Immediate enhancement of first-phase insulin secretion and unchanged glucose effectiveness in patients with type 2 diabetes after Roux-en-Y gastric bypass

Christoffer Martinussen,1,2 Kirstine N. Bojsen-Møller,1,2 Carsten Dirksen,1,2 Siv H. Jacobsen,1,2 Nils B. Jørgensen,1,2 Viggo B. Kristiansen,4 Jens J. Holst,2,3 and Sten Madsbad1

1Department of Endocrinology, Hvidovre Hospital, Hvidovre, Denmark; 2Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark; 3Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark; and 4Department of Surgical Gastroenterology, Hvidovre Hospital, Hvidovre, Denmark

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ROUX-EN-Y GASTRIC BYPASS SURGERY (RYGB) causes weight loss and has dramatic effects on glucose metabolism, leading to remission of type 2 diabetes in many patients. Interestingly, major improvements in glycemic control occur a few days after surgery, before any significant weight loss (44, 46). The surgical changes in gut anatomy especially affect postprandial glucose metabolism, leading to accelerated glucose absorption, enhanced insulin secretion, and exaggerated release of gut hormones, including glucagon-like peptide-1 (GLP-1) (26).

Increased glucagon-like peptide-1 (GLP-1) contributes importantly to increased postprandial insulin secretion after RYGB, as demonstrated in studies using pharmacological blockade of the GLP-1 receptor (29, 30, 51, 54).

However, other factors may also affect β-cell function after surgery. In patients with type 2 diabetes, fasting glucose levels are reduced immediately after RYGB, due to improved insulin sensitivity of the liver, resulting in decreased glucose production (4). The lower glucose levels per se may lead to improved function of the pancreatic β-cells, due to relief of glucotoxicity. Such an effect could contribute to improved insulin secretion in response to not only oral, but also intravenous (IV) administration of glucose. Glucotoxicity refers to the deleterious, but potentially reversible, effects of chronic hyperglycemia on β-cell function (14, 20). Accordingly, short periods of tight glycemic regulation achieved by insulin therapy or other antidiabetic medication in patients with type 2 diabetes have beneficial effects on several aspects of β-cell function, including restoration of first-phase insulin secretion in response to IV glucose (12, 14, 19, 50, 56), which is severely impaired in patients with type 2 diabetes (7, 45) and is reported to have major impact on glucose tolerance (6).

Glucotoxicity may also affect other tissues, including muscle, fat, and liver, rendering them resistant to the effects of insulin and glucose per se on glucose disposal (14a, 22). It is well established that glucose stimulates its own uptake and inhibits its own production from the liver, independently of insulin, an effect known as glucose effectiveness (GE) (2). In glucose-tolerant individuals, about 50% of glucose disposal after an oral glucose load is attributable to GE (2). In type 2 diabetes, this percentage is even higher, due to the attenuated effect of insulin (2). In light of this, it is interesting that the absolute GE is reduced in subjects with prediabetes or who manifest type 2 diabetes (2, 38) and appears to be regulated differentially from insulin sensitivity (32, 53). In two studies, GE was reported unchanged 3 wk after RYGB in patients with type 2 diabetes and normal glucose tolerance (25, 43). However, postoperative changes in GE may not be evident until months after surgery, when glucose levels have been normalized for a longer period.

Thus, we hypothesized that in patients with type 2 diabetes, and possibly also those with impaired glucose tolerance, RYGB may have immediate beneficial effects on the β-cells per se, resulting in early improvement of first-phase insulin secretion in response to IV glucose. Conversely, in normoglycemic individuals, we anticipated that the increased insulin
secretion after RYGB would be linked exclusively to the oral route of administration. Finally, we also wanted to evaluate the effects of RYGB on glucose effectiveness. We tested our hypothesis by studying subjects with type 2 diabetes, impaired glucose tolerance, and normal glucose tolerance before and 1 wk and 3 mo after RYGB, using intravenous glucose tolerance tests (IVGTT) and oral tolerance tests (meal test and OGTT).

METHODS

Participants. Thirty obese subjects scheduled for laparoscopic RYGB were recruited: 10 with type 2 diabetes (T2D group, age 46 ± 2.8 yr, median diabetes duration 2.0 yr, range 0.5–12.0 yr), 8 with impaired glucose tolerance (IGT group, age 42 ± 3.3 yr), and 12 with normal glucose tolerance (NGT group, age 41 ± 2.8 yr). Age was not significantly different between groups (P = 0.406). All participants met the inclusion criteria for bariatric surgery in Denmark (age >20 yr, BMI >50 kg/m² or BMI >35 kg/m² associated with comorbidities such as type 2 diabetes) and had accomplished a preoperative diet-induced total body weight loss of 8%, required by health authorities. Subjects with type 2 diabetes were excluded if Hb A1c >69 mmol/mol (>8.5%) or fasting C-peptide <700 pmol/l. Glucose tolerance was defined by OGTT performed after the preoperative weight loss and without glucose-lowering medications according to standard criteria (NGT: 2-h P-glucose <7.8 mmol/l; IGT: ≥7.8 mmol/l and <11.1 mmol/l; type 2 diabetes: ≥11.1 mmol/l). Patients with type 2 diabetes were treated with metformin alone (n = 6) or metformin combined with lixisenatide (n = 1), sitagliptin (n = 2), or glimepiride (n = 1) preoperatively. Liraglutide was discontinued >10 days and all other antidiabetic medication >3 days prior to experimental testing. After surgery, all antidiabetic medications were discontinued except in one subject, who still received metformin. Written informed consent was obtained from all participants, and the study was approved by the Municipal Ethics Committee of Copenhagen in accordance with the Helsinki-II declaration and by the Danish Data Protection Agency, and registered at www.clinicaltrials.gov (ID NCT01993511).

Study design. On separate days, an IVGTT and a mixed-meal test (MMT) were performed before and 1 wk and 3 mo after RYGB. The order of the tests was random. Moreover, OGTTs were performed before and 3 mo after surgery. On all examination days, participants were admitted to the ward in the morning after a 10- to 12-h fast and were weighed and placed in a reclined position in a hospital bed, allowing no strenuous activity. One (OGTT, MMT) or two (IVGTT) catheters were placed in antecubital veins.

IVGTT. An intravenous bolus of 0.5 g/ml glucose solution was injected over 60 s (0.3 g glucose/kg body wt). Blood for measurements of glucose, insulin, and C-peptide was sampled at baseline and frequently following the glucose bolus for a total of 3 h (1). An intravenous bolus (0.03 U/kg in NGT, 0.05 U/kg in IGT/T2D) of insulin (Actrapid; Novo Nordisk, Bagsværd, Denmark) was administered at t = 20 min. Insulin was dissolved in saline, to which was added blood from the participant.

MMT. A liquid meal consisting of 200 ml of Fresubin Energy Drink [300 kcal, carbohydrate (E% 49), protein (E% 16), fat (E% 35), Fresenius Kabi Deutschland, Bad Homburg, Germany] was consumed over a 30-min period. Ingestion was supervised to ensure even distribution of meal intake over the full 30-min period. Blood for measurements of glucose, insulin, C-peptide, glucagon, GLP-1, and GIP (glucose-dependent insulinnotropic polypeptide) was sampled in triplicate at fasting and frequently following the meal for a total of 3 h.

OGTT. Participants ingested 75 g of glucose dissolved in 250 ml of water in under 5 min. Blood for measurements of glucose, insulin, and C-peptide was sampled at baseline and every 30 min during the 2-h test. The test was well tolerated after surgery, except for mild degrees of nausea during the first hour.

Surgical procedure. Operations were performed at the Department of Surgical Gastroenterology, Hvidovre Hospital (Copenhagen, Denmark), using a standard laparoscopic RYGB technique as previously described (31).

Postoperative diet. A total energy intake of ~1,200 kcal was recommended for the first months after surgery, with an energy...
distribution of carbohydrate, protein, and fats of ~45, ~27, and ~28%, respectively. However, it is our experience that patients consume considerably fewer calories for some time after RYGB. Liquid meals were recommended in the first postoperative week, after which the diet was changed to pureed foods until solid foods were allowed after 4 wk.

Sample collection and analytic procedure. Blood was collected into clot activator tubes for insulin and C-peptide analysis, prechilled EDTA tubes containing a DPP-4 inhibitor (valine-pyrrolidide, 0.01 mmol/l, final concentration) for glucagon, GLP-1, and GIP analysis, and EDTA-Eppendorf tubes for glucose analysis. Clot activator tubes were left to coagulate for 30 min, whereas EDTA tubes were cooled on ice and centrifuged at 4°C. EDTA-Eppendorf tubes were immediately centrifuged and analyzed for P-glucose, using YSI model 2300 STAT plus (YSI, Yellow Springs, OH). All other samples were frozen immediately and stored at −80°C until analysis, except for glucagon, GLP-1, and GIP, which were stored at −20°C. Serum C-peptide and insulin concentrations were determined by Immulite 2000 analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY). Hb A1c was measured using high-pressure liquid chromatography (Tosoh Bioscience, Tokyo, Japan). Glucagon, total GLP-1, and GIP were analyzed as previously described (31).

Values are means ± SE. AIRg, DLins, first-phase ISR, GEZI, Sg, and S, were logarithmically transformed due to skewed distribution for better fit to the linear mixed-effects model. AIRg, acute insulin response to iv glucose; GEZI, glucose effectiveness at zero insulin; DI, disposition index; Sg, glucose effectiveness at basal insulin; S, insulin sensitivity index; ∆P-glucose-2min, incremental glucose concentration within the first 2 min after iv glucose injection. *P < 0.05, **P < 0.01 for the change from preoperative level within the group (post hoc estimates from mixed-effects model). † P < 0.05, †† P < 0.01 for differences between T2D and IGT or T2D and NGT at a given study session (post hoc unpaired t-test).

Figure 1. P-glucose (A), S-insulin (B), and insulin secretion rate (ISR; C) in response to an IVGTT in patients with type 2 diabetes (T2D; left), impaired glucose tolerance (IGT; middle) and normal glucose tolerance (NGT; right) before (solid line, filled •) and 1 wk (dotted line, •) and 3 mo (solid line, □) after Roux-en-Y gastric bypass (RYGB). Values are means ± SE.

Table 2. Insulin secretion, insulin sensitivity, and glucose effectiveness following an intravenous glucose tolerance test before and 1 wk and 3 mo after Roux-en-Y gastric bypass.

<table>
<thead>
<tr>
<th></th>
<th>Type 2 Diabetes</th>
<th>IGT</th>
<th>NGT</th>
<th>ANOVA</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>1 wk</td>
<td>3 mo</td>
<td>Before</td>
</tr>
<tr>
<td>Number (m/f)</td>
<td>10 (4/6)</td>
<td>9 (4/5)</td>
<td>8 (3/5)</td>
<td>7 (1/6)</td>
</tr>
<tr>
<td>Days from surgery</td>
<td>−5 ± 2</td>
<td>7 ± 1</td>
<td>100 ± 6</td>
<td>−5 ± 2</td>
</tr>
<tr>
<td>∆P-glucose-2min/mmol/l</td>
<td>13.0 ± 0.6</td>
<td>13.4 ± 0.8</td>
<td>12.5 ± 1.2</td>
<td>12.4 ± 0.6</td>
</tr>
<tr>
<td>AIRg, pmol/l × min</td>
<td>508 ± 85</td>
<td>904 ± 239</td>
<td>1095 ± 202**</td>
<td>1782 ± 4251</td>
</tr>
<tr>
<td>Sg, (mU/l)</td>
<td>1.96 ± 0.28</td>
<td>1.71 ± 0.19</td>
<td>2.54 ± 0.28**</td>
<td>2.31 ± 0.42</td>
</tr>
<tr>
<td>GEZI, 106/min</td>
<td>1.08 ± 0.09</td>
<td>1.12 ± 0.10</td>
<td>1.15 ± 0.14</td>
<td>1.34 ± 0.15</td>
</tr>
<tr>
<td>Si, (mU/l)</td>
<td>0.65 ± 0.11</td>
<td>0.82 ± 0.14</td>
<td>0.88 ± 0.14</td>
<td>1.05 ± 0.16</td>
</tr>
</tbody>
</table>
**β-cell function and glucose effectiveness after RYGB**

**Calculations.** Bergman’s minimal model (MINMOD Millennium 6.02 software; MinMod Millennium, Pasadena, CA) was used for analysis of IVGTT data to estimate sensitivity of glucose elimination to insulin (Si) as well as glucose-dependent glucose disposal at basal insulin (S0) and zero insulin (GEZI = S0 – S1 × basal insulin) (1, 5). Glucose values from time 0–6 min were weighted zero (5, 21). All other time points were set to a weight of 1, except in 12 of the 68 IVGTT analyses, in which weighting of certain glucose values was adjusted for better fit to the model. Fasting glucose concentration was used as basal value (21). HOMA-S and HOMA-β were obtained using the HOMA2 calculator (available at www.dtu.ox.ac.uk/homa), using fasting concentrations of glucose and insulin or C-peptide, respectively. Si primarily reflects skeletal muscle and fat tissue insulin sensitivity (37), whereas HOMA-S to a larger extent represents hepatic insulin sensitivity (39, 55). To circumvent the confounding changes in hepatic insulin clearance, prehepatic insulin secretion rates (ISR) were calculated from C-peptide by deconvolution, using the ISIC software program (settings: obese subjects, CV 4%; basal measurement feature switched on) (23). Total area under the curve (tAUC) was calculated using the trapezoidal rule and incremental AUCs (iAUC) by subtracting fasting levels. Positive iAUC (piAUC) (tAUC) was calculated using the trapezoidal rule and incremental changes in hepatic insulin clearance, prehepatic insulin secretion rates (first-phase ISR) and insulin responses to glucose (AIRg) were calculated as piAUC from 0–10 min of ISR and insulin, respectively. Early insulin secretory responses from the OGTTs and MMTs were normalized to the glycemic stimulus by calculating insulinogetic indexes (IGI = ΔISR0–30min/Δglucose0–30min, IGI MMT = ΔISR0–60min/Δglucose0–60min), using different time intervals due to the differences in ingestion times (i.e., OGTT <5 min and MMT 30 min) (42). Disposition indexes (DI) were calculated by multiplying first-phase ISR or IGI with Si (DIiv or DImmt/DoGTT, respectively) (8, 13, 28). Insulin clearance during fasting (Clfasting) and throughout the meal (ClMGMT) was calculated as the ratio of ISR to insulin concentration in the fasting state and iAUCISR/iAUCinsulin for the meal test, respectively (11).

**Statistical analysis.** Data are expressed as means ± SE. Data were analyzed by ANOVA in a linear mixed effects model, using time from surgery and group as fixed effects and individual subjects as random effect. Logarithmic transformation was used if distribution was skewed. Post hoc comparisons of group differences at a given study point were performed using unpaired t-tests. P < 0.05 was considered significant. All analyses were performed in R version 2.12.2 (www.R-project.org).

**RESULTS**

**Body weight and fasting values.** Body weight and BMI were comparable between groups before RYGB and decreased significantly 1 wk postoperatively to similar extents in all three groups, with further significant reductions at 3 mo (Table 1). Total weight loss was ~15%. Preoperatively, Hb A1c and fasting glucose concentration reflected glucose tolerance status. Fasting glucose decreased significantly 1 wk after surgery, with the largest reduction in patients with type 2 diabetes (P = 0.021 and P = 0.007 vs. IGT and NGT groups, respectively). A further significant reduction was observed after 3 mo in the T2D group, whose levels were now similar to those of IGT and NGT subjects. Hb A1c decreased at 3 mo in the T2D (P < 0.001) and IGT (P = 0.017) groups, reaching values <42 mmol/mol (<6%) in all 10 patients with type 2 diabetes. HOMA-S and fasting insulin clearance increased significantly

![Fig. 2. β-Cell function and disposition index in patients with T2D (left), IGT (middle), and NGT (right) before and 1 wk and 3 mo after RYGB assessed by IVGTT (A), mixed meal (B), and OGTT (C). Values are means ± SE. *P < 0.05, **P < 0.01 vs. baseline; †P < 0.05; ††P < 0.01 vs. T2D.](http://ajpendo.physiology.org/)

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at 1 wk and further at 3 mo. HOMA-β was improved by 26% at 1 wk ($P < 0.001$) and by 32% at 3 mo ($P < 0.001$) in patients with type 2 diabetes but was unchanged in the IGT group and decreased in NGT subjects at 3 mo ($P < 0.001$).

A transient increase in fasting glucagon was observed at 1 wk in the T2D ($P = 0.015$) and NGT ($P = 0.010$) groups, whereas fasting GLP-1 and GIP remained unchanged.

**IVGTT.** Preoperatively, insulin sensitivity ($S_I$) and glucose effectiveness ($S_g$ and GEZI) were not significantly different between groups. $S_I$ was unchanged at 1 wk but improved by 30% at 3 mo ($P = 0.015$, $P = 0.068$, and $P = 0.019$ in T2D, IGT, and NGT groups, respectively). $S_g$ was unchanged in the T2D and IGT groups but decreased by ~40% in NGT subjects ($P = 0.005$ at 1 wk and $P = 0.023$ at 3 mo). GEZI did not change significantly in any group (Table 2 and Figs. 1 and 2).

Before RYGB, first-phase $S_{IRG}$ and AIRg, as well as DI$_{IV}$, were severely impaired in patients with type 2 diabetes; ~85% lower than NGT subjects (first-phase $S_{IRG}$ $P = 0.001$, AIRg $P = 0.010$, DI$_{IV}$ $P = 0.010$). In the T2D group, first-phase $S_{IRG}$ doubled after 1 wk ($P < 0.001$), with a significant further enhancement at 3 mo (1 wk vs. 3 mo, $P = 0.031$). AIRg showed less pronounced improvements (pre vs. 1 wk, $P = 0.084$; pre vs. 3 mo, $P < 0.001$). Neither first-phase $S_{IRG}$ nor AIRg was normalized in the T2D group (T2D 3 mo vs. NGT pre: $P = 0.008$ and $P = 0.024$, respectively). At 3 mo, first-phase $S_{IRG}$ and AIRg in patients with type 2 diabetes remained ~55% lower than in the NGT group ($P < 0.001$). DI$_{IV}$ in the T2D group increased by 50% at 1 wk ($P = 0.006$) and threefold at 3 mo ($P < 0.001$) but remained significantly lower than that of IGT and NGT subjects.

First-phase $S_{IRG}$ tended to increase in the IGT group (pre vs. 1 wk, $P = 0.112$; pre vs. 3 mo, $P = 0.055$) and was unchanged in NGT subjects (pre vs. 1 wk, $P = 0.210$; pre vs. 3 mo, $P = 0.731$). DI$_{IV}$ improved markedly in subjects with IGT at 3 mo ($P = 0.015$) but remained unchanged in the NGT group.

Notably, the incremental iv glucose stimulus applied in the test ($\Delta$-glucose$_{0-2 \text{ min}}$) was comparable between groups and sessions, averaging 12.1 mmol/l (SD 3.3).

**MMT.** Overall glucose levels during the meal (IAUC glucose) decreased significantly after 3 mo in all three groups ($P < 0.001$, $P = 0.002$, and $P = 0.026$ in the T2D, IGT, and NGT groups, respectively). Postoperatively, glucose concentrations peaked faster and with higher increments and returned to baseline more rapidly (Table 3 and Figs. 2 and 3).

After RYGB, there was a brisk rise in ISR with significantly higher peak values in response to meal ingestion. Total insulin secretion (IAUC$_{ISR}$) increased considerably at 1 wk and 3 mo regardless of glucose tolerance. After 1 wk, IGI$_{MMT}$ improved by 55% in patients with type 2 diabetes ($P = 0.016$). At 3 mo, IGI$_{MMT}$ was enhanced by 90, 45, and 55% in the T2D ($P < 0.001$), IGT ($P = 0.121$), and NGT ($P = 0.005$) groups, respectively. DI$_{MMT}$ increased significantly at 3 mo by ~150% in the T2D group and 100% in IGT and NGT subjects. In the T2D group, IGI$_{MMT}$ and DI$_{MMT}$ remained significantly reduced at 3 mo compared with NGT and IGT subjects, and IGI$_{MMT}$ was not normalized (T2D 3 mo vs. NGT pre, $P = 0.008$). Postprandial insulin clearance (CI$_{MMT}$) increased only in the T2D group.

After RYGB, postprandial GLP-1 secretion was exaggerated, with fivefold increased peak concentration and ninefold increased iAUC. Preoperatively, glucagon secretion was suppressed in response to the meal (negative iAUC), but after surgery the postprandial glucagon response was altered, although it was unchanged within the first week in the T2D group. Total GIP secretion was unchanged.

### Table 3. Glucose excursions and insulin, glucagon, GLP-1, and GIP secretion in response to a liquid meal before and 1 wk and 3 mo after Roux-en-Y gastric bypass

<table>
<thead>
<tr>
<th></th>
<th>Type 2 Diabetes</th>
<th>IGT</th>
<th>NGT</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number (m/l)</strong></td>
<td>Before</td>
<td>1 wk</td>
<td>3 mo</td>
<td></td>
</tr>
<tr>
<td>tAUC glucose, mmol/l</td>
<td>158 ± 84</td>
<td>140 ± 59**</td>
<td>117 ± 26**</td>
<td></td>
</tr>
<tr>
<td>mmol/l × min</td>
<td>261 ± 27</td>
<td>304 ± 45</td>
<td>227 ± 21</td>
<td></td>
</tr>
<tr>
<td>Peak glucose, mmol/l</td>
<td>10.4 ± 0.5</td>
<td>10.8 ± 0.3</td>
<td>10.3 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Time to peak glucose,</td>
<td>72 ± 7</td>
<td>60 ± 4.4**</td>
<td>47 ± 3.3**</td>
<td></td>
</tr>
<tr>
<td>min</td>
<td>5.6 ± 6.6</td>
<td>60 ± 6</td>
<td>45 ± 9**</td>
<td></td>
</tr>
<tr>
<td>IAUC insulin × 10^7,</td>
<td>344 ± 24</td>
<td>494 ± 36*</td>
<td>413 ± 48</td>
<td></td>
</tr>
<tr>
<td>pmol/l × min</td>
<td>565 ± 53</td>
<td>861 ± 76**</td>
<td>778 ± 74*</td>
<td></td>
</tr>
<tr>
<td>Peak ISR, pmol/kg</td>
<td>8.3 ± 3.7</td>
<td>13.3 ± 10.1**</td>
<td>14.6 ± 11.1**</td>
<td></td>
</tr>
<tr>
<td>ISR, pmol/kg/min</td>
<td>895 ± 120</td>
<td>1223 ± 183**</td>
<td>896 ± 248</td>
<td></td>
</tr>
<tr>
<td>CI$_{MMT}$ × 10^7,</td>
<td>17 ± 1.0</td>
<td>2.2 ± 0.1**</td>
<td>2.0 ± 0.1**</td>
<td></td>
</tr>
<tr>
<td>pmol/l × min</td>
<td>459 ± 182</td>
<td>492 ± 817**</td>
<td>407 ± 471**</td>
<td></td>
</tr>
<tr>
<td>Peak GLP-1, pmol/l</td>
<td>12 ± 7.4</td>
<td>8.8**</td>
<td>69 ± 6.6</td>
<td></td>
</tr>
<tr>
<td>CI$_{GLP-1}$ × 10^6,</td>
<td>4864 ± 650</td>
<td>4677 ± 409</td>
<td>4296 ± 1434</td>
<td></td>
</tr>
<tr>
<td>pmol/l × min</td>
<td>65 ± 10</td>
<td>70 ± 6.8</td>
<td>71 ± 8.6</td>
<td></td>
</tr>
<tr>
<td>iAUC glucagon, pmol/l</td>
<td>-62 ± 8.4</td>
<td>14 ± 97</td>
<td>185 ± 76*</td>
<td></td>
</tr>
<tr>
<td>CI$_{glucagon}$ × 10^6,</td>
<td>15 ± 2</td>
<td>18 ± 2</td>
<td>15 ± 2</td>
<td></td>
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</tbody>
</table>

Values are means ± SE. DI$_{MMT}$ and IGI were logarithmically transformed due to skewed distribution for better fit to the linear mixed-effects model. IGI, insulinoenic index; CI$_{MMT}$, insulin clearance in MMT; IAUC, incremental area under the curve; MMT, mixed-meal test; tAUC, total AUC. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (between groups) and †$P < 0.05$. ††$P < 0.01$ for change from preoperative level within the group (post hoc estimates from mixed-effects model); ‡$P < 0.05$. †††$P < 0.01$ for differences between T2D and IGT or T2D and NGT at a given study session (post hoc unpaired t-test).
OGTT. three months after RYGB, glucose concentrations in response to the OGTT peaked faster, but the 2-h value was markedly reduced in all three groups. The tAUC and iAUC of glucose decreased significantly only in the T2D group (Table 4 and Figs. 2 and 4). ISR in response to the OGTT peaked earlier and reached higher levels after RYGB. In all three groups, iAUCISR increased significantly, and IGIOGTT improved by 115, 70, and 20% in the T2D (P < 0.004), IGT (P < 0.009), and NGT (P < 0.051) groups, respectively. DIOGTT increased significantly, by 185, 120, and 68% in the T2D, IGT, and NGT groups, respectively. In the T2D group, IGIOGTT and DIOGTT remained significantly reduced at 3 mo compared with NGT subjects, and IGIOGTT was not normalized (T2D 3 mo vs. NGT pre, P < 0.048).

DISCUSSION

In the present study, we have performed an extensive evaluation of changes in glucose metabolism in the fasting state and in response to iv glucose and two different oral tests in patients with type 2 diabetes and impaired, as well as normal, glucose tolerance 1 wk and 3 mo after RYGB. Insulin clearance and hepatic insulin sensitivity (HOMA-S) increased already at 1 wk, whereas peripheral insulin sensitivity (Si) improved at 3 mo, consistent with results obtained using the hyperinsulinemic clamp (4). We could also confirm previous findings of enhanced insulin and GLP-1 secretion postoperatively in response to orally ingested glucose, as well as mixed meals, regardless of glucose tolerance (4, 15, 30, 31, 33, 34, 41). A novel finding of the present study is the demonstration of an early enhancement of first-phase insulin secretion to iv glucose within 1 wk after surgery in patients with type 2 diabetes, before any major weight loss. Previous studies using IVGTTs have reported gradually increased first-phase insulin secretion in patients with type 2 diabetes in the first year after RYGB, attributed to weight loss (10, 18, 25, 36, 41, 43, 48). One study performed an IVGTT within the first week after RYGB and did not report significantly improved AIRg in patients with type 2 diabetes (48). However, insulin and not C-peptide measurements had been used. Since we, in line with other studies, have demonstrated enhanced insulin clearance already 1 wk after RYGB, insulin secretion may be underestimated postoperatively when evaluated from peripheral insulin concentrations instead of insulin secretion rates or C-peptide (3, 4). This was clearly illustrated in the present study by the less pronounced improvement of AIRg compared with first-phaseAIR. Two RYGB studies, one of which used the glucose-glucagon test and the other the hyperglycemic clamp, could also not demonstrate acute or long-term changes in first-phase insulin release in patients with type 2 diabetes despite using C-peptide measurements (4, 33). Glucagon injection may activate other triggering pathways in β-cells (45), and the insulin response elicited is known to be potentiated by the ambient fasting glucose levels, which decrease after RYGB (19, 45).

Fig. 3. P-glucose (A), S-insulin (B), and ISR (C) in response to a liquid mixed meal in patients with T2D (left), IGT (middle), and NGT (right) before (solid line, •), and 1 wk (dotted line, △) and 3 mo (solid line, ●) after RYGB. Values are means ± SE.
The inconsistent findings compared with the study performing a hyperglycemic clamp may be explained by differences in preoperative characteristics of the study populations (33).

In addition to enhanced first-phase insulin release, we observed an early improvement of HOMA-β in patients with type 2 diabetes. This is an important aspect of β-cell function, especially with regard to its role in regulating hepatic glucose production (13, 55). In contrast to β-cell indexes obtained by oral testing, both first-phase $\text{ISR}_{\text{IR}}$ and HOMA-β can be regarded as independent of gastrointestinal regulation, reflecting the “true” function of the β-cells (intrinsic β-cell function). Thus, the findings in this study support the hypothesis that RYGB has an early beneficial effect on β-cells per se. Based on its known relationship with fasting glucose concentrations (7), the observed improvement in first-phase insulin secretion may be explained by the acute postprandial decrease in fasting glucose levels due to lower hepatic glucose production (4). The resulting immediate relief of glucotoxicity likely contributes to the enhanced postprandial insulin secretion observed in this and other studies. However, the pronounced postoperative calorie restriction, which likely causes the improved hepatic insulin sensitivity (35), may also enhance insulin secretion by decreasing pancreatic triacylglycerol stores, as observed within 8 wk after dietary energy restriction alone (35). Three months after RYGB, first-phase insulin responses in the type 2 diabetes group improved by 185% but were still only 45% of the responses in NGT subjects despite similar fasting glucose. Notably, neither oral nor iv indexes of β-cell function were normalized in patients with type 2 diabetes despite substantial improvements of glycemic control, highlighting the importance of other contributing factors for remission of type 2 diabetes after RYGB, i.e., increased hepatic and peripheral insulin sensitivity as well as other possible mechanisms not assessed in the present study. Thus, the results demonstrate a reversible component of β-cell dysfunction but also an underlying deficit of β-cell mass and/or secretory capacity in patients with type 2 diabetes (9). It is less likely that reduced lipotoxicity plays a major role in the early metabolic improvements after RYGB, since plasma levels of free fatty acids have been reported to increase immediately after surgery (4, 27). However, in the longer-term, weight loss will improve insulin sensitivity in adipose tissue and decrease circulating free fatty acids, which may have additional beneficial effects on insulin secretion (4, 14). In line with previous findings, we did not observe improved intrinsic β-cell function in normoglycemic individuals, whose β-cells by definition are not exposed to a glucotoxic environment, further supporting the proposed role of glucotoxicity (18, 36, 41, 43, 48, 52). First-phase insulin secretion increased nonsignificantly in the IGT group. Unchanged $\text{AIR}_{\text{IR}}$ in subjects with IGT has been reported previously (41), but one study did report an improvement 7 mo after RYGB in patients with prediabetes, defined specifically as impaired fasting glucose (18), again supporting the concept that fasting glucose is important for regulation of first-phase insulin release.

The present study also illustrates convincingly that RYGB surgery leads to faster, higher, and more transient postprandial elevations of glucose, insulin, and ISRs as well as exaggerated GLP-1 and glucagon levels (26). This phenomenon is explained by an accelerated transit of food to the distal intestine resulting in faster nutrient absorption and enhanced stimulation of distal gut epithelium (15, 16). Increased postprandial glucagon levels, a common finding after RYGB (4, 30, 31, 34), are paradoxical, given the high postprandial levels of glucose, insulin, and GLP-1, all known to suppress glucagon release. As demonstrated in a recent study, the increase represents intact, fully processed glucagon, and one explanation for the paradoxical increase may be that it is secreted from the distal small intestine due to the excess postoperative nutrient stimulation (57). The exaggerated GLP-1 levels contribute importantly to increased postprandial insulin secretion after RYGB, indicated by the enhanced insulin response to oral, but not iv, stimulation.

### Table 4. Glucose excursions and insulin secretion in response to an OGTT before and 3 mo after Roux-en-Y gastric bypass

<table>
<thead>
<tr>
<th>Time Group T x G</th>
<th>Time Group T x G</th>
<th>Time Group T x G</th>
<th>Time Group T x G</th>
<th>Time Group T x G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to peak glucose, min</td>
<td>Time to peak glucose, min</td>
<td>Time to peak glucose, min</td>
<td>Time to peak glucose, min</td>
<td>Time to peak glucose, min</td>
</tr>
<tr>
<td>81 ± 6</td>
<td>363 ± 26</td>
<td>631 ± 48</td>
<td>114 ± 4</td>
<td></td>
</tr>
<tr>
<td>54 ± 6*</td>
<td>452 ± 78</td>
<td>980 ± 120</td>
<td>48 ± 8**</td>
<td></td>
</tr>
<tr>
<td>78 ± 12</td>
<td>349 ± 85</td>
<td>719 ± 147</td>
<td>102 ± 18</td>
<td></td>
</tr>
<tr>
<td>54 ± 11*</td>
<td>453 ± 89</td>
<td>1236 ± 234*</td>
<td>72 ± 15</td>
<td></td>
</tr>
<tr>
<td>52 ± 8*</td>
<td>485 ± 51</td>
<td>901 ± 76</td>
<td>63 ± 11</td>
<td></td>
</tr>
<tr>
<td>46 ± 6</td>
<td>589 ± 90</td>
<td>1203 ± 120*</td>
<td>55 ± 9</td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>0.04</td>
<td>0.28</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Values are means ± SE. DI$<em>{\text{OGTT}}$ and IGI$</em>{\text{OGTT}}$ were logarithmically transformed due to skewed distribution for better fit to the linear mixed-effects model. *$P &lt; 0.05$. **$P &lt; 0.01$ for change from preoperative level within the group (post hoc estimates from mixed-effects model). †$P &lt; 0.05$. ††$P &lt; 0.01$ for differences between T2D and IGT or T2D and NGT at a given study session (post hoc unpaired t-test).</td>
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</table>
in the IGT and NGT groups, and clearly demonstrated in studies using pharmacological blockade of the GLP-1 receptor (29, 30, 51, 54). It could be argued that the increased postprandial GLP-1 levels during the days preceding the IVGTT could in part explain the enhanced first-phase ISR and AIRg after RYGB, since pharmacological administration of GLP-1 or GLP-1 agonists have been shown to restore both first- and second-phase insulin secretion to iv glucose in patients with type 2 diabetes (17). However, experiments indicate absence of a memory effect of the insulinotropic actions of GLP-1 (40, 49). Furthermore, a similarly improved intrinsic \textit{β}-cell function was demonstrated in studies comparing RYGB patients with matched subjects treated with hypocaloric diet, who do not experience enhanced GLP-1 secretion (25). In the context of reduced glucotoxicity, it is likely that RYGB not only sensitizes \textit{β}-cells to glucose but that a positive forward cycle is initiated, resulting in improved insulinotropic actions of GLP-1 and GIP, contributing further to enhanced insulin secretion after meal intake but not in response to iv glucose (14, 24). As expected, a similar mechanism cannot be demonstrated in normoglycemic subjects (15).

Similar to \textit{β}-cell function, GE in patients with type 2 diabetes may be improved by periods of intensive glycemic regulation achieved by insulin therapy (22). However, whole body GE did not increase significantly in the present study. The lack of improvement in patients with type 2 diabetes may have been due to the relatively short duration of the disease (median 2 yr) and good glycemic regulation prior to RYGB (Hb A1c =45 mmol/mol), making them less susceptible to glucotoxicity. This may also explain why GE preoperatively was not significantly lower in patients with type 2 diabetes compared with NGT subjects. However, the study was powered to detect postoperative changes within groups, and minor differences between groups may not have reached significance. Another limitation of the study is that patients were included after a preoperative weight loss of 8%, likely to have improved several metabolic parameters. Thus, the magnitude of postoperative metabolic improvements probably would have been greater if participants had not been subjected to the preoperative diet and weight loss. Last, it is worth noting that the study does not evaluate the role of glucotoxicity per se but rather describes the natural history of glucose metabolism after RYGB.

In conclusion, RYGB has early beneficial effects on \textit{β}-cell function per se in patients with type 2 diabetes, reflected in enhanced, but not normalized, first-phase insulin secretion to iv glucose and increased HOMA-\textit{β}. These metabolic changes may, in addition to increased insulin sensitivity and exaggerated postprandial GLP-1 levels, contribute to improved glycemic control postoperatively. A major role for increased glucose effectiveness after RYGB was not supported by this study.

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Fig. 4. P-glucose (A), S-insulin (B), and ISR (C) in response to an OGTT in patients with T2D (left), IGT (middle), and NGT (right) before (solid line, •) and 3 mo (solid line, □) after RYGB. Values are means ± SE.
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Denmark) as well as Lene Bruus Albrek and Sofie Pilegaard (Department of Biomedical Sciences, the Panum Institute, University of Copenhagen, Denmark).

Trial registration: ClinicalTrials.gov ID NCT01993511.

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