Downregulation of Insulin Sensitivity After Oral Glucose Administration: Evidence for the Anti-Incretin Effect

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Intestinal nutrients stimulate insulin secretion more potently than intravenous (IV) glucose administration under similar plasma glucose levels (incretin effect). According to the anti-incretin theory, intestinal nutrients should also cause a reduction of insulin sensitivity and/or secretion (anti-incretin effect) to defend against hyperinsulinemia-hypoglycemia. An exaggerated anti-incretin effect could contribute to insulin resistance/type 2 diabetes, whereas reduction of anti-incretin signals might explain diabetes improvement after bariatric surgery. In this study, we tested some of the predictions made by the anti-incretin theory. Eight healthy volunteers and eight severely obese subjects with insulin resistance were studied. Insulin secretion, insulin sensitivity, R_a, and disposition index were measured after oral glucose tolerance test and isoglycemic IV glucose injection (IGIV). Obese subjects were studied before and after intestinal bypass surgery (biliopancreatic diversion [BPD]). The D-xylose test and lactulose-to-rhamnose ratio were used to test for possible malabsorption of glucose after surgery. Monte Carlo mathematical simulations were used to test whether insulin secretion induced by oral glucose could cause hypoglycemia when coupled with the levels of insulin sensitivity measured during IGIV. Despite isoglycemic conditions, insulin sensitivity was lower during oral than during IV glucose administration. This difference was amplified in obese subjects and reduced to normal after BPD. No evidence of glucose malabsorption was found. Mathematical simulations showed that hypoglycemia would occur if insulin sensitivity were not reduced by oral glucose stimulation. This study demonstrates an anti-incretin effect of intestinal glucose stimulation, which downregulates insulin sensitivity. The findings support a new model for how foodborne factors can induce insulin-resistance and provide a possible explanation for the improvement of insulin resistance/diabetes after gastrointestinal bypass surgery.

Insulin resistance (IR) is a critical pathophysiologic feature of type 2 diabetes and can develop decades before β-cell failure and overt hyperglycemia (1,2). IR also is common to a host of metabolic conditions, such as obesity, hypertension, dyslipidemia, and nonalcoholic steatohepatitis, and is associated with increased cardiovascular risk. The exact cause of IR remains elusive.

Over the past decade, a growing body of evidence has shown that gastrointestinal (GI) bypass procedures (e.g., gastric bypass, biliopancreatic diversion [BPD]) can cause durable remission of type 2 diabetes and dramatic improvements of IR (3,4). The exact mechanism responsible for the effects of surgery on IR and diabetes remission, however, is unclear. One hypothesis is that specific changes in GI anatomy, particularly the bypass of the proximal small intestine, reduce putative nutrient-stimulated diabetogenic signals from the gut, thus explaining the potency of the antidiabetic effect (5).This hypothesis is part of the anti-incretin theory (5,6), a theoretical model that reconciles physiologic observations, pathophysiologic aspects of IR and diabetes, and the effects of GI surgery.

Oral glucose is well known to stimulate insulin secretion more potently than intravenous (IV) glucose administration under similar plasma glucose levels, a phenomenon referred to as the incretin effect. The incretin effect is caused by the release of gut hormones that potently stimulate insulin secretion (incretins) (7). According to the anti-incretin theory,

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postprandial downregulation of insulin sensitivity and/or secretion—the anti-incretin effect—is physiologically necessary to prevent hyperinsulinemia-hypoglycemia. An exaggerated anti-incretin effect, possibly elicited by long-term exposure to certain foodborne stimuli, could result in IR and/or reduced insulin secretion, leading to type 2 diabetes (5,6). This model predicts that the exclusion of the proximal bowel from the transit of nutrients (intestinal bypass), which is characteristic of certain bariatric/metabolic procedures, may reduce anti-incretin signals. In patients with obesity/diabetes, this would restore the physiologic balance between incretin and anti-incretin mechanisms, thus explaining improvements in IR and remission of diabetes after surgery.

In this study, we tested some of the predictions made by the anti-incretin theory. We specifically investigated the hypothesis that intestinal glucose delivery can physiologically elicit an anti-incretin effect that reduces insulin sensitivity compared with IV glucose administration. We also investigated the hypothesis that such an anti-incretin effect is enhanced in obese subjects with IR and reduced after intestinal bypass surgery.

RESEARCH DESIGN AND METHODS

Study Design

To investigate the possible differences in postprandial regulation of insulin sensitivity between normal and IR individuals, we studied eight nonobese healthy control volunteers and eight obese subjects with IR. To verify whether intestinal nutrient stimuli can acutely influence insulin sensitivity, we obtained several measures of glucose metabolism (see below) during an oral glucose tolerance test (OGTT) as well as during an isoglycemic IV glucose injection (IGIV) performed in each subject on two separate sessions (1 day apart). To study the effects of the intestinal bypass, obese subjects were studied before and 8–10 months after undergoing elective BPD. R_a and glucose infusion rate (GIR) were measured to assess potential differences in the amount of glucose entering the circulation during OGTT and IGIV. The D-xylose test and lactulose-to-rhamnose ratio were measured before and after BPD to investigate whether changes in glucose metabolism after the operation were due to differences in intestinal glucose absorption. To verify whether the expected increase in insulin levels caused by intestinal glucose stimulation (incretin effect) could actually cause hypoglycemia in the absence of concurrent adjustments of insulin sensitivity, we performed Monte Carlo mathematical simulations where the values of insulin sensitivity observed during the IGIV were coupled with the levels of plasma insulin measured during the OGTT.

The study protocol was approved by the Institutional Ethics Committee of the Catholic University of Rome. All subjects provided written consent to participate.

Subjects

Eight obese individuals with IR (HOMA-IR >3.60) were recruited into this study. They were selected among patients awaiting elective bariatric surgery (BPD). Eight nonobese healthy volunteers were recruited as normal control subjects. Subject demographics and baseline characteristics are listed in Table 1.

OGTT and IGIV

Subjects underwent a 75-g OGTT and an IGIV on two separate sessions. For the OGTT, blood samples were drawn at −20, 0, 30, 60, 90, 120, 150, 180, 210, and 240 min. The first two glucose, insulin, and C-peptide concentrations were averaged to obtain a baseline level.

To match the circulating glucose concentrations measured during the OGTT, subjects underwent a graded glucose infusion at progressively increasing and then decreasing rates. Each step of the GIR was administered over 15 min. Glucose, insulin, and C-peptide levels were measured at 10-min intervals during a 20-min baseline period before the glucose infusion and every 5 min throughout the 240-min glucose infusion.

Carbohydrate Absorption and Intestinal Permeability Tests

To measure carbohydrate absorption, subjects underwent a D-xylose test. After an overnight fast and after voiding their bladder, subjects ingested 25 g D-xylose in 200 mL water. Urine was collected over 0–5 h. A 5-h cumulative urinary excretion of D-xylose >21% of the administered dose was regarded as the cutoff for normal upper-GI absorptive function (8).

To measure intestinal permeability, all subjects emptied their bladders after an overnight fast and before drinking 100 mL of a solution of 1.0 g α-L-rhamnose, 5.0 g lactulose, and 22.6 g glucose. Urine was collected for the next 5 h. Subjects were encouraged to drink water after the first 30 min and could eat after 3 h. Intestinal permeability was expressed as the excretion ratio of urinary lactulose to rhamnose. Given that the subjects underwent periodic (every 2–3 months) treatment with intestinal antibiotics postoperatively as standard practice to reduce GI symptoms associated with the operation (e.g., bloating, diarrhea), we administered rifaximin three times a day for 3 days before performing carbohydrate

Table 1—Anthropometric parameters in obese subjects before and after BPD and in healthy control subjects

Parameter	Obese subjects before BPD	Obese subjects after BPD	Control subjects
Sex			
Male	3	3	4
Female	5	5	4
Age (years)	46.2 ± 2.6	46.2 ± 2.6	44.8 ± 6.9
Height (cm)	167.4 ± 5.4	167.4 ± 5.4	173.2 ± 8.8
Weight (kg)	115.6 ± 40.2*	85.4 ± 33.8	74.2 ± 7.4
BMI (kg/m^2)	40.2 ± 12.5	30.3 ± 9.9	24.7 ± 0.5
Fat mass (kg)	56.3 ± 24.8	34.6 ± 19.2	20.8 ± 2.1
Fat-free mass (kg)	57.8 ± 17.4	52.4 ± 13.4	53.6 ± 8.0

Data are mean ± SD. *P < 0.01 before BPD vs. after BPD and control.

absorption and permeability tests both before and after BPD to have comparable experimental conditions.

Body Composition

Body weight was measured to the nearest 0.1 kg with a beam scale, and height was measured to the nearest 0.5 cm with a stadiometer (Holtain, Crosswell, Wales, U.K.). Fat-free mass and fat mass were determined by using a Lunar Prodigy whole-body scanner (GE Medical Systems, Madison, WI).

BPD

Our BPD procedure consists of an ~60% distal (horizontal) gastric resection with stapled closure of the duodenal stump. The residual volume of the stomach is ~300 mL. The small bowel is transected at 2.5 m from the ileocaecal valve, and its distal end is anastomosed to the remaining stomach. The proximal end of the ileum, comprising the remaining small bowel, is anastomosed back to the bowel ~50 cm proximal to the ileocaecal valve. After BPD, the entirety of the duodenum and jejunum are bypassed and no longer exposed to nutrient flow. The total absorbing bowel is 250 cm in length; of this, the proximal 200 cm are exposed to food but not to bile/pancreatic juice, whereas the final 50 cm (distal to the bowel-to-bowel anastomosis) is the only site where nutrient and bile mix again (common channel).

Analytical Methods

Blood samples were drawn into EDTA evacuated tubes. The plasma was immediately separated by centrifugation at 4°C and stored at −80°C until assay. Plasma glucose was measured by the glucose-oxidase method (Beckman, Fullerton, CA). Plasma insulin was assayed by microparticle enzyme immunoassay (Abbott, Pasadena, CA) with a sensitivity of 1 μU/mL and an intra-assay coefficient of variation (CV) of 6.6%. C-peptide was assayed by radioimmunoassay (MyRIA; Technogenetics, Milan, Italy), with a minimal detectable concentration of 17 pmol/L and inter- and intra-assay CVs of 3.3–5.7% and 4.6–5.3%, respectively. The D-xylose excretion was measured by high-performance liquid chromatography (8). Urinary concentrations of lactulose and rhamnose were determined by high-performance liquid chromatography as described by Miki et al. (9).

Measurements of Insulin Sensitivity and Glucose Metabolism: Minimal Models

The OGTT minimal model (10) was used to compute insulin sensitivity (S_I), glucose effectiveness (S_G), and the R_a profile. The glucose distribution volume (V_G) was estimated in the graded infusion experiment, and the same value was used for the OGTT experiment. The following relationship (Eq. 1) was obtained from the minimal model by integrating the equation of glucose kinetics from zero to the final time (T) of the experiment and multiplying by the V_G:

$$V_G[G(T) - G_b] = -V_G S_G \mathrm{AUC}(\Delta G) - V_G S_I \int_0^T Z(t)G(t)dt + \mathrm{AUC}(R_a) \quad \text{(Eq.1)}$$

where G is glucose concentration (basal value [G_b]), ΔG is $G - G_b$, $Z(t)$ is the minimal model variable related to insulin action, and AUC is the area under the curve. Equation 1 also holds for the IGIV experiment provided that R_a is replaced by the IV GIR. If the lefthand side of Eq. 1 is 0 (i.e., at time T, the glucose concentration reaches the basal value), the equation states that the exogenous glucose amount delivered to plasma in the time horizon of the experiment, AUC(R_a) or AUC(GIR), equals the sum of glucose disposed by insulin-independent uptake plus glucose disposed by insulin-dependent uptake. The profiles of the insulin secretion rate (ISR) and the indexes of β-cell sensitivity to glucose (the dynamic β-cell sensitivity [Φ_d] and the static β-cell sensitivity [Φ_s] + the total sensitivity [Φ]) were computed by the C-peptide minimal model (11).

Insulin clearance (Cl_{INS}) was computed from Eq. 2:

$$V_I[I(T) - I_b] = -Cl_{INS}\mathrm{AUC}(I) + F\ \mathrm{AUC}(ISR_t) \quad \text{(Eq.2)}$$

which was derived by integrating from zero to T and multiplying by V_G (Eq. 10 in Tura et al. [12]). I is insulin plasma concentration (basal value I_b), V_I is the insulin distribution volume, F is the posthepatic insulin fractional appearance, and ISR_t is the sum of basal plus incremental component of ISR. The values of F for obese and lean control subjects were set to the estimates reported in Tura et al. (12).

The parameters of the glucose and C-peptide minimal models were estimated by minimization of a weighted least squares index with a constrained minimization routine of the MATLAB library. The SEs of the estimates of individual parameters were evaluated by the linearization method, and the coefficients of variation were found to be <20%.

Simulations to Calculate Potential for Postprandial Hypoglycemia

To investigate whether the postprandial rise in plasma insulin levels (incretin effect) has the potential to actually induce hypoglycemia, we performed a Monte Carlo simulation that coupled the levels of plasma insulin measured during the OGTT with S_I values randomly generated within the S_I range measured during the IGIV. The number of iterations was set on the basis of a <1% difference between the average S_I estimated from our experimental data and the average S_I of the simulations in at least 100 consecutive iterations. Generally, this condition would be satisfied starting from 300 iterations; we performed 500 simulations (13).

Statistics

All data are expressed as mean ± SE unless otherwise specified. The AUC was computed by the trapezoidal rule. Nonparametric significance Wilcoxon test for two dependent samples was used with two-tailed Monte Carlo significance. Mann-Whitney test was used for intergroup comparisons, and Bonferroni correction was applied. Two-sided $P < 0.05$ was considered significant.

The individual estimates of the β-cell glucose sensitivity (Φ) were plotted against the S_I in the IGIV and the OGTT.

Although the disposition index (DI) usually is defined as the product of S_I and Φ, these data were best fitted by the function $\Phi = DI / S_I + \Phi_b$, where DI is a modified disposition index and Φ_b a basal value of the β-cell glucose sensitivity. DI and Φ_b were estimated by a nonlinear fitting routine, and the Monte Carlo method was used to find the SD of the estimates and to assess whether DI and Φ_b were significantly different in the IGIV and OGTT.

RESULTS

Insulin Secretion and Cl_{INS}

Consistent with the known incretin effect, insulin, C-peptide, and ISR were significantly higher when glucose was given orally versus IV (Fig. 1). In obese subjects (before surgery), total incremental insulin secretion (AUC_{ISR}) as well as its static and dynamic indexes (Table 2) were higher during OGTT than during IGIV. In obese subjects, the AUC_{ISR} during OGTT was more than threefold greater than that of nonobese control subjects ($P = 0.001$). After intestinal bypass surgery by BPD, the differences in total incremental ISR between the two routes of glucose administration were still significant but markedly reduced, becoming similar to values observed in the healthy control subjects (Table 2 and Fig. 1*N* and *O*).

Cl_{INS} was computed by assuming a V_I per unit weight of 78 mL/kg (12). As shown in Table 2, Cl_{INS} during the OGTT was significantly smaller in normal control subjects than for obese subjects before BPD.

Insulin Sensitivity

Obese subjects had significantly lower insulin sensitivity than the normal control subjects (Table 2). In normal control

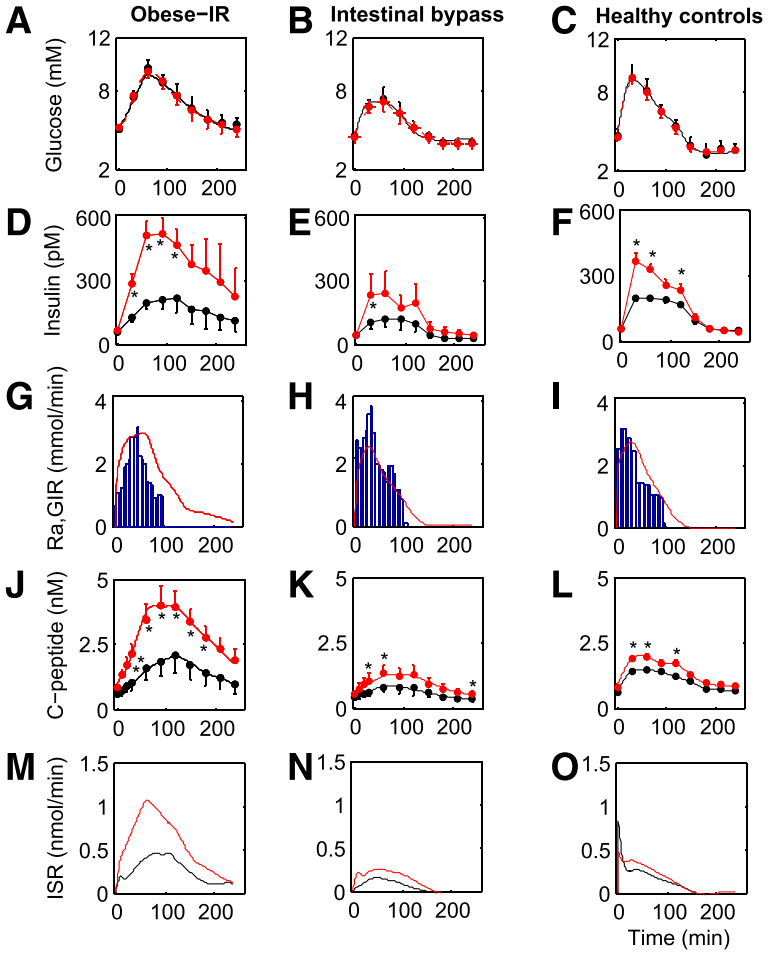

Figure 1—*A–C*: Time course of plasma glucose concentration during the OGTT (red ●) and IGIV (black ●). The two curves overlap well, showing that the glycemic excursions were similar with both routes of administration. Solid lines represent glucose minimal model fitting. *D–F*: Time course of plasma insulin during the OGTT and IGIV. As expected, insulin levels were higher during the OGTT (incretin effect) in each group. Obese subjects, however, showed much higher levels of plasma insulin when glucose was administered orally compared with healthy control subjects and after intestinal bypass surgery (BPD). *G–I*: R_a, which indicates the rate of glucose absorption from the intestine (red curves), and the GIR, which indicates the amount of glucose entering the blood circulation during the IGIV (columns). R_a is calculated by using the oral glucose minimal model; GIR is measured by the amount of glucose infusion at each time point. *J–L*: Plasma concentrations of C-peptide, which mimic those of insulin above. The solid lines represent C-peptide minimal model fitting. *M–O*: ISR computed from the C-peptide data by using the C-peptide minimal model. *$P < 0.05$.

Table 2—Metabolic parameters during IGIV and OGTT in obese subjects before and after BPD and in healthy control subjects

	Before BPD				After BPD				Control			
Parameter	IGIV	*P* value	OGTT	$P_{O/BPD}$ OGTT	IGIV	*P* value	OGTT	IGIV	*P* value	OGTT	$P_{O/C}$ OGTT	$P_{BPD/C}$ OGTT
S_G ($\times 10^2$ min^{-1})	1.46 ± 0.29	NS	2.06 ± 0.27	NS	4.71 ± 0.60	NS	4.01 ± 0.36	3.12 ± 0.32	NS	3.85 ± 0.31	0.039	NS
S_I ($\times 10^4$ min^{-1} $pmol/L^{-1}$)	0.91 ± 0.26	0.010	0.27 ± 0.06	0.001	2.70 ± 0.55	NS	2.41 ± 0.37	2.69 ± 0.22	0.021	2.03 ± 0.20	0.001	NS
AUC_{ISR} (nmoles)	59.44 ± 20.18	0.009	135.63 ± 28.30	0.001	12.92 ± 3.40	0.036	26.44 ± 7.22	28.99 ± 2.54	0.009	36.90 ± 3.42	0.001	NS
Φ_s ($\times 10^9$ min^{-1})	26.83 ± 7.06	0.010	54.58 ± 10.98	0.023	12.95 ± 2.38	0.010	18.65 ± 2.81	11.65 ± 0.76	0.014	17.77 ± 1.76	0.002	NS
Φ_d ($\times 10^9$)	273.6 ± 99.8	NS	364.7 ± 130.3	NS	156.0 ± 46.4	NS	274.0 ± 37.5	391.20 ± 61.4	NS	399.2 ± 105.3	NS	NS
Φ ($\times 10^9$ min^{-1})	29.86 ± 7.46	0.009	58.85 ± 11.44	0.023	14.70 ± 2.53	0.014	20.52 ± 2.72	16.96 ± 0.83	0.040	27.67 ± 4.15	0.031	NS
Cl_{INS} ($L \cdot min^{-1}$)	1.99 ± 0.59	NS	1.23 ± 0.12	NS	2.24 ± 0.94	NS	1.46 ± 0.35	1.09 ± 0.10	0.012	0.79 ± 0.07	0.030	NS

Data are mean ± SE. The columns between IGIV and OGTT report the statistical significance of differences observed between the two ways of glucose administration. The other three columns report statistical significance between OGTT parameters in obese subjects before and after BPD ($P_{O/BPD}$), between OGTT before BPD and control ($P_{O/C}$), and between OGTT after BPD and control ($P_{BPD/C}$).

subjects, insulin-sensitivity was lower during oral versus IGIV. The relative difference, which was computed as (IGIV S_I – OGTT S_I)/IGIV S_I, was 24.5 ± 5.43%. The difference between oral and IV insulin sensitivity was far greater in obese subjects (70.3 ± 3.7%). The amplification of the difference in insulin sensitivity between the oral and the IV route of glucose administration disappeared after BPD because S_I values were not significantly different. The S_I values after surgery for OGTT and IGIV became similar to those of normal control subjects (Table 2).

R_a

R_a in OGTT substantially matched the IV GIR in obese subjects after BPD and normal control subjects, indicating that plasma glucose concentrations during OGTT and IGIV were only determined by the levels of insulin sensitivity and secretion and not by differences in the amount of glucose entering the circulation (Figs. 1*G–I* and 2*A*).

Intestinal Absorption and Permeability Tests

The urinary recovery of D-xylose (measured value/dose) and the lactulose-to-rhamnose ratio did not differ significantly from baseline after BPD, indicating that the absorption of these mono- and disaccharides was unaffected by intestinal bypass after this type of bariatric operation (Fig. 2*B*).

DI

Oral and IV glucose administration resulted in significantly different DIs and β-cell glucose sensitivity, which also indicate lower insulin sensitivity after oral glucose administration. Figure 2*C* shows Φ versus whole-body S_I values for all subjects and experimental conditions. The OGTT and IGIV data were fitted by two separated hyperbolas, with DI (min^{-2} $pmol/L^{-1}$) and Φ_b (min^{-1}) values significantly different (IGIV: DI 8.23 ± 2.93 × 10^{-13}, Φ_b 13.02 ± 2.06 × 10^{-9}; OGTT: DI 7.20 ± 2.37 × 10^{-13}, Φ_b 21.13 ± 3.39 × 10^{-9}; $P < 0.01$). In Fig. 2*C*, the fitting lines show that a greater total β-cell glucose sensitivity is required in OGTT than in IGIV to achieve the same level of whole-body S_I when the glucose concentration profiles are kept equal. This phenomenon is shown by the upward shift of the curve of the DI after oral glucose ingestion, which is particularly visible in the lower hyperbole branch.

Insulin-Dependent Glucose Disposal

The cumulative insulin-dependent glucose disposal, computed by Eq. 1 as $V_G S_I \times AUC(ZG)$, was normalized to the cumulative R_a for the OGTT or to cumulative GIR for the IGIV. In the obese subjects with IR, the normalized glucose disposal was lower during the OGTT than during the IGIV (0.36 ± 0.05 vs. 0.53 ± 0.08; $P = 0.039$). The normalized glucose disposal measured during the OGTT after BPD and in control subjects was larger ($P = 0.014$) than with OGTT disposal measured in obese subjects with IR and not different than disposal during IGIV.

Probability of Hypoglycemia

Figure 2*D–F* reports the experimental data of the OGTT for the three groups of subjects with the model fitting (solid black

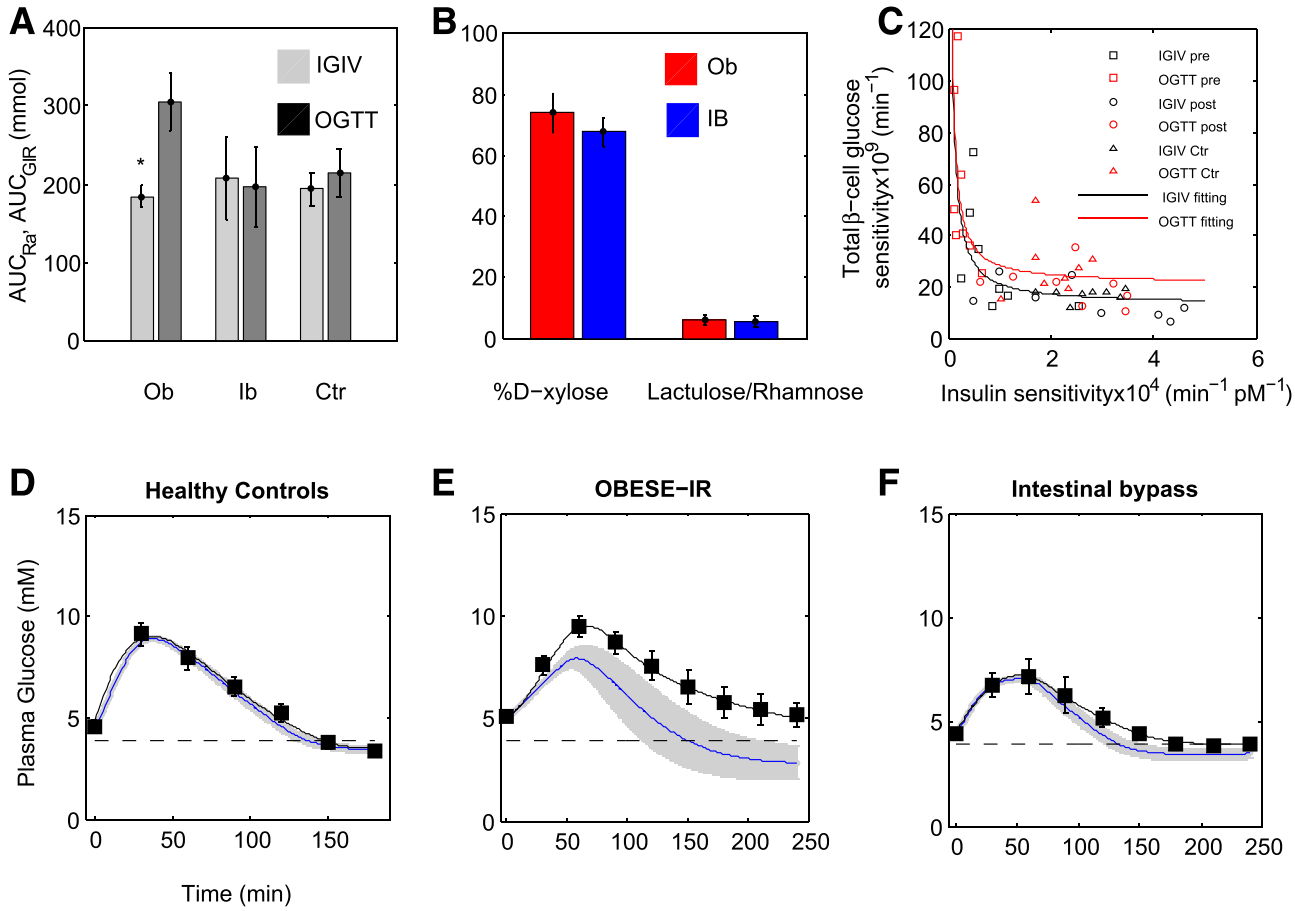

Figure 2—*A*: AUC(R_a) and AUC(GIR) during OGTT and IGIV in obese subjects with IR (Ob), subjects after BPB (Ib), and control subjects (Ctr). In Ob, AUC(R_a) was larger than AUC(GIR) (P = 0.039). *B*: Urinary recovery of D-xylose (measured value/dose) and lactulose-to-rhamnose ratio. The D-xylose test and lactulose-to-rhamnose ratio were similar before (Ob) and after (IB) BPD, indicating that malabsorption of mono- and disaccharides cannot account for the observed effects of surgery on insulin sensitivity. *C*: DI after oral vs. IV glucose administration across all study subjects. The graph shows a right shift of the DI with oral glucose administration, demonstrating that the postprandial state is associated with reduced insulin sensitivity. *D–F*: Monte Carlo simulation. The graphs show the plasma levels of glucose during the OGTT (■ and black lines) and the simulations (blue lines) with relative CIs highlighted in gray. The simulations show that if the values of S_I observed during IGIV were coupled with the levels of plasma insulin observed during the OGTT, hypoglycemia (glucose concentration below the threshold [dashed line]) would occur. Hypoglycemia would be more severe in Ob than in Ctr and IB.

lines) together with the average time course of the simulated plasma glucose concentrations reiterated 500 times for each subject (solid blue lines). The shaded area indicates the CIs.

By using the insulin sensitivity levels observed with the IGIV in obese subjects and the values of the model parameters and R_a obtained during the OGTT, a severe hypoglycemia well below the threshold of 3.9 mmol/L (14) occurred at an average time of 137 min (minimum 108 min, maximum 167 min). The frequency and degree of hypoglycemia was much less pronounced after BPD and in healthy control subjects. Accordingly, the AUCs of glucose concentration during the OGTT compared with the simulation curves were significantly different only before surgery (OGTT vs. Monte Carlo simulation: 1,574.5 ± 90.1 vs. 1,200.5 ± 215.6 mmol; $P < 0.001$).

Body Weight After BPD

Obese patients lost ~26% of their initial weight during the 8–10-month period of the study, mainly as a consequence of fat mass reduction. However, their average BMI was still in the range of obesity at the time of postoperative tests. Body weight and composition are reported in Table 1.

DISCUSSION

This study shows that in addition to the known incretin effect, oral glucose administration can elicit downregulation of insulin sensitivity, an effect that appears to be enhanced in obese subjects with IR. Mathematical simulations show that hypoglycemia would develop during an OGTT if insulin sensitivity levels were the same as measured during the IGIV, especially in obese subjects with IR, supporting the hypothesis that a state of relative IR or an anti-incretin effect is physiologically necessary to defend against the risk of hypoglycemia generated by the significant postprandial elevations of plasma insulin levels in response to intestinal nutrient passage.

In this study, intestinal bypass surgery by BPD abolished the enhancement of the anti-incretin effect observed in

obese subjects concomitantly with an overall improvement of all measures of insulin sensitivity, which became similar to those of healthy control subjects (Table 2). These findings suggest that the reduction of anti-incretin signals plays a role in the mechanism of action of BPD and possibly other procedures that similarly involve a bypass of the small intestine (e.g., Roux-en-Y gastric bypass). In fact, R_a, D-xylose test, and lactulose-to-rhamnose ratio were similar before and after BPD, indicating that malabsorption of sugars cannot account for the observed effects of surgery on insulin sensitivity. This observation is consistent with previous findings showing that simple carbohydrate absorption is unaffected by duodenal switch (15), a technical variant of the BPD procedure used in the current study. Although the obese subjects in the current study lost weight after surgery, which might theoretically contribute to their enhanced whole-body insulin sensitivity, most of the patients remained frankly obese (Table 1) despite having recovered full normalization of insulin sensitivity. The observation that glucose ingestion can acutely induce downregulation of insulin sensitivity and that bypassing a segment of small bowel can substantially revert to normal the alteration of such an effect in obese subjects supports a role of the bypassed bowel in the regulation of this mechanism of glucose homeostasis as well as in its changes observed in obese subjects.

A role for nutrient-gut interaction in the modulation of insulin-sensitivity is consistent with previous observations. Data from rodent studies have shown that the impairment of glucose tolerance and insulin sensitivity after a high-fat diet is more pronounced after oral than IV glucose administration (16). Similarly, a human study showed that S_I calculated from the oral glucose minimal model is 34% lower than that measured by euglycemic-hyperinsulinemic clamp (17). Furthermore, in perfused rat hindquarter preparation, insulin-stimulated glucose uptake in skeletal muscle is reduced in the fed versus the fasted state (18). Our group also reported that 10–100-kDa protein fractions secreted by the duodenum/jejunum of *db/db* mice and humans with IR induce IR both in vivo (normal mice) and in vitro (mouse and human myocytes) (19) and, therefore, may represent anti-incretin mechanisms.

The current study has several limitations. First, obtaining isoglycemic conditions by IV injection required a lower amount of the substrate (glucose) compared with the oral route of administration. Theoretically, the greater amount of glucose entering the circulation after oral administration could, per se, offset (or cause) the greater secretion of insulin associated with this route of administration without requiring changes in insulin sensitivity. However, several studies have shown that the liver extracts 30–60% of the glucose delivered to it in a single passage (20,21), thus substantially accounting for the difference between the amount of glucose being administered during OGTT and IGIV. This potential bias also is discounted in our study by the R_a for OGTT being closely matched to the GIR for IGIV in control and post-BPD subjects, meaning that no difference was found in the amount of glucose entering the systemic circulation between these two routes of administration.

A second limitation is that we did not use glucose tracers to measure glucose clearance and the contribution of changes in liver metabolism to the anti-incretin effect. Third, we only tested the effect of glucose ingestion; therefore, we cannot conclude whether the observations from this study extrapolate to other types of nutrients. Fourth, the study did not allow us to establish whether the enhancement of the anti-incretin effect seen in subjects with IR contributes to the cause of whole-body IR or whether it merely represents a protective mechanism against the increased risk of hypoglycemia as a result of the greater insulin response also observed in these subjects compared with nonobese control subjects. Fifth, the study did not include weight-matched control subjects (i.e., nonoperated individuals with similar BMI to post-BPD subjects), which could have confirmed that the effects of BPD are indeed weight independent.

Finally, we acknowledge that the lack of information on incretin and counterregulatory hormones may represent a limitation of our study. However, counterregulatory hormones, such as glucagon, usually are suppressed, not increased, by intestinal nutrients, thus further increasing the theoretical risk of hypoglycemia. In addition, suppression of both glucagon and (22) and cortisol (23,24) by carbohydrate ingestion is preserved in overweight and obese subjects, suggesting that no increased counterregulatory hormonal response to glucose-induced hyperinsulinemia in obesity exists and supporting the hypothesis that other types of intestinal signals capable of inducing IR counteract the action of insulin to avoid hypoglycemia. We have previously shown that glucagon-like peptide 1 levels increase after BPD in obese subjects with and without diabetes (25,26), whereas the ISR was largely reduced, which is consistent with the current study.

Despite the above limitations, this study has several important implications. First, it reveals a previously unappreciated effect of intestinal glucose stimulation that appears to play an important role in maintaining postprandial glucose homeostasis as shown by mathematical simulations. This mechanism may explain why hypoglycemia is rare despite the large increase in insulin levels normally elicited by intestinal nutrient stimulation. Second, the study documents the ability of intestinal bypass surgery to normalize an exaggerated anti-incretin response to glucose in obese subjects, which may partly explain the powerful clinical effects of bariatric/metabolic surgery on obesity and diabetes (27). A disproportionate reduction of the anti-incretin effect after bariatric surgery might explain the rare, but consistent observations of postprandial hyperinsulinemia-hypoglycemia that can complicate GI bypass surgery (28). The ability of the small intestine to elicit both incretin and anti-incretin effects in response to ingested nutrients further underscores the importance of this organ in glucose homeostasis and provides a plausible link between overnutrition and/or foodborne factors and IR syndromes.

In conclusion, the results of this study demonstrate that in addition to the known incretin-induced increase of insulin secretion, intestinal glucose delivery can downregulate insulin sensitivity to prevent hypoglycemia (anti-incretin effect). The data also show that this effect is augmented in obese subjects with IR and normalized by intestinal bypass surgery (BPD) concomitantly with a dramatic improvement of whole-body insulin sensitivity after the operation. Future studies aimed at investigating the molecular determinants of the anti-incretin effect may help us to understand the causes of IR and its relation to nutrition. This knowledge could lead to the development of novel nutritional or pharmacologic approaches for the prevention and treatment of obesity and diabetes.

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