Gastric Bypass Alters Both Glucose-Dependent and Glucose-Independent Regulation of Islet Hormone Secretion

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Objective: Roux-en-Y gastric bypass surgery (GB) is characterized by accentuated but short-lived post-prandial elevations of blood glucose and insulin. This profile has been attributed to effects of relative hyperglycemia to directly stimulate β-cells and an augmented incretin effect. An additional glucose-independent stimulation of insulin secretion in GB subjects was hypothesized.

Methods: Fifteen subjects with prior GB, six matched obese non surgical controls, and seven lean individuals were recruited. Islet hormones were measured before and after meal ingestion during hyperinsulinemic hypoglycemic clamps to minimize the direct effects of glycemia and glucose-dependent gastrointestinal hormones on insulin secretion.

Results: The GB subjects had less suppression of fasting β-cell secretion during the insulin clamp compared to controls. In addition, meal-induced insulin secretion increased in the GB subjects but not controls during fixed sub-basal glycemia. In contrast, the glucagon responses to hypoglycemia and meal ingestion were lower in the GB subjects than controls.

Conclusions: Among subjects with GB, the response of insulin and glucagon secretion to decreasing blood glucose is blunted, but meal-induced insulin secretion is stimulated even at fixed systemic sub-basal glycemia. These findings indicate that, following GB, islet hormone secretion is altered as a result of factors beyond circulatory glucose levels.

Introduction

The past decade has seen a dramatic increase in the use of bariatric surgery for weight loss and mitigation of the co-morbidities of obesity (1). Gastric bypass (GB) is one of the most common and effective procedures, involving the creation of a small gastric pouch connected to the jejunum with diversion of meal contents to the mid-gut. In addition to reducing food intake and causing weight loss, GB has dramatic effects on the regulation of blood glucose (2,3). Because individuals with GB have rapid passage of ingested nutrients into the intestine, their blood glucose levels rise rapidly after meals, achieving earlier and higher glycemic peaks followed by lower nadirs (4,5). These changes in postprandial glucose are associated with increased insulin and GLP-1 responses (6). The commonly held explanation for the meal-induced hyperinsulinemia typical of GB is that stimulation of β-cells by glucose and incretins is enhanced (6,7). However, we recently observed that subjects with GB have increased insulin secretion rates in the latter phases of meal absorption when blood glucose and GLP-1 levels have declined to near basal levels (8). This finding suggests that factors beyond direct glucose stimulation and glucose potentiation of incretins act on the β-cell in persons with GB.

In the present study we measured the islet cell response to meal ingestion during a period of fixed, mild hypoglycemia (3-3.5 mmol l$^{-1}$) using a hyperinsulinemic (80 mU m$^{-2}$ min$^{-1}$) glucose clamp (9,10) in GB subjects compared to non surgical controls. This approach eliminates the direct effects of increasing blood glucose to stimulate the β-cells and neutralizes the insulinotropic actions of the incretins, which are dependent on hyperglycemia (11-13). We hypothesized that subjects with GB would have increased meal-stimulated β-cell responses during sub-basal glycemic levels.
Methods

Subjects
Fifteen subjects with previous GB were recruited from the Endocrinology clinics at the University of Cincinnati as well as by general advertisement. Six subjects without prior surgery, matched for BMI and age of the GB subjects, were recruited as controls for the GB subjects (Obese-Controls, O-CON), as well as a group of seven lean young subjects (L-CON) to approximate the normative range independent of BMI and age in non surgical individuals. The GB subjects did not have a prior history of diabetes, had an average of 54 ± 5 kg (19-80 kg) of weight loss in a mean of 5.9 ± 0.5 years (3-8 years) since surgery, and had been weight stable for at least 6 months prior to the studies. The control subjects had no personal or family history of diabetes and had normal oral glucose tolerance tests before enrollment. All subjects were free of active gastrointestinal disease, renal dysfunction, or liver disorders and none took any medications that interfere with glucose metabolism or blood pressure for at least 1 week prior to the studies. The institutional review board of the University of Cincinnati approved the protocol, and all participants provided written informed consent before participating in any experiments.

Experimental protocols
All studies were performed at the Clinical and Translational Research Center at Cincinnati Children’s Hospital in the morning after an overnight fast. Participants were instructed to maintain normal carbohydrate ingestion for 3 days before each visit, and not to engage in excessive physical activity. On the first day of study, body composition was assessed using dual-energy X-ray absorptiometry only in GB and O-CON subjects. For glucose clamps, intravenous catheters were placed in each forearm for the withdrawal of blood and the infusion of insulin and glucose; the arm used for blood sampling was continuously warmed with a heating pad to maintain consistent blood flow.

Hyperinsulinemic clamp/MTT
The insulin infusate consisted of recombinant human insulin (Humulin 100 U ml⁻¹) diluted in isotonic saline/25% human serum albumin. After withdrawal of three fasting blood samples, a 10-min priming infusion of insulin was followed by constant administration of 80 mU m⁻² surface area per minute for the duration of the study (14). Blood was sampled at 5-min intervals and a variable infusion of 20% dextrose was infused to clamp blood glucose at a target of 3-3.5 mmol l⁻¹. At 120 min, a 140-ml liquid mixed meal containing 3 kcal kg⁻¹ distributed as 40% protein, 40% fat, and 20% glucose was consumed within 10 min. Blood samples were removed at timed intervals and stored on ice, plasma was separated within 60 min, and these were stored at −80°C until assay. Each subject’s heart rate was monitored throughout the studies using the GE Dash 3000 monitoring system (GE Healthcare, Milwaukee, WI) with values averaged over every 5-min period throughout the study.

Calculations and analysis
Fasting values of blood glucose and hormones were computed as the average of the three samples drawn before the clamp, and the pre-meal values as the average of the samples drawn in the 20 min before the test meal (100-120 min). The effects of the sub-basal hyperinsulinemic clamp on islet hormone release and heart rates were computed as: [(fasting values–premeal values)/fasting values] × 100. Islet hormone responses to the test meal were summarized as the incremental areas under the curve (AUC) over premeal values using the trapezoidal rule. Postprandial changes in heart rates were calculated as: [(postprandial values–premeal values)/premeal values] × 100. Insulin sensitivity was computed as the average of the glucose infusion rate from 100-120 min divided by mean plasma insulin levels over the same period (16). Fasting clearance rates of insulin were calculated from the insulin infusion rate divided by the steady-state insulin concentrations at 100-120 min corrected for endogenous insulin secretion (16). Systemic appearance of ingested glucose (RaOral) was computed as the integrated reduction of glucose infused after meal ingestion (9,10).

Statistical analysis
Data are presented as mean ± SEM. The parameters of interest at baseline and during the hyperinsulinemic clamp were compared using ANOVA based on pre-specified comparisons between GB and O-CON as well as GB and L-CON. Statistical analyses were performed using SPSS 22 (SPSS, Chicago, IL).

Results

Subject characteristics
The GB and the O-CON subjects had similar BMI, fat and lean body mass, age, A1C, and female to male ratio (Table 1). The L-CON subjects were younger and leaner than the GB subjects.

Glucose clamp and insulin sensitivity
Glucose levels at baseline were similar among the three groups (Table 1) and decreased rapidly to the target with infusion of insulin (GB: 3.3 ± 0.1, O-CON: 3.3 ± 0.1, and L-CON: 3.0 ± 0.1 mmol l⁻¹; Figure 1A). The mean coefficient of variation of blood glucose levels from 40-120 min was 5% ± 1% for the three groups. Basal insulin levels were significantly lower in the GB subjects compared to the O-CON individuals but they did not differ between the GB and L-CON groups (Table 1). A square wave of hyperinsulinemia was achieved in each of the three groups with mean steady-state (100-120 min) levels of 669 ± 49, 909 ± 78, and 647 ± 42 pmol l⁻¹ in the GB, O-CON, and L-CON subjects, respectively (Figure 1B), representing comparable increments over basal values in the groups.

The glucose infusion rate needed to achieve the premeal glycemic target was similar in the GB subjects compared to the L-CON subjects but there was a trend for lower value in the O-CON subjects compared to GB individuals (P = 0.13; Figure 1C). Accordingly, the GB subjects were more insulin sensitive and had greater insulin clearance measured by commercial radioimmunoassay (Millipore, Billerica, MA), and insulin (ALPCO Diagnostics, Salem, NH), GLP-1 and PP (Millipore, Billerica, MS) using commercial ELISA, according to the manufacturers’ specifications.
Islet Hormone Secretion at Sub-Basal Glucose in GB  Salehi et al.

TABLE 1 Baseline characteristics, glucose, and hormonal profile of study participants

|                     | GB (15) | O-CON (6) | L-CON (7) | GB vs. O-CON (P value) | GB vs. L-CON (P value) |
|---------------------|---------|-----------|-----------|------------------------|------------------------|
| Gender (M/F)        | 2/13    | 1/5       | 3/4       | 0.66                   | 0.05                   |
| Age (years)         | 50.9 ± 2.7 | 48.7 ± 1.9 | 20.7 ± 0.3 | 0.58               | <0.001               |
| Current BMI (kg m⁻²) | 32.0 ± 1.4 | 30.9 ± 2.7 | 23.1 ± 0.8 | 0.74               | <0.001               |
| Total fat mass (kg)  | 38 ± 4   | 33 ± 4    | -         | 0.41                 | -                     |
| Total lean mass (kg) | 56 ± 2   | 56 ± 3    | -         | 0.95                 | -                     |
| HbA1C (%)           | 5.2 ± 0.1 | 5.4 ± 0.2 | 4.8 ± 0.2 | 0.51                 | 0.08                  |
| Blood glucose (mmol l⁻¹) | 4.5 ± 0.1 | 4.8 ± 0.1 | 4.8 ± 0.1 | 0.10                 | 0.10                  |
| Insulin (pmol l⁻¹)   | 12 ± 3   | 69 ± 17   | 13 ± 5    | 0.04                 | 0.75                  |
| C-peptide (nmol l⁻¹) | 3.6 ± 0.3 | 6.0 ± 0.9 | 3.3 ± 0.6 | 0.42                 | 0.26                  |
| GLP-1 (pmol l⁻¹)     | 3.6 ± 0.3 | 3.1 ± 0.7 | 4.0 ± 0.7 | 0.59                 | 0.56                  |
| Glucagon (pg ml⁻¹)   | 33.5 ± 1.8 | 36.1 ± 1.6 | 39.6 ± 2.6 | 0.42                 | 0.06                  |
| Pancreatic polypeptide (pg ml⁻¹) | 287 ± 153 | 108 ± 56 | 34 ± 9 | 0.42                 | 0.26                  |
| Heart rate (bpm)     | 68 ± 6   | 68 ± 6    | 57 ± 2    | 0.42                 | 0.13                  |
| Insulin sensitivity (M/I) | 7.3 ± 0.9 | 4.2 ± 0.9 | 6.1 ± 0.5 | 0.05                 | 0.30                  |
| Insulin clearance (ml m⁻² min⁻¹) | 903 ± 61 | 682 ± 56 | 897 ± 52 | 0.04                 | 0.94                  |

Data are presented as mean ± SEM unless specified otherwise. P values for ANOVA or X² comparison between GB and O-CON and GB and L-CON are provided.

*Data were obtained in 10 GB and 5 O-CON subjects.

aBaseline values.

bDerived from clamp studies before meal ingestion.

compared to the O-CON subjects, while these parameters were similar among the GB subjects and L-CON individuals (Table 1).

Fasting islet hormone responses to the hyperinsulinemic glucose clamp

Fasting C-peptide values were lower in the GB compared to the matched O-CON subjects (Figure 2B, Table 1). With gradual reduction of blood glucose from basal during the clamp, endogenous insulin secretion, reflected by C-peptide levels, declined. Despite a significant difference in fasting C-peptide levels among GB and O-CON individuals, the absolute C-peptide levels from 100 to 120 min were similar in both groups. Thus the relative change in C-peptide in response to the clamp was significantly lower in GB patients compared to the O-CON subjects (GB: 76% ± 1%; P < 0.01; Figure 2B). Fasting C-peptide levels were comparable in the GB and L-CON subjects (Figure 2B, Table 1). However, the absolute C-peptide levels from 100 to 120 min were significantly greater in the GB subjects, indicative of a smaller relative rate of suppression of C-peptide as a result of the glucose clamp in the GB subjects compared to L-CON individuals (GB: 59 ± 4 and L-CON: 80% ± 3%; P < 0.01; Figure 2B).

Despite similar fasting glucagon levels among the groups (Table 1), the relative increase in premeal glucagon in response to glucose reduction during the hyperinsulinemic clamp was significantly greater in the L-CON compared to the GB subjects (GB: −21% ± 4% vs. L-CON: 49% ± 17%; P < 0.01; Figure 3B). Compared to the O-CON individuals, the GB subjects tended to reduce premeal glucagon with responses that were significantly lower than those of the matched non surgical group (GB: −21% ± 4% vs. O-CON: 0% ± 6%; P = 0.02; Figure 3B). Similarly, fasting PP levels did not differ among the three groups (Table 1), but followed a generally similar trajectory as glucagon. The L-CON subjects had the largest PP response to the premeal decrease in glycemia, with no significant differences between the GB and the O-CON groups (Figure 3A).

Fasting heart rates were similar among the surgical and nonsurgical groups (Table 1). Heart rates increased in response to the sub-basal glucose clamp in all subjects, with the largest enhancement in the L-CON individuals while the relative increase was similar in the BMI-matched controls and surgical groups (Figure 4A).

Figure 1 (A) Blood glucose and (B) insulin levels and (C) glucose infusion rates during hyperinsulinemic hypoglycemic clamp combined with MTT in GB subjects (solid black line, closed circle), matched obese controls (dashed black line, open circle), and lean controls (gray line, open circle).
Systemic appearance of ingested glucose

Following meal ingestion the glucose infusion rates were sharply reduced to compensate for glucose influx from the gut, but then gradually increased at a different pace among surgical and nonsurgical subjects (Figure 1C). Blood glucose was maintained at the target in all groups except for five subjects in the GB group, whose levels rose slightly above the target for a short period, yet remained below fasting levels (absolute difference between glycemia from 125-150 min and 100-120 min: 0.25 ± 0.07, 0.03 ± 0.1, and −0.03 ± 0.09 mmol l⁻¹ for GB, O-CON, and L-CON, respectively; Figure 1A). The average coefficient of variation for glucose concentration from 120-240 min was 9% ± 1%, 7% ± 1%, and 7% ± 1% for GB, O-CON, L-CON respectively. The completion of oral glucose absorption, marked by the return of glucose infusion rates to steady-state values, was achieved in surgical subjects over the 60 min following meal ingestion (Figure 1C). When the data are expressed as meal glucose appearance, it is apparent that postprandial glucose absorption occurred at a faster rate in the GB subjects (Figure 5).

Islet hormone responses to meal ingestion during a sub-basal hyperinsulinemic glucose clamp

Following meal ingestion, the L-CON and O-CON subjects had a minimal release of C-peptide (Figure 2B). In contrast almost all of the surgical subjects had measurable C-peptide release in the first 30-60 min after eating (Figure 2B). There was no association of meal-induced β-cell output with the small changes in postprandial glucose levels (Supporting Information Figure). Meal ingestion increased GLP-1 levels in both surgical and non surgical groups, with the largest and earliest response occurring in the GB subjects (Figure 2A).
After meal ingestion, there was a two- to threefold increase in glucagon concentrations in all groups (Figure 3B). However, meal-induced glucagon release was significantly lower in the surgical subjects compared to both O-CON and L-CON individuals (Figure 3B). Similar to the glucagon response, meal ingestion led to augmented PP values during the clamp in all subjects, with a larger response in the L-CON compared to the GB, and no differences between the surgical and O-CON subjects (Figure 3A).

Meal ingestion increased heart rates in all groups, with the largest enhancement in the surgical individuals compared to the non-surgical controls (Figure 4B).

**Discussion**

Postprandial hyperinsulinemia in subjects with GB has been attributed mainly to the rapid and substantial changes in glycemia combined with the enhanced incretin effect that is typical after surgery. In this study we assessed the GB effect on meal-induced β-cell secretion, independent of postprandial changes in systemic glucose levels. Our findings demonstrate that in contrast to persons without surgery, those with GB have meal-induced β-cell responses in the absence of stimulatory changes in blood glucose. Also, there is blunted suppression of β-cell secretion after GB in response to glucose lowering compared to non surgical individuals. Taken together with the distinct glucagon responses observed in GB subjects, these findings suggest systematic differences in islet cell regulation after surgery, and for the first time dissociate these from changes in systemic blood glucose.

To address glucose-independent regulation of islet hormone secretion we refined a meal/hyperinsulinemic clamp protocol similar to one previously used to demonstrate abnormal insulin secretion in patients with insulinoma (17). We selected a glucose target of 3.3-3.5 mmol l⁻¹ to prevent glycemic stimulation of insulin and to neutralize the effect of incretins, which are glucose-dependent in non-surgical cohorts (11-13). Subjects were given a mixed nutrient meal with relatively low carbohydrate content to minimize postprandial glucose excursions. As validated in previous studies (9,10), we used the meal/clamp method to measure systemic meal glucose appearance, although this parameter may have been underestimated in the GB subjects given the differences in postprandial insulin and glucagon response in these individuals compared to the controls.

To determine the lowest absolute C-peptide levels reached during the clamp, GB subjects were also compared with lean non-operated individuals, whose fasting C-peptide levels were expected to match those of the GB subjects (18). Despite differences in fasting C-peptide between the lean and obese control groups, exogenous insulin infusion suppressed plasma C-peptide to the same extent in both groups as described by previous investigators (16); certainly the sub-basal glucose target used here contributed to this effect. However, the GB subjects had less suppression of β-cell output before meal consumption compared to both lean and obese controls, suggesting abnormal glucose sensing with declining glycemia in these individuals. This observation is consistent with our previous findings that GB subjects with hyperinsulinemic hypoglycemia maintain higher rates of insulin secretion in the latter phases of a MTT when glucose concentrations approach basal and sub-basal levels (8). Previous studies have identified pancreatic denervation as causing a similar lack of β-cell response to relative hypoglycemia (19,20), and altered neural control of insulin secretion is plausible in GB subjects. However, despite the attenuated response to declining glycemia, there was a 2.5-fold increase in postprandial C-peptide in the GB cohorts at fixed, sub-basal glycemia, an insulin response that was not associated with the extent of glycemic deviation from the clamp target. In contrast, the non surgical controls had virtually no β-cell secretion after eating. This finding supports factors other than glycemia to promote the elevated rates of postprandial insulin secretion typical of individuals with GB. Potential mechanisms include differential β-cell stimulation by non-glucose nutrients (21,22) or neural signals as a result of enhanced pace of nutrient flux, increased sensitivity to the effects of the incretins, or a combination of these factors. Any of these mechanisms, and others as well, are best accounted for by chronic effects of a reconfigured GI anatomy and an altered metabolic milieu. The important implication of the results presented here is that the effect of GB on β-cell function is not wholly explained by acute changes in systemic glycemia.

It is notable that although we maintained blood glucose 20-30% below fasting levels, we did not see the robust glucagon response typical of hypoglycemic counter-regulation among the surgical and obese non surgical controls. Moreover, fasting heart rates did not increase by >10% of basal in the majority of GB and O-CON subjects whereas heart rates in lean controls rose by ~25%. In contrast, the L-CON individuals had marked increases in glucagon and PP secretion as well as heart rate. The lean subjects were significantly younger than the GB and O-CON subjects, which may contribute to this difference, but given that the study population as a whole consisted of young and middle-aged adults, these findings suggest blunted activation of the autonomic nervous system by reductions in blood glucose in obese subjects, with and without GB.

The role of autonomic nervous system activation of the β-cell has been extensively studied during the preabsorptive phase of insulin secretion (23,24) and as an anticipatory response to food intake or to oral nutrient sensory stimulation (25). However, beyond premeal insulin secretion, parasympathetic nervous system (PNS) activation has been found to make an important contribution to the β-cell response to food intake (26,27). Circulating PP concentrations have been generally taken to be a marker of PNS input to the islet (28).
PP secretion from islet F-cells is under cholinergic control and the PP levels tend to increase reliably during hypoglycemia (29,30). In our cohorts, plasma PP measures do not provide clear insights into islet neural signaling. The PP responses to hypoglycemia and meal ingestion in the L-CON subjects were compatible with previous studies, increasing significantly in response to both stimuli (29-31). However, the O-CON and GB subjects did not have a rise in PP during glucose lowering with the first 2 h of the glucose clamp. Overall the pattern of PP secretion followed very closely the glucagon profiles in these studies, with blunted postprandial responses in the GB groups. Therefore, plasma PP does not support increased parasympathetic stimulation of the β-cell response to the meal in the GB subjects. On the other hand cholinergic inputs are not the only neural stimuli to insulin secretion (24), and recent work has demonstrated that prandial insulin and PP secretion can be dissociated (32). Therefore, the profile of altered PP responses in the GB subjects does not exclude neural stimulation in the response to meal ingestion at sub-basal glucose.

It is conceivable that our findings could be explained by effects of meal-induced GLP-1 given the relatively large and early secretion of the peptide after eating in the GB subjects. The glucose dependency of gut hormones at normal glucose levels has been established in meal studies with fixed basal, glucose levels (11) or intravenous GLP-1 infusion studies in non-operated individuals (12,13). Therefore, these findings may not apply to GB where large amounts of GLP-1 are secreted into the portal vein after eating, causing an exaggerated discrepancy between portal and peripheral levels (33), and potentially activating portal GLP-1 sensors (34). These possibilities do not exclude, and even support, an eventual neural contribution to meal-induced insulin secretion.

It is surprising that following meal ingestion the GB subjects had relatively smaller glucagon responses than the non surgical controls. Several studies have reported that the glucagon response to meal ingestion is enhanced after GB (5,35), and is similar in post-surgical subjects with and without hypoglycemia (8,36). While the mechanism(s) underlying distinct glucagon responses of GB subjects has not been explained, several possibilities could be considered to explain our findings. A diminished glucagon response to hypoglycemia could be due to antecedent hypoglycemia (37,38), the short but extreme glycemic excursion with lower nadir glucose levels, in GB subjects might affect subsequent α-cell responses. In addition, it has been demonstrated previously that carbohydrate ingestion during hyperinsulinemic clamps reduces the glucagon response to hypoglycemia in healthy subjects (29), likely via neutrally mediated process initiated by nutrient sensing in the GI tract. While this mechanism does not explain the different responses in our surgical and nonsurgical subjects, it is possible that rapid nutrient flux from the gut in the GB individuals exaggerates this neutrally mediated response. Finally, inhibition of the α-cell due to paracrine regulation by factors released from the β-cell has been well established (39,40). Thus, less meal-induced glucagon release could be attributed to greater suppression by β-cell products. On the basis of our current findings it appears that α-cell responses in GB subjects are glucose dependent, with hypersecretion during meals or other conditions with elevated blood glucose (7,8), but muted in the setting described here where glucose levels are held fixed and low. The present study adds to the case that α-cell, as well as β-cell, function is significantly changed by GB.

In summary, these findings extend previous studies demonstrating distinct patterns of islet hormone secretion in persons with GB. In particular, this study indicates that the insulin and glucagon responses to declining blood glucose are blunted, and that a component of meal-induced hyperinsulinemia after GB is attributable to factors beyond glucose. Taken together these factors support distinct regulation of the islet following GB. While there are many possible explanations for these observations, they implicate chronic effects of surgery, in addition to acute shifts in circulating regulatory factors, on islet function.

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