Impaired Regulation of the Incretin Effect in Patients with Type 2 Diabetes

Jonatan I. Bagger, Filip K. Knop, Asger Lund, Henrik Vestergaard, Jens J. Holst, and Tina Vilsbøll

Diabetes Research Division (J.I.B., F.K.K., A.L., T.V.), Department of Internal Medicine F, Gentofte Hospital; Center for Endocrinology and Metabolism (J.I.B., H.V.), Department of Medicine O, Herlev Hospital; and Department of Biomedical Sciences (J.J.H.), The Panum Institute, University of Copenhagen, DK-2900 Copenhagen, Denmark

Objective: In healthy subjects, the incretin effect during an oral glucose tolerance test increases with the size of glucose load, resulting in similar glucose excursions independently of the glucose loads. Whether patients with type 2 diabetes mellitus (T2DM) are able to regulate their incretin effect is unknown.

Research Design and Methods: Incretin effect was measured over 6 d by means of three 4-h oral glucose tolerance test with increasing glucose loads (25, 75, and 125 g) and three corresponding isoglycemic iv glucose infusions in eight patients with T2DM [fasting plasma glucose, mean 7.7 (range 7.0–8.9) mM; glycosylated hemoglobin, 7.0% (6.2–8.4%)] and eight matched healthy control subjects [fasting plasma glucose, 5.3 (4.8–5.7) mM; glycosylated hemoglobin, 5.4% (5.0–5.7%)].

Results: Patients with T2DM exhibited higher peak plasma glucose in response to increasing oral glucose loads, whereas no differences in peak plasma glucose values among control subjects were observed. The incretin effect was significantly ($P < 0.003$) lower in patients with T2DM (0 ± 7, 11 ± 9, and 36 ± 5%) as compared with control subjects (36 ± 5, 53 ± 6, and 65 ± 6%). Equal and progressively delayed gastric emptying due to the increasing loads was found in both groups. Incretin hormone responses were similar.

Conclusions: Up-regulation of the incretin effect in response to increasing oral glucose loads seems to be crucial for controlling glucose excursions in healthy subjects. Patients with T2DM are characterized by an impaired capability to regulate their incretin effect, which may contribute to the exaggerated glucose excursions after oral ingestion of glucose in these patients. **(*J Clin Endocrinol Metab* 96: 737–745, 2011)**

The incretin effect refers to the phenomenon that oral glucose elicits a higher insulin response than iv glucose at identical plasma glucose profiles (isoglycemia) (1, 2). The incretin effect is conveyed by the two incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (3, 4). They are secreted from intestinal endocrine cells in response to ingestion of nutrients, and both are highly insulinotropic at their physiological plasma concentrations in a strictly glucose-dependent manner (4, 5). The incretin effect is gauged by comparing insulin responses after oral ingestion of glucose and isoglycemic iv glucose infusion (IIGI), respectively (6).

Nauck *et al.* (7) estimated the incretin effect in healthy subjects during different glucose loads (25, 50, and 100 g) and showed that the incretin effect increases with increased amounts of oral glucose, resulting in similar plasma glucose excursions during the three loads. This regulatory capability was partly explained by increased GIP responses to the larger oral glucose loads, but re-

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2010-2435 Received October 14, 2010. Accepted December 22, 2010.
First Published Online January 20, 2011

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Abbreviations: AUC, Area under the curve; BMI, body mass index; FPG, fasting plasma glucose; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; IIGI, isoglycemic iv glucose infusion; ISR, insulin secretion rate; NS, not significant; OGTT, oral glucose tolerance test; T2DM, type 2 diabetes mellitus.

sponses of the other incretin hormone GLP-1 were not measured. It is uncertain whether patients with type 2 diabetes mellitus (T2DM) are able to increase their incretin effect in response to lager oral glucose loads equivalent to healthy subjects. With the present study, we aimed to quantify the incretin effect and incretin hormone responses in patients with T2DM and in healthy control subjects during increasing oral glucose challenges.

Subjects and Methods

The protocol was approved by the Scientific-Ethical Committee of the Capital Region of Denmark (registration no. H-A-2007-0048), the Danish Data Protection Agency (registration j.nr. 2007-41-1058) and at www.ClinicalTrials.gov (ID NCT00529048). The study was conducted according to the principles of the Helsinki Declaration II.

Subjects

Eight patients (three males) with T2DM [mean age, 57 (range 40–75) yr; body mass index (BMI), 29 (25–34) kg/m^2; fasting plasma glucose (FPG): 7.7 (7.0–8.9) mM; glycosylated hemoglobin (HbA_{1c}), 7.0% (6.2–8.4%); duration of diabetes, 8 (6–36) months] and eight gender-, age-, and BMI-matched healthy control subjects [age, 57 (38–74) yr; BMI, 29 (26–33) kg/m^2; FPG, 5.3 (4.8–5.7) mM; and glycosylated hemoglobin (HbA_{1c}), 5.4% (5.0–5.7%)] were studied. All patients with T2DM were diagnosed according to the criteria of the World Health Organization (8). The patients were treated with diet and exercise only. None of the patients had impaired renal function, microalbuminuria, proliferative retinopathy, or impaired liver function. All healthy control subjects were without family history of diabetes and had normal glucose tolerance according to a 75-g oral glucose tolerance test (OGTT) performed immediately before inclusion in the study. All participants were negative with regard to islet cell autoantibodies and glutamic acid decarboxylase-65 autoantibodies. None of the subjects used drugs suspected to influence glucose, insulin, C-peptide, or incretin hormone responses during oral and iv glucose administration. Hypercholesterolemia and hypertension, if any, were well treated in both groups. All subjects agreed to participate after receiving oral and written information.

Experimental design

All participants were studied on six different occasions: 25-, 75-, and 125-g OGTTs and three corresponding IIGIs over a 4-wk period. On all occasions, the subjects were studied in the morning in a recumbent position after an overnight (10 h) fast including medication and use of tobacco. On OGTT days (d 1A, 2A, and 3A, performed in randomized order), a cannula was inserted in a cubital vein for collection of arterialized blood samples. The cannulated forearm was placed in a heating box (50 C) throughout the experiment. The participants ingested 25 g (d 1A), 75 g (d 2A), or 125 g (d 3A) of water-free glucose, dissolved in 300 ml water containing 1.5 g acetaminophen (Panodil). Blood samples were drawn 15, 10, and 0 min before and 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 90, 120, 150, 180, and 240 min after ingestion of glucose. Blood was distributed into chilled tubes containing EDTA and aprotinin (500 kIU/ml blood; Trasylol; Bayer, Leverkusen, Germany) for analyses of GLP-1 and GIP. For analyses of insulin and C-peptide, blood was distributed into chilled tubes containing heparin. EDTA and heparin tubes were immediately cooled and kept on ice until centrifugation. Blood for analysis of acetaminophen was distributed into dry tubes for coagulation (20 min at room temperature). All samples were centrifuged for 20 min at 1200 × *g* and 4 C. Plasma samples for GLP-1 and GIP analyses, and serum samples for acetaminophen analysis were stored at −20 C, and plasma samples for insulin and C-peptide analyses were stored at −80 C until analysis. For bedside measurement of plasma glucose, blood was distributed into fluoride tubes and centrifuged immediately at 7400 × *g* for 2 min at room temperature. After the three OGTTs, three corresponding IIGIs were performed in randomized order (d 1B, 2B, and 3B). On IIGI days, cannulas were inserted into cubital veins in both arms: one for collection of arterialized blood samples (see above) and one for glucose infusion. IIGI was performed using a sterile 20% wt/vol glucose infusion. The infusion rate was adjusted aiming at duplication of the plasma glucose profile determined during the corresponding OGTT (plasma glucose profiles achieved on A days were copied on corresponding B days). Blood was sampled as on OGTT days (d 1A, 2A, and 3A), except for more frequent plasma glucose sampling to adjust the glucose infusion rate. Before each experiment, the urinary bladder was emptied, and total urine production during each experiment was collected to measure renal glucose excretion.

Analyses

Plasma concentrations of glucose were measured by the glucose oxidase method, using a glucose analyzer (Yellow Springs Instrument model 2300 STAT plus analyzer; YSI Inc., Yellow Springs, OH). Plasma insulin and C-peptide concentrations were measured using a two-sided electrochemiluminescence immunoassay (Roche/Hitachi Modular analytics; Roche Diagnostic GmbH, Mannheim, Germany) (9, 10). Plasma concentrations of total GLP-1 and GIP were measured by RIAs as previously described (11, 12). Serum acetaminophen was measured by the Vitros ACET slide method based on an aryl acyl amidase reaction linked to a color shift reaction using liquid chromatography for quantification as described elsewhere (13, 14). Concentrations of glucose in urine were measured by a hexokinase method (Konelab PRIME 60; Thermo Fisher Scientific Inc., Pittsburgh, PA).

Statistical analyses and calculations

Baseline, peak, area under the curve (AUC), and incretin effect values are expressed as mean ± SEM. Differences resulting in P values <0.05 were considered significant. For analysis of interload variations, repeated-measurement ANOVA after Greenhouse-Geisser (G-G) or Huynh-Feldt (H-F) depending on sphericity assumption ($\varepsilon < 0.75 \rightarrow$G-G; $\varepsilon > 0.75 \rightarrow$H-F) (15) was used. As *post hoc* analysis, Bonferroni adjustments were used. For analysis of OGTT *vs.* IIGI days or intergroup variation, paired-samples *t* test (two-tailed) was used. Differences between time courses were compared using two-factor repeated-measurement ANOVA. One-sample *t* test (two-tailed) was used to test for significant incretin effects (different from 0). Paired and unpaired two-sample *t* tests (two-tailed) were used as required to test parameters within and between the groups. AUC values were calculated using the trapezoidal rule and are presented as total AUC if nothing else is stated. Insulin secretion rate (ISR) values were

calculated by deconvolution of measured C-peptide concentrations and application of population-based parameters for C-peptide kinetics as described previously (16–18). ISR is expressed as picomoles insulin secreted per minute per kilogram body weight. Homeostasis model assessment was used to assess insulin resistance (19). Incretin effects were calculated by relating the difference in integrated β-cell secretory responses (AUC for insulin and C-peptide, respectively; and ISR $\times$ time) between stimulation with OGTT and IIGI to the response after OGTT, which was taken as 100% [incretin effect (percent) = 100% $\times$ ($AUC_{OGTT} - AUC_{IIGI}$)/AUC_{OGTT}] (6).

Results

Glucose

FPG was similar on all experimental days in the two groups, but higher in the patients than in the healthy control subjects (Table 1). As illustrated in Fig. 1, similar peak plasma glucose values in response to 25-, 75-, and 125-g OGTTs were observed in healthy control subjects (Table 1). Nevertheless, a protracted decrease in plasma glucose with increasing oral glucose loads was observed (Fig. 1 and Table 1). The patients with T2DM displayed increasing plasma glucose excursions with increasing glucose load and, accordingly, increasing AUC and peak plasma glucose values (Fig. 1 and Table 1). Glucose infusions resulted in isoglycemia compared with the respective oral response in both groups [P = not significant (NS)]. Urinary glucose excretion was similar during OGTTs and corresponding IIGIs in both groups. In the patients with T2DM, a significant and increasing amount of glucose was excreted with increasing amount of glucose given, although the maximal excretion amounted to only 4.0 g despite the different glucose loads and plasma glucose profiles (Table 1). Urinary glucose excretion was calculated based on pooled data from both OGTT and corresponding IIGI.

TABLE 1. Glucose

	T2DM	Control	*t* test (*P*)
Baseline			
FPG (mM)	7.7 ± 0.1	5.4 ± 0.1	<0.001
Peak values (mmol/liter)			
25-g OGTT	12.8 ± 0.5[a]	8.9 ± 0.5	<0.001
75-g OGTT	17.5 ± 0.8	10.4 ± 0.9	<0.001
125-g OGTT	17.7 ± 1.2	10.2 ± 0.9	<0.001
ANOVA (*P*)	0.001	NS	
PG response (mM × min)			
25-g OGTT	2.1 ± 0.3[a]	1.4 ± 0.1[a]	<0.001
75-g OGTT	3.0 ± 0.1	1.5 ± 0.1[a]	<0.001
125-g OGTT	3.3 ± 0.2	1.8 ± 0.1[a]	<0.001
ANOVA (*P*)	<0.001	<0.001	
Glucose infusions (g)			
IIGI (25 g)	23.4 ± 2.0[a]	14.5 ± 2.9[a]	0.022
IIGI (75 g)	63.4 ± 3.3	36.0 ± 3.7[a]	<0.001
IIGI (125 g)	78.6 ± 5.7	44.4 ± 3.6[a]	<0.001
ANOVA (*P*)	<0.001	<0.001	
Total urinary glucose excretion (g)			
25 g	0.06 ± 0.03[a]	0.02 ± 0.02	NS
75 g	3.01 ± 0.73	0.32 ± 0.27	0.001
125 g	4.00 ± 0.86	0.65 ± 0.43	0.001
ANOVA (*P*)	0.002	NS	

Data for healthy control subjects and patients with T2DM are mean values ± SEM. *P* values are derived from two-tailed *t* test for intergroup variation and repeated-measurement ANOVA for interload variations.

[a] Significant differences ($P < 0.05$) in the interload observations (*post hoc* analysis) from both of the other glucose loads compared. Total plasma glucose responses are calculated as total AUC.

Insulin, C-peptide, and ISR

Time courses for plasma insulin and C-peptide and ISR are shown in Fig. 2, and AUC, peak values, and incretin effect are given in Table 2. Similar fasting values of insulin and C-peptide, respectively, were observed on the two experimental days in each group (P = NS), but higher fasting insulin and C-peptide levels and ISR values were seen in patients with T2DM compared with healthy control subject (Table 2). Homeostasis model assessment for insulin resistance was significantly greater in patients with T2DM compared with healthy control subjects (Table 3). In both groups, increasing oral glucose loads resulted in significant enhancement of insulin and C-peptide excursions and ISR responses (Fig. 2). Significant differences between OGTT and IIGI days with regard to time courses for insulin, C-peptide, and ISR were evident among healthy control subjects. No such differences between OGTT and IIGI time courses were found in patients with T2DM except for a small, but significant difference in C-peptide and ISR responses between d 2A (75-g OGTT) and 2B (corresponding IIGI). The control group exhibited similar peak concentrations for insulin during the OGTT as patients with T2DM, but they were reached after 30–70 min, which was significantly ($P \leq 0.01$) earlier compared with patients with T2DM (30–150 min) (Table 2 and Fig. 2). The same pattern of protracted excursions among patients with T2DM was observed with regard to C-peptide and ISR (Fig. 2).

Incretin effect

As indicated in Table 2, significant increases in incretin effect based on insulin, C-peptide, and ISR data were observed in both groups in response to increasing oral glucose loads. Nevertheless, during the 125-g OGTT, patients with T2DM only managed to reach an incretin effect similar to what healthy control subjects exhibited with a fifth of the amount of glucose (25 g). The incretin effect based on insulin, C-peptide, and ISR was significantly lower in the group of patients with T2DM during all three

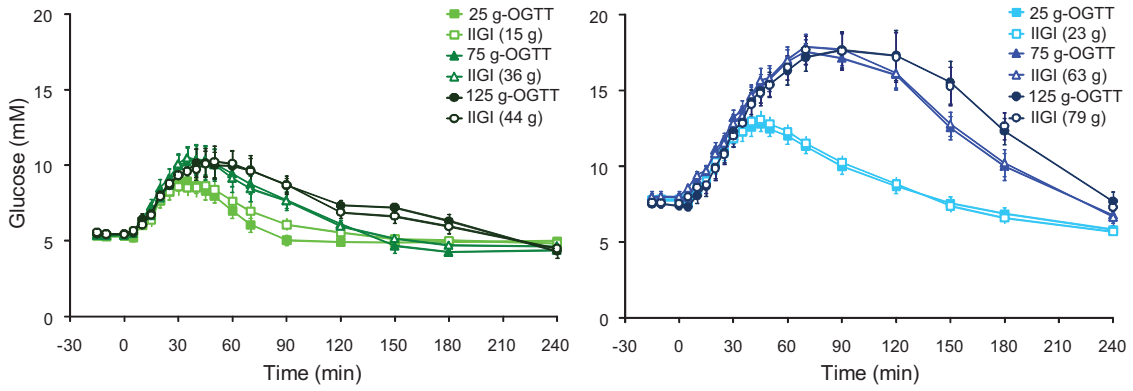

FIG. 1. Time courses for plasma glucose concentrations in patients with T2DM (*blue curves*) and in healthy control subjects (*green curves*). Color tone indicates each set of OGTT and IIGI [*light*, 25-g OGTT (*closed symbols*) and corresponding IIGI (*open symbols*); *medium*, 75-g OGTT (*closed symbols*) and corresponding IIGI (*open symbols*); and dark, 125-g OGTT (*closed symbols*) and corresponding IIGI (*open symbols*)].

experiments compared with the healthy control subjects (Table 2). Absolute incretin effects were measured as $(AUC_{OGTT} - AUC_{IIGI})/AUC_{OGTT})$ for the three β-cell secretory parameters. Calculated in this way, the incretin effect (whether based on insulin, C-peptide, or ISR) was not significantly different from zero in the patients at either 25- or 75-g glucose loads but was significant in response to 125-g OGTT, whereas a significant effect was observed in the controls at all doses.

GIP

No significant differences in baseline levels between OGTTs or IIGIs in either group were observed. As indicated in Table 3, fasting levels of GIP were significantly higher in patients with T2DM compared with healthy control subjects. No differences in GIP responses during OGTTs were observed between the groups (P = NS). Both groups displayed brisk rises in GIP concentration in response to increasing oral glucose loads; the excursions were identical during the first 45 min, and thereafter, differences were observed (Fig. 3). Similarly, AUC values showed significantly increasing GIP responses with increasing oral glucose loads (Table 3). As expected, no significant GIP responses occurred during IIGIs in any of the two groups (Fig. 3).

GLP-1

There were no differences in mean fasting values between the groups. As shown in Fig. 3 and Table 3, increasing GLP-1 responses were obtained in both groups during increasing oral glucose loads with no differences between the groups (P = NS). AUC values were significantly larger during OGTTs compared with corresponding IIGIs, during which no significant GLP-1 responses occurred, in any of the groups. No significant differences in peak values were observed between the groups.

Gastric emptying

There were no significant differences in gastric emptying (assessed by serum acetaminophen excursions) between the groups during any of the three different glucose loads. However, a markedly prolonged emptying time was observed with increasing oral glucose loads in both groups ($P < 0.05$) as assessed by time to peak of plasma acetaminophen [25 g, 41 ± 4 (T2DM) *vs.* 36 ± 5 min (healthy control subjects), P = NS; 75 g, 92 ± 9 *vs.* 105 ± 15 min, P = NS; 125 g, 131 ± 11 *vs.* 150 ± 16 min, P = NS) (Fig. 4).

Discussion

The novel findings of the present study include 1) a markedly reduced capability to regulate the incretin effect in response to increasing oral glucose loads among patients with T2DM, 2) similar and progressively prolonged responses of GIP and GLP-1 with increasing oral glucose load in both patients with T2DM and healthy control subjects, and 3) identification of delayed gastric emptying as an efficient mean of reducing postprandial glucose excursions in both patients with T2DM and healthy control subjects.

In healthy subjects, the increasing incretin effect in response to larger oral glucose loads is the most probable explanation for their ability to limit the plasma glucose excursions to such stimuli. In 1986, Nauck and co-workers (7) published data demonstrating identical plasma glucose excursions after different oral glucose loads (25, 50, and 100 g) in healthy control subjects. From that study, it was concluded that the ability to increase the incretin effect ensures that hyperglycemia does not occur regardless of the size of the glucose load (7). This adaptive regulation of the incretin effect, as assessed by the isoglycemic clamp technique, has never previously been evaluated in patients with T2DM, nor have both of the incretin hormone re-

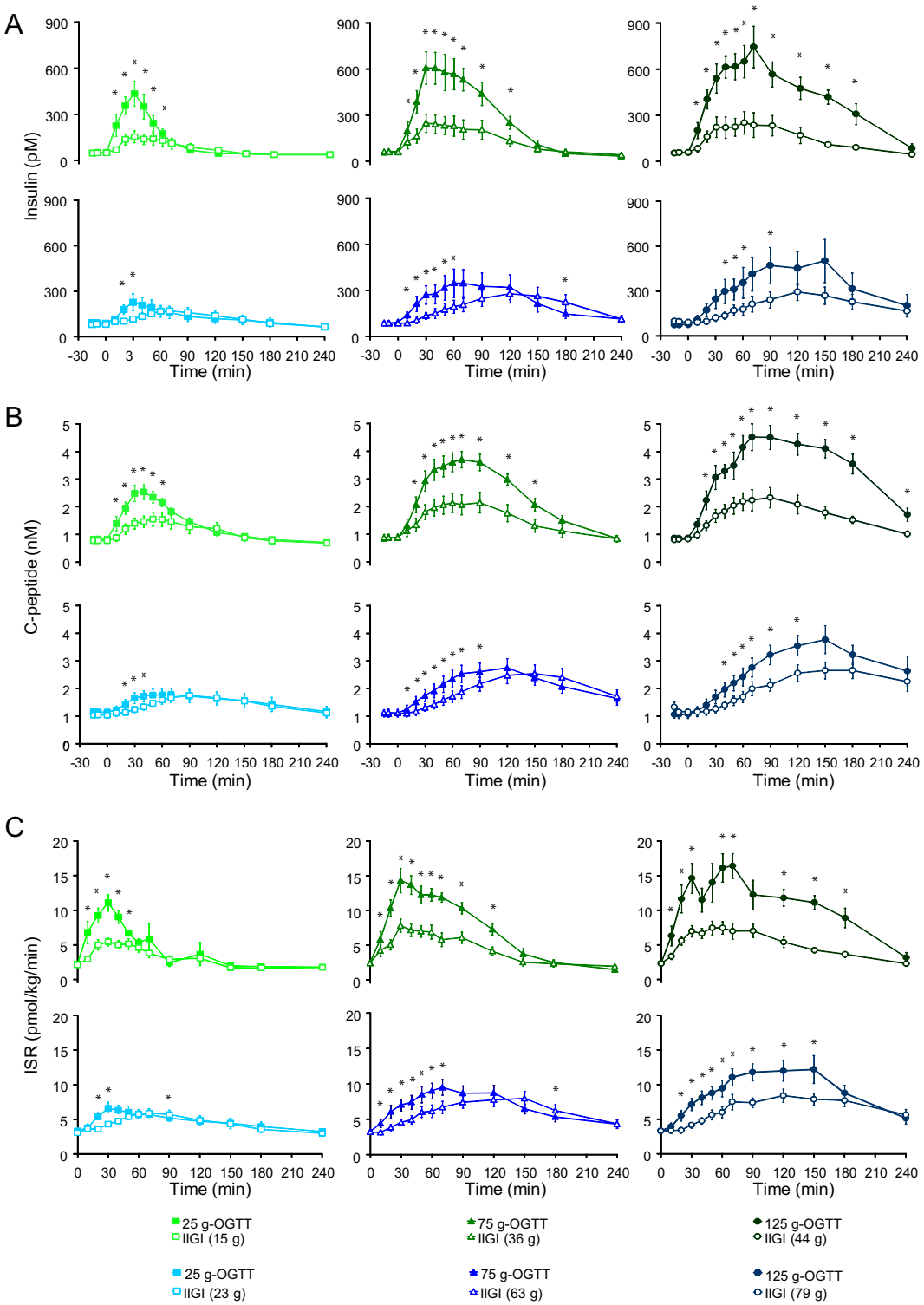

FIG. 2. Time courses for plasma concentrations of insulin (A) and C-peptide (B) and ISR (C) in patients with T2DM (*blue curves*) and in healthy control subjects (*green curves*). Color tone indicates each set of OGTT and IIGI [*light*, 25-g OGTT (*closed symbols*) and corresponding IIGI (*open symbols*); *medium*, 75-g OGTT (*closed symbols*) and corresponding IIGI (*open symbols*); and dark, 125-g OGTT (*closed symbols*) and corresponding IIGI (*open symbols*)]. *Asterisks* indicate significant differences on behalf of paired-samples *t* test referring to the specific time points.

sponses during increasing oral glucose loads in healthy subjects and patients with T2DM been investigated. Our results confirm the findings of Nauck *et al.* (7) that similar plasma glucose excursions in response to a wide range of different oral glucose challenges occur in healthy subjects. Interestingly, in the healthy control subjects volunteering for the present study, we observed equal plasma glucose peak values despite a 5-fold increase in oral glucose loads (from 25–125 g). However, a progressively protracted elevated plateau profile was observed with

TABLE 2. β-cell responses

	Insulin			C-peptide			ISR		
	T2DM	Control	*t* test (*P*)	T2DM	Control	*t* test (*P*)	T2DM	Control	*t* test (*P*)
Baseline	86.3 ± 5.8	55.4 ± 3.3	<0.001	1.1 ± 0.1	0.8 ± 0.0	<0.001	3.2 ± 0.1	2.3 ± 0.1	<0.001
Peak values									
25-g OGTT	226 ± 55	434 ± 81	NS	1.8 ± 0.2[b]	2.5 ± 0.3[b]	NS	7 ± 1[b]	11 ± 1[b]	0.02
(IIGI)	(169 ± 32)	(154 ± 38)	(NS)	(1.7 ± 0.2)	(1.6 ± 0.3)	(NS)	(6 ± 1)	(6 ± 1)	(NS)
75-g OGTT	348 ± 92	608 ± 106	NS	2.7 ± 0.3[b]	3.7 ± 0.3	NS	10 ± 1	14 ± 2	NS
(IIGI)	(278 ± 58)	(248 ± 59)	(NS)	(2.5 ± 0.3)	(2.1 ± 0.4)	(NS)	(8 ± 1)	(8 ± 1)	(NS)
125-g OGTT	502 ± 144	745 ± 135	NS	3.8 ± 0.5[b]	4.5 ± 0.5	NS	12 ± 2	16 ± 2	NS
(IIGI)	(295 ± 57)	(251 ± 73)	(NS)	(2.7 ± 0.3)	(2.3 ± 0.4)	(NS)	(8 ± 1)	(8 ± 1)	(NS)
ANOVA (*P*)	NS	0.042		<0.001	<0.001		0.038	0.016	
Plasma response (4 h × nM)									
25-g OGTT	30 ± 7[b]	27 ± 4[b]	NS	366 ± 51[b]	315 ± 25[b]	NS	2.0 ± 0.3[b]	1.7 ± 0.2[b]	NS
(IIGI)	(28 ± 5)	(18 ± 4)	(NS)	(343 ± 40)	(257 ± 38)	(NS)	(1.8 ± 0.2)	(1.3 ± 0.1)	(0.049)
75-g OGTT	56 ± 12[b]	63 ± 9[b]	NS	515 ± 58[b]	561 ± 42[b]	NS	2.9 ± 0.3[b]	2.9 ± 0.2[b]	NS
(IIGI)	(47 ± 9)	(31 ± 8)	(NS)	(481 ± 57)	(360 ± 57)	(NS)	(2.6 ± 0.3)	(1.8 ± 0.2)	(NS)
125-g OGTT	82 ± 20[b]	99 ± 11[b]	NS	680 ± 71[b]	823 ± 65[b]	NS	3.9 ± 0.4[b]	4.5 ± 0.5[b]	NS
(IIGI)	(50 ± 10)	(35 ± 8)	(NS)	(515 ± 56)	(412 ± 49)	(NS)	(2.8 ± 0.3)	(2.1 ± 0.2)	(NS)
ANOVA (*P*)	0.008	<0.001		<0.001	<0.001		<0.001	<0.001	
Incretin effect (%)									
25-g OGTT	0 ± 7	36 ± 5	0.002	4 ± 4	20 ± 7[b]	0.044	0 ± 20	17 ± 16	NS
75-g OGTT	11 ± 9	53 ± 6	0.002	6 ± 5	37 ± 6	0.005	-3 ± 26	35 ± 10	NS
125-g OGTT	36 ± 5[b]	65 ± 6[b]	0.003	24 ± 3[b]	49 ± 5	0.001	21 ± 17	48 ± 8	NS
ANOVA (*P*)	0.016	0.001		0.010	<0.001		0.041	0.019	

Data for patients with T2DM and healthy control subjects are mean values ± SEM. Values are compared using two-tailed *t* test for intergroup variation and repeated-measurement ANOVA for interload variations.

[a] Baseline and peak values are picomolar for insulin and C-peptide and picomoles per kilogram per minute for ISR.

[b] Significant differences ($P < 0.05$) in the interload observations (*post hoc* analysis) from both of the other glucose loads compared. Total plasma glucose responses are calculated as total AUC.

increasing oral glucose loads. Looking meticulously at plasma glucose profiles from Nauck *et al.* (7), the same tendency is apparent (although not to the same extent). The tight control of the plasma glucose profiles was ascribed by Nauck *et al.* (7) to the effects of GIP secretion. Although confirming the GIP responses, our data demonstrate a similar regulation of GLP-1 secretion. In the present study, patients with T2DM were found to be characterized by a severely reduced capability to regulate the incretin effect. With the smaller glucose loads (25 and 75 g), the incretin effect was not significantly different from zero and increased to 36% only with the highest oral glucose load (125 g), similar to that seen with 25 g glucose in the healthy control subjects. Our data clearly show that the incretin effect in patients with T2DM as well as in healthy subjects cannot be presented as a single figure but must be related to the oral glucose load.

TABLE 3. Incretin hormones

	GLP-1			GIP		
	T2DM	Control	*t* test (*P*)	T2DM	Control	*t* test (*P*)
Baseline (pM)	21 ± 1	22 ± 1	NS	12.0 ± 0.9	10.1 ± 0.8	0.014
Peak values (pM)						
25-g OGTT	35 ± 2	32 ± 5	NS	79 ± 9	83 ± 13	NS
75-g OGTT	51 ± 6	69 ± 17	NS	108 ± 10	142 ± 34	NS
125-g OGTT	58 ± 14	67 ± 26	NS	91 ± 11	106 ± 24	NS
ANOVA (*P*)	NS	NS		0.021	NS	
Plasma response (4 h × nM)						
25-g OGTT	5.2 ± 0.7[a]	4.0 ± 0.5[a]	0.012	6.2 ± 0.7[a]	6.1 ± 0.9[a]	NS
75-g OGTT	6.9 ± 0.9[a]	7.2 ± 0.9	NS	10.8 ± 1.2[a]	12.3 ± 1.8	NS
125-g OGTT	9.9 ± 1.2[a]	11.3 ± 2.0	NS	15.7 ± 1.4[a]	20.5 ± 3.7	NS
ANOVA (*P*)	0.002	0.006		<0.001	0.001	

Data for patients with T2DM and healthy control subjects are mean values ± SEM. *P* values are from two-tailed *t* test for intergroup variation and repeated-measurement ANOVA for interload variations. Total plasma responses are calculated as total AUC.

[a] Significant difference ($P < 0.05$) from both of the other glucose loads compared, derived from *post hoc* analysis.

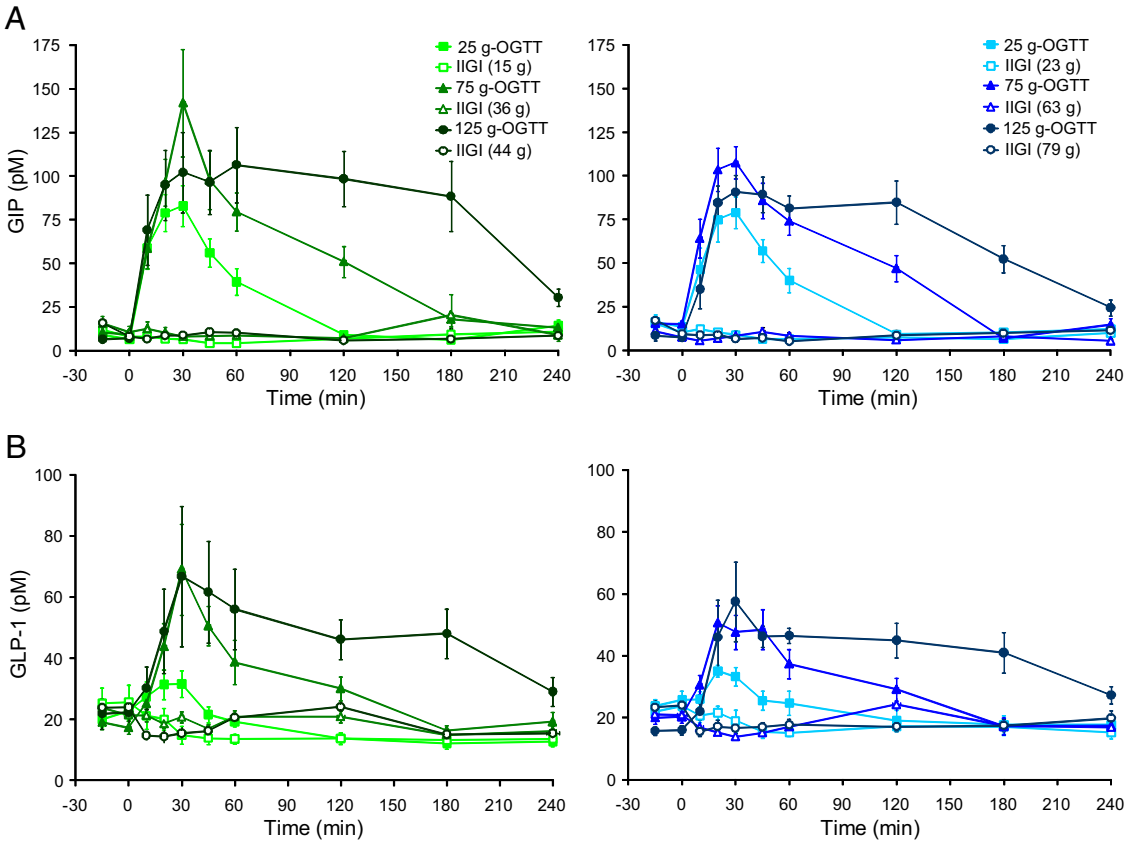

FIG. 3. Time courses for plasma concentrations of GIP (A) and GLP-1 (B) in patients with T2DM (*blue curves*) and in healthy control subjects (*green curves*). Color tone indicates each set of OGTT and IIGI [*light*, 25-g OGTT (*closed symbols*) and corresponding IIGI (*open symbols*); *medium*, 75-g OGTT (*closed symbols*) and corresponding IIGI (*open symbols*); and dark, 125-g OGTT (*closed symbols*) and corresponding IIGI (*open symbols*)].

The somewhat protracted glucose profiles after larger oral glucose loads demonstrated by both Nauck *et al.* (7) and us among healthy subjects are likely to result from deceleration of gastric emptying in response to the larger oral glucose loads as indicated by the acetaminophen results of the present study. Interestingly, with increasing glucose loads, a pronounced decrease in gastric emptying was observed in both healthy control subjects and patients with T2DM. This mechanism clearly serves to limit the amount of glucose emptied into the small intestine. Thereby, glucose excursions are dampened and prolonged, perhaps serving to avoid osmotic overload of the proximal small intestine. Conversely, accelerated gastric emptying, as observed after gastric surgery, is frequently associated with the dumping syndrome. Importantly, this mechanism is completely preserved in the patients with T2DM, but in these subjects, a severely impaired insulin secretion during oral glucose is seen, probably due to an impaired capability to regulate the incretin effect. The regulation of gastric emptying may well be the main regulator of the glucose excursions in patients with T2DM. It has been speculated that differences in the incretin effect ob-

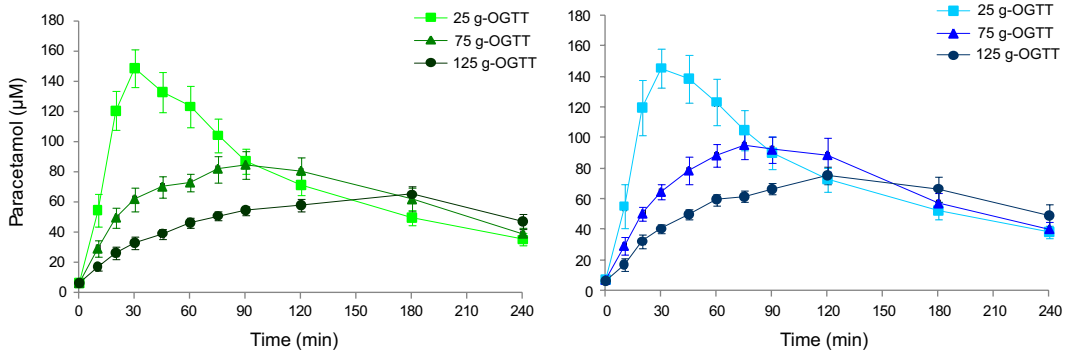

FIG. 4. Time courses for plasma concentrations of acetaminophen in patients with T2DM (*blue curves*) and in healthy control subjects (*green curves*). Color tone indicates each OGTT (*light*, 25-g OGTT; *medium*, 75-g OGTT; and *dark*, 125-g OGTT).

served among healthy control subjects and patients with T2DM could be ascribed to different gastric emptying rates (20–23). In the current study, the acetaminophen absorption technique for the measurement of gastric emptying was used. This method is a simple, noninvasive, and well-validated method that correlates well with the gold standard, the scintigraphic method (24). Therefore, the method is often used in clinical studies despite some limitations (*e.g.* provides an indirect estimation of gastric emptying and some inter-individual variation) (24, 25). However, in the present study, we found similar rates of gastric emptying between the two groups. Increased insulin resistance was seen in our patients with T2DM compared with the healthy control subjects, a phenomenon that is thought to contribute to an impaired incretin effect (26, 27). Furthermore, reduced postprandial GLP-1 responses (20, 28), decreased insulinotropic potency of GLP-1 (29) and an almost complete loss of insulin secretion in response to GIP (21, 30) have been proposed as potential contributors to the impaired incretin effect in T2DM. Because one of the aims of the present study was to evaluate the secretion of incretin hormones after increasing oral glucose loads, both GIP and GLP-1 were measured. The incretin hormone responses in the present study correspond to previously reported findings: no differences in GIP or GLP-1 responses to OGTT between healthy control subjects and patients with T2DM (31, 32). Both incretin hormones show rapid increases to similar peak concentrations after all three glucose loads but progressively protracted responses to increasing loads. This finding is probably best explained by the effect on gastric emptying of the larger glucose loads (the larger the load the slower the gastric emptying), providing almost equal amounts of glucose per unit of time to the upper small intestine. The similar incretin hormone responses in patients with T2DM and healthy control subjects are consistent with the notion that reduced insulinotropic potencies of the incretin hormones are the most important mechanisms behind the reduced incretin effect in patients with T2DM (33). Increased urinary excretion of glucose could also contribute to the regulation of the glucose profiles and thereby mask a deficit in the incretin effect, but only small amounts of glucose were excreted by the kidneys during the high glucose loads, which therefore do not explain the results. The minor glucose excretion found in the patients with T2DM in this study possibly result from increased renal glucose reuptake, potentially amplifying the postprandial glucose excursions (34, 35).

In conclusion, our results suggest that patients with T2DM are characterized by a reduced ability to increase their incretin effect to levels that allow accommodation of large glucose loads, thereby resulting in exaggerated postabsorptive glucose excursions. This pathophysiological trait may contribute to the relative insulin deficiency and hyperglycemia in the postprandial state, especially during large meals, in patients with T2DM.

Acknowledgments

We are grateful to our volunteers whose availability made this work possible and to Ulla K. Hansen, Tonni L. Hansen, Dorthe Mogensen, and Lone Bagger.

Address all correspondence and requests for reprints to: Dr. Tina Vilsbøll, M.D., D.M.Sc., Head of Diabetes Research Division, Department of Internal Medicine F, Gentofte Hospital, University of Copenhagen, Niels Andersens Vej 65, DK-2900 Hellerup, Denmark. E-mail: t.vilsboll@dadlnet.dk.

This work was supported by an unrestricted research grant (no. 34851) from the Investigator Initiated Studies Program of Merck & Co., Inc. The opinions expressed in this paper are those of the authors and do not necessarily represent those of Merck & Co., Inc. The funding source had no involvement in the study design; in the collection, analysis, or interpretation of the data; or in the decision to submit the paper for publication.

The study is registered at www.ClinicalTrials.gov as NCT00529048.

The study was presented in 2009 at the American Diabetes Association 69th Scientific Session (abstract P-1421).

Disclosure Summary: The authors had no conflicts of interests in relation to this present paper.

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