Higher Endogenous Glucose Production During OGTT vs Isoglycemic Intravenous Glucose Infusion

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Context: Oral glucose ingestion elicits a larger insulin response and delayed suppression of glucagon compared to isoglycemic IV glucose infusion (IIGI).

Objective: We studied whether these differences translate into effects on endogenous glucose production (EGP) and glucose disposal in patients with type 2 diabetes and nondiabetic control subjects.

Design: This was a single-blinded, randomized, crossover study.

Setting: The study was conducted at a specialized research unit.

Participants: Ten patients with type 2 diabetes (age, [mean ± SD] 57.1 ± 6.7 years; body mass index, 29.0 ± 4.3 kg/m^2; hemoglobin A_{1c}, 53.8 ± 11.0 mmol/mol; duration of diabetes, 9.2 ± 5.0 years) and 10 matched nondiabetic control subjects (age, 56.0±10.7 years; body mass index, 29.8 ± 2.9 kg/m^2; hemoglobin A_{1c}, 33.8 ± 5.5 mmol/mol) participated.

Interventions: Three experimental days: 75 g-oral glucose tolerance test (OGTT), IIGI, and IIGI+glucagon (IIGI with a concomitant IV glucagon infusion [0.8 ng/kg/min from 0 to 25 minutes] designed to mimic portal glucagon concentrations during OGTT in the type 2 diabetic group) were undertaken.

Main Outcome Measures: Glucose kinetics were assessed by tracer methodology.

Results: Glucose rate of disappearance was higher during the OGTT vs IIGI in the control group, but similar on all days in the diabetic group. Surprisingly, in both groups, EGP was more suppressed during IIGI than during OGTT, and exogenous glucagon infusion during IIGI did not restore EGP to the levels observed during OGTT.

Conclusion: EGP was less suppressed during OGTT than during IIGI in both patients with type 2 diabetes and in nondiabetic control subjects. Based on the present experimental design, it was not possible to attribute this difference to the delayed glucagon suppression observed in the initial phase of the OGTT. **(*J Clin Endocrinol Metab* 101: 4377–4384, 2016)**

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Abbreviations: BMI, body mass index; bsAUC, baseline-subtracted area under curve; EGP, endogenous glucose production; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; HbA_{1c}, hemoglobin A_{1c}; IIGI, isoglycemic intravenous glucose infusion; OGTT, oral glucose tolerance test; R_a, rate of appearance; R_d, rate of disappearance.

Attenuated suppression of endogenous glucose production (EGP) during ingestion of glucose has been shown to contribute to the postprandial hyperglycemia characterizing patients with type 2 diabetes (1–3). EGP is tightly controlled by a variety of nutrient and hormonal factors, with glucose and insulin (suppressors) and glucagon (stimulator) being the best described (4). Because of the incretin effect (ie, the greater insulin response observed during oral glucose tolerance test [OGTT] compared to isoglycemic IV glucose infusion [IIGI]) (5, 6), EGP would be expected to be lower during OGTT than during IIGI. However, we and others have shown that plasma glucagon concentrations are higher during OGTT compared to during IIGI in patients with type 2 diabetes (and to some extent also in nondiabetic subjects) (7–9). This phenomenon could be expected to counteract or perhaps outweigh any suppression of EGP resulting from the incretin-stimulated insulin secretion during OGTT and perhaps explain the attenuated suppression of EGP in patients with type 2 diabetes.

We studied how the differential insulin and glucagon responses during OGTT and IIGI affect EGP in patients with type 2 diabetes and nondiabetic control subjects. Furthermore, we aimed to evaluate the isolated effect of attenuated glucagon suppression on EGP. To address these questions, we used tracer methodology (10, 11) during OGTT and IIGI and during an additional IIGI in which glucagon suppression was prevented by an infusion of glucagon. This was done both in patients with type 2 diabetes and in nondiabetic control subjects.

Materials and Methods

The protocol was approved by the Scientific-Ethical Committee of the Capital Region of Denmark, and registered with ClinicalTrials.gov (ID: NCT02010827). The study was performed in accordance with the principles of the Helsinki Declaration (Seventh revision, 2013). Oral and written informed consent was obtained from all participants before inclusion.

Study participants

Ten patients (five women) with type 2 diabetes diagnosed according to the World Health Organization criteria (12) (age, [mean ± SD] 57.1 ± 6.7 years; body mass index [BMI], 29.0 ± 4.3 kg/m^2; hemoglobin A_{1c} [HbA_{1c}], 53.8 ± 11.0 mmol/mol; duration of diabetes, 9.2 ± 5.0 years) and 10 matched nondiabetic control subjects (five women) (age, 56.0 ± 10.7 years; BMI, 29.8 ± 2.9 kg/m^2; HbA_{1c}, 33.8 ± 5.5 mmol/mol) without any family history of diabetes were studied. Nine of the patients were treated with diet plus metformin and one was treated with diet alone. None of the subjects was treated with other drugs known to affect glucose levels.

Experimental procedures

After a screening visit, subjects were studied on three separate occasions (separated by at least 72 hours) over a minimum of 4 weeks: OGTT, IIGI, and IIGI+glucagon infusion (IIGI with a concomitant IV glucagon infusion [0.8 ng/kg/min from 0 to 25 minutes] designed to mimic portal glucagon concentrations during OGTT in the type 2 diabetic group). Patients with diabetes were instructed not to take glucose-lowering medication for 1 week before each experimental day. On all occasions, participants were studied after an overnight (10-hour) fast having avoided strenuous physical activity and alcohol the day before. On the first experimental day, a 4-hour 75 g-OGTT was performed. Participants were placed in a recumbent position and cannulas were inserted in cubital veins, one for collection of arterialized blood with the hand wrapped in a heating pad (~50 C) and one in a contralateral vein for infusion of stable isotopes. At time –120 minutes, a primed continuous infusion of [6,6-D_2]-glucose (priming dose of 17.6 μmol×kg^{-1}×γ/5, where γ stands for fasting plasma glucose in mmol/liter, and continuous infusion of 0.6 μmol × kg^{-1} × min^{-1}) (Cambridge Isotope Laboratories) was started. At time 0 minutes, the participants ingested 75 g of glucose (71.5 g of water-free glucose added 3.5 g of [U-13C6]glucose) dissolved in 300 ml of water over 5 minutes. Arterialized blood samples were drawn at times: –30, –15, 0, 10, 20, 30, 50, 70, 90, 120, 150, 180, and 240 minutes relative to the ingestion of glucose. During the IIGI and IIGI+glucagon days (performed in randomized order following the OGTT), glucose (20% wt/vol) was infused at a rate aiming at duplicating the plasma glucose profile observed in the same individual during the OGTT. On the IIGI+glucagon day, a concomitant glucagon infusion (0.8 ng/kg/min during 0–25 minutes) (GlucaGen, Novo Nordisk A/S), designed to give rise to glucagon concentrations equivalent to the levels in the portal vein during the OGTT (according to a ratio of approximately 2 between portal and peripheral glucagon concentrations) (13), but without a notable effect on serum insulin concentrations, was carried out. Other procedures were as on the first study day.

Analytical procedures

Plasma glucose concentrations were measured bedside using the glucose oxidase method (Yellow Springs Instrument Model 2300 STAT plus analyzer). Plasma concentrations of glucagon, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) were measured with RIAs as previously described (9, 14, 15). C-peptide concentrations were measured using a two-sided electrochemiluminescence immunoassay (Siemens Healthcare). Plasma enrichment of [6,6-D2]glucose, [U-13C6]glucose was determined using liquid chromatography tandem-mass spectrometry, as previously described (11).

Calculations and statistics

Data are reported as mean ± SEM unless otherwise stated. Baseline-subtracted area under curve (bsAUC) was calculated by the trapezoidal method. Glucose rate of appearance (R_a) and glucose rate of disappearance (R_d) were calculated from changes in glucose enrichment using the one-compartment, fixed-volume, nonsteady-state model of Steele and modified for use with stable isotopes (16) and a varying pool fraction ranging from 70 ml × kg^{-1} initially, increasing step-wise to 200 ml × kg^{-1} at the end of trial. During OGTT, we applied a double-tracer approach with one glucose tracer infused IV ([6,6-D_2]glucose) and one

orally ingested glucose tracer ([U-$^{13}C_6$]glucose). The IV glucose tracer was used to calculate total glucose R_a and the ingested tracer to calculate the oral R_a of glucose. The EGP was then calculated by subtracting oral R_a of glucose from total R_a of glucose. In the IIGI studies, we used a single IV glucose tracer ([6,6-D_2]glucose) and, here, the EGP was calculated by subtracting the glucose infusion rate from the total R_a of glucose. Group differences in baseline characteristics were evaluated using two-sample Student's *t* test (two-tailed). For analysis of variations and differences between AUC values, repeated measures ANOVA with Tukey´s post hoc test was used. A two-sided *P* value <0.05 was chosen to indicate significant differences. Statistical analysis was carried out using GraphPad prism, version 6.0, for Windows/Mac (GraphPad, Software).

Results

Plasma glucagon

In the diabetic group, fasting levels of glucagon were 9.8 ± 0.8, 9.9 ± 0.8 ,and 10.9 ± 0.9 pmol/liter on the OGTT, IIGI, and IIGI+glucagon infusion days, respectively (P = .27), and the corresponding values in the control group were (8.7 ± 1.0, 8.9 ± 1.2, and 8.0 ± 1.5 pmol/liter, respectively, P = .51), with no significant differences between groups (P = .23). Higher plasma glucagon concentrations were observed during the initial phase (0–90 minutes) of the OGTT compared to the IIGI in both groups (Figure 1, A–C), but the increase during the initial phase of the OGTT was greater in the diabetic group compared to the control group (P = .02). During the IIGI+glucagon day, higher peak levels of glucagon were obtained compared to the OGTT (22.2 ± 1.9 vs 12.9 ± 1.3 pmol/liter [diabetic group], P < .001; 20.4 ± 2.0 vs 9.1 ± 1.3 pmol/liter [control group], P < .001), but bsAUC (0–90 minutes) of glucagon was not significantly different between the OGTT and the IIGI+glucagon day in the diabetic group (Figure 1c).

Plasma insulin

Fasting concentrations of insulin were not different between experimental days in any of the groups, and not different between groups (Figure 1, D and E). Insulin concentrations were significantly greater during OGTT in both groups compared to the IIGI and IIGI+glucagon day, and concentrations during the initial phase (0–90 minutes) of the OGTT were significantly greater in the control group compared to the diabetic group (bsAUC: 48.9 ± 12.2 vs 18.6 ± 3.3 nmol/liter × min, P = 0.04, respectively). Time courses of insulin concentrations during the IIGI and the IIGI+glucagon day were not different in any of the groups (Figure 1, D-F).

Incretin hormones

Concentrations of GIP and GLP-1 were higher during the OGTT day in both groups compared to the IIGI and IIGI+glucagon day (Figure 1G-L). Peak concentrations of GIP during the OGTT were higher in the diabetic group compared to the control group (85.7 ± 9.8 vs 60.8 ± 7.4 pmol/liter, respectively P = .047), but overall responses (bsAUC) of GIP during OGTT were not different between groups (Figure 1G–I). Similar GLP-1 responses during OGTT were observed in the two groups (Figure 1J–L).

Glucose kinetics

Isoglycemia during all three study days was obtained in both groups (Figure 2, A-C). Fasting concentrations of glucose were higher in the diabetic group compared to the control group (8.5 ± 0.4 vs 5.6 ± 0.1 mmol/liter, P < .001) and peak concentrations were higher in the diabetic group compared to the control group (17.2 ± 0.8 mmol/liter (time 90 minutes) vs 9.8 ± 0.6 mmol/liter (time 50 minutes), P < .001). Glucose tracer-to-tracee ratio was still increasing slightly at the end of the prime-period, suggesting that steady-state was not complete before initiation of the OGTT and IIGI, respectively, as otherwise intended (Supplemental Figure 1). Baseline levels of total glucose R_a and total glucose R_d were higher in the diabetic group compared to the control group (P = .003 for both measures) (Figure 2, D-I). Total glucose R_a and R_d (AUC) were similar between all experimental days in the diabetic group, whereas total glucose R_a and R_d were higher during OGTT in the control group compared to the IIGIs (Figure 2, F,I). Oral glucose R_a and IV glucose R_a (ie, the glucose infusion rate) during the IIGI and IIGI+glucagon interventions were not different in the diabetic group, whereas the oral glucose R_a was greater than the IV glucose R_a during both the IIGI and IIGI+glucagon days in the control group (Figure 2J–L). In both groups, the total IV glucose R_a was greater during the IIGI compared to the IIGI+glucagon day; only in the control group, however, was this difference significant (205 ± 14 vs 179 ± 13 mmol × min, P = .011 [control group]; 332 ± 22 vs 319 ± 22 mmol × minutes, P = .19 [diabetic group]). Baseline levels of EGP were higher in the diabetic group compared to the control group (17.8 ± 1.0 vs 13.5 ± 0.6 μmol/kg/min, P = .008) and all glucose interventions in both groups suppressed EGP over time (Figure 2, M and N). The initial (0–90 minutes) AUC of EGP during OGTT was higher in the diabetic group compared to the control group (AUC, 79.6 ± 4.1 vs 61.8 ± 5.1 mmol × min, P = .014) (Figure 2O). This difference, however, was due to the higher basal EGP in the diabetic group because bsAUCs were similar in the diabetic and the control groups (−56.8 ± 4.3 vs −47.1 ± 2.1 mmol × min, P = .08). EGP from 0 to 90 minutes was significantly less suppressed during OGTT compared to during IIGI in both groups (Figure 2O); a difference that was more pronounced in the diabetic group compared to the control group (ΔEGP 50.6 ± 3.8 vs 36.2 ± 5.0 mmol, P = .05). EGP during 0–90

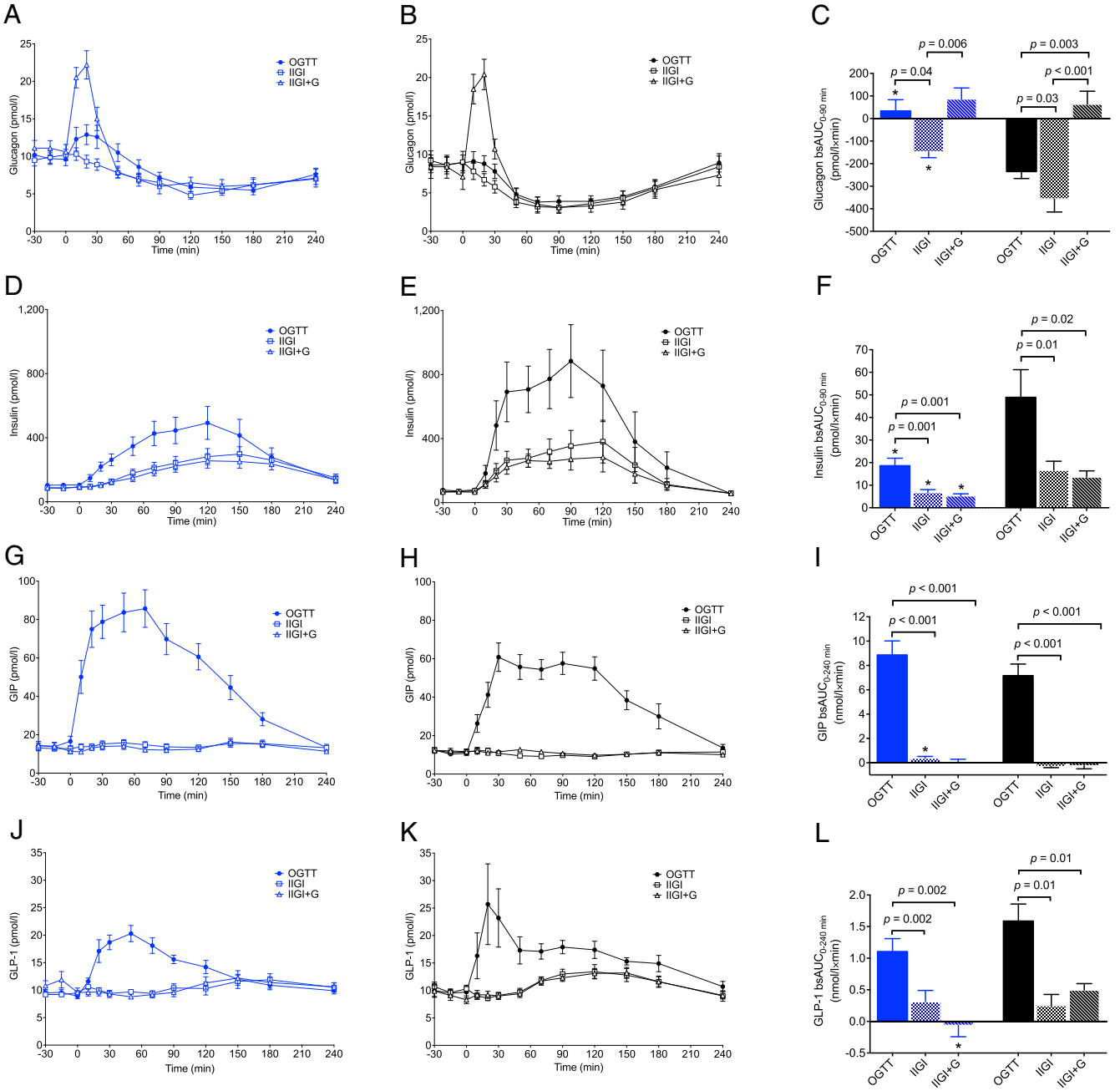

Figure 1. Time courses for plasma/serum concentrations and bsAUC for glucagon (A-C), insulin (D-F), GIP (G-I), and GLP-1 (J-L) during 75 g-OGTT, IIGI, and IIGI+glucagon infusion (IIGI+G), respectively, in patients with type 2 diabetes (blue) and nondiabetic control subjects (black). Data are mean ± SEM. *Significant ($P < .05$) differences between the two groups.

minutes was not significantly different during the IIGI day and the IIGI+glucagon day in any of the groups (AUC, 29.1 ± 5.9 vs 35.2 ± 6.3 mmol × min; $P = .52$ [diabetic group]; 25.6 ± 6.2 vs 35.9 ± 6.1 mmol × min, $P = .087$ [control group]).

Discussion

In this study, we evaluated the impact of the oral vs the IV route of glucose administration on EGP and glucose disappearance. We found that EGP was less suppressed during OGTT than during IIGI in patients with type 2 diabetes as well as in matched nondiabetic control subjects. Furthermore, we found that glucose disappearance was similar during the two glucose administration routes in patients with type 2 diabetes, but greater during OGTT compared to during IIGI in nondiabetic control subjects.

Several studies have previously established that patients with type 2 diabetes have increased EGP in the fasting state and attenuated suppression of EGP following carbohydrate ingestion (2). It is important to note that it

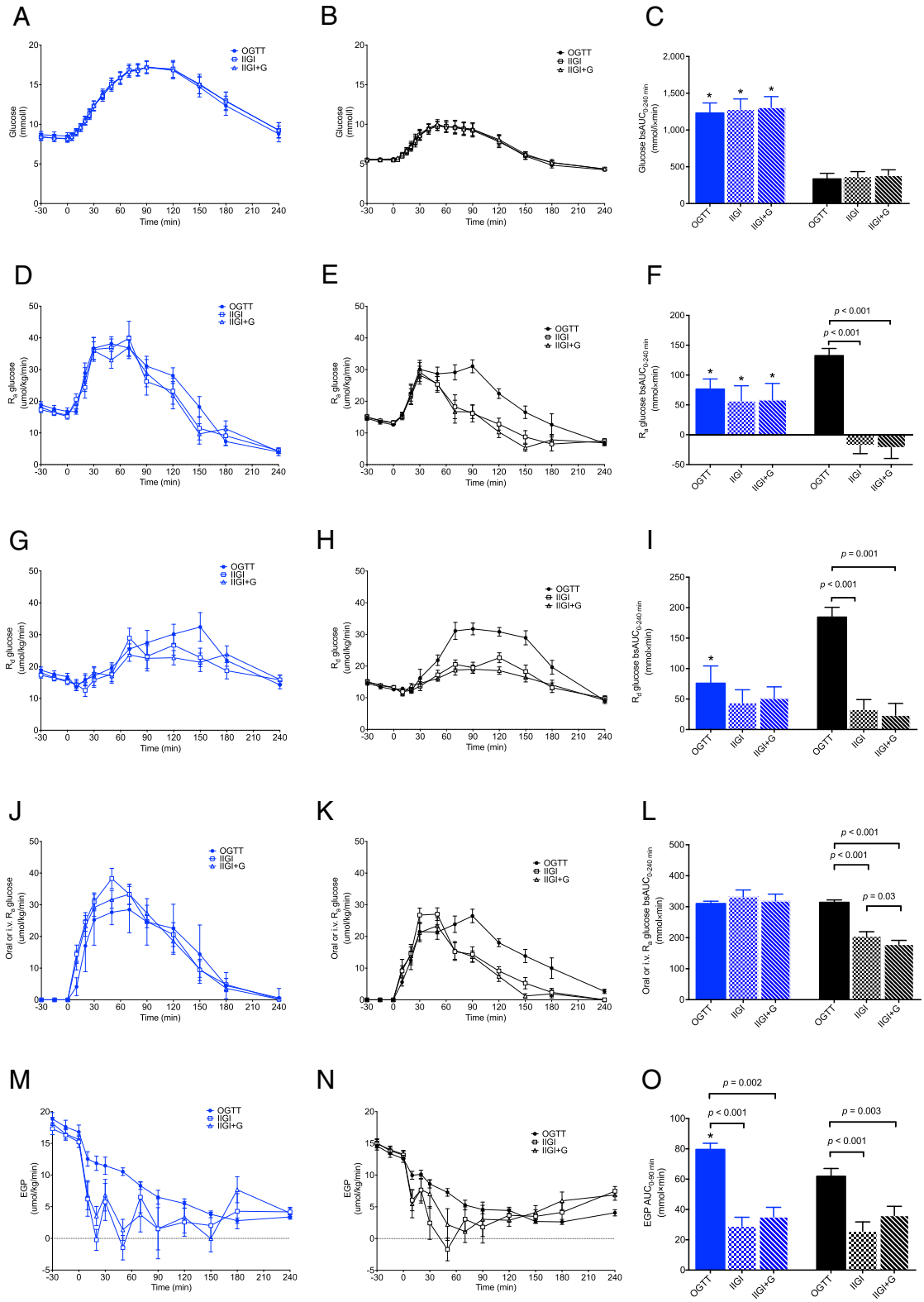

Figure 2. Time courses and AUC/bsAUC for plasma glucose concentrations (A-C), total glucose R_a (D-F), total glucose R_d (G-I), oral and IV glucose R_a (J-L), and EGP (M-O) during 75 g-OGTT, IIGI, and IIGI+G, respectively, in patients with type 2 diabetes (blue) and nondiabetic control subjects (black). Data are mean ± SEM. *Significant ($P < .05$) differences between the two groups.

is in the context of elevated basal rate of EGP and hyperglycemia that suppression of EGP is attenuated in patients with type 2 diabetes as "percent suppression" of EGP from baseline may (17) or may not (2, 3, 18–20) be different from nondiabetic control subjects. To our knowledge, this study represents the first evaluation of glucose kinetics

using glucose tracer methodology during OGTT and IIGI in patients with type 2 diabetes and nondiabetic control subjects. Our study corroborates previous findings of increased EGP in the fasting state and attenuated suppression of EGP following OGTT in patients with type 2 diabetes.

The finding of less suppressed EGP during oral compared to IV glucose administration might seem counterintuitive because it occurred despite significantly larger responses of insulin during the OGTT (21). Also, GLP-1, which is increased during the OGTT, inhibits hepatic glucose productions via its effects on insulin and glucagon secretion (22) and has also been claimed to inhibit hepatic glucose production during pancreatic clamping with somatostatin (ie, independent of endogenous insulin and glucagon secretion) (23). As glucose per se is known to suppress EGP (24), the higher hepatoportal concentrations of glucose during OGTT (compared to IIGI, during which the peripheral glucose concentrations from the OGTT are copied) would also be expected to suppress EGP more than during IIGI. However, the opposite was seen. This draws attention to the discrepant glucagon concentrations during the two glucose administration routes. Glucagon is a potent stimulator of EGP (25). It is generally thought that glucagon has little effect on EGP when insulin concentrations are high (26). Nevertheless, here we show that EGP is higher in the setting of high insulin and "high" glucagon concentrations (during the OGTT) as compared to a setting of moderate insulin and "low" glucagon concentrations (during IIGI). To test whether these differences in glucagon concentrations during the two glucose administration routes might be responsible for the difference in EGP, we included the IIGI+glucagon day. We aimed at doubling the plasma glucagon concentrations of the OGTT day to reflect the peripheral-to-portal concentration ratio of approximately 2 (13). In the control group, the peripheral glucagon concentration during the IIGI+glucagon day was augmented as intended (notably without any increase in serum insulin concentration compared to the IIGI), and here the addition of glucagon led to a 26 mmol (~5 g) (14%) lower need for glucose administration to obtain isoglycemia. In the diabetic group, however, the glucagon infusion resulted in the intended "portal" concentrations only during the first 30 minutes of the experiment. Thus, correct portal glucagon concentration may not have been achieved in this experiment, which may explain why we did not see a difference in the amount of glucose administered between the IIGI and the IIGI+glucagon day in the diabetic group. Neither did we detect a difference in EGP between the two IIGI days in any of the groups although a tendency toward greater EGP was seen in the control subjects during the IIGI+glucagon day compared to the IIGI day.

The finding of greater glucose R_d in nondiabetic control subjects during oral compared to IV glucose administration was expected because of the incretin effect (ie, the greater insulin response on the OGTT day). The increased R_d of glucose on the OGTT day explains why the oral glucose R_a and the IV glucose R_a differ between the OGTT and the IIGI, because less glucose needs to be infused to obtain isoglycemia when R_d of glucose is low. In the diabetic group, R_d values of glucose were similar during the OGTT and the IIGI days, although insulin concentrations were higher during OGTT. However, in line with the known reduced incretin effect in type 2 diabetes, the difference between insulin responses during OGTT and IIGI was not as pronounced in the diabetic group as in the control group, which likely, together with increased insulin resistance in these patients, explains why the R_d of glucose was similar between the two different glucose administration routes in patients with type 2 diabetes. In line with this, we observed no difference between oral glucose R_a and the IV glucose R_a in the diabetic group.

The present study has limitations: first of all, the real glucagon concentrations in portal venous plasma in our patients were unknown. The assumption of a 2-fold elevation compared to peripheral levels is based on direct measurements, but in patients with cirrhosis of the liver. The real porto-peripheral gradient may be greater as proposed by others (27). Furthermore, we do not know whether endogenous glucagon secreted from the pancreas (or extrapancreatic glucagon secreted from the gut, as novel findings from our group suggest, might occur during OGTT (28)) affects EGP in other ways than the classical endocrine pathway. If endogenous glucagon exerts some of its hepatic effect via neural pathways emanating in close relation to its secretion site, the design of replacing portal glucagon concentrations by a peripheral IV infusion of glucagon is inherently wrong, and might underestimate the impact of the glucagon abundance during OGTT. Also, our tracer data should be interpreted with some caution. First, we did not achieve complete steady-state according to our tracer-to-tracee ratio at baseline, which may contribute to the decreasing R_a of glucose in the fasting state we observed in both patients with type 2 diabetes and the nondiabetic control subjects. However, this was evident in all the experimental settings and not different between interventions in each group and thus does not likely explain the differences in glucose kinetics between the interventions in each group. Furthermore, during the IIGIs, the rate of EGP was fluctuating and slightly negative EGP rates (though not significantly different from zero) were observed at time 20 and 50 minutes during the IIGI

in the diabetic group and at time 50 minutes during IIGI in the control group. Similar findings have previously been observed in situations of large fluctuations in the tracer-to-tracee ratio (29, 30). In this study, large abrupt changes in glucose infusion rates occurred during this period, especially in the diabetic group (when copying the rapidly rising plasma glucose concentrations registered during the OGTT), which probably, in this time-frame, violated the assumptions of total and instantaneous tracer distribution in the glucose pool (mixing) and a stable distribution volume (Steel's pool fraction) (30). Last, we did not measure adrenal hormone responses and thus cannot exclude that the observed differences in glucose kinetics and EGP between the two glucose administration routes may have been influenced by differential adrenal hormone responses.

In summary, we show a blunted difference in R_d of glucose between oral and IV glucose administration in patients with type 2 diabetes in accordance with the well-described insulin resistance, and blunted incretin effect in these patients. Furthermore, we show that EGP is less suppressed during OGTT compared to during IIGI in both patients with type 2 diabetes and in nondiabetic control subjects. However, on the basis of our IIGI+glucagon results, it was not possible to explain this difference by differences in the glucagon responses between OGTT and IIGI.

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A.L., J.I.B., M.C., T.V., and F.K.K. designed the study and wrote the manuscript. A.L. and M.G. performed the experiments. G.v.H. performed analysis of glucose tracer data, and contributed with calculations of glucose kinetic data. J.J.H. provided RIA analyses of glucagon, glucagon-like peptide-1, and glucose-dependent insulinotropic polypeptide. All authors contributed to discussion and critically reviewed the manuscript.

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Disclosure Summary: The authors have nothing to disclose in relation to this manuscript.

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