The Role of Endogenous Incretin Secretion as Amplifier of Glucose-Stimulated Insulin Secretion in Healthy Subjects and Patients With Type 2 Diabetes

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In order to quantify the role of incretins in first- and second-phase insulin secretion (ISR) in type 2 diabetes mellitus (T2DM), a double-blind, randomized study with 12 T2DM subjects and 12 healthy subjects (HS) was conducted using the hyperglycemic clamp technique together with duodenal nutrition perfusion and intravenous infusion of the glucagon-like peptide 1 (GLP-1) receptor antagonist exendin(9-39). Intravenous glucose alone resulted in a significantly greater first- and second-phase ISR in HS compared with T2DM subjects. Duodenal nutrition perfusion augmented both first- and second-phase ISR but first-phase ISR more in T2DM subjects (approximately eight- vs. twofold). Glucose-related stimulation of ISR contributed only 20% to overall ISR. Infusion with exendin(9-39) significantly reduced first- and second-phase ISR in both HS and T2DM subjects. Thus, both GLP-1 and non–GLP-1 incretins contribute to the incretin effect. In conclusion, both phases of ISR are impaired in T2DM. In particular, the responsiveness to glucose in first-phase ISR is blunted. GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) secretions are unaltered. The absolute incretin effect is reduced in T2DM; its relative importance, however, appears to be increased, highlighting its role as an important amplifier of first-phase ISR in T2DM. ***Diabetes*** **61:2349–2358, 2012**

Type 2 diabetes mellitus (T2DM) largely results as a consequence of impaired β-cell function and increased insulin resistance (1). In order to assess the magnitude and contribution of those components, various in vivo methods to assess insulin secretion (ISR) and resistance have been developed (2,3). Those methods include hyperglycemic clamp techniques, intravenous glucose tolerance tests, and modeling approaches, as well as oral glucose and mixed-meal testing. The most physiological approach to assess insulin secretion certainty represents mixed-meal testing (4). Interpretations of those data, however, are confounded by absolute differences in glucose concentrations as well as changes over time and differences in gastric emptying (5). To overcome these limitations, the hyperglycemic glucose clamp method has been developed, where plasma glucose concentrations are maintained stable over time through a variable intravenous glucose infusion. Under such conditions, a clear separation of the two phases of insulin responses can be identified (3). It is thought that the first-phase ISR results from the mobilization of stored insulin, whereas the second phase resembles the release of newly produced insulin and thus lasts as long as hyperglycemia exists (1). Using hyperglycemic clamp technique, impairment of both phases of ISR can be detected in T2DM. However, it appears that first-phase ISR may be more altered than second-phase ISR (6). A potential disadvantage of this method is that only the response of glucose can be assessed, whereas with the method of oral mixed-meal testing, ISR is the result of various factors, including lipid and amino acid efflux (7). Ingested nutrients lead to a stimulation of insulinotropic gut-born hormones usually termed incretins, where glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) seem to be the most important representatives (8,9). The incretin effect, in particular the role of GLP-1, is well described in the literature and is reduced in T2DM patients (10). However, our knowledge in patients with T2DM is largely based on experiments where either intravenous and oral glucose alone were given, thus not taking into account that lipids can also profoundly stimulate the incretin effect or on pharmacological experiments where mostly supraphysiological administration, in particular GLP-1, has been shown to improve insulin and to inhibit glucagon secretion (11). Previous studies on the incretin effect used either glucose as the sole stimulus or a pharmacological stimulation and, thus, did not examine the physiological situation, e.g. the additional effect of lipids and the action of endogenous incretins. The data on GIP appear to be less conclusive, and a role for GIP as an operative incretin in T2DM has been questioned (12–16). At present, however, no studies have been conducted to assess the effects of endogenous incretin secretion in first- and second-phase insulin release in humans and the extent that endogenous incretin release may amplify the magnitude of first- and second-phase ISR in T2DM.

To assess the impact of the incretin effect on first- and second-phase ISR, we combined the above-mentioned hyperglycemic clamp technique with a constant intraduodenal nutrient infusion. To determine the role of GLP-1, we conducted experiments with and without exendin(9-39), a specific GLP-1 receptor antagonist (17), which allowed us to estimate the GLP-1– and the non-GLP-1–mediated effects on first- and second-phase ISR.

RESEARCH DESIGN AND METHODS

Subjects. Written informed consent was obtained from 12 healthy subjects (HS) and 12 subjects with T2DM. The protocol was approved by the Munich Institutional Review Board of the Ludwig Maximilians University. Healthy subjects (seven men and five women, 40 ± 10 years of age, BMI = 27.8 ± 3.2 kg/m^2, HbA_{1c} = 5.1 ± 0.1%) had normal routine laboratory blood test results as well as no family history of T2DM and normal glucose tolerance as assessed by oral glucose tolerance tests. Patients with T2DM (six men and six women,

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60 ± 7 years of age, BMI = 28.1 ± 4.4 kg/m^2, HbA_{1c} = 6.4 ± 0.3%, known T2DM duration 4.5 ± 0.6 years) had normal physical examinations, no gastrointestinal symptoms, and no history of gastrointestinal diseases. Patients with previous treatment with GLP-1–based therapies, thiazolidinediones, or insulin were excluded from the studies. None had symptoms or a history of cardiac disease, gastrointestinal neuropathy, or evidence for nephropathy as assessed by microalbumin excretion. Previous treatment with no more than one oral antidiabetic medication was allowed and discontinued at least 1 week prior to study entry.

Experimental protocol. HS and patients with T2DM were studied on three occasions under hyperglycemic isoglycemic clamp conditions: on 2 days, a duodenal meal perfusion was performed with either intravenous saline or intravenous exendin(9-39), and on a third day, duodenal perfusion of saline and intravenous saline served as isoglycemic fasting control. Subjects were restrained from food intake at least 10 h before admission to the Clinical Research Unit between 7:00 and 7:30 A.M. on the study day. The subjects swallowed a duodenal probe for recording of antroduodenal motility and for duodenal meal perfusion. The catheter was placed with its tip at the ligament of Treitz. The correct position was checked either by fluoroscopy or manometry. A dorsal hand vein was cannulized, and temperature was maintained at 40°C with a thermo-regulated lamp for arterialized venous blood sampling (8). The contralateral forearm was used for intravenous infusions of glucose (hyperglycemic clamp) and the test solutions (exendin[9-39] or saline).

All experiments were performed in a double-blind, randomized fashion separated by time intervals of at least 3 days (Fig. 1). After collection of basal blood samples, an intravenous infusion of exendin(9-39) at 600 pmol · kg^{-1} · min^{-1} or saline was started and continued for 3 h. After 30 min, an intraduodenal meal perfusion (77% lipids [long-chain triglycerides 20%, Deltalipid; AlleMan Pharma] and 23% glucose) designed to stimulate incretin hormone release (18,19) was started at 2.03 kcal/min (3 mL/min) and continued throughout the study. After an additional 30 min, isoglycemic hyperglycemic clamp experiments were initiated with a target value of 180 mg/dL and continued for 2 h. After an initial bolus, glucose 20% was intravenously infused, and infusion rate adjustments were performed based on 5-min blood glucose determinations (17). Blood samples were collected at 15-min intervals throughout each experiment and at 2.5-min intervals during the first 10 min of the hyperglycemic clamp for determination of the first phase of ISR. Blood was collected in ice-chilled EDTA tubes containing aprotinin (500 KIU/mL) and diprotin A (3 mM, 50 μL/mL) and centrifuged immediately. Plasma was stored at −30°C until assayed.

Peptides and assays. Exendin(9-39) acetate was purchased as a lyophilized sterile powder at pharmaceutical grade from Bachem (Clinalfa, Läufelfingen, Switzerland). Blood glucose was measured using the glucose oxidase method (glucose analyzer; HemoCue GmbH, Ängelholm, Sweden).

The immunoreactivities of bioactive GLP-1(7-36) were determined by a double-sandwich immunoluminiscence assay using specific antibodies for capture (HYP147-06) and detection (ABS033-10-biot, free N-terminus specific; BioPorto Diagnostics, Gentofte, Denmark) after alcohol extraction of the plasma. The GLP-1 assay cross-reacts 100% with human GLP-1(7-36)amide with no measurable cross-reactivity with GLP-1(7-37)amide, GLP-1(9-36)amide, GLP-2(1-33)amide, GIP(3-42)amide, glucagon(1-29)amide, and exendin(9-39)amide. The lower detection limit is 0.4 pmol/L. Intra- and interassay coefficients of variations are <6 and <15%, respectively. Specific sandwich immunoluminiscence assays were also carried out for determining immunoreactivities of insulin and C-peptide. Commercial kits for glucagon (Human Glukagon RIA Kit, Linco Research) and GIP (ELISA, Human GIP(total) ELISA Kit, Linco Research) were obtained from Biotrend (Hamburg, Germany).

Calculations and statistical analysis. Power calculations were performed based on a two-tailed paired *t* test at the 5% significance level. A sample size of 12 subjects in each group ensured a power of 88% to yield a statistically significant difference in the incretin effect of at least 15%. This calculation was based on an intersubject coefficient of variation of 0.43 (20). Data are expressed as mean ± SEM. Blood glucose concentrations, the total glucose load and the plasma concentrations of hormones before hyperglycemic clamp are given as absolute values. The total glucose load represents the sum of the intestinal glucose perfusion and the intravenous glucose during clamps. Changes of plasma hormone concentrations during hyperglycemic clamp were calculated as incremental areas under the curve (AUCs) above the individual baseline levels according to the trapezoidal rule. Baseline levels of plasma hormones were calculated as the mean of levels at −70 and −60 min. The AUCs of plasma hormones were determined for the first 10 min of the clamp experiments to express first phase of ISR and between 60 and 120 min to assess the second phase of ISR. The incretin effects for first and second phase were calculated for insulin and C-peptide (10,20). Experiments with duodenal perfusion of saline and intravenous saline correspond to first- and second-phase ISR attributable to glucose alone. The difference between experiments with duodenal meal perfusion without exendin(9-39) and the respective isoglycemic experiments without intestinal nutrient perfusion represents the overall incretin effect, whereas the difference between meal perfusion without exendin(9-39) and meal perfusion with exendin(9-39) represents the incretin effect attributable to GLP-1. In addition, the ratio of the incretin effect to ISR was expressed as percent proportion to assess its relative contribution. Normality of distribution was assessed by the Kolmogoroff-Smirnov test. Effects of exendin(9-39) as well as differences between HS and T2DM subjects were analyzed using a two-way ANOVA using the intravenous infusions (intravenous saline/duodenal meal,

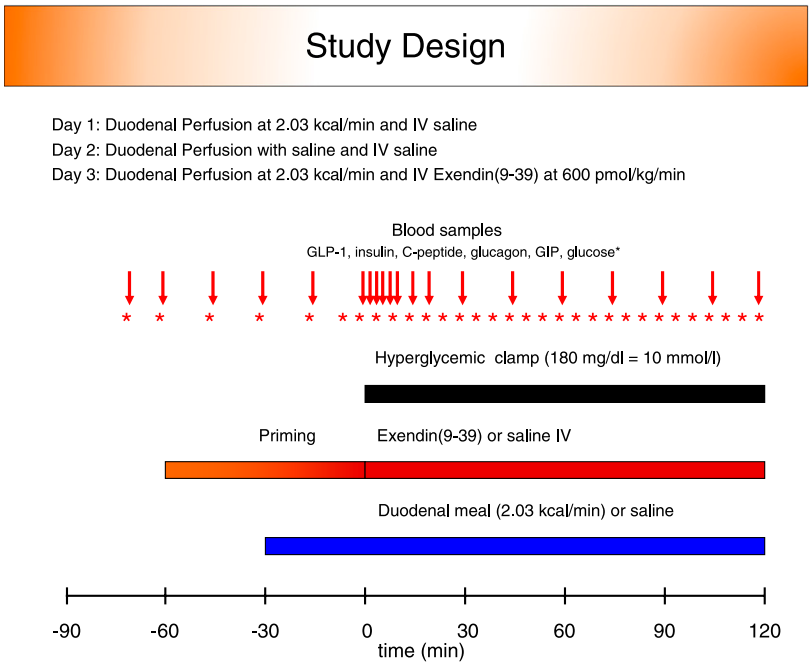

FIG. 1. Overview on study design. Red arrows indicate the blood samples for measuring plasma hormones; red asterisks indicate the time points of blood samples for glucose measurement. The asterisk in the term "glucose*" represents the time points of blood samples for glucose measurement that are indicated as asterisks in contrast to the arrows, which indicate the blood samples for measuring plasma hormones. (A high-quality color representation of this figure is available in the online issue.)

intravenous exendin(9-39)/duodenal meal, or intravenous saline/duodenal saline = isoglycemic control) as well as the health state as independent factors. If ANOVA indicated significant differences within the infusion subgroup or a significant interaction between intravenous infusion and health state, the Student-Newman-Keuls multicomparison test was used as post hoc test. Differences were considered significant at $P < 0.05$.

RESULTS

Plasma glucose and hormone concentrations prior to clamp experiments. All subjects tolerated the studies and no adverse events were reported. At study start, blood glucose levels of HS were within the normal range whereas the T2DM patients displayed elevated fasting glucose concentrations ($P < 0.001$). Exendin(9-39) significantly ($P < 0.01$) increased blood glucose concentrations during fasting in both groups. Exendin(9-39) did not alter the plasma concentrations of insulin and C-peptide in HS whereas T2DM subjects displayed significantly reduced C-peptide ($P < 0.001$) and moderately lowered plasma insulin ($P = 0.691$). During the same period, fasting plasma glucagon increased under exendin(9-39) in both HS and T2DM subjects ($P = 0.007$). The duodenal nutrient perfusion led to a small, but significant, rise of blood glucose, insulin, and C-peptide concentration, which was more pronounced in patients with T2DM ($P < 0.05$). Fasting plasma concentrations of GLP-1 and GIP did not differ between groups and increased significantly after duodenal meal perfusion in both groups ($P < 0.05$), by approximately five to sixfold (Table 1).

Hyperglycemic isoglycemic clamp experiments

First-phase insulin and C-peptide secretion rates. Administration of the intravenous glucose bolus led to an immediate increase of blood glucose concentrations, to a mean of ~190 mg/dL, in both HS and T2DM subjects. The total required glucose load was significantly lower in T2DM subjects. The typical first-phase ISR was not observed in patients with T2DM and thus was significantly lower compared with first-phase ISR in HS ($P < 0.01$) (Fig. 2). In both HS and T2DM subjects, plasma concentrations of insulin and C-peptide were significantly greater when nutrients were perfused compared with saline experiments. The difference resembles the incretin effect, which was, in absolute terms, twice as high in HS compared with T2DM subjects (Table 2 and Figs. 2 and 3), but plasma insulin increased to a lesser extent within HS (approximately twofold) compared with T2DM subjects (approximately eightfold); both $P < 0.05$ within the groups. In HS, the contribution of the incretin effect to the total insulin and C-peptide amounted to 62 ± 6.6 and 50 ± 8.1% for insulin and C-peptide, respectively. T2DM subjects starting off from lower levels exhibited a relatively greater increase in insulin and C-peptide, which results in a significantly greater contribution of the incretin effect (insulin, 90 ± 6.3%; C-peptide, 74 ± 8.5%) compared with HS ($P < 0.01$).

The proportion of meal experiment–related ISR over ISR in saline experiments represents the contribution of the incretin-independent effect to total ISR, i.e., the sole glucose-dependent stimulation in ISR. It amounted to 42.6 and 51.4% for insulin and C-peptide in HS, respectively, whereas it was only 12.6 and 6.9% in T2DM subjects, respectively, indicating that glucose-dependent first-phase ISR was largely impaired in T2DM subjects.

Administration of exendin(9-39) resulted in a significant reduction in plasma insulin and C-peptide secretion ($P < 0.05$), which was still significantly greater ($P < 0.05$) compared with isoglycemic fasting control experiments in both groups. Accordingly, the absolute incretin effect was significantly reduced with exendin(9-39) in HS and T2DM subjects. Plasma insulin increased to a greater extent within T2DM subjects compared with HS during the duodenal meal when exendin(9-39) was infused. Accordingly, the incretin effect accounted for 83 ± 9.6% (insulin) and 69 ± 9.8% (C-peptide) of the postprandial ISR in T2DM subjects, which was significantly greater than in HS (53 ± 8.1% [insulin], 33 ± 5.8% [C-peptide]; both $P < 0.05$ vs. T2DM).

Second-phase insulin and C-peptide secretion rates. During the steady state of the hyperglycemic clamp experiments, glucose concentrations were stable throughout and

TABLE 1
Effect of intravenous exendin(9-39) and duodenal nutrient perfusion on blood glucose and plasma hormones in HS and T2DM subjects prior to initiating hyperglycemic clamp

	Basal		Intravenous saline or exendin(9-39)			
	Fasting		Fasting		Duodenal meal	
	−70/−60 min		−30 min		0 min	
	Saline	Saline	Saline	Exendin(9-39)	Saline	Exendin(9-39)
Healthy subjects						
Blood glucose (mg/dL)	84 ± 3	85 ± 2	85 ± 3	92 ± 3*	92 ± 3§	98 ± 3§
Insulin (μU/mL)	9.1 ± 2.0	10.8 ± 1.6	8.8 ± 2.0	10.0 ± 1.8	16.1 ± 2.0§	13.7 ± 2.3§
C-peptide (pg/mL)	1.9 ± 0.2	1.9 ± 0.2	1.9 ± 0.2	1.6 ± 0.2	3.2 ± 0.3§	2.7 ± 0.4§
Glucagon (pg/mL)	38.7 ± 7.5	33.2 ± 6.6	33.5 ± 8.6	41.4 ± 7.3*	35.9 ± 7.2	37.3 ± 4.9
GLP-1 (pmol/L)	0.29 ± 0.06	0.3 ± 0.06	0.53 ± 0.1	0.45 ± 0.08	2.5 ± 0.6§	3.0 ± 0.8§
GIP (pg/mL)	27 ± 4	26 ± 3	23 ± 5	25 ± 3	141 ± 18§	146 ± 22§
T2DM						
Blood glucose (mg/dL)	122 ± 6#	122 ± 5#	118 ± 6#	129 ± 5*,#	126 ± 6#,§	145 ± 4*,#,§
Insulin (μU/mL)	4.9 ± 1.9#	3.7 ± 2.4#	5.3 ± 2.2#	2.5 ± 1.4#	10.1 ± 3.1§	11.5 ± 5.0§
C-peptide (pg/mL)	4.2 ± 0.5#	4.3 ± 0.8#	4.2 ± 0.6#	3.3 ± 0.5*,#	5.0 ± 0.7§	5.7 ± 1.7§
Glucagon (pg/mL)	44 ± 6	46 ± 7	47 ± 5	60 ± 7 *	54 ± 7#	64 ± 7#
GLP-1 (pmol/L)	0.22 ± 0.05	0.3 ± 0.07	0.7 ± 0.3	0.9 ± 0.2	2.4 ± 0.7§	4.0 ± 1.3§
GIP (pg/mL)	24 ± 4	26 ± 6	24 ± 5	23 ± 6	166 ± 34§	221 ± 50§

Mean ± SEM of total data of 12 HS and 12 patients with T2DM. *$P < 0.05$, significant difference between saline and exendin(9-39) considering effects of T2DM state. #$P < 0.05$, significant difference between T2DM and HS considering effects of intravenous infusion. §$P < 0.05$, significant difference between duodenal meal (0 min) and fasting state (−30 min), considering effects of intravenous infusion.

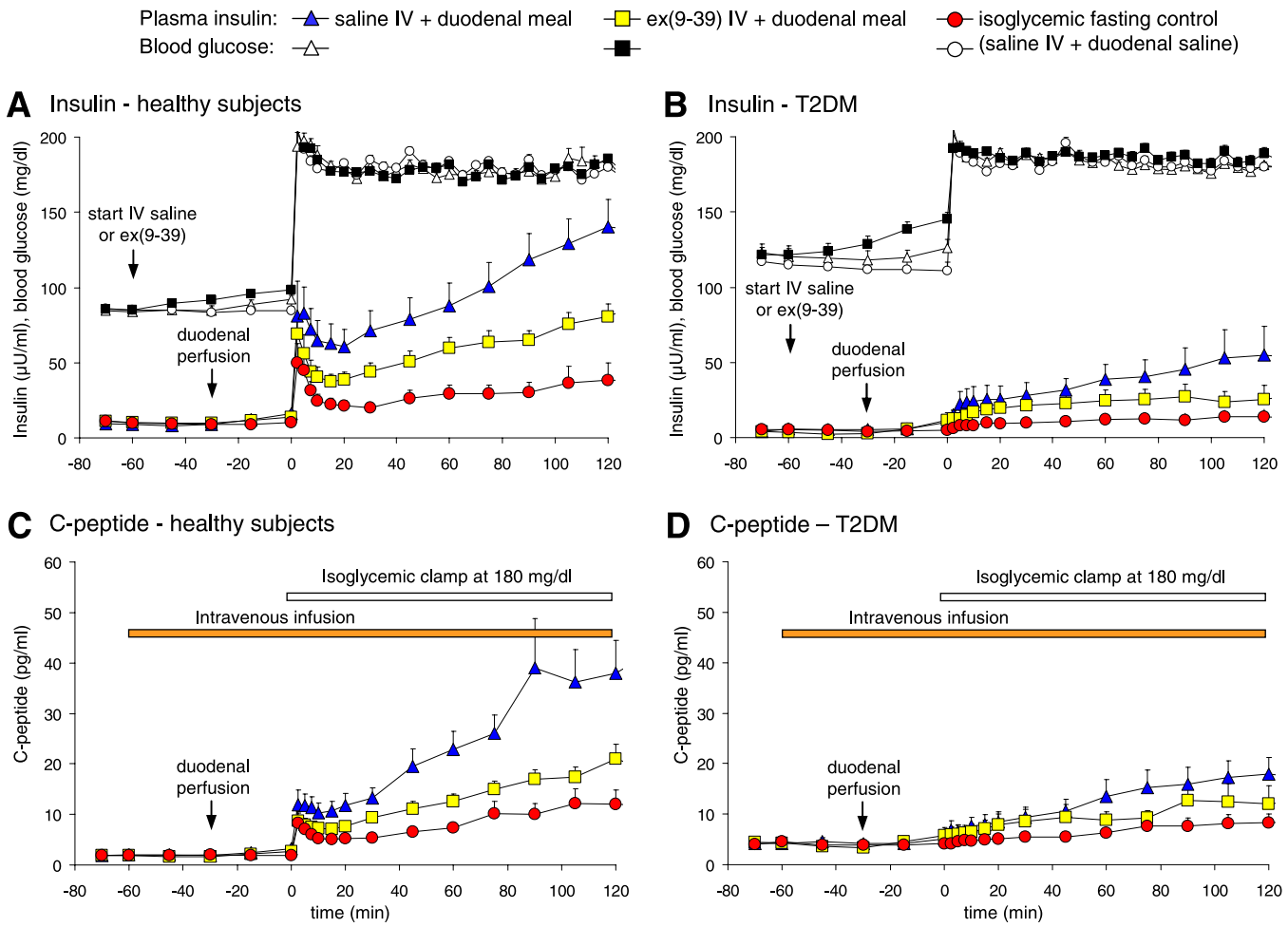

FIG. 2. Plasma insulin (*A* and *B*) and C-peptide (*C* and *D*) in response to hyperglycemic isoglycemic clamp experiments with intravenous (IV) saline plus duodenal meal (blue triangles), exendin(9-39) (ex[9-39]) IV plus duodenal meal (yellow squares), and isoglycemic IV glucose experiments without intraduodenal meal perfusion (red circles) in HS (*A* and *C*) and patients with T2DM (*B* and *D*). Blood glucose concentrations are shown in *A* and *B* (black and white symbols). *n* = 12 per group; mean ± SEM. See Tables 2 and 3 for statistical analysis.

averaged ~180 mg/dL between 60 and 120 min in all experiments. In both HS and T2DM subjects, plasma concentrations of insulin and C-peptide were significantly augmented in experiments with duodenal meal compared with the hyperglycemic isoglycemic fasting experiments. Second-phase ISR increased to a similar extent by approximately four- to fivefold in both HS and T2DM subjects (both $P <$ 0.05); in absolute terms, however, second-phase ISR was approximately twofold greater in HS compared with T2DM subjects ($P < 0.05$) (Table 3 and Figs. 2 and 3). Therefore, the absolute incretin effect was significantly reduced in the patients with T2DM compared with the HS by ~60%. In HS, the contribution of the incretin effect to the total ISR amounted to 80 ± 5.9 and 68 ± 6.1% for insulin and C-peptide, respectively. In T2DM subjects, the incretin effect contributed 85 ± 4.1 and 70 ± 6.6% to ISR in the second phase for insulin and C-peptide, respectively, which was similar to HS.

The glucose-dependent secretion rates for insulin and C-peptide were similar, with 20.3 and 26.3% in HS and 20.0 and 28.5% in T2DM subjects for insulin and C-peptide, respectively.

Exendin(9-39) significantly reduced postprandial insulin and C-peptide as well as the incretin effect during second-phase secretion in both groups. Still, hormone levels remained significantly elevated compared with the isoglycemic fasting control. The reduction of the incretin effect amounted to ~60% in both HS and T2DM subjects. In the T2DM subjects, the remaining incretin effect under GLP-1R blockade contributed 74 ± 6.8% (insulin) and 46 ± 7.7% (C-peptide) to the postprandial ISR and was not different from the HS (68 ± 9.4%, $P = 0.540$, and 44 ± 6.9%, $P = 0.825$).

Plasma GLP-1, GIP, and glucagon. Plasma GLP-1 and GIP concentrations increased promptly with initiation of the intraduodenal meal perfusion and were not significantly different between HS and T2DM subjects (Fig. 4 and Table 3). Of note, no meaningful changes in GLP-1 concentrations occurred when no nutrients where perfused into the duodenum. A small, but significant, increase in GLP-1 secretion was seen when exendin(9-39) was infused intravenously, which was more pronounced in T2DM subjects (Fig. 4*A* and *B*).

In contrast to HS, plasma concentrations of glucagon under duodenal meal perfusion were not suppressed in patients with T2DM (Fig. 5 and Table 3), resulting in significantly greater glucagon excursions ($P < 0.05$). These findings mirror the pathophysiologically relevant failure of

TABLE 2
Effect of duodenal nutrient perfusion and intravenous exendin(9-39) on blood glucose and plasma hormone levels and the incretin effect during first-phase ISR in HS and T2DM subjects

	Duodenal meal		Isoglycemic control: duodenal saline
	Saline IV	Exendin(9-39) IV	Saline IV
Healthy subjects			
Blood glucose (mg/dL)	194 ± 4	193 ± 3	190 ± 4
Total glucose load (mg/kg)	211 ± 9	188 ± 7*	204 ± 6
Insulin (μU/mL · min)	599 ± 158§	383 ± 82*,§	255 ± 81
C-peptide (pg/mL · min)	85.2 ± 19.4§	53.9 ± 10.1*,§	43.8 ± 11.0
Incretin effect			
Insulin (μU/mL · min)	345 ± 100	166 ± 39*	—
%	61.5 ± 6.6%	52.7 ± 8.1%*	
C-peptide (pg/mL · min)	45.4 ± 13.9	17.1 ± 4.7*	—
%	50.3 ± 8.1%	33.2 ± 5.8%*	
T2DM			
Blood glucose (mg/dL)	192 ± 3	191 ± 2	191 ± 4
Total glucose load (mg/kg)	129 ± 13§,#	86 ± 10*,§,#	148 ± 13#
Insulin (μU/mL · min)	145 ± 52§,#	97.5 ± 35.8*,§,#	18.2 ± 17.9#
C-peptide (pg/mL · min)	23.3 ± 10.0§,#	17.3 ± 8.5*,§,#	1.6 ± 3.1#
Incretin effect			
Insulin (μU/mL · min)	127 ± 44	79.4 ± 28.1*	—
%	90.1 ± 6.3%#	82.9 ± 9.6%*,#	
C-peptide (pg/mL · min)	21.7 ± 7.9	15.9 ± 6.6*	—
%	74.3 ± 8.5%#	68.5 ± 9.8%*,#	

Mean ± SEM during the first 10 min of the hyperglycemic clamp (first-phase ISR). Blood glucose concentration and total glucose load are given as total data. Plasma hormone concentrations are calculated as incremental AUCs above fasting individual baseline. IV, intravenous. *$P < 0.05$, significant difference compared with intravenous saline/duodenal meal. §$P < 0.05$, significant difference compared with isoglycemic control. #$P < 0.05$, significant difference between HS and T2DM subjects.

glucagon inhibition by hyperglycemia. Under GLP-1 receptor blockage, postprandial glucagon excursions significantly increased in both groups. This indicates that suppression of glucagon secretion by endogenous GLP-1 is also active in T2DM subjects.

DISCUSSION

The incretin effect describes the proportion of insulin being released as a consequence of additional gut hormone stimulation not being attributable to glucose as a stimulus. It is well known that pharmacological concentrations of the incretin hormone GLP-1 augment glucose-dependent insulin release (11). Under such conditions, GLP-1 concentrations are raised several-fold and thus one cannot necessarily equate to its relevance in human physiology and the pathophysiology of T2DM. Hence, it is not clear to what extent endogenously secreted incretins, and in particular GLP-1, contribute to the augmentation of ISR in patients with T2DM. Accordingly, the purpose of the present study was to evaluate the effects of endogenous secreted incretin hormones in first- and second-phase ISR in HS and patients with T2DM, and to quantify the incretin effect independent of confounding factors such as changes in glucose concentration or differences in gastric emptying.

The main findings of this study are as follows. *1*) Both phases of ISR are significantly impaired in T2DM subjects. In particular, the responsiveness of first-phase ISR to glucose was largely blunted. *2*) Stimulation of endogenous incretins resulted in a profound augmentation of ISR and resulted in an improvement in first-phase ISR in T2DM subjects. *3*) Approximately 60% of the secreted insulin was attributable to the incretin effect in both HS and T2DM subjects. However, its importance for first-phase insulin is greater in T2DM. *4*) In T2DM subjects, given the inability of glucose to stimulate first-phase ISR, incretins accounted for 80% of overall first-phase ISR and thus play a crucial role in facilitating immediate insulin release to a glucose stimulus. *5*) The incretin effect is operative in T2DM subjects; however, its magnitude is reduced by 60%. *6*) Endogenous GLP-1 is an important component of the incretin effect and accounts for ~60% of the incretin effect. *7*) Endogenous GLP-1 inhibits glucagon secretion in both HS and T2DM subjects, indicating that suppression of glucagon secretion by endogenous GLP-1 is also active in T2DM subjects.

As expected, and previously described (21), we found glucose-dependent ISR largely impaired in T2DM subjects with an almost absent first-phase ISR. Perfusion of nutrients into the duodenum was associated with a significant increase in plasma GLP-1 and GIP concentrations as well as a robust increase in both first- and second-phase insulin release both in HS and patients with T2DM. Thus, these data document impressively that the stimulation of the enteroinsulinar axis plays an important role in facilitating postprandial ISR in humans and are fully in line with previous studies. Interestingly, we found the incretin effect in first- and second-phase ISR to be different in HS and T2DM subjects. Insulin secretion was increased by approximately four- to fivefold during second-phase ISR in both HS and T2DM subjects. In contrast, incretins augmented first-phase ISR to a greater extent in T2DM subjects as compared with HS (approximately eight- vs. twofold) and thus at least partially overcame the inability of glucose to stimulate first-phase ISR in T2DM subjects where only ~10% of first-phase ISR was directly related to the glucose stimulus. Various factors, such as differences in impact of glucose toxicity and reduction in sensitivity of

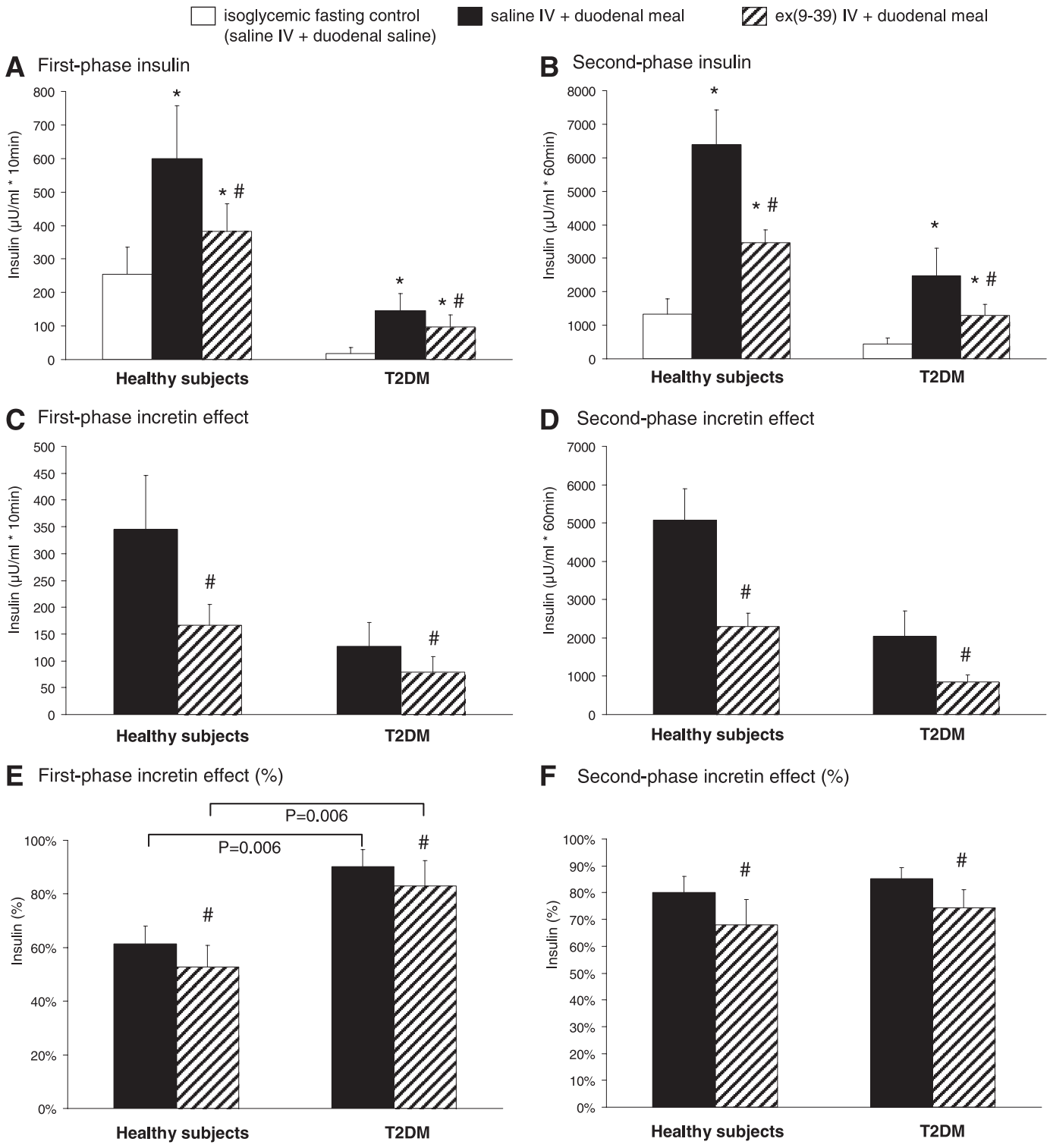

FIG. 3. Plasma insulin (*A* and *B*), incretin effect (*C* and *D*), and percentage contribution of the incretin effect to the total insulin response (*E* and *F*) in response to hyperglycemic isoglycemic clamp experiments during first-phase ISR (*A*, *C*, and *E*) and second-phase ISR (*B*, *D*, and *F*) with intravenous (IV) isoglycemic glucose experiments without intraduodenal meal perfusion (isoglycemic fasting control, white bars), with saline IV plus duodenal meal (black bars), and with exendin(9-39) (ex[9-39]) IV plus duodenal meal (striated bars) in HS and patients with T2DM. *n* = 12 per group; mean ± SEM of incremental AUC. **P* < 0.05 vs. isoglycemic fasting control; #*P* < 0.05 vs. saline IV plus duodenal meal. See Tables 2 and 3 for further statistical analysis.

the β-cell as well differences in responsiveness to various stimuli in first- and second-phase insulin release, may be involved. For example, sulfonylurea seems to predominantly stimulate the second phase of ISR in hyperglycemic clamp conditions (22,23), whereas others such as GLP-1, exenatide, and arginine seem to have a greater impact on the first phase of ISR (24–26).

Earlier studies described the incretin effect as being reduced both in absolute as well as relative terms (10). In accordance with these data, we also find the incretin effect

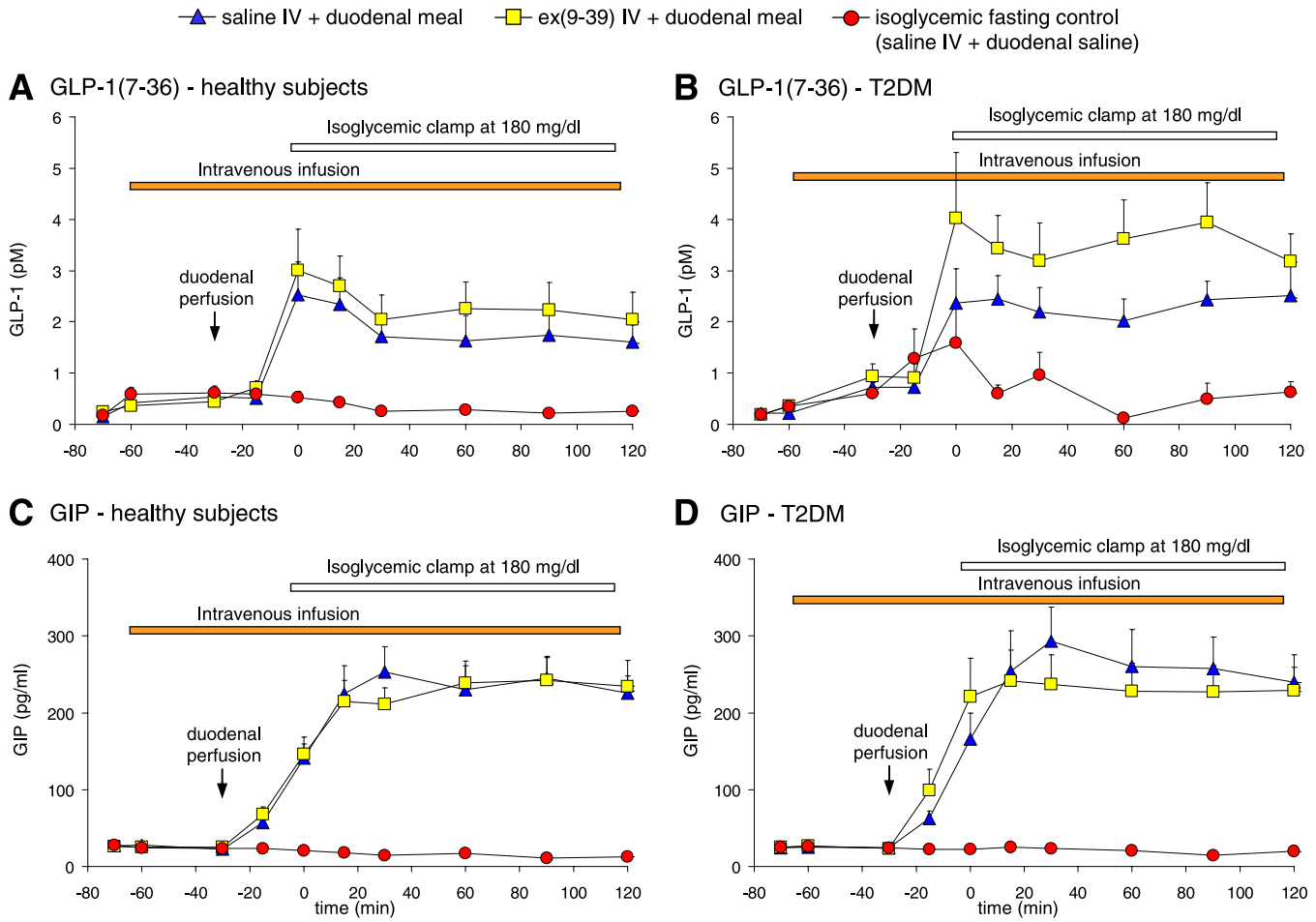

FIG. 4. Plasma GLP-1 (*A* and *B*) and GIP (*C* and *D*) concentration in response to hyperglycemic isoglycemic clamp experiments with intravenous (IV) saline plus duodenal meal (blue triangles) or exendin(9-39) (ex[9-39]) IV plus duodenal meal (yellow squares) and isoglycemic IV glucose experiments without intraduodenal meal perfusion (red circles) in HS (*A* and *C*) and patients with T2DM (*B* and *D*). *n* = 12 per group; mean ± SEM. See Table 3 for statistical analysis.

reduced by ~60% compared with HS. In contrast, however, we find the relative responsiveness to be either comparable regarding second-phase ISR or even increased for first-phase ISR. These findings argue against an incretin-specific defect in T2DM and argue more toward a general β-cell defect, be it β-cell mass or others. The major difference between previous studies and ours is that we assessed insulin responses under hyperglycemic clamp conditions, representing the gold standard to simultaneously assess first- and second-phase insulin release, the latter not necessarily possible in oral glucose or mixed-meal testing. A distinct β-cell stimulation by oral glucose challenges (10) may preclude an additional stimulation by incretins. Further arguments that the incretin effect may be reduced in T2DM comes from several studies (27–29) suggesting that postprandial secretion of GLP-1 may be reduced in T2DM. We, however, find GLP-1 concentrations to be unaltered. These, on a first glance, conflicting results may again be explained by the different experimental design. We have chosen to perfuse nutrients directly into the duodenum in order to exclude potential impact of differences on gastric emptying. Hyperglycemia is a potent inhibitor of gastric emptying, and T2DM is frequently associated with some delay in gastric emptying (30–32). However, nutrient flux is one of the most important components in determining incretin release (8,18,19). Thus, even though our data provide strong evidence that GLP-1 secretion is not impaired in T2DM, it does not exclude that GLP-1 concentrations may be reduced due to the effects of hyperglycemia on gastric emptying. Irrespective of the cause, the prevailing study results provide strong evidence that incretin hormone secretion and the relative contribution of the incretin effect is not impaired in T2DM (given the differences in insulin secretory capacity) and thus are fully in line with a recent assessment that the reduction of the incretin effect in patients with T2DM may simply be an epiphenomenon of impaired β-cell function (33). It appears, however, that an operative enteroinsulinar incretin effect is crucial to overcoming reduced glucose-dependent first-phase insulin release.

To further elucidate the potential role of GLP-1 as part of the incretin effect, we performed a third set of experiments where, in addition to intravenous glucose infusion and duodenal meal perfusion, the incretin action was blocked with the GLP-1 receptor antagonist (17,34). Accordingly, the prevailing experiments allowed us to distinguish GLP-1 and non–GLP-1 incretin effects on first- and second-phase ISR. GLP-1 receptor blockade with exendin (9-39) significantly reduced both phases of ISR and thus clearly supports previous studies suggesting the concept that endogenous GLP-1 secretion is a critical component of the incretin-mediated augmentation in ISR (20,35). To

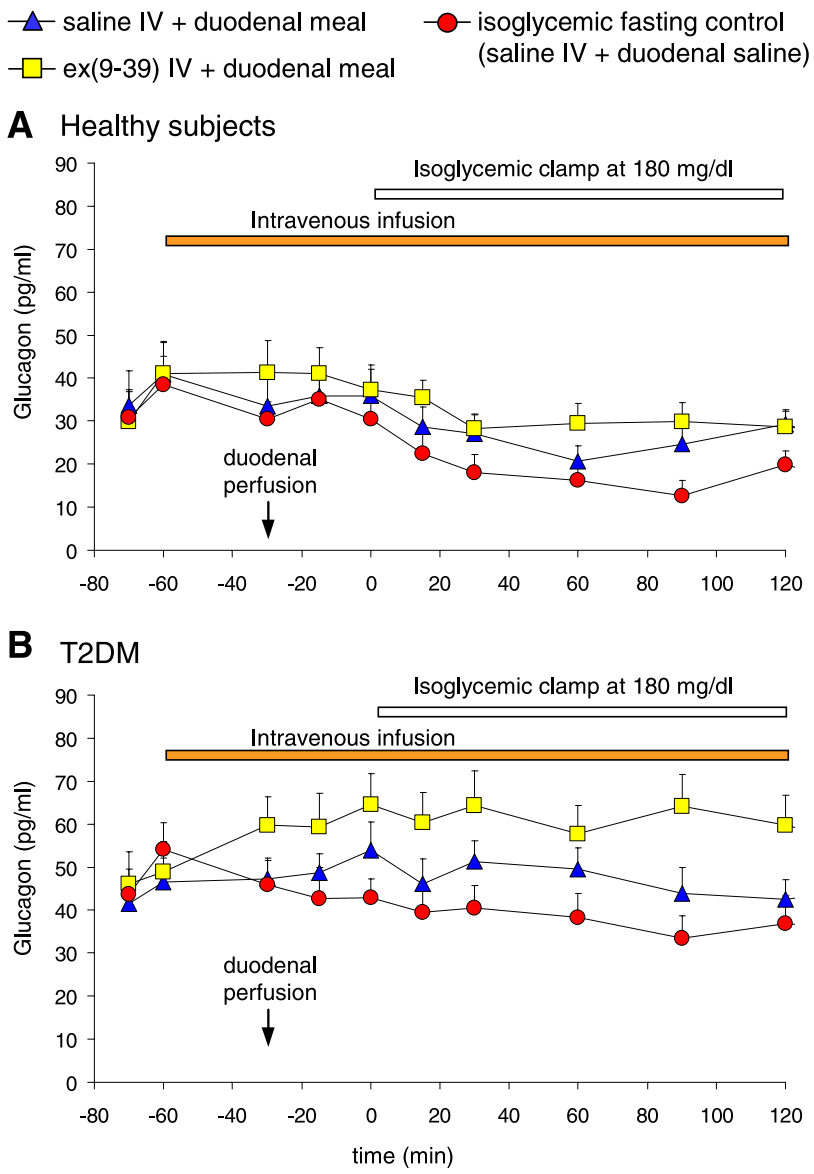

FIG. 5. Plasma glucagon concentration in response to hyperglycemic isoglycemic clamp experiments with intravenous (IV) saline plus duodenal meal (blue triangles) or exendin(9-39) (ex[9-39]) IV plus duodenal meal (yellow squares) and isoglycemic IV glucose experiments without intraduodenal meal perfusion (red circles) in HS (*A*) and patients with T2DM (*B*). *n* = 12 per group; mean ± SEM. See Table 3 for statistical analysis.

our knowledge, these are the first studies providing strong evidence that indeed endogenous GLP-1 plays a crucial role in incretin-related augmentation of both first- and second-phase ISR. According to the prevailing data, ~60% of the incretin-related effect on first- and second-phase ISR is attributable to GLP-1. It has been previously reported that only pharmacological, but not physiological, levels of GLP-1 enhance ISR in patients with T2DM (36). Our findings are certainly contrary to those reports. In accordance with Salehi et al. (35), who investigated the effect of exendin (9-39) on ISR during fasting and during an oral meal under hyperglycemic clamp conditions, we find that in the digestive state, GLP-1 contributes significantly to the overall incretin effect.

A still-controversial candidate for the residual insulinotropic action in T2DM appears to be GIP, because some studies report insulinotropic action of synthetic GIP to be impaired (12–14,16) whereas others provide evidence that synthetic GIP, at least at higher pharmacological dosages, exerted insulinotropic effects (15). Even though it has been shown that exendin(9-39) dose-dependently inhibits the actions of GLP-1 (17) and that the dosage being used in the prevailing studies should have inhibited the insulinotropic action of synthetic GLP-1 by ~95% (34), we cannot exclude that the non–GLP-1 proportion may be overestimated. Apart from the humoral pathway of incretin action, animal studies suggested neurally mediated effects of endogenous GLP-1 on glucose homeostasis and B-cell function (37,38). High local, endogenous GLP-1 concentrations on neurons within the intestinal wall or within portal vessels may not have been fully antagonized with the dose of exendin(9-39) presently used, although their contribution to the incretin effect is unclear. Furthermore, it can only be speculated to what extent GIP may have contributed to the overall incretin effect. However, based on the current experiments, GIP remains a potential candidate for incretin-stimulated

TABLE 3
Effect of duodenal nutrient perfusion and intravenous exendin(9-39) on blood glucose and plasma hormone levels and the incretin effect during second-phase ISR in HS and T2DM subjects

	Duodenal meal		Isoglycemic control: duodenal saline
	Saline IV	Exendin(9-39) IV	Saline IV
Healthy subjects			
Blood glucose (mg/dL)	178 ± 1.2	177 ± 0.5	178 ± 0.4
Total glucose load (mg/kg)	770 ± 98§	675 ± 50§	343 ± 52
Insulin (mU/mL · min)	6.4 ± 1.0§	3.5 ± 0.4*,§	1.3 ± 0.5
C-peptide (ng/mL · min)	1.9 ± 0.4§	0.9 ± 0.1*,§	0.5 ± 0.1
Glucagon (pg/mL · min)	−837 ± 361§	−224 ± 283*,§	−1,091 ± 220
GLP-1 (pM · min)	83.3 ± 24.9§	113.7 ± 30.0*,§	−8.2 ± 5.2
GIP (ng/mL · min)	12.6 ± 1.6§	12.8 ± 1.8§	−0.8 ± 0.1
Incretin effect			
Insulin (mU/mL · min)	5.1 ± 0.8	2.3 ± 0.3*	—
%	80.0 ± 5.9%	68.0 ± 9.4%*	
C-peptide (ng/mL · min)	1.3 ± 0.3	0.4 ± 0.08*	—
%	67.9 ± 6.1%	44.0 ± 6.9%*	
T2DM			
Blood glucose (mg/dL)	180 ± 1.0	187 ± 2.7*,§,#	181 ± 0.8
Total glucose load (mg/kg)	180 ± 26#	121 ± 14#	110 ± 11#
Insulin (mU/mL · min)	2.5 ± 0.8§,#	1.3 ± 0.4*,§,#	0.5 ± 0.2
C-peptide (ng/mL · min)	0.7 ± 0.2 §,#	0.4 ± 0.1*,§	0.2 ± 0.08
Glucagon (pg/mL · min)	54 ± 216§,#	942 ± 213*,§,#	−838 ± 220#
GLP-1 (pM · min)	128 ± 24§	204 ± 38*,§	10.2 ± 9.3
GIP (ng/mL · min)	13.8 ± 2.3§	12.1 ± 1.7§	−0.5 ± 0.2
Incretin effect			
Insulin (mU/mL · min)	2.0 ± 0.7#	0.8 ± 0.2 *,#	—
%	85.3 ± 4.1%	74.4 ± 6.8%*	
C-peptide (ng/mL · min)	0.5 ± 0.1#	0.2 ± 0.06 *,#	—
%	69.7 ± 6.6%	46.2 ± 7.7%*	

Mean ± SEM during 60–120 min of the hyperglycemic clamp. Blood glucose concentration and total glucose load are given as total data. Plasma hormone concentrations are calculated as incremental AUCs above fasting individual baseline. IV, intravenous. *$P < 0.05$, significant difference compared with intravenous saline/duodenal meal. §$P < 0.05$, significant difference compared with isoglycemic control. #$P < 0.05$, significant difference between HS and T2DM subjects.

ISR. This, however, is in contrast to recent studies suggesting that only supraphysiological GIP concentration may exhibit insulinotropic actions (16).

Plasma concentrations of bioactive GLP-1 during the duodenal meal were increased under GLP-1R blockade in both HS and T2DM subjects. This effect was not observed for GIP. Similar elevations of total GLP-1 under exendin(9-39) were reported in previous studies (20,35,39). It is consistent with the hypothesis that there is an auto-feedback inhibition whereby GLP-1 restrains L-cell secretion.

Glucagon plays an essential role in glucose regulation. In HS, the pancreatic A cell is under a tonic inhibitory control of GLP-1 (17,20,39), which suppresses glucagon secretion through a paracrine action by somatostatin (40,41). Inhibition of glucagon takes a substantial part within the antidiabetic actions of GLP-1–based therapies. In the present study, plasma glucagon increased under GLP-1R blockade even in T2DM subjects both during fasting and the duodenal meal. Hence, our data demonstrate that endogenous GLP-1 suppresses glucagon secretion in T2DM patients, thus not providing evidence that the pathological hypersecretion of glucagon in T2DM subjects is related to dysfunctional GLP-1 inhibition.

In summary, the current study quantifies for the first time the contribution of incretins to first- and second-phase insulin release under standardized clamp excluding confounding factors such as changes in plasma glucose concentration over time and differences in rates of gastric emptying. Overall, the enteroinsulinar axis appeared to be operative in both HS and T2DM subjects. Stimulation of incretin hormone release resulted in a profound augmentation of ISR not only in HS but also in patients with T2DM, where, in particular, first-phase ISR was found to be largely augmented. Our findings do not support a specific defect of the enteroinsulinar axis within the pathophysiology of T2DM but rather support the concept that the absolute incretin effect is reduced as a consequence of the underlying β-cell defect and suggest that GLP-1–based therapies may in particular be advantageous for improving impairments in first-phase ISR in patients with T2DM.

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H.J.W., L.C., and J.S. wrote the manuscript and researched data. A.D. researched data and reviewed and edited the manuscript. B.G. contributed to discussion and reviewed and edited the manuscript. J.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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