Comparative Effects of Proximal and Distal Small Intestinal Glucose Exposure on Glycemia, Incretin Hormone Secretion, and the Incretin Effect in Health and Type 2 Diabetes

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OBJECTIVE

Cells releasing glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) are distributed predominately in the proximal and distal gut, respectively. Hence, the region of gut exposed to nutrients may influence GIP and GLP-1 secretion and impact on the incretin effect and gastrointestinal-mediated glucose disposal (GIGD). We evaluated glycemic and incretin responses to glucose administered into the proximal or distal small intestine and quantified the corresponding incretin effect and GIGD in health and type 2 diabetes mellitus (T2DM).

RESEARCH DESIGN AND METHODS

Ten healthy subjects and 10 patients with T2DM were each studied on four occasions. On two days, a transnasal catheter was positioned with infusion ports opening 13 cm and 190 cm beyond the pylorus, and 30 g glucose with 3 g 3-*O*-methylglucose (a marker of glucose absorption) was infused into either site and 0.9% saline into the alternate site over 60 min. Matching intravenous isoglycemic clamp studies were performed on the other two days. Blood glucose, serum 3-*O*-methylglucose, and plasma hormones were evaluated over 180 min.

RESULTS

In both groups, blood glucose and serum 3-*O*-methylglucose concentrations were higher after proximal than distal glucose infusion (all $P < 0.001$). Plasma GLP-1 increased minimally after proximal, but substantially after distal, glucose infusion, whereas GIP increased promptly after both infusions, with concentrations initially greater, but less sustained, with proximal versus distal infusion (all $P < 0.001$). Both the incretin effect and GIGD were less with proximal than distal glucose infusion (both $P \leq 0.009$).

CONCLUSIONS

The distal, as opposed to proximal, small intestine is superior in modulating postprandial glucose metabolism in both health and T2DM.

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The incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) are secreted from the gut in response to nutrient ingestion. In health, they account for the augmented insulin response to enteral, versus intravenous (i.v.), glucose administration—known as the incretin effect—and play a major role in gastrointestinal-mediated glucose disposal (GIGD) (1). These effects are attenuated with the progression of glucose intolerance and insulin resistance (2) and impaired in type 2 diabetes mellitus (T2DM) (1,3), related in part to the diminished insulinotropic action of GIP (4,5). By contrast, the insulinotropic effect of GLP-1 is better maintained in T2DM (5). Moreover, GLP-1 has a physiological role in slowing gastric emptying (6) and suppressing glucagon secretion (7) and energy intake (8). Accordingly, GLP-1 is more important than GIP in driving the incretin effect and GIGD in T2DM.

GIP is secreted from enteroendocrine K cells, which predominate in the duodenum and jejunum (9), whereas GLP-1–releasing L cells are most densely distributed in the ileum and colon (10). Although indirect neuroendocrine loops arising from the duodenum could contribute to GLP-1 secretion (11), compelling evidence suggests that direct exposure of L cells to enteral stimuli is a primary route for GLP-1 stimulation (12). Interventions that delay absorption of glucose from dietary carbohydrates, such as administration of acarbose (an α-glucosidase inhibitor), enhance the exposure of nutrients to the distal gut and are associated with increased, albeit delayed, GLP-1 secretion (13). Furthermore, a formulation of lauric acid designed to release a relatively small load of fatty acid in the ileum and colon induces substantial stimulation of GLP-1 and reduces postprandial glycemia, without affecting plasma GIP levels, in patients with T2DM (14). Conversely, GLP-1 was minimally stimulated when intraduodenal glucose (3.5 kcal/min) was restricted only to the proximal 60-cm segment of the small intestine (i.e., the duodenum and proximal jejunum) in healthy individuals, in contrast to a substantial response when glucose was also allowed to access the distal small intestine (15). These observations suggest that the secretion of GLP-1 and associated metabolic effects in response to enteral nutrients are dependent on the region of the gut exposed.

Patients with T2DM who have undergone Roux-en-Y gastric bypass (16) or placement of a duodenojejunal bypass liner (17) exhibit improved glycemic control, associated with enhanced GLP-1 secretion and the incretin effect. However, it remains unclear as to whether these changes reflect a superior glucose-lowering capacity of the distal gut, disrupted regulation of gastric emptying or small intestinal transit (18), and/or dietary restrictions associated with the procedures (19). We have reported that intrajejunal infusion of glucose elicited greater GIP and GLP-1 secretion than did intraduodenal infusion (50 cm vs. 12 cm beyond the pylorus; 2 kcal/min), associated with enhanced insulin release and a tendency for diminished blood glucose excursion (20), although these observations were derived from a retrospective comparison of two different groups of healthy subjects, with insufficient numbers to detect differences in the glycemic response. To better characterize the glucose-regulating capacity of the proximal and distal small intestine, we compared the glycemic and incretin hormone responses to glucose administered directly into the proximal or distal small intestine (13 or 190 cm beyond the pylorus [i.e., the duodenum or ileum]) and quantified the respective incretin effect and GIGD using i.v. isoglycemic clamps, in both healthy subjects and subjects with T2DM in the current study. Our hypothesis was that administration of glucose into the ileum, compared with the duodenum, would result in enhanced GLP-1 secretion, greater incretin effect, and GIGD and, hence, lower blood glucose excursions.

RESEARCH DESIGN AND METHODS

Participants

Ten healthy subjects and 10 patients with T2DM managed by diet or metformin monotherapy (Supplementary Table 1) were studied after providing written informed consent. Patients on metformin (n = 4) were instructed to withhold the dose for 48 h before each study. None had impaired liver or renal function, had diabetic microvascular complications (including autonomic dysfunction, as assessed by standardized cardiovascular reflex tests [21]), was a smoker, or was taking any medication known to affect gastrointestinal function. The protocol (Supplementary Fig. 1) was approved by the Royal Adelaide Hospital Human Research Ethics Committee.

Protocol

Subjects were studied on 4 days each, separated by $\geq$7 days, including 2 enteral glucose infusion days and 2 i.v. isoglycemic glucose infusion days, in a crossover design. The order of the enteral infusions was randomized and double-blinded, facilitated by a biostatistician who generated the randomization code and a research officer who prepared the study solutions but was not involved in data collection or analysis. After a standardized evening meal ($\sim$1900 h) and overnight fast from solids and liquids (other than water), subjects attended the laboratory at $\sim$0800 h.

On the 2 enteral glucose infusion days, a customized multilumen silicone catheter (Dentsleeve International, Mississauga, Ontario, Canada) was inserted through an anesthetized nostril to the small intestine by peristalsis. The catheter was 3 m long, incorporated 23 lumens within its 4.2-mm diameter, and included: *1*) a 10-mL balloon near the tip, which was inflated to facilitate passage of the catheter; *2*) two infusion ports spaced 177 cm apart for administration of glucose or saline; and *3*) 20 side holes spaced 10 cm apart to allow continuous monitoring of the position of the catheter by perfusing with saline and measuring the antral and duodenal transmucosal potential difference, as described (15). The catheter was positioned with the two infusion ports located at 13 cm (i.e., duodenum) and 190 cm (i.e., ileum) beyond the pylorus (22), with the subject lying supine. If the catheter could not be positioned before 1500 h, the study was terminated and repeated 7 days later. Otherwise, enteral infusions all commenced at 1500 h (defined as t = 0 min), when a 120-mL aqueous solution containing 30 g glucose and 3 g 3-*O*-methylglycose (as a marker of glucose absorption) was infused into either the duodenum or ileum during t = 0–60 min (i.e., 2 mL/min; 2 kcal/min) in randomized order, while infusing 0.9% saline at the alternate site (2 mL/min). Subsequent to each enteral infusion day, an i.v. isoglycemic clamp study (1) was performed without the intestinal catheter in site at $\sim$1500 h.

On each study day, an i.v. cannula was inserted into a forearm vein and the arm was kept warm with a heat pad for sampling of arterialized blood at frequent intervals to measure blood glucose, serum 3-*O*-methylglucose, plasma C-peptide, insulin, glucagon, and total GLP-1 and GIP concentrations. Blood samples were collected into ice-chilled serum and EDTA tubes and centrifuged immediately at 3,200 rpm for 15 min at 4°C. Serum and plasma were separated and stored at −80°C until analyzed.

Measurements

Blood glucose was measured using the glucose oxidase technique (2300 STAT Plus; YSI, Yellow Springs, OH). Plasma insulin and C-peptide were measured by ELISA (10-1113 and 10-1136-01, respectively; Mercodia, Uppsala, Sweden). Plasma glucagon and total GLP-1 were measured by radioimmunoassay (GL-32K and GLPIT-36HK, respectively; Millipore, Billerica, MA). Plasma total GIP was measured by radioimmunoassay using modifications of a previously published method (23). Serum 3-*O*-methylglucose was measured by liquid chromatography and mass spectrometry (24).

Statistical Analysis

Basal measurements on the 4 study days in both groups were compared using one-factor repeated-measures ANOVA. Intergroup differences were compared using unpaired Student *t* tests. Integrated areas under the curve (iAUCs), reflecting changes from baseline, were calculated using the trapezoidal rule. Intragroup differences between proximal and distal glucose infusions, and between the enteral and respective i.v. isoglycemic infusion days, were compared using paired Student *t* test. Changes in blood glucose, plasma hormones, and serum 3-*O*-methylglucose were assessed using two-factor repeated-measures ANOVA, with treatment and time as factors. Post hoc comparisons were adjusted by Holm–Bonferroni correction. The incretin effect was calculated from the iAUCs for plasma insulin and C-peptide as $([iAUC_{enteral\ glucose} - iAUC_{i.v.\ glucose}]/iAUC_{enteral\ glucose}) \times 100\%$. GIGD was calculated as 100% × (30 − i.v. glucose [g])/30 (25). The Matsuda index, to estimate whole-body insulin sensitivity, was calculated as 10,000/square root of (fasting glucose [mg/dL] × fasting insulin [mU/L] × mean $glucose_{0–120\ min}$ × mean $insulin_{0–120\ min}$) (26). The HOMA of insulin resistance (HOMA-IR) was calculated as fasting insulin (mU/L) × fasting glucose (mmol/L)/22.5. Insulin clearance was calculated as molar ratio of $AUC_{180\ min}$ (C-peptide) and $AUC_{180\ min}$ (insulin) (27). The insulinogenic index was calculated as $C\text{-peptide}_{(0–30\ min)}/glucose_{(0–30\ min)}$ to evaluate β-cell responsiveness (28). Based on our previous work, a sample size of 10 subjects was calculated to have at least 80% power (at $\alpha = 0.008$ to enable correction for multiple post hoc testing) to detect a difference in the iAUC for plasma total GLP-1 of 80 pmol/L × h with an SD of 45 pmol/L × h between treatments (20,29). All analyses were performed using SPSS 25.0 (IBM, Armonk, NY). Data are means ± SEM; $P < 0.05$ was considered statistically significant.

RESULTS

All subjects tolerated the study well.

Blood Glucose and GIGD

Fasting blood glucose concentrations did not differ among the 4 study days in either group (Table 1). In response to enteral glucose infusion, blood glucose concentrations increased before returning to baseline, with lower concentrations in response to distal than proximal infusion in both healthy subjects and subjects with T2DM (iAUC: $P = 0.038$ and 0.006, respectively; ANOVA: $P < 0.001$ each for treatment × time interaction, with significant differences in blood glucose concentrations between $t = 15–60$ min in healthy subjects and $t = 30–120$ min in subjects with T2DM [$P < 0.05$ for each]) (Table 2 and Fig. 1*A* and *B*). In both groups, i.v. isoglycemic glucose infusion closely replicated the blood glucose profile observed with proximal or distal small intestinal glucose infusion, with the total glucose load administered being less for the latter ($P = 0.009$ and <0.001, respectively). Accordingly, GIGD was greater with distal versus proximal small intestinal glucose infusion (Table 2).

Both fasting glucose and the iAUCs after proximal and distal small intestinal glucose infusions were higher in subjects with T2DM than healthy subjects ($P < 0.01$ for each). However, GIGD following either proximal or distal small intestinal glucose infusion was numerically, but not statistically, less in subjects with T2DM than healthy subjects (Tables 1 and 2).

Plasma Insulin, C-Peptide, HOMA-IR, Matsuda Index, Insulinogenic Index, Incretin Effect, and Insulin Clearance

Fasting plasma insulin and C-peptide concentrations, as well as HOMA-IR values, did not differ among the 4 study days in either group (Table 1). In response to enteral glucose infusion, plasma insulin and C-peptide concentrations increased before returning to baseline, with lower concentrations in response to distal than proximal infusion in healthy subjects (iAUCs: $P < 0.001$ for each; ANOVA: $P < 0.001$ each for treatment × time interaction, with significant differences in plasma concentrations during $t = 15–60$ min [$P < 0.05$ for each]), but without any difference between proximal and distal infusions in subjects with T2DM (Table 2 and Fig. 1*C–F*). Both Matsuda and insulinogenic indices were higher with distal versus proximal small intestinal glucose infusion in both groups ($P < 0.05$ for each) (Table 2), indicating greater whole-body insulin sensitivity and β-cell responsiveness following distal infusion. Insulin clearance was less in response to enteral than i.v. glucose infusion ($P < 0.05$ for each), but did not differ between the two enteral or i.v. glucose infusion days within each group or under the same conditions between the two groups (Table 2). Compared with i.v. isoglycemic glucose infusion, enteral glucose was associated with higher plasma insulin and C-peptide concentrations (both $P < 0.001$), such that the incretin effect, based on either insulin (each $P < 0.05$) or C-peptide (each $P < 0.001$), was greater with distal versus proximal small intestinal glucose infusion in both groups (Table 2).

Fasting insulin concentrations tended to be higher ($P = 0.078$), and HOMA-IR values ($P = 0.022$) and fasting C-peptide concentrations ($P < 0.001$) were higher, in subjects with T2DM than healthy subjects (Table 1). The iAUC for insulin following either proximal or distal small intestinal glucose infusion did not differ between the two groups. The iAUC for C-peptide also did not differ between the two groups following proximal small intestinal glucose infusion, but was greater in subjects with T2DM than healthy subjects following distal

Table 1—Basal levels of blood glucose, plasma insulin (HOMA-IR), C-peptide, glucagon, total GIP, and GLP-1 on both the enteral (proximal and distal) and respective i.v. isoglycemic glucose infusion days in healthy subjects and subjects with T2DM

	Enteral glucose (P)	Intravenous glucose (P)	Enteral glucose (D)	Intravenous glucose (D)	Means	*P* value
Healthy subjects (*n* = 10)						
Glucose (mmol/L)	5.0 ± 0.2	4.9 ± 0.1	4.9 ± 0.1	5.1 ± 0.1	5.1 ± 0.1	0.411
Insulin (mU/L)	2.2 ± 0.6	1.7 ± 0.5	2.5 ± 0.5	2.5 ± 0.7	2.2 ± 0.5	0.156
HOMA-IR (mIU · mmol/L^2)	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.6 ± 0.2	0.5 ± 0.1	0.134
C-peptide (pmol/L)	261.0 ± 28.5	249.8 ± 35.3	247.9 ± 21.5	299 ± 38.2	264.5 ± 28.5	0.116
Glucagon (pg/mL)	61.1 ± 4.7	61.3 ± 4.6	62.8 ± 4.9	62.3 ± 5.7	61.9 ± 4.6	0.924
GIP (pmol/L)	9.4 ± 2.0	11.7 ± 3.2	9.6 ± 2.0	9.2 ± 1.8	9.9 ± 1.7	0.947
GLP-1 (pmol/L)	21.5 ± 1.9	23.8 ± 2.4	22.7 ± 1.6	21.6 ± 1.8	22.4 ± 1.4	0.636
Subjects with T2DM (*n* = 10)						
Glucose (mmol/L)	6.1 ± 0.4	6.0 ± 0.3	5.9 ± 0.3	6.2 ± 0.3	6.1 ± 0.3*	0.375
Insulin (mU/L)	3.6 ± 0.5	4.2 ± 0.8	3.7 ± 0.7	2.7 ± 0.3	3.6 ± 0.5	0.062
HOMA-IR (mIU · mmol/L^2)	0.9 ± 0.1	1.1 ± 0.2	1.0 ± 0.2	0.7 ± 0.1	0.9 ± 0.1*	0.127
C-peptide (pmol/L)	518.1 ± 55.8	608.9 ± 78.3	474.4 ±56.9	517.1 ± 60.0	526.2 ± 58.3*	0.007
Glucagon (pg/mL)	65.4 ± 6.2	64.0 ± 5.6	68.2 ± 5.9	62.8 ± 6.9	65.1 ± 5.8	0.447
GIP (pmol/L)	14.9 ± 0.9	17.2 ± 2.0	13.4 ± 1.3	15.5 ± 1.6	15.2 ± 1.2*	0.167
GLP-1 (pmol/L)	24.5 ± 2.8	25.5 ± 2.9	25.9 ± 3.0	24.9 ± 2.3	25.2 ± 2.6	0.817

One-factor repeated-measures ANOVA was used to determine statistical difference among the 4 study days in both groups. Unpaired Student *t* test was used to determine statistical difference between healthy subjects and subjects with T2DM. Data are means ± SEM. Enteral glucose (D), glucose infusion into the distal small intestine; Enteral glucose (P), glucose infusion into the proximal small intestine; Intravenous glucose (D), isoglycemic i.v. glucose infusion matching blood glucose concentrations on the enteral glucose (D) day; Intravenous glucose (P), isoglycemic i.v. glucose infusion matching blood glucose concentrations on the enteral glucose (P) day. *$P \leq 0.003$.

infusion ($P = 0.049$). However, the incretin effect (based on either insulin or C-peptide) induced by either proximal or distal small intestinal glucose infusion did not differ between the two groups (Table 2).

Plasma Glucagon

Fasting plasma glucagon concentrations did not differ among the 4 study days in either group (Table 1). In response to enteral glucose infusion, plasma glucagon concentrations were relatively stable on the distal infusion days, but decreased before returning to baseline on the proximal infusion days ($P < 0.001$), with concentrations being higher in response to distal than proximal infusion in healthy subjects (iAUC: $P = 0.019$; ANOVA: $P < 0.001$ for treatment × time interaction, with significant differences in plasma concentrations during t = 30–150 min [$P < 0.05$ for each]). However, in subjects with T2DM, plasma glucagon increased initially, followed by a gradual decline on both enteral glucose infusion days ($P < 0.001$ for each), with concentrations being higher in response to distal than proximal infusion (iAUC: $P = 0.007$; ANOVA: $P < 0.001$ for treatment × time interaction, with significant differences in plasma concentrations during t = 30–150 min [$P < 0.05$ for each]) (Table 2 and Fig. 1*G* and *H*). By contrast, plasma glucagon concentrations decreased during i.v. glucose infusions in both groups, without any difference between the 2 days.

Neither fasting glucagon concentrations nor the iAUC for glucagon in response to proximal or distal small intestinal glucose infusion differed between the two groups (Tables 1 and 2).

Plasma Total GIP

Fasting plasma total GIP concentrations did not differ among the 4 study days in either group (Table 1). In response to enteral glucose infusion, plasma GIP concentrations increased promptly on both days, with concentrations being initially higher, but less sustained, with proximal than distal infusion in both groups (ANOVA: $P < 0.001$ each for treatment × time interaction, with significant differences in plasma concentrations during t = 15–60 min and 120–180 min in healthy subjects and during t = 15–60 min in subjects with T2DM [$P < 0.05$ for each]). By contrast, plasma GIP concentrations remained unchanged during i.v. glucose infusions (Table 2 and Fig. 2*A* and *B*).

Fasting GIP concentrations were higher in subjects with T2DM than healthy subjects ($P = 0.017$). However, the iAUC for GIP in response to proximal or distal small intestinal glucose infusion did not differ between the two groups (Tables 1 and 2).

Plasma Total GLP-1

Fasting plasma total GLP-1 concentrations did not differ among the 4 study days in either group (Table 1). In response to enteral glucose, plasma GLP-1 concentrations increased minimally with proximal infusion, but substantially with distal infusion, such that GLP-1 concentrations were higher in the latter in both groups (iAUC: $P < 0.001$ for each; ANOVA: $P < 0.001$ each for treatment × time interaction, with significant differences in plasma concentrations during t = 30–150 min in healthy subjects and t = 15–180 min in subjects with T2DM [$P < 0.05$ for each]). By contrast, plasma GLP-1 concentrations remained unchanged during i.v. glucose infusions (Table 2 and Fig. 2*C* and *D*).

Neither fasting GLP-1 concentrations nor the iAUC for GLP-1 in response to proximal or distal small intestinal glucose infusion differed between the two groups (Tables 1 and 2).

Serum 3-*O*-Methylglucose

During and after enteral glucose infusion, serum 3-*O*-methylglucose concentrations increased steadily before a gradual decline, with lower concentrations in response to distal than proximal infusion in both groups (iAUC: $P < 0.001$ and $P = 0.002$, respectively; ANOVA: $P < 0.001$ each for treatment × time interaction, with significant differences in serum

Table 2—GIGD and integrated responses (during t = 0–180 min) of glucose, insulin, C-peptide, glucagon, GIP, and GLP-1 to the enteral (proximal or distal) and respective isoglycemic i.v. glucose in healthy subjects and patients with T2DM

	Healthy subjects (n = 10)			Subjects with T2DM (n = 10)		
	Proximal infusion	Distal infusion	P value	Proximal infusion	Distal infusion	P value
Amount of glucose (g/experiment)						
Enteral	30 ± 0	30 ± 0	—	30 ± 0	30 ± 0	—
Intravenous	22.0 ± 1.5	16.0 ± 1.0	0.009	24.6 ± 1.4	18.4 ± 1.5	<0.001
GIGD (%)	26.8 ± 4.9	46.5 ± 3.4	0.009	18.0 ± 4.7	38.6 ± 4.9	<0.001
Glucose iAUC (mmol/L · h)						
Enteral	5.3 ± 0.9	3.2 ± 0.3	0.038	9.2 ± 0.9	6.4 ± 0.9	0.006
Intravenous	6.3 ± 0.7	2.9 ± 0.6	<0.001	9.8 ± 1.2	5.9 ± 1.0	<0.001
Insulin iAUC (mU/L · h)						
Enteral	33.5 ± 4.3	25.4 ± 2.8	<0.001	32.5 ± 11.5	33.2 ± 7.1	0.960
Intravenous	16.0 ± 2.4	6.8 ± 0.7	<0.001	14.3 ± 2.7	9.8 ± 1.3	0.091
Incretin effect$_{insulin}$ (%)	48.6 ± 7.6	71.9 ± 2.8	0.025	43.3 ± 8.2	62.0 ± 5.9	0.042
Matsuda index (dL/mg · L/mU)	14.8 ± 4.7	21.0 ± 8.2	0.019	11.9 ± 3.1	16.9 ± 6.3	0.012
C-peptide iAUC (pmol/L · h)						
Enteral	1,782.3 ± 135.0	1,506.0 ± 150.3	<0.001	2,261.2 ± 384.2	2,223.5 ± 305.9	0.886
Intravenous	1,047.2 ± 113.0	510.4 ± 78.0	<0.001	1,245.3 ± 150.9	844.8 ± 115.3	0.002
Incretin effect$_{C\text{-}peptide}$ (%)	41.8 ± 3.8	67.1 ± 2.7	<0.001	41.1 ± 5.7	58.9 ± 5.7	<0.001
Insulinogenic index 1 (pmol/mmol)	225.8 ± 38.7	374.8 ± 44.9	0.029	198.0 ± 43.5	480.6 ± 92.7	0.030
Insulin clearance						
Enteral	10.6 ± 1.3	10.7 ± 1.3	0.873	9.1 ± 1.0	8.6 ± 1.0	0.869
Intravenous	14.1 ± 1.9	17.0 ± 3.5	0.140	18.6 ± 2.1	19.8 ± 1.0	0.490
Glucagon iAUC (pg/mL · h)						
Enteral	−20.7 ± 6.9	3.5 ± 5.1	0.019	−24.1 ± 6.1	3.3 ± 7.2	0.007
Intravenous	−35.4 ± 5.6	−31.8 ± 6.4	0.378	−31.6 ± 5.8	−31.6 ± 7.9	1.000
GIP iAUC (pmol/L · h)						
Enteral	48.5 ± 4.7	41.6 ± 7.3	0.157	54.5 ± 5.9	49.1 ± 4.5	0.272
Intravenous	−3.1 ± 3.1	1.3 ± 2.7	0.240	−10.6 ± 3.9	−1.8 ± 2.8	0.002
GLP-1 iAUC (pmol/L · h)						
Enteral	0.4 ± 3.6	51.6 ± 8.6	<0.001	−2.6 ± 4.3	46.6 ± 8.4	<0.001
Intravenous	−21.3 ± 4.3	−15.4 ± 4.2	0.135	−17.2 ± 4.1	−14.1 ± 3.4	0.403
3-*O*-Methylglucose iAUC (mmol/L · min)	57.4 ± 3.1	31.6 ± 3.9	<0.001	67.2 ± 5.1	45.2 ± 5.0	0.002

Differences between proximal and distal small intestinal glucose infusion and between the enteral and respective i.v. glucose infusion days were compared using paired Student t test in both healthy subjects and subjects with T2DM. Data are means ± SEM.

concentrations during t = 30–120 min in healthy subjects and t = 60–90 min in subjects with T2DM [$P < 0.05$ for each]) (Table 2 and Fig. 2*E* and *F*).

The iAUC for 3-*O*-methylglucose was greater (P = 0.035) in response to distal small intestinal glucose infusion and tended to be greater (P = 0.095) in response to proximal infusion in subjects with T2DM than healthy subjects (Table 2).

CONCLUSIONS

The gastrointestinal tract is pivotal to the regulation of glucose metabolism, particularly in the postprandial phase. However, the relative importance of exposure of different regions of the gut to nutrients is poorly characterized. In the current study, we evaluated the glycemic and incretin responses to glucose administered directly into either the proximal or distal small intestine (i.e., duodenum or ileum) and also quantified the corresponding incretin effect and GIGD as well as glucose absorption in both health and T2DM. The experimental approach was novel and allowed a detailed comparison of glucose-regulating capacity between the proximal and distal small intestine. The key findings were that, in both groups, blood glucose concentrations were substantially lower in response to distal than proximal small intestinal glucose infusion, associated with greater GLP-1 secretion, incretin effect, and GIGD and slower intestinal glucose absorption, although the stimulation of GIP was less and glucagon concentrations were augmented. These observations support the superiority of the distal, compared with the proximal, small intestine in modulating postprandial glucose metabolism in both health and T2DM and may contribute to an improved understanding of the mechanisms underlying the metabolic benefits of both bariatric surgery and the duodenojejunal bypass liner.

Strengths of our study are that both the site and rate of enteral glucose administration were carefully standardized, and the volume infused at the alternate site was controlled for using 0.9% saline. The rate of glucose infusion (i.e., 2 kcal/min, which is within the physiological range of gastric emptying [30]) was chosen to allow a clear differentiation of proximal and distal intestinal effects, because the proximal glucose load would be expected to be absorbed within 30 cm in healthy individuals (31–33) and an even shorter distance in patients with T2DM (24). The advantage of infusing glucose, rather than mixed nutrients, was that potential confounding related to different rates of intraluminal digestion was removed.

As expected, the incretin hormone responses following intraduodenal and intraileal glucose infusion differed substantially in both healthy subjects and subjects with T2DM, and i.v. isoglycemic

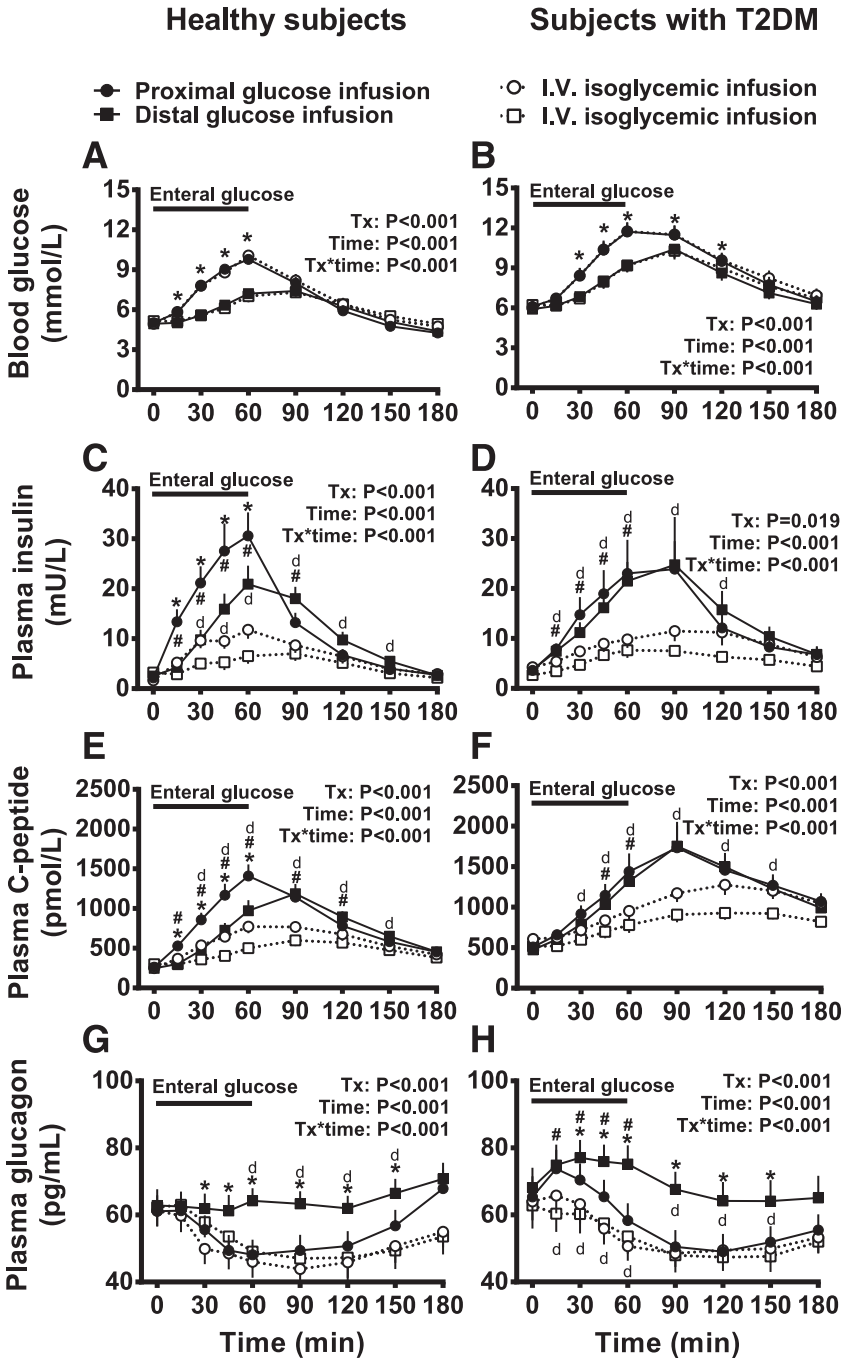

Figure 1—Effects of enteral (proximal or distal) or i.v. isoglycemic glucose infusion on blood glucose (*A* and *B*), plasma insulin (*C* and *D*), plasma C-peptide (*E* and *F*), and plasma glucagon (*G* and *H*) in healthy subjects ($n = 10$) and patients with T2DM ($n = 10$), respectively. Repeated-measures ANOVA was used to determine statistical difference. Results of ANOVA are reported as *P* values for differences by treatment (Tx), differences over time (time), and differences due to interaction of experiment and time (Tx*time). Post hoc comparisons were adjusted for multiple comparisons by Holm–Bonferroni correction. *$P < 0.05$ for proximal vs. distal enteral glucose infusion; #$P < 0.05$ for proximal enteral vs. corresponding i.v. glycemic glucose infusion; $^{d}P < 0.05$ for distal enteral vs. corresponding i.v. glycemic glucose infusion. Data are mean values ± SEM.

glucose infusion had no effect on either GIP or GLP-1. In keeping with our previous findings (4,29), intraduodenal glucose infusion at the rate of 2 kcal/min induced prompt and substantial GIP but minimal GLP-1 secretion in both groups. By contrast, ileal glucose infusion was associated with a marked increase in GLP-1. Interestingly, there was still considerable GIP secretion induced by ileal glucose, although the initial response was less than for proximal administration. That plasma GIP decreased promptly after the end of intraduodenal, but more slowly with intraileal, glucose infusion and that the stimulation of GLP-1 was sustained following ileal glucose infusion are likely to reflect the differences in the rate of glucose absorption, as assessed by serum 3-*O*-methylglucose, affecting the duration/length of gut exposed to glucose in these two regions. These observations highlight the potential for targeted delivery of intraluminal stimuli to optimize endogenous GLP-1 secretion and also establish that even the distal small intestine represents an important source of GIP production. Based on double immunohistochemistry and in situ hybridization, it has been reported that GLP-1 and GIP are colocalized in a subset of enteroendocrine cells throughout the small intestine of pigs, rats, and humans (34), suggesting that the concept of discrete K and L cells is inaccurate. It should also be noted that the magnitude of GLP-1 and GIP responses to enteral glucose infusion did not differ substantially between the healthy group and two groups with T2DM, although basal GIP levels were slightly higher in the groups with T2DM, possibly related to impaired GIP signaling in these patients (35).

In support of our hypothesis, the increment in blood glucose induced by intraileal glucose infusion was substantially lower (~3 mmol/L) than that associated with intraduodenal infusion, reflected as a doubling of GIGD in both healthy subjects and subjects with T2DM. Furthermore, whole-body insulin sensitivity, β-cell responsiveness, and incretin effect were greater following intraileal than intraduodenal glucose infusion in both groups, without any difference in insulin clearance between the two enteral glucose infusion days or between the two groups. As expected (27), insulin clearance induced by enteral glucose infusion was less compared with i.v. glucose infusion, which may somewhat contribute to augmented insulin concentrations during enteral glucose infusion. Further studies are needed to better understand the mechanisms underlying this phenomenon. That concentrations of plasma insulin and C-peptide were modestly higher following intraduodenal than intraileal glucose infusion is likely to reflect the difference in blood glucose levels between the two study days, given that the insulinotropic effects

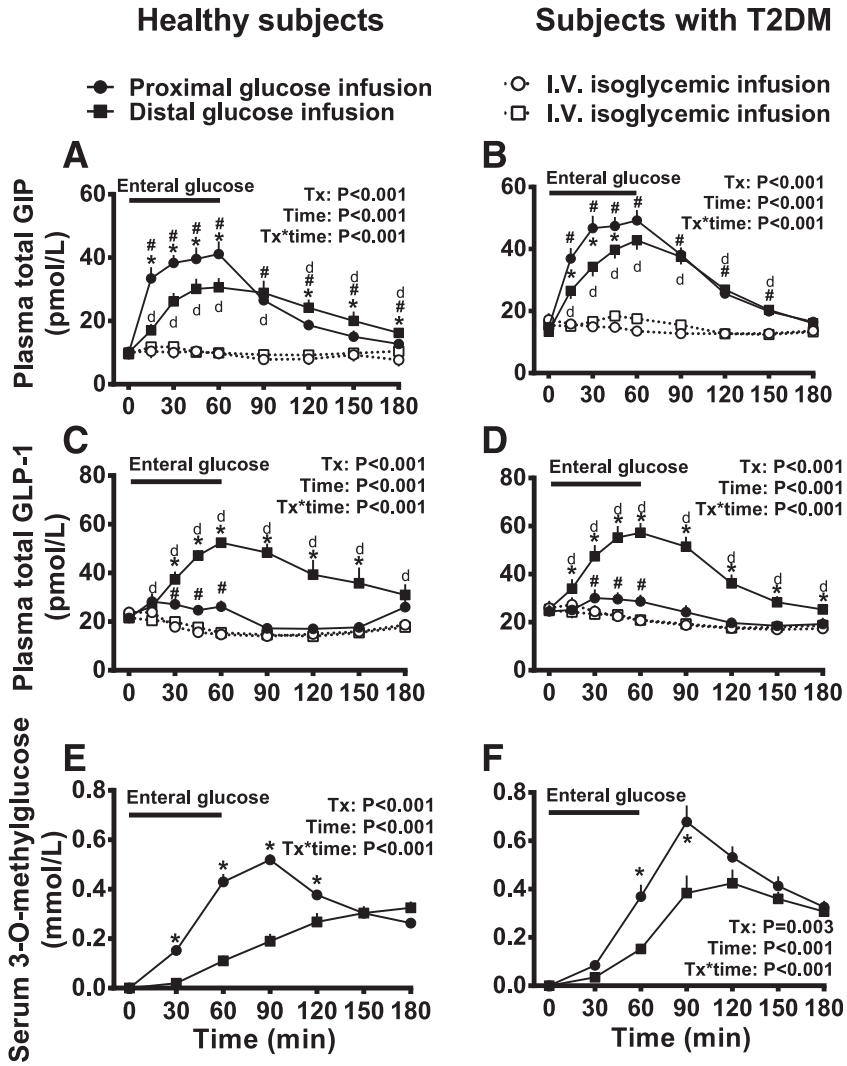

Figure 2—Effects of enteral (proximal or distal) or i.v. isoglycemic glucose infusion on plasma total GIP (*A* and *B*), plasma total GLP-1 (*C* and *D*), and serum 3-*O*-methylglucose during proximal or distal enteral glucose infusion (*E* and *F*) in healthy subjects (n = 10) and patients with T2DM (n = 10), respectively. Repeated-measures ANOVA was used to determine statistical difference. Results of ANOVA are reported as P values for differences by treatment (Tx), differences over time (time), and differences due to interaction of experiment and time (Tx*time). Post hoc comparisons were adjusted for multiple comparisons by Holm–Bonferroni correction. *$P < 0.05$ for proximal vs. distal enteral glucose infusion; #$P < 0.05$ for proximal enteral vs. corresponding i.v. glycemic glucose infusion; $^{d}P < 0.05$ for distal enteral vs. corresponding i.v. glycemic glucose infusion. Data are mean values ± SEM.

of both GIP and GLP-1 are glucose dependent (5). The latter may have also accounted, at least partly, for the lack of significant difference in the incretin effect and GIGD between the two groups. Moreover, our subjects with T2DM maintained excellent glycemic control with diet or metformin monotherapy (mean HbA_{1c} of 6.2%) and presented almost normal basal glucose levels (~6 mmol/L) after ~20 h fasting, so that the insulinotropic and glucose-lowering effects of GIP may have been retained to some extent (36). Finally, the current study was not powered to compare differences between the two groups, so a type 2 error cannot be ruled out.

The differences in serum 3-*O*-methylglucose concentrations indicate that intraileal glucose infusion was associated with a slower rate of active glucose absorption via sodium–glucose cotransporter 1 (SGLT-1), and this would be complementary to the enhanced incretin effect in limiting the blood glucose response to intraileal glucose. Although a functional SGLT-1 pathway is present in the human ileum and accounts for glucose-induced GLP-1 release from human ileal tissues (37), a detailed comparison of this system at different sites along the human gastrointestinal tract has not been reported. Our observations suggest that, relative to the duodenum and jejunum, the expression and/or the function of SGLT-1 is decreased in the ileum. In addition, the iAUC for serum 3-*O*-methylglucose also suggested more rapid glucose absorption, particularly within the distal small intestine, in subjects with T2DM than healthy subjects. However, given that serum 3-*O*-methylglucose concentrations had not returned to baseline by the end of each study, we cannot be certain as to the completeness of glucose absorption within the small intestine, particularly when infused into the ileum. Nonetheless, none of the subjects experienced diarrhea or flatulence that would be expected upon colonic fermentation of incompletely absorbed glucose, so we doubt that substantial quantities of glucose entered the colon. A hydrogen breath test may have helped confirm this assumption.

The difference in blood glucose profiles between intraduodenal and intraileal glucose infusion does not seem to be driven by glucagon, because intraileal glucose infusion was associated, paradoxically, with a modest increase in plasma glucagon, whereas intraduodenal glucose suppressed glucagon to a similar degree to that observed with i.v. glucose infusion in both groups. The mechanisms accounting for these distinct glucagon responses remain to be understood. There is emerging evidence that the gastrointestinal tract represents an extrapancreatic source of glucagon production (38). We have also reported that intraduodenal infusion of glucose at a higher rate (4 kcal/min) than in the current study, although inducing substantial GLP-1 and GIP secretion, increased plasma glucagon concentrations in patients with T2DM (29). It should also be noted that GLP-1 (which causes suppression) and GIP (which induces stimulation) counterregulate glucagon levels in a glucose-dependent manner (39); in the context of lower blood glucose concentrations after intraileal than intraduodenal glucose infusion, it would be expected that the glucagonostatic effect of GLP-1 would be less and the glucagonotropic effect of GIP would be greater.

Several limitations should be noted. First, the sample sizes were relatively small and calculated to provide power for comparisons of the responses to proximal and distal small intestinal glucose infusion within each group, rather than differences between the two groups. Second, the subjects with T2DM were essentially uncomplicated and well controlled by diet and/or metformin, so that any extrapolation of our findings, particularly to those with morbid obesity and/or poorly controlled T2DM, should be circumspect. Third, this proof-of-concept study used a single, fixed rate of glucose infusion to control for potential confounding factors, such as meal composition and variations in nutrient transit across the different gut regions, so the effects of other macronutrients and different rates of infusion remain to be determined. Fourth, the overall load of glucose used (30 g over 60 min) was relatively small; a larger load (e.g., 60 g) may have been more representative, but would have prolonged the technically demanding protocol, substantially compromising feasibility. Moreover, our previous studies indicate the incretin response to intraduodenal glucose is dependent primarily on the rate, rather than the load, of glucose infusion (4,24,29). Furthermore, the two groups were not matched for age or BMI, although intergroup comparisons were not primary aims of the study. Finally, the isoglycemic clamps were conducted without the enteral infusion catheter, which would have compromised the feasibility substantially. This is most unlikely to be a major confounder, because intraduodenal infusion of 0.9% saline has no effect on GIP, GLP-1, or insulin secretion in healthy subjects (40).

In summary, in both health and T2DM, distal small intestinal glucose exposure is associated with a much lower blood glucose excursion, slower glucose absorption, markedly greater plasma GLP-1, slightly lower but more sustained GIP responses, and substantially greater incretin effect and GIGD when compared with proximal glucose infusion. These observations support the concept of diverting nutrients from the proximal to the distal gut, such as by nutritional (complex carbohydrates), pharmacological (α-glucosidase inhibitors), or surgical (Roux-en-Y bypass) means, for the management of T2DM.

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