Mikkel Christensen,[1,2] Salvatore Calanna,[1,3] Alexander H. Sparre-Ulrich,[1,4] Peter L. Kristensen,[5] Mette M. Rosenkilde,[4] Jens Faber,[6] Francesco Purrello,[3] Gerrit van Hall,[7] Jens J. Holst,[2] Tina Vilsbøll,[1] and Filip K. Knop[1,2]

# Glucose-Dependent Insulinotropic Polypeptide Augments Glucagon Responses to Hypoglycemia in Type 1 Diabetes

*Diabetes 2015;64:72–78 | DOI: 10.2337/db14-0440*

**Glucose-dependent insulinotropic polypeptide (GIP) is glucagonotropic, and glucagon-like peptide-1 (GLP-1) is glucagonostatic. We studied the effects of GIP and GLP-1 on glucagon responses to insulin-induced hypoglycemia in patients with type 1 diabetes mellitus (T1DM). Ten male subjects with T1DM (C-peptide negative, age [mean ± SEM] 26 ± 1 years, BMI 24 ± 0.5 kg/m$^2$, HbA$_{1c}$ 7.3 ± 0.2%) were studied in a randomized, double-blinded, crossover study, with 2-h intravenous administration of saline, GIP, or GLP-1. The first hour, plasma glucose was lowered by insulin infusion, and the second hour constituted a "recovery phase." During the recovery phase, GIP infusions elicited larger glucagon responses (164 ± 50 [GIP] vs. 23 ± 25 [GLP-1] vs. 17 ± 46 [saline] min · pmol/L, $P < 0.03$) and endogenous glucose production was higher with GIP and lower with GLP-1 compared with saline ($P < 0.02$). On the GIP days, significantly less exogenous glucose was needed to keep plasma glucose above 2 mmol/L (155 ± 36 [GIP] vs. 232 ± 40 [GLP-1] vs. 212 ± 56 [saline] mg · kg$^{-1}$, $P < 0.05$). Levels of insulin, cortisol, growth hormone, and noradrenaline, as well as hypoglycemic symptoms and cognitive function, were similar on all days. Our results suggest that during hypoglycemia in patients with T1DM, exogenous GIP increases glucagon responses during the recovery phase after hypoglycemia and reduces the need for glucose administration.**

In recent years, it has become evident that glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) have glucose-dependent effects on glucagon secretion (1). While GLP-1 lowers glucagon levels during fasting and elevated blood glucose (1–3), GIP raises glucagon levels during fasting and hypoglycemic conditions (4–6). In most patients with type 1 diabetes mellitus (T1DM), the glucagon counterregulatory response to insulin-induced hypoglycemia is deficient (7,8), and it is unknown to what extent the incretin hormones might affect this response. We therefore studied the individual effects of GIP and GLP-1 on the glucagon response to insulin-induced hypoglycemia in patients with T1DM without endogenous insulin secretion.

## RESEARCH DESIGN AND METHODS

### Study Design

This was a double-blinded, randomized, crossover study comparing the effects of GIP, GLP-1, and placebo (saline)

[1]Center for Diabetes Research, Department of Medicine, Gentofte Hospital, University of Copenhagen, Hellerup, Denmark
[2]Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[3]Department of Clinical and Molecular Biomedicine, University of Catania, Catania, Italy
[4]Department of Neuroscience and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[5]Department of Cardiology, Nephrology and Endocrinology, Hillerød Hospital, University of Copenhagen, Hillerød, Denmark
[6]Department of Medicine, Herlev Hospital, University of Copenhagen, Copenhagen, Denmark
[7]Clinical Metabolomics Core Facility, Department of Infectious Diseases, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
Corresponding author: Mikkel Christensen, mch@dadlnet.dk.

Received 18 March 2014 and accepted 14 July 2014.
Clinical trial reg. no. NCT01739283, clinicaltrials.gov.
M.C. and S.C. contributed equally to this study.

on the glucagon response to insulin-induced hypoglycemia in patients with T1DM. The study was conducted at Gentofte Hospital, University of Copenhagen. The study was approved by the Scientific-Ethical Committee of the Capital Region of Denmark (registration no. H-D-2009-0078) and was conducted according to the principles of the Declaration of Helsinki (fifth revision, Edinburgh, 2000).

### Participants

We included 10 male patients (C-peptide negative, age [mean ± SEM] 26 ± 1 years, BMI 24 ± 0.5 kg/m$^2$, $HbA_{1c}$ 7.3 ± 0.2%) with T1DM (positive islet cell and/or GAD-65 antibodies), treated with multiple doses of insulin ($N$ = 9) or insulin pump ($N$ = 1), without late diabetes complications, hypoglycemia unawareness, or residual β-cell function (i.e., C-peptide negative after a 5-g arginine stimulation test [9]).

### Experimental Procedures

Each patient underwent three separate study days in randomized order with intervals of at least 1 week. Subjects were instructed to maintain a regular diet and avoid alcohol and strenuous physical activity for 3 days prior to each study day. Subjects were asked to wear a Guardian REAL-time continuous glucose monitor (Medtronic A/S, Copenhagen, Denmark) for 2–4 days before each study day to monitor glycemic variability and facilitate therapeutic decisions in relation to food intake and insulin delivery. If patients had fasting plasma glucose levels between 5 and 9 mmol/L at the morning of the study day, two cannulae were inserted bilaterally into the cubital veins for infusions and blood sampling, respectively. Two hours before study start (i.e., time −120 min), an infusion of stable isotopes was initiated ($D_2$-glucose [priming dose 17.6 μmol · kg$^{-1}$ and continuous 0.5 μmol · kg$^{-1}$ · min$^{-1}$] and $D_5$-glycerol [priming dose 3 μmol · kg$^{-1}$ and continuous 0.1 μmol · kg$^{-1}$ · min$^{-1}$]) and at the same time basal insulin (Actrapid; Novo Nordisk, Bagsværd, Denmark) intravenous infusion was initiated (dose 0.1 mU · kg$^{-1}$ · min$^{-1}$). At time 0 min, a 60 min infusion of human insulin (1 mU · kg$^{-1}$ · min$^{-1}$ i.p.) was initiated to lower plasma glucose. Also, at time 0 min, similar appearing infusions of GIP (4 pmol · kg$^{-1}$ · min$^{-1}$ i.p.), GLP-1 (1 pmol · kg$^{-1}$ · min$^{-1}$ i.p.), or placebo (saline) were initiated and continued for the remainder of the study that day. Plasma glucose was measured bedside every 5 min. An adjustable continuous infusion of 20% dextrose (w/v) was administered by a physician (S.C.) unaware of randomization allocation to keep plasma glucose >2 mmol/L in the time interval 0–75 min and >3.5 mmol/L in the interval 75–120 min. Hypoglycemic symptoms and cognitive function were assessed at baseline (time −60 min) and during hypoglycemia (time 65 min) using validated rating scales (10).

### Analytical Procedures

Steady-state glucose and glycerol concentrations and stable isotope enrichment were determined at time −45, −30, and −15 min. Arterialized blood was drawn at time −30, −15, 0, 5, 10, 20, 30, 45, 60, 75, 90, 105, and 120 min. Bedside glucose concentrations were measured by the glucose oxidase method, and concentrations of intact GIP, total GLP-1, and glucagon were measured by specific immunoassays as previously described (5,11). We used the assay for total GLP-1 to allow for comparison between GLP-1 concentrations in healthy volunteers (12) and during subcutaneous treatment with GLP-1 (13). Blood enrichment of $D_2$-glucose and $D_5$-glycerol was determined using gas chromatography–mass spectrometry as previously described (14). Plasma insulin, C-peptide, cortisol, noradrenaline, and growth hormone were measured with commercial immunoassays.

### Calculations and Statistical Analyses

For detection of a real difference in glucagon responses between days of at least 2.5 pmol/L with a two-sided 5% significance level, a power of 80%, and a within-patient SD of plasma glucagon measurements of 1.7 pmol/L (estimated from a previous study [5]), a sample size of 10 patients was found to be necessary. Area under the curve (AUC) and incremental AUC (iAUC), i.e., baseline level–subtracted AUC values, were calculated using the trapezoidal rule. Glucose $R_a$ and glucose $R_d$ were calculated from the changes in glucose enrichment using a one-compartment, fixed-volume, non–steady-state model modified for use with stable isotopes and a pool fraction of 70 mL · kg$^{-1}$ (15). To test for differences in concentration time courses, we used two-way repeated-measures ANOVA followed by Holm-Šídák corrected posttests. For comparisons of AUC values, we used Friedman test followed by paired comparisons using Wilcoxon test. Statistics and graphical presentations were performed in GraphPad Prism, version 6.01 (GraphPad Software, La Jolla, CA). A two-sided $P$ value <0.05 was used to indicate statistically significant differences. Results are reported as means ± SEM unless otherwise stated.

## RESULTS

### Plasma Glucose

Mean plasma glucose concentrations during each of the three experimental days are displayed in Fig. 1*A*. Baseline plasma glucose concentrations (time −90 to 0 min) did not differ significantly between study days ($P > 0.79$), and the overall mean fasting value was 6.9 ± 0.5 mmol/L. Mean plasma glucose excursions from time 0 to 120 min also did not differ between days, with a mean nadir of 2.7 ± 0.1 mmol/L reached at time 70 min. The total amount of glucose needed to prevent hypoglycemia differed significantly between days (156 ± 35 [GIP] vs. 234 ± 41 [GLP-1] vs. 214 ± 56 [saline] mg · kg body wt$^{-1}$, $P < 0.05$).

### GIP and GLP-1

Plasma concentrations over time of GIP and GLP-1 are shown in Fig. 1. The respective plasma concentrations peaked at 134 ± 4 pmol/L during GIP infusion (Fig. 1*B*) and 219 ± 19 pmol/L during GLP-1 infusion (Fig. 1*C*).

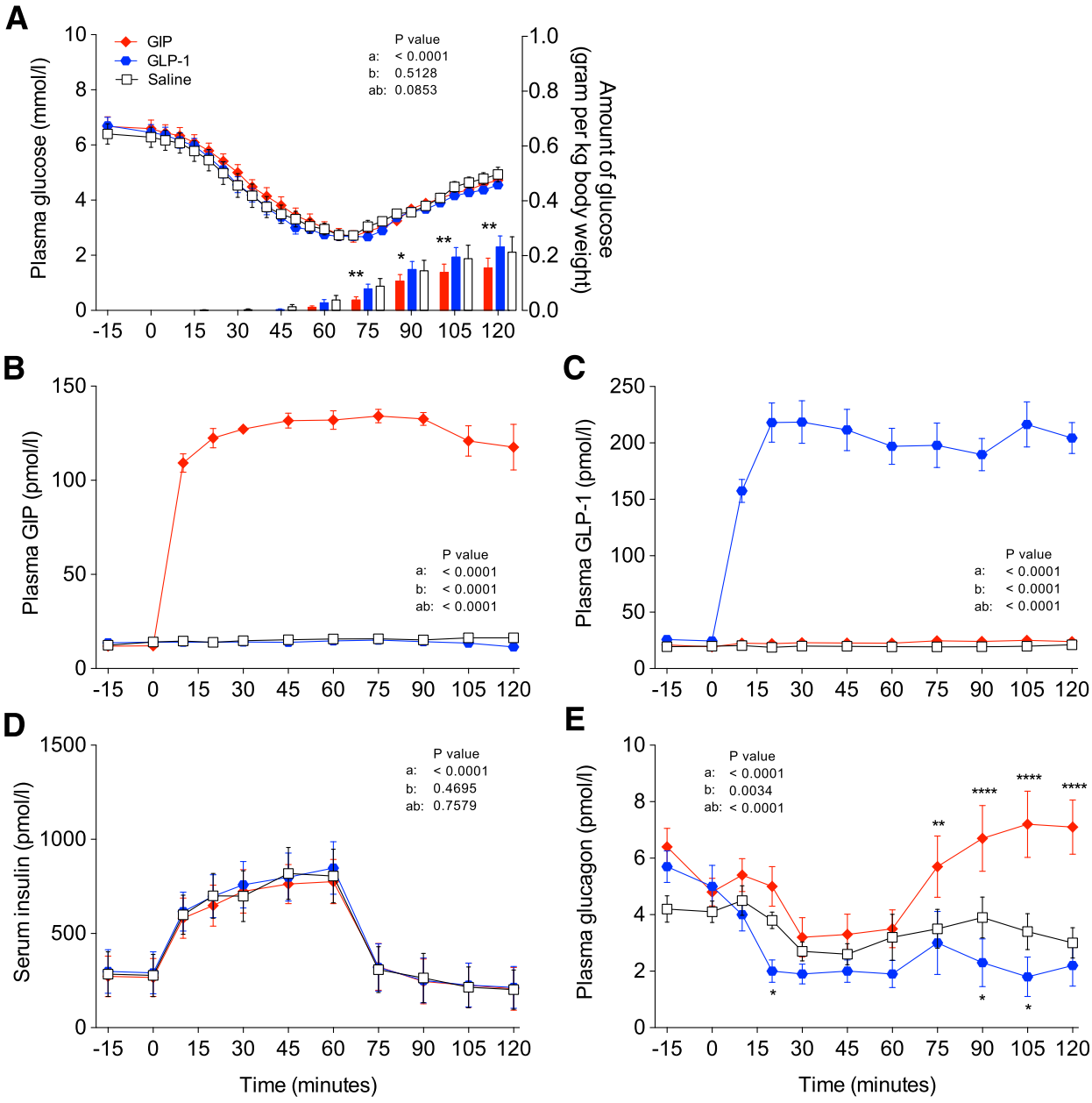


**Figure 1**—Plasma glucose and hormones. Plasma/serum glucose (*A*), intact GIP (*B*), total GLP-1 (*C*), insulin (*D*), and glucagon (*E*) during insulin-induced hypoglycemia with intravenous infusion of GIP (red diamonds), GLP-1 (blue hexagons), or saline (white squares). Data are means ± SEM. Cumulated glucose infusions are depicted as bar graphs (*A*). Statistical analyses were done with repeated-measures ANOVA; [a]differences over time, [b]differences between the groups, [ab]differences owing to interaction between group and time. Posttests at individual time points were by Holm-Šídák multiple comparisons tests. Significant differences are indicated by asterisks: *$P < 0.05$, **$P = 0.001$ to 0.01, ****$P < 0.0001$.

### Insulin and C-Peptide

Serum insulin concentrations over time are presented in Fig. 1*D*. Overall basal concentrations of serum insulin were similar on study days ($P = 0.58$). After the start of insulin infusion, levels of serum insulin increased significantly to overall mean peak levels of 810 ± 129 pmol/L, with no difference in mean values between study days ($P = 0.52$). Mean C-peptide concentrations were below the detection limit at all time points (data not shown).

### Glucagon

Plasma glucagon concentrations are presented in Fig. 1*E*. Baseline levels of glucagon were similar on all study days ($P = 0.29$). Glucagon responses expressed as AUC differed between study days ($P < 0.001$). During the first hour, the glucagon iAUCs amounted to −88 ± 38 (GIP) vs. −166 ± 28 (GLP-1) vs. −49 ± 20 (saline) min · pmol/L ($P < 0.006$), and during the second hour, the glucagon iAUCs amounted to 164 ± 50 (GIP) vs. 23 ± 25 (GLP-1)

vs. 17 ± 46 (saline) min · pmol/L ($P < 0.036$). Thus, during the insulin infusion (time 0–60 min), the glucagon iAUC was significantly lower on the GLP-1 days ($P < 0.01$ vs. saline), whereas during the "recovery period" (i.e., after termination of insulin infusion), there was a higher glucagon response on the GIP days ($P < 0.02$ vs. saline).

### Glucose Kinetics

The time courses of glucose $R_a$ and glucose $R_d$ were similar on study days (Fig. 2*A* and *B*). GIP infusion increased endogenous $R_a$ (significant at 75 min), whereas GLP-1 reduced endogenous $R_a$ compared with saline (time 90–120 min) (Fig. 2*C*). The glucose infusion rates also differed between days, with lower infusion rates on the days of GIP infusion at time 75 min (Fig. 2*D*).

### Free Fatty Acids, Glycerol, and Glycerol Kinetics

The levels of free fatty acids (FFAs) were equally suppressed during the first hour of the study, but during the recovery phase they increased more on the days of GIP infusion compared with saline (Fig. 3*A*) and FFA iAUC was higher on the GIP days ($P < 0.04$). Plasma glycerol concentrations and glycerol $R_a$ and $R_d$ did not differ between study days (Fig. 3*C* and *D*).

### Hypoglycemia Symptom Score and Cognitive Function

There were no differences in hypoglycemia symptom scores or cognitive function scores between the study days (Fig. 4*A* and *B*).

### Plasma Cortisol, Noradrenaline, and Growth Hormone Responses

The plasma cortisol, noradrenaline, and growth hormone concentrations did not differ significantly between study days (data not shown).

## DISCUSSION

We report that in patients with T1DM without residual β-cell function, GIP augments glucagon responses to experimental hypoglycemia and reduces the need for exogenous glucose administration (to prevent severe hypoglycemia) accompanied by a higher rate of endogenous glucose production (significantly higher at the time around maximal hypoglycemia, 75 min after study start) compared with placebo. We also report that GLP-1 infusions slightly suppress plasma glucagon during induction of hypoglycemia, with no additional need for exogenous glucose administration.

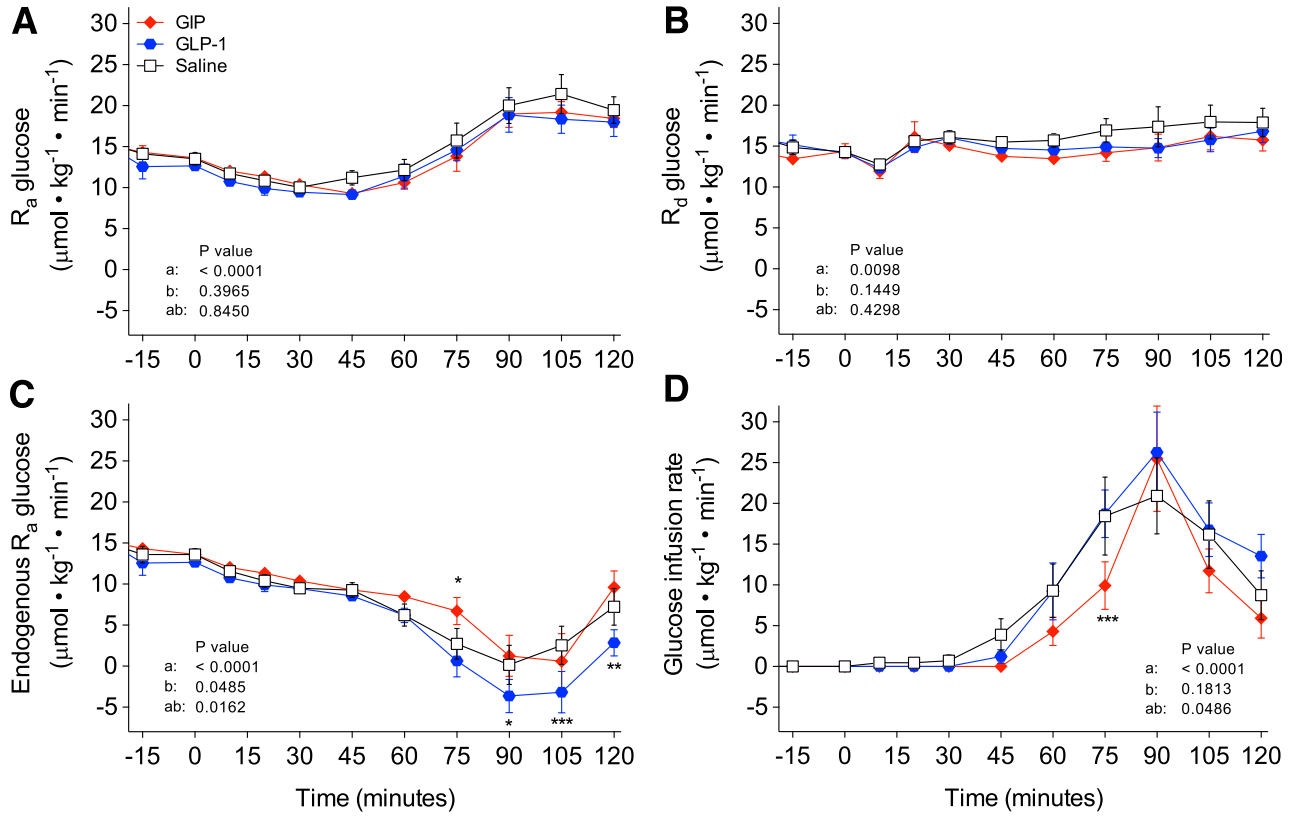


**Figure 2**—Glucose kinetics. $R_a$ (*A*), glucose $R_d$ (*B*), rate of endogenous glucose production (*C*), and glucose infusion rate in 15-min intervals (*D*) during insulin-induced hypoglycemia with intravenous infusion of GIP (red diamonds), GLP-1 (blue hexagons), or saline (white squares). Data are means ± SEM. Statistical analyses were done with repeated-measures ANOVA; [a]differences over time, [b]differences between the groups, [ab]differences owing to interaction between group and time. Posttests at individual time points were by Holm-Šídák multiple comparisons tests. Significant differences are indicated by asterisks: *$P < 0.05$, **$P = 0.001$–$0.01$, ***$P = 0.0001$–$0.001$.

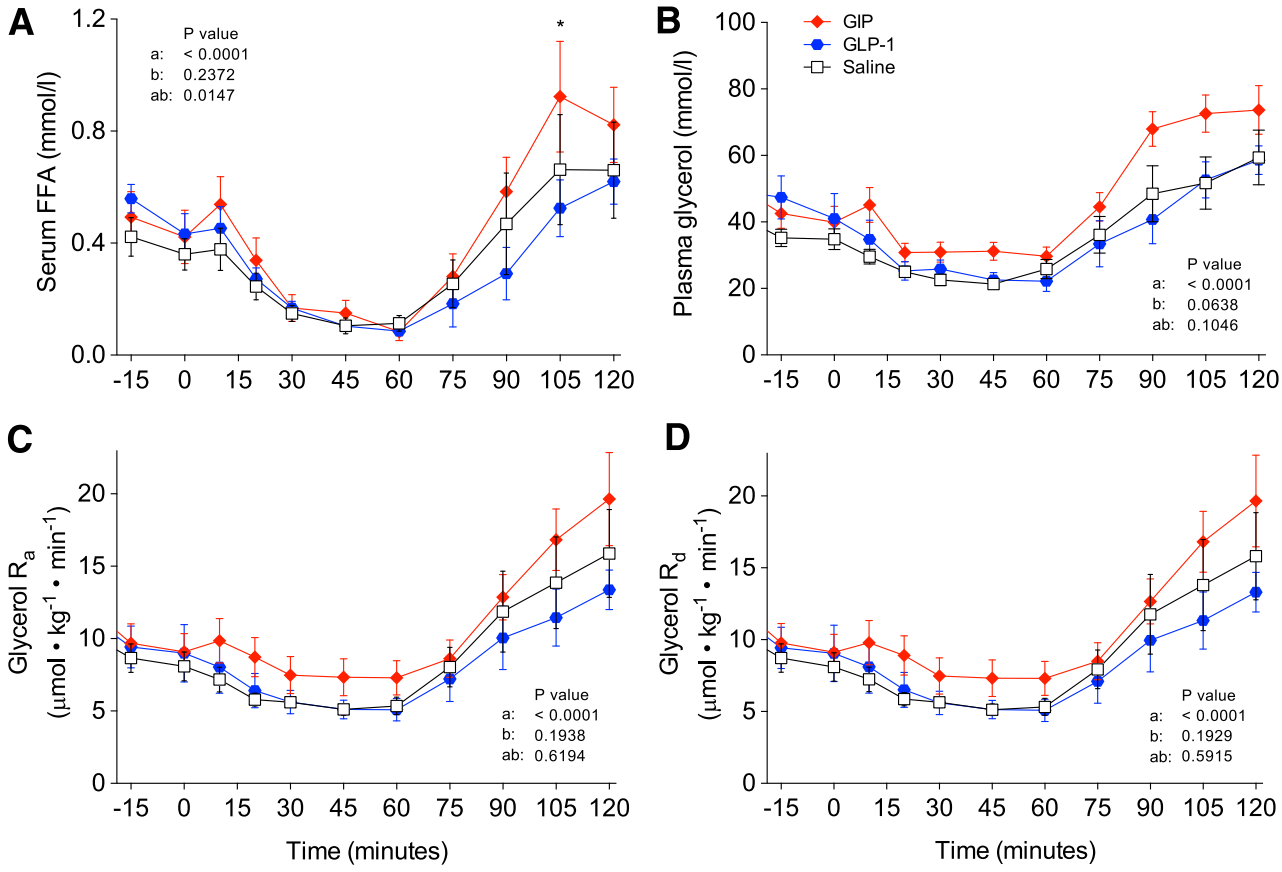


**Figure 3**—Serum FFAs and glycerol kinetics. Plasma/serum FFAs (*A*), glycerol (*B*), glycerol $R_a$ (*C*), and glycerol $R_d$ (*D*) during insulin-induced hypoglycemia with intravenous infusion of GIP (red diamonds), GLP-1 (blue hexagons), or saline (white squares). Data are means ± SEM. Statistical analyses were done with repeated-measures ANOVA; [a]differences over time, [b]differences between the groups, [ab]differences owing to interaction between group and time. Posttests at individual time points were by Holm-Šídák multiple comparisons tests. *Significant ($P < 0.05$) difference.

The mechanism underlying these findings could be the direct action of the incretin hormones on the pancreatic α-cells, as demonstrated in perfused rat pancreata (16). During perfusion with low levels of glucose (1.5 mmol/L), GIP had stimulatory and GLP-1 had inhibitory effects on glucagon secretion (16). The glucagonotropic effect of GIP is presumed to be mediated by activation of GIP receptors present on pancreatic α-cells (17,18). The glucagon

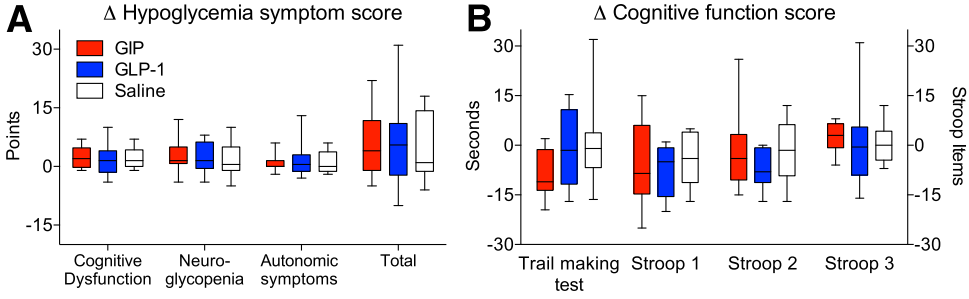


**Figure 4**—Hypoglycemia symptoms and cognitive function score. Hypoglycemia symptom scores (*A*) and cognitive function scores (*B*) depicted as the difference between baseline (time −60 min) and hypoglycemia (time 65 min) during infusion of GIP (red), GLP-1 (blue), or saline (white). Hypoglycemia symptoms were graded using the Edinburgh Hypoglycemia Scale, which is a Likert scale of symptom scores from 1 (not present) to 7 (very intense). Cognitive functions scores are based on the Trail Making Test, which measures time to finish in seconds (*B* [left *y*-axis]), and the Stroop test, which measures items named during a 45-s period (*B* [right *y*-axis]). Results are depicted as box and whiskers (minimum to maximum). No significant differences between study days were observed.

inhibitory effects of GLP-1 have been attributed to paracrine inhibitory signaling (somatostatin) from neighboring δ-cells (16) or—more controversially—direct effects via GLP-1 receptors present on α-cells (1,19).

We were not able to detect any differences between interventions in cognition, hypoglycemia symptoms, or other counterregulatory hormones. This is not surprising, as the glucose levels were similar on the three experimental days. Notably, the fact that glucose levels were kept >2 mmol/L could explain the limited symptoms and hormonal responses to hypoglycemia. We demonstrated a minor GIP-dependent increase in FFAs and a nonsignificant increase in glycerol during the recovery phase from hypoglycemia. This was unexpected, as a preponderance of evidence including human in vivo data supports a role for GIP combined with insulin in stimulating postprandial lipogenesis (20,21). The observed paradoxical lipolytic effect could therefore have depended on the low plasma glucose levels in combination with fairly low insulin levels and stimulated glucagon levels during the recovery phase after hypoglycemia.

A few considerations are worth mentioning. Firstly, we used doses of GIP and GLP-1 resulting in supraphysiological plasma levels. The plasma intact GIP levels of ~125 pmol/L were in the range that can be reached postprandially during dipeptidyl peptidase-4 inhibition (22). The total GLP-1 levels of ~200 pmol/L were similar or slightly lower than what can be used therapeutically in patients with type 2 diabetes (13). Secondly, even the maximal glucagon responses (i.e., on the GIP days) were deficient compared with the responses observed in healthy subjects under similar conditions (5) and in the range of what can be considered basal levels. Important for the interpretation of these modest changes, basal glucagon levels are located on the steep part of the dose response curve relating glucagon concentration to hepatic glucose output, as evidenced by studies where as little as 2–3 pmol/L changes in basal levels of portal glucagon significantly increase hepatic glucose output (23). Lastly, the negative (albeit not statistically different from zero) endogenous glucose $R_a$ values from time 75 to 90 min on the GLP-1 days and around zero on the GIP and saline days were likely caused by large abrupt glucose infusions leading to analytical and modeling limitations (24). Although these limitations may have been the same on all study days, the endogenous glucose production estimates should be interpreted with caution.

The present findings may have clinical relevance. Iatrogenic hypoglycemia is a major safety concern in patients with diabetes, and consequently minor improvements in hypoglycemia risk have attracted great public and pharmaceutical interest. The mean difference in glucose administration between the days of GIP versus saline in the current study amounted to 4 g. For comparison, 15–20 g glucose is commonly recommended as self-treatment of mild-to-moderate symptomatic hypoglycemia (25). Thus, both the hypoglycemia-limiting effect of adding GIP to insulin treatment and glucagon-reducing effect of GLP-1 (and GLP-1 receptor agonists) during low levels of glycemia merit further investigation.

**Acknowledgments.** The authors are grateful to the participating patients and for laboratory assistance from J. Purtoft, N. Kjeldsen, and S.M. Schmidt from the Center for Diabetes Research; S.P. Olesen and L. Albæk from the Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen; and Lene Foged at the Clinical Metabolomics Core Facility, Rigshospitalet.

**Duality of Interest.** S.C. became an employee in the Novo Nordisk Pharmaceutical Medicine Programme after the study was finished (September 2013). J.J.H. has served as a consultant or advisor to Novartis Pharmaceuticals, Novo Nordisk, Merck Sharp & Dohme, and Roche and has received fees for lectures from Novo Nordisk, Merck Sharp & Dohme, and GlaxoSmithKline. T.V. has received fees for being part of an advisory board from AstraZeneca, Boehringer Ingelheim Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly and Company, GI Dynamics, Merck Sharp & Dohme, Novo Nordisk, Sanofi, and Takeda; has received fees for lectures from AstraZeneca, Boehringer Ingelheim Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly and Company, Merck Sharp & Dohme, Novo Nordisk, Novartis, Sanofi, Takeda, and Zealand Pharma; and has received research support from Novo Nordisk. F.K.K. has received fees for consultancy or being part of an advisory board from AstraZeneca, Bristol-Myers Squibb/AstraZeneca, Eli Lilly, Gilead Sciences, Ono Pharmaceuticals, Sanofi, and Zealand Pharma; has received fees for lectures from AstraZeneca, Boehringer Ingelheim Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly and Company, Gilead Sciences, Merck Sharp & Dohme, Novo Nordisk, Ono Pharmaceuticals, Sanofi, and Zealand Pharma; and has received research support from Sanofi. No other potential conflicts of interest relevant to this article were reported.

**Author Contributions.** M.C. designed the study, performed the data analysis, contributed to the clinical experiments, drafted the manuscript, and revised and approved the final manuscript. S.C. contributed to the study design and data analysis, performed most of the clinical experiments, and revised and approved the final manuscript. A.H.S.-U. contributed to the clinical experiments and biochemical analyses and revised and approved the final manuscript. P.L.K. contributed to the study design and data analysis and revised and approved the final manuscript. M.M.R., F.P., and T.V. revised and approved the final manuscript. J.F. analyzed plasma for noradrenaline and revised and approved the final manuscript. G.v.H. contributed to the study design and data analysis of stable isotope kinetics and revised and approved the final manuscript. J.J.H. contributed to the study design and measurements of peptide hormones and revised and approved the final manuscript. F.K.K. designed the study, contributed to the data analysis, and revised and approved the final manuscript. M.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

## References

1. Holst JJ, Christensen M, Lund A, et al. Regulation of glucagon secretion by incretins. Diabetes Obes Metab 2011;13(Suppl. 1):89–94

2. Vilsbøll T, Krarup T, Madsbad S, Holst JJ. Both GLP-1 and GIP are insulinotropic at basal and postprandial glucose levels and contribute nearly equally to the incretin effect of a meal in healthy subjects. Regul Pept 2003;114:115–121

3. Kielgast U, Holst JJ, Madsbad S. Antidiabetic actions of endogenous and exogenous GLP-1 in type 1 diabetic patients with and without residual β-cell function. Diabetes 2011;60:1599–1607

4. Meier JJ, Gallwitz B, Siepmann N, et al. Gastric inhibitory polypeptide (GIP) dose-dependently stimulates glucagon secretion in healthy human subjects at euglycaemia. Diabetologia 2003;46:798–801

5. Christensen M, Vedtofte L, Holst JJ, Vilsbøll T, Knop FK. Glucose-dependent insulinotropic polypeptide: a bifunctional glucose-dependent regulator of glucagon and insulin secretion in humans. Diabetes 2011;60:3103–3109

6. 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

7. Gerich JE, Langlois M, Noacco C, Karam JH, Forsham PH. Lack of glucagon response to hypoglycemia in diabetes: evidence for an intrinsic pancreatic alpha cell defect. Science 1973;182:171–173

8. Arbelaez AM, Xing D, Cryer PE, et al.; Diabetes Research in Children Network (DirecNet) Study Group. Blunted glucagon but not epinephrine responses to hypoglycemia occurs in youth with less than 1 yr duration of type 1 diabetes mellitus. Pediatr Diabetes 2014;15:127–134

9. Christensen M, Knop FK, Vilsbøll T, et al. Glucagon-like peptide-2, but not glucose-dependent insulinotropic polypeptide, stimulates glucagon release in patients with type 1 diabetes. Regul Pept 2010;163:96–101

10. Kristensen PL, Pedersen-Bjergaard U, Kjær TW, et al. Influence of erythropoietin on cognitive performance during experimental hypoglycemia in patients with type 1 diabetes mellitus: a randomized cross-over trial. PLoS ONE 2013;8:e59672

11. Ørskov 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

12. Nauck MA, Heimesaat MM, Behle K, et al. Effects of glucagon-like peptide 1 on counterregulatory hormone responses, cognitive functions, and insulin secretion during hyperinsulinemic, stepped hypoglycemic clamp experiments in healthy volunteers. J Clin Endocrinol Metab 2002;87:1239–1246

13. Zander M, Madsbad S, Madsen JL, Holst JJ. Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. Lancet 2002;359:824–830

14. Wolsk E, Mygind H, Grøndahl TS, Pedersen BK, van Hall G. IL-6 selectively stimulates fat metabolism in human skeletal muscle. Am J Physiol Endocrinol Metab 2010;299:E832–E840

15. Wolfe RR. *Isotope Tracers in Metabolic Research: Principles and Practice of Kinetic Analysis*. 2nd ed. Hoboken, NJ, Wiley-Liss, 2005, p. 474

16. de Heer J, Rasmussen C, Coy DH, Holst JJ. Glucagon-like peptide-1, but not glucose-dependent insulinotropic peptide, inhibits glucagon secretion via somatostatin (receptor subtype 2) in the perfused rat pancreas. Diabetologia 2008;51:2263–2270

17. Chia CW, Carlson OD, Kim W, et al. Exogenous glucose-dependent insulinotropic polypeptide worsens post prandial hyperglycemia in type 2 diabetes. Diabetes 2009;58:1342–1349

18. Lyssenko V, Eliasson L, Kotova O, et al. Pleiotropic effects of GIP on islet function involve osteopontin. Diabetes 2011;60:2424–2433

19. De Marinis YZ, Salehi A, Ward CE, et al. GLP-1 inhibits and adrenaline stimulates glucagon release by differential modulation of N- and L-type Ca2+ channel-dependent exocytosis. Cell Metab 2010;11:543–553

20. Asmar M, Simonsen L, Madsbad S, Stallknecht B, Holst JJ, Bülow J. Glucose-dependent insulinotropic polypeptide may enhance fatty acid re-esterification in subcutaneous abdominal adipose tissue in lean humans. Diabetes 2010;59:2160–2163

21. Yip RG, Wolfe MM. GIP biology and fat metabolism. Life Sci 2000;66:91–103

22. Aaboe K, Knop FK, Vilsbøll T, et al. KATP channel closure ameliorates the impaired insulinotropic effect of glucose-dependent insulinotropic polypeptide in patients with type 2 diabetes. J Clin Endocrinol Metab 2009;94:603–608

23. Cherrington AD. Banting Lecture 1997. Control of glucose uptake and release by the liver in vivo. Diabetes 1999;48:1198–1214

24. Vella A, Rizza RA. Application of isotopic techniques using constant specific activity or enrichment to the study of carbohydrate metabolism. Diabetes 2009;58:2168–2174

25. American Diabetes Association. Standards of medical care in diabetes–2014. Diabetes Care 2014;37(Suppl. 1):S14–S80