

BRIEF REPORT

## Hyperinsulinemic Hypoglycemia with Nesidioblastosis after Gastric-Bypass Surgery

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### SUMMARY

We describe six patients (five women and one man; median age, 47 years; range, 39 to 54) with postprandial symptoms of neuroglycopenia owing to endogenous hyperinsulinemic hypoglycemia after Roux-en-Y gastric bypass surgery. Except for equivocal evidence in one patient, there was no radiologic evidence of insulinoma. Selective arterial calcium-stimulation tests, positive in each patient, were used to guide partial pancreatectomy. Nesidioblastosis was identified in resected specimens from each patient, and multiple insulinomas were identified in one. Hypoglycemic symptoms diminished postoperatively. We speculate that hyperfunction of pancreatic islets did not lead to obesity but that beta-cell trophic factors may have increased as a result of gastric bypass.

**A** CONSEQUENCE OF THE OBESITY EPIDEMIC IN THE UNITED STATES<sup>1</sup> IS the increasing use of gastric bypass surgery for patients with severe, medically complicated obesity.<sup>2</sup> Some patients who have undergone this procedure have postprandial symptoms that have been ascribed to rapid emptying of gastric contents.<sup>3</sup> This phenomenon, referred to as the dumping syndrome, is characterized by vasomotor symptoms of diaphoresis, weakness, dizziness, and flushing, but not neuroglycopenia.<sup>4</sup> In the past five years, we have treated six patients in whom postprandial symptoms of neuroglycopenia developed as a result of endogenous hyperinsulinemic hypoglycemia after gastric bypass. Their clinical presentation typified that of the non-insulinoma pancreatogenous hypoglycemia syndrome (postprandial neuroglycopenic hyperinsulinemic hypoglycemia and pancreatic nesidioblastosis)<sup>5,6</sup> and is also seen in some patients with insulinoma.<sup>7</sup> We attempted to determine whether hyperfunction of pancreatic islets as a result of nesidioblastosis, which is characteristic of the noninsulinoma pancreatogenous hypoglycemia syndrome, or insulinoma was the basis for the hypoglycemia and to determine the possible role of gastric bypass in the genesis of the abnormal islets.

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### METHODS

#### SUBJECTS

From 2000 to 2004, six patients (five women and one man; median age, 47 years; range, 39 to 54) who had undergone Roux-en-Y gastric bypass for extreme obesity were referred for evaluation of repeated episodes of postprandial hypoglycemia associat-

ed with symptoms of profound neuroglycopenia, which could not be controlled by lifestyle modification. Their median body-mass index (the weight in kilograms divided by the square of the height in meters) at the time of this evaluation was 28.2 (range, 23.1 to 39.5), in contrast to a median body-mass index of 50.1 (range, 44.4 to 62.5) before bypass, representing a median loss of 44 percent (range, 20 to 51 percent) of the preoperative weight. In the bypass circuit the alimentary limbs were a median of 100 cm in length (range, 80 to 250) from the anastomosis. Historical details regarding the development of symptoms in relation to gastric bypass and meals and the type of symptoms are provided in Table 1. The timing of symptoms in relation to gastric bypass was self-reported, since there was no documentation of hypoglycemia until the present evaluation. One patient recalled symptoms antedating gastric bypass that had worsened considerably after the procedure. Confirmation of the history of postprandial hypoglycemia was obtained by waiting for a spontaneous episode to occur, at which point venous blood samples were obtained either at our facility (four patients) or elsewhere (two patients). All laboratory analyses were performed at our facility.

Data relevant to the episodes of spontaneous postprandial hypoglycemia are shown in Table 1. Each patient was confirmed to have endogenous

postprandial (one to four hours after eating) hyperinsulinemic hypoglycemia, defined as a serum insulin level of at least 3  $\mu$ U per milliliter (18 pmol per liter) and a serum C-peptide level of at least 0.6 ng per milliliter (199 pmol per liter) with a concomitant serum glucose level of less than 55 mg per deciliter (3.1 mmol per liter) and the absence of sulfonlylurea in the plasma (measured in five of the six patients).<sup>7</sup> Although postprandial hypoglycemia is an unusual occurrence in patients with insulinoma, the fact that it is a possibility warranted radiologic localization procedures, such as triple-phase spiral computed tomography and transabdominal ultrasonography of the pancreas. Endoscopic ultrasonography is often neither feasible nor useful in patients with a small, remnant gastric pouch. When the results of these conventional imaging procedures are negative or equivocal, the selective arterial calcium-stimulation test is recommended for the identification and regionalization of potentially hyperfunctioning beta cells.

#### LABORATORY ANALYSES

Serum levels of insulin<sup>8</sup> and C peptide<sup>9</sup> were measured with the use of immunochemiluminometric assays with a lower limit of detection of 0.1  $\mu$ U per milliliter (0.6 pmol per liter) and 0.1 ng per milliliter (33 pmol per liter), respectively. Plasma sulfonlylurea levels were measured by liquid chro-

**Table 1. Historical Symptoms and Laboratory Values Obtained during Episodes of Spontaneous Postprandial Hypoglycemia.\***

Patient No.	Historical Symptoms			Observed Episode of Spontaneous Postprandial Hypoglycemia			
	Timing after Gastric Bypass	Timing after Meals†	Type	Serum Glucose	Serum Insulin	Serum C Peptide	Plasma Sulfonlylurea
	yr	hr		mg/dl	$\mu$ U/ml	ng/ml	
1	2	1–3	Confusion	53	16.0	1.8	Not measured
2	1	2–3	Confusion	38	4.2	3.3	Undetectable
3	0.5	2	Loss of consciousness	31	28.0	1.4	Undetectable
4	1	1.5–2	Loss of consciousness	42	8.3	7.6	Undetectable
5	—‡	2	Confusion	39	3.1	3.6	Undetectable
6	8	1	Tunnel vision	44	3.4	3.7	Undetectable

\* Criteria for endogenous hyperinsulinemic hypoglycemia are a serum insulin level of at least 3  $\mu$ U per milliliter and a C peptide level of at least 0.6 ng per milliliter with a concomitant serum glucose level of less than 55 mg per deciliter and the absence of sulfonlylurea in the plasma. To convert the values for glucose to millimoles per liter, multiply by 0.05551. To convert the values for insulin to picomoles per liter, multiply by 6. To convert the values for C peptide to picomoles per liter, multiply by 331.

† The timing is in the context of ad libitum feeding.

‡ This patient reported having symptoms three years before gastric bypass.

matographic tandem mass spectroscopy.<sup>10,11</sup> Serum glucose levels were measured according to a standard hexokinase method on a Hitachi Chemistry Analyzer (model 747-200, Roche Diagnostics).<sup>12</sup>

#### *Selective Arterial Calcium-Stimulation Test*

When selective arterial calcium-stimulation tests are performed as previously described,<sup>5</sup> a doubling of the basal insulin level in the right hepatic vein in response to the sequential injection of 0.025 mEq of calcium per kilogram of body weight into the splenic, superior mesenteric, and gastroduodenal arteries is considered to indicate hyperfunction of the beta cells in the vascular distribution of the artery studied. Although overlap can occur across vascular territories, such overlap can be identified from the angiographic findings. In general, the body and tail of the pancreas are within the splenic-artery distribution; the head and, secondarily, the uncinate process are within the gastroduodenal-artery distribution; and the uncinate process and, secondarily, the head are within the distribution of the superior mesenteric artery. The pattern of response to the intraarterial injection of calcium is expressed as a difference in response (gradient) from artery or arteries with a positive response to artery or arteries with a negative response. For instance, a positive response to the injection of calcium into the splenic artery, but not to injections into the superior mesenteric or gastroduodenal arteries, creates a gradient between the former and latter arterial distributions that can guide the surgeon in the extent of pancreatic resection.

#### *Pathological Analysis*

The resected pancreatic tissues were sectioned into 1-mm slices, fixed in buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin. Histologic criteria for nesidioblastosis in adults (hypertrophic beta cells within enlarged or normal-appearing islets; small, scattered clusters of endocrine cells; and ductuloinsular complexes)<sup>13</sup> were used to evaluate the sections.

Immunohistochemical staining was performed on paraffin sections 5  $\mu$ m thick with the use of the avidin-biotin-peroxidase complex system as previously described.<sup>14</sup> The antibodies used included chromogranin A (Boehringer Mannheim) at a dilution of 1:100, insulin (Dako) at a dilution of 1:750, glucagon (Dako) at a dilution of 1:3000, somatostatin (Dako) at a dilution of 1:1000, and gastrin

(Dako) at a dilution of 1:1000. Positive controls for immunostaining consisted of normal pancreatic tissues. Negative controls consisted of sections in which the primary antibody had been omitted during the immunostaining procedure. The immunostained slides were analyzed for staining within the islets, ducts, and interacinar locations within the pancreas.

The size of the islets in each patient was estimated by measuring 50 islets per patient with a micrometer in the ocular of the microscope; the largest islet in each of the 50 randomly selected fields was selected. Then, the mean diameter of the largest islets was calculated. Pancreatic tissues with normal-sized islets from four obese patients (three women and one man; median body-mass index, 34.1; range, 33.2 to 36.3) without known endocrine disease were also analyzed as controls.

#### **CONDUCT OF THE STUDY**

Each author vouches for the data and analyses. Four provided direct care of each patient; one contributed to the bariatric aspects of the manuscript; and one, a surgical resident, assisted in the design, data acquisition, and writing of the manuscript. The institutional review board approved this minimal-risk study with waiver of informed consent in accordance with the Code of Federal Regulations (45 CFR 46.116), as noted in the Federal Policy for the Protection of Human Subjects from the Department of Health and Human Services.

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## RESULTS

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#### **CLINICAL INVESTIGATION**

During spontaneous postprandial episodes, each patient had hypoglycemia related to endogenous hyperinsulinemia (Table 1). Because of the long half-life of C peptide (30 minutes), a finding of elevated postprandial levels is not proof of excessive secretion by beta cells; rather, it rules out the possibility of exogenous insulin administration, in which case the levels would be low or undetectable. Patient 1 had equivocal evidence of insulinoma on triple-phase spiral CT and transabdominal ultrasonography of the pancreas; the results of these procedures were entirely negative in the other five patients. Each patient therefore underwent selective arterial calcium-stimulation testing, with positive responses in one, two, or three arterial distributions (Table 2). Each patient underwent pancreatic exploration with complete mobilization and pal-

**Table 2. Results of Selective Arterial Calcium Stimulation Tests.\***

Artery Injected	Peak Hepatic-Vein Insulin					
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
	<i>multiple of the basal level</i>					
Splenic	2.0	5.9	3.7	6.4	3.6	2.6
Superior mesenteric	1.0	2.5	2.8	1.1	2.9	1.1
Gastroduodenal	1.0	4.5	1.9	3.7	2.1	2.9

\* Multiples of 2 or greater are considered to indicate a positive response.

pation of the pancreas and intraoperative ultrasonography. The latter showed an insulinoma in Patient 1 in the tail of the pancreas, as suggested by the preoperative radiologic assessment. Ultrasonography revealed no abnormalities in the other five patients.

#### SURGERY

Patient 1 underwent a spleen-preserving distal pancreatectomy. No insulinoma was detected by palpation and intraoperative ultrasonography in the other five patients; therefore, with guidance by the gradient between positive and negative responses in the selective arterial calcium-stimulation test, distal pancreatectomy in which the superior mesenteric vein was used as the distinguishing landmark was performed. When the positive response to the intraarterial injection of calcium was confined to the splenic-artery distribution, the distal resection was carried to the left of the superior mesenteric vein. When the distribution of the gastroduodenal artery, superior mesenteric vein, or both were also involved, a safe, subtotal distal resection was performed to the right of the superior mesenteric vein. With the use of this gradient guidance, Patients 2, 4, 5, and 6 underwent extended distal pancreatectomy. Although warranted in the case of Patient 3, dissection to the right of the superior mesenteric vein was thought to be unsafe because it would place the retrogastric Roux-en-Y limb at risk for devitalization; therefore, a distal pancreatectomy was performed.

#### PATHOLOGICAL FINDINGS

Patient 1 had multiple islet-cell tumors, some of which stained positive for insulin and were therefore considered to be functional insulinomas. Patient 5 had a 0.4-cm, nonfunctional islet-cell tumor with no staining for pancreatic islet-cell hormones

but with staining for chromogranin A (a general neuroendocrine marker). The islets in most patients showed a variable pattern of islet-cell hypertrophy and hyperplasia. The mean ( $\pm$ SE) size of the islets was significantly larger in all the patients than in the obese controls ( $214\pm 7.7$  vs.  $151\pm 7.3$   $\mu$ m,  $P=0.001$ ) (Fig. 1A and 1B).

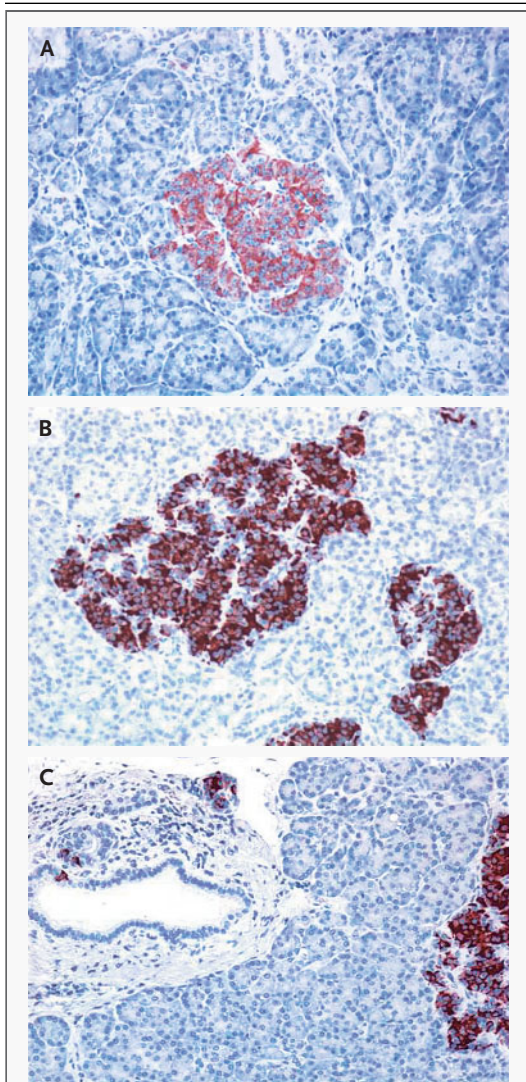
Immunohistochemical staining of the islets for insulin, glucagon, somatostatin, and chromogranin A indicated that 60 to 80 percent of islets from both patients and controls were positive for insulin. Insulin-positive cells budding off the pancreatic ducts were noted in Patients 2, 3, 4, 5, and 6 and were more numerous (median, 7 per slide; range, 1 to 11) than in the controls (median, 3 per slide; range, 1 to 5), although the difference was not significant (Fig. 1C). Chromogranin A staining revealed more cells budding off the ducts than did insulin staining, suggesting that this was a subgroup of the chromogranin A-positive cells.

#### FOLLOW-UP

Over a median period of 20 months after partial pancreatectomy (range, 5 to 51), three patients were entirely free of any postprandial symptoms, and two patients had occasional mild, nondescript symptoms but no hypoglycemia. After almost a year of being symptom-free, Patient 3 had a recurrence of symptoms of postprandial hypoglycemia, which was confirmed by findings of low glucose levels on a reflectance meter, although the symptoms were less severe and frequent than they had been preoperatively. The recurrence of symptoms is probably due to the more conservative distal pancreatectomy performed in Patient 3, which probably did not remove all affected pancreatic tissue.

#### DISCUSSION

There is emerging recognition that postprandial hypoglycemia may be due to endogenous hyperinsulinemia from abnormal islets, as a result of either nesidioblastosis or insulinoma. Our finding extends that observation to patients with postprandial neuroglycopenia who have undergone gastric bypass as a treatment for severe obesity. The need for caution in ascribing postprandial symptoms to the dumping syndrome in patients who have undergone gastric bypass without considering the possibility of organic hyperinsulinism was emphasized by the authors of a case report of insulinoma after gastric bypass.<sup>15</sup>



**Figure 1.** Islets from an Obese Control Subject (Panel A) and Patient 2 (Panel B) and Pancreatic Ducts from Patient 2 (Panel C).

Panel A shows a normal islet from an obese control subject with immunohistochemical staining for insulin. Panel B shows an enlarged islet from Patient 2 with immunohistochemical staining for insulin. The islet is about three times as large as the normal-sized islet in the lower right side of the figure. Panel C shows pancreatic ducts with insulin-positive cells (nesidioblastosis) from Patient 2. A portion of a hypertrophic islet with immunohistochemical staining for insulin is on the right. All three specimens are shown at the same magnification.

We initially considered our index case, in a patient who had functioning insulinomas and islet hypertrophy after gastric bypass, to be an unusual coincidence (postprandial symptoms and two types

of pathological islet lesions). However, subsequent experience with patients who had postprandial hyperinsulinemic hypoglycemia as a result of nesidioblastosis after gastric bypass led us to raise the possibility of a link between the islet hyperfunction and the bypass surgery. The frequency of nesidioblastosis after gastric bypass exceeds that in the general population, since only nine adult patients without a history of gastric bypass had surgically confirmed nesidioblastosis at our institution during the same period in which the six patients in the present report were evaluated and treated. Thus, the latter group of patients accounted for 40 percent of our patients with confirmed cases of nesidioblastosis during that time, but only about 0.1 percent of the U.S. population has undergone gastric bypass procedures.<sup>2</sup>

It is possible that hyperinsulinemia from islet-cell hyperfunction led to the development of severe obesity or, alternatively, that the hyperinsulinemia was a consequence of the gastric bypass. Patients with insulinomas may gain weight, but rarely to a degree that would warrant gastric bypass. The median body-mass index of 58 patients with surgically confirmed insulinoma consecutively treated at our institution during the same five-year period in which the patients in this report were seen was 29.1 (range, 18.4 to 53.8). Six patients (10 percent) had a body-mass index of at least 40, and 12 patients (21 percent) had a body-mass index of at least 35. Among 17 patients at our institution who had the noninsulinoma pancreatogenous hypoglycemia syndrome and who did not undergo gastric bypass (some of whom have been described previously<sup>5,6</sup>), the median body-mass index was 27.2 (range, 19.5 to 35.6). These observations argue against islet hyperfunction, especially nesidioblastosis, as the trigger for severe obesity. Conversely, obesity does not appear to cause islet hypertrophy, as attested to by the normal size of islets in obese patients without hypoglycemia.

Persons with the dumping syndrome as a result of previous gastric surgery have been reported to have increased levels of glucagon-like peptide 1,<sup>16-18</sup> possibly owing to the rapid presentation of nutrients (a stimulus for the secretion of this peptide) to the distal ileum, the site of L cells, which are the source of glucagon-like peptide 1. Glucagon-like peptide 1 increases beta-cell mass in rodents through neogenesis and proliferation<sup>19-22</sup> and decreases apoptosis of islets in humans.<sup>23</sup> These findings provide support for the possibility

that beta-cell trophic factors may be brought into play after bypass surgery, leading to the growth of pancreatic beta cells and consequent hyperfunction of islets, ultimately culminating in postprandial hypoglycemia. Such a phenomenon, if ultimately confirmed, would affect not only the continued use of gastric bypass, but also the proposed use of glucagon-like peptide 1 or its analogues for the treatment of diabetes.<sup>24</sup> Furthermore, a potential disturbance in the negative association between insulin and grehlin<sup>25</sup> as a result of the expected reduction in grehlin levels after gastric bypass surgery may aggravate the propensity to hypoglycemia.

Postprandial hyperinsulinemic hypoglycemia and nesidioblastosis may occur in patients who have undergone Roux-en-Y gastric bypass for extreme obesity. Increased levels of a beta-cell-trophic polypeptide, such as glucagon-like peptide 1, may contribute to the hypertrophy of pancreatic beta cells in these patients.

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