Separate Impact of Obesity and Glucose Tolerance on the Incretin Effect in Normal Subjects and Type 2 Diabetic Patients

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OBJECTIVE—To quantitate the separate impact of obesity and hyperlycemia on the incretin effect (i.e., the gain in β-cell function after oral glucose versus intravenous glucose).

RESEARCH DESIGN AND METHODS—Isoglycemic oral (75 g) and intravenous glucose administration was performed in 51 subjects (24 with normal glucose tolerance [NGT], 17 with impaired glucose tolerance [IGT], and 10 with type 2 diabetes) with a wide range of BMI (20–61 kg/m^2). C-peptide deconvolution was used to reconstruct insulin secretion rates, and β-cell glucose sensitivity (slope of the insulin secretion/glucose concentration dose-response curve) was determined by mathematical modeling. The incretin effect was defined as the oral-to-intravenous ratio of responses. In 8 subjects with NGT and 10 with diabetes, oral glucose appearance was measured by the double-tracer technique.

RESULTS—The incretin effect on total insulin secretion and β-cell glucose sensitivity and the GLP-1 response to oral glucose were significantly reduced in diabetes compared with NGT or IGT ($P \leq 0.05$). The results were similar when subjects were stratified by BMI tertile ($P \leq 0.05$). In the whole dataset, each manifestation of the incretin effect was inversely related to both glucose tolerance (2-h plasma glucose levels) and BMI (partial $r = 0.27–0.59$, $P \leq 0.05$) in an independent, additive manner. Oral glucose appearance did not differ between diabetes and NGT and was positively related to the GLP-1 response ($r = 0.53$, $P < 0.01$). Glucagon suppression during the oral glucose tolerance test was blunted in diabetic patients.

CONCLUSIONS—Potentiation of insulin secretion, glucose sensing, glucagon-like peptide-1 release, and glucagon suppression are physiological manifestations of the incretin effect. Glucose tolerance and obesity impair the incretin effect independently of one another. ***Diabetes*** **57:1340–1348, 2008**

Type 2 diabetes results from the interaction of insulin insensitivity and β-cell dysfunction (1). The relative contribution of reduced β-cell mass and β-cell dysfunction to hyperglycemia is still debated (2,3), but mounting evidence indicates that gastrointestinal factors play an important role. In fact, it has long been known that oral glucose stimulates insulin secretion over and above the stimulus that is provided by rising glucose levels (4,5). This potentiation of β-cell function by the route of nutrient administration has been termed the incretin effect (6). Among a host of factors and signals originating from the absorptive process, concentrations of glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic polypeptide (GIP) have received special attention (7). These hormones are released in parallel with insulin following oral glucose or meals, and each has been shown to potentiate glucose-dependent insulin release. The key observation that the GLP-1 response is blunted and that the β-cell response to GIP is grossly impaired in diabetes (7–11) has led to the notion that an impaired incretin effect contributes to the β-cell incompetence of diabetes (11). In recent years, the clinical data showing that GLP-1 analogs can normalize glycemia by stimulating insulin secretion in diabetic patients (12,13) has strengthened the incretin theory.

The impact of obesity on the incretin effect is uncertain. Obese subjects, especially those with visceral fat accumulation, frequently are insulin resistant and insulin hypersecretors, in proportion to the degree of overweight (14). The incretin effect has been reported to be increased in obese adolescents (15) but normal in obese adults (16). In some studies, the GLP-1 response of obese subjects has been found to be normal, whereas the GIP response was increased in the fasting state and early after a meal (17,18). In others studies (19–21), however, GLP-1 levels in response to oral carbohydrate or a meal were reduced in obese patients. Because diabetes is strongly associated with obesity, the question of the separate impact of obesity and hyperglycemia on the incretin effect has full pathophysiologic relevance. The primary aim of the present work was to answer this question.

Previous work from our laboratory has shown that the incretin effect, as tested with the use of the isoglycemic protocol (22), can be quantitated by measuring not only the plasma insulin response, as per the original definition (6), but also the two main parameters describing β-cell function, namely, absolute insulin secretion and β-cell glucose sensitivity (i.e., insulin secretion in relation to the concomitant plasma glucose concentration). We therefore set forth to measure β-cell function and hormones in

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AUC, area under the time concentration curve; FFM, fat-free mass; GIP, glucose-dependent insulinotropic polypeptide; GLP, glucagon-like peptide; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; TTR, tracer-to-tracee ratio.

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response to oral glucose and isoglycemic intravenous glucose in a large group of volunteers, including subjects with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), or diabetes over a wide range of body mass. Because gastrointestinal hormone responses are linked with the rate of nutrient absorption (23), a secondary aim of the study was to measure the rate of appearance of ingested glucose in the systemic circulation in subjects with different incretin effect (i.e., NGT and diabetes).

RESEARCH DESIGN AND METHODS

Fifty-one subjects, selected from the outpatient clinic, volunteered for the study. None of them had lost weight or changed dietary habits during the 3 months preceding the study. Three diabetic patients were on treatment with metformin alone and one with acarbose, and both were withheld 3 weeks before the study. All subjects had resting arterial blood pressure ≤140/90 mmHg and normal results for liver and renal function tests. Fat-free mass (FFM) was measured by electrical bioimpedance using a body composition analyzer model TB-300 (Tanita, Tokyo, Japan) (24); fat mass was then obtained as the difference between body weight and FFM. On the oral glucose tolerance test (OGTT) (Table 1), 24 had NGT, 17 had IGT, and 10 had type 2 diabetes according to American Diabetes Association criteria (25). Partial data from 11 subjects with NGT and 10 with IGT have been published previously (22). The study was approved by an institutional review board, and all subjects gave informed, written consent to the study.

Two studies were carried out in each subject after an overnight (12- to 14-h) fast at 1-week intervals. In the first study, subjects underwent a 3-h OGTT (75 g), with measurements of plasma glucose concentrations at 10-min intervals. In the second study (isoglycemic test), the plasma glucose profile was reproduced by a variable intravenous glucose (20% dextrose) infusion by using an ad hoc–developed algorithm. In both studies, venous blood was sampled at −30, 0, 10, 20, 30, 40, 60, 90, 120, 150, and 180 min for plasma insulin, C-peptide, glucagon, GLP-1, and GIP measurements.

In a subgroup of 18 participants (8 with NGT and 10 with diabetes), glucose fluxes were measured by the double-tracer technique (26). With this protocol, a primed-constant infusion of [6,6-$^2H_2$]-glucose (Cambridge Isotype Laboratories, Boston, MA) ([28 μmol/kg × [fasting glycemia/5] − prime followed by a 0.28 μmol/kg infusion) was administered throughout the basal period (−180 to 0 min) and during the OGTT (0–180 min). At time 0, subjects drank a 75-g glucose solution containing 1.5 g [1-^{2}H]-glucose.

Assays. Plasma glucose was measured by the glucose oxidase technique (Beckman Glucose Analyzers; Beckman, Fullerton, CA). Plasma insulin was measured in duplicate by radioimmunoassay using a kit for human insulin with negligible cross-reactivity with proinsulin and its split products (Linco Research, St. Louis, MO). Glucagon and C-peptide were measured by radioimmunoassay (Linco Research). Plasma triglyceride and serum HDL cholesterol were assayed in duplicate by standard spectrophotometric methods on a Synchron Clinical System CX4 (Beckman). Total COOH-terminal amidated GLP-1 was assayed by radioimmunoassay using the polyclonal antiserum no. 89390 (raised in rabbits), which has an absolute requirement for the amidated C-terminus of GLP-1 and does not cross-react with C-terminally truncated metabolites or with the glycine-extended forms. The assay cross-reacts <0.01% with GLP-1 (7–35) and GLP-1 (7–37), 83% with GLP-1 (9–36) amide, and 100% with GLP-1 (1–36) amide, GLP-1 (7–36) amide, and GLP-1 (8–36) amide. The assay has a detection limit of ~1 pmol/l and an ED_{50} of 25 pmol/l. Intra- and interassay coefficients of variation are <6 and <15%, respectively (27,28). The active (NH_2-terminal) GIP was assayed by radioimmunoassay using polyclonal antiserum 98171 (raised in rabbits) that is NH_2-terminally directed and does not recognize NH_2-terminally truncated peptides. It has a cross-reactivity of 100% with human GIP 1–42 and <0.1% with human GIP 3–42, GLP-1 (7–36) amide, GLP-1 (9–36) amide, GLP-2 (1–33), GLP-2 (3–33), and glucagon. Detection limit is ~ 5 pmol/l with an ED_{50} of 48 pmol/l. Intra- and interassay coefficients of variation were <6 and <15%, respectively (29). 6,6-[$^2H_2$]glucose and [1-^{2}H]-glucose enrichment were measured by gas chromatography/mass spectrometry.

Calculations. Insulin sensitivity was estimated from the plasma glucose and insulin responses to oral glucose loading by calculating the oral glucose insulin sensitivity index, which has previously been shown to be well correlated with the *M* value from the euglycemic-hyperinsulinemic clamp (30). Areas under the time concentration curves (AUCs) were calculated by the trapezium rule. To estimate the size of the incretin effect, we used the ratio of oral to intravenous measures (6). This calculation cancels the impact of glucose levels, per se, which were matched by protocol.

All glucose fluxes were expressed per kilogram of FFM, since this normalization has been shown to minimize differences due to sex, obesity, and age (26). During the last 20 min of the basal tracer equilibration period, plasma glucose concentrations and 6,6-[^{2}H]glucose enrichment were stable in all subjects. Therefore, endogenous glucose production was calculated as the ratio of the 6,6-[$^2H_2$]glucose infusion rate to the plasma tracer enrichment (tracer-to-tracee ratio $[TTR]_{6.6}$, mean of three determinations). After glucose ingestion, the total glucose rate of appearance was calculated from $TTR_{6.6}$ using Steele's equation, as previously described (26). Before applying Steele's equation, plasma $TTR_{6.6}$ data were smoothed using a spline-fitting approach to stabilize the calculation of the derivative of enrichment. The plasma glucose concentration resulting from the absorption of ingested glucose (exogenous glucose concentration) was calculated from the product of total plasma glucose concentration and the ratio of plasma [1-^{2}H]-glucose TTR to the [1-^{2}H]-glucose TTR of the ingested glucose. The plasma glucose concentration resulting from endogenous glucose release was obtained as the difference between total and exogenous glucose concentration. TTR_{end} of endogenous glucose and oral glucose rate of appearance were calculated as described (26). The tracer-determined rate of glucose disappearance (R_d) provided a measure of insulin-mediated total-body glucose disposal.

β-Cell function modeling. The model used to reconstruct insulin secretion and its control by glucose has been previously described (31). In brief, the model consists of three blocks: *1*) a model for fitting the glucose concentration profile, the purpose of which is to smooth and interpolate plasma glucose concentrations; *2*) a model describing the dependence of insulin (or C-peptide) secretion on glucose concentration; and *3*) a model of C-peptide kinetics (i.e., the two-exponential model proposed by Van Cauter et al. [32]), in which the model parameters are individually adjusted to the subject's anthropometric data. In particular, with regard to the insulin secretion block (block 2), the relationship between insulin release and plasma glucose concentrations is modeled as the sum of two components: *1*) The first component is the relationship between insulin secretion and glucose concentration (i.e., a dose-response function). The dose-response function is modulated by a time-varying factor, expressing a potentiation effect on insulin secretion, which was calculated as the ratio of the 2-h to zero time value. The mean slope of the dose-response function is taken to represent β-cell glucose sensitivity, and *2*) the second insulin secretion component represents a dynamic dependence of insulin secretion on the rate of change of glucose concentration. This component, termed rate sensitivity, accounts for anticipation of insulin secretion as glucose levels rise (data not reported here). Total insulin secretion is the sum of the two components described above, and is calculated every 10 min for the whole 3-h period.

Statistical analysis. Data are given as means ± SD or median (interquartile range) for nonnormally distributed variables. The latter were transformed into their natural logarithms for use in statistical testing. Group differences were analyzed by ANOVA; individual group differences were analyzed by the Bonferroni-Dunn test. Paired group values were compared by the Wilcoxon test. Differences in time course between groups were analyzed by 2-way ANOVA for repeated measures. Linear regression models were tested by standard techniques. Adjustment for covariates was carried out by ANCOVA. A P value ≤0.05 was considered statistically significant; when post hoc performing multiple comparisons; the P value was divided by the number of comparisons.

RESULTS

The groups with NGT, IGT, and diabetes had similar age and sex distribution, BMI, and fat mass. A1C and serum triglycerides were higher in diabetes, and insulin sensitivity was reduced in both IGT and diabetes (Table 1). The fasting and post-OGTT plasma glucose concentrations were higher in IGT and diabetes than in NGT and by design were virtually identical during OGTT and the isoglycemic test in each group (Fig. 1). The plasma insulin response to oral glucose was higher in IGT and lower in diabetes compared with NGT. Fasting insulin secretion rates were increased in diabetes (median 115 [interquartile range 53]) versus subjects with NGT (86 [81] pmol · min^{-1} · m^{-2}; $P <$ 0.05). The insulin secretory response to oral glucose was similar in NGT, IGT, and diabetes (60 [27] nmol/m^2 vs. 67 [21] nmol/m^2 vs. 67 [38] nmol/m^2) but delayed in diabetes ($P <$ 0.0001 by repeated-measures ANOVA). The total secretory response to intravenous glucose was significantly lower than to oral glucose in NGT and IGT but not in diabetes. In contrast, β-cell glucose sensitivity was progres-

TABLE 1
Anthropometric and metabolic characteristics of the study subjects by glucose tolerance status

	NGT	IGT	Diabetes	P*
n	24	17	10	
Male/female	10/14	5/12	9/1	NS
Age (years)	41 ± 11	47 ± 13	50 ± 9	NS
BMI (kg/m^2)	33.1 ± 10.5	35.9 ± 8.0	35.5 ± 11.5	NS
Waist circumference (cm)	98 ± 20	102 ± 12	112 ± 19	NS
Fat mass (%)	35 ± 13	42 ± 6	38 ± 15	NS
A1C (%)	5.3 ± 0.4	5.5 ± 0.1	6.8 ± 0.6†	<0.0001
LDL cholesterol (mmol/l)	2.90 ± 0.83	3.37 ± 0.74	3.00 ± 0.73	NS
HDL cholesterol (mmol/l)	1.35 ± 0.47	1.19 ± 0.29	1.19 ± 0.22	NS
Triglycerides (mmol/l)	1.26 ± 0.69	1.32 ± 0.68	1.99 ± 0.97†	<0.02
Insulin sensitivity (ml · min^{-1} · m^{-2})	380 ± 51	319 ± 49†	305 ± 32†	<0.0001

Data are means ± SD. *ANOVA. †$P \leq 0.05$ vs. NGT by Bonferroni-Dunn test. NS, not significant.

sively impaired in IGT and diabetes compared with NGT on both the OGTT and isoglycemic test. However, β-cell glucose sensitivity was better with oral than intravenous glucose in NGT and IGT but not in diabetes (Fig. 2). Rate sensitivity was significantly higher during the oral than intravenous study in all groups (all $P < 0.001$) and was impaired in diabetes (749 [671] pmol · min^{-1} · m^{-2} · mmol^{-1} · l, $P = 0.03$, vs. 1,482 [1,589] pmol · min^{-1} · m^{-2} · mmol^{-1} · l for NGT); however, the incretin effect (ratio of oral to intravenous values) did not differ across glucose tolerance status. Likewise, the incretin effect on potentiation (as a single value at 2 h versus baseline) was similar across groups.

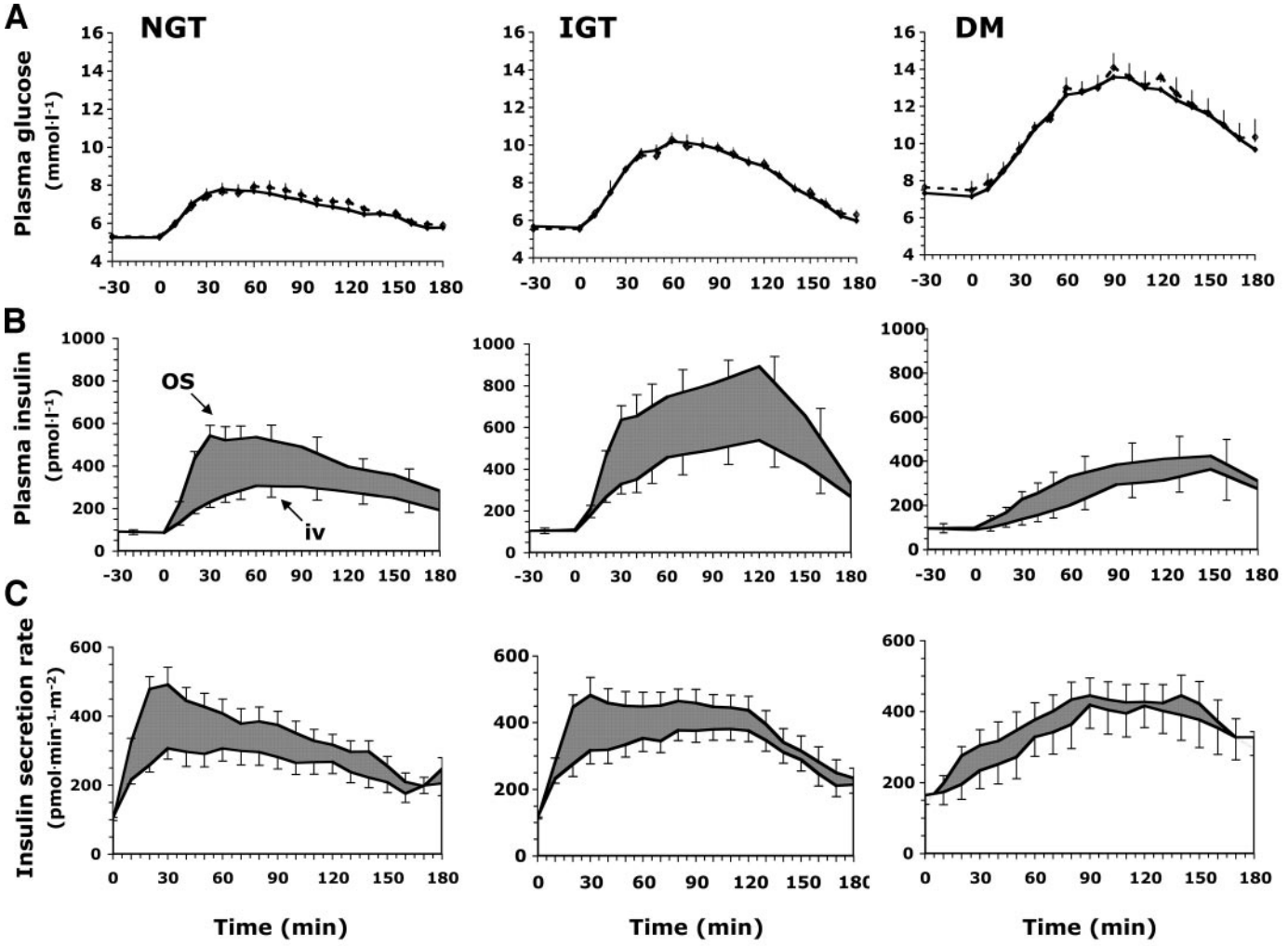

FIG. 1. Time-course of plasma glucose (*A*), insulin concentrations (*B*), and insulin secretion rates (*C*), as reconstructed from C-peptide deconvolution, in nondiabetic patients (NGT) and patients with IGT and type 2 diabetes (DM), following oral glucose (continuous line) and isoglycemic intravenous glucose administration (dashed line). The plasma glucose profiles are significantly higher in both IGT and diabetes than in NGT ($P < 0.0001$ for the time × group interaction by two-way ANOVA). Compared with NGT, the plasma insulin concentration and secretion responses to oral glucose were higher in IGT ($P < 0.0001$ and $P = 0.08$, respectively) and lower in diabetes ($P < 0.0001$ for both). The stippled areas visualize the incretin effect. Data are means ± SE.

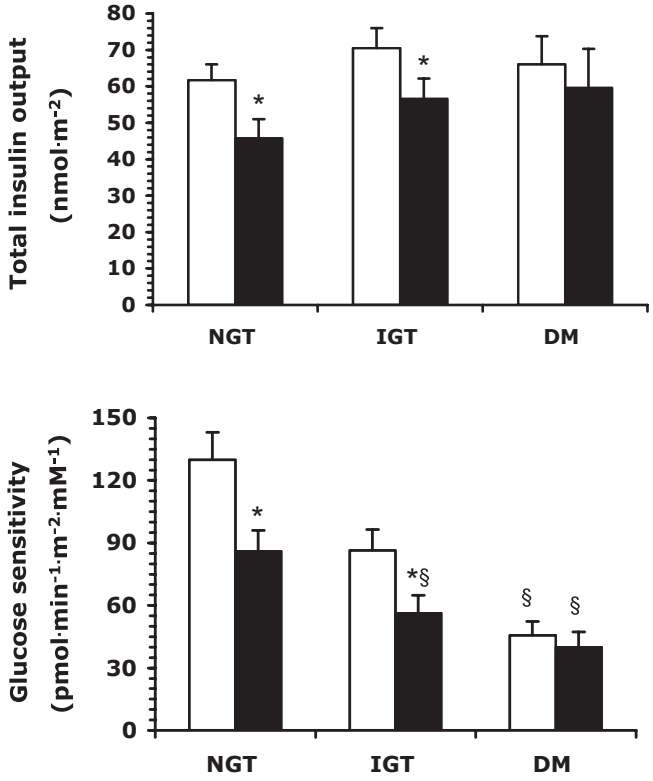

FIG. 2. Total insulin secretion and β-cell glucose sensitivity in response to oral (□) and intravenous (■) glucose in the three groups. *$P \leq 0.05$ for the difference between oral and intravenous (Wilcoxon test). §$P \leq 0.05$ for the difference from the group with NGT (Bonferroni-Dunn test).

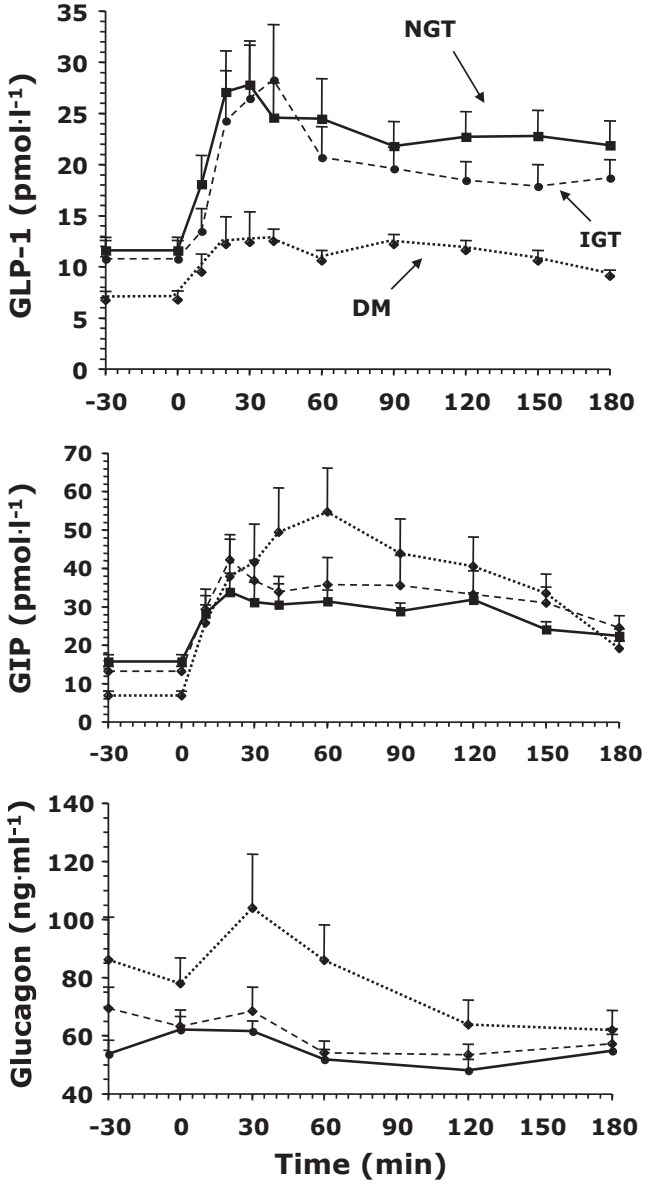

FIG. 3. Time-course of plasma GLP-1, GIP, and glucagon concentrations in response to oral glucose in the three groups. The GLP-1 response is significantly ($P < 0.01$) reduced, and the GIP and glucagon responses are significantly higher in diabetes versus NGT ($P = 0.03$ and $P = 0.002$, respectively).

With the OGTT, plasma GLP-1 levels were similar in IGT and NGT but markedly reduced in diabetes, whereas plasma GIP concentrations were higher in diabetes than in either NGT or IGT, with a prompt response and a delayed peak. Plasma glucagon levels were similar in NGT and IGT but were significantly higher in diabetes, which showed a paradoxical rise 30 min into the OGTT (Fig. 3). Neither GLP-1 nor GIP changed significantly during the intravenous test, whereas glucagon was equally suppressed in all groups (data not shown). The analysis of the hormonal AUCs is reported in Table 2.

In the subgroup receiving the double-tracer protocol, oral glucose was still appearing in the systemic circulation at 180 min at sizeable rates (averaging 16 ± 10 μmol · min^{-1} · $kg_{FFM}{}^{-1}$). The amount of oral glucose appearing over the 3 h of the OGTT totaled 43 ± 7 g in diabetes and 47 ± 11 g in NGT (P = NS); over the same time period, plasma glucose clearance was markedly reduced in diabetes (2.3 ± 0.4 vs. 4.2 ± 1.4 ml · min^{-1} · $kg_{FFM}{}^{-1}$; $P < 0.001$). Oral glucose appearance was positively related to the GLP-1 incremental AUC ($r = 0.53$, $P < 0.01$).

When the study population was stratified into BMI tertiles, groups had similar A1C levels and glucose AUCs and an approximately equal proportion of diabetic subjects ($\chi^2 = 5.6$, $P = 0.2$) (Table 3). As expected, insulin sensitivity was progressively lower and insulin levels and secretion rates (fasting and postglucose) were progressively higher with increasing BMI (Table 4). However, β-cell glucose sensitivity was similar across BMI tertiles with oral glucose and increased somewhat with intravenous glucose. Rate sensitivity and potentiation were similar in BMI groups. The GLP-1 response, but not the GIP or glucagon response, was impaired with increasing BMI.

Incretins and the incretin effect. The incretin effect was analyzed separately for total insulin secretion and β-cell glucose sensitivity. For both parameters, the incretin effect was markedly attenuated in association with diabetes (Fig. 2). When analyzed by BMI tertiles, the incretin effect on total insulin secretion was progressively lower with higher BMI (oral-to-intravenous ratio = 1.8 ± 0.6 vs. 1.4 ± 0.3 vs. 1.1 ± 0.2; $P = 0.0002$); the same was true of the incretin effect on β-cell glucose sensitivity (2.1 ± 1.0 vs. 1.6 ± 0.8 vs. 1.3 ± 0.5; $P = 0.02$). In bivariate analysis, the impact of BMI and glucose tolerance were independent of each other. Using continuous variables, the incretin effect on total insulin secretion was a simultaneous function of BMI (partial $r = -0.59$, $P < 0.0001$) and 2-h plasma glucose levels (partial $r = -0.36$, $P < 0.01$). Likewise, the incretin effect on β-cell glucose sensitivity

TABLE 2
Hormone AUC by glucose tolerance status

	NGT	IGT	Diabetes	*P**
AUC_I (nmol · l^{-1} · h)				
OGTT	75 ± 40	118 ± 64†	59 ± 42	0.007
Intravenous	45 ± 36	73 ± 53†	40 ± 29	0.05
P‡	<0.0001	0.0003	0.005	
AUC_{CP} (nmol · l^{-1} · h)				
OGTT	441 ± 159	494 ± 159	450 ± 177	NS
Intravenous	330 ± 186	401 ± 166	409 ± 247	NS
P‡	<0.0001	0.003	NS	
AUC_{Glg} (ng · l^{-1} · h)				
OGTT	10.3 ± 4.1	9.5 ± 2.7	13.9 ± 5.9*	<0.03
Intravenous	9.2 ± 2.6	8.7 ± 3.0	11.1 ± 5.0	NS
P‡	0.02	NS	0.008	
$AUC_{GLP\text{-}1}$ (nmol · l^{-1} · h)				
OGTT	4.1 ± 2.3	3.4 ± 1.5	2.0 ± 0.5†	0.01
Intravenous	2.4 ± 1.4	2.3 ± 1.3	1.0 ± 0.3†	0.02
P‡	<0.0001	0.0005	0.005	
AUC_{GIP} (nmol · l^{-1} · h)				
OGTT	5.9 ± 4.2	5.0 ± 1.9	7.4 ± 4.0	NS
Intravenous	2.5 ± 1.4	2.8 ± 1.4	1.3 ± 0.7†	0.02
P‡	<0.0001	0.0003	0.005	

Data are means ± SD. *ANOVA. †$P \leq 0.05$ vs. NGT by Bonferroni-Dunn test. ‡Wilcoxon's signed-rank test, OGTT versus intravenous. NS, not significant.

was reciprocally related to both BMI (partial $r = -0.41$, $P = 0.003$) and 2-h glucose levels (partial $r = -0.27$, $P = 0.05$). In both these models, sex and age were not significant covariates; furthermore, replacing 2-h plasma glucose levels with the glucose AUC did not change the results, and there was no evidence of interaction between BMI and glucose levels.

Using the regression coefficients of the above models, incretin effects (in percent) were calculated for BMIs of 25, 30, and 45 kg/m^2 and for 2-h plasma glucose levels corresponding to the median of the groups with NGT, IGT, and diabetes. The predicted values clearly illustrate the additive effect of obesity and IGT on the incretin effects on total insulin secretion and β-cell glucose sensitivity (Fig. 4).

The GLP-1, but not the GIP, response to oral glucose was independently related to both BMI and 2-h plasma glucose levels (Fig. 5). The GLP-1, but not the GIP, AUC was directly related to the incretin effect on both insulin output and β-cell glucose sensitivity ($r = 0.51$, $P < 0.001$ and $r = 0.28$, $P = 0.05$). The incretin effect on glucagon, on the other hand, was unrelated to BMI but was significantly ($P = 0.02$) higher in diabetes (1.4 ± 0.3) than in IGT (1.1 ± 0.3) or NGT (1.1 ± 0.3) when using the oral-to-intravenous ratio of the 0- to 60-min AUC.

DISCUSSION

The main finding of the present study is that obesity and glucose tolerance each attenuate the incretin effect on β-cell function and GLP-1 response independently of one another. The incretin effect, assessed as the plasma insulin response gradient during an isoglycemic protocol, is blunted in diabetes as previously demonstrated (11). Our results specify the mechanisms of this defect. First, the oral-to-intravenous ratio in total insulin output was narrower in diabetes compared with control subjects (Fig. 2). Of note, the insulin secretory response to intravenous glucose was, if anything, greater in diabetic patients than subjects with NGT, on account of the higher plasma glucose levels. Therefore, in diabetes the incretin defect consisted of an inability to increment insulin release when

TABLE 3
Anthropometric and metabolic characteristics of the study subjects by BMI tertile

	OB 1	OB 2	OB 3	*P**
n	17	17	17	
Male/female	8/9	9/8	7/10	NS
NGT/IGT/diabetes	11/2/4	6/8/3	7/7/3	NS
Age (years)	46 ± 11	50 ± 13	46 ± 10	0.02
BMI (kg/m^2)	25.1 ± 2.7	32.1 ± 2.7†	46.3 ± 6.5†	—
Waist circumference (cm)	84 ± 10	104 ± 9†	121 ± 15†	<0.0001
Fat mass (%)	27 ± 10	38.7 ± 8†	47 ± 5†	<0.0001
A1C (%)	5.7 ± 0.8	5.7 ± 0.7	6.0 ± 0.8	NS
LDL cholesterol (mmol/l)	2.85 ± 0.57	3.28 ± 0.99	3.00 ± 0.72	NS
HDL cholesterol (mmol/l)	1.55 ± 0.55	1.18 ± 0.22†	1.17 ± 0.26†	0.03
Triglycerides (mmol/l)	1.12 ± 0.86	1.61 ± 0.76	1.40 ± 0.73	NS
Insulin sensitivity (ml · min^{-1} · m^{-2})	381 ± 47	328 ± 58†	327 ± 52†	0.005

Data are means ± SD. *ANOVA. †$P \leq 0.05$ vs. OB 1 by Bonferroni-Dunn test. NS, not significant; OB, obese subject.

TABLE 4
Hormone AUC and β-cell function parameters by BMI tertile

	OB 1	OB 2	OB 3	*P**
AUC_G ($\mu mol \cdot l^{-1} \cdot h^{-1}$)				
OGTT	1,447 ± 436	1,583 ± 402	1,425 ± 216	NS
Intravenous	1,478 ± 435	1,594 ± 403	1,438 ± 203	NS
P†	0.02	NS	NS	
AUC_I ($nmol \cdot l^{-1} \cdot h^{-1}$)				
OGTT	51 ± 23	86 ± 47‡	122 ± 62‡	0.0003
Intravenous	24 ± 10	48 ± 28‡	88 ± 51‡	<0.0001
P†	0.0003	0.0003	0.003	
AUC_{CP} ($nmol \cdot l^{-1} \cdot h^{-1}$)				
OGTT	324 ± 81	471 ± 133‡	585 ± 143‡	<0.0001
Intravenous	204 ± 79	358 ± 129‡	546 ± 176‡	<0.0001
P†	0.0004	0.007	0.04	
AUC_{Glg} ($ng \cdot l^{-1} \cdot h^{-1}$)				
OGTT	10.1 ± 3.4	9.8 ± 2.5	12.3 ± 6.2	NS
Intravenous	8.6 ± 2.6	8.8 ± 2.2	10.9 ± 4.5‡	NS
P†	0.009	NS	0.06	
$AUC_{GLP\text{-}1}$ ($nmol \cdot l^{-1} \cdot h^{-1}$)				
OGTT	4.7 ± 2.3	3.5 ± 1.5‡	2.2 ± 0.8‡	0.0002
Intravenous	2.9 ± 1.4	2.1 ± 1.1‡	1.3 ± 1.1‡	0.001
P†	0.0004	0.0003	0.005	
AUC_{GIP} ($nmol \cdot l^{-1} \cdot h^{-1}$)				
OGTT	6.5 ± 2.8	5.8 ± 2.1	5.2 ± 5.1	NS
Intravenous	3.0 ± 2.8	2.6 ± 1.5	1.5 ± 0.8‡	0.004
P†	0.0003	0.0003	0.003	
Fasting ISR ($pmol \cdot min^{-1} \cdot m^{-2}$)				
OGTT	65 ± 25	108 ± 41‡	160 ± 57‡	<0.0001
Intravenous	70 ± 31	111 ± 42‡	183 ± 82‡	<0.0001
P†	NS	NS	NS	
Total IS ($nmol/m^2$)				
OGTT	48 ± 13	69 ± 20‡	80 ± 21‡	<0.0001
Intravenous	30 ± 13	51 ± 18‡	75 ± 25‡	<0.0001
P†	0.0004	0.0007	0.04	
β-Cell glucose sensitivity ($pmol \cdot min^{-1} \cdot m^{-2} \cdot mmol^{-1} \cdot l$)				
OGTT	101 ± 82	101 ± 49	95 ± 46	NS
Intravenous	48 ± 26	77 ± 60‡	78 ± 36‡	NS
P†	0.0006	0.05	0.04	

Data are means ± SD. *By ANOVA. †Wilcoxon's signed-rank test, OGTT versus intravenous. ‡$P \leq 0.05$ vs. OB 1 by Bonferroni-Dunn test. NS, not significant; OB, obese subject.

the plasma glucose profile was the result of glucose ingestion. In addition, β-cell glucose sensitivity was progressively worse in IGT and diabetes on both intravenous and oral glucose, but subjects with NGT and IGT retained the ability to enhance β-cell glucose sensitivity when the stimulus came by mouth, whereas diabetic patients failed to do so. There was no major impact of IGT or diabetes on the incretin effect on rate sensitivity or potentiation.

As previously reported (8,9), the GLP-1 secretory response was depressed in diabetes and the GIP response was enhanced (at least early during absorption) (Fig. 3). By pooling data from all groups, we found a positive correlation between the GLP-1 response and the incretin effect (insulin output and β-cell glucose sensitivity). Of note, the GLP-1 response accounted for a relatively small fraction (~25%) of the variance of these incretin effects, suggesting that other factors contribute to the incretin effect or that circulating GLP-1 concentrations are a distant reflection of its biological activity. Like others (11), we found no correlation between the GIP response and incretin effects. In summary, each aspect of the incretin effect (quantitative insulin response, β-cell glucose sensing, and GLP-1 secretory response) was impaired in diabetes. Whether this defect is inherent in the diabetic state or secondary to diabetic hyperglycemia is still somewhat uncertain. The weight of available evidence, however, favors the view that the defective incretin function is a secondary phenomenon. Thus, one or the other aspect of incretin function has been reported to be normal in first-degree relatives of subjects with diabetes (33,34), in nondiabetic twins of diabetic probands (9), and in nondiabetic patients with chronic pancreatitis (while a reduced incretin effect is demonstrable in patients with chronic pancreatitis and secondary diabetes) (35). Furthermore, preliminary data suggest that normalization of glycemia in patients with diabetes with insulin treatment improves insulin secretion in response to GLP-1 infusion (36). Whether correction of chronic hyperglycemia reverses all manifestations of the incretin effect as measured by the isoglycemic protocol remains to be proven.

When the study population was stratified by obesity, the incretin effect (on insulin output, β-cell glucose sensitivity, and GLP-1 response) was gradedly depressed across increasing degrees of overweight despite the fact that the clinical (Table 3) and metabolic (Table 4) features of BMI groupings were different from those of the glucose toler-

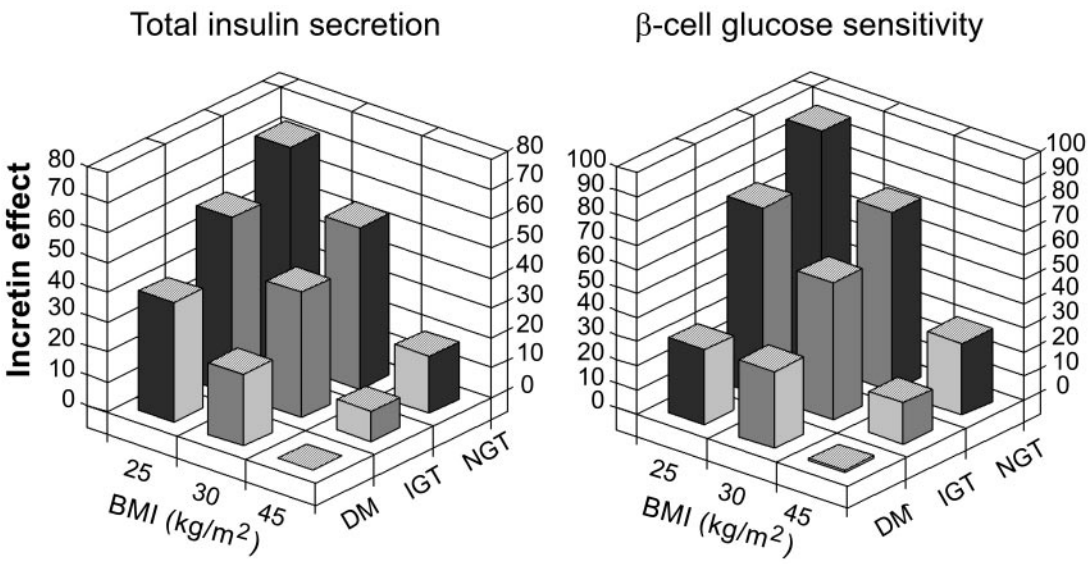

FIG. 4. Predicted percent changes in the incretin effect on total insulin secretion and β-cell glucose sensitivity as a simultaneous function of BMI and 2-h plasma glucose levels in the whole study group.

ance groupings. Thus, fasting and total insulin release rose markedly across BMI tertiles, whereas β-cell glucose sensitivity did not change (Table 4) (i.e., the opposite of the glucose tolerance ranking). As a consequence, bivariate analysis of the whole dataset convincingly showed an independent contribution of BMI and 2-h glucose levels on all manifestations of incretin function (insulin release and β-cell glucose sensitivity [Fig. 4] and GLP-1 response [Fig. 5]). Previous studies (15,16,20,21) of incretin function in obesity have been largely inconclusive. In obese diabetic patients undergoing gastric bypass surgery (37), GLP-1 and GIP release and insulin secretion were enhanced early postoperatively at a time when body weight was unchanged but glycemia was improved. In nondiabetic obese subjects, dietary-induced weight reduction was associated with a small (~9%), albeit statistically significant, increase in the GLP-1 response to a mixed meal (21). All in all, previous studies have not separated out the impact of obesity, per se, from that of hyperglycemia on the incretin effect. Which feature of the obese state is causally related to the incretin defect remains unknown. Circulating free fatty acids have been suggested to inhibit GLP-1 release and stimulate GIP secretion (19). However, Verdich et al. (21) and Toft-Nielsen et al. (8) did not find a correlation between plasma free fatty acids and GLP-1 response. In pancreatectomized, hyperglycemic rats, both GLP-1 and GIP receptor expression in islets was downregulated (38). Whether a similar phenomenon occurs in spontaneous human diabetes or as a result of obesity is unknown.

Short of changes in splanchnic glucose uptake, the pattern of appearance of orally derived glucose is the integrated result of gastric emptying and intestinal glucose absorption (39), the former being rate limiting. If the release of GLP-1 were delaying gastric emptying (40), as occurs when exogenously GLP-1 is given by constant infusion (41,42), a defective incretin effect should be manifested as accelerated gastric transfer of ingested glucose. However, in the current study, appearance of ingested glucose in the systemic circulation occurred at similar rates and in similar time course in NGT and diabetes despite the largely different incretin effect. There-

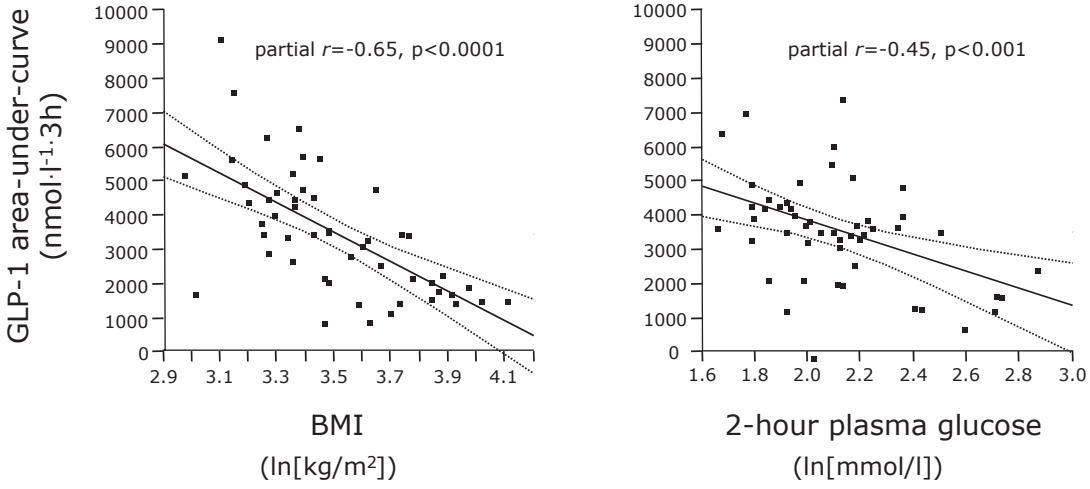

FIG. 5. Dual dependence of plasma GLP-1 response on BMI and 2-h plasma glucose concentrations. The graphs plot the residuals of one independent variable against the other.

fore, we can conclude that during an OGTT, changes in oral glucose appearance (or, at least major detectable differences) are not part of the incretin effect. The positive relation of GLP-1 response to oral glucose appearance (also found by others [23]) is best explained by the fact that the rate of glucose transfer across the intestinal mucosa is a quantitative determinant of the release of gastrointestinal hormones (43). This conclusion is indirectly supported by the observation that dipeptidyl-peptidase intravenous inhibitors, which cause modest increments in endogenous GLP-1 levels, have been shown not to alter gastric emptying (44), whereas the use of GLP-1 analogs delays gastric emptying (45).

In contrast to oral glucose appearance, the observed changes in plasma glucagon concentrations between oral and intravenous glucose administration do imply an incretin effect. In fact, during the early phase of glucose absorption, the oral-to-intravenous ratio of glucagon level was significantly higher in diabetes than in either IGT or NGT. A paradoxical, short-lived rise in glucagon levels following oral glucose has been documented in diabetic patients long ago (46) and has been held responsible for the inappropriately high rate of endogenous glucose production that is seen in diabetes following oral glucose (47) or mixed meals (48). Thus, in agreement with previous data (49), a defective incretin effect on glucagon release may explain, at least in part, the paradoxical hyperglucagonemia of diabetes and participate in the genesis of postprandial hyperglycemia in these patients.

In summary, using the isoglycemic protocol the incretin effect can be described as the glucose-independent stimulation of total insulin secretion, β-cell glucose sensitivity, and GLP-1 and glucagon release induced by oral glucose administration. This complex response is significantly impaired in association with both obesity, per se, and glucose intolerance in an independent and additive manner.

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