Gastric bypass surgery is associated with near-normal insulin suppression of lipolysis in nondiabetic individuals

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Gastric bypass surgery is associated with near-normal insulin suppression of lipolysis in nondiabetic individuals. We hypothesized that individuals who have undergone gastric bypass have greater insulin sensitivity that obese subjects but less compared with lean. We measured free fatty acid (FFA) and glucose kinetics during a two-step, hyperinsulinemic euglycemic clamp in nondiabetic subjects who were 38 ± 5 mo post-gastric bypass surgery (GB; n = 15), in lean subjects (L; n = 15), and in obese subjects (O; n = 16). Fasting FFAs were not significantly different between the three study groups but during both doses of insulin were significantly higher in GB than either O or L. The effective insulin concentration resulting in half-maximal suppression of FFA was similar in L and GB and significantly less in both groups compared with O. Glucose infusion rates during low-dose insulin were not significantly different in GB compared with either L or O. During high-dose insulin, glucose infusion rates were significantly greater in GB than in O but less than in L. Endogenous glucose production in GB was significantly lower than O only during low dose of insulin. We conclude that gastric bypass is associated with improvements in adipose tissue insulin sensitivity to levels similar to lean, healthy persons and also with improvements in the response of glucose metabolism to insulin. These changes may be due to preferential reduction in visceral fat and decreased FFA availability. However, some differences in insulin sensitivity in GB remain compared with L. Residual insulin resistance may be related to excess total body fat or abnormal lipolysis and requires further study.

insulin sensitivity; glucose; body composition; obesity

Obesity is frequently accompanied by insulin resistance (5). Elevated circulating free fatty acid (FFA) concentrations, due to increased adipose tissue lipolysis, are thought to mediate insulin resistance in both obesity and type 2 diabetes (4). Weight loss via a hypocaloric diet has been shown to reduce lipolysis in obese individuals (14). However, although gastric bypass surgery has been shown to improve insulin action on glucose metabolism, relatively little is known about the effect of gastric bypass surgery on the insulin sensitivity of adipose tissue.

We measured FFA kinetics during a two-step hyperinsuline- mic euglycemic clamp in nondiabetic individuals who had undergone gastric bypass surgery and in nondiabetic lean and obese subjects. We hypothesized that gastric bypass subjects have greater insulin sensitivity with respect to lipolysis compared with obese subjects but less than lean subjects. We also hypothesized that gastric bypass subjects would have greater insulin sensitivity with respect to glucose kinetics than obese subjects but less than lean subjects and that there would be a relationship between adipose tissue insulin sensitivity and the sensitivity of glucose metabolism to insulin.

Research design and methods. The Mayo Institutional Review Board approved the study, and subjects gave informed consent. The Mayo Clinical Research Unit (CRU) was used for this study. Three groups of nondiabetic subjects participated in a cross-sectional study: post-gastric bypass (GB; n = 15), lean (L; n = 15), and obese (O; n = 16). Male and female subjects between the ages of 18 and 45 were eligible for this study. Gastric bypass subjects were recruited from a database of patients that had undergone open or laparoscopic proximal Roux-en-Y gastric bypass operations at the Mayo Clinic for medically complicated obesity refractory to behavioral modification. Gastric bypass subjects were eligible if they had had undergone surgery ≥12 mo prior to enrollment and had undergone at least a 10-kg weight loss. O and L subjects were recruited from the surrounding Rochester, NY, area through poster advertisements. Each subject underwent a history and a physical exam by a study physician and had fasting lipids, glucose, and creatinine measured and a resting electrocardio- gram performed. Individuals that were allergic to the drugs used in this study, pregnant, or known to have been or being actively treated for renal insufficiency, diabetes, or other major endocrinological disorders or serious pulmonary or cardiovascular diseases were ineligible for the study. Persons taking part in an endurance-training program (aerobic exercise >60 min/day for >5 days/wk) or actively losing weight were also excluded.

Despite efforts to balance the subject groups, no male gastric bypass subjects qualified for the study. The average body mass index (BMI) of the gastric bypass subjects at the time of surgery was 44.7 ± 1.0 kg/m². The average time from surgery to their study day was 38 ± 5 mo, and their weight at the time of study was 34 ± 2% less than their presurgical weight. Of the 15 gastric bypass participants, seven had impaired fasting glucose preoperatively, but none had diabetes. Fasting glucose levels had normalized in all but one (6.3 mmol/l) of these subjects at the time of the study. Four of the obese subjects had impaired fasting glucose at the time of the study (5.8 ± 0.2 mmol/l).

Body composition measurements were performed on a separate day prior to the clamp study. Whole body and regional fat mass and fat-free mass (FFM) were measured with dual-energy X-ray absorptiometry. Single-slice computed tomography scans were performed at the abdomen (2nd lumbar vertebrae) and quadriceps (mid-thigh) levels to measure visceral and thigh fat content (10).
For 3 days preceding inpatient admission, all subjects received weighed weight maintenance meals provided by the CRU metabolic kitchen with a macronutrient distribution of 50% carbohydrate, 20% protein, and 30% fat. Total meal energy was calculated as the sum of basal energy expenditure (estimated from the Harris-Benedict equation) and estimated energy expenditure from activity. Subjects were admitted to the CRU the evening before the study and fasted overnight after a snack at 2000. On the morning of the study, an infusion catheter was placed in a hand or forearm vein. A retrograde catheter was placed in a contralateral hand vein, and the hand was heated for sampling of arterialized venous blood.

A continuous infusion of \([^{3}H]\)palmitate (~0.3 µCi/min) and a primed, continuous infusion of \([^{3}H]\)glucose (2.4 mg/kg FFM followed by 0.04 mg·kg·FFM\(^{-1}\)·min\(^{-1}\)) were started 30 and 180 min, respectively, prior to the start of a euglycemic hyperinsulinemic clamp and continued throughout. Beginning at 0730, insulin was infused at 0.25 mU·kg·FFM\(^{-1}\)·min\(^{-1}\) for 3 h [low-dose insulin (LDI)] and then at 1.0 mU·kg·FFM\(^{-1}\)·min\(^{-1}\) for 3 h [high-dose insulin (HDI)]. Plasma glucose was measured every 10–15 min, and the rate of an infusion of 40% dextrose containing \([^{3}H]\)glucose was adjusted to maintain a target plasma glucose concentration of 5 mmol/l. Blood samples for determination of total FFA concentration, palmitate concentration and specific activity, glucose and \([^{3}H]\)glucose concentration, and insulin concentration were drawn every 10 min during the final 30 min of each clamp interval. Indirect calorimetry was used to measure resting energy expenditure at baseline and during the clamp at steady state. Palmitate and total FFA concentration and specific activity were measured with a modification (19) of a high-performance liquid chromatography method (18). Palmitate rate of appearance (Ra) was calculated using mean specific activities (18). Plasma \([^{3}H]\)glucose concentration and enrichment were determined using gas chromatography-mass spectrometry (26). Endogenous glucose production (EGP) at steady state was calculated as described previously (27) and normalized to lean body mass.

All data are presented as means ± SE. Variables were compared across the three groups using one-way analysis of variance (ANOVA), with \(P < 0.05\) used to denote statistical significance. In cases where the ANOVA finding was statistically significant, pairwise group differences were assessed using the Tukey multiple comparison test.

**RESULTS**

Characteristics of the subject groups and baseline laboratory values are shown in Table 1. Notably, fasting plasma glucose was similar among all groups, but fasting plasma insulin concentrations were significantly greater in O compared with both L and GB. GB had significantly lower total abdominal, abdominal subcutaneous, and thigh fat mass and BMI than O but significantly higher total abdominal, abdominal subcutaneous, and thigh fat mass than L subjects. In contrast, visceral fat in GB was not significantly different from L but was significantly less than O. Lean body mass was significantly lower in GB compared with O but not significantly different from L subjects.

Steady-state conditions for plasma palmitate, \([^{3}H]\)palmitate, glucose, and \([^{3}H]\)glucose are shown in Fig. 1, A–D. Plasma total FFA concentrations were not significantly different between the three study groups at baseline (Fig. 2). However, during both doses of insulin, FFA in both GB and L were significantly lower than O. During LDI, palmitate Ra was significantly lower in L (28.7 ± 4.0 µmol/min) than in GB (51.6 ± 4.9 µmol/min) and O subjects (66.4 ± 7.6 µmol/min), whereas GB and O subjects were not statistically different. During HDI, palmitate Ra in GB (19.9 ± 1.6 µmol/min) and L (13.5 ± 2.2 µmol/min) were both significantly lower than O (33.8 ± 4.6 µmol/min) but not significantly different from each other.

During the clamp, significant differences in plasma insulin concentrations were present among the groups despite the fact that infusions rates were based on FFM (Table 2). To account for these differences, the effective insulin concentration that resulted in half-maximal suppression of fasting FFA concentrations (EC\(_{50}\)) was determined for each subject using linear regression after log transformation of data, as described previously (9). The EC\(_{50}\) was significantly greater in O compared with both L and GB, with no differences between L and GB subjects (Fig. 3). Additionally, log-log linear regression of insulin vs. palmitate Ra was performed in each subject. The average plasma insulin concentration measured at steady state during each insulin infusion in the L subjects was then used to calculate palmitate Ra during LDI and HDI (Fig. 4). Using this approach, we found that the normalized palmitate Ra during LDI was significantly greater in O than in GB and L and significantly greater in GB than in L subjects. During HDI, the normalized palmitate Ra was significantly greater in O than in both GB and L, with no significant differences between GB and L.

During LDI, glucose infusion rate (GIR) at steady state was not significantly different between O and GB subjects and was significantly greater in L subjects than in both groups (Table 2). During HDI, the steady-state GIR was significantly greater in GB compared with O but significantly less than in L subjects. In light of poor enrichment of \([^{3}H]\)glucose in several subjects, analysis of EGP was performed, excluding these subjects \((n = 13\) L, 15 O, and 7 GB). Basal EGP was significantly lower in L compared with O subjects, but there was no difference in GB from either L or O subjects (Table 2). EGP was significantly less in

**Table 1. Baseline subject characteristics and fasting laboratory values**

<table>
<thead>
<tr>
<th>GB</th>
<th>Lean</th>
<th>Obese</th>
</tr>
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<tbody>
<tr>
<td>Age, yr</td>
<td>37.1 ± 1.6</td>
<td>32.7 ± 2.3</td>
</tr>
<tr>
<td>Sex (female/male)</td>
<td>15/0</td>
<td>10/17</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>82.3 ± 3.2†</td>
<td>65.8 ± 8.1†</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29.7 ± 1.2†</td>
<td>22.9 ± 0.5†</td>
</tr>
<tr>
<td>%Body fat</td>
<td>42.9 ± 2.1*</td>
<td>26.7 ± 2.2*</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>42.6 ± 1.3†</td>
<td>45.9 ± 2.5</td>
</tr>
<tr>
<td>Total abdominal fat, cm²</td>
<td>371 ± 36†</td>
<td>143 ± 19†</td>
</tr>
<tr>
<td>Visceral fat, cm²</td>
<td>56 ± 9†</td>
<td>34 ± 5†</td>
</tr>
<tr>
<td>Subcutaneous abdominal fat, cm²</td>
<td>315 ± 29†</td>
<td>109 ± 17†</td>
</tr>
<tr>
<td>Thigh fat, cm²</td>
<td>294 ± 31†</td>
<td>154 ± 18†</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>5 ± 0.1</td>
<td>5.1 ± 0.1</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>168 ± 6†</td>
<td>171 ± 9 †</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>87 ± 7†</td>
<td>100 ± 13</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>65 ± 4†</td>
<td>67 ± 9†</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>85 ± 6†</td>
<td>89 ± 7†</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>26.1 ± 3.8*†</td>
<td>8.7 ± 1.6†</td>
</tr>
</tbody>
</table>

Values are means ± SE. GB, gastric bypass; BMI, body mass index. *\(P < 0.05\) vs. lean; †\(P < 0.05\) vs. obese.
L compared with O subjects during both LDI and HDI and significantly less in GB than in O subjects during LDI. The rate of disappearance of glucose during HDI was significantly greater in L compared with both O and GB subjects (Table 2).

We found a significant relationship between visceral fat and EC_{50} \((r^2 = 0.36)\) and visceral fat and GIR during LDI \((r^2 = 0.23)\) and HDI \((r^2 = 0.30)\). We also found that FFA concentrations were significantly related to GIR during LDI and HDI \((r^2 = 0.32\) and 0.42, respectively). There was also a small but significant relationship between fasting FFA and GIR during LDI and HDI \((r^2 = 0.11\) and 0.19, respectively). Finally, the EC_{50} was significantly correlated with GIR particularly during HDI \((r^2 = 0.44)\). Serum leptin concentrations were lower in the GB compared with O subjects but higher than in L subjects (Table 1). The leptin levels were related not only to body fat \((r^2 = 0.75)\) but also to EC_{50} \((r^2 = 0.34)\) and palmitate Ra and GIR, particularly during the higher dose of insulin \((r^2 = 0.28\) and 0.37, respectively).

**DISCUSSION**

Roux-en-Y gastric bypass is widely performed for the treatment of medically complicated obesity and results in marked and sustained weight loss (2) with marked (24) but incomplete (20) improvement in insulin resistance. However, the sensitivity of adipose tissue to insulin has not been studied previously. We used a two-step euglycemic clamp to determine the insulin sensitivity of adipose tissue lipolysis in individuals that have undergone gastric bypass surgery more closely resembles that of lean subjects than obese subjects. The finding that the suppression of circulating FFA by insulin, even after adjusting for differing insulin
The improvement of insulin sensitivity after gastric bypass in terms of peripheral glucose disposal has been studied using a number of well-established techniques, including calculated indices of insulin sensitivity such as homeostasis of model assessment (3), intravenous (16) and oral glucose tolerance tests (24), and glucose clamps (as we used in our study) (1, 3). However, there is only one report showing that lipolysis is increased visceral lipolysis after gastric bypass may be major
tions in the development of insulin resistance (4). Additionally, endogenous glucose production tended to be greater in obese subjects during insulin infusions than in individuals that had undergone gastric bypass surgery. Finally, we found that there is a highly significant relationship between the sensitivity of adipose tissue lipolysis and whole body glucose insulin sensitivity. Taken together, our results show that insulin sensitivity in individuals that have undergone gastric bypass surgery is overall more similar to lean rather than to obese individuals.

We found that the total body fat and subcutaneous abdominal fat in gastric bypass subjects was lower than in obese subjects but still higher than in lean subjects. In contrast, the amount of visceral fat in the gastric bypass subjects was not statistically different from lean controls and was approximately one-third of that of the obese subjects. Thus, it appears that post-gastric bypass surgery is to some extent a model of isolated subcutaneous obesity (22). The strong relationship we found between insulin sensitivity in adipose tissue and for glucose is consistent with a role for elevated FFA concentrations in the development of insulin resistance (4). Additionally, we found highly significant relationships between visceral fat and measurements of insulin sensitivity. This relationship between visceral fat and insulin sensitivity is well known (6), and thus preferential loss of visceral fat and, by implication, decreased visceral lipolysis after gastric bypass may be major factors in the improvement in insulin sensitivity after gastric bypass.

Table 2. Plasma insulin concentrations, GIRs required to maintain euglycemia, EGP, and Rd at baseline and at steady state during LDI and HDI infusions

<table>
<thead>
<tr>
<th></th>
<th>Insulin, pmol/l</th>
<th>GIR, µmol·kg⁻¹·min⁻¹</th>
<th>EGP, µmol·kg⁻¹·min⁻¹</th>
<th>Rd, µmol·kg⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>LDI</td>
<td>HDI</td>
<td>Baseline</td>
</tr>
<tr>
<td>GB</td>
<td>31 ± 4*</td>
<td>48 ± 4*</td>
<td>133 ± 6†</td>
<td>8.0 ± 1.4</td>
</tr>
<tr>
<td>Lean</td>
<td>24 ± 2*</td>
<td>59 ± 4*</td>
<td>191 ± 14</td>
<td>12.2 ± 2.1*</td>
</tr>
<tr>
<td>Obese</td>
<td>75 ± 7</td>
<td>101 ± 11</td>
<td>206 ± 18</td>
<td>4.6 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. GIR, glucose infusion rate; EGP, endogenous glucose production; Rd, rate of disappearance; LDI, low-dose insulin; HDI, high-dose insulin. *P < 0.05 vs. obese; †P < 0.05 vs. lean.

concentrations, was similar between lean subjects and gastric bypass patients was surprising because gastric bypass subjects had a significantly greater total body fat mass, total abdominal fat, abdominal subcutaneous fat, and percent body fat. The EC₅₀ has been used previously to analyze differences in the sensitivity of FFA release with varying plasma concentrations of insulin (11) and in our study demonstrates a dramatic improvement in adipose tissue insulin sensitivity. Adjusting palmitate Ra for differences in insulin concentrations produced similar results, particularly at HDI, where insulin concentrations were similar in lean and post-gastric bypass subjects.

A secondary finding of this study was that whole body insulin sensitivity, as measured by glucose infusion rate, was greater in gastric bypass subjects compared with obese subjects but still not as high as in lean subjects. Correspondingly, endogenous glucose production tended to be greater in obese subjects during insulin infusions than in individuals that had undergone gastric bypass surgery. Finally, we found that there is a highly significant relationship between the sensitivity of adipose tissue lipolysis and whole body glucose insulin sensitivity. Taken together, our results show that insulin sensitivity in individuals that have undergone gastric bypass surgery is overall more similar to lean rather than to obese individuals.

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Fig. 3. Effective insulin concentration required for half-maximal suppression of plasma FFA concentrations (EC₅₀) during 2-step hyperinsulinenic glucose clamp. *P < 0.05 vs. gastric bypass and lean. Black bars, gastric bypass.

Fig. 4. Lipolysis [palmitate rate of appearance (Ra)] normalized using log-log linear regression to the average insulin concentrations in lean subjects during LDI and HDI infusions of the hyperinsulinenic euglycemic clamp. *P < 0.05 vs. gastric bypass and lean. Black bars, gastric bypass; open bars, lean; hatched bars, obese.
Unfortunately, even longitudinal studies have not been able to provide satisfactory mechanistic explanations of the improvement in insulin sensitivity after gastric bypass surgery. Although it is well known that insulin sensitivity improves within days after gastric bypass surgery, this initial improvement is likely related to caloric restriction (8). In fact, there is evidence that FFAs increase immediately after surgery, presumably because of increased lipolysis as reductions in adipose tissue occur (12). Long-term insulin sensitivity improvements are less well understood but appear to be more than expected for the amount of weight lost (3), and preferential loss of fat may be a key factor (7). In support of this, we found that the improvement in adipose tissue insulin sensitivity was greater than might be expected based on the weight and overall body fat of the gastric bypass subjects and suggests that the preferential loss of visceral fat is important in the improvement of insulin sensitivity. However, we recognize that abnormalities in visceral lipolysis do not necessarily correlate with visceral fat mass (13) and that subtle abnormalities in visceral lipolysis are difficult to detect with measurement of systemic kinetics (21). Therefore, it remains possible that visceral lipolysis is not completely normal in gastric bypass patients, which could explain the abnormal sensitivity of glucose disposal that is still present to some degree in the gastric bypass subjects (25).

Direct measurements of adipose tissue lipolysis would be required to determine whether there are differences in metabolic activity. Finally, other adipose tissue-related factors may also be important. Circulating hormones associated with adipose tissue (e.g., leptin) may also play a role in insulin sensitivity (17). We found that leptin is decreased after gastric bypass and was correlated with insulin sensitivity, but whether this is simply due to a reduction in leptin-secreting adipose tissue or provides a mechanistic explanation is not known.

Although this is the first report of adipose tissue insulin sensitivity in individuals that have undergone gastric bypass surgery, our study was not a prospective study of patients before and after gastric bypass. Thus, further studies are needed to determine the relationship between longitudinal changes in adipose tissue insulin sensitivity and visceral fat loss. However, we were able to demonstrate that adipose tissue insulin sensitivity is greater compared with obese subjects and almost identical to lean subjects 3 yr after surgery. Additionally, the BMI of our gastric bypass subjects was significantly different from both control groups, which makes direct comparisons difficult. We also found differences in plasma insulin concentrations between subject groups, including during the clamp despite our infusion rates for FFM being normalized. Our data cannot address whether this is due to decreased endogenous insulin production or increased clearance after gastric bypass surgery, but if a reduction in the visceral fat depot leads to lower portal venous plasma FFA concentrations, improvement in hepatic insulin clearance would be a plausible consequence (23).

However, considering that analysis of insulin sensitivity of lipolysis demonstrated improved sensitivity in gastric bypass subjects compared with obese subjects, the impact of these lower insulin levels does not alter interpretation of our results and in fact, if anything, leads to a conservative error. Finally, most of the subjects in this study were women. Men may have different weight loss responses to gastric bypass surgery, and this may be important in the insulin sensitivity response to the surgery. Future studies are needed to determine whether there are differences between men and women in the sensitivity of lipolysis to insulin after gastric bypass surgery.

In summary, the well-described, sustained weight loss after gastric bypass surgery is characterized by improvements in adipose tissue insulin sensitivity to levels near that of lean, healthy persons and also by significant improvements in the response of glucose metabolism to insulin. This improvement in insulin sensitivity is associated with a preferential reduction in visceral fat. A reduction in visceral lipolysis may be responsible for the overall improvement in insulin sensitivity, although further research on the relative contribution of abnormalities in lipolysis in visceral vs. subcutaneous fat to insulin resistance is needed to fully understand the role of circulating FFAs.

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DISCLOSURES

The contents of this article are solely the responsibility of the authors and do not necessarily represent the official views of the Mayo Foundation or the National Institutes of Health. No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES