Effect of Exenatide on Splanchnic and Peripheral Glucose Metabolism in Type 2 Diabetic Subjects

E. Cersosimo,* A. Gastaldelli,* A. Cervera, E. Wajcberg, A. Sriwijilkamol, M. Fernandez, P. Zuo, R. Petz, C. Triplitt, N. Musi, and R. A. DeFronzo

Division of Diabetes (E.C., A.G., A.C., E.W., A.S., M.F., P.Z., C.T., N.M., R.A.D.), Department of Medicine, University of Texas Health Science Center, and the Texas Diabetes Institute (E.C., C.T., N.M., R.A.D.), San Antonio, Texas 78229; and Institute of Clinical Physiology (A.G., R.P.), National Research Council, 56124 Pisa, Italy

Objective: Our objective was to examine the mechanisms via which exenatide attenuates postprandial hyperglycemia in type 2 diabetes mellitus (T2DM).

Study Design: Seventeen T2DM patients (44 yr; seven females, 10 males; body mass index = 33.6 kg/m^2; glycosylated hemoglobin = 7.9%) received a mixed meal followed for 6 h with double-tracer technique ([1-^{14}C]glucose orally; [3-^{3}H]glucose iv) before and after 2 wk of exenatide. In protocol II (n = 5), but not in protocol I (n = 12), exenatide was given in the morning of the repeat meal. Total and oral glucose appearance rates (RaT and RaO, respectively), endogenous glucose production (EGP), splanchnic glucose uptake (75 g − RaO), and hepatic insulin resistance (basal EGP × fasting plasma insulin) were determined.

Results: After 2 wk of exenatide (protocol I), fasting plasma glucose decreased (from 10.2 to 7.6 mM) and mean postmeal plasma glucose decreased (from 13.2 to 11.3 mM) ($P < 0.05$); fasting and meal-stimulated plasma insulin and glucagon did not change significantly. After exenatide, basal EGP decreased (from 13.9 to 10.8 μmol/kg · min, $P < 0.05$), and hepatic insulin resistance declined (both $P < 0.05$). RaO, gastric emptying (acetaminophen area under the curve), and splanchnic glucose uptake did not change. In protocol II (exenatide given before repeat meal), fasting plasma glucose decreased (from 11.1 to 8.9 mM) and mean postmeal plasma glucose decreased (from 14.2 to 10.1 mM) ($P < 0.05$); fasting and meal-stimulated plasma insulin and glucagon did not change significantly. After exenatide, basal EGP decreased (from 13.4 to 10.7 μmol/kg · min, $P = 0.05$). RaT and RaO decreased markedly from 0–180 min after meal ingestion, consistent with exenatide's action to delay gastric emptying.

Conclusions: Exenatide improves 1) fasting hyperglycemia by reducing basal EGP and 2) postmeal hyperglycemia by reducing the appearance of oral glucose in the systemic circulation. ***(J Clin Endocrinol Metab* 96: 1763–1770, 2011)**

Individuals with type 2 diabetes mellitus (T2DM) are characterized by defects in insulin secretion, peripheral (muscle) glucose uptake, suppression of endogenous (hepatic) glucose production (EGP), and splanchnic (primarily hepatic) glucose uptake (SGU) (1). It follows that interventions that reverse specific pathogenic abnormalities in T2DM are most likely to exert beneficial and durable effects to improve glycemic control. Glucagon-like peptide-1 (GLP-1) receptor agonists represent a new class of antidiabetic agents that have gained widespread use (2, 3). In T2DM, there is both deficiency of GLP-1 (4, 5) and β-cell resistance to the stimulatory effect of GLP-1 on in-

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* E.C. and A.G. contributed equally to the study.

Abbreviations: AUC, Area under the curve; EGP, endogenous glucose production; FPG, fasting plasma glucose; GLP-1, glucagon-like peptide-1; HGB, hepatic glucose balance; HGP, hepatic glucose production; HGU, hepatic glucose uptake; HIRI, hepatic insulin resistance index; ISR, insulin secretory rate; MTT, meal tolerance test; NS, not significant; RaO, rate of oral glucose appearance; RaT, rate of total glucose appearance; Rd, rate of glucose disappearance; SGU, splanchnic glucose uptake; T2DM, type 2 diabetes mellitus.

sulin secretion (6). Treatment of T2DM patients with GLP-1 receptor agonists augments insulin secretion and inhibits glucagon release, leading to reductions in both fasting and postprandial plasma glucose concentrations (7–10). However, no previous study has examined the physiological mechanisms via which chronic treatment with GLP-1 receptor agonists improve glucose homeostasis in humans with T2DM.

After mixed meal ingestion, acute iv exenatide administration in T2DM markedly reduces the postmeal plasma glucose excursion by inhibiting EGP and delaying gastric emptying (8). Approximately half of the decline in EGP was due to inhibition of glucagon secretion and half was due to increased insulin secretion (8). Because gastric emptying was inhibited, an effect of exenatide to augment SGU could not be examined. A single dose of the dipeptidyl peptidase-4 inhibitor vildagliptin, which increased endogenous GLP-1 levels in T2DM subjects, also reduced postmeal plasma glucose concentration in association with increased plasma insulin and decreased plasma glucagon concentrations and a decrease in EGP; no effect on peripheral tissue insulin sensitivity or gastric emptying was observed (11). In rodents, GLP-1 receptors are expressed on nerve terminals in the portal circulation and function to augment hepatic glucose uptake (HGU) in response to elevated portal vein GLP-1 levels (12, 13). Enhanced HGU also has been demonstrated after acute portal vein GLP-1 administration in dogs (14–16).

Despite their introduction into the clinical market for the treatment of T2DM over 6 yr ago, no study in man has examined the effect of chronic GLP-1 receptor agonists on the physiological mechanisms via which they improve glucose homeostasis after meal ingestion. The present study was undertaken to 1) provide qualitative/quantitative information about the physiological mechanisms [enhanced suppression of hepatic glucose production (HGP), increased peripheral tissue glucose disposal, increased SGU, and altered glucose absorption rate] responsible for improved postprandial hyperglycemia (mixed meal) after exenatide administration in T2DM individuals and 2) contrast the effect of chronic (2 wk) exenatide administration when exenatide is given *vs.* not given in the morning of study. Because exenatide administration before the meal produced physiological changes that closely paralleled those after acute iv exenatide (8), we enrolled more subjects in the protocol in which exenatide was not administered immediately before the meal to examine whether there was a long-lasting effect of the exenatide administered on the previous day.

Subjects and Methods

Subjects

Seventeen T2DM (43 ± 2 yr; seven females, 10 males; body mass index = 33.8 ± 1.5 kg/m^2; glycosylated hemoglobin = 7.9 ± 0.4%; diabetes duration = 6 ± 4 yr) without glutamic acid decarboxylase (GAD65) antibodies or diabetic complications received meal tolerance test (MTT) before and after 2 wk of exenatide. Subjects were in good health as determined by medical history, physical exam, screening blood tests, urinalysis, and electrocardiogram. Diabetes was treated with metformin (five in protocol I, two in protocol II), sulfonylurea (three in protocol I, two in protocol II), metformin/sulfonylurea (one in protocol I, one in protocol II), or diet (three in protocol I, none in protocol II). Oral hypoglycemic agents were not given on the day of study. No subject was taking any other medication known to affect glucose metabolism. Body weight was stable (within ±1 kg) for 6 months before study. No subject participated in any strenuous exercise program. The study was approved by the Institutional Review Board at the University of Texas Health Science Center at San Antonio, and informed written consent was obtained from each subject before participation.

Study design

Subjects participated in two protocols that were identical with one exception. After the baseline MTT, subjects were treated with exenatide for 2 wk, at which time the MTT was repeated. In protocol I, on the morning of the repeat MTT (n = 12) subjects did not take exenatide; in protocol II (n = 5), subjects received their usual exenatide dose in the morning of the repeat MTT. Subjects in both protocols I and II continued to take their regular oral antidiabetic medication except on the morning of the MTT. At screening, subjects received dietary guidance to consume a weight-maintaining diet (50% carbohydrate, 30% protein, 20% fat).

MTT with double tracer

Subjects reported to the Clinical Research Center of the Texas Diabetes Institute at 0700 h after a 10-h overnight fast. A catheter was inserted into an antecubital vein, and a prime [25 μCi × fasting plasma glucose (FPG)/100[and continuous (0.25 μCi/min) [3-^{3}H]glucose infusion was started and maintained for the entire study (9 h). A second catheter was inserted retrogradely into a vein on the dorsum of the hand, which was placed in a thermo-regulated box (70 C) for blood sampling. At 0930 h, blood samples were obtained at −30, −20, −10, −5, and 0 min for FPG, insulin, glucagon, and acetaminophen concentrations and plasma tritiated glucose radioactivity. At time zero (1000 h), subjects voided and ingested a standardized meal containing 75 g glucose, 25 g fat, and 20 g protein. There was no other carbohydrate source in the meal. The solid component of the meal contained 600 kcal, made with eggs and cheese. The oral glucose load was mixed with 100 μCi 1-^{14}C-labeled glucose and 1000 mg acetaminophen elixir (protocol I only) to monitor gastric emptying (17). Patients consumed the meal within 15 min, and blood was obtained every 15–30 min for plasma [^{14}C/^{3}H]glucose specific activities and plasma glucose, insulin, glucagon, C-peptide, and acetaminophen concentrations. Upon study termination, urine was collected and glucose concentration measured.

On the day after MTT, subjects self-administered exenatide, 5 μg sc before breakfast and dinner, for 7 d. Exenatide was

increased to 10 μg sc twice daily for the subsequent 7 d (14 d of therapy). In protocol II, exenatide was injected sc 15 min before meal ingestion. In protocol I, the last exenatide dose was given at 1800 h on the evening before the repeat MTT; no exenatide was given on the morning of the MTT.

Analytical determinations

Plasma glucose was determined with glucose oxidase (Beckman Glucose Analyzer, Fullerton, CA). Plasma insulin, C-peptide, and glucagon concentrations were measured by RIA (Diagnostics Products Corp., Los Angeles, CA). Plasma acetaminophen was determined by liquid chromatography spectrometry (PPD Laboratories, Middleton, WI). Plasma [^{3}H]glucose and [^{14}C]glucose radioactivity was determined on barium hydroxide/zinc sulfate-precipitated plasma extracts.

Calculations

In the postabsorptive state, steady-state conditions prevail and EGP (equals rate of total-body glucose disposal) was measured as [3-^{3}H]glucose infusion rate (decay per minute) ÷ steady-state plasma [3-^{3}H]glucose specific activity (decay per milligram). After glucose ingestion, non-steady-state conditions prevail and rate of total glucose appearance (RaT) and rate of glucose disappearance (Rd) were computed from [3-^{3}H]glucose data using Steele's two-compartment model. Glucose clearance was calculated as Rd divided by plasma glucose concentration.

To calculate the rate of oral glucose appearance (RaO), [1-^{14}C]glucose plasma radioactivity was divided by the specific activity of the glucose drink to calculate the plasma oral glucose concentration that would be attained in the systemic circulation if the sole source of glucose was the oral load and by subtracting the plasma endogenous glucose concentration. The calculated endogenous glucose concentration and [3-^{3}H]glucose data were then used to compute EGP in peripheral plasma. RaO was obtained as the difference between total and endogenous glucose appearance rates (18). Splanchnic (represents hepatic plus gastrointestinal tissues) glucose uptake was calculated as the difference between ingested glucose (75 g) and RaO.

Hepatic insulin resistance index (HIRI) was calculated as baseline EGP (milligrams per kilogram per minute) × fasting plasma insulin (picomoles per liter) (19). Insulin secretory rate was calculated by plasma C-peptide deconvolution (20). β-Cell function was assessed by dividing the insulin secretory rate (picomoles per kilogram per minute) by plasma glucose concentration (milligrams per deciliter) (21). Insulin secretion/insulin resistance (disposition) index was calculated as [Δ area under the curve (AUC)-insulin secretory rate (ISR)/ΔAUC-glucose] × Matsuda index of insulin sensitivity (21, 22).

Statistics

Differences between metabolic parameters (plasma glucose, insulin, C-peptide, glucagon, glucose kinetic measurements, HIR, and indices of β-cell function) before and after MTT studies were compared using ANOVA with repeated measures and time course during the MTT. Significant differences were confirmed with a Bonferroni test. Differences between baseline (fasting) and postmeal values within protocol I and within protocol II were compared using paired *t* test. *P* value <0.05 was considered significant. Data are presented as mean ± SE.

Results

Because no differences in any clinical, laboratory. or metabolic parameters were noted between metformin- and sulfonylurea-treated subjects in protocols I and II, data from all subjects were combined for analysis. Body weight did not change after 2 wk of exenatide in either protocol I or II [93.9 ± 3.7 to 93.3 ± 3.7 kg, P value not significant (NS)].

Protocol I

Plasma glucose, insulin, and glucagon concentrations and insulin secretion and insulin sensitivity indices

FPG was 10.2 ± 0.8 mmol/liter and increased to 13.2 ± 0.8 mmol/liter (mean, 0–360 min) during the preexenatide MTT (Table 1 and Fig. 1). After 2 wk of exenatide, FPG decreased to 7.6 ± 0.7 mmol/liter, and mean plasma glucose (0–360 min) during MTT declined to 11.3 ± 0.7 μmol/liter (both $P < 0.005$, pre *vs.* post) (Table 1 and Fig. 1). After exenatide treatment, FPI decreased from 61 ± 6 to 46 ± 6 pmol/liter (*P* value NS) (Table 1 and Fig. 1), and the mean plasma insulin from 0–360 min increased slightly from 122 ± 16 to 150 ± 20 pmol/liter (Table 1 and Fig. 1). Both fasting and mean postmeal plasma glucagon concentration did not change significantly after exenatide (Table 1).

The fasting insulin secretory rate tended to increase (*P* value NS), whereas the ISR during the meal (1665 ± 151 to 1951 ± 120 pmol/min, $P < 0.01$) increased significantly after exenatide. The insulin secretion/insulin resistance (disposition) index during MTT performed after exenatide increased by 69% from 563 ± 126 to 951 ± 167 ($P = 0.04$) (Table 1).

The Matsuda index of insulin sensitivity during MTT tended to increase after exenatide (4.0 ± 0.5 to 5.4 ± 0.6), but the increase did not reach significance. The mean glucose clearance (0–360 min) did not change (Table 1).

Glucose kinetics (Table 1)

Neither the RaT nor the RaO (Table 1) in the systemic circulation after the meal was changed after 2 wk of exenatide. After exenatide for 2 wk, basal EGP declined from 13.4 ± 1.5 to 10.7 ± 0.8 μmol/kg · min ($P < 0.05$) (Table 1), and the decrement in basal EGP correlated closely with the decrement in FPG ($r = 0.70$; $P = 0.01$). After 14 d of exenatide, the mean rate of EGP during the meal did not change (4.0 ± 0.8 to 4.3 ± 0.9, $P = 0.60$) (Table 1).

The appearance of acetaminophen (AUC) in the systemic circulation over the 0–360 min after the meal was similar in the meal test performed before (1481 ± 22 μg/ml) and after (1487 ± 49 μg/ml) exenatide (Supplemental

TABLE 1. Clinical and metabolic parameters at baseline and after 2 wk of exenatide treatment

	Protocol I, no exenatide			Protocol II, with exenatide			
	Pre	Post	*P* value (pre *vs.* post)	Pre	Post	*P* value (pre *vs.* post)	*P* value (protocol I *vs.* II)
Number	12			5			
Age (yr)	42 ± 2			46 ± 4			
Gender (male/female)	8/4			2/3			
Weight (kg)	95.8 ± 4.5	95.1 ± 4.5		89.4 ± 6.8	89.0 ± 6.7		NS
BMI (kg/m^2)	33.3 ± 1.3	33.1 ± 1.3		34.4 ± 3.8	34.2 ± 3.7		NS
HbA_{1c} (%)	9.1 ± 0.7			9.4 ± 0.7			0.0005
Fasting							
Plasma glucose (nmol/liter)	10.2 ± 0.8	7.6 ± 0.7	0.0001	11.1 ± 0.6	8.9 ± 0.5	0.04	NS
Plasma insulin (pmol/liter)	61 ± 6	46 ± 6		73 ± 22	88 ± 39		NS
Plasma glucagon (pg/liter)	68 ± 6	75 ± 8		47 ± 7	54 ± 7		NS
EGP (μmol/kg · min)	13.9 ± 0.8	10.8 ± 0.6	0.0005	13.4 ± 1.5	10.7 ± 0.8	<0.05	NS
ISR (pmol/min)	498 ± 51	581 ± 71		341 ± 46	434 ± 47		NS
HIRI (μmol/kg · min × pmol/liter)	852 ± 97	492 ± 59	0.01	1105 ± 479	1016 ± 515	0.69	NS
Glucose clearance (ml/kg · min)	1.5 ± 0.1	1.5 ± 0.2		1.4 ± 0.3	1.2 ± 0.1		NS
Mixed meal							
Plasma glucose (nmol/liter), 0–360 min	13.2 ± 0.8	11.3 ± 0.7[a]	0.0001	14.2 ± 0.6	10.1 ± 0.3[a]	0.04	
Plasma insulin (pmol/liter), 0–360 min	122 ± 16	150 ± 20[a]	0.04	127 ± 33	148 ± 23		NS
Plasma glucagon (pmol/liter), 0–360 min	68 ± 6	72 ± 5		53 ± 6	51 ± 9		NS
EGP (μmol/kg · min), 0–360 min	5.5 ± 0.7	4.4 ± 0.7		4.0 ± 0.8	4.3 ± 0.9		NS
0–180 min	6.3 ± 1.0	4.9 ± 0.8		3.9 ± 1.0	4.7 ± 1.2		NS
180–360 min	4.7 ± 0.5	4.0 ± 0.6		4.1 ± 0.6	3.9 ± 0.7		NS
RaO (μmol/kg · min), 0–360 min	10.7 ± 1.3	10.4 ± 1.0		12.4 ± 0.9	10.2 ± 1.1		0.12
0–180 min	16.2 ± 1.7	16.8 ± 1.3		19.8 ± 1.2	9.5 ± 3.5	<0.05	0.002
180–360 min	5.1 ± 1.7	4.0 ± 0.7		5.0 ± 0.8	10.9 ± 3.4	0.05	0.008
RaT (μmol/kg · min), 0–360 min	17.0 ± 1.1	15.3 ± 0.9	0.10	16.4 ± 1.4	14.5 ± 1.2	0.10	NS
0–180 min	23.8 ± 1.1	22.6 ± 1.1		23.7 ± 1.8	14.3 ± 2.5	<0.05	0.015
180–360 min	10.1 ± 1.3	7.9 ± 0.8	0.04	9.1 ± 1.2	14.8 ± 3.6		0.008
Glucose clearance (ml/kg · min), 0–360 min	1.4 ± 0.1	1.5 ± 0.2		1.2 ± 0.1	1.5 ± 0.1	0.04	NS
0–180 min	1.4 ± 0.1	1.4 ± 0.1		1.2 ± 0.1	1.4 ± 0.1		NS
180–360 min	1.4 ± 0.1	1.5 ± 0.2		1.2 ± 0.1	1.6 ± 0.1	0.007	0.06
ISR (pmol/min), 0–360 min	1665 ± 151	1951 ± 120[a]	0.01	1155 ± 74	1492 ± 80[a]	0.01	NS
ISI (Matsuda)							
0–180 min	3.5 ± 0.4	4.7 ± 0.5	0.07	3.3 ± 0.6	4.4 ± 1.0		NS
0–360 min	4.0 ± 0.5	5.4 ± 0.6	0.10	3.7 ± 0.7	4.2 ± 1.0		NS
Insulin secretion index [ISR/(G × ISI)]							
0–180 min	447 ± 97	749 ± 118	0.02	262 ± 50	612 ± 151	0.04	NS
0–360 min	563 ± 126	951 ± 167	0.04	296 ± 63	618 ± 143	0.01	
Splanchnic glucose retention (g), 0–360 min	11.3 ± 5.4	12.3 ± 4.4		4.5 ± 1.1	17.6 ± 5.3	0.05	NS
Urinary glucose excretion (g), 0–360 min	7.4 ± 0.0	2.1 ± 0.7[a]	0.0003	7.5 ± 1.4	3.4 ± 0.3[a]	0.02	NS
Urinary glucose excretion (μmol/min · kg), 0–360 min	1.23 ± 0.14	0.34 ± 0.1	0.0002	1.37 ± 0.31	0.62 ± 0.09	0.03	NS

BMI, Body mass index; ISI, insulin sensitivity index; G, glucose.

[a] $P < 0.05$ *vs.* basal.

Fig. 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). This indicates that gastric emptying of ingested glucose was complete and was not altered when the last exenatide dose was given in the evening before the meal. SGU was similar in the preexenatide (63.7 ± 5.4 g) and postexenatide (62.7 ± 4.4 g) studies. Urinary glucose excretion during MTT declined significantly after exenatide (Table 1).

Protocol II

Plasma glucose, insulin, and glucagon, and insulin secretory indices

FPG was 11.1 ± 0.6 nmol/liter and increased to 14.2 ± 0.6 μmol/liter (mean, 0–360 min) during the preexenatide MTT (Table 1 and Fig. 1). After 2 wk of exenatide, FPG decreased to 8.9 ± 0.5 nmol/liter ($P < 0.05$) and mean PG during MTT declined to 10.1 ± 0.3 nmol/liter ($P < 0.05$

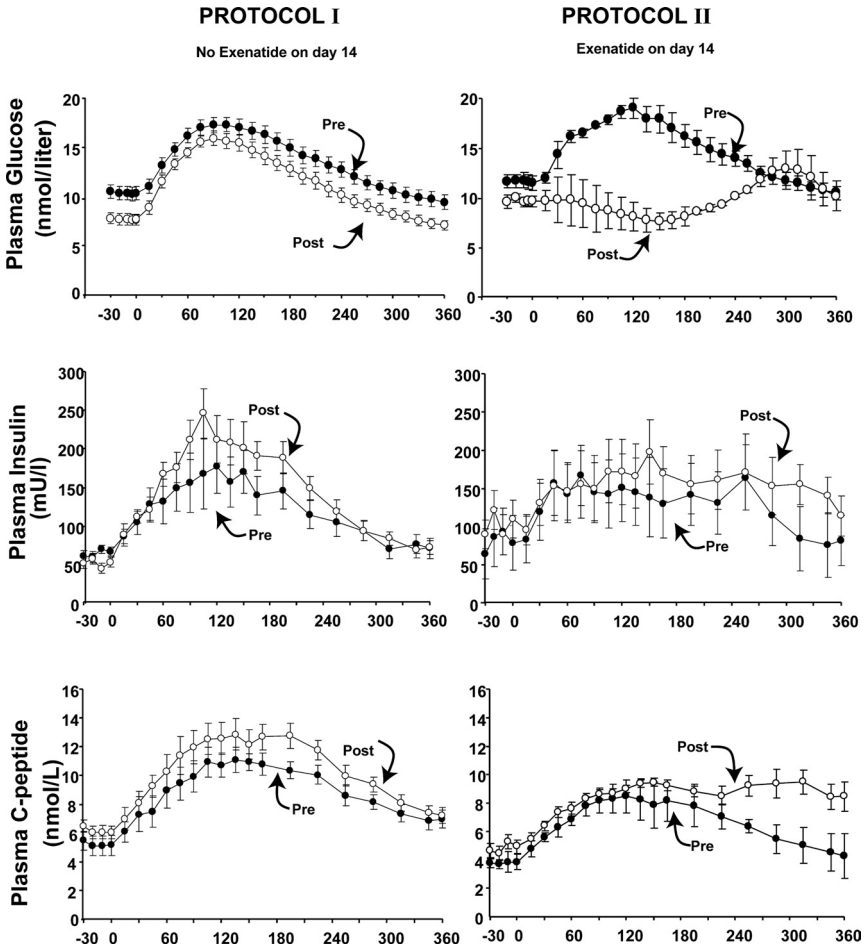

FIG. 1. Plasma glucose, insulin, C-peptide, and glucagon concentrations in T2DM patients after ingestion of a mixed meal before and after 2 wk of exenatide administration. In protocol I, but not in protocol II, exenatide was administered before the repeat meal. See Table 1 for statistical analysis.

vs. baseline MTT and mean postmeal PG in protocol I). The incremental plasma glucose response was markedly reduced ($P < 0.0001$) in protocol II (exenatide given in morning of MTT) *vs.* protocol I (exenatide not given) (Fig. 1). Neither FPI nor mean plasma insulin concentration during the meal (Table 1) changed significantly during the postexenatide MTT (Fig. 1). Fasting plasma glucagon and plasma glucagon during the meal were similar in pre- and postexenatide studies (Table 1 and Fig. 1).

Fasting insulin secretory rate tended to increase (*P* value NS) (Table 1), whereas ISR during the meal increased significantly (1155 ± 74 to 1492 ± 80 pmol/min, $P < 0.02$) (Table 1 and Fig. 1) after exenatide. The insulin secretion/insulin resistance (disposition) index increased 2-fold (296 ± 63 to 618 ± 143, $P < 0.02$) during the postexenatide meal test (Table 1).

Neither the Matsuda index of insulin sensitivity nor the glucose clearance changed significantly during the meal test performed after exenatide (Table 1).

Glucose kinetics (Table 1)

Both the RaT in the systemic circulation (23.7 ± 1.8 to 14.3 ± 2.5 μmol/min · kg) as well as RaO (19.8 ± 1.2 to 9.5 ± 3.5 μmol/min · kg) were reduced ($P < 0.05$) during the initial 0–180 min during the meal after exenatide treatment (Table 1). From 180–360 min, both RaT and RaO increased to values above those in the preexenatide MTT (Table 1), yet the plasma glucose concentration during this time period rose only slightly (Fig. 1). After 2 wk of exenatide, basal rate of EGP decreased from 13.4 ± 1.5 to 10.7 ± 0.8 μmol/kg · min ($P < 0.05$). When all 17 subjects (protocols I and II) were considered collectively, the decline in FPG correlated strongly with the decline in EGP ($r = 0.74$; $P < 0.001$). After 14 d of exenatide, the mean rate of EGP during MTT was similar to the preexenatide MTT. Unlike the RaO, which increased from 180–360 min, EGP declined, thus minimizing the rise in plasma glucose concentration from 180–360 min. During the postexenatide study, glucose clearance increased modestly ($P = 0.04$) (Table 1) and urinary glucose excretion decreased ($P = 0.03$) (Table 1).

During 0–360 min, approximately 75% (57.4 ± 5.3 of 75 g) of the oral glucose load appeared in the systemic circulation (compared with 70.5 ± 1.1 g during the preexenatide study). Thus, 17.6 g of the ingested glucose was retained within the splanchnic region during the 6-h MTT.

Exenatide was well tolerated in both protocols I and II. Six of 17 T2DM patients experienced mild nausea, which disappeared by the end of the 2-wk treatment period. One sulfonylurea-treated patient developed mild symptomatic hypoglycemia (FPG = 45 mg/dl), and the sulfonylurea was discontinued.

Discussion

In both protocols I and II, 2 wk of exenatide treatment improved glycemic control in T2DM by reducing both the fasting and postprandial plasma glucose concentrations (Table 1 and Fig. 1) without change in body weight. In protocols I and II combined, the decrease in FPG correlated positively with the reduction in basal EGP ($r = 0.74$;

$P < 0.001$). In neither protocol I nor protocol II was there a significant increase in fasting plasma insulin concentration despite the significant reduction in basal EGP. However, the fasting ISR tended to increase in both protocols I and II. We interpret these findings as follows. Initially, exenatide augments insulin secretion leading to decreased EGP. As basal EGP falls over the subsequent 2 wk, FPG declines because basal EGP is the primary determinant of FPG (1). The decline in FPG leads to a decrease in fasting plasma insulin concentration, explaining the failure to observe any increase in FPI or any correlation between fasting FPI and reduced EGP. Because the basal HIRI improved by 33% (Table 1), an unchanged FPI concentration was able to maintain a reduced rate of basal EGP. In contrast, when exenatide was given on d 14 (protocol II), fasting plasma insulin tended to increase, and this may have obscured any change in HIRI. Furthermore, although a small but significant decrease in EGP was noted in protocol II, the large variation in plasma insulin concentration (~40%), which directly affects calculation of the HIRI, also could have contributed to the failure to observe a decline in HIRI. Because fasting plasma glucagon concentration did not change and because glucagon extraction by the liver is minimal (<10%), reduced plasma glucagon concentration cannot account for the decrease in basal EGP. A direct inhibitory effect of exenatide on HGP, as recently suggested, cannot be excluded (4). The failure to observe a reduction in plasma glucagon concentration after exenatide administration before the MTT differs from previous results by us (8) and may be related to the route (iv *vs.* sc) and/or duration (acute *vs.* 2 wk) of exenatide administration.

In protocol I (no exenatide given in the morning of the repeat MTT), the decrement in FPG (by 2.6 μmol/liter) accounted for all of the decrement in postmeal plasma glucose concentration (Fig. 1). In protocol II (exenatide given before the repeat MTT), the increment in postmeal plasma glucose concentration was markedly reduced by more than 90% compared with protocol I, and this was due primarily to a marked decrease in the RaO in the systemic circulation (Table 1).

In contrast to the basal HIRI, which was significantly improved after 2 wk of exenatide, we did not observe any significant effect of exenatide on peripheral tissue insulin sensitivity as assessed by the Matsuda insulin sensitivity index. A small increase in glucose clearance during the MTT was observed when exenatide was given immediately before the meal (protocol II) but not when the last dose of exenatide was given on the previous evening (protocol I) (Table 1). However, the difference in glucose clearance during the MTT was not significant between protocols I and II and may represent an artifact of the calculation due to the greater decline in postmeal plasma glucose concentration in protocol II. The failure to observe a major effect of exenatide on insulin-mediated glucose disposal is consistent with previous observations (5, 8, 13, 23–26). Because urinary glucose excretion was small and decreased only slightly (by 0.7–0.9 μmol/kg · min) after exenatide in protocols I and II (Table 1), changes in renal glucosuria cannot explain the improvement in FPG or meal glucose tolerance after exenatide in T2DM.

T2DM subjects have reduced circulating GLP-1 levels (3, 27) and a marked defect in the ability of the liver to dispose of an ingested glucose load (18, 28–30). The lack of effect of exenatide on gastric emptying in protocol I allowed us to examine the effect of this GLP-1 analog on splanchnic (liver plus gastrointestinal tissues) glucose uptake. In the postexenatide study, splanchnic glucose retention was similar to that in the preexenatide study (12.3 ± 4.4 *vs.* 11.3 ± 5.4 g, *P* value NS). Thus, GLP-1 does not appear to be the long sought after gut factor that explains enhanced SGU after glucose ingestion (29, 30) and deficiency of which accounts for impaired SGU after oral glucose in T2DM (29–31). This conclusion may appear to be at variance with recently published studies in dogs (6, 14–16). However, in addition to species differences (man *vs.* dog), there were a number of other differences between the present and previous studies. In the studies by Johnson *et al.* (6) and Dardevet *et al.* (14), net hepatic glucose balance (HGB) (sum of HGP and HGU) was measured, GLP-1 was infused directly into the portal vein, and the increase in net HGB was quite small and not significant in one of the two studies (6). In the study by Edgerton *et al.* (15), both glucose and GLP-1 were delivered into the portal vein and vildagliptin (dipeptidyl peptidase-4 inhibitor) was given orally. Net HGB increased after vildagliptin in association with a rise in plasma GLP-1 and insulin levels and a decrease in plasma glucagon concentration. Whether the increase in net HGB is due to increased HGU or decreased HGP and what role vildagliptin *per se vs.* increased GLP-1 *vs.* the changes in plasma insulin and glucagon played in the increase in net HGB is difficult to determine. Ionut *et al.* (16) also suggested that GLP-1 may augment HGU, but no measures of HGU, HGP, or net HGB were made to explain the increase in whole-body glucose clearance after intraportal GLP-1 infusion.

One could argue that the lack of stimulatory effect of exenatide on SGU (protocol I) is not surprising, because exenatide was not administered on the day of MTT and plasma exenatide levels were undetectable. In an attempt to examine this question, we quantitated splanchnic glucose retention (sum of SGU and gastric retention of glucose) when exenatide was administered in the morning of the mixed meal (protocol II). Of the 75-g glucose load,

only 57.4 g, or 73% of the administered glucose load, was recovered in the systemic circulation (RaO). However, because exenatide delays gastric emptying (8), it is not possible to determine whether the decrease in RaO is explained by increased SGU or delayed gastric emptying and sequestration of the ingested glucose within the stomach. Because the inhibitory effect of exenatide on gastric emptying is related to the prevailing plasma exenatide concentration and is not observed until pharmacological levels of the GLP-1 analog are reached (32), it will be difficult in man to evaluate the effect of exenatide on SGU.

GLP-1 and GLP-1-mimetic agents are potent insulin secretagogues (2–3, 7, 23, 27, 33). In this study, the insulin secretion/insulin resistance index increased by 69% in protocol I compared with 109% in protocol II. Thus, in protocol II, exenatide still exerted a stimulatory effect on insulin secretion 16 h after administration, although the stimulatory effect on insulin secretion already had begun to wane by the following morning. Because exenatide treatment for 2 wk similarly reduced the fasting plasma glucose concentration in both protocols I and II (Table 1), it is unlikely that an improvement in glucotoxicity can explain the persistent effect of exenatide on insulin secretion 16 h after its administration. Bunck *et al.* (7) have shown that within 1 month after stopping exenatide (after treatment of T2DM patients for 1 yr), the stimulatory effect of exenatide on insulin secretion had completely disappeared. The precise time course of loss of effect of exenatide on insulin secretion remains to be determined, but the present results, when combined with those of Bunck *et al.* (7), clearly document that the stimulatory effect of exenatide on insulin secretion is lost within a relatively short time after its discontinuation. Baseline antidiabetic therapy (metformin, sulfonylurea, and metformin plus sulfonylurea) was similar in subjects in protocols I and II and, thus, cannot explain the stimulatory effect of exenatide on insulin secretion. Lastly, the improvement in insulin secretion index with exenatide in the three diet-treated subjects was similar to that in oral agent-treated individuals (data not shown).

In summary, the present study represents the first comprehensive evaluation of the physiological mechanisms via which chronic exenatide treatment in man improves glucose homeostasis after a typical mixed meal. Exenatide treatment of T2DM subjects for 2 wk decreased the FPG by inhibiting basal EGP. When exenatide is administered immediately before the meal, there is a marked reduction in postprandial glucose excursion due to a decrease in the rate of appearance of ingested glucose in the systemic circulation. We previously have shown that this decrease in RaO is due to inhibition of gastric emptying (8). This explains the much greater reduction in postmeal plasma glucose excursion compared with studies performed when exenatide is omitted before meal ingestion.

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Address all correspondence and requests for reprints to: Ralph A. DeFronzo, M.D., Division of Diabetes, Department of Medicine, Mail Code 7886, 7703 Floyd Curl Drive, San Antonio, Texas. E-mail: albarado@uthscsa.edu.

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