Acute and long-term effects of Roux-en-Y gastric bypass on glucose metabolism in subjects with Type 2 diabetes and normal glucose tolerance

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Jørgensen NB, Jacobsen SH, Dirksen C, Bojesen-Møller KN, Naver L, Hvolris L, Clausen TR, Wulff BS, Worm D, Hansen DL, Madsbad S, Holst JJ. Acute and long-term effects of Roux-en-Y gastric bypass on glucose metabolism in subjects with Type 2 diabetes and normal glucose tolerance. Am J Physiol Endocrinol Metab 303: E122–E131, 2012. First published April 24, 2012; doi:10.1152/ajpendo.00073.2012.—Our aim was to study the potential mechanisms responsible for the improvement in glucose control in Type 2 diabetes (T2D) within days after Roux-en-Y gastric bypass (RYGB). Thirteen obese subjects with T2D and twelve matched subjects with normal glucose tolerance (NGT) were examined during a liquid meal before (Pre), 1 wk, 3 mo, and 1 yr after RYGB. Glucose, insulin, C-peptide, glucagon-like peptide-1 (GLP-1), glucagon-dependent-insulinotropic polypeptide (GIP), and glucagon concentrations were measured. Insulin resistance (HOMA-IR), β-cell glucose sensitivity (β-GS), and disposition index (DIβ-GS = β-GS × 1/HOMA-IR) were calculated. Within the first week after RYGB, fasting glucose [T2D Pre: 8.8 ± 2.3, 1 wk: 7.0 ± 1.2 (P < 0.001)], and insulin concentrations decreased significantly in both groups. At 129 min, glucose concentrations decreased in T2D [Pre: 11.4 ± 3, 1 wk: 8.2 ± 2 (P = 0.003)] but not in NGT. HOMA-IR decreased by 50% in both groups. β-GS increased in T2D [Pre: 1.03 ± 0.49, 1 wk: 1.70 ± 1.2, (P = 0.012)] but did not change in NGT. The increase in DIβ-GS was 3-fold in T2D and 1.5-fold in NGT. After RYGB, glucagon secretion was increased in response to the meal. GIP secretion was unchanged, while GLP-1 secretion increased more than 10-fold in both groups. The changes induced by RYGB were sustained or further enhanced 3 mo and 1 yr after surgery. Improvement in glycemic control in T2D after RYGB occurs within days after surgery and is associated with increased insulin sensitivity and improved β-cell function, the latter of which may be explained by dramatic increases in GLP-1 secretion.

glucagon-like-peptide-1; incretins; β-cell function; insulin sensitivity; glucagon

Besides obesity, Type 2 Diabetes (T2D) pathophysiology comprises several other components, including hepatic and muscle insulin resistance, decreased β-cell mass and function, and abnormal glucagon secretion. The incretin effect exerted by glucagon-like-peptide-1 (GLP-1) and glucagon-dependent insulinotropic polypeptide (GIP), which is responsible for one-half to two-thirds of the insulin secretion during a meal in normal healthy subjects, is also severely impaired (3, 8, 34, 36, 46). Weight loss will ameliorate many of these defects in subjects with T2D, but maintaining a weight loss and the resultant improvements in glycemic control are difficult over years (49).

Bariatric surgery has proven effective in inducing a weight loss, and the procedure most often used, Roux-en-Y gastric bypass (RYGB), produces sustained mean weight losses of 30–40% (47). Such weight losses have profound effects on glucose metabolism in subjects with T2D, and T2D remission rates have been reported as high as 75–85% (1, 32, 47). Surprisingly, in most subjects with T2D, the improvement in glycemic control occurs within days after surgery and before any major weight loss (41, 45). The mechanisms responsible for this early improvement in glycemic control are still incompletely understood.

Using a mixed-meal test, Isbell et al. (18) found increased insulin sensitivity and an unchanged insulin secretion in spite of an increased GLP-1 response in a mixed group of subjects with T2D or normal glucose tolerance (NGT) within 1 wk after RYGB. Le Roux and colleagues (42) examined acute effects of RYGB in subjects with T2D and with NGT and found the insulin response 15 min after a meal to be increased already at 2 days after surgery, but because changes in glucose profiles were not reported in this study, evaluating β-cell function is difficult (42). Kashyap et al. (20) reported increased insulin sensitivity, as well as improved β-cell function 1 mo after RYGB during a mixed-meal test, but at a time when patients had already lost 11% of their preoperative body weight (20). Other investigators have evaluated insulin sensitivity and β-cell function using oral or intravenous tests, and it seems that peripheral insulin sensitivity and β-cell function increase with time from surgery, in parallel with weight loss, but these studies do not address the changes taking place within the first week after surgery (5, 6, 27, 28, 30, 33).

In the present study, we describe the changes in plasma glucose, insulin, and C-peptide concentrations, prehepatic insulin secretion rates (ISR), and gut hormone concentrations in the fasting state and during a mixed-meal in a well-defined group of subjects with T2D within the first week after RYGB, at a time when weight loss is minimal. We evaluate the effect of RYGB on β-cell glucose sensitivity (β-GS), and we compare the results to a matched group of subjects with NGT also undergoing RYGB. In addition, results from follow-up studies carried out 3 mo and 1 yr after surgery are reported.

METHODS

Subjects. Subjects with T2D and with NGT were recruited from the Hvidovre Hospital bariatric surgery program (Hvidovre, Denmark). All patients had accomplished a preoperative 8% diet-induced total body
weight loss before inclusion and met the criteria for bariatric surgery: >20 years old and a body mass index (BMI) > 40 or > 35 kg/m^2 with Type 2 diabetes or hypertension. Subjects were excluded if they had been treated with incretin-based therapies or insulin, thyroid hormone substitution therapy, antithyroid medication, or anorectics within 3 mo prior to experiments. Written informed consent was obtained from all participants, and the study was approved by the Municipal Ethical Committee of Copenhagen in accordance with the Helsinki-II declaration and by the Danish Data Protection Agency, and it was registered at wwwclinicaltrials.gov (ClinicalTrials.gov ID NCT002808122).

NGT was assumed, if participants at the time of entry into the bariatric surgery program and before the 8% preoperative weight loss, had a fasting plasma glucose <6.1 mM and 2 h plasma glucose of <7.8 mM after a 75-g oral glucose load.

A diagnosis of T2D was accepted for participants treated with ≥1 antidiabetic agent, who presented with a fasting plasma glucose of >7.0 mM on the first day of experiments before the operation. An oral glucose tolerance test (OGTT), 2 wk before surgery, was used to confirm the diagnosis of T2D (2 h plasma glucose of ≥11.1 mM) in diet-treated subjects with T2D.

Study design. Subjects were studied before, 1 wk, 3 mo, and 1 yr after RYGB. All antidiabetic medications were paused in individuals with T2D 72 h prior to the preoperative meal test. After surgery, none of the subjects in the T2D group received any antidiabetic medication. On each study day after a 12-h fast, participants were weighed (Tanita, Tokyo, Japan) and then subjected to a liquid meal test consisting of 200 ml of Fresubin Energy Drink [300 kcal, carbohydrate (E% 50), protein (E% 15), fat (E% 35), Fresenius Kabi Deutschland, Bad Homburg, Germany]. The meal was ingested over a 30-min period before and at all study visits after RYGB to avoid dumping. Subjects were supervised during meal ingestion to ensure that meal intake was equally distributed over the full 30-min period. Blood was sampled from a catheter in an antecubital vein before and frequently following the meal for a total of 4 h (10, −5, 0, 15, 30, 45, 60, 90, 120, 180, and 240 min relative to meal start). During each test, subjects sat in a reclined position in a hospital bed. No strenuous activity was allowed.

Surgical procedure. Surgery was performed at the Department of Gastroenterology at Hvidovre Hospital (Hvidovre, Denmark) by either of two surgeons (L. Naver or L. Hvolis), using a standard laparoscopic RYGB technique, resulting in a gastric pouch with a volume of about 25 ml, a 100-cm-long Roux-limb and a 75-cm-long biliopancreatic limb.

Sample collection and laboratory analyses. Blood was collected into clot activator tubes for insulin and C-peptide analysis, prechilled EDTA tubes containing a DPP-IV inhibitor (valine-pyrrolidine; 0.01 mmol/l, final concentration) for GLP-1, GIP, and glucagon analysis, and prechilled EDTA 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. Plasma glucose was measured with the glucose oxidase technique (YSI model 2300 STAT Plus; Yellow Springs Instruments, Yellow Springs, OH). All other samples were frozen and stored at −80°C until analysis except for GLP-1, GIP, and glucagon, which were stored at −20°C.

Serum insulin and C-peptide concentrations were determined by AutoDELFI fluoroimmunoassay (Wallac OY, Turku, Finland). Plasma samples were assayed for total GLP-1 immunoreactivity, as described previously (38). Total GIP was measured using the COOH-terminally directed antisera R65 (23, 24). The glucagon assay was directed against the COOH-terminus of the glucagon molecule (antibody code no. 4305) and, therefore, measured glucagon of mainly pancreatic origin (37). HbA1c was measured using HPLC with a cation exchange column (Tosoh Bioscience, Tokyo, Japan).

Calculations and statistical analyses. Data are expressed as means ± SD, except for figures, where means ± SE are used.

Total area-under-the-curve (T-AUC) was calculated using the trapezoidal model. Incremental AUC (I-AUC) was calculated as T-AUC minus baseline × 240 min. Positive I-AUC (PI-AUC) was calculated as the AUC above baseline values. PI-AUC was reported when a meal-stimulated increase was observed, whereas I-AUC was reported when a meal-stimulated decrease or a biphasic response was observed.

Insulin resistance was calculated using the homeostasis model assessment of insulin resistance (HOMA-IR) as 𝐼𝑛𝑠𝑢𝑙𝑖𝑛_(fasting) × Glucose(fasting) / (22.5 × 6.945).

Prehepatic ISR were calculated by deconvolution of peripheral C-peptide concentrations and application of population-based parameters for C-peptide kinetics (7) using the ISEC software (17). ISR is expressed as picomoles per kilogram per minute.

To characterize the dose-response relationship between glucose concentration and ISR during the meal, we first identified the time point at which peak glucose concentrations were reached for each subject on each experimental day (tpeak glucose); then we plotted ISR values from time point 0 min to tpeak glucose against the corresponding plasma glucose concentrations. The slopes of these linear relationships were evaluated by cross-correlation analysis, as previously described (21) and expressed as picomoles per kilogram per minute per millimolar, reflecting the change in ISR per millimolar increase in plasma glucose. This was regarded as the β-cell glucose sensitivity (β-GS).

To adjust for different insulin sensitivities both between the groups and before and after RYGB, the individual β-cell glucose sensitivities were related to the ambient HOMA-IR by calculating the disposition index (DI) (β-GS × 1/HOMA-IR).

Statistical analyses were carried out using Wilcoxon matched-pairs signed-rank test for comparing the results within groups. For comparison between groups, the Mann-Whitney U-test or χ²-test was used as appropriate. A P value <0.05 was considered significant. The analyses were carried out using R 2.11.1 statistical software package (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

A total of 30 patients were included in the study. Three subjects did not wish to participate following surgery and two were not operated.

Thirteen subjects with T2D and 12 subjects with NGT were examined before and after RYGB [age: T2D: 52 ± 9 yr; NGT: 43 ± 13 yr (P = 0.2); height: T2D: 1.73 ± 0.08 m; NGT: 1.75 ± 0.07 m (P = 0.7), sex (male/female): T2D: 7/6; NGT: 3/9 (P = 0.4)]. One subject with T2D could not be studied 1 wk post-RYGB because of anemia. One subject with NGT was excluded from the 3 mo follow-up data set, due to excessively high fasting insulin and C-peptide concentrations, indicating a nonfasting state. One subject with NGT was not examined at 1 yr follow-up due to pregnancy.

Twelve subjects with T2D were treated with ≥1 oral antidiabetic medication, and one subject was diet-treated only. All presented with fasting blood glucose concentrations above 7 mM on the first day of experiments, except for the diet-treated subject, who presented with fasting plasma glucose of 6.7 mM. This subject had 2-h plasma glucose level of 11.6 mM following a 75-g OGTT 2 wk prior to the first experiment. The mean time from diagnosis of T2D was 5.4 ± 3.6 yr. Study visit times and group weight, BMI, and HbA1c are given in Table 1.

Weight. Both groups lost weight after RYGB. One week after surgery, the relative weight loss in both groups was small, about 2% of preoperative body weight, but after 1 yr, subjects had lost about 25% of preoperative body weight (T2D: 1 wk: 2.0 ± 1.6% (P < 0.001), 3 mo: 13 ± 3.6% (P < 0.001), 1 yr: 22 ± 8.8% (P < 0.001); NGT: 1 wk: 2.5 ± 1.3% (P < 0.001),
3 mo: 15 ± 4.5% (P < 0.001), 1 yr: 25 ± 8.2% (P < 0.001).

The relative weight loss after RYGB did not differ between the two groups at any postoperative study time, nor did BMI or absolute body weight (Table 1).

**Fasting state.** In the group of subjects with T2D, fasting glucose concentrations decreased 1.9 ± 1.8 mM 1 wk after RYGB (P < 0.001) (Table 2). This decrease was maintained at 3 mo and 1 yr follow-up. In the group of subjects with NGT, fasting glucose concentrations also decreased within the first week following RYGB, but remained unchanged subsequently. Both fasting insulin and C-peptide concentrations were significantly lower within 1 wk after surgery in both groups (Table 2).

Fasting GLP-1 concentrations were significantly elevated by 1 yr in the T2D group. No changes were detected in the NGT group. Concentrations were not different between the groups.

Fasting GIP concentrations were lower immediately after RYGB in subjects with NGT (T2D: P = 0.067, NGT: P = 0.021) but returned to baseline values thereafter.

Fasting glucagon concentrations did not differ within the first week after surgery but were significantly reduced both 3 mo and 1 yr after RYGB in both groups.

**Postprandial state.** Changes in postprandial glucose and hormone responses are reported in Table 3. In the T2D group, peak plasma glucose concentrations decreased and occurred earlier after RYGB [Pre: 83 ± 18 min, 1 wk: 59 ± 12 min (P = 0.008), 3 mo: 49 ± 7 min (P = 0.001), 1 yr: 44 ± 7 min (P = 0.001)] (Fig. 1A). Two-hour postprandial glucose concentrations were lower immediately after surgery, and this effect was even more pronounced 3 mo and 1 yr post-RYGB (Fig. 1A, Table 3). Glucose I-AUC showed a trend toward a decrease immediately after RYGB (P = 0.077) and was significantly decreased 3 mo and 1 yr after surgery.

In the NGT group, peak glucose concentrations increased gradually and were higher 3 mo and 1 yr after surgery (Fig. 1A). Time to peak glucose concentration did not change after RYGB [pre: 45 ± 16 min, 1 wk: 51 ± 8 min (P = 0.28), 3 mo: 42 ± 11 min (P = 0.71), 1 yr: 40 ± 8 min (P = 0.34)]. Two-hour postprandial glucose concentrations were unaltered directly after RYGB and significantly lower 3 mo and 1 yr after surgery (Table 3). Glucose I-AUC increased immediately after RYGB only to return to preoperative levels 3 mo and 1 yr after surgery. I-AUC glucose in subjects with NGT were significantly reduced before and 1 yr after RYGB compared with subjects with T2D.

In the T2D group, peak insulin concentrations increased 3 mo and 1 yr post-RYGB, while peak C-peptide concentrations did not increase significantly (1 wk: P = 0.42, 3 mo: P = 0.16, 1 yr: P = 0.89) (Table 3, Fig. 1, B and C). Time to peak insulin and peak C-peptide concentrations decreased with time from surgery [C-peptide: Pre: 105 ± 43 min, 1 wk: 71 ± 17 min (P = 0.018), 3 mo: 55 ± 7 min (P = 0.003), 1 yr: 55 ± 7 min (P = 0.005)]. PI-AUC C-peptide increased within the first week after surgery, but not 3 mo (P = 0.057) or 1 yr (P = 0.74) after RYGB.

In the NGT group, peak insulin and C-peptide concentrations increased by 3 mo and 1 yr (Fig. 1C). Time to peak insulin concentrations did not change postoperatively, but by 1 yr, peak C-peptide concentrations were reached earlier than before the operation [Pre: 64 ± 25 min, 1 yr: 46 ± 5 min (P = 0.037)]. PI-AUC insulin levels increased by 1 wk and 3 mo, but they had returned to preoperative levels by 1 yr. PI-AUC C-peptide increased 1 wk, 3 mo, and 1 yr after RYGB (Table 3).

In the T2D group, the peak prehepatic insulin secretion rate (ISR) increased 3 mo and 1 yr after RYGB (Table 3, Fig. 2). The ISR response to the meal, PI-AUC ISR, was increased 1 wk and 3 mo following RYGB, but 1 yr after surgery, PI-AUC ISR were not significantly different from preoperative levels (P = 0.094) (Fig. 2, Table 3). In the NGT group, effects of RYGB on peak ISR were similar to that seen in subjects with T2D (Fig. 2, Table 3), whereas PI-AUC ISR remained significantly increased at all postoperative study visits (Table 3).

### Table 2. Fasting glucose and hormone concentrations in subjects with T2D and with NGT before (Pre) and 1 wk, 3 mo, and 1 yr after Roux-en-Y gastric bypass

<table>
<thead>
<tr>
<th>T2D</th>
<th>Pre</th>
<th>1 wk</th>
<th>3 mo</th>
<th>1 yr</th>
<th>T2D</th>
<th>Pre</th>
<th>1 wk</th>
<th>3 mo</th>
<th>1 yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/l</td>
<td>8.8 ± 2.3</td>
<td>7.0 ± 1.2**</td>
<td>6.8 ± 1.6**</td>
<td>6.2 ± 1.6**</td>
<td>5.5 ± 0.6i**</td>
<td>5.0 ± 0.6ii**</td>
<td>4.9 ± 0.4iii**</td>
<td>4.9 ± 0.3iiv**</td>
<td></td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>125 ± 77</td>
<td>73 ± 32**</td>
<td>58 ± 35**</td>
<td>47 ± 27**</td>
<td>82 ± 28</td>
<td>49 ± 14**</td>
<td>43 ± 14**</td>
<td>36 ± 16**</td>
<td></td>
</tr>
<tr>
<td>C-peptide, pmol/l</td>
<td>1483 ± 543</td>
<td>1175 ± 595**</td>
<td>1049 ± 501**</td>
<td>796 ± 345**</td>
<td>1098 ± 227</td>
<td>834 ± 187**</td>
<td>816 ± 191**</td>
<td>602 ± 198**</td>
<td></td>
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<tr>
<td>Total GLP-1, pmol/l</td>
<td>11 ± 3</td>
<td>11 ± 13</td>
<td>10 ± 2</td>
<td>16 ± 7**</td>
<td>10 ± 3</td>
<td>12 ± 6</td>
<td>11 ± 3</td>
<td>12 ± 5</td>
<td></td>
</tr>
<tr>
<td>Total GIP, pmol/l</td>
<td>7 ± 5</td>
<td>4 ± 2</td>
<td>9 ± 5</td>
<td>7 ± 5</td>
<td>8 ± 7</td>
<td>4 ± 4**</td>
<td>6 ± 3</td>
<td>9 ± 3</td>
<td></td>
</tr>
<tr>
<td>Glucagon, pmol/l</td>
<td>13 ± 7</td>
<td>14 ± 5</td>
<td>8 ± 2**</td>
<td>8 ± 4**</td>
<td>10 ± 4</td>
<td>11 ± 3</td>
<td>6 ± 1**</td>
<td>6 ± 2**</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as means ± SD. *P < 0.05, **P < 0.01 compared to baseline. †P < 0.05, ††P < 0.01 compared to T2D.
Table 3. Postprandial glucose and hormone concentrations in subjects with T2D and with NGT before and after RYGB.

<table>
<thead>
<tr>
<th></th>
<th>T2D</th>
<th>NGT</th>
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<tbody>
<tr>
<td>Pre</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 wk</td>
<td></td>
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<td>3 mo</td>
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<tr>
<td>1 yr</td>
<td></td>
<td></td>
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<tr>
<td><strong>Pre</strong></td>
<td>2.6 ± 0.81**</td>
<td>8.7 ± 0.81**</td>
</tr>
<tr>
<td><strong>1 wk</strong></td>
<td>11.3 ± 1.0**</td>
<td>8.6 ± 0.9**</td>
</tr>
<tr>
<td><strong>3 mo</strong></td>
<td>11.0 ± 1.0**</td>
<td>6.3 ± 0.9**</td>
</tr>
<tr>
<td><strong>1 yr</strong></td>
<td>120 ± 0.81**</td>
<td>120 ± 0.81**</td>
</tr>
<tr>
<td><strong>I-AUC glucose, mmol/l</strong></td>
<td>11.4 ± 0.81**</td>
<td>11.4 ± 0.81**</td>
</tr>
<tr>
<td><strong>I-AUC glucagon, nmol·min</strong></td>
<td>0.59* ± 0.37</td>
<td>0.59* ± 0.37</td>
</tr>
<tr>
<td><strong>Peak ISR, pmol·kg</strong></td>
<td>2.0 ± 10.7</td>
<td>7.8** ± 10.7</td>
</tr>
<tr>
<td><strong>Peak tGLP-1, pmol/l</strong></td>
<td>19 ± 106</td>
<td>57** ± 106</td>
</tr>
<tr>
<td><strong>Peak tGIP, pmol/l</strong></td>
<td>95 ± 196</td>
<td>91 ± 196</td>
</tr>
<tr>
<td><strong>PI-AUC ISR, pmol/kg</strong></td>
<td>505</td>
<td>505</td>
</tr>
<tr>
<td><strong>PI-AUC GLP-1, nmol·min</strong></td>
<td>0.36 6.2</td>
<td>0.36 6.2</td>
</tr>
<tr>
<td><strong>PI-AUC tGIP, nmol·min</strong></td>
<td>0.36 6.2</td>
<td>0.36 6.2</td>
</tr>
<tr>
<td><strong>PI-AUC glucagon, nmol·min</strong></td>
<td>0.59 0.37</td>
<td>0.59 0.37</td>
</tr>
</tbody>
</table>

Interestingly, following RYGB, the prehepatic insulin secretion profile in the T2D group resembled that of the subjects with NGT before surgery, and a significantly greater proportion of the total prehepatic insulin secretion occurred in the first 60 min after meal start after RYGB than before [AUC ISR 0–60 min/AUC ISR: Pre: 24% ± 4%; 1 wk: 31 ± 10% (P < 0.001); 3 mo: 37 ± 10% (P < 0.001); 1 yr: 42 ± 12%, (P < 0.001)] (Fig. 2).

The GLP-1 response to a meal was dramatically changed after RYGB (Fig. 3A). In the T2D group a 5.5-fold increase in peak GLP-1 concentrations was observed within the first week after surgery, and after 1 yr, concentrations were eight-fold higher. Accordingly, PI-AUC GLP-1 was 11-fold increased within the first week and almost 16 times greater 1 yr after surgery (Table 3). Similar responses were seen in the NGT group with peak GLP-1 concentrations increasing by a factor of 1.5. In the T2D group, a glucagon peak was observed postprandially and this peak remained after RYGB (Fig. 3C). Peak glucagon concentrations were increased within the first week after RYGB but then decreased subsequently (Table 3). The NGT group had no postprandial peak, and peak glucagon concentrations were lower compared with the T2D group at all study visits (Fig. 3C, Table 3).

In the T2D group, peak GIP concentrations did not differ before and after surgery. PI-AUC GIP concentrations were unchanged at 1 wk and 3 mo following RYGB, but they had decreased significantly after 1 yr compared with before surgery. The NGT group followed the same pattern, except for the 3-mo follow-up, at which time peak GIP concentrations increased compared with presurgical concentrations (Fig. 3B, Table 3).

In the T2D group, the glucagon response to a meal was dramatically changed, and this peak remained after RYGB (Fig. 3C). Peak glucagon concentrations were increased within the first week after RYGB but then decreased subsequently (Table 3). Glucagon I-AUC was negative before the operation, but at all postoperative study visits, the glucagon I-AUC was positive and approximately equal (Table 3).

The NGT group had no postprandial peak, and peak glucagon concentrations were lower compared with the T2D group at all study visits (Fig. 3C, Table 3).

Insulin resistance. HOMA-IR decreased by about 50% in both groups 1 wk after RYGB, and by 1 yr, HOMA-IR had decreased by almost 70% in subjects with T2D and 60% in the group of subjects with NGT (T2D: Pre: 6.6 ± 3.6, 1 wk: 3.2 ± 1.5 (P < 0.001), 3 mo: 2.5 ± 1.7 (P < 0.001), 1 yr: 1.8 ± 1.1 (P < 0.001); NGT: Pre: 2.9 ± 1.1, 1 wk: 1.6 ± 0.51 (P < 0.001), 3 mo: 1.3 ± 0.46 (P = 0.002), 1 yr: 1.1 ± 0.54 (P < 0.001)]. HOMA-IR was higher in subjects with T2D both before and in the first months after surgery but approached that of operated subjects with NGT by 1 yr. After 3 mo, HOMA-IR in the group of subjects with T2D did not differ from that of subjects with NGT before surgery (P = 0.098), and by 1 yr, it was lower than that of nondiabetic subjects before surgery (P = 0.011).

Measures of β-cell function. β-cell glucose sensitivity (β-GS) was increased by 74% 1 wk (P = 0.012) and 90% 3 mo (P < 0.001) after surgery in the T2D group, and by 1 yr, β-GS was still increased by 69% compared with before surgery (P < 0.001) (Fig. 4A). Still, β-GS was more than 50% reduced in the subjects with T2D at all study visits compared with subjects with NGT. In the NGT group, β-GS did not change significantly following RYGB (Fig. 4A).

In the T2D group, DI_0-GS increased within 1 wk after surgery and continued to increase with time from surgery (Fig.
Thus, DI$_{H252}$-GS increased three-, five-, and almost eight-fold 1 wk, 3 mo, and 1 yr after RYGB, respectively. In the NGT group, DI$_{H252}$-GS increased 1.5 (1 wk), 2.2 (3 mo), and 2.8 times (1 yr) compared with baseline levels (Fig. 4B). Though still numerically smaller, DI$_{H252}$-GS in subjects with T2D after 1 yr approached that of subjects with NGT before surgery ($P = 0.32$). However, at all study times, DI$_{H252}$-GS in subjects with NGT was more than three times greater than that of subjects with T2D (Fig. 4B).

**DISCUSSION**

In the present study, glucose metabolism was radically changed within the first week following RYGB in subjects with T2D and with NGT, with decreased insulin resistance, leftward shifts of the glucose and insulin curves and an improved $\beta$-cell function as evaluated by $\beta$-GS and disposition index. Concurrently, we found GLP-1 secretion grossly increased, whereas GIP secretion was almost unaffected by RYGB. Fasting glucagon concentrations were reduced months after RYGB, but postprandial glucagon secretion increased after surgery.

A decreased HOMA-IR weeks to months following RYGB has been observed in several studies of both subjects with T2D and with NGT (18, 33, 42). Isbell et al. (18) reported a reduced HOMA-IR in a mixed group of subjects with NGT or T2D 4 days after RYGB. In the present study, we observed similar relative decreases in HOMA-IR in subjects with both T2D and NGT.
within the first week after surgery, and HOMA-IR continued to decrease during the first year after surgery. After 3 mo, the HOMA-IR in subjects with T2D did not differ significantly from that of subjects with NGT before the operation. However, HOMA-IR probably reflects hepatic insulin sensitivity to a greater extent than peripheral insulin sensitivity (31), and correspondingly, in a recent study, basal endogenous glucose production and hepatic insulin resistance were reduced 1 mo after RYGB, while peripheral insulin sensitivity, assessed by euglycemic hyperinsulinemic clamp, remained unchanged (10).

The mechanism behind the rapid decrease in hepatic insulin resistance after RYGB is not yet clear, but the hypocaloric diet immediately following surgery could well be responsible. In the study by Isbell et al. (18), HOMA-IR decreased equally in a group of RYGB-operated subjects and a group of matched obese controls on an identical 4-day hypocaloric diet. Similarly, in a group of subjects with T2D, a 600 kcal/day diet brought about decreases in HOMA-IR, as well as decreases in hepatic insulin sensitivity, as measured by tracer infusion and euglycemic hyperinsulinemic clamp within 1 wk (26). These changes were accompanied by a decrease in liver fat, a mechanism that has earlier been proposed as a key explanation for the decreased insulin resistance in liver following a minor weight loss (40).

The leftward shift of the glucose profile in subjects with T2D observed here may be due to an accelerated delivery of nutrients to the small intestine with a subsequent increase in the rate of nutrient absorption, as has been previously reported in RYGB-operated subjects with NGT (11). Prior to surgery, subjects with NGT reached peak glucose concentrations 45 min after meal start, and because meal ingestion took 30 min, there was little chance of detecting a shift to the left of the glucose profile in these subjects after RYGB. However, the increased peak glucose levels and I-AUC immediately after RYGB-operated subjects with NGT (11). Prior to surgery, subjects with NGT reached peak glucose concentrations 45 min after meal start, and because meal ingestion took 30 min, there was little chance of detecting a shift to the left of the glucose profile in these subjects after RYGB. However, the increased peak glucose levels and I-AUC immediately after surgery, yet PI-AUC C-peptide increased only modestly directly after surgery, yet β-GS increased. This is most likely due to the C-peptide secretory profile being changed after RYGB, with a greater part of the insulin secretion taking place within the first hour after the meal start and peak concentrations being reached earlier. These changes in the dynamic insulin secretion after the operation in subjects with T2D could potentially be one of the explanations for the improved glucose tolerance (4).

It is worth noting that β-GS in subjects with T2D were reduced at all time points compared with subjects with NGT, even when changes in insulin resistance were compensated for by calculating the disposition indices. After 1 yr, the β-GS of subjects with T2D approached that of subjects with NGT before RYGB (Fig. 4, B and C). The results demonstrate that RYGB improves β-cell function very early after the operation and even more during the 1-yr follow-up period.

In the study by Falkén et al. (11), subjects with NGT following RYGB had increased 1-yr peak insulin and C-peptide concentrations compared with earlier postoperative study visits. However, in that study, the meal consisted of carbohydrate (89 E%) and some protein (11 E%), with no fat at all, so differences in the oral stimulus may explain this discrepancy. Isbell et al. (18) reported no changes in insulin or C-peptide secretion following a meal 1 wk after RYGB, but the lack of measure points for the first 60 min after the end of a meal in that study can explain the contradictory finding (19). Two further studies have reported the acute effects of RYGB on glucose metabolism in response to a mixed meal, but in neither of them do the authors report an improvement in β-cell

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Fig. 2. Prehepatic insulin secretion rates (ISR) in response to a meal in subjects with T2D and with NGT, before (Pre) and 1 wk, 3 mo, and 1 yr after RYGB. Data are presented as means ± SE.
function within the first week after RYGB (43, 48). This may again be a consequence of differences in the oral stimulus (both meal composition and ingestion time) and the fact that both studies were less well powered than the present study (n = 9 and n = 10, respectively).

What causes this improved β-cell function? Laferrère et al. (25) reported a several-fold increase of the incretin effect after RYGB surgery when subjects were first given an oral glucose tolerance test and a subsequent isoglycemic intravenous glucose challenge (25). In a recent study, further support for the role of GLP-1 in the post-RYGB glucose metabolism has emerged: a greater amount of the insulin response following a meal could be blocked using the GLP-1 receptor specific blocker, exendin (9–39), in RYGB-operated subjects compared with nonoperated controls (44). Furthermore, Dirksen et al. (9) found a close association between the increased GLP-1 secretion and insulin responses demonstrated in an RYGB-operated subject with T2D (9).

One could speculate that, since fasting glucose concentrations decrease so dramatically, a reduced glucotoxicity could, in part, explain the improved β-cell function observed here, but in the study by Morinigo et al. (33), 1.5 mo after RYGB, β-cell
function in subjects with T2D increased only in response to oral, not to intravenous, glucose stimulation, despite fasting plasma glucose concentrations decreasing from 8.5 mM to 5.8 mM (33). Similarly, in a study in which subjects with T2D were kept on a hypocaloric diet and fasting plasma glucose concentrations decreased from 9.2 to 5.9 mM within 1 wk, first-phase insulin response to an intravenous glucose challenge did not significantly increase in the same time interval, reflecting an unchanged \( \beta \)-cell function (26). Thus, it seems that the improved \( \beta \)-cell function observed in this study 1 wk after RYGB is more likely due to the greatly increased GLP-1 secretion demonstrated than to a reduced glucotoxicity or a hypocaloric diet.

While the increase in GLP-1 secretion after RYGB is well documented, reports on the effects of RYGB on the secretion of the other major incretin, GIP, are less consistent. Laferrère et al. (25) reported an increased GIP, as well as GLP-1, secretion 1 mo after RYGB in subjects with T2D in response to an oral glucose load, implying that increased GIP secretion could play a role in the improved glucose metabolism after surgery. In the present study, GIP secretion was uninfluenced by RYGB in the group of subjects with T2D, whereas peak GIP concentrations in subjects with NGT were transiently increased 3 mo after surgery. In both groups, 1 yr after RYGB, peak GIP concentrations had returned to presurgical levels, and PI-AUC GIP was reduced. Taken together, these findings do not suggest that RYGB causes a substantial increase in meal-induced GIP secretion, which is in line with previous reports (11, 20). The discrepancies in the reported effects of RYGB on meal-induced GIP secretion may reflect differences in the oral stimulus (glucose vs. mixed meal), but one could further speculate whether minor differences in surgical technique with resulting differences in e.g., Roux-limb length could also explain such divergent results. However, our results do not indicate that improved \( \beta \)-cell function in subjects with T2D immediately after RYGB is due to an increase in GIP secretion. For GIP to positively influence \( \beta \)-cell function in our subjects, this would require a postsurgery increase in \( \beta \)-cell GIP sensitivity.

In subjects with NGT, increasing \( \beta \)-cell function could potentially induce hypoglycemia. Although we did not register any symptoms of hypoglycemia during any of the meal tests, subjects in the NGT group did show significantly lower values of plasma glucose 2 h after meal start postoperatively, and earlier studies have demonstrated increased insulin and GLP-1 secretion in response to a meal in RYGB-operated subjects with severe neuroglycopenia compared with operated subjects without severe neuroglycopenia (15). In the present study, it is obvious from Fig. 4C that the \( \beta \)-cell function of subjects with NGT has not adapted to the pronounced improvement in insulin sensitivity after RYGB, which could pose a risk factor for postprandial hypoglycemia.
Another potential benefit of the greatly increased GLP-1 secretion on the glucose metabolism of subjects with T2D could be a suppression of glucagon secretion (16), but RYGB produces an increase in meal-stimulated glucagon secretion, as illustrated by the significant increase in I-AUC in both groups from negative to positive values. This change reflects an inability to adequately suppress glucagon release from ~60 min after the beginning of the meal to the end of the observational period. It has previously been suggested that this could be due to “pancreatic type processing” of proglucagon in the intestinal L-cell with subsequent glucagon release (11). Our observation is consistent with earlier reports and makes glucagon an unlikely candidate for explaining the improved glucose metabolism immediately after surgery (11, 44). Still, fasting glucagon concentrations do decrease in the months following RYGB, and so this could potentially explain some of the long-term benefits of RYGB on glucose metabolism.

The strengths of the present study are that we have thoroughly tested the β-cell function in both subjects with T2D and with NGT comparable in BMI before and in weight loss after the operation. We have compared the β-cell glucose sensitivity using DIβ-GS, which represents an integrated measure of insulin secretion over a longer time period in response to a meal, and this is a sensitive index of β-cell responsiveness to glucose, incretin hormones, and nutrients (12, 13, 21). Finally, we obtained accurate estimates of insulin secretion by calculating prehepatic ISR to circumvent potential problems with hepatic and posthepatic insulin clearance before and after the operation and between the two groups of participants.

A limitation is that insulin sensitivity was estimated by use of HOMA-IR, which primarily reflects liver insulin sensitivity (31); therefore, our study provides limited information on the development of peripheral insulin sensitivity.

In conclusion, RYGB has a very pronounced effect on glucose metabolism in both subjects with T2D and with NGT. Insulin sensitivity and β-cell function are improved, and a dramatically increased GLP-1 secretion is observed concurrently, suggesting a role for this potent insulin secretagogue in the post-RYGB glucose metabolism. Effects are rapid within a week after surgery and are sustained for at least 1 yr.

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AUTHOR CONTRIBUTIONS


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