5 years [mean ± SD], age 67 ± 5 years, BMI 27.4 ± 2.6 kg/m^2, fasting plasma glucose 165 ± 52 mg/dL [9.2 ± 2.9 mmol/L], HbA_{1c} 7.1 ± 1.4% [54 ± 15 mmol/mol]) (Table 1). All recruited participants completed the study, and their data were analyzed.

GIP(3-30)NH_2 Infusion

Infusion with the GIPR antagonist GIP(3-30)NH_2 resulted in mean ± SD steady-state concentrations of 94 ± 17 nmol/L during placebo treatment and 102 ± 16 nmol/L during sitagliptin treatment before ingestion of the liquid mixed meal (Fig. 1*G*). The result was an antagonist/agonist ratio >1,100-fold previously shown to significantly inhibit GIP(1-42)-induced actions (24). We observed no adverse reactions to the infusions.

Table 1—Baseline characteristics of the study participants ($N = 12$)

Characteristic	Mean ± SD	Range	Median
Sex, *n*			
Male	8		
Female	4		
Age (years)	67 ± 5	59 – 73	67.5
BMI (kg/m^2)	27.4 ± 2.6	24.2 – 33.8	27.5
Body fat (%)	35.7 ± 6.2	22.9 – 45.4	37.2
Systolic blood pressure (mmHg)	138 ± 17	108 – 172	135
Diastolic blood pressure (mmHg)	87 ± 15	71 – 123	81
Heart rate (beats/min)	71 ± 13	44 – 90	71
Fasting plasma glucose (mg/dL)	165 ± 52	106 – 272	148
Fasting plasma glucose (mmol/L)	9.2 ± 2.9	5.9 – 15.1	8.2
HbA_{1c}			
%	7.1 ± 1.4	5.8 – 10.3	6.35
mmol/mol	54 ± 15	40 – 89	46
Duration of diabetes (years)	11 ± 5	4 – 22	10.5
Metformin dose (mg/day) ($n = 12$)	1,683 ± 654	500 – 3,000	2,000
Triglycerides (mmol/L)	1.6 ± 0.6	0.78 – 2.87	1.58
Cholesterol (mmol/L)			
Total	4.6 ± 1.4	2.7 – 7.5	4.1
HDL	1.3 ± 0.6	0.9 – 3.0	1.1
LDL	2.6 ± 1.2	0.9 – 5.1	2.4
VLDL	0.7 ± 0.3	0.4 – 1.3	0.7
Estimated glomerular filtration rate (mL/min/1.73 m^2)	85 ± 8	63 – 90	87.0

Plasma Glucose

Ten days of placebo treatment reduced fasting plasma glucose by −0.5 ± 0.5 mmol/L ($P = 0.335$), and sitagliptin treatment lowered fasting plasma glucose by −1.6 ± 0.6 mmol/L ($P = 0.016$), corresponding to a between-treatment reduction of fasting plasma glucose of −1.1 ± 0.2 mmol/L ($P < 0.001$). During the mixed meal, sitagliptin reduced the AUC of the glucose excursion and the peak plasma glucose concentration compared with placebo, but bsAUC was unchanged by sitagliptin (Fig. 1*A–C* and Table 2). During placebo treatment, GIP(3-30)NH_2 increased postprandial plasma glucose AUC by 7.3 ± 2.8% ($P = 0.017$) and increased peak plasma glucose concentration by 0.8 ± 0.2 mmol/L ($P = 0.003$) compared with saline infusion. During sitagliptin treatment, GIP(3-30)NH_2 did not change postprandial plasma glucose AUC or peak glucose concentration compared with saline infusion. Compared with placebo, sitagliptin delayed the time to peak plasma glucose by 20 ± 6.1 min ($P = 0.008$), but the time to peak plasma glucose was unaffected by GIP(3-30)NH_2 infusion during both placebo and sitagliptin treatment ($P = 0.731$ and $P = 0.220$, respectively).

Gastric Emptying Assessed by Acetaminophen Absorption

Serum acetaminophen concentrations were undetectable at baseline. Postprandial serum acetaminophen concentrations reached similar peak concentrations during all four interventions (Fig. 1*D* and Table 2). During sitagliptin treatment and saline infusion, the time to peak for serum acetaminophen was slowed by −19 ± 9.3 min ($P = 0.068$) compared with placebo and saline infusion. The time to peak concentration of serum acetaminophen was not affected by infusion of the GIPR antagonist.

Insulin and C-Peptide Secretory Responses and Insulinogenic Indices

Fasting levels of insulin and C-peptide were similar during both treatment periods. During placebo treatment, GIP(3-30)NH_2 caused a significant −20 ± 8% decrease in mean bsAUC for insulin and a −19 ± 6% decrease in mean bsAUC for C-peptide concentrations after the mixed meal (Table 2). In addition, during placebo treatment, GIP(3-30)NH_2 infusion lowered the C-peptide/glucose ratio by −35 ± 6%. During sitagliptin treatment, GIP(3-30)NH_2 caused a significant decrease in mean bsAUC for insulin (−6 ± 13%, $P = 0.028$) despite lower glycemia, but mean bsAUC for C-peptide was not significantly changed.

Sitagliptin improved the β-cell function assessed by ISR/plasma glucose ratio and serum C-peptide/plasma glucose ratio. This improvement was partially due to endogenous GIP, since GIP(3-30)NH_2 infusion lowered the serum C-peptide/plasma glucose ratio by −11 ± 7.3% during sitagliptin treatment (Fig. 2*G* and Table 2). The contribution of endogenous GIP to the insulinotropic effect of sitagliptin was found to be 34 ± 10% of the potential maximum

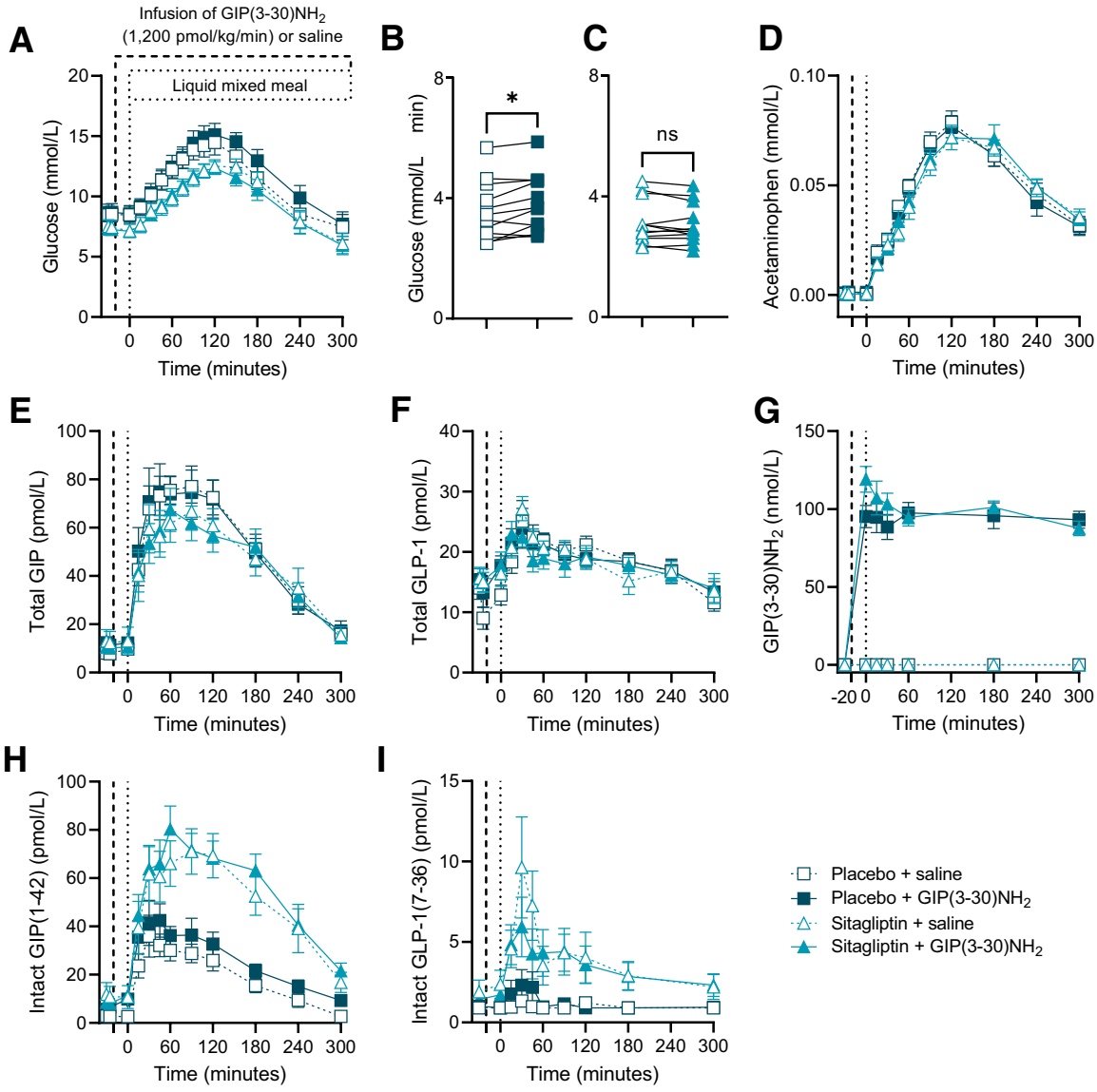

Figure 1—Plasma glucose (*A*), AUC for plasma glucose (*B* and *C*), serum acetaminophen (*D*), total GIP (*E*), total GLP-1 (*F*), GIP(3-30)NH_2 (*G*), intact GIP(1-42) (*H*), and intact GLP-1(7-36) (*I*) during mixed-meal tests performed during sitagliptin and placebo treatment [with GIP(3-30)NH_2 infusion and saline infusion] in 12 metformin-treated patients with type 2 diabetes. Data are mean ± SEM ($n = 12$). Statistical analyses are Student *t* tests for pairwise comparisons. *$P < 0.05$.

based on serum C-peptide/plasma glucose ratios and 37 ± 12% of the potential maximum based on ISR/plasma glucose ratios (Fig. 2*I*).

Plasma proinsulin was similarly reduced by infusion of GIP(3-30)NH_2 during the two treatment periods (Fig. 3*A*–*E* and Table 2). The baseline values of the proinsulin/insulin ratio, a measure of β-cell stress, were nonsignificantly increased by 3% following sitagliptin treatment (Fig. 3*D* and Table 2). However, assessment of the proinsulin/insulin ratio controlled for the chronological order of the interventions revealed that patients undergoing sitagliptin treatment in the second treatment period ($n = 5$) had a −20% decrease in the proinsulin/insulin ratio ($P = 0.005$) following sitagliptin treatment compared with placebo treatment, reflecting reduced β-cell stress. Sitagliptin significantly lowered baseline values of the proinsulin/C-peptide ratio compared with placebo tablet treatment, and during sitagliptin treatment, compared with saline placebo, GIPR antagonism significantly reduced β-cell stress assessed by the proinsulin/C-peptide ratio (Fig. 3*E* and Table 2).

Glucagon

During placebo treatment, GIP(3-30)NH_2 infusion reduced the AUC for plasma glucagon compared with saline (Table 2 and Fig. 4*A*). Postprandial plasma glucagon concentrations reached similar peak concentrations during all four interventions. When taking into account the prevailing glucose levels, endogenous GIP significantly increased postprandial

Table 2—Overview of postprandial plasma and serum measurements

	Placebo + saline	Placebo + GIP(3-30)NH_2	*P*	Sitagliptin + saline	Sitagliptin + GIP(3-30)NH_2	*P*	*P* saline vs. saline
Glucose							
Baseline (mmol/L)	8.5 ± 2.2	8.6 ± 2.4	0.546	7.4 ± 1.9	7.2 ± 1.4	0.566	0.001
Peak (mmol/L)	14.8 ± 3.4	15.5 ± 3.3	0.003	13.1 ± 2.1	12.8 ± 2.1	0.317	0.033
Time to peak (min)	118 ± 24	121 ± 27	0.731	138 ± 32	128 ± 40	0.220	0.008
AUC (mmol/L × min)	3,559 ± 975	3,794 ± 975	0.017	3,130 ± 734	3,068 ± 673	0.277	0.008
bsAUC (mmol/L × min)	847 ± 337	971 ± 321	0.095	746 ± 310	739 ± 265	0.914	0.268
Acetaminophen							
Peak (μmol/L)	78.9 ± 17.9	80.7 ± 16.9	0.466	75.4 ± 16.9	79.4 ± 21.0	0.381	0.241
Time to peak (min)	126 ± 19.7	128 ± 34	0.862	145 ± 31	140 ± 37	0.551	0.068
Insulin							
Baseline (pmol/L)	66.0 ± 25.8	72.7 ± 36.9	0.164	85.3 ± 73.3	67.3 ± 36.1	0.295	0.308
Peak (pmol/L)	362 ± 240	274 ± 199	0.009	409 ± 291	367 ± 281	0.099	0.123
Time to peak (min)	105 ± 20	110 ± 43	0.698	134 ± 56	139 ± 48	0.761	0.091
bsAUC (nmol/L × min)	39.5 ± 28.8	27.8 ± 19.8	0.014	47.6 ± 39.4	40.6 ± 32.8	0.028	0.113
C-peptide							
Baseline (pmol/L)	682 ± 28	711 ± 90	0.304	854 ± 94	714 ± 65	0.282	0.215
Peak (pmol/L)	2,251 ± 857	1,936 ± 717	0.016	2,664 ± 1,121	2,338 ± 971	0.020	0.016
Time to peak (min)	140 ± 30	175 ± 31	0.027	186 ± 50	175 ± 40	0.441	0.024
bsAUC (nmol/L × min)	275 ± 140	209 ± 95	0.005	305 ± 181	272 ± 143	0.104	0.253
C-peptide/glucose							
Baseline (pmol/mmol)	81.5 ± 23.4	82.4 ± 24.4	0.823	111.7 ± 46.1	99.2 ± 30.8	0.210	0.030
Peak (pmol/mmol)	207 ± 82	167 ± 63	0.012	287 ± 127	265 ± 121	0.103	0.002
Time to peak (min)	190 ± 43	230 ± 43	0.054	220 ± 64	235 ± 40	0.429	0.191
AUC (nmol/mmol × min)	47.5 ± 17.2	39.7 ± 14.8	0.009	64.8 ± 25.3	57.1 ± 23.9	0.005	0.001
$AUC_{0–30\ min}$ (pmol/mmol × min)	2,785 ± 478	2,546 ± 363	0.143	3,746 ± 642	3,066 ± 460	0.013	0.016
ISR							
Baseline (pmol/kg × min^{-1})	2.0 ± 0.6	2.0 ± 0.8	0.535	2.4 ± 1.4	2.0 ± 0.6	0.222	0.200
Peak (pmol/kg × min^{-1})	7.7 ± 3.4	6.5 ± 3.0	0.027	8.8 ± 3.7	7.9 ± 3.8	0.014	0.016
Time to peak (min)	133 ± 30	163 ± 41	0.074	168 ± 62	165 ± 37	0.889	0.084
bsAUC (pmol/kg)	931 ± 522	742 ± 341	0.020	1,044 ± 647	969 ± 526	0.336	0.210
ISR/glucose							
Baseline (mmol/L)	0.2 ± 0.1	0.2 ± 0.1	0.814	0.3 ± 0.1	0.3 ± 0.1	0.228	0.021
Peak (mmol/L)	0.8 ± 0.3	0.6 ± 0.3	0.012	1.1 ± 0.5	1.0 ± 0.5	0.168	0.004
Time to peak (min)	163 ± 52	205 ± 31	0.016	185 ± 65	205 ± 31	0.339	0.384
AUC (mmol/L × min)	118 ± 46	95 ± 35	0.004	153 ± 68	141 ± 66	0.080	0.003
Proinsulin							
Baseline (pmol/L)	17.9 ± 11.2	21.0 ± 14.6	0.097	21.7 ± 26.5	16.3 ± 11.1	0.327	0.462
Peak (pmol/L)	65.6 ± 29.8	54.1 ± 26.1	0.006	72.9 ± 40.2	56.1 ± 32.5	0.002	0.233
Time to peak (min)	145 ± 31	150 ± 31	0.674	165 ± 37	185 ± 31	0.039	0.104
bsAUC (pmol/L × min)	7,952 ± 4,090	5,913 ± 2,839	0.004	8,248 ± 5,924	6,793 ± 4,740	0.010	0.749
Proinsulin/C-peptide							
Baseline (pmol/pmol)	0.025 ± 0.010	0.028 ± 0.010	0.112	0.022 ± 0.009	0.022 ± 0.009	0.777	0.016
AUC (pmol/pmol × min)	9.0 ± 2.6	9.3 ± 2.4	0.481	8.1 ± 2.6	7.6 ± 2.4	0.050	0.026
Glucagon							
Baseline (pmol/L)	7.4 ± 4.0	6.7 ± 4.0	0.591	7.4 ± 4.7	7.5 ± 3.7	0.945	0.994
Peak (pmol/L)	14.9 ± 5.6	14.0 ± 4.6	0.614	14.3 ± 4.9	14.5 ± 4.5	0.843	0.869
Time to peak (min)	31.9 ± 12.0	36.3 ± 11.9	0.393	34.1 ± 16.6	32.5 ± 21.1	0.211	0.593
AUC (pmol/L × min)	2,497 ± 872	2,110 ± 766	0.069	2,430 ± 1,085	2,091 ± 979	0.084	0.711
Glucagon/glucose							
Baseline (pmol/mmol)	0.9 ± 0.5	0.8 ± 0.6	0.657	1.1 ± 0.7	1.1 ± 0.5	0.953	0.422
Peak (pmol/mmol)	1.51 ± 0.57	1.3 ± 0.4	0.400	1.7 ± 0.7	1.7 ± 0.3	0.914	0.470
Time to peak (min)	28.75 ± 10.03	32.7 ± 11.3	0.432	28.5 ± 13.1	28.8 ± 11.9	0.780	1.000
AUC (pmol/mmol × min)	236.5 ± 25.5	195.6 ± 32.6	<0.05	266.6 ± 41.9	230.6 ± 34.5	0.143	0.178

Data are mean ± SD ($N = 12$). Differences between groups were evaluated using Student paired *t* tests.

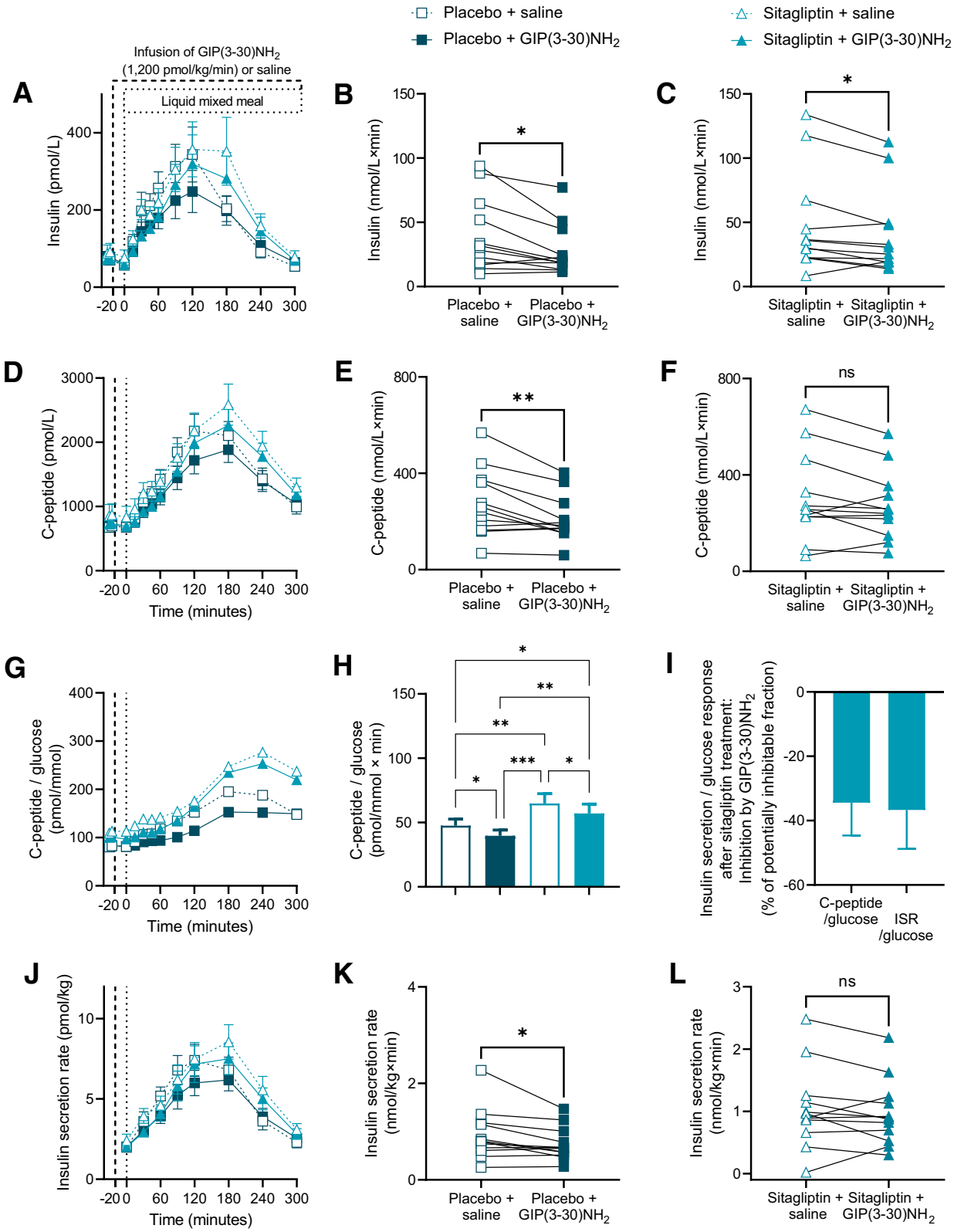

Figure 2—Serum insulin (*A*), bsAUC for serum insulin (*B* and *C*), serum C-peptide (*D*), bsAUC for serum C-peptide (*E* and *F*), serum C-peptide/plasma glucose ratio (*G*), AUC for serum C-peptide/plasma glucose ratio (*H*), percent reduction in postprandial insulinogenic indices (serum C-peptide/plasma glucose and ISR/plasma glucose (*I*), ISR (*J*), and bsAUC for ISR (*K* and *L*) during mixed-meal tests performed during sitagliptin and placebo treatment [with GIP(3-30)NH_2 infusion or saline infusion] in 12 metformin-treated patients with type 2 diabetes. The baseline is the mean of values at time −30, −25, and 0 min, when available. Data are mean ± SEM ($n = 12$). Statistical analyses are Student *t* tests for pairwise comparisons and repeated-measures one-way ANOVA for comparisons of more than two groups. *$P < 0.05$, **$P < 0.005$, ***$P < 0.0005$.

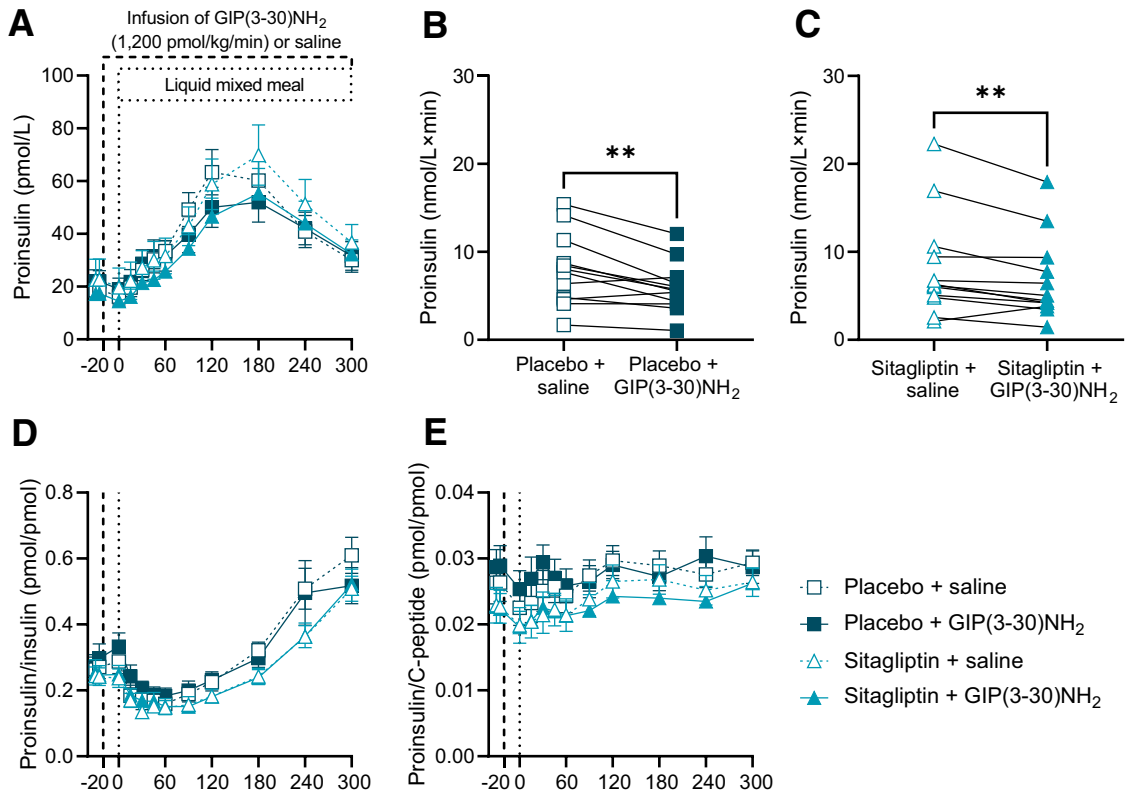

Figure 3—Proinsulin (*A*), bsAUC for proinsulin (*B* and *C*), proinsulin/insulin ratio (*D*), and proinsulin/C-peptide ratio (*E*) during mixed-meal tests performed during sitagliptin and placebo treatment [with GIP(3-30)NH_2 infusion or saline infusion] in 12 metformin-treated patients with type 2 diabetes. Data are mean ± SEM (n = 12). Statistical analyses are Student t tests for pairwise comparisons. **$P < 0.005$.

glucagon levels during placebo treatment but not during sitagliptin treatment (Fig. 4*D–F* and Table 2).

Appetite Ratings

Visual analog scale evaluations of hunger, prospective food consumption, satiety, fullness, thirst, nausea, well-being, and tiredness were similar between the interventions (data not shown).

GIP and GLP-1

Sitagliptin treatment increased plasma levels of intact, active GIP and GLP-1 substantially during the mixed-meal test (Fig. 1*F–I* and Table 3) and the postprandial response of total GIP. Infusion of GIP(3-30)NH_2 did not change total GIP and GLP-1 during the mixed meal, but during sitagliptin treatment, the GIP(3-30)NH_2 infusion increased levels of intact GIP compared with the saline infusion.

Heart Rate and Blood Pressure

During both placebo and sitagliptin treatment, infusion of GIP(3-30)NH_2 increased systolic and diastolic blood pressure, increased MAP, and decreased heart rate (Fig. 5). Differences were most pronounced during sitagliptin treatment, with significant increases of 8 ± 2, 4 ± 1, and 5 ± 1 mmHg in systolic pressure, diastolic blood pressure, and MAP, respectively, at time 150 min (P = 0.008, P = 0.015, and P = 0.001) (Fig. 5*B* and *F* and Supplementary Table 2). Moreover, MAP was increased by 5 ± 2 mmHg at time 245 min (P = 0.031). Infusion of GIP(3-30)NH_2 lowered heart rate by 5 ± 1 beats/min at time 15 min during sitagliptin (P = 0.009) and at time 75 min during both placebo and sitagliptin treatment (5 ± 1 [P = 0.007] and 5 ± 1 [P = 0.005], respectively) (Fig. 5*A* and *E* and Supplementary Table 2).

DISCUSSION

In this study, we used the selective GIPR antagonist GIP(3-30)NH_2 during a 5-h liquid mixed-meal test in 12 patients with type 2 diabetes treated with the DPP-4 inhibitor sitagliptin and placebo in a crossover design. We observed *1*) an insulinotropic and glucose-lowering effect of endogenous GIP in patients with type 2 diabetes during placebo treatment and *2*) that endogenous GIP contributes with 37 ± 12% of the sitagliptin-induced improvement in β-cell function.

Endogenous GIP Is Insulinotropic and Lowers Plasma Glucose in Patients With Type 2 Diabetes During Placebo Treatment

The current study shows that during placebo treatment, endogenous GIP is insulinotropic and significantly increases

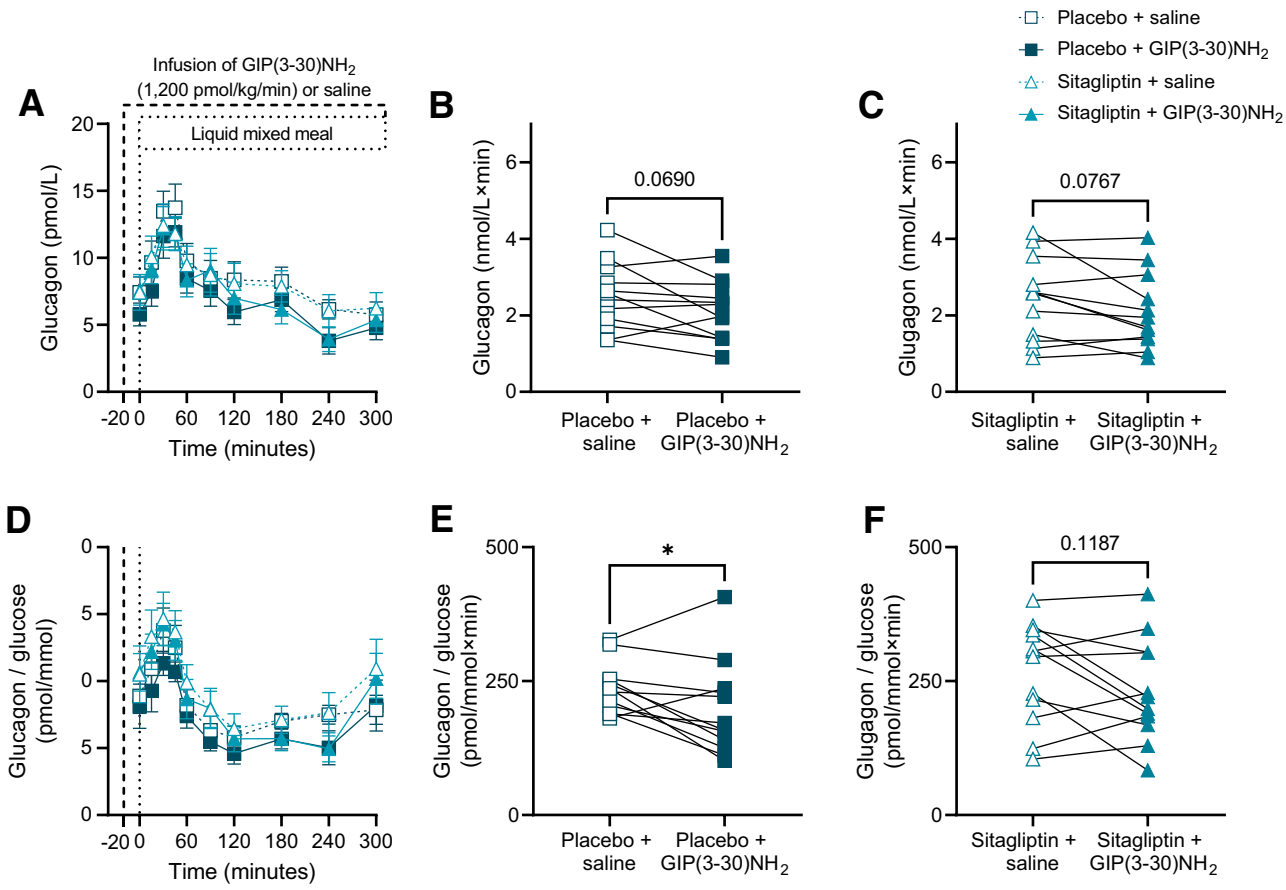

Figure 4—Plasma glucagon (*A*), AUC for plasma glucagon (*B* and *C*) and glucagon/glucose ratio (*D*), and AUC for glucagon/glucose ratio (*E* and *F*) during mixed-meal tests performed during sitagliptin and placebo treatment [with GIP(3-30)NH_2 infusion or saline infusion] in 12 metformin-treated patients with type 2 diabetes. Data are mean ± SEM (n = 12). Statistical analyses are Student t tests for pairwise comparisons. *$P < 0.05$. Nonsignificant P values are displayed on the plots.

both C-peptide and insulin secretion and consequently lowers plasma glucose in patients with type 2 diabetes. This effect was not associated with diabetes duration (before or after 10 years) or HbA_{1c} levels (less than or greater than 7.5% [58 mmol/mol]) (data not shown). We previously demonstrated an insulinotropic effect of endogenous GIP in 10 patients with type 2 diabetes, but the GIP(3-30)NH_2-induced increase of plasma glucose in that study did not reach statistical significance (10). In the current study of 12 patients with type 2 diabetes, we observed a significant glucose-lowering effect of endogenous GIP. Furthermore, endogenous GIP contributed to β-cell function assessed by serum C-peptide/plasma glucose ratio in these patients. During sitagliptin treatment, GIPR antagonism did not change plasma glucose, which could be due to increased GLP-1 effects blunting the effects of GIP, but further investigation is warranted to clarify this matter.

Quantification of the Contribution by Endogenous GIP to the Effects of Sitagliptin

In the current study, we used similar calculations to quantify the contribution of GIP to the glucose-lowering effects of DPP-4 inhibition as were used in two previous studies that used the GLP-1R antagonist exendin(9-39)NH_2 to quantify the contribution of GLP-1 to the glucose-lowering effects of DPP-4 inhibition. Aulinger et al. (17) investigated the contribution of GLP-1 to the insulinotropic effect of sitagliptin (initiated 1 day before the experimental day) during an oral glucose tolerance test in participants with type 2 diabetes (mean HbA_{1c} 6.2 ± 0.2%). Nauck et al. (16) investigated the contribution of GLP-1 to the insulinotropic effect of 9–10 days of vildagliptin treatment during a mixed-meal test in participants with type 2 diabetes (mean HbA_{1c} 7.2 ± 0.5%) and healthy control subjects. In the current study, we used GIP(3-30)NH_2 to investigate the contribution of GIP to the insulinotropic effect of 13 days of sitagliptin treatment during a mixed-meal test in participants with type 2 diabetes (mean HbA_{1c} 7.1 ± 1.4%). Unlike Aulinger et al. and Nauck et al., our study was randomized and double blinded. Nevertheless, despite some minor differences in study designs, the results and calculations should be comparable, and DPP-4 inhibition influenced incretin hormone responses similarly in all three. It is well known that the feedback inhibition of GIP secretion is less prominent compared with that of GLP-1 secretion during sitagliptin treatment; still, in the current

Table 3—Overview of incretin hormone concentrations

	Placebo + saline	Placebo + GIP(3-30)NH_2	*P*	Sitagliptin + saline	Sitagliptin + GIP(3-30)NH_2	*P*	*P* saline vs. saline
Total GIP							
Baseline (pmol/L)	8.5 ± 3.7	11.9 ± 5.6	0.014	12.8 ± 17.6	10.4 ± 4.2	0.601	0.349
Peak (pmol/L)	92.4 ± 24.3	93.1 ± 40.3	0.935	82.3 ± 25.5	80.3 ± 31.3	0.741	0.268
Time to peak (min)	71.3 ± 43.4	63.8 ± 46.2	0.546	53.8 ± 32.9	78.8 ± 43.9	0.117	0.253
bsAUC (nmol/L × min)	12.9 ± 4.7	11.9 ± 4.9	0.231	10.2 ± 3.9	10.7 ± 4.9	0.510	0.002
Intact GIP							
Baseline (pmol/L)	2.7 ± 2.1	7.3 ± 7.4	0.017	11.1 ± 16.1	9.3 ± 7.5	0.648	0.079
Peak (pmol/L)	47.6 ± 25.9	55.8 ± 26.6	0.089	88.1 ± 30.5	91.2 ± 31.2	0.538	0.001
Time to peak (min)	43.8 ± 22.6	52.5 ± 45.0	0.430	57.5 ± 34.3	75.0 ± 51.6	0.359	0.363
bsAUC (nmol/L × min)	4.9 ± 2.5	5.9 ± 3.0	0.201	11.9 ± 5.7	13.8 ± 5.2	0.013	<0.001
Total GLP-1							
Baseline (pmol/L)	11.9 ± 5.8	15.5 ± 6.1	0.061	15.7 ± 5.6	16.2 ± 6.0	0.777	0.008
Peak (pmol/L)	28.9 ± 9.5	29.2 ± 9.2	0.940	28.5 ± 6.9	26.1 ± 7.3	0.221	0.899
Time to peak (min)	62.5 ± 66.4	59.3 ± 68.4	0.916	50.0 ± 45.4	51.3 ± 66.9	0.960	0.602
bsAUC (nmol/L × min)	2.1 ± 1.1	1.4 ± 1.1	0.120	1.0 ± 0.6	1.0 ± 0.8	0.996	0.011
Intact GLP-1							
Baseline (pmol/L)	0.9 ± 0.0	1.6 ± 1.0	0.044	2.1 ± 2.8	1.6 ± 1.0	0.543	0.150
Peak (pmol/L)	1.7 ± 1.5	3.5 ± 4.7	0.199	11.4 ± 10.9	8.0 ± 6.4	0.175	0.010
Time to peak (min)	60.0 ± 52.0	30.0 ± 15.0	NA	45.0 ± 43.8	60.0 ± 91.2	0.730	0.742
bsAUC (nmol/L × min)	0.02 ± 0.05	0.05 ± 0.01	0.309	0.04 ± 0.05	0.04 ± 0.04	0.410	0.019

Data are mean ± SD. Differences between groups were evaluated using Student paired *t* test. NA, not applicable.

study, sitagliptin increased levels of active GIP and lowered total GIP, likely reflecting a negative feedback mechanism acting on the GIP-secreting enteroendocrine K cells, as previously suggested (25). Like Aulinger et al. and Nauck et al., we focused on changes in the β-cell secretory response during DPP-4 inhibition. As sitagliptin lowers plasma glucose, the β-cell secretory response needs to be interpreted relative to prevailing glycemia (i.e., assessed by insulinogenic indices [serum C-peptide/plasma glucose and ISR/plasma glucose ratios]). In the current study, we confirm that DPP-4 inhibition increases insulinogenic indices in patients with type 2 diabetes. Using a similar way of calculating the contribution of an antagonized incretin receptor as Nauck et al., and as a key finding, we demonstrate for the first time in human physiology that endogenous GIP is responsible for 37 ± 12% of sitagliptin-induced improvement in β-cell function (Fig. 2*I*). Both Aulinger et al. and Nauck et al. found that approximately one-half of DPP-4 inhibitor–mediated improvements in β-cell function could be ascribed to endogenous GLP-1. Thus, we cannot rule out the existence of other mediators contributing to the effect of DPP-4 inhibitors, and a future study with simultaneous antagonism of both incretin receptors during DPP-4 inhibition could perhaps contribute to clarifying this matter further.

Sitagliptin Effects Beyond Postprandial Modulation

Sitagliptin has effects beyond improved postprandial insulin secretion, likely because of improved β-cell function postprandially and in the fasting state (26) and increased insulin-stimulated peripheral glucose utilization (27). In the current study, sitagliptin treatment resulted in lower fasting plasma glucose, as previously shown (28,29). Since DPP-4 inhibitors increase circulating levels of active incretin hormones, we expected reduced postprandial glucose excursion irrespective of the fasting plasma glucose levels due to increased postprandial insulin secretion. Thus, it is puzzling that the postprandial glucose excursions assessed by bsAUC were unchanged compared with placebo treatment. Some studies, however, have found that DPP-4 inhibitor treatment does not affect postprandial glucose tolerance as measured by bsAUC (16,30), while others have found that bsAUC of plasma glucose is slightly lower (17,31–33). The current study does not allow for a direct evaluation of an increased sitagliptin-mediated effect of GIP on fasting glucose and fasting insulin levels.

β-Cell Stress

We found a secretory increase in insulin following just 13 days of sitagliptin treatment, confirming that DPP-4 inhibitor treatment increases insulin secretion early on (32). Glucotoxic stress during chronic hyperglycemia is proposed to impair β-cell responsiveness to GIP by downregulation of GIPR gene expression (34,35), and an extended sitagliptin treatment period with improved glycemic control to relieve β-cell stress might have improved the insulinotropic effect of GIP further (5). The proinsulin/insulin ratio is a highly sensitive marker of β-cell stress (36), and circulating proinsulin levels are increased in type 2 diabetes (37,38). The dual GIPR/GLP-1R agonist tirzepatide reduced the proinsulin/insulin ratio by 26–37% after 26 weeks of treatment (39). In the current study, we observed a 20% decrease in the proinsulin/insulin ratio after sitagliptin treatment with

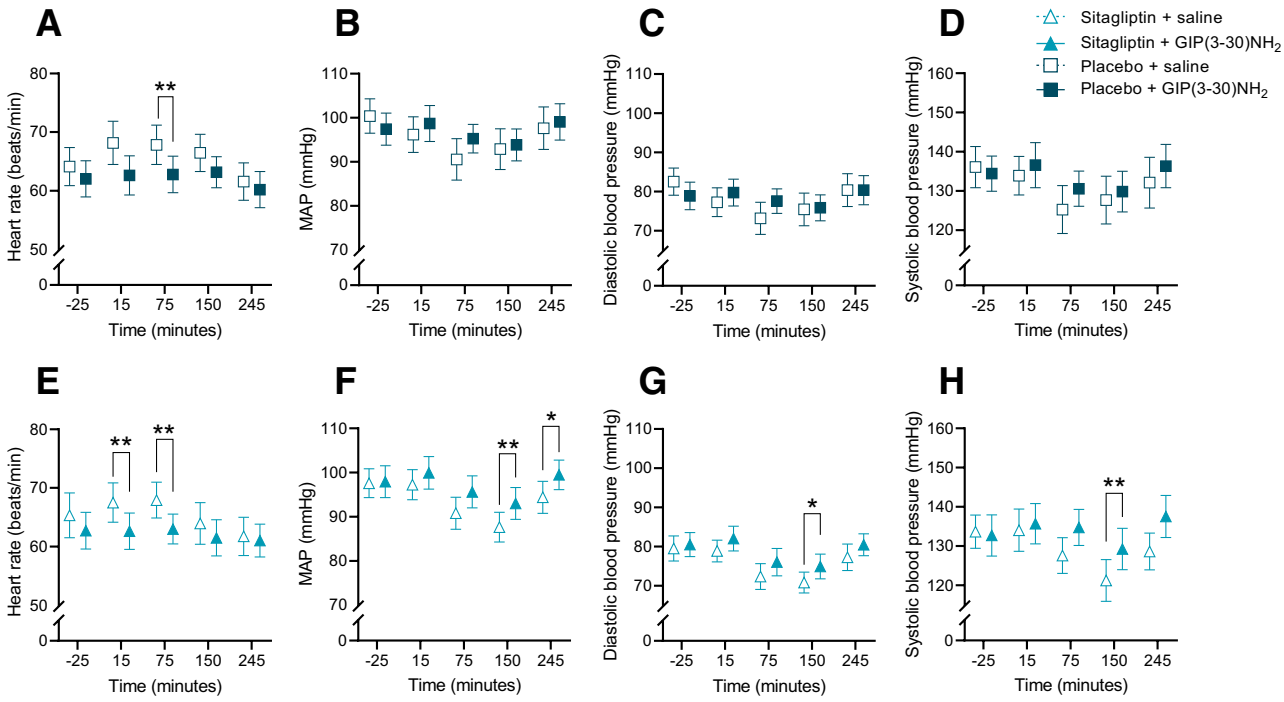

Figure 5—Heart rate and blood pressure during GIP(3-30)NH_2 infusion (*A–D*) compared with saline during placebo and sitagliptin treatment (*E–H*). Comparisons are made by two-way ANOVA with Tukey correction for multiple comparisons. *$P < 0.05$, **$P < 0.005$.

increased endogenous GIP and GLP-1 (intact, active forms), as previously described (40).

GIPR Antagonism Reduced Plasma Glucagon Levels

GIPR antagonism inhibited the glucagonotropic actions of endogenous GIP in patients with type 2 diabetes. Postprandial glucagon concentrations were reduced, which was most pronounced when plasma glucose was declining (3–5 h postmeal), confirming a glucagonotropic action of GIP during normal to low plasma glucose levels (41). The effect was most pronounced during placebo treatment when the prevailing glucose levels were considered in a glucagon/glucose ratio. On the contrary, the effect was blunted during sitagliptin treatment, which could be explained by increased levels of active GLP-1 inhibiting glucagon secretion (4).

Gastric Emptying

Nauck et al. (16) and Aulinger et al. (17) reported that gastric emptying was not affected (oral glucose tolerance test) or slightly accelerated (mixed-meal test) by exendin(9-39)NH_2, whereas in our study, sitagliptin lowered the time to peak by ~20 min with no effect of GIPR antagonism. This likely reflects elevated circulating levels of intact GLP-1 decelerating gastric emptying (42), whereas GIP exerts no influence on gastric emptying (43).

Hemodynamic Effects

It has been hypothesized that postprandial elevated GIP levels increase mesenteric blood flow to facilitate nutrient digestion and utilization, resulting in decreased blood pressure and increased heart rate (44). Consistent with this hypothesis, and as previously reported (24,45,46), GIPR antagonism increased blood pressure and slowed the heart rate during the mixed-meal test in the current study.

Limitations

It is uncertain whether GIP(3-30)NH_2 blocks GIPR completely (7,24). If not, this would lead to an underestimation of the effects of endogenous GIP, which is a limitation to the interpretation of the results.

Conclusions

Using GIPR antagonism, we demonstrate that endogenous GIP contributes to postprandial glucose homeostasis in patients with type 2 diabetes by increasing glucose-stimulated insulin secretion and glucagon secretion and is responsible for part of the improved β-cell function observed during DPP-4 inhibition by sitagliptin.

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Duality of Interest. L.S.G. has been a speaker for Eli Lilly and is cofounder of Antag Therapeutics ApS. B.H. is cofounder of Bainan Biotech ApS.

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Author Contributions. S.S. performed the clinical study. S.S., L.S.G., M.M.R., T.V., J.J.H., M.B.C., and F.K.K. designed the clinical study. S.S., L.S.G., and F.K.K. performed and are responsible for the data analysis and wrote the manuscript. J.J.H. and B.H. performed the radioimmunoassay and ELISA analyses. All authors contributed to the interpretation of data, reviewed and edited the manuscript, and approved the final version. S.S., L.S.G., and F.K.K. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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References

1. Mentlein R, Gallwitz B, Schmidt WE. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36)amide, peptide histidine methionine and is responsible for their degradation in human serum. Eur J Biochem 1993;214:829–835
2. Deacon CF. Dipeptidyl peptidase 4 inhibitors in the treatment of type 2 diabetes mellitus. Nat Rev Endocrinol 2020;16:642–653
3. Krarup T, Saurbrey N, Moody AJ, Kühl C, Madsbad S. Effect of porcine gastric inhibitory polypeptide on β-cell function in type I and type II diabetes mellitus. Metabolism 1987;36:677–682
4. Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W. Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. J Clin Invest 1993;91:301–307
5. Aaboe K, Akram S, Deacon CF, Holst JJ, Madsbad S, Krarup T. Restoration of the insulinotropic effect of glucose-dependent insulinotropic polypeptide contributes to the antidiabetic effect of dipeptidyl peptidase-4 inhibitors. Diabetes Obes Metab 2015;17:74–81
6. Højberg PV, Vilsbøll T, Rabøl R, et al. Four weeks of near-normalisation of blood glucose improves the insulin response to glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide in patients with type 2 diabetes. Diabetologia 2009;52:199–207
7. Hansen LS, Sparre-Ulrich AH, Christensen M, et al. N-terminally and C-terminally truncated forms of glucose-dependent insulinotropic polypeptide are high-affinity competitive antagonists of the human GIP receptor. Br J Pharmacol 2016;173:826–838
8. Gasbjerg LS, Helsted MM, Hartmann B, et al. GIP and GLP-1 receptor antagonism during a meal in healthy individuals. J Clin Endocrinol Metab 2020;105:1–14
9. Gasbjerg LS, Helsted MM, Hartmann B, et al. Separate and combined glucometabolic effects of endogenous glucose-dependent insulinotropic polypeptide and glucagon-like peptide 1 in healthy individuals. Diabetes 2019;68:906–917
10. Stensen S, Gasbjerg LS, Krogh LL, et al. Effects of endogenous GIP in patients with type 2 diabetes. Eur J Endocrinol 2021;185:33–45
11. Waget A, Cabou C, Masseboeuf M, et al. Physiological and pharmacological mechanisms through which the DPP-4 inhibitor sitagliptin regulates glycemia in mice. Endocrinology 2011;152:3018–3029
12. Marguet D, Baggio L, Kobayashi T, et al. Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. Proc Natl Acad Sci U S A 2000;97:6874–6879
13. Flock G, Baggio LL, Longuet C, Drucker DJ. Incretin receptors for glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide are essential for the sustained metabolic actions of vildagliptin in mice. Diabetes 2007;56:3006–3013
14. Hutch CR, Roelofs K, Haller A, et al. The role of GIP and pancreatic GLP-1 in the glucoregulatory effect of DPP-4 inhibition in mice. Diabetologia 2019; 62:1928–1937
15. Hansotia T, Baggio LL, Delmeire D, et al. Double incretin receptor knockout (DIRKO) mice reveal an essential role for the enteroinsular axis in transducing the glucoregulatory actions of DPP-IV inhibitors. Diabetes 2004;53:1326–1335
16. Nauck MA, Kind J, Köthe LD, et al. Quantification of the contribution of GLP-1 to mediating insulinotropic effects of DPP-4 inhibition with vildagliptin in healthy subjects and patients with type 2 diabetes using exendin [9-39] as a GLP-1 receptor antagonist. Diabetes 2016;65:2440–2447
17. Aulinger BA, Bedorf A, Kutscherauer G, et al. Defining the role of GLP-1 in the enteroinsulinar axis in type 2 diabetes using DPP-4 inhibition and GLP-1 receptor blockade. Diabetes 2014;63:1079–1092
18. Medhus AW, Sandstad O, Bredesen J, Husebye E. Delay of gastric emptying by duodenal intubation: sensitive measurement of gastric emptying by the paracetamol absorption test. Aliment Pharmacol Ther 1999;13:609–620
19. Deacon CF, Nauck MA, Meier J, Hücking K, Holst JJ. Degradation of endogenous and exogenous gastric inhibitory polypeptide in healthy and in type 2 diabetic subjects as revealed using a new assay for the intact peptide. J Clin Endocrinol Metab 2000;85:3575–3581
20. Wewer Albrechtsen NJ, Bak MJ, Hartmann B, et al. Stability of glucagon-like peptide 1 and glucagon in human plasma. Endocr Connect 2015;4:50–57
21. Orskov C, Rabenhøj L, Wettergren A, Kofod H, Holst JJ. Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. Diabetes 1994;43:535–539
22. Gasbjerg LS, Christensen MB, Hartmann B, et al. GIP(3-30)NH_2 is an efficacious GIP receptor antagonist in humans: a randomised, double-blinded, placebo-controlled, crossover study. Diabetologia 2018;61:413–423
23. Kjems LL, Christiansen E, Vølund A, Bergman RN, Madsbad S. Validation of methods for measurement of insulin secretion in humans in vivo. Diabetes 2000;49:580–588
24. Gasbjerg LS, Bari EJ, Stensen S, et al. Dose-dependent efficacy of the glucose-dependent insulinotropic polypeptide (GIP) receptor antagonist GIP(3-30)NH_2 on GIP actions in humans. Diabetes Obes Metab 2021;23:68–74
25. Vardarli I, Arndt E, Deacon CF, Holst JJ, Nauck MA. Effects of sitagliptin and metformin treatment on incretin hormone and insulin secretory responses to oral and "isoglycemic" intravenous glucose. Diabetes 2014;63:663–674
26. D'Alessio DA, Denney AM, Hermiller LM, et al. Treatment with the dipeptidyl peptidase-4 inhibitor vildagliptin improves fasting islet-cell function in subjects with type 2 diabetes. J Clin Endocrinol Metab 2009;94:81–88
27. Azuma K, Rádiková Z, Mancino J, et al. Measurements of islet function and glucose metabolism with the dipeptidyl peptidase 4 inhibitor vildagliptin in patients with type 2 diabetes. J Clin Endocrinol Metab 2008;93:459–464
28. Ahrén B, Landin-Olsson M, Jansson PA, Svensson M, Holmes D, Schweizer A. Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes. J Clin Endocrinol Metab 2004;89:2078–2084

29. Aaboe K, Knop FK, Vilsbøll T, et al. Twelve weeks treatment with the DPP-4 inhibitor, sitagliptin, prevents degradation of peptide YY and improves glucose and non-glucose induced insulin secretion in patients with type 2 diabetes mellitus. Diabetes Obes Metab 2010;12:323–333
30. Scherbaum WA, Schweizer A, Mari A, et al. Evidence that vildagliptin attenuates deterioration of glycaemic control during 2-year treatment of patients with type 2 diabetes and mild hyperglycaemia. Diabetes Obes Metab 2008;10:1114–1124
31. Rosenstock J, Foley JE, Rendell M, et al. Effects of the dipeptidyl peptidase-IV inhibitor vildagliptin on incretin hormones, islet function, and postprandial glycemia in subjects with impaired glucose tolerance. Diabetes Care 2008;31:30–35
32. Herman GA, Bergman A, Stevens C, et al. Effect of single oral doses of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on incretin and plasma glucose levels after an oral glucose tolerance test in patients with type 2 diabetes. J Clin Endocrinol Metab 2006;91:4612–4619
33. Herman GA, Bergman A, Liu F, et al. Pharmacokinetics and pharmacodynamic effects of the oral DPP-4 inhibitor sitagliptin in middle-aged obese subjects. J Clin Pharmacol 2006;46:876–886
34. Xu G, Kaneto H, Laybutt DR, et al. Downregulation of GLP-1 and GIP receptor expression by hyperglycemia: possible contribution to impaired incretin effects in diabetes. Diabetes 2007;56:1551–1558
35. Lynn FC, Pamir N, Ng EHC, McIntosh CHS, Kieffer TJ, Pederson RA. Defective glucose-dependent insulinotropic polypeptide receptor expression in diabetic fatty Zucker rats. Diabetes 2001;50:1004–1011
36. Pfützner A, Kunt T, Hohberg C, et al. Fasting intact proinsulin is a highly specific predictor of insulin resistance in type 2 diabetes. Diabetes Care 2004;27:682–687
37. Sun J, Cui J, He Q, Chen Z, Arvan P, Liu M. Proinsulin misfolding and endoplasmic reticulum stress during the development and progression of diabetes. Mol Aspects Med 2015;42:105–118
38. Russo GT, Giorda CB, Cercone S, De Cosmo S, Nicolucci A; BetaDecline Study Group. Beta cell stress in a 4-year follow-up of patients with type 2 diabetes: A longitudinal analysis of the BetaDecline Study. Diabetes Metab Res Rev 2018;34:e3016
39. Thomas MK, Nikooienejad A, Bray R, et al. Dual GIP and GLP-1 receptor agonist tirzepatide improves beta-cell function and insulin sensitivity in type 2 diabetes. J Clin Endocrinol Metab 2021;106:388–396
40. Asai S, Ohta A, Kato H, et al. Effect of sitagliptin on glycemic control and beta cell function in Japanese patients given basal-supported oral therapy for type 2 diabetes. Endocr J 2014;61:1213–1220
41. Christensen MB, Calanna S, Holst JJ, Vilsbøll T, Knop FK. Glucose-dependent insulinotropic polypeptide: blood glucose stabilizing effects in patients with type 2 diabetes. J Clin Endocrinol Metab 2014;99:E418–E426
42. Deane AM, Nguyen NQ, Stevens JE, et al. Endogenous glucagon-like peptide-1 slows gastric emptying in healthy subjects, attenuating postprandial glycemia. J Clin Endocrinol Metab 2010;95:215–221
43. Meier JJ, Goetze O, Anstipp J, et al. Gastric inhibitory polypeptide does not inhibit gastric emptying in humans. Am J Physiol Endocrinol Metab. 2004;286:E621–E625
44. Asmar M, Asmar A, Simonsen L, et al. The gluco- and liporegulatory and the vasodilatory effects of glucose-dependent insulinotropic polypeptide (GIP) are abolished by an antagonist of the human GIP receptor. Diabetes 2017;66:2363–2371
45. Wang S, Oestricker LZ, Wallendorf MJ, et al. Cholinergic signaling mediates the effects of xenin-25 on secretion of pancreatic polypeptide but not insulin or glucagon in humans with impaired glucose tolerance. PLoS One 2018;13:e0192441
46. Karstoft K, Mortensen SP, Knudsen SH, Solomon TPJ. Direct effect of incretin hormones on glucose and glycerol metabolism and hemodynamics. Am J Physiol Endocrinol Metab 2015;308:E426–E433