Increased Hepatic Insulin Clearance After Roux-en-Y Gastric Bypass


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Context: Roux-en-Y gastric bypass (RYGB) improves glucose tolerance and ameliorates fasting hyperinsulinemia within days after surgery. Improvements in hepatic insulin sensitivity and insulin clearance could contribute importantly to these effects.

Objective: The objective of the investigation was to study changes in insulin clearance after RYGB.

Design: This was a prospective study of fasting hepatic insulin clearance and, in a subgroup of patients, postprandial insulin clearance after a meal test before and 1 week, 3 months, and 1 year after RYGB.

Setting: The study was conducted at Hvidovre Hospital (Hvidovre, Denmark).

Patients: Patients included 2 groups of obese RYGB-patients: 1) type 2 diabetes (T2D) group: 32 patients with T2D (meal test, n = 13), 2) normal glucose tolerance (NGT) group: 32 patients with NGT (meal test, n = 12).

Intervention: The intervention was RYGB.

Main Outcome Measure: Fasting hepatic insulin clearance (fasting C-peptide/fasting insulin). Postprandial insulin clearance (incremental areas under the curve of insulin secretion rates/incremental areas under the curve of insulin).

Results: Fasting hepatic insulin clearance increased after 1 week (P < .01) and further at 3 months (P < .01), remaining elevated 1 year postoperatively (P < .01) with no difference between the T2D and NGT groups. Postprandial insulin clearance changed only in the T2D group with an increase at 1 week (P < .01) that was maintained at 3 months (P = .06) and 1 year (P < .01).

Conclusions: RYGB increases insulin clearance within 1 week after surgery, highlighting the liver as a key organ involved in the early beneficial effect on glucose metabolism. Postprandial insulin secretion may be underestimated postoperatively in patients with type 2 diabetes when evaluated by peripheral insulin concentrations instead of insulin secretion rates or C-peptide. (J Clin Endocrinol Metab 98: E1066–E1071, 2013)

Roux-en-Y gastric bypass (RYGB) is a surgical procedure inducing weight loss in severely obese patients; however, it also has marked effects on type 2 diabetes (1, 2), often with reductions in glucose levels within days after surgery (3). Understanding the mechanisms behind this rapid improvement in glycemic control after RYGB is a potential source to new knowledge of the pathophysiology of type 2 diabetes.

Abbreviations: CI ratio, C-peptide to insulin ratio; FFA, free fatty acid; HGP, hepatic glucose production; iAUC, incremental area under the curve; ISR, insulin secretion rate; NGT, normal glucose tolerance; RYGB, Roux-en-Y gastric bypass.
In the early postoperative period after RYGB, reports of reductions in fasting insulin and glucose concentrations in patients with type 2 diabetes and normal glucose tolerance (NGT) indicate an early improvement in hepatic insulin sensitivity (4–8). Nevertheless, tracer studies of hepatic glucose production (HGP) have been inconclusive with one study reporting no change at 2 weeks and another reduced basal HGP at 1 month after RYGB (9, 10). None of the studies assessed changes in hepatic insulin clearance, although closely related to hepatic insulin action, as degradation of insulin primarily is initiated by receptor-mediated endocytosis (11).

Hepatic insulin clearance can be estimated using simple measurements of the ratio of C-peptide to insulin (CI ratio), based on the assumption that C-peptide is secreted equimolarly with insulin but is not subjected to hepatic first-pass metabolism (12). Due to the different elimination kinetics of insulin and C-peptide, the use of the CI ratio is valid only during steady-state conditions, eg, in the fasting state, whereas the estimation of insulin clearance during non-steady-state conditions requires modeling of C-peptide kinetics to calculate prehepatic clearance during non-steady-state conditions requires modeling of C-peptide kinetics to calculate prehepatic insulin secretion rates (ISRs) to which peripheral insulin can be compared (11).

In the present study, we report changes in hepatic insulin clearance as measured by the fasting CI ratio in patients with type 2 diabetes and NGT before and 1 week, 3 months, and 1 year after RYGB. In addition, insulin clearance is estimated during a meal test in a subgroup.

**Materials and Methods**

**Patients**

We recruited 32 patients (17 males and 15 females) with type 2 diabetes (T2D group; oral glucose tolerance test, 2 hour plasma glucose ≥ 11.1 mmol/L and/or treated with ≥ 1 antidiabetic agent, diabetes duration 5.4 ± 3.6 (SD) years) and 32 patients (11 males and 21 females) with NGT (NGT group; oral glucose tolerance test, 2 hour plasma glucose < 7.8 mmol/L, and glycosylated hemoglobin < 6%) scheduled for laparoscopic RYGB at Hvidovre Hospital (Hvidovre, Denmark). All patients completed a mandatory preoperative diet-induced total body weight loss of 8%. Antidiabetic agents were discontinued 3 days or longer prior to the first study day, and none received antidiabetic medication postoperatively. Patients participated in 4 different investigational protocols but underwent the same sampling schedule in fasting at our research facility. Six did not complete the 1-week study due to postoperative complications, but all completed the 3-month follow-up, except 1 patient who violated the fasting requirement. Not all protocols included a 1-year post-surgery study visit and 1 patient (NGT group) was excluded due to pregnancy.

A subgroup of 13 patients with type 2 diabetes and 12 patients with NGT were also subjected to a liquid meal test. Results from this study have been published previously (13), but in the present study, data were reanalyzed to examine changes in insulin clearance.

Written informed consent was obtained from all patients; protocols were approved by the Municipal Ethical Committee of Copenhagen in accordance with the Helsinki II declaration and by the Danish Data Protection Agency and were registered at www.ClinicalTrials.gov (NCT 01202526, NCT01559779, NCT00810823, NCT01579981).

**Sampling**

After an overnight fast (10-12 hours), 3 fasting blood samples (5 minute intervals) were obtained from a catheter in an antecubital vein.

**Meal test**

Details are reported elsewhere (13). In brief, patients ingested a liquid meal containing carbohydrate, protein, and fat (Fresubin Energy drink 200 mL, 300 kcal; Fresenius Kabi, Bad Homburg, Germany) over 30 minutes. Blood was sampled frequently for 4 hours.

**Analysis**

Clot-activator tubes were left at room temperature to coagulate before centrifugation. Serum was stored at −80°C. Serum C-peptide and insulin were analyzed using an AutoDELFIA fluorometric immunoassay (Wallac OY, Turku, Finland). Median intra- and interassay coefficient of variation for C-peptide was 4.2% and 2.6%, respectively, and the median coefficient of variation of the 3 fasting samples was 4.2% (interquartile range 2.4–8.0%). EDTA tubes were immediately centrifuged, plasma glucose measured using YSI model 2300 STAT plus (Yellow Springs Instruments, Yellow Springs, Ohio), and plasma free fatty acids (FFAs) measured using an enzymatic colorimetric assay (Wako Chemicals Gmbh, Neuss, Germany).

**Surgery**

RYGB was performed at the Department of Surgical Gastroenterology at Hvidovre Hospital (Hvidovre, Denmark) as previously described (13).

**Postoperative diet**

Patients were on a liquid diet (1200 kcal/d) until 14 days after surgery at which time the diet gradually changed toward solid foods.

**Calculations and statistics**

The CI ratio was calculated as the ratio of the mean C-peptide concentration and the mean insulin concentration at fasting. Prehepatic ISR was calculated from C-peptide concentrations using the ISEC software program (14). Incremental areas under the curve (iAUCs) were calculated using the trapezoidal model, subtracting fasting values. The postprandial insulin clearance was calculated as the ratio between the iAUC ISR and the iAUC insulin.

Postoperative values were compared with preoperative within the group using paired t tests, whereas unpaired t tests were used for comparisons between groups. The relationship between fasting insulin and C-peptide concentrations was further assessed using a linear mixed-effects model with zero intercept; C-peptide, diabetes status, and time from surgery as fixed
effects; and individual subjects as random effect. A Wald test was used to compare differences between postoperative time points. $P < .05$ was considered significant. Analyses were performed in R 2.11.1 (www.r-project.org).

**Results**

**Fasting state (Table 1)**

Fasting insulin and C-peptide were significantly lower postoperatively in both groups; however, the relative decline in insulin was greater than the relative decline in C-peptide at all time points (all comparisons $P < .01$).

Consequently, the CI ratio increased in both groups 1 week postoperatively and further at 3 months ($P < .05$), with no significant change from 3 months to 1 year ($P > .30$). This was confirmed in a linear mixed-effects analysis, with a significant change from before to 1 week ($P < .01$), a further change from 1 week to 3 months ($P < .01$), and no change from 3 months to 1 year ($P = .61$). In the model, diabetes status was not a significant covariable at any time point ($P = .42$).

### Table 1. Glucose, C-Peptide, Insulin, and FFAs Before and 1 Week, 3 Months, and 1 Year After RYGB in Patients With Type 2 Diabetes and NGT

<table>
<thead>
<tr>
<th></th>
<th>Type 2 Diabetes</th>
<th>NGT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>1 week</td>
</tr>
<tr>
<td>Fasting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>32</td>
<td>29</td>
</tr>
<tr>
<td>Days from surgery</td>
<td>−6.7 (7.9)</td>
<td>6.2 (1.3)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>40.6 (5.9)</td>
<td>39.4 (6.1)</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>8.6 (2.1)</td>
<td>6.9 (1.2)</td>
</tr>
<tr>
<td>Fasting insulin, pmol/L</td>
<td>111 (60)</td>
<td>72 (36)</td>
</tr>
<tr>
<td>Fasting C-peptide, pmol/L</td>
<td>1377 (431)</td>
<td>1187 (515)</td>
</tr>
<tr>
<td>Fasting CI-ratio</td>
<td>14.4 (5.9)</td>
<td>17.6 (5.9)</td>
</tr>
<tr>
<td>Meal test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Days from surgery</td>
<td>−2.7 (2.8)</td>
<td>5.4 (1.1)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>43.1 (5.1)</td>
<td>42.4 (5.3)</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>8.8 (2.3)</td>
<td>7.0 (1.2)</td>
</tr>
<tr>
<td>Fasting insulin, pmol/L</td>
<td>125 (77)</td>
<td>73 (32)</td>
</tr>
<tr>
<td>Fasting ISR, pmol/kg/min</td>
<td>3.3 (1.1)</td>
<td>2.7 (1.2)</td>
</tr>
<tr>
<td>Fasting FFA, mmol/L</td>
<td>0.8 (0.2)</td>
<td>0.9 (0.2)</td>
</tr>
<tr>
<td>iAUC glucose, mmol/min/L</td>
<td>350 (193)</td>
<td>284 (257)</td>
</tr>
<tr>
<td>iAUC insulin, pmol/min/mL</td>
<td>31.7 (18.2)</td>
<td>35.0 (17.1)</td>
</tr>
<tr>
<td>iAUC ISR, pmol/kg</td>
<td>489 (186)</td>
<td>664 (249)</td>
</tr>
<tr>
<td>iAUC FFA, mmol/min/L</td>
<td>−78 (39)</td>
<td>−72 (36)</td>
</tr>
</tbody>
</table>

Abbreviation: BMI, body mass index. Values are expressed as mean ± SD. Fasting refers to changes in the fasting state. Meal test refers to changes in a subgroup subjected to a meal test.

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| a | $P < .01$, compared with before with paired $t$ test. |
| b | $P < .01$, compared with patients with type 2 diabetes at the same time point using an unpaired $t$ test. |
| c | $P < .05$, compared with patients with type 2 diabetes at the same time point using an unpaired $t$ test. |
| d | $P < .05$, compared with before with paired $t$ test. |
Relative declines in fasting insulin, C-peptide, and the CI ratio were similar in patients with type 2 diabetes and NGT at all time points (all comparisons $P > .25$).

**Meal test (Table 1 and Figure 1)**

Insulin secretion (iAUC ISR) during the meal increased in both groups at 1 week and 3 months after RYGB, whereas the change at 1 year was not significantly different from preoperatively in the T2D group ($P = .09$). No changes in iAUC insulin were observed, except in the NGT group at 3 months postoperatively.

Postprandial insulin clearance increased in patients with type 2 diabetes at 1 week after RYGB and remained higher postoperatively, whereas no change in postprandial clearance of insulin was seen in the NGT group (Figure 1B). Changes in the ratio between fasting ISR and fasting insulin (Figure 1A) were comparable with the changes in the CI ratio in the whole data set (Table 1). Compared with fasting insulin clearance, postprandial clearance was reduced at all time points in both groups ($P < .01$, all comparisons) with the exception of 1 week postoperatively in the T2D group ($P = .11$).

In both groups, fasting levels of FFA decreased after 1 year, whereas postprandial suppression was unchanged throughout the study period.

**Discussion**

We studied insulin clearance in patients with type 2 diabetes and NGT throughout the first year after RYGB surgery. The main finding was an increase in fasting hepatic insulin clearance after RYGB in both patients with type 2 diabetes and NGT, whereas postprandial insulin clearance increased only in patients with type 2 diabetes. The most pronounced changes occurred within the first week after surgery, although fasting insulin clearance had improved further at 3 months postoperatively.

Hepatic insulin clearance is believed to be mediated by receptor binding and thus likely to be coupled to hepatic insulin sensitivity (11). Short-term calorie restriction in patients with type 2 diabetes improves hepatic insulin sensitivity independently of weight loss (15, 16) and is associated with reductions in liver fat content (17, 18). A similar mechanism could be responsible for a rapid change in hepatic insulin sensitivity after RYGB, which we propose to be essential for the immediate improvement of glycemic control (19), although final conclusions from tracer studies are warranted (9, 10). Thus, postoperative calorie restriction could be the possible mechanism behind the increase in insulin clearance after RYGB.

Insulin clearance is a saturable process (11), which is important to consider during conditions with high portal insulin concentrations, ie, when evaluating postprandial clearance. Indeed, we observed postprandial suppression of insulin clearance both before and after RYGB. Further supporting the hypothesis of saturation in this study is the lack of postoperative changes in postprandial insulin clearance in the NGT group. On the contrary, in the T2D group, postprandial insulin clearance increased concomitantly with increased ISR and therefore is not explained by receptor saturation. Although we cannot determine whether postprandial insulin clearance increased at hepatic or peripheral sites, increased insulin binding to hepatocytes could be a likely mechanism as RYGB seems to induce early changes in hepatic rather than peripheral insulin action (19). Another contributing mechanism could be the very
marked change in the insulin secretion profile observed in the T2D group postoperatively (13) as hepatic insulin clearance has been shown to respond rapidly to dynamic changes in insulin secretion (20). Increased postprandial clearance of insulin in patients with type 2 diabetes must be taken into consideration when interpreting studies using peripheral insulin in the estimation of insulin secretion after RYGB because postoperative changes will be underestimated if not evaluated by ISR or C-peptide.

In this study, FFAs did not seem to contribute to the results because fasting and postprandial suppression of FFAs did not change immediately after RYGB. Changes in incretin hormones and glucagon secretion in response to the meal were reported previously (13) but did not seem to contribute to our findings either; secretion of the hormones were unchanged in fasting in the immediate postoperative period and changes in postprandial hormone secretion after RYGB were comparable in patients with type 2 diabetes and NGT. In contrast, the greatest change in insulin clearance was observed at fasting and changes in postprandial clearance differed between the 2 groups.

The study has limitations. First, insulin clearance was measured via measurements of C-peptide concentrations, which rely on the assumption of unchanged C-peptide elimination after RYGB. Second, we did not perform measurements of hepatic insulin sensitivity, eg, tracer studies of HGP, which would be of major interest. On the other hand, the strengths of the study are the large number of patients followed prospectively and the use of triplicate sampling in fasting.

In conclusion, RYGB increases fasting hepatic insulin clearance within 1 week postoperatively in both patients with type 2 diabetes and NGT, highlighting the liver as a key organ involved in the early improvement in fasting glucose metabolism after surgery. Three months postoperatively fasting hepatic insulin clearance is further enhanced and the improvement is sustained for at least 1 year. Furthermore, postprandial insulin clearance is increased in patients with type 2 diabetes already 1 week after RYGB.

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