Improvement in β-cell function in patients with normal and hyperglycemia following Roux-en-Y gastric bypass surgery

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Lin E, Liang Z, Frediani J, Davis SS Jr, Sweeney JF, Ziegler TR, Phillips LS, Gletsu-Miller N. Improvement in β-cell function in patients with normal and hyperglycemia following Roux-en-Y gastric bypass surgery. Am J Physiol Endocrinol Metab 299: E706–E712, 2010. First published August 17, 2010; doi:10.1152/ajpendo.00405.2010.—Glycemic disorders resolve following Roux-en-Y gastric bypass (RYGB) surgery, but early and longer-term mechanisms regarding effects on β-cell dysfunction as well as relationships with decreasing adiposity are not well understood. We evaluated longitudinal changes in peripheral insulin sensitivity (Si), the acute insulin response to glucose (AIRg), and the composite estimate of β-cell function, the disposition index (DI), over 24 mo via frequently sampled intravenous glucose tolerance testing in severely obese women who had fasting normoglycemia (n = 16) and hyperglycemia (n = 11) before RYGB surgery; homeostatic model assessment (HOMA-IR) estimated insulin resistance; air displacement plethysmography determined adipose tissue mass. At baseline, subjects with normoglycemia had adequate DI associated with elevated AIRg, but DI was markedly reduced in subjects with hyperglycemia. Within 1–6 mo post-RYGB, glycemic control was normalized in subjects with hyperglycemia related to reduced HOMA-IR (−54% at 1 mo, P < 0.005) and increased DI (23-fold at 6 mo vs. baseline, P < 0.05). Over 24 mo, DI improved in subjects with hyperglycemia (15-fold vs. baseline, P < 0.005) and also modestly in subjects with normoglycemia (58%, P < 0.05), due largely to increased Si. Decreasing adiposity correlated with longer-term HOMA-IR and Si values at 6 and 24 mo, respectively. In patients exhibiting fasting hyperglycemia before surgery, β-cell function improved early following RYGB, due largely to increases in insulin secretion. For both normoglycemic and hyperglycemic subjects, further improvement or stabilization of β-cell function over the 2 yr is due largely to improved Si associated with reduced adiposity.

disposition index; weight loss; adipose tissue mass

SEVERE OBESITY, DEFINED AS body mass index greater than 40 kg/m², is associated with an increased prevalence of type 2 diabetes (T2DM) (5). Bariatric surgery is the most efficient method of producing substantial weight loss in severely obese patients (42). Surgical procedures induce weight loss by reducing the volume of the stomach, thereby restricting food intake, or by diverting the flow of ingested food to the ileum and bypassing the duodenum and the proximal jejunum, which promotes malabsorption (39). Roux-en-Y gastric bypass (RYGB) is a common and restrictive plus malabsorptive procedure; other procedures include adjustable gastric banding, a restrictive-only surgery, and biliopancreatic diversion, a primarily malabsorptive procedure that is less common in the US. (39).

As has been found in overweight and obese individuals (47), severely obese persons with prediabetes and diabetes have a decline in β-cell function such that insulin secretion is inadequate to compensate for reduced insulin sensitivity (Si) (19, 37). A special feature of bariatric surgery is its ability to induce remission of T2DM (9). Following adjustable gastric banding, remission of diabetes is strongly related to weight loss (15, 38). However, a greater effect of diabetes resolution is seen with malabsorptive surgeries RYGB and biliopancreatic diversion (9), which can induce normalization of glucose within weeks, before appreciable weight loss occurs. This suggests that rapid remission of diabetes is related to mechanisms that are independent of weight loss.

Diabetes remission early following surgery, observed exclusively following RYGB and biliopancreatic diversion, has been shown to be related to dramatic improvements in insulin secretion (25, 34, 41) and decreases in the HOMA-IR index (4, 34, 36), a surrogate of Si that primarily reflects hepatic insulin resistance. Improvement in β-cell function is also thought to be the basis for improvement in glucose levels following bariatric surgery, but little information currently exists on effects of bariatric surgery on β-cell function. β-Cell function can be assessed in vivo using the disposition index (DI) constant, which describes an individual’s insulin secretion response for a prevailing level of Si. However, rarely have both insulin secretion and Si been measured longitudinally following bariatric surgery using detailed methods (19, 22, 41). In patients with prediabetes and diabetes undergoing RYGB and biliopancreatic diversion, improvement in the DI was found in subjects early (1–7 mo) following surgery (19, 41). The longer-term effects of bariatric surgery on patients with euglycemia and hyperglycemia remain unanswered.

Given the popularity of RYGB, the mechanisms responsible for its antidiabetic effects are needed. The purpose of this study was to determine the early (1 and 6 mo) and longer-term (24 mo) impact of RYGB on the DI, as a primary end point, in severely obese women who were normoglycemic or exhibited hyperglycemia (prediabetes and T2DM) at baseline. The contribution of decreasing adiposity to Si and secretion outcomes following surgery was also examined.

MATERIALS AND METHODS

Patients. Subjects in the study were 27 consecutively enrolled severely obese female patients who had weight loss treatment at the Emory Bariatrics Center via laparoscopic RYGB surgery by a single surgeon (E. Lin), as has been described (28). Each patient served as her own control, and subjects were evaluated at baseline (before
surgery) and at 1 mo (38 ± 2 days, n = 27), 6 mo (177 ± 12 days, n = 27), and 24 mo (823 ± 36 days, n = 15) following surgery. Based on baseline fasting blood glucose concentrations, subjects were categorized as having normoglycemia (<5.56 mmol/l, n = 16) or hyperglycemia (≥5.56 mmol/l, n = 11; 4 patients were categorized as having prediabetes, and 11 had T2DM [3]). For the study’s primary end point, the DI, a prior study of subjects with T2DM showed an improvement in DI of 83-fold during 7 mo following RYGB and BPD, suggesting a sample size of n = 6, β = 0.89, α = 0.05 (19). The Emory University Institutional Review Board approved the study, and all patients gave informed consent (IRB no. 333-2002). Subjects were monitored throughout the study.

Glucose tolerance testing. The insulin-modified frequently sampled intravenous glucose tolerance test (FSIGTT) (29) was chosen to assess insulin action in vivo, as it provides information about peripheral SI and first-phase insulin secretion (AIRg) for the calculation of the DI in a single test. Patients were admitted to the Emory General Clinical Research Center on the night before FSIGTT testing and fasted overnight (12 h). Before testing, diabetes medication use was adjusted so that baseline glucose levels were close to normal, but medications were withheld from patients on the morning of testing. Serum glucose was quantified at the Emory University Hospital Laboratory using the Beckman Coulter Alex 20 automated system; assay limit 0.17 mM (Beckman Coulter, Brea, CA). Insulin was measured by regular and ultrasensitive immunoassay; assay limits 1 μU/ml and 0.07 μU/ml, respectively, with less than 1% cross-reactivity to proinsulin and C-peptide (Mercordia, Winston Salem, NC). Minimal-modeling analysis (6) was used to quantify SI, AIRg, and DI (MinMod Millennium, Los Angeles, CA; http://research.vet.upenn.edu/biomath/CurrentProjects/DiabetesGlucoseMetabolism/tabid/1622/Default.aspx). HOMA-IR was calculated using fasting insulin (mU/l) × fasting glucose (mM)/22.5 (32). To distinguish insulin secretion and hepatic insulin clearance, C-peptide concentrations were measured for a limited number of subjects during each of the time points for a 24-mo period (ARUP Laboratories, Salt Lake City, UT). The assay limit is 0.1 ng/ml, and the coefficient of variation is <10%. Insulin and C-peptide concentrations during first-phase insulin response to the IVGTT are plotted for a representative subject in Supplemental Fig. S3 (Supplemental materials are found in the online version of this paper at the journal website).

Responses of insulin and C-peptide in the first 10 min of the FSIGTT occurred in parallel, suggesting a close approximation between determined AIRg and insulin secretion. Comparisons with published values of reference controls (23, 44) were possible, as similar populations and FSIGTT methodology were used, and glucose/insulin assays had comparable specificity and sensitivity.

Anthropometry, body composition and fat distribution. Body fat composition was measured by air plethysmography (BOD-POD; Life Measurement Instruments, Concord, CA). We found that the coefficient of variation and the measurement error were similar to published values (33). Waist circumference was obtained by tape measure at the smallest point of the torso below the most inferior rib. Body height was measured without shoes. Body weight was measured with subjects in light clothing, in the fasting state, and immediately after voiding in the morning.

Statistical analysis. The statistical software packages STATISTICA (StatSoft, Tulsa, OK) was used for analysis. Analysis of variance with repeated measures was used to determine group differences and changes over time; post hoc comparisons used Tukey’s test. Only when indicated in the text, a paired t-test was used to test for differences between time points within the same group, but this analysis is less conservative. Relationships among measurements were examined as standard multiple regression analysis, covariates tested were age, glycemic status, and race/ethnicity group at baseline. Data are presented separately for 0–6 mo for the entire group of 27 subjects and from 0–24 mo for the subgroup of 15 subjects, although similar trends were observed over the 0–6 mo period for all subjects; data were compared using unpaired t-tests. χ² Analysis was used to compare baseline proportions race and glycemic status of 24-mo completers vs. noncompleters. Results are expressed as means ± SE.

RESULTS

Baseline patient characteristics. Patient characteristics are described in Table 1. Twenty-seven subjects completed longitudinal analysis up to 6 mo postsurgery, and fifteen of these were evaluated again at 24 mo. The average age, BMI, postmenopausal status, and race/ethnicity were comparable between normoglycemic and hyperglycemic groups. Of the 12 patients not measured at 24 mo, two were ineligible due to pregnancy, two were undergoing serious illnesses unrelated to surgery, five were lost to follow-up due to moving from the area or inability to contact, and three had not reached the 24-mo time point. However, we found no differences in baseline or in 6-mo characteristics and measurements in subjects who completed vs. those who did not complete the 24-mo assessment. For example, there was no difference in weight loss at 6 mo for 24-mo completers vs. noncompleters (P = 0.51). Also, the glycemic status of completers vs. noncompleters was comparable (P = 0.48).

Changes in adiposity following weight loss surgery. Anthropometric values at baseline and changes following RYGB for normoglycemic and hyperglycemic subjects are shown in Table 2 for 27 subjects followed out to 6 mo and in Supplemental Table S1 for the 15 subjects followed out to 24 mo. At baseline, there were no differences in adiposity between the normoglycemic and hyperglycemic groups. For all subjects, body weight, body mass index, and body fat mass decreased as early as 1 mo and continued to decrease at 6 and 24 mo postsurgery (P < 0.05 for 1 mo, P < 0.005 for 6 and 24 mo). For both groups, decreases in waist circumference were evident at 6 and 24 mo following surgery (both P < 0.005). Body fat mass decreased similarly among normoglycemic and hyperglycemic subjects (average -12.3% and -14.2%, respectively, at 1 mo, and -43.1% and -43.2% at 6 mo, vs. baseline). Also, over the 24-mo

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics</th>
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<tbody>
<tr>
<td>Normal Glycemia</td>
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<tr>
<td>n</td>
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<tr>
<td>Age, yr</td>
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<tr>
<td>Body mass index, kg/m²</td>
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<tr>
<td>Ethnicity, %total population: black; white; Hispanic</td>
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<tr>
<td>Postmenopausal status, %total population</td>
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<tr>
<td>Antihyperglycemic medication use, %population</td>
</tr>
</tbody>
</table>

Values are means ± SE. Baseline characteristics of 27 subjects completed longitudinal analysis ≥6 mo postsurgery, of these subjects, 15 were evaluated again at 24 mo. Patients exhibiting normal glycemia had fasting plasma glucose measures <5.6 mmol/l; patients exhibiting hyperglycemia had measures >5.6 mmol/l.
Anthropometric and glycemic responses during 6 mo following RYGB

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1 mo</th>
<th>Δ 1 mo</th>
<th>6 mo</th>
<th>Δ 6 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>Normal glycemia</td>
<td>47.4 ± 0.9</td>
<td>−4.5 ± 0.4</td>
<td>34.1 ± 1.0</td>
<td>−13.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Hyperglycemia</td>
<td>48.5 ± 1.3</td>
<td>−4.8 ± 0.4</td>
<td>35.0 ± 1.8</td>
<td>−13.5 ± 0.9</td>
</tr>
<tr>
<td><strong>Body weight, kg</strong></td>
<td>Normal glycemia</td>
<td>126.6 ± 2.7</td>
<td>−12.2 ± 1.0</td>
<td>114.4 ± 2.6x</td>
<td>−35.5 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>Hyperglycemia</td>
<td>125.3 ± 3.7</td>
<td>−12.6 ± 1.3</td>
<td>112.7 ± 3.5x</td>
<td>−34.7 ± 2.3</td>
</tr>
<tr>
<td><strong>Body fat mass, kg</strong></td>
<td>Normal glycemia</td>
<td>71.2 ± 2.4</td>
<td>−12.0 ± 3.3</td>
<td>63.1 ± 2.3x</td>
<td>−30.4 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>Hyperglycemia</td>
<td>71.8 ± 3.0</td>
<td>−10.3 ± 1.2</td>
<td>61.5 ± 2.7x</td>
<td>−30.3 ± 2.1</td>
</tr>
<tr>
<td><strong>Waist circumference, cm</strong></td>
<td>Normal glycemia</td>
<td>132.0 ± 4.1</td>
<td>−4.9 ± 1.3</td>
<td>126.6 ± 4.3</td>
<td>−21.5 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>Hyperglycemia</td>
<td>132.4 ± 4.9</td>
<td>−5.7 ± 2.3</td>
<td>126.7 ± 3.7</td>
<td>−22.4 ± 2.1</td>
</tr>
<tr>
<td><strong>Glucose, mmol/l</strong></td>
<td>Normal glycemia</td>
<td>4.63 ± 0.11</td>
<td>−0.68 ± 0.11</td>
<td>3.94 ± 0.11x</td>
<td>−0.60 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>Hyperglycemia</td>
<td>6.94 ± 0.33b</td>
<td>−1.79 ± 0.44</td>
<td>5.15 ± 0.38x</td>
<td>−2.43 ± 0.42</td>
</tr>
<tr>
<td><strong>Hemoglobin A₁c, %</strong></td>
<td>Normal glycemia</td>
<td>5.2 ± 0.2a</td>
<td>−0.1 ± 0.2</td>
<td>5.1 ± 0.1</td>
<td>−0.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Hyperglycemia</td>
<td>7.6 ± 0.7b</td>
<td>−0.9 ± 0.5</td>
<td>6.8 ± 0.4</td>
<td>−1.8 ± 0.8</td>
</tr>
<tr>
<td><strong>Insulin, mU/l</strong></td>
<td>Normal glycemia</td>
<td>13.49 ± 1.44</td>
<td>−7.50 ± 1.23</td>
<td>6.00 ± 0.77x</td>
<td>−3.47 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>Hyperglycemia</td>
<td>13.63 ± 1.29</td>
<td>−5.63 ± 1.52</td>
<td>7.99 ± 0.94x</td>
<td>−5.74 ± 1.37</td>
</tr>
<tr>
<td><strong>HOMA-IR, mU/l·mM</strong></td>
<td>Normal glycemia</td>
<td>2.79 ± 0.32a</td>
<td>−1.74 ± 0.28</td>
<td>1.06 ± 0.14x</td>
<td>−0.62 ± 0.08x</td>
</tr>
<tr>
<td></td>
<td>Hyperglycemia</td>
<td>4.27 ± 0.50b</td>
<td>−2.32 ± 0.57</td>
<td>1.95 ± 0.38x</td>
<td>−1.28 ± 0.43</td>
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</table>

Values are means ± SE. Parameters were determined in 27 subjects with normal glycemia (n = 16) and hyperglycemia (n = 11) who were measured during 6 mo, following Roux-en-Y gastric bypass (RYGB) surgery. *P < 0.05, †P < 0.01, ‡P < 0.005 vs. baseline. "Changes in insulin action following surgery. Peripheral SI occurred at 1 mo, associated with dramatic decreases in values with further decreases occurring at 6 and 24 mo following surgery (all time points, P < 0.005 vs. baseline)."
sentiment by the DI is well described (7, 24); changes to the DI result from increases in AIRg or Si or both. Graphic representation of this relationship based on longitudinal measurements of both parameters is presented in Fig. 2 (for subjects followed out to 24 mo). For subjects with normoglycemia, DI changed little over 6 mo, reflecting reciprocal changes in Si and AIRg. In contrast, DI improved over 6 mo in subjects with hyperglycemia (23-fold vs. baseline, $P < 0.05$), largely associated with improved AIRg and Si. Over 24 mo, DI improved further, as seen by shifts to the right in the curve in both groups (by 58% vs. baseline, $P < 0.05$ in the normoglycemic group; 15-fold in the hyperglycemic group, $P < 0.005$), reflecting consistent improvement in Si without further change in AIRg.

Cross-sectional associations between fat mass and peripheral Si at baseline and 1, 6, and 24 mo following surgery were determined using multiple regression analysis; β-statistic, and associated $P$ values are presented ($n = 27$). Longitudinal association between change ($Δ$) in fat mass and the final value of Si at 24 mo following surgery. Significant associations are highlighted in boldface ($n = 15$). Associations remained significant after adjusting for race and glycemic status at baseline, but not age.

### Associations among adiposity and measures of insulin action.

We tested for cross-sectional and longitudinal associations between adiposity and in vivo measurements of insulin action using multiple regression analysis. At baseline, no cross-sectional relationships were found between fat mass and AIRg, Si, or DI. Associations between fat mass and Si grew stronger as time progressed following surgery, so that at 24 mo a significant negative association was observed ($β = −0.56, P = 0.04$; Table 4). Also, a greater adipose tissue decrease over 24 mo negatively predicted Si at that time point ($β = −0.58, P = 0.038$). These relationships remained significant after adjustment for race and baseline glycemic status, but not age (data not shown). As found for Si, associations between HOMA-IR and fat mass grew progressively stronger with weight loss (Table 5), with significant cross-sectional associations independent of baseline age, race, and glycemic status at 6 mo ($β = 0.60, P = 0.001$). At 24 mo, the cross-sectional association between adipose tissue mass and HOMA-IR was also significant ($β = 0.71, P = 0.004$), but this was not independent of age, race, and glycemic status. Greater decrease in adipose tissue mass at 6 and 24 mo was a determinant of HOMA-IR values at those respective time points ($β = 0.53, P < 0.004$, independent of age, race, and glycemic status; $β = 0.61, P = 0.02$, respectively). No cross-sectional or longitudi-
Improvements in Si occurred as subjects continued to lose weight, primarily related to an increase in insulin secretion. The present data show, within 6 mo post-RYGB surgery, an acute resistance. Consistent with these studies (19, 21, 41) and RYGB surgery (19, 25, 27, 46) as well as lifestyle interventions (13, 43) and pharmacotherapy (10, 45). However, only a few longitudinal studies of bariatric surgery (19, 21, 41) have simultaneously measured both insulin secretion and peripheral Si and thus derived the DI, an estimate that adjusts for the response of β-cell function following therapy, our finding is novel in that it shows that improvement in β-cell function is sustained in patients with varying presurgery glycemic status. Previous findings in normoglycemic subjects at 24 mo following biliopancreatic diversion did not show changes in β-cell glucose sensitivity measured by OGTT (12, 30). The reasons for this discrepancy are unclear, since the increase in peripheral Si in the present study was similar in magnitude to that observed in patients undergoing biliopancreatic diversion (11). It is likely that variables measured by OGTT may provide different characteristics of β-cell function than those estimated by IVGTT (31). Improvement in DI was demonstrated in subjects treated with the dipeptidyl peptidase-4 inhibitor vildaglaptin for 6 wk (45) and also with exenatide for 1 yr (10), but these effects were not sustained after washout of the drugs. No change in β-cell function was observed in subjects with impaired glucose tolerance following lifestyle intervention for 24 mo (13). A recent study demonstrated improved DI in normoglycemic subjects who underwent exercise training for 8 mo (43); since very little weight loss was reported in this study, a longer follow-up of these individuals would be important to determine whether these improvements were maintained. In the present study, improvements in β-cell function and expansion of β-cell reserve appear to be sustained over the long term, associated with dramatic weight loss and improvements in peripheral insulin action. This finding therefore highlights the unique ability for bariatric surgery to protect against future risk of diabetes.

The early improvement in glycemic status observed following RYGB surgery was also related to decreases in HOMA-IR in all groups. Parallel decreases were observed over the first 6 mo in normo- and hyperglycemic subjects, and steady states were achieved by 24 mo. Early and long-term decreases in HOMA-IR following various bariatric surgeries have been frequently reported (4, 8, 34, 36). Although fat mass decreased by −13% from baseline to 1 mo following surgery, cross-sectional and longitudinal associations between fat mass and HOMA-IR over that time period were not observed, suggesting that mechanisms responsible for improvement in hepatic Si may be related to caloric restriction rather than decreasing adiposity (17). Laferriere et al. (25) demonstrated equivalent decreases in HOMA-IR in severely obese individuals following weight loss of 9% (initial body weight) via RYGB or dietary restriction; thus, mechanisms responsible for improvement in HOMA-IR may not be limited to gastric bypass surgery.

Although the negative relationship between adiposity and Si is well described (1), we did not find that body fat mass was correlated with HOMA-IR or Si values at baseline. An explanation may be that effects of adiposity on Si may be nonlinear
in the severely obese state (35). We found that correlations among body fat and both peripheral and hepatic SI grew stronger as subjects approached normal weight, suggesting that other mediators confound the relationship when fat mass is excessive. Consistent with other studies (25, 35, 41), our findings demonstrate that during weight loss early improvements in SI and insulin secretion do not appear to be related to loss of adipose tissue. Subjects who experienced greater loss of adipose tissue at 6 and 24 mo had a lower HOMA-IR and higher peripheral SI at those respective time points, which suggests that decreasing adipose tissue had longer-term effects on both hepatic and peripheral SI.

Our study has limitations. Only 56% of initial subjects were followed to 24 mo, and this is a substantial limitation of the study, as it may have led to the error of selection bias. However, at baseline and 1- and 6-mo time points, there were no differences in study end points between patients studied at 6 mo and those followed up to 24 mo. For example, subjects who were followed up at 24 mo did not experience greater weight loss at 6 mo than those who were not followed. Also, similar proportions of normoglycemic and hyperglycemic subjects were followed for the entire study. Finally, given the individual reasons presented for lack of follow-up, it does not appear that some members were more likely to be measured than others. We did not determine the contribution of an incretin effect over the short term following RYGB, although an increase compared with baseline measurements was observed in glucagon-like peptide-1 at 24 mo following surgery, and this was not correlated to changes in AIRg, SI, or DI (data not shown). Glycemic status at baseline was assessed using fasting glucose and not by OGTT, which could distinguish patients who were truly glucose tolerant from those who had impaired glucose tolerance but normal fasting glucose (2). Although our study was adequately powered to measure changes in the DI (19), apparent increases in AIRg early following surgery might not have reached significance due to small sample size. We did not subject patients to a substantial washout of diabetes medications; thus, improvements in insulin action may be underestimated. Changes in diet may contribute to improvements in insulin action following RYGB surgery; although we have reported decreases in energy intake following RYGB (20), we did not collect dietary information on subjects in the current study. Our findings may also be limited to subjects who are severely obese and perhaps only to females within this population. However, the study population is fairly representative of those undergoing bariatric surgery in the United States in terms of sex, ethnicity, and ranges in body mass index (BMI) with a somewhat lower BMI compared with the national sample (10). Factors that may have contributed to this lower BMI include the use of detailed screening methods and the definition of severe obesity used in the study. In addition, we were able to follow subjects for up to 24 mo, and this is a substantial limitation of the study, as it may have led to the error of selection bias.

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REFERENCES


