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Prospective Evaluation of Gastric Acid Secretion and Cobalamin Absorption Following Gastric Bypass for Clinically Severe Obesity

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The pathophysiologic mechanism(s) responsible for cobalamin deficiency after Roux-en-Y gastric bypass for clinically severe obesity remains unexplained. Inadequate secretion of intrinsic factor has been postulated, but decreased gastric acid secretion resulting in maldigestion and inadequate liberation of free cobalamin from its native protein-bound form is also possible. The aim of this study was to determine prospectively secretion of gastric acid and absorption of crystalline (free) and protein-bound cobalamin before and after gastric bypass. Eight patients (two men, six women) underwent orogastric intubation of the intact stomach preoperatively and the proximal gastric pouch postoperatively. Gastric acid secretion in the basal and stimulated (pentagastrin, 6 μg/kg) states was determined by a perfused, nonabsorbable marker technique to quantitate recovery of gastric secretion. Absorption of radiolabeled (^{57}Co) crystalline and protein-bound cobalamin was assessed on separate days by 24-hr urinary excretion. After gastric bypass, acid secretion ($\bar{x} \pm SEM$) was markedly reduced in basal (9.1 ± 3.6 vs 0.005 ± 0.003 meq/hr; P = 0.04) and stimulated (12.8 ± 1.8 vs 0.008 ± 0.003 meq/30 min; P = 0.002) states. Absorption of crystalline cobalamin was decreased (15.8 ± 2.5 vs 9.4 ± 1.4%; P = 0.08) to a lesser extent than was protein-bound cobalamin (5.9 ± 1.0 vs 1.1 ± 0.3%; P = 0.004). In summary, gastric acid secretion from the gastric pouch is negligible after gastric bypass, and food-bound cobalamin is maldigested and subsequently malabsorbed presumably due to pouch achlorhydria. Decreased absorption of free cobalamin suggests decreased cobalamin–intrinsic factor complex formation. This study suggests that cobalamin deficiency after Roux-en-Y gastric bypass results both from inadequate digestion of food-bound cobalamin and from insufficient secretion of intrinsic factor.

KEY WORDS: bariatric surgery; obesity; gastric acid secretion; intrinsic factor; cobalamin; absorption; morbid obesity; clinically severe obesity.

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Biochemical and clinically symptomatic cobalamin deficiency is a well-recognized, late complication of gastric bypass surgery for clinically severe obesity (1, 2). Decreased hepatic stores and low serum concentrations of cobalamin may result in megaloblastic anemia (3) and potentially severe neurologic disease. In health, cobalamin homeostasis is maintained by ingestion of animal products rich in pro-

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tein-bound cobalamin. Gastric acid/pepsin digestion liberates cobalamin from ingested protein for intragastric binding with R-protein. The R-protein–cobalamin complex is cleaved by pancreatic proteases in the lumen of the upper gut where cobalamin combines with intrinsic factor secreted by the stomach for specific, receptor-mediated active transport in the ileum (4, 5). Following gastric bypass, cobalamin deficiency may potentially result from: (1) insufficient protein, and, thus, cobalamin intake; (2) maldigestion of protein-bound cobalamin; (3) insufficient secretion of intrinsic factor; or (4) malabsorption of the cobalamin–intrinsic factor complex.

Since dietary intake of cobalamin is adequate except in strict vegetarians, maldigestion of protein-bound cobalamin due to lack of acid/pepsin secretion and/or malabsorption secondary to decreased cobalamin–intrinsic factor complex formation appear to be the likely mechanisms for cobalamin deficiency after gastric bypass. Previous studies have demonstrated decreased absorption of cobalamin in gastric bypass patients compared to healthy individuals (6) and in volunteers with drug-induced hypochlorhydria (7). In addition, a relative deficiency of luminal intrinsic factor following gastric bypass has been demonstrated by Marcuard et al (8). No studies, however, have evaluated prospectively gastric acid secretion and addressed its relationship to cobalamin absorption in obese patients before and after gastric bypass.

The aims of this study were to determine prospectively acid secretion from the proximal gastric pouch and absorption of free and food-bound cobalamin before and after vertical Roux-en-Y gastric bypass. We hypothesized that minimal gastric acid secretion from the proximal gastric pouch would result in maldigestion of protein-bound cobalamin, decreased liberation of free cobalamin, and, thus, decreased absorption of the native form of cobalamin.

MATERIALS AND METHODS

Patients. Eight patients (two men, six women) with a mean age of 47 ± 5 years (mean ± SEM) were studied prior to and six to eight weeks after vertical Roux-en-Y gastric bypass. The mean preoperative weight, body mass index [BMI = weight (kg)/height (m^2)], and percent above ideal body weight were 161 ± 11 kg, 50.9 ± 2.7 kg/m^2, and 146 ± 11.6%, respectively. At the time of restudy, postoperative weight loss averaged 18 ± 1 kg, and body weight and body mass index decreased to 143 ± 10 kg and 45.1 ± 2.6 kg/m^2, respectively. After the study was reviewed and approved by the Institutional Review Board of the Mayo Clinic, the patients were enrolled prospectively and consent was obtained. A normal serum beta-HCG was obtained from all premenopausal females prior to inclusion in the study. All patients were screened for a history of peptic ulcer disease and anemias, and medications interfering with gastric acid secretion were discontinued two weeks prior to the study. No patients were receiving parenteral injections of cobalamin preoperatively.

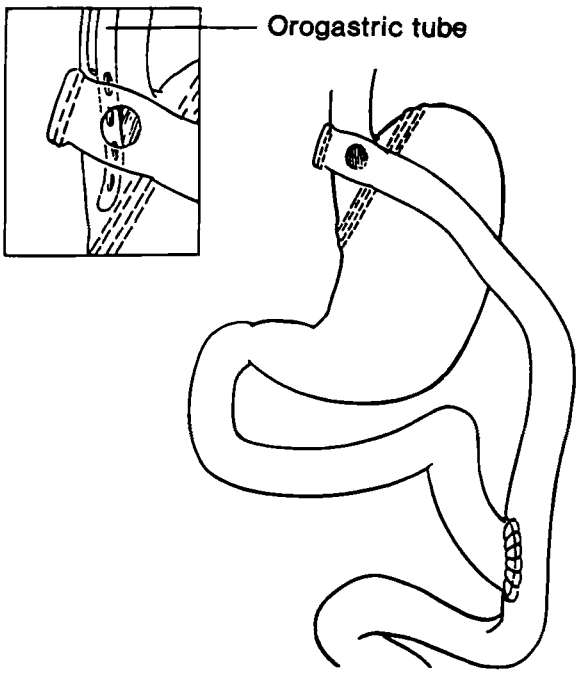

Fig 1. Vertical Roux-en-Y gastric bypass. Inset shows orogastric sump tube positioned in the proximal gastric pouch (Fig 1 copyrighted in 1992 by the Mayo Foundation).

Operative Technique. Through a midline celiotomy, the gastric cardia was isolated and stapled across vertically using a double staple line (TA90B Stapler, Autosuture, US Surgical Corporation, Norwalk, Connecticut) from the angle of His on the greater curvature to 2 cm distal to the gastroesophageal junction along the lesser curvature (Figure 1). The capacity of the proximal gastric pouch was estimated at <10 ml. A 70- to 150-cm Roux-en-Y limb of proximal jejunum was anastomosed to this proximal gastric pouch with a No. 21 end-to-end stapler (ILS Stapler, Ethicon Corporation, Cincinnati, Ohio) leaving an anastomosis only 11 mm in diameter. This operation excludes the majority of the stomach from anything ingested orally, thus, "bypassing" the stomach.

Conduct of Experiments. Following an overnight fast, patients were intubated orally with a modified 18-French sump tube assembly with the proximal infusion port positioned 5 cm proximal to multiple distal side holes for aspiration. The tube was positioned using fluoroscopy along the greater curvature in preoperative experiments and in the proximal gastric pouch in postoperative studies (Figure 1, inset). Immediately thereafter, the contents of the stomach or the pouch were aspirated and discarded.

A solution of 154 mM NaCl containing 5 g/liter of polyethylene glycol (PEG), a nonabsorbable marker, was infused at 5 ml/min via the proximal port; this facilitated collection of gastric secretion and calculation of the corrected volume of recovered gastric acid. Gastric content was collected in 15-min intervals for 1-hr under basal secretory conditions. Acid secretion was then stimulated with pentagastrin (6 μg/kg) subcutaneously, and gastric secretion was collected in 15-min intervals for an additional 90 min.

Cobalamin absorption was assessed measuring urinary excretion of cobalamin with a modified Schilling test (9). Each patient ingested, in random fashion, either 1 μg of crystalline (free) cyanocobalamin labeled with 0.5 μCi of ^{57}Co (Mallinckrodt, St. Louis, Missouri) in 20 ml of water or a protein-bound ^{57}Co-labeled cyanocobalamin preparation (0.5 μCi) incorporated into scrambled egg yolk as described previously (9). A flushing dose of 1 mg of nonradioactive cobalamin (Warner-Chilcott, Morris Plains, New Jersey) was administered intramuscularly to hasten urinary excretion of ingested cobalamin. Patients then collected urine for 24 hr. Subsequently, patients ingested the other cobalamin preparation and collected urine over the ensuing 24 hr. Preoperative and postoperative experiments were conducted in an identical fashion.

Analysis of Gastric Acid Secretion and Cobalamin Absorption. For each 15-min interval of gastric acid collection, the volume was measured, and the concentration of PEG was determined by a modification of the turbidimetric method (10). Percent recovery of PEG was used to calculate the "corrected volume" of each gastric collection period. The acid content of each sample was determined by titrating an aliquot with 0.1 N NaOH to a pH of 7.0. The output of HCl (milliequivalents per 15 min) was calculated by multiplying the concentration of HCl by the corrected volume.

The urinary excretion of ^{57}Co-labeled cobalamin was determined by measuring the volume of the 24-hr urine output and measuring radioactivity of a 4-ml aliquot using a well-type gamma counter. Percent urinary recovery (absorption) was calculated by the ratio of urinary recovery to ingested oral dose × 100.

Data and Statistical Analysis. For the basal period, the HCl secretion for the four 15-min periods was summed and the basal acid output (milliequivalents per hour) calculated. The acid output for each of the six 15-min intervals during pentagastrin stimulation was analyzed and expressed in two ways: first, as total integrated output over 90 min, and, second, as the peak acid output by summing the two consecutive intervals with the greatest outputs in milliequivalents per 30 min. Comparisons of basal and stimulated acid outputs and percent absorption (percent urinary excretion) of crystalline and protein-bound cobalamin preoperatively and postoperatively were assessed with a two-tailed Student's *t* test for paired data or with a Wilcoxon signed rank test where appropriate. Correlation of gastric acid secretion and cobalamin absorption was determined with a model of linear regression. Differences were considered significant when $P < 0.05$. Summary values in the text are expressed as the means ± SEM.

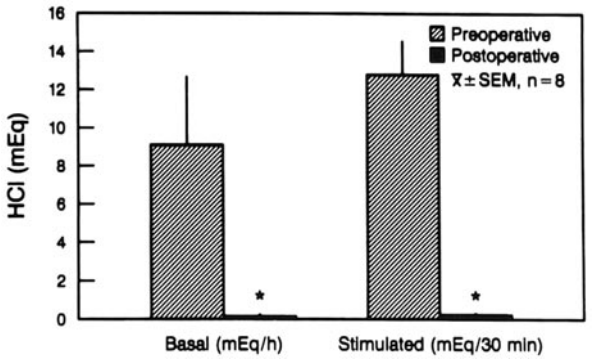

Fig 2. Preoperative and postoperative gastric acid secretion in the basal and stimulated (pentagastrin, 6 μg/kg) states (*$P < 0.05$ from preop).

RESULTS

Acid Secretion. Gastric acid secretion into the proximal gastric pouch after vertical Roux-en-Y gastric bypass was markedly less in all patients when compared to acid secretion into the entire stomach preoperatively. Preoperatively, secretion of HCl was 9.1 ± 3.6 meq/hr in the basal state, but decreased to 0.005 ± 0.003 meq/hr ($P = 0.04$) after gastric bypass (Figure 2). Following pentagastrin stimulation, peak HCl output increased to 12.8 ± 1.8 meq/30 min in the intact stomach but remained negligible in the pouch at 0.008 ± 0.003 meq/30 min ($P = 0.0002$). In addition, the 90-min integrated acid output after pentagastrin stimulation was much greater from the entire stomach preoperatively than from the proximal gastric pouch postoperatively (30.1 ± 0.7 vs 0.018 ± 0.001; $P < 0.05$) (Figure 3).

Cobalamin Absorption. The absorption (percent urinary excretion) of crystalline ^{57}Co-labeled cobalamin postoperatively (Figure 4) was less but was not significantly different from preoperative values

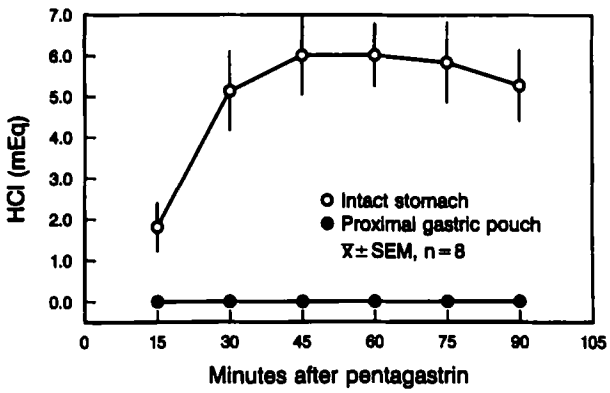

Fig 3. Gastric acid secretion after pentagastrin stimulation (6 μg/kg) of the native stomach and the gastric pouch.

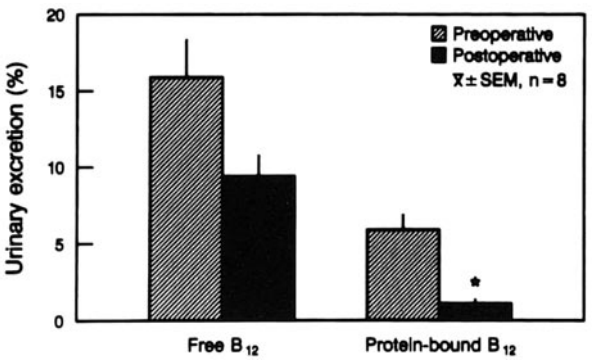

Fig 4. Crystalline and protein-bound cobalamin absorption before and after vertical Roux-en-Y gastric bypass (*$P < 0.05$ from preop).

(15.9 ± 2.5 vs 9.4 ± 1.4%; $P = 0.08$). The absorption of protein-bound cobalamin, however, decreased markedly (5.9 ± 1.0 vs 1.1 ± 0.3%; $P = 0.004$). Six of eight patients had decreased absorption of free cobalamin postoperatively, while seven of eight patients had markedly decreased absorption of protein-bound cobalamin after gastric bypass (Table 1).

Correlation of Gastric Acid Secretion and Cobalamin Absorption. There were no detectable relationships between preoperative and postoperative basal or stimulated HCl secretion and crystalline or food-bound cobalamin absorption; all correlation coefficients were <0.4.

DISCUSSION

The pathophysiologic mechanism(s) whereby biochemical or clinically significant cobalamin deficiency occurs in up to 70% (2) of patients following gastric exclusion surgery for clinically severe obesity remains unknown. This study demonstrates that absorption of both crystalline and protein-bound cobalamin is decreased postoperatively, but only food-bound cobalamin is decreased to a significant extent. In addition, functionally important gastric acid secretion (ie, acid/peptic secretions that mix with ingested food) is markedly reduced after gastric bypass. These data suggest strongly that cobalamin deficiency results primarily from inadequate release of cobalamin from ingested proteins (its native form) because of decreased acid/pepsin digestion; however, absorption of crystalline cobalamin was also decreased somewhat postoperatively (albeit not significantly), suggesting that binding of free cobalamin to intrinsic factor may also be impaired after gastric bypass.

TABLE 1. PERCENT URINARY EXCRETION OF COBALAMIN BEFORE AND AFTER VERTICAL ROUX-EN-Y GASTRIC BYPASS

	Free cobalamin		*Protein-bound cobalamin*	
Patient	*Preoperative*	*Postoperative*	*Preoperative*	*Postoperative*
1	4	12	3	0.4
2	16	4	6	0.6
3	23	9	8	2
4	20	6	7	0.8
5	19	14	10	0.6
6	17	15	2	2
7	21	5	8.1	0.6
8	6	10	3.1	2.1

The necessity of acid/pepsin digestion of ingested protein for release of protein-bound cobalamin was demonstrated by Doscherholmen and Swaim (9) in 1973. These authors found that patients with hypochlorhydria or achlorhydria, either of the idiopathic variety or after gastric resection, absorb crystalline cobalamin normally, and, therefore, may have a normal classic Schilling test; however, absorption of food-bound cobalamin was only 9% of control values. Their work suggested that intrinsic factor is present in sufficient quantity for normal absorption of crystalline cobalamin, but that malabsorption of the naturally occurring, protein-bound cobalamin is secondary to decreased acid/pepsin secretion, which impairs release of cobalamin for subsequent binding to R-protein. They compared cobalamin absorption in healthy controls to patients with a gastric resection, whereas our study examined cobalamin absorption in obese patients before and after gastric bypass. Further work supporting a role for gastric acid secretion in cobalamin deficiency was produced by Salom et al (7), who showed that cimetidine impaired the absorption of food-bound cobalamin but did not affect absorption of crystalline cobalamin. Kittang and Schjonsby (11), however, failed to demonstrate decreased cobalamin release from ingested proteins when omeprazole was given to inhibit gastric acid secretion. The reasons for these disparate findings are not evident but may be related to inadequate dosage of omeprazole to completely halt acid secretion in all subjects in the latter study. Our study demonstrates that both basal and peak acid output are markedly decreased after Roux-en-Y gastric bypass and that postoperative absorption of protein-bound cobalamin is also significantly decreased. These findings suggest that impaired absorption of protein-bound cobalamin results from insufficient acid/peptic digestion of native cobalamin. A potential methodologic limitation of our study, however, is inade-

quate recovery of the acid secreted from the proximal gastric pouch. The short (5 cm) distance between the infusion and aspiration ports of the orogastric catheter may have precluded adequate mixing of gastric acid and PEG and, thereby, altered HCl recovery. Inadequate mixing is unlikely, however, because approximately 65% of infused PEG was recovered.

Ileal site-specific absorption of cobalamin is dependent on the formation of a cobalamin–intrinsic factor complex. Until recently, cobalamin was believed to bind to intrinsic factor in the stomach for eventual active transport in the ileum; however, a gastric R-protein has been described that has higher binding affinity for cobalamin than does intrinsic factor (4, 5). The R-protein–cobalamin complex is cleaved in the duodenum and proximal jejunum by pancreatic proteases, and the liberated cobalamin binds to intrinsic factor. Although the role of R-proteins in cobalamin homeostasis is still incompletely understood, evidence that free intrinsic factor is susceptible to gastric acid proteolysis and, therefore, unavailable for binding with cobalamin in the jejunum (12), has led to speculation that cobalamin deficiency after gastric bypass is the result of malabsorption from a deficiency of intrinsic factor. In fact, Marcuard et al (8) have shown that the concentration of intrinsic factor intragastrically in the bypassed stomach is decreased in patients with cobalamin deficiency after gastric bypass. These authors, however, did not study patients after stimulation with pentagastrin or histamine, and only nine of the 19 patients with cobalamin deficiency had an abnormal Schilling test, suggesting that adequate intrinsic factor was present for the absorption of crystalline cobalamin in some patients. Although our study suggests that the major reason for cobalamin absorption after gastric bypass is related to maldigestion of food-bound cobalamin, a relative deficiency of cobalamin-intrinsic factor complexes may coexist since the absorption of crystalline cobalamin after gastric bypass was decreased (albeit not significantly).

In addition, this study demonstrates that secretion of gastric acid from the pouch after vertical Roux-en-Y gastric bypass is negligible and, therefore, the risk of acid/pepsin-mediated marginal ulceration, a potentially serious complication, should be low in contrast to previous reports (13, 14). Indeed, in our practice we have yet to identify any patient with a marginal ulcer in the 100 patients undergoing vertical gastric bypass over the last seven years. The present study corroborates and extends our previous preliminary findings in which gastric acid secretion and absorption of free and food-bound cobalamin absorption were studied in gastric bypass patients and compared to historic controls and healthy subjects, respectively (15).

In summary, biochemical and clinically symptomatic cobalamin deficiency in patients after Roux-en-Y gastric bypass results from decreased acid/pepsin digestion of protein-bound cobalamin. Low or absent gastric acid/pepsin secretion from the proximal gastric pouch fails to liberate cobalamin for eventual binding with intrinsic factor. Although absorption of crystalline cobalamin is decreased slightly postoperatively, the extent of malabsorption is undetermined and requires further study. After gastric bypass, patients should be supplemented with monthly parenteral injections of cobalamin or by daily oral ingestion of crystalline, free cobalamin preparation. We did not modify our Schilling test to include the use of intrinsic factor. In theory, the addition of intrinsic factor to the protein-bound cobalamin preparation would not have enhanced the absorption of food-bound cobalamin, but evidence to support this hypothesis is lacking. Additionally, intrinsic factor may have provided enhanced absorption of free cobalamin postoperatively.

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