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Effects of Sitagliptin and Metformin Treatment on Incretin Hormone and Insulin Secretory Responses to Oral and "Isoglycemic" Intravenous Glucose

Dipeptidyl peptidase-4 (DPP-4) inhibitors prevent degradation of incretin hormones (glucagon-like peptide 1 [GLP-1] and glucose-dependent insulinotropic polypeptide [GIP]), whereas metformin may increase GLP-1 levels. We examined, in a four-period crossover trial, the influence of metformin (2,000 mg/day), sitagliptin (100 mg/day), or their combination, on GLP-1 responses and on the incretin effect in 20 patients with type 2 diabetes, comparing an oral glucose challenge (75 g, day 5) and an "isoglycemic" intravenous glucose infusion (day 6). Fasting total GLP-1 was significantly increased by metformin and not changed by sitagliptin. After oral glucose, metformin increased and sitagliptin significantly decreased (by 53%) total GLP-1. Fasting and postload intact GLP-1 increased with sitagliptin but not with metformin. After oral glucose, only sitagliptin, but not metformin, significantly augmented insulin secretion, in monotherapy and as an add-on to metformin. The incretin effect was not changed numerically with any of the treatments. In conclusion, sitagliptin increased intact GLP-1 and GIP through DPP-4 inhibition but reduced total GLP-1 and GIP (feedback inhibition) without affecting the numerical contribution of the incretin effect. Insulin secretion with sitagliptin treatment was similarly stimulated with oral and "isoglycemic" intravenous glucose. This points to an important contribution of small changes in incretin concentrations within the basal range or to additional insulinotropic agents besides GLP mediating the antidiabetic effects of DPP-4 inhibition.

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The incretin effect denotes the phenomenon whereby oral glucose stimulation elicits a higher insulin secretory response compared with "isoglycemic" intravenous glucose and is explained by the actions of the intestinally derived incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (1). In patients with type 2 diabetes, this incretin effect is impaired (2), mainly because diabetic β-cells no longer respond to GIP (3,4). However, the effects of GLP-1 in type 2 diabetes are impaired (5) but are better retained compared

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with healthy control subjects (4), and incretin-based medications successfully lower plasma glucose in type 2 diabetic patients (1). Activation of GLP-1 receptors stimulates insulin secretory responses (4,5) and suppresses glucagon (4,6), with additional effects on gastrointestinal motility (7,8) and on the central regulation of appetite (9,10), food intake (9,11), and body weight (12). Incretin mimetics are injectable GLP-1 receptor agonists that result in pharmacological concentrations of the agonist (1) (compared with physiological concentrations of endogenous GLP-1 [4,13,14]). In contrast, dipeptidyl peptidase-4 (DPP-4) inhibitors prevent the proteolytic degradation and inactivation of GLP-1 and GIP (1,15).

These hormones are primarily thought to contribute to the insulin secretory response after meals or oral glucose challenges while playing only a minor role at low, basal, non–nutrient-stimulated plasma concentrations (16–18). Therefore, it could be postulated that enhancement of endogenous intact incretin levels with DPP-4 inhibitors may ameliorate the defective incretin effect seen in type 2 diabetes. Despite the plausibility of this hypothesis, our previous study using vildagliptin treatment did not show any change in the numerical contribution of the incretin effect to insulin secretory responses after oral glucose challenges (19). A similar study, using mixed-meal stimulation of insulin secretion, also came to a similar conclusion (20).

DPP-4 inhibitors are most often used in conjunction with metformin (21,22). Metformin, according to some clinical observations, may elevate concentrations of GLP-1 through a stimulation of production/secretion (23) or through an inhibition of DPP-4 activity (24,25), which, however, has been disputed (26,27). Accordingly, it is possible that a combined treatment with metformin plus a DPP-4 inhibitor might result in a meaningful interaction at the level of L-cell (GLP-1) secretion. This was not explored in our previous study using vildagliptin treatment (19).

The aim, therefore, of the current study was to explore the influence of treatment with the DPP-4 inhibitor sitagliptin, with metformin, or with a combination of both, on the secretion of incretin hormones and on the incretin effect in patients with type 2 diabetes. Preliminary data have been communicated in abstract form (28).

RESEARCH DESIGN AND METHODS

Study Protocol

The study protocol was approved by the Georg August University Göttingen Ethics Committee before the study (registration number 1/4/08; date of approval: 10 October 2008). Written informed consent was obtained from all participants.

Patients

Twenty-one patients with type 2 diabetes participated in the current study. One patient withdrew due to dizziness and uncontrolled hyperglycemia. The characteristics of the 20 patients who completed the study are presented in Table 1. They were drug-naïve or treated with oral antihyperglycemic monotherapy before entering the study. Key inclusion criteria were *a*) Patients with type 2 diabetes not on antihyperglycemic medication or patients treated with metformin or a sulfonylurea (monotherapy), who were willing to discontinue their medication for the duration of the study; *b*) age in the range of 30–75 years (inclusive); *c*) BMI in the range of 25–35 kg/m^2 (inclusive); *d*) HbA_{1c} of $\geq$6.5 and $\leq$9.0% inclusive (if drug-naïve) or $\geq$6.0 and $\leq$8.5% (if being treated with metformin or sulfonyurea); *e*) fasting plasma glucose of $\geq$110 mg/dL ($\geq$6.1 mmol/L) and $\leq$220 mg/dL ($\leq$12.2 mmol/L) before and after a 2-week placebo run-in period; *f*) male or nonfertile female (women of childbearing potential using a medically approved birth control

Table 1—Patient characteristics

Parameter	Value
Sex	
Male	16 (80)
Female	4 (20)
Age, years	59 ± 7 (44–73)
BMI, kg/m^2	30.6 ± 3.0 (24.1–36.5)
HbA_{1c}, %	7.0 ± 0.6 (6.0–7.9)
Diabetes duration, years	5 ± 3 (1–10)
OAD pretreatment	
Yes	16 (80)
No	4 (20)
Metformin pretreatment	
Yes	15 (75)
No	5 (25)
Metformin dose, mg/day	1,480 ± 585 (500–2,000)
Glimepiride pretreatment	
Yes	1 (5)
No	19 (95)
Glimepiride dose, mg/day	3
Serum creatinine, μmol/L	89 ± 12 (62–106)
Triglycerides, mg/dL	169 ± 115 (47–572)
LDL cholesterol, mg/dL	127 ± 51 (70–169)
Blood pressure, mmHg	
Systolic	141 ± 11 (124–170)
Diastolic	86 ± 11 (48–95)
Arterial hypertension	
Yes	16 (80)
No	4 (20)
Pulse rate, min^{-1}	70 ± 12 (48–95)

Data are presented as mean ± SD (range) or *n* (%). OAD, oral antidiabetic agent.

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method could be included if they were willing to use the same method of contraception during the full course of the study); *g*) written informed consent to participate in the study.

At a screening visit, body height and weight were measured to calculate the BMI (Table 1), and blood was drawn in the fasting state for measurements of standard hematological and clinical chemistry parameters. Spot urine was sampled for the determination of albumin, protein, and creatinine by standard methods. Eligible patients entered a 6-week washout period if previously treated with oral antidiabetic agents. All patients entered a 2-week single-blind run-in period before randomized treatment was started.

Study Design

After the run-in period, eligible patients entered the crossover study period, which consisted of four double-blind treatment periods lasting 6 days each (order randomized) with three 4-week (tolerance −3/+7 days) washout periods between treatments. The incretin effect was quantified after oral treatment with sitagliptin (100 mg daily), metformin (dose escalation day 1, 500 mg, daily; day 2, 500 mg twice daily; day 3, 500 mg three times daily; days 4 to 6, 500 mg four times daily, i.e., 2,000 mg/day), and the metformin/sitagliptin combination or matching placebo tablets.

Experimental Procedures

The tests were performed in the morning after an overnight fast. On day 5 of each treatment period, an oral glucose challenge (75 g glucose and glucose oligomers; Roche O.G.T.) was given at 0 min. On day 6, 20% glucose was administered intravenously to copy the glycemic excursions obtained after the oral glucose ("isoglycemic" intravenous glucose infusion), as previously described (19). After basal blood specimens were drawn at –15 and 0 min, blood was taken at 15, 30, 45, 60, 90, 120, 180, and 240 min.

Laboratory Determinations

Glucose, insulin, C-peptide, total GLP-1 (C-terminally directed assay), intact GLP-1 (sandwich ELISA), total GIP (C-terminally directed assay), intact GIP (N-terminally directed assay), and glucagon were determined as previously described (19).

Calculations

Integrated values (areas under the curve) were calculated using the trapezoidal rule. Incremental responses describe changes above baseline, whereas "total" responses describe the response above 0.

Insulin secretion rates were calculated from C-peptide concentrations using ISEC 3.4a software supplied by Dr. Roman Hovorka, London, U.K. (29). Population-derived coefficients of transition between compartments were used as described (30,31).

The incretin effect was calculated based on the integrated incremental responses (trapezoidal rule) of plasma insulin, C-peptide, or insulin secretion rates after oral and "isoglycemic" intravenous glucose administration. The difference was related to the respective response after oral glucose, which was taken as 100%. Therefore, incretin effects were expressed as the percent contribution to the total β-cell secretory response after oral glucose, as previously described (2,19).

Statistical Analysis

Patient characteristics are reported as mean ± SD, and results are reported as mean ± SEM. The significance of any differences was assessed using repeated-measures ANCOVA or ANOVA, as appropriate, using Statistica 5.0 software (StatSoft Europe, Hamburg, Germany). When comparing experiments with the oral versus intravenous administration of glucose, the type of experiment was used as the independent variable. When the four experimental treatments were to be compared (baseline values or integrated responses), the treatments (sitagliptin or metformin, either yes or no) were used as independent variables. Mean values of the parameter of interest as determined with placebo treatment were imputed as the covariate. Results of ANCOVA are reported as *P* values for (A) differences by experiment, (B) differences over time, and (AB) differences due to the interaction of experiment and time. If a significant interaction was documented ($P < 0.05$), values at single time points were compared by one-way ANOVA for repeated measurements and Duncan's post hoc test. *P* values for the influence of treatment on basal and integrated parameters are presented for sitagliptin treatment, metformin treatment, or the interaction between sitagliptin and metformin treatment. A two-sided *P* value of <0.05 was taken to indicate significant differences.

RESULTS

Clinical Effectiveness

Sitagliptin, metformin, and the sitagliptin/metformin combination led to reductions in fasting glucose. Compared with placebo (8.2 ± 0.4 mmol/L), sitagliptin treatment alone reduced fasting plasma glucose to 7.4 ± 0.3 mmol/L ($P < 0.0001$), metformin treatment alone reduced fasting plasma glucose to 7.1 ± 0.2 mmol/L ($P < 0.0001$), and the combination of sitagliptin and metformin further reduced fasting plasma glucose to 6.5 ± 0.2 mmol/L ($P < 0.0001$). Fasting glucose concentrations were similar before oral and intravenous glucose administration for all experiments ($P = 0.30$–0.82; Fig. 1). After oral glucose administration, the integrated incremental glucose concentration was reduced, relative to placebo treatment, by 26.8% with sitagliptin ($P < 0.0001$), by 23.4% with metformin ($P < 0.0001$), and by 46.4% with the metformin/sitagliptin combination ($P < 0.0001$; Table 2).

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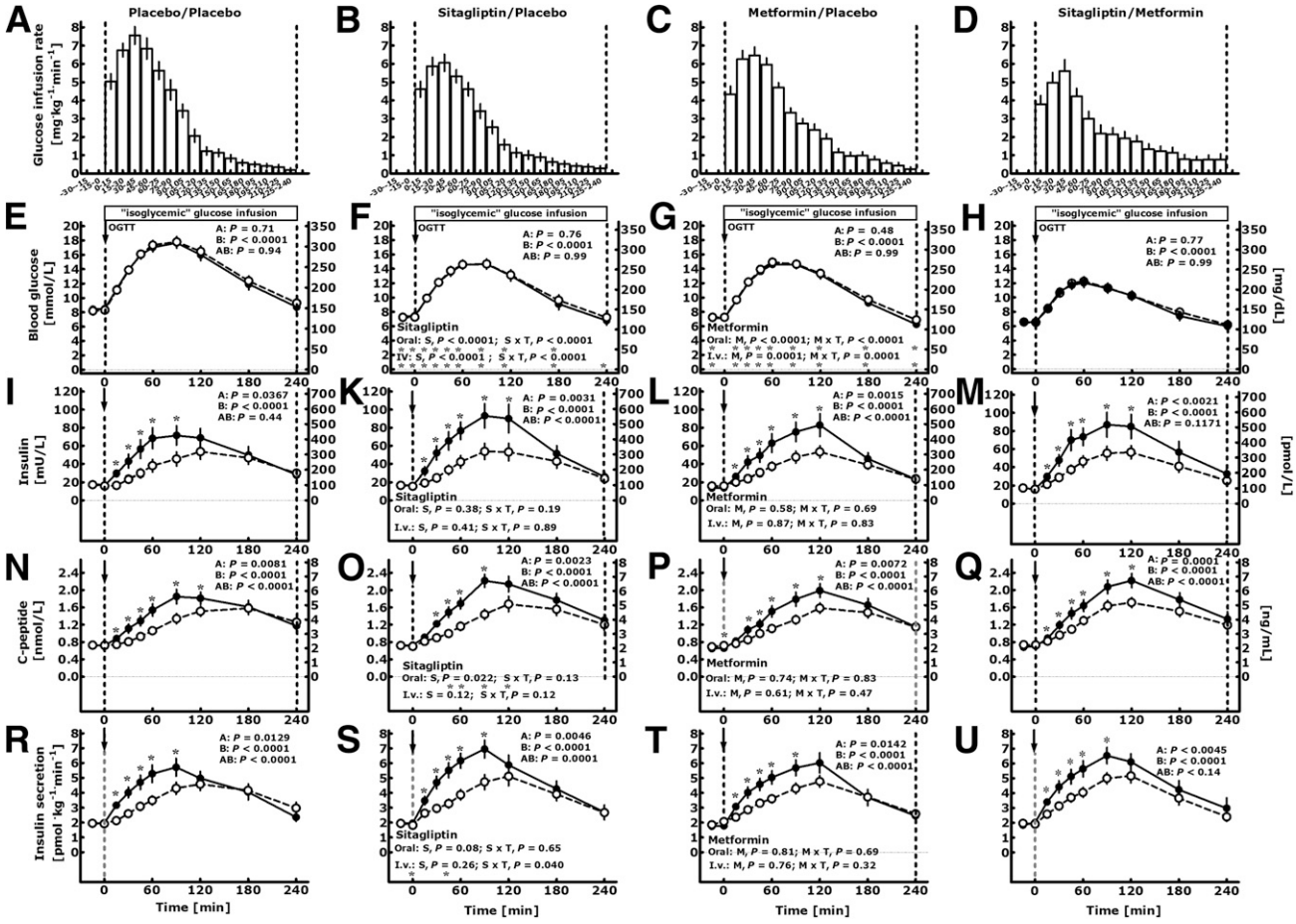

Figure 1—Time course of glucose infusion rates (*A–D*), concentrations of plasma glucose (*E–H*), insulin (*I–M*), C-peptide (*N–Q*), and insulin secretion rates (*R–U*) with placebo (left panels *A*, *E*, *I*, *N*, *R*), sitagliptin (panels *B*, *F*, *K*, *O*, *S*), metformin (panels *C*, *G*, *L*, *P*, *T*), and the combination of sitagliptin and metformin (right panels *D*, *H*, *M*, *Q*, *U*) after stimulation with oral glucose, 75 g (filled symbols), and "isoglycemic" intravenous glucose (open symbols), respectively, in patients with type 2 diabetes. Mean ± SEM. When comparing experiments with the oral vs. intravenous administration of glucose, the type of experiment was used as the independent variable. When the four experimental treatments were to be compared, treatments (sitagliptin: yes/no, metformin: yes/no) were used as independent variables. Values of the parameter of interest as determined with placebo treatment were imputed as the covariate. Results of ANCOVA are reported as *P* values for (A) differences by experiment, (B) differences over time, and (AB) differences due to the interaction of experiment and time. If a significant difference by treatment or a significant interaction or treatment and time was documented ($P < 0.05$), values at single time points were compared by one-way ANCOVA. Asterisks in the bottom of the figures indicate that the respective treatment (sitagliptin or metformin) had a significant influence on the parameter in question at this particular time point.

GLP-1/GIP

Fasting total GLP-1 was increased by metformin treatment ($P = 0.0001$) and not changed by sitagliptin treatment ($P = 0.62$; Fig. 2). Total GLP-1 was stimulated by oral but not by "isoglycemic" intravenous glucose ($P < 0.0001$; Fig. 3*C*). Metformin treatment increased total GLP-1 responses significantly after oral ($P = 0.0026$) and after "isoglycemic" intravenous glucose ($P = 0.0014$) in the fasting state and after glucose administration (see asterisks in Fig. 3*C*). In contrast, sitagliptin treatment decreased total GLP-1 responses significantly, both without and with a metformin background ($P < 0.0001$; Fig. 3*B* and *D* and Table 3).

Although fasting intact GLP-1 was not significantly changed by metformin treatment (Fig. 2*C*), fasting intact GLP-1 increased with sitagliptin treatment ($P < 0.0001$; Fig. 2*C*). Likewise, intact GLP-1 responses were significantly increased by sitagliptin treatment before and after the oral glucose load ($P < 0.0001$; Fig. 3*F*). Again, intact GLP-1 concentrations were stimulated by oral but not by "isoglycemic" intravenous glucose.

Integrated incremental responses after oral glucose increased by 1.7-fold (both without and with a metformin background; Table 3).

Metformin treatment had no significant effect on fasting total or intact GIP (Fig. 2*B* and *D* and Fig. 3*L* and *P*). However, sitagliptin raised fasting intact GIP ($P < 0.0001$; Fig. 2*D*) as well as responses before and after oral glucose (Fig. 3*O*). As expected, GIP concentrations were raised considerably more after oral glucose stimulation than during "isoglycemic" intravenous glucose infusions, when incretin levels remained in the basal

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Table 2—Integrated incremental responses of glucose, insulin, C-peptide, insulin secretion rates (calculated by deconvolution), and glucagon after oral glucose stimulation and during the "isoglycemic" intravenous infusion of glucose in type 2 diabetic patients treated with placebo, sitagliptin, metformin, or sitagliptin plus metformin and amount of glucose administered per experiment

	Treatment										
	Placebo		Sitagliptin only		Metformin only		Sitagliptin + Metformin		*P* value†		
Parameter	Mean ± SEM	*P* value*	Mean ± SEM	*P* value*	Mean ± SEM	*P* value*	Mean ± SEM	*P* value*	Sitagliptin	Metformin	Interaction
Glucose, mmol · L^{-1} · min											
OR	23.4 ± 1.6	0.42	17.2 ± 1.4	0.034	18.0 ± 1.0	0.35	12.6 ± 1.2	0.43	<0.0001	<0.0001	0.64
IV	24.4 ± 1.4		18.5 ± 1.5		18.6 ± 1.2		13.0 ± 1.2		<0.0001	<0.0001	0.85
Insulin, IU · L^{-1} · min											
OR	45.9 ± 8.9	0.0097	64.2 ± 10.2	<0.0001	56.0 ± 8.8	<0.0001	65.1 ± 9.4	0.0005	0.014	0.31	0.40
IV	32.9 ± 6.7		33.9 ± 7.5		30.5 ± 4.7		35.5 ± 5.6		0.27	0.87	0.45
$IE_{insulin}$, %	35.8 ± 4.6		49.7 ± 5.0		42.4 ± 3.4		46.0 ± 5.3		0.96	0.59	0.17
C-peptide, nmol · L^{-1} · min											
OR	185 ± 13	0.0005	232 ± 23	<0.0001	205 ± 21	<0.0001	238 ± 21	0.0002	0.0032	0.31	0.59
IV	134 ± 18		151 ± 20		135 ± 14		155 ± 15		0.07	0.79	0.88
$IE_{C\text{-}peptide}$, %	31.5 ± 4.8		41.4 ± 3.6		32.6 ± 3.5		39.8 ± 4.3		0.60	0.80	0.22
ISR, pmol/kg											
OR	557 ± 72	0.0032	705 ± 83	<0.0001	620 ± 75	<0.0001	697 ± 77	0.0008	0.0076	0.51	0.39
IV	426 ± 64		474 ± 65		400 ± 43		468 ± 57		0.11	0.66	0.77
IE_{ISR}, %	30.5 ± 4.9		34.6 ± 4.2		33.1 ± 3.4		38.7 ± 5.1		0.84	0.75	0.14
Glucagon, pmol · L^{-1} · min											
OR	−1,429 ± 193	0.046	−1,687 ± 248	0.045	−1,144 ± 162	<0.0001	−1,426 ± 210	0.072	0.11	0.10	0.94
IV	−1,790 ± 231		−1,836 ± 239		−2,113 ± 242		−1,731 ± 199		0.33	0.53	0.22
Glucose administration, g/experiment											
OR	75 ± 0	0.024	75 ± 0	<0.0001	75 ± 0	0.0047	75 ± 0	<0.0001	0.64	0.53	0.44
IV	69.1 ± 2.5		57.4 ± 3.4		60.9 ± 3.8		53.3 ± 3.4		0.0011	0.020	0.40

IE, incretin effect; ISR, insulin secretion rate; IV, "isoglycemic" intravenous glucose administration; OR, oral glucose load (75 g). *ANOVA. Experiments (OR vs. IV) were used as fixed variables; subjects were used as random variables. †Repeated-measures ANCOVA. Treatments were used as independent variables; individual areas under the curve with placebo treatment were imputed as covariates.

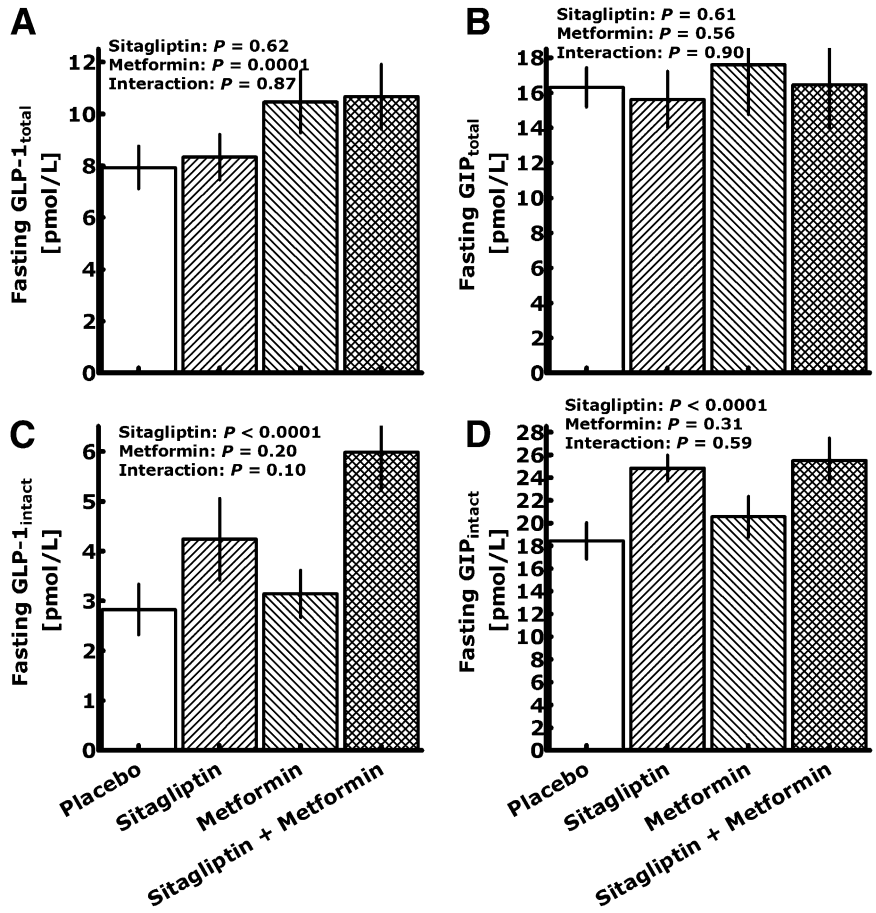

Figure 2—Fasting concentrations of total GLP-1 (panel *A*), intact GLP-1 (panel *C*), total GIP (panel *B*), and intact GIP (panel *D*). Depicted are mean values from samples drawn before stimulation with 75 g oral glucose or before "isoglycemic" intravenous glucose infusions were initiated in patients with type 2 diabetes. Mean ± SEM. *P* values are the result of two-factor ANCOVA. Experiments (sitagliptin: yes/no, metformin: yes/no) were used as independent variables; values of the parameter in question determined with placebo treatment were imputed as covariates. Results are reported as *P* values for the influence of sitagliptin and metformin and of their interaction.

range. Thus, intact GLP-1 and intact GIP were raised slightly, but significantly, after sitagliptin treatment. The concentrations of intact GLP-1 remained in the "basal" (i.e., low picomolar) range (Fig. 2).

As with total GLP-1 (Fig. 3*A–D*), total GIP responses after oral glucose were significantly reduced with sitagliptin treatment by 28% ($P < 0.0001$; Fig. 3*I–M* and Table 3), but were not significantly changed by metformin treatment.

Glucose Concentrations

It was possible to closely match the glucose excursions after oral and intravenous glucose administrations (Fig. 1*E–H*). Thus, the conditions of "isoglycemia" necessary to accurately quantify the incretin effect were met. Irrespective of treatment, oral glucose elicited a significantly higher insulin secretory response, whether based on insulin, C-peptide, or the calculation of insulin secretion rates (Fig. 1*I–U* and Table 4).

Insulin Secretion

After oral glucose, there was no significant stimulation of insulin secretory responses (insulin, C-peptide, or insulin secretion rates) by sitagliptin or metformin treatment (Fig. 1*K*, *L*, *O*, *P*, *S*, and *T*), except for a slight increase of C-peptide with sitagliptin treatment ($P = 0.022$; Fig. 1*O*, asterisks). However, integrated incremental responses were higher with sitagliptin treatment (Table 3: insulin, $P = 0.0014$; C-peptide, $P = 0.0032$; insulin secretion rates, $P = 0.0076$), and not significantly changed with metformin treatment (Table 3). The stimulatory effect of sitagliptin was observed when used in monotherapy or in combination with metformin (Fig. 1 and Table 3). As a result of the glucose-lowering effects of sitagliptin and metformin treatments, these enhanced insulin secretor responses occurred at lower glucose concentrations. This applied to responses after oral glucose and "isoglycemic" intravenous glucose infusions because these were matched to those after oral glucose. Judging insulin secretory responses (insulin, C-peptide, insulin secretion rates) relative to the increment in glucose concentrations revealed a stimulation after sitagliptin treatment that occurred not only after oral glucose stimulation (when intact GLP-1 and GIP concentrations were in the

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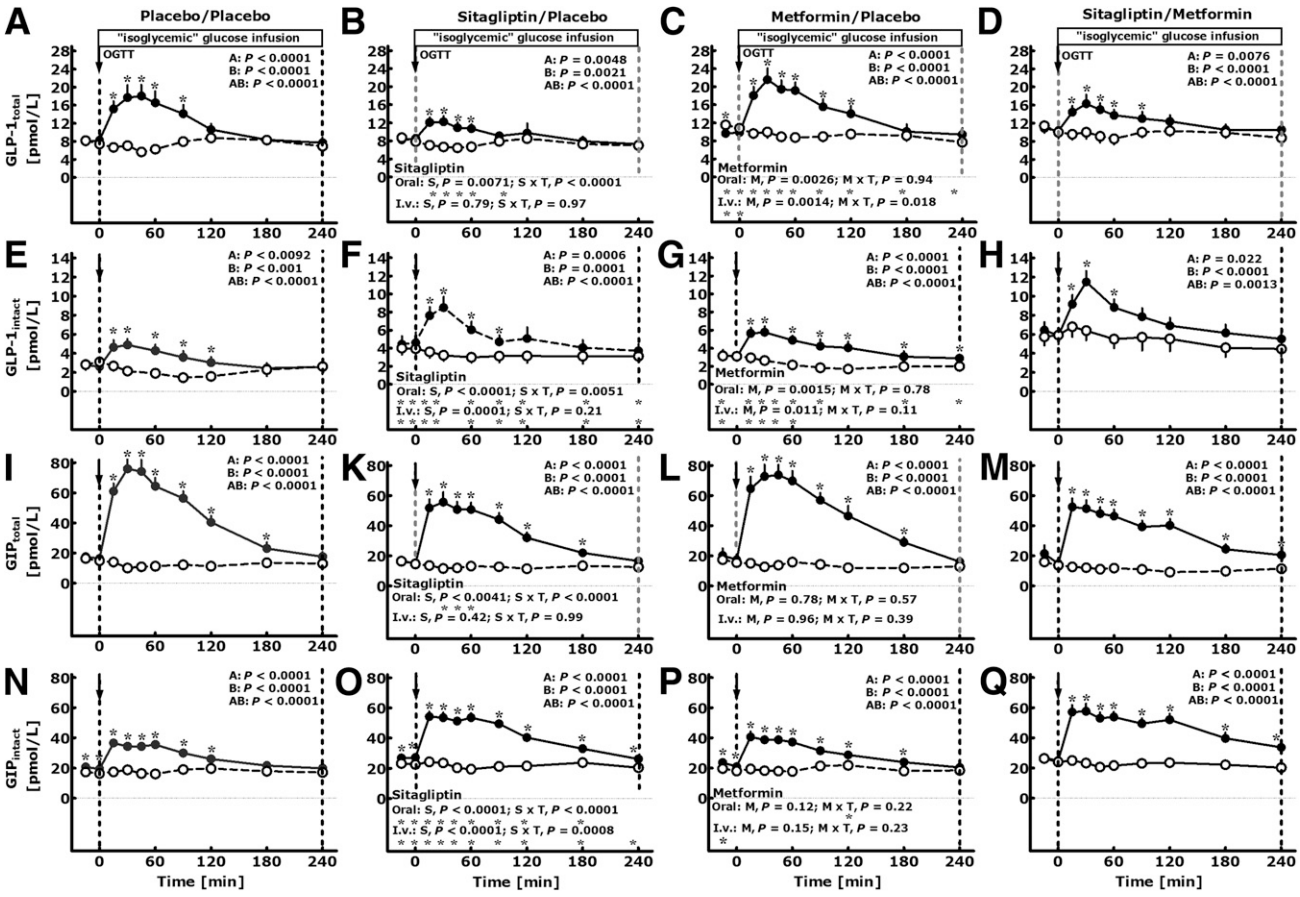

Figure 3—Time course of concentrations of total GLP-1 (*A–D*), intact GLP-1 (*E–H*), total GIP (*I–M*), and intact GIP (*N–Q*) with placebo (left panels *A, E, I, N*), sitagliptin (panels *B, F, K, O*), metformin (panels *C, G, L, P*), and with sitagliptin and metformin (right panels *D, H, M, Q*), after stimulation with oral glucose, 75 g (filled symbols), and "isoglycemic" intravenous glucose (open symbols), respectively, in patients with type 2 diabetes. Mean ± SEM. For details regarding the statistical analysis, see legend to Fig. 1.

"nutrient-stimulated" range of concentrations; Fig. 3) but also during "isoglycemic" intravenous glucose infusions (i.e., at just slightly elevated basal concentrations of intact GLP-1 and GIP; Figs. 2 and 3). Although responses were significantly lower with "isoglycemic" intravenous glucose infusions than after oral glucose stimulation, the pattern of stimulation by sitagliptin was similar, resulting in comparable relative increments in insulin secretory responses relative to glycemic rises for both ways of administering glucose (Table 4). When the same analysis was based in integrated incremental responses of insulin, C-peptide, or insulin secretion rates, and of glucose responses, the conclusions were unchanged (Supplementary Table 1).

Incretin Effect

Insulin secretion in response to oral glucose was higher than with "isoglycemic" intravenous glucose with all treatments ($P < 0.0001$; Table 2). Thus numerically, the incretin effect (placebo: 31 ± 5%; insulin secretion, calculated from C-peptide responses) was not significantly changed by sitagliptin ($P = 0.84$) or metformin treatment ($P = 0.75$, interaction $P = 0.14$; Table 2). The reason is that sitagliptin augmented insulin secretory responses (relative to glucose increments) not only after oral glucose stimulation but also with "isoglycemic" intravenous glucose infusions (Table 4).

Glucagon

Metformin raised fasting glucagon concentrations significantly (Supplementary Fig. 1). Glucagon concentrations were suppressed with oral and intravenous glucose and in a similar manner with placebo, sitagliptin, metformin, and the combination of sitagliptin and metformin treatment (Supplementary Figs. 1 and 2 and Table 4). Intravenous glucose led to a significantly earlier suppression of glucagon than did oral glucose (Supplementary Fig. 2).

DISCUSSION

The present analysis confirms that metformin leads to an augmented secretion of GLP-1, as previously shown after 2 days of treatment in nondiabetic (27) and in type 2 diabetic subjects (23). Furthermore, although DPP-4 inhibitor administration raises plasma concentrations of intact GLP-1 and GIP, the numerical contribution of the

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Table 3—Integrated incremental responses after oral glucose load (75 g) and during the "isoglycemic" intravenous infusion of total GLP-1, intact, biologically active GLP-1, total GIP, and intact, biologically active GIP induced by placebo, sitagliptin, metformin, or sitagliptin plus metformin administration in patients with type 2 diabetes

	Treatment										
	Placebo		Sitagliptin only		Metformin only		Sitagliptin + Metformin		*P* value†		
Parameter	Mean ± SEM	*P* value*	Mean ± SEM	*P* value*	Mean ± SEM	*P* value*	Mean ± SEM	*P* value*	Sitagliptin	Metformin	Interaction
$GLP\text{-}1_{total}$, pmol · L^{-1} · min											
OR	1,025 ± 164	<0.0001	482 ± 105	<0.0001	1,247 ± 168	0.0083	667 ± 142	0.0086	<0.0001	0.10	0.88
IV	194 ± 52		147 ± 49		83 ± 31		215 ± 56		0.36	0.65	0.62
% Reduction vs. placebo‡	—		−53.0 ± 36.0		21.7 ± 2.4		−46.5 ± 15.5				
$GLP\text{-}1_{intact}$, pmol · L^{-1} · min											
OR	200 ± 45	0.0083	342 ± 89	0.0002	295 ± 56	<0.0001	495 ± 126	0.029	0.044	0.14	0.73
IV	44 ± 28		89 ± 88		16 ± 7		179 ± 79		0.09	0.61	0.33
Fold increase vs. placebo§	—		1.71 ± 1.98				1.68 ± 2.25				
GIP_{total}, pmol · L^{-1} · min											
OR	6,437 ± 695	<0.0001	4,608 ± 514	<0.0001	6,814 ± 838	<0.0001	4,681 ± 539	<0.0001	0.0001	0.65	0.76
IV	299 ± 96		338 ± 139		307 ± 84		151 ± 45		0.45	0.25	0.21
% Reduction vs. placebo‖	—		−28.4 ± 26.0		5.9 ± 20.6		−31.3 ± 35.7				
GIP_{intact}, pmol · L^{-1} · min											
OR	1,986 ± 301	<0.0001	3,600 ± 362	<0.0001	2,082 ± 315	<0.0001	4,978 ± 436	<0.0001	<0.0001	0.029	0.43
IV	523 ± 115		415 ± 122		422 ± 95		227 ± 70		0.11	0.08	0.66
Fold increase vs. placebo¶	—		1.81 ± 1.20				2.39 ± 1.38				

For time courses, see Fig. 2. IV, "isoglycemic" intravenous glucose administration; OR, oral glucose load (75 g). *ANOVA. Experiments (OR vs. IV) were used as fixed variables; subjects were used as random variables. †Repeated-measures ANCOVA. Treatments were used as independent variables; individual areas under the curve with placebo treatment were imputed as covariates. ‡Relation to the integrated incremental response of total GLP-1 with placebo concentration (oral glucose administration). §Relation to the integrated incremental response of intact GLP-1 with placebo concentration (oral glucose administration). ‖Relation to the integrated incremental response of total GIP with placebo concentration (oral glucose administration). ¶Relation to the integrated incremental response of intact GIP with placebo concentration (oral glucose administration).

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Table 4—Ratio of integrated total responses of insulin, C-peptide, and insulin secretion rates to integrated total glucose responses after an oral glucose load (75 g) and during the "isoglycemic" intravenous infusion of glucose (*n* = 20)

	Treatment								*P* value†		
	Placebo		Sitagliptin only		Metformin only		Sitagliptin + Metformin				
Parameter	Mean ± SEM	*P* value*	Mean ± SEM	*P* value*	Mean ± SEM	*P* value*	Mean ± SEM	*P* value*	Sitagliptin	Metformin	Interaction
Insulin/glucose, pmol/mmol											
OR	26.2 ± 3.1	0.0129	36.9 ± 5.7	0.0004	30.3 ± 5.0	0.0004	43.0 ± 6.1	0.0006	0.026	<0.0001	0.67
IV	19.1 ± 2.0		22.6 ± 3.8		20.7 ± 2.9		27.7 ± 3.8		0.0012	<0.0001	0.089
C-peptide/glucose, pmol/mmol											
OR	119.4 ± 9.6	0.0084	163.6 ± 16.2	<0.0001	141.9 ± 14.5	<0.0001	192.7 ± 15.5	0.0002	0.0003	<0.0001	0.63
IV	101.6 ± 8.8		125.0 ± 2.8		116.6 ± 11.2		153.0 ± 11.8		<0.0001	<0.0001	0.14
ISR/glucose, pmol · kg^{-1} · min^{-1}											
OR	0.34 ± 0.03	0.010	0.47 ± 0.05	<0.0001	0.41 ± 0.05	0.0002	0.55 ± 0.05	0.0002	0.0002	<0.0001	0.73
IV	0.29 ± 0.03		0.36 ± 0.04		0.33 ± 0.04		0.43 ± 0.04		<0.0001	<0.0001	0.28

Integration was carried out from 0–240 min. IV, "isoglycemic" intravenous glucose administration; OR, oral glucose load (75 g). *ANOVA. Experiments (OR vs. IV) were used as fixed variables; subjects were used as random variables. †Repeated-measures ANCOVA. Treatments were used as independent variables; individual areas under the curve with placebo treatment were imputed as covariates.

incretin effect after oral glucose is unchanged, as shown in our previous study using vildagliptin treatment (another DPP-4 inhibitor (19) and confirmed by Muscelli et al. (20) studying the effects of sitagliptin on mixed meal–stimulated insulin secretory responses compared with those after "isoglycemic" intravenous glucose. We believe this is explained by the fact that in the current study, as in our previous study (19) and that of Muscelli et al. (20), DPP-4 inhibitor administration stimulated insulin secretion not only after oral glucose (at high, nutrient-stimulated incretin hormone concentrations) but also at much lower incretin levels in the basal range (i.e., as typical for the absence of nutrient stimulation) (Fig. 3), and it did so both when studied in monotherapy and as an add-on to metformin.

This important combination had not been examined in the previous studies on this topic. This is best illustrated by analyzing the ratio of insulin secretory responses and glycemic increments, which—by definition—are very similar comparing oral glucose and "isoglycemic" intravenous glucose stimulation. The similarity of results obtained by looking at insulin, C-peptide, and insulin secretion rates (deconvolution) attests to the robustness of these analyses. The surprising observation is that the small but significant rises in fasting levels of intact, biologically active GLP-1 and GIP (Fig. 3 and Table 3) introduced by DPP-4 inhibition with sitagliptin are associated with significant augmentations in insulin secretion, not only after oral glucose stimulation but also with "isoglycemic" intravenous glucose infusions, resulting in no net change in the size of the incretin effect (Table 4). Thus, small variations in the low concentrations of basal incretin hormone levels may determine insulin secretory responses to a greater degree than previously thought. These findings challenge the view that incretins are important in the postload, nutrient-stimulated situation only, but have little if any influence as long as their plasma concentrations are low, as in the fasting state (16,17). However, we can only describe associations, and this cannot be used as a proof of causality.

Our finding is well compatible with the observation by Salehi et al. (32) that a receptor antagonist at the GLP-1 receptor is able to reduce hyperglycemia-induced insulin secretory responses in the absence of nutrient stimulation; that is, at low ("basal") concentrations of incretin hormones, in particular GLP-1, again indicating a relatively strong insulinotropic effect at these low concentrations.

An alternative explanation could be the involvement of other mediators in addition to GLP-1. Whether GIP contributes much in patients with type 2 diabetes may be viewed as highly unlikely, given the inability of exogenous GIP to stimulate insulin secretion in these subjects (3,4). Nevertheless, there appears to be some potential interaction at the level of GLP-1– and GIP-mediated effects on insulin secretion, as indicated by the fact that the endocrine pancreas in GLP-1 receptor knockout mice hyper-responds to GIP stimulation (33). Thus, hyporesponsiveness to one incretin may cause hyperresponsiveness to the other incretin, allowing for the possibility that another manipulation of the enteroinsular axis, the inhibition of DPP-4 activity, may have consequences regarding the ability of GIP to elicit insulin secretory responses. This, at present, is a mere hypothesis that needs experimental validation.

Another explanation could be a predominant action of DPP-4 inhibition on DPP-4 in the intestinal compartment, with little or no the importance of DPP-4 inhibition in the circulation (where it affects plasma concentrations of intact GLP-1 and GIP), as shown in mouse experiments by Waget et al. (34). This would require mediation by the autonomous nervous system rather than by changes in circulating incretin concentrations. However, establishing these mechanisms experimentally in human subjects will be difficult.

Muscelli et al. (20) reported, in addition to effects on insulin secretion, that sitagliptin treatment reduced the amount of glucose appearing in the general circulation, pointing to metabolic effects over and above the changes induced in insulin and glucagon secretion.

In principle, other mediators may include hitherto undiscovered gut peptides with incretin activity or neuropeptides that reach an effective concentration only when close to the neurons they act upon, which normally does not lead to high plasma concentrations, and thus are much more difficult to identify compared with incretin hormones with a more or less humoral mode of action. To our knowledge, there is no "hot" candidate among the known neuropeptides for this role. However, the existence of non–GLP-1–mediated mechanisms of action of DPP-4 inhibitors is also suggested from studies from our laboratory (35) as well as from other groups (36) indicating that clinical effects of DPP-4 inhibition can be partially but not fully blocked by exendin, a GLP-1 receptor antagonist [9–39].

A surprising finding was a small but significant rise in fasting glucagon concentrations with metformin treatment (Supplementary Fig. 1). The difference was small, and this may be a chance finding. Alternatively, the rise in fasting glucagon may be a response to the glucose-lowering effect of metformin (Fig. 1 and Table 1). It probably was not observed with sitagliptin (lowering glucose by a similar degree) because the elevation in intact GLP-1 (Figs. 2 and 3) induced a concomitant suppression in glucagon (1).

Our study confirms the feedback inhibition that elevated intact GLP-1 concentrations exert on L-cell secretion (19,37–39). Sitagliptin reduced total GLP-1 responses by ~50% and total GIP responses to a somewhat lesser degree, ~30%. This was not prevented by metformin cotreatment, despite metformin's activity to augment GLP-1 secretion (both regarding basal concentrations [Fig. 2] and oral glucose-stimulated levels [Table 3]). This feedback effect, whereby increased intact GLP-1

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levels restrain L-cell secretion, is one explanation of why DPP-4 inhibitors enhance intact GLP-1 concentrations by only 2- to 3-fold (15,39), even though, in the absence of DPP-4 inhibition, intact concentrations of GLP-1 are only 10–20% of the respective total concentrations (40), indicating a potential to increase these levels by 5- to 10-fold. This raises the possibility that interfering with the mechanism of this feedback inhibition may further enhance intact GLP-1 concentrations and potentially increase the clinical effectiveness of DPP-4 inhibitors. Nevertheless, despite total incretin levels being reduced, sitagliptin and, to a greater extent, the combination with metformin led to an increase in intact GLP-1 levels, which likely contributes to the greater clinical effectiveness of the DPP-4 inhibitor plus metformin combination (41). Thus, insulin secretory responses relative to the glycemic increment were highest with sitagliptin plus metformin treatment compared with either treatment alone (Table 4; Supplementary Table 1).

In conclusion, treatment of type 2 diabetes patients with metformin increases GLP-1 secretion. Treatment with the DPP-4 inhibitor sitagliptin raises plasma concentrations of intact GLP-1 and GIP in the basal (fasting) state and after stimulation with oral glucose. This gives rise to augmented insulin secretory responses after oral glucose and "isoglycemic" intravenous glucose infusions, resulting in the numerical size of the incretin effect being unchanged. Overall, our results indicate a prominent insulinotropic effect of small variations in incretin hormone levels in the low, "basal" range, or alternatively, other mediators; that is, hitherto undescribed peptides that are subject to degradation/inactivation by DPP-4 and have the ability to stimulate insulin secretion at elevated glucose concentrations.

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