

# Effect of Endogenous GLP-1 on Insulin Secretion in Type 2 Diabetes

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**OBJECTIVE**—The incretins glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) account for up to 60% of postprandial insulin release in healthy people. Previous studies showed a reduced incretin effect in patients with type 2 diabetes but a robust response to exogenous GLP-1. The primary goal of this study was to determine whether endogenous GLP-1 regulates insulin secretion in type 2 diabetes.

**METHODS**—Twelve patients with well-controlled type 2 diabetes and eight matched nondiabetic subjects consumed a breakfast meal containing D-xylose during fixed hyperglycemia at 5 mmol/l above fasting levels. Studies were repeated, once with infusion of the GLP-1 receptor antagonist, exendin-(9–39) (Ex-9), and once with saline.

**RESULTS**—The relative increase in insulin secretion after meal ingestion was comparable in diabetic and nondiabetic groups ( $44 \pm 4\%$  vs.  $47 \pm 7\%$ ). Blocking the action of GLP-1 suppressed postprandial insulin secretion similarly in the diabetic and nondiabetic subjects ( $25 \pm 4\%$  vs.  $27 \pm 8\%$ ). However, Ex-9 also reduced the insulin response to intravenous glucose ( $25 \pm 5\%$  vs.  $26 \pm 7\%$ ; diabetic vs. nondiabetic subjects), when plasma GLP-1 levels were undetectable. The appearance of postprandial ingested D-xylose in the blood was not affected by Ex-9.

**CONCLUSIONS**—These findings indicate that in patients with well-controlled diabetes, the relative effects of enteral stimuli and endogenous GLP-1 to enhance insulin release are retained and comparable with those in nondiabetic subjects. Surprisingly, GLP-1 receptor signaling promotes glucose-stimulated insulin secretion independent of the mode of glucose entry. Based on rates of D-xylose absorption, GLP-1 receptor blockade did not affect gastric emptying of a solid meal. *Diabetes* 59:1330–1337, 2010

**G**lucagon-like peptide 1 (GLP-1) is a gut-brain peptide that is a major component of the incretin effect and is essential for normal glucose tolerance (1). Based on studies in which synthetic GLP-1, or GLP-1 receptor (GLP-1r) agonists, is administered to humans, GLP-1 has a broad range of actions that promote glucose homeostasis, including stimulating insulin secretion (2), suppressing glucagon release (3–4), delaying gastric emptying (5), and increasing hepatic glucose balance (6–7). Importantly, and unlike other

insulinotropic gut peptides, the effects of GLP-1 on glucose metabolism are retained in people with diabetes (8–10). This has led to the development of novel therapeutic compounds for use in diabetic patients that are based on GLP-1r signaling (11).

The physiologic role of GLP-1 in individuals with diabetes has not been determined. However, there are several reasons to question whether the GLP-1 system is fully functional in this patient group. First, there is some evidence that GLP-1 secretion in response to meal ingestion in type 2 diabetes is impaired (12–15), although this finding has not been uniform (16–17). Second, the sensitivity of insulin secretion to exogenous GLP-1 is reduced in diabetic individuals (18). Finally, it has long been believed that the augmentation of glucose-stimulated insulin secretion during enteral glucose absorption, the incretin effect, is severely attenuated in type 2 diabetes, implying that incretins such as GLP-1 are not normally active in this group of subjects.

In this study, we tested the hypothesis that the effect of endogenous GLP-1 to promote insulin secretion after meal ingestion is reduced in people with diabetes. Diabetic subjects and age- and weight-matched nondiabetic subjects were studied with and without infusion of the specific GLP-1r antagonist, exendin-(9–39) (Ex-9), during fixed hyperglycemia before and after a breakfast meal.

## RESEARCH DESIGN AND METHODS

**Subjects.** Twelve subjects with established type 2 diabetes (five females and seven males) and eight age- and BMI-matched nondiabetic subjects (six females and two males) were studied on two separate days (Table 1). All subjects were weight stable for 3 months prior to the experiments. Diabetic patients had good glycemic control with a mean A1C level of  $6.3 \pm 0.1\%$  (range 5.9–7.6%). Normal glucose tolerance was confirmed in the nondiabetic subjects by a 2-h venous plasma glucose level of  $<7.8$  mmol/l after a 75-g oral glucose tolerance test. The control subjects had no family history of type 2 diabetes, were free of any chronic medical conditions such as coronary artery disease, uncontrolled dyslipidemia, or hypertension, and received no medications for any of these conditions. The studies were approved by the institutional review board of the University of Cincinnati, and all participants provided written informed consent prior to the studies.

**Peptides.** Synthetic exendin-(9–39) (Cinalfa; Merck Biosciences AG, Läufelfingen, Switzerland) was greater than 95% pure, sterile, and free of pyrogens. Lyophilized peptide was prepared in 0.25% human serum albumin on the day of study. The use of synthetic Ex-9 is approved under the U.S. Food and Drug Administration Investigational New Drug 65,837.

**Experimental procedures.** Subjects were instructed to consume greater than 200 g of carbohydrate for 3 days before each visit and not to engage in vigorous physical activity. Subjects with diabetes withheld their oral antidiabetic medication for 3 days before each study. They were admitted to the General Clinical Research Center at Cincinnati Children's Hospital on separate occasions after an overnight fast. Intravenous catheters were placed in each forearm for the withdrawal of blood and the infusion of glucose and Ex-9; the arm used for blood sampling was continuously warmed using a heating pad to arterialize the venous blood. After removal of fasting blood samples, a primed continuous infusion of 20% glucose was started to achieve and maintain a target blood glucose concentration of 5 mmol/l greater than fasting levels (19). At 30 min, subjects received either 1) an intravenous bolus of synthetic Ex-9 (7,500 pmol/kg) for 1 min followed by a continuous infusion

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TABLE 1  
Characteristics of the study participants

	Patients with type 2 diabetes	Nondiabetic subjects
Age (year)	54 (41–63)	49 (34–60)
BMI (kg/m <sup>2</sup> )	34 (26–44)	32 (24–41)
Sex (F/M)	5/7	6/2
Duration of diabetes (month)	50 (1–200)	
Sulfonylurea/metformin/diet	4/9/3	

Data are presented as mean (range) unless otherwise noted.

(750 pmol/kg/min) for the remainder of the study, or 2) saline, as a control (19). The order of the infusions was balanced so that half the subjects received saline or Ex-9 as their first infusion, and the two experiments were separated by an interval of at least 1 week. At 90 min, subjects consumed a mixed nutrient breakfast (300 kcal with a calorie distribution of 40% carbohydrate, 20% protein, and 40% fat) consisting of scrambled eggs, English muffin, margarine, chocolate pudding mixed with 10 g D-xylose, and milk that was eaten within 10 min. Subjects were instructed to consume the pudding in the middle of the meal. The rate of intravenous glucose infusion was adjusted to maintain the blood glucose at the target rate throughout the meal and for the remainder of the study. Blood samples were drawn at -10, -5, 0, 15, 30, 45, 60, 70, 75, 80, 85, 90, 95, 100, 105, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 255, 260, 265, and 270 min; the plasma was separated within 60 min of blood withdrawal and stored at -80°C until assay. Blood samples were collected in tubes containing heparin, 50 mmol/L EDTA, and 500 kallikrein inhibitory units per milliliter of aprotinin.

**Assays.** Blood glucose concentrations were determined by a glucose oxidase method using a glucose analyzer (YSI 2300 STAT Plus; Yellow Springs Instruments, Yellow Springs, OH). Insulin, glucagon, total GLP-1, and C-peptide were measured by radioimmunoassays as described previously (19). D-xylose was measured by colorimetric assay (20), and total glucose-dependent insulinotropic polypeptide (GIP) was measured by ELISA (Linco Research, St. Charles, MO).

**Calculations and analysis.** Insulin secretion rates (ISRs) were derived from plasma C-peptide concentrations using deconvolution with population estimates of C-peptide clearance (21–22). Fasting concentrations of blood glucose, hormones, and ISR were taken as the mean of the three samples drawn at -10, -5, and 0 min. Preprandial insulin secretory responses were computed as the mean increments above fasting values of ISR from 60 to 90 min; these were used to determine the responses to intravenous glucose-induced hyperglycemia. Postprandial insulin secretory responses were calculated as the mean increments above the 60–90 min values of ISR from 95 to 270 min. This measure of postprandial insulin secretion reflects the augmentation of ISR, beyond 5 mmol/L hyperglycemia, by meal-induced stimuli, and has been used previously as a surrogate for the incretin effect (23–25). The D-xylose, GLP-1, and GIP concentrations from 80 to 90 min were taken as baseline, and levels after meal consumption (95–270 min for D-xylose and GLP-1; 95–240 min for GIP) were used to compute postprandial areas under the curve (AUC) above baseline. The glucagon levels from 80 to 90 min (preprandial) were compared with fasting as well as values of glucagon from 250 to 260 min. The stability of the glucose clamps was computed as the mean of coefficients of variation for each study from 60 to 270 min. The reproducibility of the clamps was computed as the difference in mean glucose from 60 to 270 for each subject.

The contribution of endogenous GLP-1 to insulin secretion was determined by comparing the mean values of ISR for each subject during infusion of saline or Ex-9. Separate comparisons were made for the preprandial and postprandial periods.

The parameters obtained from each subject in the two studies and from the diabetic and nondiabetic groups were compared using one- and two-way ANOVA with repeated measures. Spearman correlation was used to seek relationships between measured outcomes and subject characteristics such as A1C, and paired *t* test was used to compare measured variables within each study. Data are presented as the mean  $\pm$  SEM.

## RESULTS

Fasting glucose was significantly higher in the diabetic than in the nondiabetic subjects ( $P < 0.001$ ), but fasting insulin and C-peptide concentrations were comparable, and the values for each subject were similar on the days of the saline and Ex-9 studies (Table 2). Blood glucose

concentrations were raised by  $\sim 5$  mmol/L from fasting values to hyperglycemic levels from 60 to 270 min that were comparable during the Ex-9 and saline studies (Fig. 1, Table 2). The average clamped glucose levels (60–270 min) for nondiabetic subjects during the saline and Ex-9 studies were  $9.3 \pm 0.2$  and  $9.3 \pm 0.2$  mmol/L, respectively, with a mean difference of  $0.02 \pm 0.03$  mmol/L. For the diabetic subjects, the mean glucose concentrations (60–270 min) were  $12.0 \pm 0.4$  and  $12.1 \pm 0.4$  mmol/L for the control and Ex-9 clamps, respectively, and the mean difference was  $0.09 \pm 0.07$  mmol/L. The average coefficient of variation of the glucose concentrations during the hyperglycemic clamp control studies was  $4.4 \pm 0.4\%$  for the nondiabetic and  $4.9 \pm 0.5\%$  for the diabetic subjects.

The glucose infusion rates needed to reach target glycemia were higher in the nondiabetic compared with diabetic subjects during the preprandial (60–90 min;  $P < 0.01$ ) and postprandial (95–270 min;  $P < 0.05$ ) periods. Infusion of Ex-9 was associated with a significant reduction of the glucose infusion rates necessary to maintain target glycemia from 60 to 90 min in both groups ( $P < 0.05$  for both comparisons). After ingestion of the breakfast meal, the glucose infusion rate was initially reduced in some subjects to compensate for glucose influx from the gut, but was significantly higher than preprandial infusions by the end of the study (Fig. 1, Table 2).

In both the diabetic and nondiabetic groups,  $\beta$ -cell secretion rose in response to intravenous glucose administration to a plateau from 60 to 90 min (Fig. 2, Table 2); preprandial insulin, C-peptide, and ISR were greater in the nondiabetic than the diabetic group ( $P < 0.05$ ). Among the diabetic subjects, there were significant inverse correlations between the preprandial insulin secretory response and fasting glucose ( $r = -0.7$ ,  $P < 0.01$ ) and A1C ( $r = -0.8$ ,  $P < 0.05$ ). Compared with the study with saline infusion, GLP-1r blockade with Ex-9 caused a significant reduction of insulin secretion before meal ingestion (60–90 min), as indicated by significantly lower mean values of insulin, C-peptide, and ISR (Table 2). The GLP-1 effect on intravenous glucose-stimulated insulin secretion, taken as the percentage difference in mean ISR from 60 to 90 min with and without Ex-9, was  $25 \pm 5\%$  in diabetic subjects and  $26 \pm 7\%$  in the nondiabetic group (Fig. 3).

After meal consumption (95–270 min),  $\beta$ -cell secretion increased significantly over preprandial (60–90 min) values in both the nondiabetic ( $P < 0.01$ ) and diabetic ( $P < 0.05$ ) subjects; although the absolute responses were significantly lower in the diabetic group ( $P < 0.05$ ; Fig. 2, Table 2). Among the diabetic subjects, there were significant inverse correlations between postprandial insulin secretory responses and A1C ( $r = -0.6$ ,  $P < 0.05$ ). During the saline infusion study, meal ingestion significantly enhanced ISR, by  $47 \pm 7\%$  in the nondiabetic and  $44 \pm 4\%$  for diabetic subjects, above preprandial ISR. During the Ex-9 infusion, postprandial ISR was  $44 \pm 6\%$  and  $45 \pm 4\%$  higher than preprandial ISR in the nondiabetic and diabetic groups, respectively. Blocking endogenous GLP-1 with Ex-9 diminished postprandial ISR by  $27 \pm 8\%$  in nondiabetic and  $25 \pm 4\%$  in diabetic subjects, compared with the studies with saline infusion (Fig. 3).

Fasting glucagon concentrations in the diabetic subjects were similar during the saline and Ex-9 studies (Table 2). During both the saline and Ex-9 infusions, the hyperglycemic clamp suppressed glucagon levels from 80 to 90 min ( $P < 0.05$ ). Glucagon values at the conclusion of the experiments remained suppressed during the saline infu-

TABLE 2

Effect of meal ingestion during hyperglycemic clamp on  $\beta$ -cell hormonal response and gastrointestinal peptides in studies with and without intravenous Ex-9 in subjects with and without diabetes

Time interval (min)	Diabetic subjects		Nondiabetic subjects		Statistical effects ( <i>P</i> values)		
	Saline	Ex-9	Saline	Ex-9	Ex-9 vs. Saline	Diabetic vs. nondiabetic subjects	Interaction
Glucose (mmol/l)							
Fasting	6.7 ± 0.3	6.5 ± 0.3	4.8 ± 0.2	4.9 ± 0.1	NS	<0.001	NS
60–90	12.3 ± 0.3	12.2 ± 0.3	9.3 ± 0.2	9.5 ± 0.2	NS	<0.001	NS
95–270	12.0 ± 0.4	12.1 ± 0.4	9.3 ± 0.2	9.3 ± 0.2	NS	<0.001	NS
GINF (mg/kg/min)							
60–90	3.9 ± 0.4	3.5 ± 0.4	6.0 ± 0.5	5 ± 0.5	<0.05	<0.01	NS
95–270	6.1 ± 1.6	4.6 ± 1.4	11.5 ± 0.7	9.6 ± 0.9	<0.01	<0.05	NS
Insulin (pmol/l)							
Fasting	128 ± 22	142 ± 28	111 ± 20	116 ± 21	NS	NS	NS
60–90	363 ± 69	207 ± 32	719 ± 134	490 ± 107	<0.001	<0.01	NS
95–270	894 ± 220	577 ± 94	2,243 ± 365	1,531 ± 287	<0.001	<0.01	<0.05
C-peptide (nmol/l)							
Fasting	1.2 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.2	0.09	NS	NS
60–90	2.1 ± 0.3	1.8 ± 0.2	3.2 ± 0.4	2.6 ± 0.4	<0.001	<0.05	NS
95–270	4.6 ± 1.1	3.4 ± 0.6	7.6 ± 0.9	5.9 ± 1.1	<0.01	<0.05	NS
ISR (nmol/min)							
Fasting	0.34 ± 0.03	0.35 ± 0.04	0.47 ± 0.11	0.49 ± 0.13	NS	NS	NS
60–90	0.71 ± 0.1	0.53 ± 0.08	1.20 ± 0.17	0.95 ± 0.2	<0.001	<0.05	NS
95–270	1.43 ± 0.32	1.03 ± 0.18	2.42 ± 0.32	1.83 ± 0.36	<0.01	<0.05	NS
Glucagon (pg/ml)							
Fasting	48 ± 4	49 ± 4	46 ± 4	51 ± 5	NS	NS	NS
80–90	41 ± 3	41 ± 4	43 ± 2	42 ± 3	NS	NS	NS
250–260	43 ± 4	58 ± 8	40 ± 4	41 ± 5	<0.05	NS	0.06
GLP-1 (pmol/l)							
80–90	3.6 ± 0.3	3.6 ± 0.2	3.1 ± 0.1	3.0 ± 0.0	NS	NS	NS
AUC <sub>GLP-1</sub> (pmol/l/min)							
95–270	438 ± 92	1,423 ± 340	154 ± 73	560 ± 230	<0.05	0.08	NS
GIP (μmol/l)							
80–90	0.07 ± 0.0	0.06 ± 0.0	0.05 ± 0.0	0.04 ± 0.0	NS	NS	NS
AUC <sub>GIP</sub> (μmol/l/min)							
95–240	12.7 ± 1.4	17.1 ± 1.4	7.9 ± 1.9	7.7 ± 2.3	NS	<0.05	NS

GINF, glucose infusion rate; NS, not significant.

sion, but were significantly higher when Ex-9 was given ( $P < 0.05$ ). In nondiabetic subjects, plasma glucagon concentrations decreased slightly, but not significantly, between the fasting and preprandial periods, and did not change further after the meal, with either saline or Ex-9.

Plasma concentrations of D-xylose increased steadily after meal intake and peaked at 60 min after meal consumption in both diabetic and nondiabetic subjects during both saline and Ex-9 studies (Fig. 4). The area under plasma D-xylose curve in the studies with saline and Ex-9 infusion, respectively, were  $105 \pm 8.2$  and  $102 \pm 9.1$  mmol/l/min in diabetic and  $71 \pm 8.5$  and  $70 \pm 8$  mmol/l/min in nondiabetic subjects, indicating that GLP-1 receptor blockade had minimal effects on the passage of D-xylose from the stomach to the intestine. D-xylose AUC was significantly greater in the diabetic compared with the nondiabetic group ( $P < 0.05$ ).

Fasting plasma GIP levels were comparable before meal ingestion in the studies with saline and Ex-9 infusion in the diabetic ( $16 \pm 1$  and  $16 \pm 2$  pmol/l) and nondiabetic ( $20 \pm 1$  and  $20 \pm 2$  pmol/l) subjects. In both the nondiabetic and diabetic groups, meal consumption caused a similar rise of GIP concentrations in the saline and Ex-9 studies (Fig. 4, Table 2), suggesting that GLP-1r blockade had no effect on GIP secretion. The GIP response was significantly greater

in the diabetic compared with nondiabetic group ( $P < 0.05$ ). Fasting plasma GLP-1 were not different in the diabetic ( $3.6 \pm 0.3$  and  $3.6 \pm 0.2$  pmol/l) and nondiabetic ( $3.1 \pm 0.1$  and  $3.0 \pm 0.0$  pmol/l) groups, during the saline and Ex-9 studies, but levels were undetectable in ~80% of the subjects. Plasma GLP-1 increased after meal ingestion in both groups (Fig. 4, Table 2), and Ex-9 infusion significantly increased these responses in the nondiabetic and diabetic subjects ( $P < 0.05$ ).

Subjects tolerated the experiments without notable problems, and there were no adverse events associated with Ex-9 infusion.

#### DISCUSSION

The current study investigated the role of endogenous GLP-1 on islet hormone secretion and gastric emptying in patients with type 2 diabetes. Although previous studies have demonstrated that diabetic patients respond to pharmacologic amounts of GLP-1, the effect of endogenous GLP-1 has not been previously demonstrated. Our results indicate that in diabetic subjects with good glycemic control, there is an enhancement of glucose-stimulated insulin secretion after meal ingestion that is similar on a relative basis to nondiabetic individuals. Moreover, the

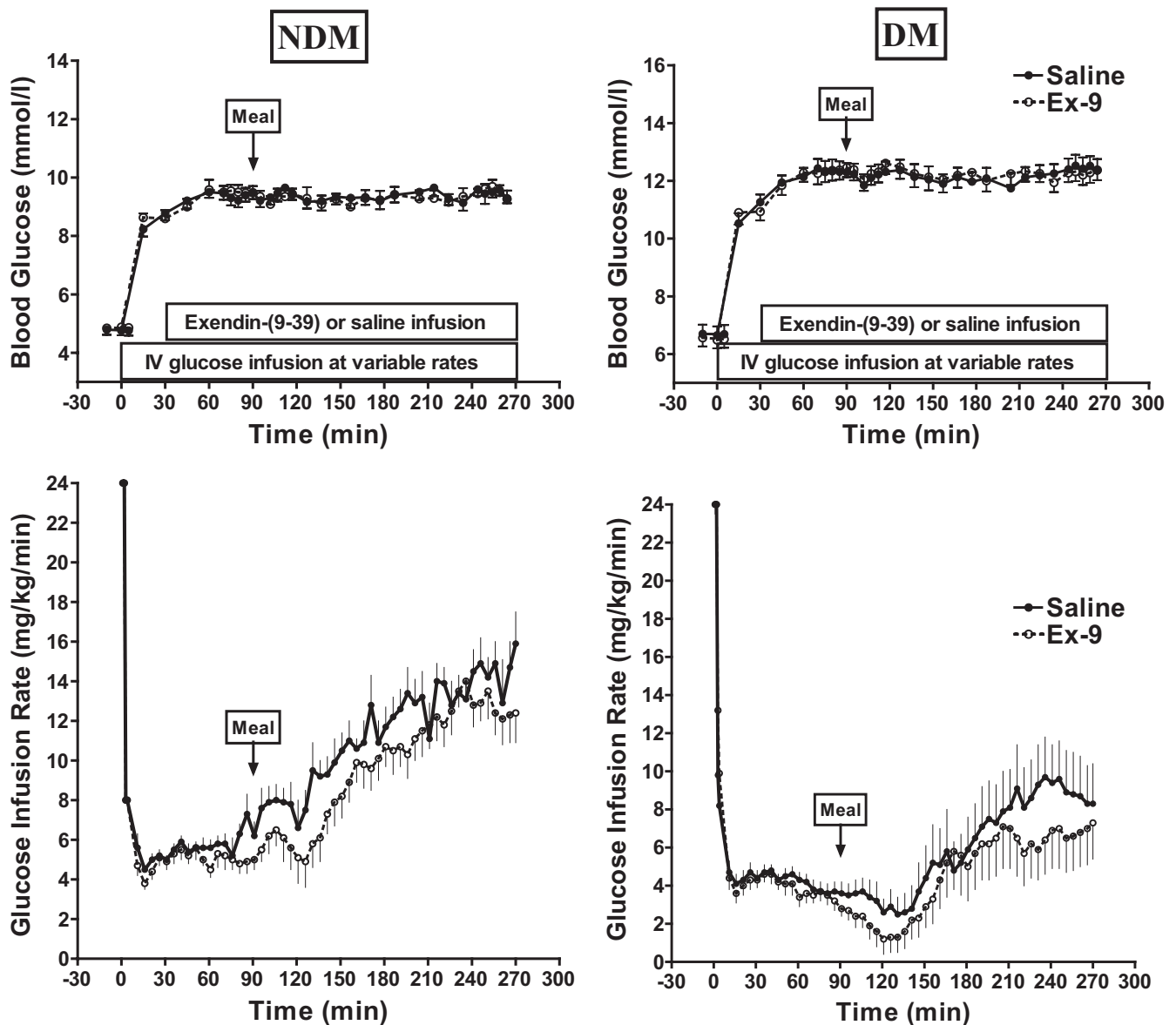


FIG. 1. Blood glucose concentrations and glucose infusion rates during intravenous-meal clamps with Ex-9 or saline infusions in nondiabetic (*left*) and diabetic (*right*) subjects. Data are presented as mean  $\pm$  SEM.

impact of GLP-1r blockade was also comparable in the groups, indicating that endogenous GLP-1 contributes significantly to postprandial insulin secretion in diabetic subjects. Surprisingly, GLP-1r blockade reduced insulin secretion in response to intravenous glucose alone, and the magnitude of this effect was as great as that on postprandial  $\beta$ -cell responses. Gastric emptying, as reflected by D-xylose uptake, was not affected by blocking GLP-1r. These findings support an important physiologic role for GLP-1 to amplify glucose-stimulated insulin secretion, and demonstrate that this effect is retained in at least a subset of type 2 diabetic patients.

Our group and others (4,19,26–27) have previously used Ex-9 to demonstrate the effects of GLP-1r signaling in human subjects. In our previous report, we demonstrated that doses of Ex-9 identical to those used in the present study were effective for blocking supraphysiologic infusions of GLP-1 nearly completely, and for reducing postprandial insulin secretion by  $\sim$ 30%. Although no one has established definitively a dose of Ex-9 beyond which no

further inhibition of GLP-1 action can be detected, based on previously published results (4,19,26) we believe that the dose used in this study was near maximal for what can be achieved in an acute infusion.

The classic method for determining the incretin effect is a 2-day method with an oral glucose tolerance test on day 1 followed on day 2 by an intravenous glucose infusion isoglycemic to arterialized venous glucose levels after glucose ingestion (28). Using this method, Nauck et al. demonstrated a severe impairment of the incretin effect in subjects with type 2 diabetes (29), a much cited finding that has been very influential in shaping the understanding of enteroinsular physiology. We elected to use an alternative method, the hyperglycemic clamp-meal test, as a means of comparing the relative increase in insulin secretion when carbohydrate is absorbed enterally, because the test can be performed in a single morning, reducing day-to-day variability and the time commitment of research subjects. Variations of this method have been used previously by a number of investigators (23–25). Our

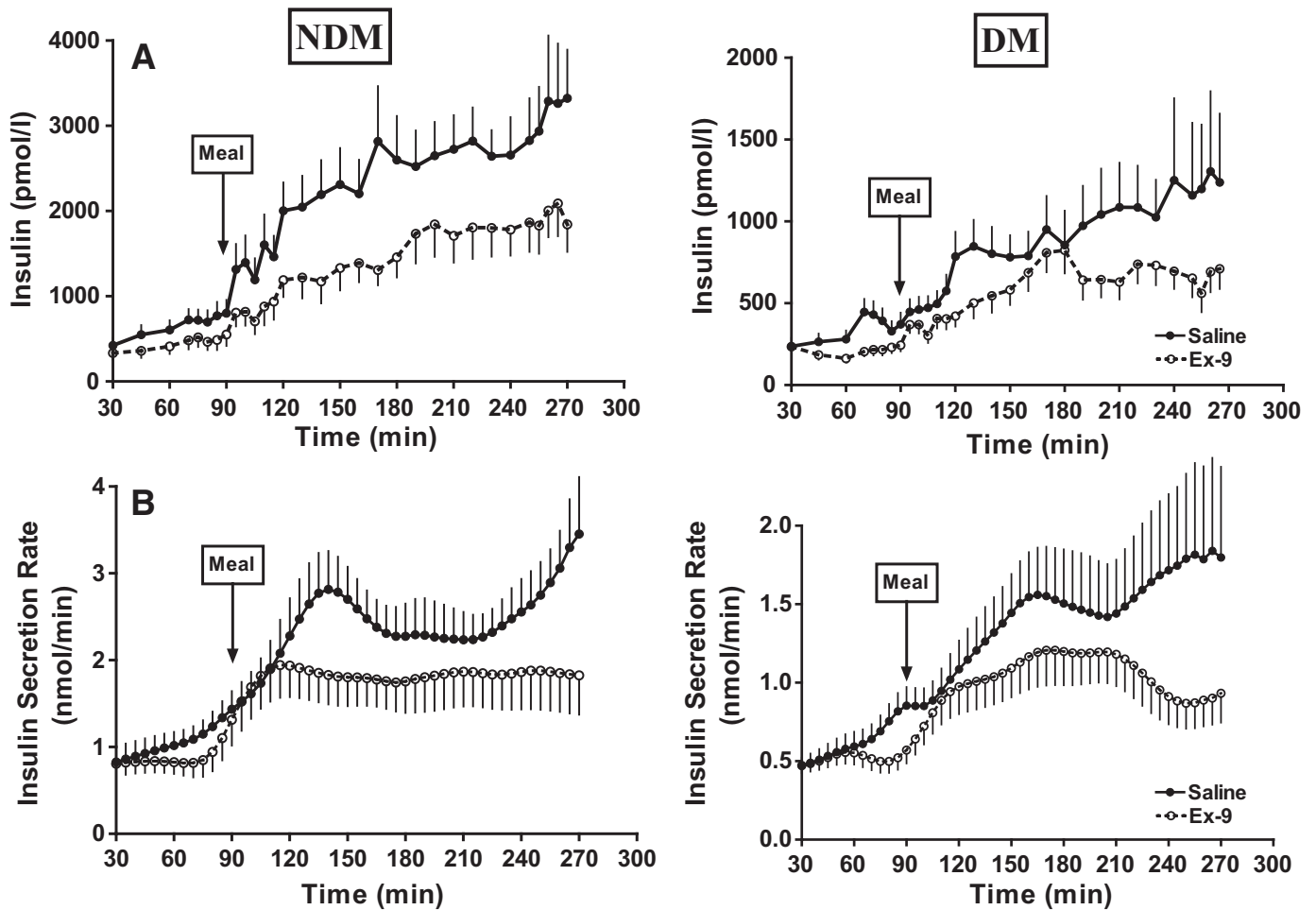


FIG. 2.  $\beta$ -cell secretion in response to hyperglycemia and meal ingestion with and without Ex-9 infusion. Plasma insulin concentrations (A) and insulin secretion rates (B) for nondiabetic (left) and diabetic (right) subjects. Data are presented as mean  $\pm$  SEM.

preliminary data for normal glucose-tolerant subjects demonstrate that the estimation of the incretin effect using the 1-day and 2-day methods is comparable with a strong and significant within-subject correlation (30). In the present study, we used a mixed nutrient meal as the enteral stimulus during the hyperglycemic clamp to assess the nutrient, incretin, and neural responses activated by typical food consumption. We targeted the clamp as 5 mmol/l

above fasting glucose to achieve levels that were slightly greater than what we expected as a result of meal ingestion alone. Overall, this design permitted a measure of the enhancement of insulin secretion by meal ingestion, and the GLP-1 component of it. Without further validation, this measure cannot be equated to the classically derived incretin effect, although we believe that the two methods assess similar physiologic processes.

In contrast to what was described originally by Nauck et al. (29), and confirmed in more recent studies (13), the cohort of diabetic subjects described here had a comparable degree of meal enhancement of insulin secretion to the nondiabetic group. Although  $\beta$ -cell secretion was clearly abnormal in diabetic compared with the nondiabetic subjects, relative to the ISR or plasma insulin levels achieved during stimulation with intravenous glucose alone, meal ingestion caused equivalent augmentation in the diabetic and nondiabetic groups (Table 2). This finding is consistent with the results reported by Perley and Kipnis more than three decades ago (31), showing that obese subjects with mild type 2 diabetes had preserved stimulation of insulin secretion by alimentary factors relative to nondiabetic control subjects. In contrast to the findings of Nauck et al. (29), the plasma GIP responses were increased in our diabetic compared with nondiabetic subjects. Increased GIP secretion has been reported previously in some studies of diabetic subjects (reviewed

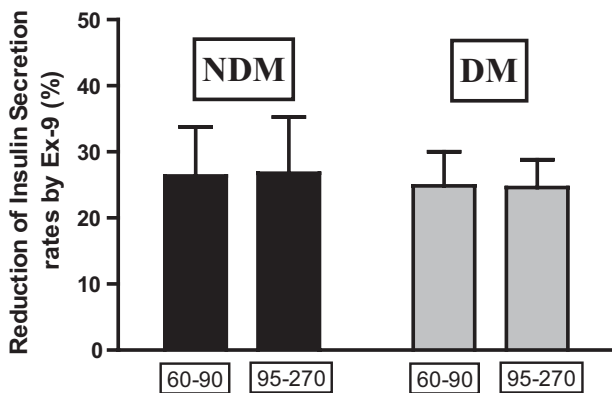


FIG. 3. The contribution of GLP-1 to preprandial and postprandial insulin secretion. The percentage reduction of insulin secretion rates by Ex-9 is shown for the preprandial (60–90 min) and postprandial (95–270 min) periods in the nondiabetic (black bars) and diabetic (gray bars) groups. Data are presented as mean  $\pm$  SEM.

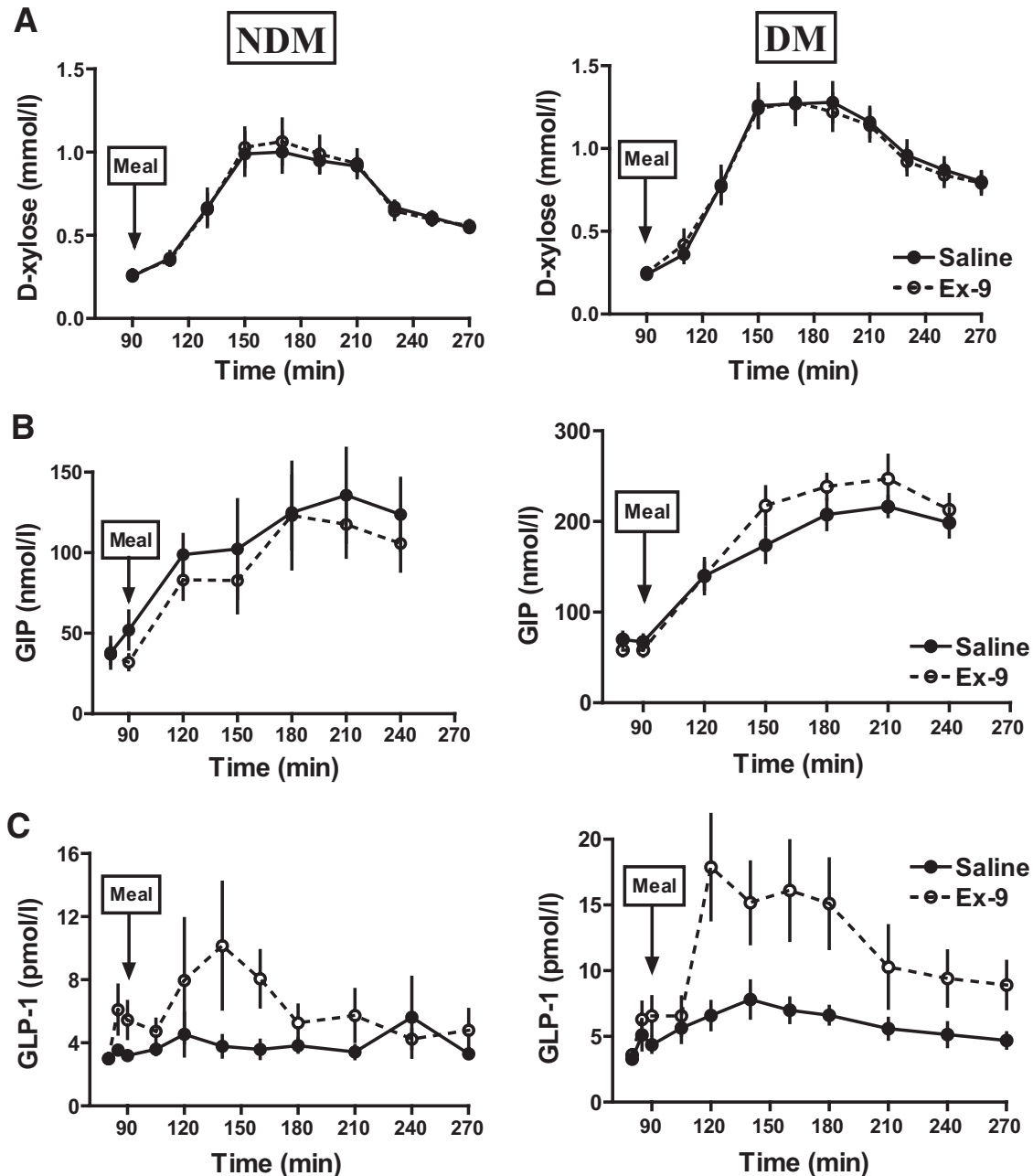


FIG. 4. Postprandial concentrations of D-xylose (A), GIP (B), and GLP-1 (C) in nondiabetic (left) and diabetic (right) subjects. Data are presented as mean  $\pm$  SEM.

in [32]). Although an increased effect of GIP could account for a bigger augmentation of insulin secretion by meal ingestion in our diabetic subjects, most previous studies indicate that GIP has very modest effects on insulin secretion in type 2 diabetes (8).

Just as the relative increase of ISR with meal ingestion was similar in the diabetic and nondiabetic subjects, so too was the contribution of GLP-1 action to postprandial  $\beta$ -cell responses. Based on the effects of Ex-9, GLP-1 accounted for  $\sim$ 25% of the insulin response to the mixed meal at a fixed level of hyperglycemia. This is consistent with what we reported recently as the GLP-1 effect in healthy lean subjects during an oral glucose tolerance test (19). The finding of intact effects of GLP-1 in the diabetic group was unexpected, in that previous work had indicated that type 2 diabetic subjects have a blunted response

to exogenous GLP-1 infused to supraphysiologic levels (18). Although there was a weak trend toward the diabetic group having a greater postprandial GLP-1 response, there was considerable heterogeneity in the AUC among the subjects and no correlation between this measure and insulin secretion or the magnitude of insulin suppression by GLP-1 receptor blockade. These results suggest that circulating GLP-1 may not be a good predictor of the physiologic response to endogenous peptide.

The most surprising finding in this study was that Ex-9 diminished insulin secretion in response to intravenous glucose-induced hyperglycemia before meal consumption, at a time when circulatory levels of GLP-1 were often undetectable. In fact, the relative effect of GLP-1r blockade on preprandial and postprandial ISR was nearly identical. These findings indicate that GLP-1r effects on the  $\beta$ -cell are

not restricted to the potentiation of insulin secretion after enteral nutrient absorption when circulating levels of GLP-1 increase. It is important to note that in previous studies blockade of the GLP-1 receptor did not affect fasting plasma insulin levels (4,27,33), but that Schirra et al. noted a reduction in plasma insulin, similar to what we describe here, during a hyperglycemic clamp when Ex-9 was infused (27). Thus, when considered together, the current evidence supports a role for GLP-1r signaling to potentiate glucose-stimulated but not basal insulin secretion in humans. This is consistent with studies in mice with engineered deletion of the GLP-1r gene in which similar degrees of glucose intolerance have been described to both oral and intraperitoneal glucose challenges (34), conditions with elevated and basal plasma GLP-1 levels, respectively. The mechanism whereby Ex-9 affects insulin secretion in the absence of elevated circulating levels of GLP-1 is unclear. However, recent studies raise the possibility of neural (35–36) and paracrine (37) modes of GLP-1 signaling, either of which could be affected by GLP-1r blockade. In addition, *in vitro* work suggests that Ex-9 can act as an inverse agonist in isolated mouse islets (38). Overall, our findings fit with an expanded model of  $\beta$ -cell regulation by GLP-1 that stretches beyond endocrine effects after eating.

Although several investigators have suggested that the predominant effect of GLP-1 on glucose control is based on its effect on gastric emptying (39–40), this conjecture is based on the results of studies using exogenous administration of GLP-1. Given the identical pattern of postprandial plasma D-xylose in our subjects with and without Ex-9, endogenous GLP-1 has no detectable effect on the rate of passage of nutrients from the stomach to the intestine. Appearance of ingested D-xylose followed a similar time course in diabetic and nondiabetic subjects, but D-xylose reached higher concentrations in the diabetic group, suggesting more rapid gastric emptying in patients with early or mild diabetes, compatible with previous reports (41–42). The lack of effect of Ex-9 to alter D-xylose appearance is similar to what we have reported previously in studies with GLP-1 blockade in healthy humans and nonhuman primates given liquid glucose solution (19,33). We have extended these findings in this study by using a solid meal, and our data stand against an important physiologic role for GLP-1 in the regulation of prandial gastric motility in humans. However, Deane et al. have recently published evidence that endogenous GLP-1 delays gastric emptying in healthy subjects after a solid carbohydrate meal (43). Because these investigators used scintigraphy, the gold standard for measuring gastric emptying, our findings must be interpreted cautiously. Nonetheless, our results are consistent with a recent report that gastric emptying in diabetic patients was not affected by administration of a dipeptidyl peptidase-4 inhibitor that enhanced endogenous GLP-1 by threefold (44).

In previous studies, we and others (4,19) have found that infusion of Ex-9 during oral or intestinal glucose administration increases postprandial plasma glucagon, supporting an important role for GLP-1 to regulate the  $\alpha$ -cell. This effect was seen here, but only in the diabetic group. The diabetic subjects had equivalent suppression of glucagon in response to intravenous glucose during the Ex-9 and control experiments, but after the meal there was a significant rise in plasma levels when the GLP-1r was blocked. Interestingly, we did not see this latter effect in the nondiabetic subjects. Although this outcome could

indicate differential regulation of prandial  $\alpha$ -cell secretion in diabetes, this conclusion needs more rigorous confirmation.

In summary, we report here that administration of Ex-9 reduces insulin secretion proportionately in response to intravenous glucose and enteral stimuli, and that this effect is similar in diabetic and nondiabetic subjects. These findings indicate that the effect of endogenous GLP-1 on postprandial insulin release is preserved in patients with well-controlled type 2 diabetes. Moreover, GLP-1r signaling is important for stimulated insulin secretion independent of the mode of glucose entry. Based on these results, it appears that the function of the enteroinsular axis is intact, at least in some people with type 2 diabetes, and that the role of endogenous GLP-1 to regulate islet function is not mediated entirely by an endocrine mechanism.

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