Glucose-dependent Insulinotropic Polypeptide: Blood Glucose Stabilizing Effects in Patients With Type 2 Diabetes

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Context: Patients with type 2 diabetes mellitus (T2DM) have clinically relevant disturbances in the effects of the hormone glucose-dependent insulinotropic polypeptide (GIP).

Objective: We aimed to evaluate the importance of the prevailing plasma glucose levels for the effect of GIP on responses of glucagon and insulin and glucose disposal in patients with T2DM.

Design and Setting: We performed a single center, placebo-controlled, cross-over, experimental study.

Patients: We studied twelve patients with T2DM (age: 62 ± 1 years [mean ± SEM], body mass index: 29 ± 1 kg/m^2; glycosylated hemoglobin A1c: 6.5 ± 0.1% [48 ± 2 mmol/mol]).

Intervention: We infused physiological amounts of GIP (2 pmol × kg^{-1} × min^{-1}) or saline.

Main Outcome Measures: We measured plasma concentrations of glucagon, glucose, insulin, C-peptide, intact GIP, and amounts of glucose needed to maintain glucose clamps.

Results: During fasting glycemia (plasma glucose ~8 mmol/L), GIP elicited significant increments in both insulin and glucagon levels, resulting in neutral effects on plasma glucose. During insulin-induced hypoglycemia (plasma glucose ~3 mmol/L), GIP elicited a minor early-phase insulin response and increased glucagon levels during the initial 30 minutes, resulting in less glucose needed to be infused to maintain the clamp (29 ± 8 vs 49 ± 12 mg × kg^{-1}, $P < .03$). During hyperglycemia (1.5 × fasting plasma glucose ~12 mmol/L), GIP augmented insulin secretion throughout the clamp, with slightly less glucagon suppression compared with saline, resulting in more glucose needed to maintain the clamp during GIP infusions (265 ± 21 vs 213 ± 13 mg × kg^{-1}, $P < .001$).

Conclusions: In patients with T2DM, GIP counteracts insulin-induced hypoglycemia, most likely through a predominant glucagonotropic effect. In contrast, during hyperglycemia, GIP increases glucose disposal through a predominant effect on insulin release. **(*J Clin Endocrinol Metab* 99: E418–E426, 2014)**

In healthy subjects, the effects of the gut-derived hormone glucose-dependent insulinotropic polypeptide (GIP) are reliant on the prevailing plasma glucose levels. At elevated glucose levels GIP augments insulin release (early- and late-phase responses) and has little or no effect on glucagon release (1–3). In contrast, at fasting plasma glucose values or during insulin-induced hypoglycemia, GIP has glucagon-releasing properties with no effect on phase

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Abbreviations: AUC, area under the curve; DPP-4, dipeptidyl peptidase 4; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; HbA_{1c}, glycated hemoglobin A1c; iAUC, incremental area under the curve; ISR, insulin secretion rate; T2DM, type 2 diabetes mellitus.

insulin secretion (3, 4). Thus, within physiological blood glucose levels GIP could act to appropriately lower or elevate plasma glucose levels (ie, act as a plasma glucose stabilizer). However, in type 2 diabetes mellitus (T2DM) there is considerable evidence that at least the insulinotropic effect of GIP is compromised (1, 5–11). In particular the GIP-dependent amplification of late-phase insulin secretion in response to hyperglycemia seems impaired (10, 11). This defect is considered an important determinant for the diminished incretin effect (ie, a reduction in the normal clear-cut difference between insulin release following oral as compared with isoglycemic iv administration of glucose) and the impairment of postprandial glucose tolerance that characterizes patients with T2DM (12, 13). Recently, GIP has also been implicated in the inappropriate postprandial glucagon responses observed in patients with T2DM (14–16). However, the role of GIP on insulin and glucagon levels in situations where plasma glucose is not raised or even lowered (as would be the case following meals with only lipid or protein content [17] or overdosing of antidiabetic therapy) is unknown. A possible contribution of GIP to inappropriate glucagon responses could be important, as excessive glucagon secretion, in the context of elevated glucose levels and inadequate insulin signaling, has been demonstrated to contribute substantially to hyperglycemia in T2DM (16, 18). Also, several lines of evidence support the reduction of glucagon action as a therapeutic target of antidiabetic treatment (19). Therefore, we aimed to investigate to what extent GIP and the prevailing plasma glucose contributes to excessive glucagon secretion and impaired insulin secretion in patients with T2DM. Hence, we infused (iv) high physiological concentrations of GIP at three distinct glycemic levels (ie, fasting [diabetic] glycemia, insulin-induced hypoglycemia, and hyperglycemia [1.5 × fasting glycemia]) and measured the glucose-dependent effects of GIP on insulin secretion, glucagon responses, and glucose disposal in patients with T2DM.

Materials and Methods

Approval and registration of study protocol

The study was approved by the Scientific-Ethical Committee of the Capital Region of Denmark (registration number: H-D-2009–0078), registered with http://clinicaltrials.gov/show/NCT01414556 (ID: NCT01414556) and conducted according to the principles of the Helsinki Declaration (Fifth revision, Edinburgh, 2000). Oral and written informed consent were obtained from all participants before inclusion.

Subjects

We included 12 male patients with T2DM (age: 62 ± 1 y [mean ± SEM[rwqb]; body mass index: 29 ± 1 kg/m^2; glycated hemoglobin A1c [HbA_{1c}]: 6.5 ± 0.1%, HbA_{1c}: 48 ± 2 mmol/L; fasting plasma glucose 7.9 ± 0.3 mM; diabetes duration: 51 ± 11 mo). All subject characteristics are presented in Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org. At screening all potential subjects went through a physical examination, had standard hematological and clinical biochemistry parameters measured, and had urine sampled for the determination of albumin-creatinine ratio. Patients on antidiabetic treatment with a HbA_{1c} <6.5% (48 mmol/mol) underwent a 75-g oral glucose tolerance test preceded by a full week without antidiabetic medication to confirm the diagnosis of T2DM. Exclusion criteria were fasting plasma glucose >12 mmol/L on screening day, HbA_{1c} >9% (>75 mmol/mol), liver disease (alanine aminotransferase >2× upper normal limit), diabetic nephropathy, proliferative diabetic retinopathy, severe atherosclerosis or heart failure (New York Heart Association Functional Classification group III and IV), anemia (hemoglobin <130 g/L), treatment with a glucagon-like peptide-1 (GLP-1) receptor agonist, dipeptidyl peptidase 4 (DPP-4) inhibitor, insulin, or medication that could not be paused for 12 hours before the experimental days. All participants were well-treated with regards to glycemia, plasma lipids, and blood pressure and were without overt diabetic complications. All patients had an oral antidiabetic drug washout period of a minimum of 7 days before each experimental day.

Study design

The study design was a placebo-controlled, crossover study. Each subject underwent six experimental days in randomized order according to a prespecified random numbers table within a 6-month period. For each subject either GIP or placebo (saline) was infused on paired days during clamps of plasma glucose at fasting levels, at hyperglycemic levels aiming at 1.5 × fasting glycemia, and at hypoglycemic levels (induced with iv insulin infusion) aiming at a plasma glucose level of 3–3.5 mmol/L. To avoid carryover bias, intervals between examinations were at least 2 days after days with fasting and hyperglycemia and 1 week after days with hypoglycemia. Subjects were instructed to maintain a regular diet and avoid alcohol and excessive eating for 3 days before each experimental day. Subjects arrived at the laboratory after an overnight (10 h) fast having avoided strenuous physical activity from the day before. They were placed in a recumbent position and had a cannula inserted into a cubital vein. The forearm was placed in a heating box (55°C) throughout the experiment for collection of arterialized blood samples. Another cannula was inserted into a contralateral cubital vein for glucose and hormone infusions. At time 0 minutes, a primed iv infusion of either GIP or placebo (saline) was initiated. Initial priming infusion was with 4 pmol × kg^{-1} × min^{-1}, and at time 15 minutes, the infusion rate was adjusted to 2 pmol × kg^{-1} × min^{-1}, which was kept until time 90 minutes, when the infusion was stopped. The variation in infusion rate was done with the intent to attain plasma levels of GIP observed following ingestion of a mixed meal (20). Plasma glucose was measured bedside every 5 minutes, allowing the plasma glucose level to be clamped using an adjustable continuous infusion of 20% glucose (w/v).

Hypoglycemia days: to induce hypoglycemia, insulin (Actrapid, Novo Nordisk) mixed with 1% human albumin was infused at a rate of 1 mU × kg^{-1} × min^{-1} from time −25 minutes until end of the study period.

Hyperglycemia days: the glucose bolus used to elevate plasma glucose to 1.5 × fasting values during the hyperglycemic clamps was calculated as follows: volume (mL) of 50% (w/v) glucose needed = [elevation from fasting plasma glucose needed (in mmol/L)] × [body weight (in kg)] × [0.07 (mL × mmol/L^{-1} × kg^{-1})].

Peptides

Synthetic GIP (PolyPeptide Laboratories) was prepared for infusion by the Capital Region Pharmacy in Denmark. The peptide was dissolved in sterilized water containing 2% human albumin (Statens Serum Institut), subjected to sterile filtration, dispensed into vials, and microbiologically tested.

Blood specimens

Arterialized blood was drawn at time −30, −15, 0, 5, 10, 20, 30, 45, 60, 75, and 90 minutes into chilled tubes containing EDTA plus aprotinin (500 KIU/mL blood; Trasylol, Bayer Corp) and a specific DPP-4 inhibitor (valine pyrrolidide, final concentration of 0.01 mmol/L, a gift from Novo Nordisk) for analyses of glucagon and intact GIP. Blood for analyses of insulin and C-peptide was sampled into chilled tubes containing heparin. All tubes were immediately cooled on ice and centrifuged for 20 minutes at 1200*g* and 4°C. Plasma for GIP and glucagon analyses was stored at −20°C and plasma samples for insulin and C-peptide analyses were stored at −80°C until analysis. For bedside measurements of plasma glucose, blood was added to fluoride tubes and centrifuged at room temperature immediately for 2 minutes at 7400*g*.

Analytical procedures

Plasma glucose concentrations were measured by the glucose oxidase method and plasma concentrations of GIP and glucagon were measured by specific RIA as previously described (21). Plasma insulin and C-peptide concentrations were measured using a two-sided electrochemiluminescence immunoassay (Roche/Hitachi Modular Analytics; Roche Diagnostic GmbH).

Calculations and statistical analyses

Results are reported as mean ± SEM unless otherwise stated. Area under the curve (AUC) and incremental area under the curve (iAUC) values (ie, baseline levels subtracted) were calculated using the trapezoidal rule. Insulin secretion rate (ISR) values were calculated by deconvolution of measured C-peptide concentrations and application of population-based parameters for C-peptide kinetics as previously described (22–24). ISR is expressed as picomoles of insulin secreted per minute per kilogram body weight. Two-way repeated-measures ANOVA followed by multiple comparison test with the Holm-Šídák correction was used to test for differences in plasma concentrations between days (25). For paired comparisons between single values (eg, between AUC values), we used parametric or nonparametric *t* tests as appropriate. For comparisons of more than two single AUC values, we used one-way ANOVA followed by paired comparisons. Statistics and graphical presentation were performed in Graphpad Prism version 6.0 (GraphPad Software). A two-sided *P* value < .05 was used to indicate statistically significant differences.

Results

Glucose

Mean plasma glucose concentrations during each of the six experimental days are displayed in Figure 1 (*top row*).

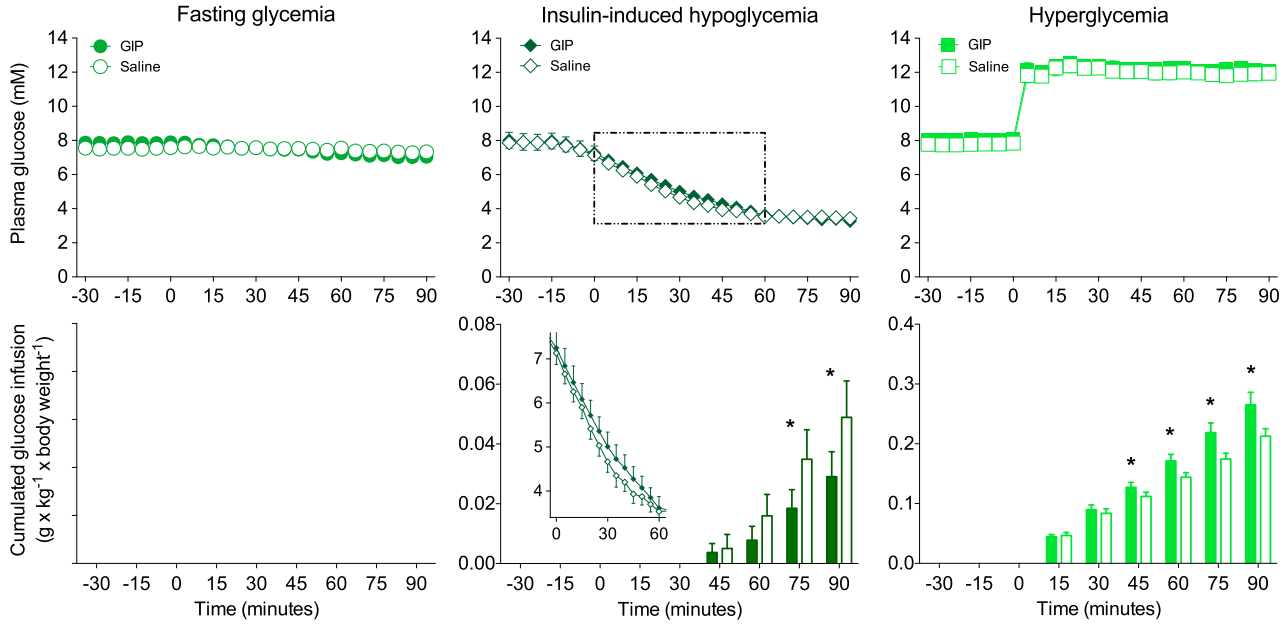

Figure 1. Glucose. Plasma concentrations of glucose (*top row*) during fasting glycemia (*left, green curves, circles*), insulin-induced hypoglycemia (*center column, dark green curves, diamonds*), and hyperglycemia (*right column, light green curves, squares*) on days with GIP infusions (*filled symbols*) and days with saline infusion (*open symbols*). Concomitant glucose infusions (*bottom row*) are depicted as bar graphs (gram per kilogram of body weight per 15-min time intervals). *Insets in middle row, center column* depict the plasma glucose values during the initial 60 minutes of infusion. Data are means ± SEM. Statistical analysis was done by two-way repeated measures ANOVA and paired nonparametric *t* tests (glucose infusions). *, Significant ($P < .05$) differences.

Baseline plasma glucose concentrations did not differ between study days and the overall mean value was 7.8 ± 0.3 mmol/L. When comparing the paired days (GIP vs saline) of matching levels, AUC for plasma glucose did not differ (P = NS).

Fasting glycemia days: no glucose was infused.

Hypoglycemia days: during insulin infusions a trend toward a slower drop in mean plasma glucose was apparent during the initial 45 minutes with GIP infusion compared with the days of saline infusion (P = .06). During the final 30 minutes, the plasma glucose levels were clamped on similar mean levels of 3.5 mmol/L. In 3 of the 12 patients, no exogenous glucose infusion was needed to prevent plasma glucose dropping below 3 mmol/L. Despite the tendency toward a slower drop in glycemic levels on the days of GIP infusion, significantly less glucose was infused on these days (total amounts 29 ± 9 and 49 ± 12 mg glucose per kilogram body weight for GIP and saline, respectively; P = .039) (Figure 1, *bottom row*).

Hyperglycemia days: the amount of glucose needed to maintain the clamp was higher on the days of GIP infusion (total amounts: 262 ± 21 vs 213 ± 15 mg glucose per kilogram body weight for GIP and saline, respectively; P < .001).

GIP

Time courses for plasma concentrations of intact GIP are shown in Figure 2. The overall mean baseline concentrations were 20 ± 1 pmol/L with no significant differences between paired days with similar glycemic levels. During GIP infusions, plasma intact GIP concentrations reached similar peak values of 98 ± 2 pmol/L (P = .72) and mean steady-state (45–90 min) concentrations of 70 ± 3 pmol/L (P = .10). No significant changes in GIP concentrations occurred during saline infusions.

Insulin and ISR

Time courses for serum insulin concentrations and ISR values are presented in Figure 3. Overall fasting concentrations of serum insulin and C-peptide were 149 ± 27 with no difference in mean values between study days (P > .45).

Fasting glycemia days (Figure 3, left column, bottom row*)*

GIP infusion resulted in a short-lasting (0–20 min) increment in ISR compared with saline (peaking at 10 min with 6.0 ± 0.6 [GIP] vs 2.8 ± 0.4 [saline] pmol × kg^{-1} × min^{-1}, P = .0002).

Hypoglycemia days

During the exogenous insulin infusion, serum insulin levels increased to levels around 1200 pmol/L during both saline and GIP infusion. Initially before plasma glucose dropped (time 0–20 min), ISR was greater during GIP infusion compared with saline (peaking at 10 min with 3.9 ± 0.5 [GIP] vs 1.4 ± 0.2 [saline] pmol × kg^{-1} × min^{-1}), but thereafter ISR was equally suppressed during both GIP and saline (Figure 3, *center column, bottom row*). The initial increase in insulin secretion with GIP infusion gave rise to slightly higher serum insulin with GIP compared with saline infusion during the initial 30 minutes (P = .01).

Hyperglycemia days

Insulin responses increased during the entire study period with both GIP and saline infusions (Figure 3, *right column*). Only during concomitant GIP infusion, an initial ISR response (time 0–30 min) was established (peaking at 10 min with 10.6 ± 1.4 [GIP] vs 4.6 ± 0.7 [saline] pmol × kg^{-1} × min^{-1}, P = .0004). After the initial 30 minutes, GIP potentiated ISR to a minor, but stable degree (time 60 min: 7.9 ± 1.4 [GIP] vs 5.9 ± 1.0 [saline] pmol × kg^{-1} × min^{-1}, P = .014).

Glucagon

Time courses for plasma glucagon concentrations are presented in Figure 4. Fasting levels of glucagon were sta-

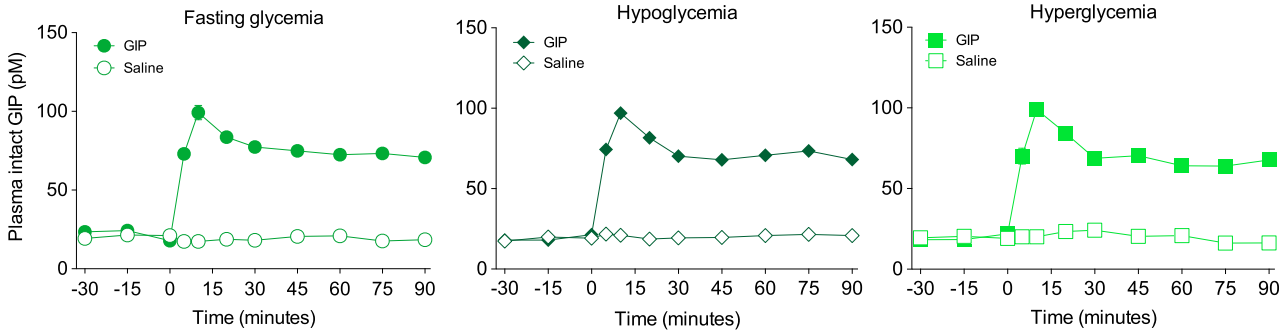

Figure 2. GIP. Plasma concentrations of GIP during fasting glycemia (*left, green curves, circles*), insulin-induced hypoglycemia (*center column, dark green curves, diamonds*), and hyperglycemia (*right column, light green curves, squares*) on days with GIP infusions (*filled symbols*) and days with saline infusion (*open symbols*).

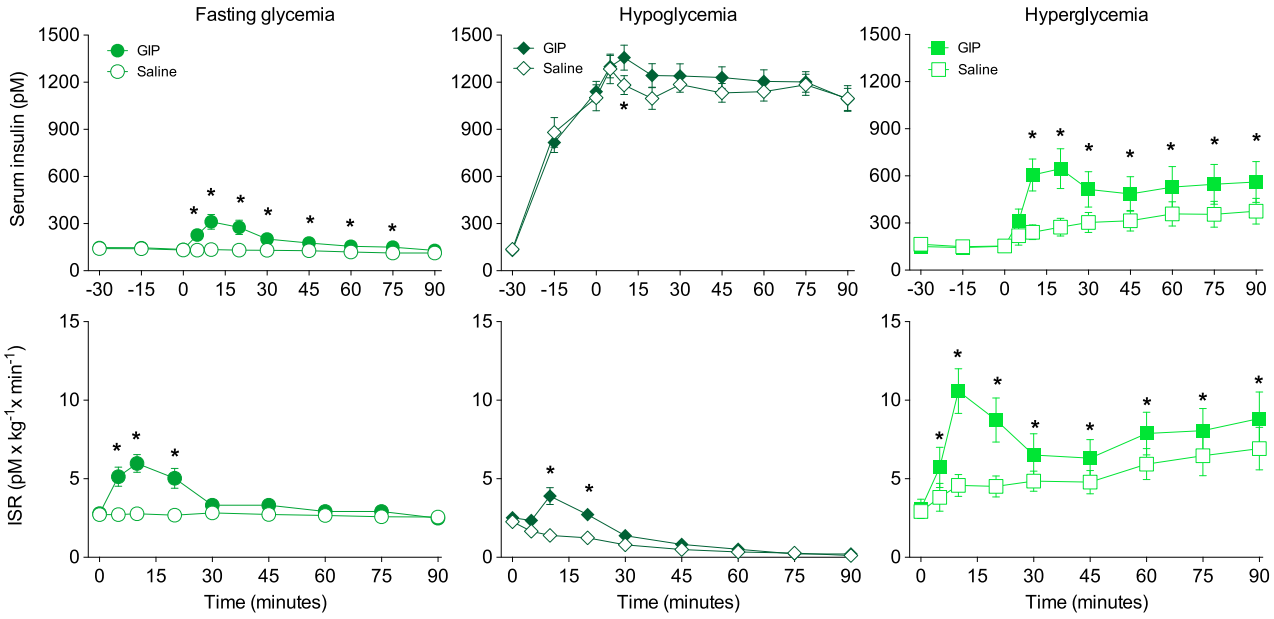

Figure 3. Insulin and ISR. Responses of serum insulin (*top row*) and insulin secretion rate (ISR; derived by deconvolution analysis of C-peptide concentrations) (*bottom row*) over 90 minutes of GIP infusions (*filled symbols*) and saline infusions (*open symbols*) during fasting glycemia (*left column, green curves, circles*), insulin-induced hypoglycemia (*center column, dark green curves, diamonds*), and hyperglycemia (*right column, light green curves, squares*). Data are means ± SEM. Statistical analysis was done by two-way repeated measures ANOVA followed by Holm-Sidak's multiple comparisons test. *, Significant ($P < .05$) differences.

tistically similar on all study days with an overall mean value of 12.5 ± 1.5 pmol/L.

Fasting glycemia days: As evident from Figure 4 (*left column*), GIP infusion resulted in an initial increase in glucagon response compared with saline ($P = .0001$) and a few individual time points were statistically higher (20 min, $P = .0001$, and 30 min, $P = .045$) on the GIP days. The area under the plasma glucagon curve for the initial 30

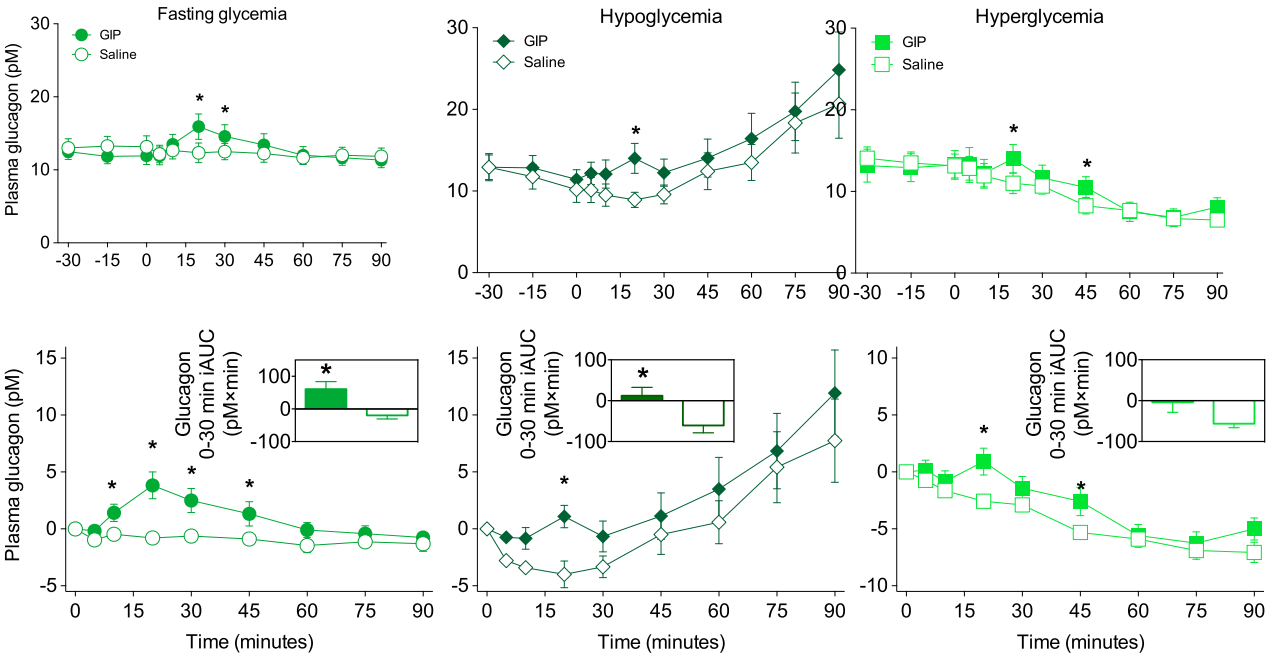

Figure 4. Glucagon. Plasma concentrations of glucagon as either absolute (*top row*) or incremental values (*bottom row*) during 90 minutes of GIP infusions (*filled symbols*) or saline infusions (*open symbols*) during fasting glycemia (*left column, green curves, circles*), insulin-induced hypoglycemia (*center column, dark green curves, diamonds*), and hyperglycemia (*right column, light green curves, squares*). Insets in bottom rows are baseline subtracted area under curves (iAUCs) of glucagon concentrations for the initial 30 minutes. Data are means ± SEM. Statistical analysis was done by two-way repeated measures ANOVA followed by Holm-Sidak's multiple comparisons test and paired *t* tests (*insets*). *, Significant ($P < .05$) differences.

minutes (61 ± 23 vs −20 ± 11 pmol/L × min, $P = .002$) and for the entire 90-minute study period was higher with GIP infusion compared with saline (86 ± 65 vs −87 ± 38 pmol/L × min, $P = .01$).

Hypoglycemia days: The mean concentrations of plasma glucagon increased significantly with time ($P = .0001$) and was statistically higher with GIP compared with saline infusion at 30 minutes ($P = .02$). Accordingly, during the initial 30 minutes, the incremental glucagon responses were significantly larger during GIP infusion (12 ± 21 [GIP] vs −60 ± 18 [saline] pmol/L × min, $P = .03$). Similar peak levels of glucagon were reached after 90 minutes with both saline and GIP (mean C_{max}: 24.8 ± 4.7 pmol/L [GIP] vs 20.7 ± 4.2 pmol/L [saline], $P = .3$). The differences in iAUC for the entire study period did not differ statistically (300 ± 143 [GIP] vs 134 ± 121 [saline] pmol/L × min, $P = .15$).

Hyperglycemia days: Plasma glucagon levels differed between GIP and saline days ($P = .01$), with statistically higher values during GIP infusion at two individual time points (20 min, $P = .002$, and 45 min, $P = .045$). However, iAUC differences during the initial 30 minutes (−4 ± 24 [GIP] vs −56 ± 10 [saline] pmol/L × min, $P = .09$), and during the entire study period did not differ statistically (−270 ± 79 [GIP] vs −403 ± 121 [saline] pmol/L × min, $P = .07$).

Discussion

We show that the effects of GIP on insulin and glucagon secretion in typical overweight, middle-aged patients with T2DM are highly dependent on the prevailing plasma glucose level: the insulinotropic effect of GIP dominates during hyperglycemic conditions, whereas the glucagonotropic effect of GIP seems to be most important during lower plasma glucose concentrations.

The present findings bear resemblance to results of similar studies in healthy subjects (3), but some novel findings and differences compared with healthy individuals and in relation to bibliographical evidence are worth considering. First, the plasma levels of intact GIP attained during the present experiment could be considered high physiological levels with a profile similar to those observed in a similar cohort of patients with T2DM after ingestion of a standardized mixed meal (20). Interestingly, our data are the first to show that high physiological levels of GIP counteract hypoglycemia induced by a fixed weight-adjusted insulin infusion in patients with T2DM. Thus, although plasma glucose values were lowered, we observed higher mean plasma glucose values (nonsignificant trend) during concomitant GIP infusion despite significantly less exogenous glucose being infused. This net effect occurred with higher mean levels of circulating insulin during GIP infusion. As GIP receptors are present on pancreatic α cells (16), our results could be compatible with the notion that GIP-induced glucagon responses overruled the minor effects on insulin secretion in these patients. This would be similar to what was observed in a meal test study in patients with T2DM (16). The insulin dose and the insulin resistance typical for patients with T2DM (26) are probably also important for the observed net effect on plasma glucose (and need for glucose administration). Thus, in insulin-sensitive healthy subjects GIP did not affect hypoglycemic excursions during infusion of a 50% higher dose of insulin than used in the present study (3). The glucose infusions, in the patients needing such, can be interpreted as an estimate of the difference in endogenous glucose production during hypoglycemia with GIP compared with saline. In this perspective, based on our data, a typical 90-kg patient with T2DM would produce an additional approximately 3 g (range 0–8 g) of glucose with GIP (vs saline) during insulin-induced hypoglycemia. This difference could seem of minor relevance from a therapeutic perspective, but could nonetheless be of some clinical relevance in preventing hypoglycemia (eg, as a physiological postprandial defense mechanism or in insulin-treated patients with T2DM). It is important to note that due to the aforementioned slightly higher insulin levels on the days of GIP infusion, the difference in endogenous glucose production could be even higher than our glucose infusion data indicate. Thus, an increased (insulin-mediated) peripheral glucose disposal could have been counteracted by endogenous glucose production, leaving plasma glucose relatively unaffected.

In normal glucose-tolerant individuals glucose is a strong suppressor of glucagon (2, 3, 27, 28), and the specific α-cell dysfunction associated with diabetes progression has been described as resistance of the α cell to glucose- and insulin-induced suppression of glucagon secretion (27–30). The glucagon responses to hyperglycemia observed in the present study (Figure 3, *right column*) suggest that the already impaired glucose-induced suppression of glucagon secretion in patients with T2DM (28, 31) further deteriorates in the presence of physiological amounts of GIP (Figure 3, *left column*). Therefore, as the ISR increased during the GIP infusions, our data are in-line with the notion that inhibitory paracrine signals from β cells are inadequate in reducing α-cell secretion in T2DM and that GIP could play a causal role the inappropriate glucagon levels during hyperglycemia in patients with T2DM.

Concerning the quantification of the late-phase insulin responses to GIP, the results of the present study point to

a previously unnoticed, but rather important aspect. Indeed, our results demonstrate that coadministration of glucose is essential for the late-phase insulin response to GIP in T2DM. We investigated the insulinotropic effect of GIP during both fasting glucose values (ie, without any glucose administration at baseline plasma glucose between 6.2 and 9.8 mmol/L) as well as hyperglycemic values (ranging from 10.6 to 14.5 mmol/L). Notably, at fasting plasma glucose values (mean 7.7 ± 0.2 mmol/L), GIP elicited only a minor short-lived early-phase insulin response (Figure 2, *left column*). In contrast, at hyperglycemia, GIP infusion augmented ISR also during the time interval of 30–90 minutes (ie, corresponding to "late-phase" insulin secretion). These findings illustrate a further differentiating feature between the two incretin hormones, as GLP-1 in contrast to GIP has been shown to elicit a robust late-phase insulin response also at fasting glycemia in patients with T2DM (32).

As previously outlined, many studies have shown severely reduced late-phase C-peptide responses to both physiological and supraphysiological infusions of GIP during hyperglycemic clamps in patients with T2DM (1, 7–11, 33). Meier and Nauck (12) recently advocated, based on a post-hoc analysis of clinical studies, that the "GIP defect" is closely related to the impairment in β-cell function. Bearing in mind the importance of concomitant glucose administration, some of the previous studies carried out at plasma glucose levels similar to or only slightly higher than the fasting levels in diabetic patients (eg, plasma glucose values of 7.8 mmol/L [11], 8 mmol/L [7], 8.75 mmol/L [1] could therefore perhaps underestimate the actual insulinotropic effect of GIP). Nevertheless, the combined evidence from the present study and other crossover studies in patients without overt dysregulation of T2DM (ie, same patients with and without GIP administration) and with clear-cut glucose administration (5, 9, 33, 34) seem to corroborate an impaired, but nevertheless clinically relevant, effect of GIP on second-phase insulin secretion and glucose disposal. In the present study, when relating the glucose-corrected ISR (GIP vs saline) in the second half hour of the hyperglycemic clamps, GIP augmented late-phase insulin secretion by a factor of 1.37. This potentiation of insulin secretion, despite the previously mentioned co-occurring deteriorations in glucagon response, translated into a 25% increase in glucose disposal measured as exogenous glucose needed to maintain the clamp (Figure 1, *right column, bottom row*). In comparison, the glucose-corrected ISR in a group of healthy subjects in response to similar GIP doses at similar hyperglycemia were augmented by a factor of 2.2, and glucose disposal was increased by approximately 100% (3).

Our study has some limitations. First, we recruited a limited number of patients (n = 12). Second, we cannot fully exclude potential bias from carryover effects despite our randomization procedures and washout of antidiabetic medication 1 week before study days. Last, we chose to perform the hyperglycemic clamping in an untraditional way by elevating glucose levels to 1.5 × fasting values instead of to an arbitrary uniform hyperglycemic level. This could impede the comparison to other studies but offered the benefit that all patients during "hyperglycemia" received exogenous glucose stimuli of similar relative size.

Besides providing some clarification on the physiological role of GIP, our results also provide some mechanistic insight into possible GIP-mediated effects of DPP-4 inhibitors. Recently, it has been demonstrated that 10 days of treatment with the DPP-4 inhibitor vildagliptin in T2DM patients confers insulinotropic effects other than those mediated through GLP-1 (34). Therefore, it is particularly interesting that during treatment with the DPP-4 inhibitor, sitagliptin, the initial increases in intact GLP-1 levels disappeared with sustained treatment, whereas the intact GIP responses were perpetually elevated during the course of treatment (20). Thus, we speculate that the often disregarded GIP responses following DPP-4 inhibition might contribute substantially to the relatively sustained efficacy of these pharmacologic agents in clinical practice (35). Another important aspect of treatment with DPP-4 inhibitors is the improved glucagon counterregulation observed during postprandial hypoglycemia (36), and the low incidence of hypoglycemia when combining DPP-4 inhibitors with exogenous insulin (37, 38). Our data suggest that both of these traits, at least in part, might be contingent on GIP-augmented glucagon responses to hypoglycemia.

Conclusions

In patients with T2DM, 1) GIP counteracts insulin-induced lowering of plasma glucose conceivably by inducing a glucagon response and only a minor early-phase insulin response; 2) GIP has negligible effect on plasma glucose at fasting glycemia through balanced effects on insulin and glucagon; and 3) GIP retains insulinotropic effects during hyperglycemia (when plasma glucose is elevated by glucose administration), thereby exerting a glucose-lowering action. Collectively, our results demonstrate that in patients with relatively well-regulated T2DM, GIP retains clinically relevant glucose-stabilizing effects at a level around fasting (diabetic) glycemia through diverging effects on insulin and glucagon.

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Disclosure Summary: The authors declare no conflicts of interest in relation to the present article. M.C. has no affiliation with any pharmaceutical company. S.C., F.K.K., T.V., and J.J.H. have served as consultants for various pharmaceutical companies (including Gilead, Ono Pharmaceuticals, Novo Nordisk, Merck Sharp & Dohme, Bristol-Myers Squibb, Eli Lilly, and Sanofi-Aventis).

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