Hyperinsulinemic Hypoglycemia After Gastric Bypass Surgery Is Not Accompanied by Islet Hyperplasia or Increased β-Cell Turnover

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OBJECTIVE — The purpose of this study was to establish whether hypoglycemia after gastric bypass surgery (GBS) for morbid obesity is due to increased fractional β-cell area or inappropriately increased insulin secretion.

RESEARCH DESIGN AND METHODS — We examined pancreata obtained at partial pancreatectomy from 6 patients with post-GBS hypoglycemia and compared these with 31 pancreata from obese subjects and 16 pancreata from lean control subjects obtained at autopsy. We addressed the following questions. In patients with post-GBS hypoglycemia, is β-cell area increased and is β-cell formation increased or β-cell apoptosis decreased?

RESULTS — We report that in patients with post-GBS hypoglycemia, β-cell area was not increased compared with that in obese or even lean control subjects. Consistent with this finding, there was no evidence of increased β-cell formation (islet neogenesis and β-cell replication) or decreased β-cell loss in patients with post-GBS hypoglycemia. In control subjects, mean β-cell nuclear diameter correlated with BMI ($r^2 = 0.79$, $P < 0.001$). In patients with post-GBS hypoglycemia, β-cell nuclear diameter was increased ($P < 0.001$) compared with that for BMI in matched control subjects but was appropriate for BMI before surgery.

CONCLUSIONS — We conclude that post-GBS hypoglycemia is not due to increases in β-cell mass or formation. Rather, postprandial hypoglycemia after GBS is due to a combination of gastric dumping and inappropriately increased insulin secretion, either as a failure to adaptively decrease insulin secretion after GBS or as an acquired phenomenon.

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Gastric bypass surgery (GBS) is a common therapy for patients with morbid obesity (1). Recently, Service et al. (2) reported six patients with postprandial hyperinsulinemic hypoglycemia that developed after Roux-en-Y GBS. The largest islet size was greater in these patients compared with that in control subjects with pancreatic cancer. Also, insulin-staining cells were noted related to exocrine ducts. Together these findings were interpreted as consistent with nesidioblastosis (2). Three additional patients presenting with similar clinical symptoms and presumed increased new islet formation were subsequently reported by Patti et al. (3,4). These reports prompted speculation that increased secretion of gastrointestinal hormones, such as glucagon-like peptide 1 (GLP-1), consequent to GBS might have led to increased β-cell mass as a result of increased β-cell formation. It was therefore proposed that the hyperinsulinemic hypoglycemia was likely secondary to this presumed excessive concentration of GLP-1 (5). In support, in vitro and animal studies reported that GLP-1 may increase β-cell replication and inhibit β-cell apoptosis (6,7), and it has been reported that GLP-1 concentrations are increased after GBS (8–10).

Hyperinsulinemic hypoglycemia has been reported both in infants (11,12) and more rarely in adults (13,14) in the absence of insulinoma. In infants, this condition was originally termed nesidioblastosis (15) as it was thought that the hyperinsulinemia arose as a consequence of increased islet formation from putative islet precursors adjacent to pancreatic ducts. However, it is now known that a marked increase in islet fractional area in the infant pancreas is normal and that the hyperinsulinemic hypoglycemia of infancy is due to genetic defects, most commonly affecting the ATP-sensitive K^+ channel, leading to dysregulated insulin secretion (16). As a consequence, this syndrome was renamed persistent hyperinsulinemic hypoglycemia of infancy (17). Recent studies of pancreata of adults with hyperinsulinemic hypoglycemia in the absence of an insulinoma have revealed that the most striking abnormality is hyperplastic β-cells with enlarged nuclei, consistent with chronically increased insulin secretion, analogous to persistent hyperinsulinemic hypoglycemia of infancy (14).

There is increasing interest in the actions of GLP-1 on β-cell mass and insulin secretion because a GLP-1 mimetic is now available as therapy for type 2 diabetes (18). In the report of post-GBS hypoglycemia, pancreata from these patients were compared with pancreata obtained from patients who had undergone pancreatectomy for pancreatic cancer (2). Insulin secretion is disturbed in patients with pancreatic cancer, probably reflecting changes in nutrition and/or local effects of the cancer (19). We therefore performed additional morphometric analyses of the pancreatic tissue of the six patients with

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Abbreviations: GBS, gastric bypass surgery; GLP-1, glucagon-like peptide 1.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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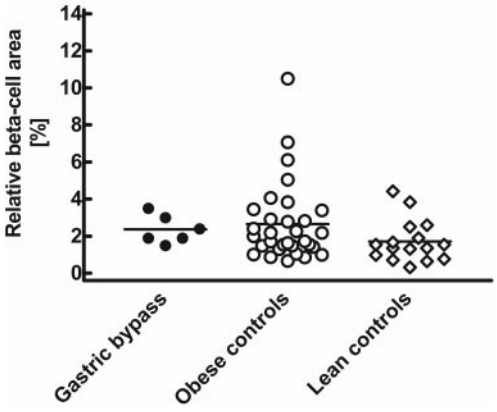

Figure 1—*Relative β-cell area (percentage of total pancreatic area) in 6 patients after GBS, 31 obese nondiabetic control subjects, and 16 lean nondiabetic control subjects. Solid lines indicate mean values. There were no significant differences between patients after GBS and obese (P = 0.70) or lean (P = 0.21) control subjects.*

post-GBS hyperinsulinemic hypoglycemia compared with pancreata obtained at autopsy from individuals with a wide range of BMIs to encompass those present in patients undergoing GBS before the GBS and at the time the pancreas was resected to address the following questions. In post-GBS hyperinsulinemic hypoglycemia: *1*) Is the fractional β-cell area increased? *2*) Is β-cell formation increased or β-cell apoptosis decreased?

RESEARCH DESIGN AND METHODS

— Specimens from the body and tail of the pancreata from six patients (five women and one man; aged 46 ± 7 years) who had developed hyperinsulinemic hypoglycemia within 8 years after Roux-en-Y GBS were assessed (2). All patients presented with repeated episodes of postprandial hypoglycemia associated with symptoms of hypoglycemia. Their mean (±SD) BMI was 51.7 ± 6.1 kg/m^2 before GBS and 30.8 ± 6.9 kg/m^2 at the time of evaluation. Mean plasma glucose concentrations measured during episodes of postprandial hypoglycemia were 41.2 ± 7.3 mg/dl (2.3 ± 0.4 mmol/l), and corresponding insulin levels were 10.5 ± 9.9 mU/l.

Pancreatic tissue was obtained from partial pancreatectomy as previously described. Sections (5 μm thick) were stained for insulin using DAB labeling (guinea-pig anti-insulin, lot 50381573; Dako, Grostrup, Denmark), for insulin, Ki67 (mouse anti-Ki67, MIB-1, lot 00014101; Dako), and DAPI using immunofluorescence and for insulin, cleaved caspase-3 (rabbit anti-cleaved caspase-3, lot 040704; Biocare Medical, Concord, CA) and DAPI using immunofluorescence as described (20). The relative β-cell area per pancreatic section was quantified using an Olympus IX70 inverted system microscope by scanning the tissue area using ×4 objective magnification as described (20). The total tissue area within this region was quantified, followed by the insulin-positive area to generate the ratio of insulin staining to total pancreas area using Image-Pro Plus software (Media Cybernetics, Silver Springs, MD). Tissue sections from 31 obese (BMI 36.4 ± 1.2 kg/m^2) and 16 lean nondiabetic (BMI 22.5 ± 0.5 kg/m^2) individuals obtained at autopsy, as described in detail when they were reported previously (20), were used as controls.

To measure nuclear diameter, insulin-stained sections of pancreas (by immunohistochemistry) counterstained with hematoxylin were used. Five islets per subject selected at random were photographed at ×40 magnification on an Olympus IX70 inverted system microscope (Olympus America, Melville, NY). These islets were then examined to identify five representative β-cell nuclei in each. Selection criteria included the clear presence of the nucleus within a β-cell, the ability to clearly visualize nuclear boundaries, a circular shape (similar dimensions in all directions), and the appearance to the observer that the nucleus had been sectioned through its maximum diameter. Once the identified nucleus was encircled, measurement of 180 nuclear diameters per β-cell nucleus was made using Image Pro Plus software version 4.5.1 (Media Cybernetics, Silver Springs, MD) that quantified these 180 diameters at 2° angles throughout the circumference of the nucleus. Thus, the mean of 4,500 single measurements per subject was used to compute the mean nuclear diameter per subject. To minimize the potential impact of an investigator bias, the representative images of each section were stored on a compact disc by one investigator (J.J.M.) and provided to another investigator (A.E.B.) for evaluation.

To measure the frequency of β-cell replication, 10 random fields per slide stained for insulin, Ki67, and DAPI were imaged at ×20 objective magnification. The number of cells costaining for Ki67 and insulin was quantified and related to the total number of insulin-positive cells per islet.

To measure the frequency of β-cell apoptosis, 10 random islets per slide stained for insulin, cleaved caspase-3, and DAPI were imaged at ×20 objective magnification. The number of cells costaining for insulin and cleaved caspase-3 was quantified and expressed in relation to the total number of insulin-positive cells per islet.

To estimate the frequency of β-cells in relation to exocrine ducts, 10 random locations per field that contained exocrine ducts were imaged. The number of insulin-positive ductal cells was quantified in each field and expressed as a proportion of the total number of ductal cells. Moreover, the appearance of "microislets," defined as a cluster of ≤5 insulin-positive cells, as well as of "macroislets," defined as a cluster of >6 insulin-positive cells in close proximity (<5 nuclei distance to the ductal cells), was quantified in each slide. For these analyses, pancreatic specimens from five lean (three women and two men, aged 81.6 ± 8.8 years with BMI 21 ± 3 kg/m^2 and fasting plasma glucose 95.5 ± 10.8 mg/dl) and five obese (two women and three men, aged 57.4 ± 24.7 years with BMI 43 ± 9 kg/m^2 and fasting plasma glucose 96.0 ± 3.7 mg/dl) individuals without a history of diabetes were included.

Results are presented as means ± SEM. Statistical comparisons were carried out using Student's *t* test or one-way ANOVA and Duncan's post hoc tests.

RESULTS

— Fractional pancreatic β-cell area was not increased in patients after GBS compared with BMI-matched control subjects ($P = 0.7$) (Fig. 1). The percentage of insulin-positive ductal cells did not differ between patients after GBS and control subjects ($P = 0.19$) (Fig. 2). There was also no difference in the frequency of microislets or macroislets around ducts between the groups

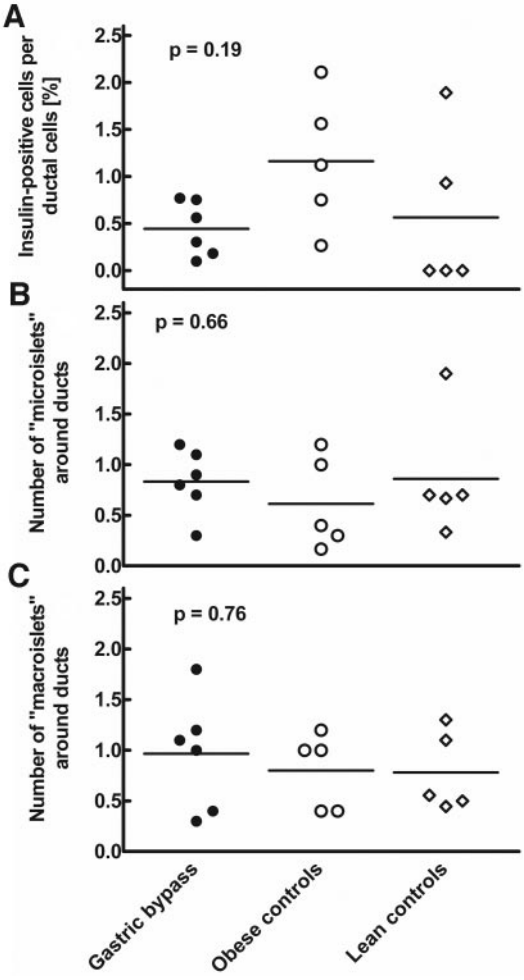

Figure 2—*Percentage of ductal cells positive for insulin* (A)*, as well as number of microislets, defined as clusters of five or less β-cells* (B)*, and macroislets, defined as clusters of more than six β-cells* (C) *around (less than five nuclei away) exocrine ducts in six patients after GBS, five obese nondiabetic control subjects, and five lean nondiabetic control subjects. Solid lines indicate mean values.*

($P = 0.66$ and $P = 0.76$, respectively; Fig. 2).

Consistent with previous reports, the frequency of β-cell replication, as measured by Ki67, was low in all groups, and this was not increased after GBS. Ki67 labeling was occasionally observed in acinar tissue and in exocrine ducts and frequently in spleen from the same subjects (data not shown), assuring that Ki67 staining successfully identified replication. Cleaved caspase-3 was identified in islets from patients after GBS as well as from obese and lean control subjects. There was, however, no difference in the frequency of cleaved caspase-3–positive β-cells between the groups (3.8 ± 0.4, 4.2 ± 0.4, and 5.7 ± 0.6%, respectively; $P = 0.1$). Notably there was no increased frequency of β-cell apoptosis in pancreata obtained at autopsy versus those obtained at surgery, assuring that pancreata obtained at autopsy were well preserved.

An unexpected finding was that the mean β-cell nuclear diameter was greater in the patients after GBS (8.0 ± 0.1 μm) compared with lean (5.8 ± 0.1 μm; $P < 0.0001$ vs. after GPS) and obese control subjects (7.4 ± 0.1 μm; $P = 0.014$ vs. after GPS). There was an impressive linear relationship between the mean nuclear diameter and the BMI in control subjects ($r^2 = 0.79$, $P < 0.001$; Fig. 3). The mean β-cell nuclear diameter in patients after GBS fell well within this relationship if the BMI for each patient before surgery was used ($r^2 = 0.84$, $P < 0.0001$). In contrast, if the actual BMI that was present in each patient at the time the pancreas was collected was used (after GBS-induced weight loss), the mean β-cell nuclear diameter was inappropriately high compared with control subjects for this relationship (Fig. 3*B*).

CONCLUSIONS — The present studies were undertaken to further characterize islet morphology in six previously reported patients with hyperinsulinemic hypoglycemia after GBS with a view to distinguishing whether the hyperinsulinemia could be attributed to increased β-cell formation or dysregulated insulin secretion (2).

We did not find increased β-cell mass as estimated by the fractional β-cell area in patients with post-GBS hyperinsulinemic hypoglycemia compared with BMI-matched control subjects. This finding appears to contradict the conclusions of the prior study of these patients (2). However, fractional insulin area was not measured in that study. Also, there were important differences in the control subjects used. In the prior study, pancreata removed from patients with pancreatic cancer was used, whereas in the present study, we obtained pancreata collected at autopsy from the same institution. Pancreatic cancer is known to disturb islet function (19) and might also be expected to disturb islet morphology, given the markedly decreased food intake typically present in pancreatic cancer. Autopsy pancreas is prone to post-mortem autolysis, but the specimens used in this report were deliberately selected for a short period between death and autopsy and were screened to exclude those with autolysis as described in detail before (20). The ideal controls for these studies would be pancreata from the majority of people who undergo GBS without developing hypoglycemia. However, because these patients do not require partial pancreatectomy, these pancreata are unavailable.

The appearance of islets in relation to exocrine ducts has been recognized for many years (20–22) and has been considered evidence for new islet formation from putative islet precursors at this site (so-called islet neogenesis) (23). This concept has been challenged by lineage studies in mice suggesting that new β-cells arise from existing β-cells rather than from newly formed islets (24). Others have reported the presence of islet progenitor cells in the human pancreas (25), raising the possibility that there are important differences in regulation of β-cell mass in humans versus rodents. There is no way of directly measuring so-called islet neogenesis in human pancreas. Here, we used conventional methods of quantifying the percentage of

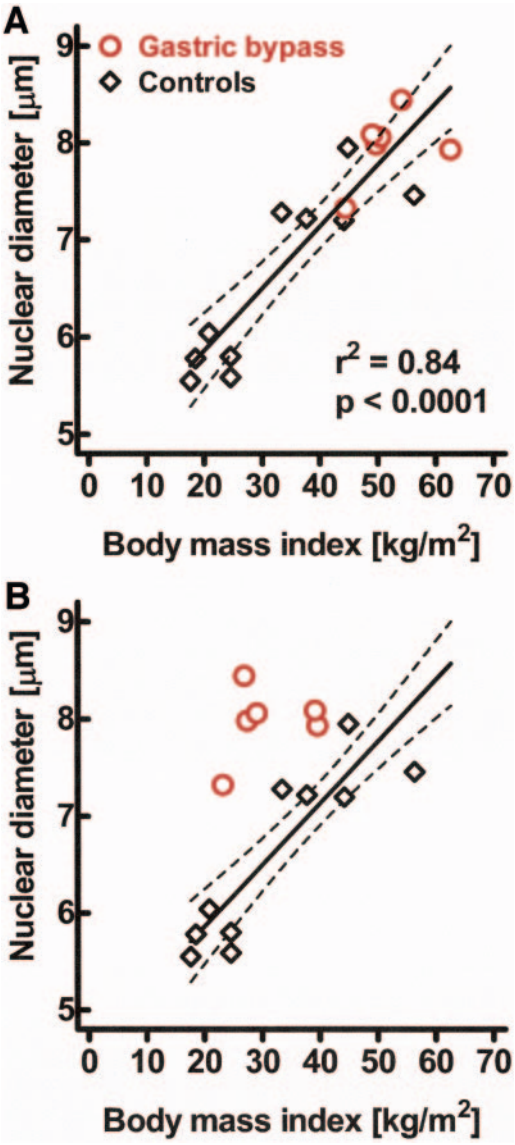

Figure 3—*Relationship between the mean β-cell nuclear diameter and BMI in 6 patients after GBS and in 10 nondiabetic control subjects (5 lean and 5 obese subjects).* A*: This relationship when using the pre-GBS BMI values in the GBS patients (red circles).* B*: Same data but now using the BMI in the GBS patients at the time of post-GBS hypoglycemia when the partial pancreatectomy was performed and the pancreas samples were obtained. As expected, the GBS led to substantial weight loss (red circles moved to the left), but in these patients, there was no concurrent adaptive decrease in β-cell nuclear diameter. The dotted lines indicate the upper and lower 95% CIs in control subjects.*

exocrine duct cells positive for insulin and the number of micro- or macroislets adjacent to exocrine ducts. None of these measures were higher in patients after GBS, but given the indirect nature of this approach, increased new islet formation cannot be ruled out. However, because there was no significant difference in the relative β-cell area between the specimens from patients after GBS and those from obese control subjects obtained at autopsy (20), increased β-cell formation does not seem to be a likely mechanism to account for the hyperinsulinemic hypoglycemia in these patients.

Theoretically, there could be increased new islet formation from islet precursors in the absence of increased β-cell mass in patients after GBS if there was a concurrent increased rate of β-cell apoptosis, i.e., increased β-cell turnover. However, the frequency of activated caspase-3–positive β-cells was not increased after GBS. Taken together, these findings imply that increased β-cell formation was probably not responsible for the hyperinsulinemic hypoglycemia after GBS.

An alternative explanation is that the insulin secretory rate per β-cell was inappropriately high in patients after GBS. Given the comparable β-cell mass in patients after GBS and obese control subjects, this explanation appears to be the most plausible. A new and unexpected finding in the present study is that the β-cell nuclear diameter is closely correlated with BMI in humans, consistent with the concept that the nuclear diameter of an endocrine cell provides an index of secretory activity (26,27). It is therefore of particular interest that the β-cell nuclear diameter in patients after GBS appears to be more appropriate for their pre–weight loss BMI. This raises the possibility that the hyperinsulinemia in these patients is analogous to hyperparathyroidism after reversal of chronic renal failure (28). Metabolic acidosis in chronic renal failure leads to a low ionized Ca^{2+} level and chronic stimulation of parathyroid glands to secrete parathyroid hormone. When this acidosis is rapidly corrected after renal transplantation, persistent "dysregulated" increased parathyroid hormone secretion can lead to posttransplant hypercalcemia. It is conceivable that a comparable mechanism occurs in post-GBS hyperinsulinemic hypoglycemia. The relatively rapid increased insulin sensitivity previously reported after GBS might unmask dysregulated insulin secretion arising as a consequence of chronic stimulation due to long-term morbid obesity and manifesting as hypoglycemia (2,29). However, in contrast to the documented relationship between the nuclear diameter of parathyroid hormone–secreting cells and parathyroid hormone secretion rates, there is not yet an established relationship between the β-cell nuclear diameter and insulin secretion. The remarkably close correlation between β-cell nuclear diameter and BMI suggests that such a relationship might exist and is worthy of further study.

It should also not be overlooked that postprandial hypoglycemia caused by rapid emptying of the gastric remnant has long been recognized as a common complication of gastric resections (dumping syndrome) (30). It has generally been held that as a consequence of GBS, there is accelerated entry of nutrients into the small intestine with rapid absorption prompting rapid and marked insulin secretion that is then not countered by sustained glucose delivery from the stomach, leading to reactive hypoglycemia (30). The present findings are not inconsistent with this long-held hypothesis that does not require any additional gut hormones such as GLP-1 or changes in β-cell mass.

An alternative postulate that increased secretion of gastrointestinal hormones, such as GLP-1, promoted the proliferation of islet β-cells in patients with post-GBS hypoglycemia was proposed (5). That postulate arose from the observation that GLP-1 inhibits β-cell apoptosis and stimulates β-cell replication in vitro in rodents (6,18). The present studies showing neither increased β-cell

replication, decreased β-cell apoptosis, nor an increased β-cell fractional area after GBS negates that postulate. Alternatively, the increased insulin secretion in these patients could be due to the actions of increased GLP-1 concentrations after GBS (31). Against this postulate, hypoglycemia has not been observed in humans exposed to long-term GLP-1 or GLP-1 mimetic treatment (18,32), and the insulinotropic effect of GLP-1 is absent at low glucose concentrations (33). Also, although some studies reported increased postprandial GLP-1 concentrations after GBS (9,34), this finding has not been confirmed by others (35,36). Moreover, the increases in GLP-1 concentrations observed in the studies with positive results were modest and usually of limited duration (~2 h after meal ingestion) (10). Also, the reported GLP-1 plasma levels were severalfold lower than those used to demonstrate GLP-1 effects on β-cell turnover in animal studies (6,34). Finally, enhanced GLP-1 secretion after meal ingestion is also typically found in patients after GBS not presenting with postprandial hypoglycemia (10). Whether increased secretion of GLP-1 was a factor in the genesis of hypoglycemia after GBS is therefore still an open question.

A limitation of histological studies of the human pancreas is that β-cell mass cannot be directly quantified in the absence of measures of the total pancreatic weight (which is usually not determined at autopsy). Therefore, we used the fractional β-cell area as a surrogate marker for β-cell mass. This parameter has previously been used as an estimate of β-cell mass (3,20,37), and adaptive changes in the fractional β-cell area have been described in obese subjects (20). Moreover, previous autopsy studies, in which the pancreatic volume was determined in a subset of subjects, demonstrated a close association between actual β-cell mass and the fractional β-cell area (37).

In summary, we report that neither β-cell area nor β-cell turnover is increased in humans with post-GBS hypoglycemia. Unexpected findings reported here are that there is a close correlation between BMI and β-cell nuclear diameter in humans and that the β-cell nuclear diameter in the patients with post-GBS hyperinsulinemic hypoglycemia appears to be more appropriate for the preoperative BMI than the BMI at onset of hypoglycemia. These findings imply that the mechanism subserving the hyperinsulinemia after GBS is most likely a combination of gastric dumping and inappropriately increased insulin secretion. The latter might be a consequence of a failure to adaptively decrease insulin secretion after GBS or due to an acquired phenomenon (29).

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