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# Perioperative changes in prouroguanylin hormone response in severely obese subjects after bariatric surgery

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ABSTRACT

*Background:* Prouroguanylin is a gut hormone converted into uroguanylin in the hypothalamus. Uroguanylin induces satiety through guanylyl-cyclase-2C receptor signaling. However, little is known about the role of this hormone in regulating human food intake.

*Methods:* In prospective-cohort study, prouroguanylin profile changes were determined during meal stimulation in obese patients 2 weeks before and 2 weeks after Roux-en-Y gastric bypass surgery. We also investigated whether these changes play a role in the anorexigenic effect of Roux-en-Y gastric bypass.

*Results:* The study enrolled 8 healthy lean volunteers and 10 obese patients with type 2 diabetes. Prouroguanylin levels were postprandially decreased at 30 minutes ($P = .04$) and 60 minutes ($P = .008$) in obese patients before surgery, and they were increased at 60 minutes ($P = .003$), 90 minutes ($P = .008$), and 120 minutes ($P = .009$) after surgery. We observed a significant difference ($P = .001$) in fasting prouroguanylin levels before (8.82 ± 1.2 ng/mL) and after (6.05 ± 1.2 ng/mL) Roux-en-Y gastric bypass. Hunger ratings in the fasted state did not change after Roux-en-Y gastric bypass. Instead, subjects demonstrated significantly ($P = .01$) lower hunger visual analog scale scores than before Roux-en-Y gastric bypass. No correlations between circulating prouroguanylin levels and hunger perception were found before or after Roux-en-Y gastric bypass.

*Conclusion:* Prouroguanylin levels decrease after meal stimulation in obese patients, and they increase after Roux-en-Y gastric bypass, but no correlations exist with hunger visual analog scale scores.

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## Introduction

Prouroguanylin is a gut hormone that is released, both apically and basolaterally, from the intestinal cells.[1] Apically secreted prouroguanylin is rapidly converted to uroguanylin by proteases residing within the intestinal lumen. Uroguanylin, acting through a paracrine mechanism, activates the guanylyl cyclase 2C receptor (GUCY2C).[2] This stimulates chloride channel activity in crypt epithelial cells and inhibits sodium/proton exchange in villous epithelial cells, decreasing net intestinal sodium absorption.[3,4] Basolaterally secreted prouroguanylin enters the circulation acting as an endocrine hormone. In the kidney, prouroguanylin is filtered and metabolized to peptide fragments and free amino acids by brush border proteases located within the proximal tubule. One of the peptide fragments (very likely uroguanylin) acts through an unidentified receptor within proximal and/or distal nephron segments to decrease sodium and potassium reabsorption. This accelerates renal salt excretion, which, in conjunction with the delayed intestinal salt absorption, reduces the impact of ingested salt on body fluid compartments.[5] In the hypothalamus, prouroguanylin is locally converted into uroguanylin that induces GUCY2C signaling, activating the appetite-suppressing neuropeptide pro-opiomelanocortin, and therefore decreasing appetite.[6]

Valentino et al.[6] demonstrated that uroguanylin has an anorexigenic effect in mice and that prouroguanylin is secreted in

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response to a meal in both mice and lean, healthy humans. In this report, the prouroguanylin-uroguanylin-GUCY2C system seems to play an important role in food intake and energy homeostasis, representing a novel component of the gut-brain axis. In the commentaries, the data interpretation has generated several speculations about the potential clinical role of this prouroguanylin-uroguanylin-GUCY2C system in human obesity and metabolic syndrome.[7,8] To our knowledge, there are no published studies addressing the role of this system in human obesity.

The purpose of this study is to evaluate whether there are differences in prouroguanylin production and/or function in response to a meal stimulation test in obese individuals before and after Roux-en-Y gastric bypass (RYGB). To determine whether this gut hormone plays a significant role in the physiopathology of obesity and in the well-known anorexigenic effect of bariatric surgery, we measured hunger perception and fasting and postprandial prouroguanylin levels in obese patients before and after RYGB. We also measured fasting and postprandial prouroguanylin levels in lean, healthy subjects to have normative controls.

## Subjects and Methods

### *Study design*

A total of 8 lean, healthy volunteers and 10 morbidly obese patients (aged 49.6 ± 11.2 y; 7/10 females) were enrolled in a prospective study. In the obese group, the mean preoperative body mass index (BMI) was 45.6 ± 7.6 kg/m$^2$. Exclusion criteria for the obese group included previous esophageal, gastric, pancreatic, small bowel, or large bowel operations, hemoglobin A1c higher than 10.0%, use of insulin, dipeptidyl peptidase-IV inhibitors, or Glucagon-like peptide-1 (GLP-1) analogues, tobacco use, known alcohol or substance abuse within 6 months of enrollment, inability to provide informed consent. All the obese patients had a diagnosis of type 2 diabetes mellitus. The study's protocol has been described elsewhere.[9] Briefly, all obese subjects completed a mixed-nutrient meal stimulation study within 2 weeks before (baseline) and 2 weeks after RYGB. The RYGB was performed using a laparoscopic approach. A linear stapler was used to create a 30-ml gastric pouch. An ante-colic, retro-gastric Roux-en-Y gastrojejunostomy, 100-cm long Roux limb, and 30-cm biliopancreatic limb were created. All subjects were able to consume the entire volume of the meal. Venous blood samples were collected immediately before (fasting state) drinking the liquid meal and every 30 minutes thereafter for 2 h. Obese subjects completed a visual analog scale (VAS) assessment of hunger at baseline and 30-, 60-, and 120-minute blood sample collections, as validated elsewhere.[10] A higher VAS score indicates a greater sensation of hunger. We measured circulating prouroguanylin levels, using a human prouroguanylin enzyme-linked immunosorbent assay kit (BioVendor, Candler, NC).

### *Statistical analysis*

A one-way within-subjects analysis of variance, with multiple paired comparisons, was performed to evaluate differences in circulating prouroguanylin levels between each time point in the healthy group and in the obese group pre and post RYGB. Paired Student *t*-tests were used to compare pre- and post-RYGB data. Pearson correlation coefficients were calculated to determine the relationship between circulating prouroguanylin levels and hunger VAS scores in response to fasting and meal stimulation (at 30, 60, and 120 minutes) pre and post RYGB. Repeated-measure general linear modeling (GLM) was used to compare meal stimulation curves (prouroguanylin and hunger VAS score) pre and post RYGB. Data are presented as means ± standard errors of the mean.

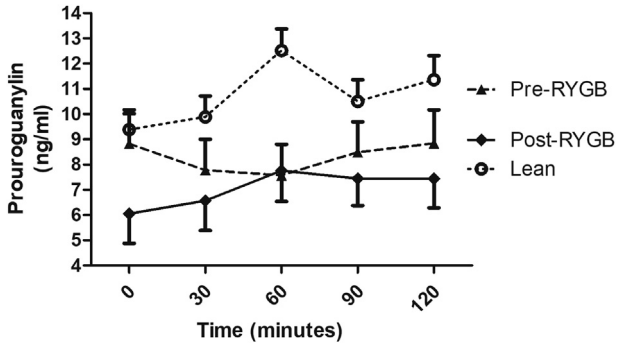


**Fig 1.** Postprandial prouroguanylin levels in lean subject and before and after RYGB. *RYGB*, Roux-en-Y gastric bypass.

*P* values of less than.05 were considered to indicate statistical significance. Statistical analyses were performed using SPSS v 22 (IBM Corp, Armonk, NY, USA).

## Results

The mean 2-week postoperative change in BMI was −3.0 ± 1.4 kg/m$^2$ at 14.6 days (standard errors of the mean = 0.43). After the meal tolerance test, the time course of changes in prouroguanylin levels from the basal levels in healthy subjects were different ($F = 29.747$, $P < .001$). Polynomial contrasts indicated that the relationship between the within factor (time) and prouroguanylin levels is quadratic ($F = 21.417$, $P = .002$). As shown in Figure 1, in lean, healthy volunteers, multiple paired comparisons showed a significant increase between fasting and 60 minutes ($P < .001$) and between 30 and 60 minutes ($P < .001$). We observed a significant decrease between 60 and 90 minutes ($P = .001$), which then increased significantly again at 120 minutes ($P = .039$). At 120 minutes, the levels of prouroguanylin were significantly higher than at baseline.

After the meal tolerance test, the time course of changes in prouroguanylin levels from the basal levels in obese patients were different ($F = 3.2$, $P = .023$). Polynomial contrasts indicated that the relationship between the within factor (time) and prouroguanylin levels is quadratic ($F = 11.8$, $P = .007$). As shown in Figure 1, in pre-RYGB subjects, multiple paired comparisons showed a significant decrease between fasting and 30 minutes ($P = .04$) and between fasting and 60 minutes ($P = .008$). We observed a significant increase between 60 and 120 minutes ($P = .03$).

After the meal tolerance test, the time course of changes in prouroguanylin levels from the basal levels in obese patients post RYGB were different ($F = 6.9$, $P = .001$). Polynomial contrasts indicated that the relationship between the within factor (time) and prouroguanylin levels is linear ($F = 11.7$, $P = .008$). Multiple paired comparisons found a significant increase between fasting and 60 minutes ($P = .003$), fasting and 90 minutes ($P = .008$), fasting and 120 minutes ($P = .009$), and 30 and 60 minutes ($P = .005$).

We observed no statistical difference between prouroguanylin curves pre and post RYGB, using repeated-measure GLM ($P = .09$). However, fasting circulating prouroguanylin levels before (8.82 ± 1.2 ng/mL) and after (6.05 ± 1.2 ng/mL) RYGB differed significantly ($P = .001$). There was also a significant group (pre-post RYGB) * time (0–120 minute) interaction ($P = .009$).

Subjects post RYGB demonstrated significantly ($P = .01$) lower hunger VAS scores than before RYGB, using repeated-measure GLM (Fig 2). However, in the fasted state, there was no difference in hunger VAS scores before and after RYGB.

We did not find any correlation between circulating prouroguanylin levels and hunger VAS scores at any time point before or

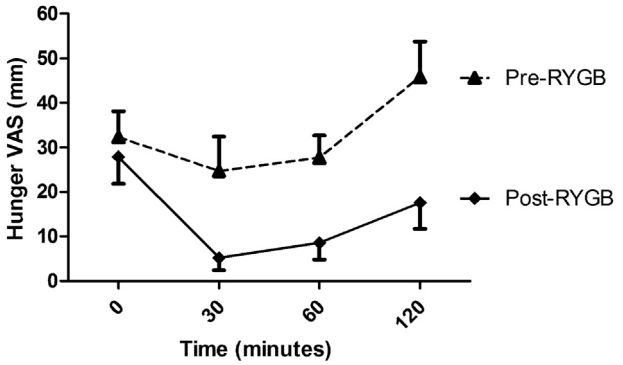


**Fig 2.** Hunger VAS score before and after RYGB. *VAS*, visual analog scale; *RYGB*, Roux-en-Y gastric bypass.

after RYGB in our obese group. Also, no correlations were found between prouroguanylin and BMI.

## Discussion

This pilot study presents the first data on prouroguanylin before and after RYGB. We measured circulating prouroguanylin pre- and post-meal in obese and in lean, healthy subjects. We found that, in this group of obese subjects, the response of prouroguanylin to meal stimulation is dysregulated compared with that in lean, healthy subjects. Two weeks after RYGB, the same obese subjects have a significantly different postprandial profile. Furthermore, we found that the postprandial prouroguanylin profile does not correlate with hunger perception before or after RYGB.

The regulation of food intake and energy metabolism involves a series of complex interactions between nutrients, gut hormones, adipokines, and the brain.[11] Changes in several of these factors have been shown to play a role in the metabolic effects of RYGB.[12] Prouroguanylin is a recently discovered gut hormone that has been shown to be involved in food-intake regulation in mice.[6]

In humans, the uroguanylin gene is mainly expressed in somatostatin-containing D cells of the ileum and colon.[13] In rats, prouroguanylin is secreted intact from the intestine into the circulation, is not processed intravascularly, and is cleared from the plasma by the kidneys.[1,5] Qian et al[5] hypothesized that the intestinal release of prouroguanylin represents an adaptive mechanism that augments renal salt and fluid excretion in response to pathologic salt and fluid retention. To support this hypothesis is the finding that plasma and urinary levels of uroguanylin and prouroguanylin are elevated in patients with kidney disease, and urinary uroguanylin levels are elevated in congestive heart failure patients.[14–16] In lean, healthy individuals, Valentino et al[6] found an increase in circulating prouroguanylin levels after a meal by comparing the circulating prouroguanylin levels at fasting and after meal stimulation (mean postprandial 30–150 minutes). Our data from lean, healthy subjects support these findings.

In obese patients before RYGB, we found a significant decrease in prouroguanylin levels from fasting to 30 and 60 minutes after meal stimulation, and levels returned to fasting levels after 90 minutes. Our finding in obese subjects is quite different from what we found in lean individuals. Therefore, we suggest that obesity is characterized by an impaired postprandial prouroguanylin secretion. Because prouroguanylin has been reported to have an anorexigenic effect, we were expecting an inverse correlation between postprandial prouroguanylin levels and hunger perception. However, our data did not support this hypothesis, confuting the anorexigenic effect of the prouroguanylin-uroguanylin-GUCY2C system.

We found a significant increase in prouroguanylin levels after RYGB from fasting to 60, 90, and 120 minutes after a meal. This postprandial response differs from what we found before surgery and shows a profile similar to lean, healthy subjects. It appears that RYGB restores the physiologic postprandial prouroguanylin response found in lean subjects. We infer that the increased postprandial prouroguanylin levels after RYGB is explained by the accelerated transit of nutrients into the prouroguanylin-secreting cells of the ileum and colon. The nutrient accelerated transit (ileal-brake) is a well-described phenomenon after RYGB.[17] Despite that RYGB restored the postprandial prouroguanylin response, we did not observe any correlation with the anorexigenic effect that follows RYGB. In our earlier study, we showed that RYGB, before substantial weight loss and independent of caloric restriction, resulted in an augmented postprandial Peptide YY and GLP-1 response.[9] We also found a negative correlation between GLP-1 levels and hunger perception, supporting the theory that increased secretion of this incretin after RYGB contributes to decreased sensations of hunger, and may ultimately be a mechanism involved in the long-term success of the surgery. These conclusions cannot be drawn for prouroguanylin.

Further supporting that the prouroguanylin-uroguanylin-GUCY2C system is not clinically involved in the anorexigenic effect of RYGB is the finding that fasting prouroguanylin levels decreased significantly post RYGB. This is in contrast with the prolonged satiety between meals that patients experience after RYGB.[18] Moss et al[1] have demonstrated that the intestine is the likely source of the circulating prouroguanylin by showing that systemic prouroguanylin levels decrease rapidly after intestinal resection in rats. Based on this observation, we hypothesize that bypassing approximately 150 cm of alimentary tract after RYGB can result in a decreased basal secretion of prouroguanylin.

To summarize, this study is the first to demonstrate that prouroguanylin levels decrease after meal stimulation in obese patients but increase after RYGB surgery. However, we found no correlations between hunger perception and prouroguanylin levels, making it difficult to support the hypothesis that the prouroguanylin-uroguanylin-GUCY2C system plays a significant role in the anorexigenic effect of bariatric surgery. This lack of correlation can be explained by the limitations of our study: the small sample size of the groups, and the follow up limited to 2 weeks. Further studies are needed to better understand the role of the prouroguanylin-uroguanylin-GUCY2C system in human obesity.

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## Conflict of interest/Disclosure

None of the authors have any conflicts of interest to disclose.

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# Discussion


**Dr Bestoun Ahmed** (Pittsburgh, PA): Thank you so much, Dr Shoup and the CSA leadership, for inviting me as a discussant for this mechanistic basic science study to further investigate pathophysiology of obesity.

Dr Torquati and co-authors have continued their previous effort to investigate hormonal derangement in the disease of obesity and how that would change following the Roux-en-Y gastric bypass. Their previous study published in *The Journal of Endoscopy* in 2012 found correlation between appetite, Glucagon-like peptide-1, and polypeptide-Y levels. This time, it's about prouroguanylin and uroguanylin levels.

The effect of this gut hormone has not been studied much in the human population. This is one of the earliest studies checking that relationship. It is thought that prouroguanylin and uroguanylin regulate food intake and energy expenditure through the CNS effect. Animal studies have suggested that these hormones may induce satiety.

Prouroguanylin increases lipolysis, and this may explain its low levels in obese people.

Randy Seeley from the University of Cincinnati, in his paper published in *Diabetes* in 2014, concluded that prouroguanylin has no central effect on satiety, and the process is much more complex than that. Understanding the brain-gut-adipose axis may clear the path for targeted nutritional therapies.

I have three questions and one comment.

Prouroguanylin levels in lean, healthy subjects: Is it quadratic or linear? Also, I wonder why the level drops at 90 minutes and then goes up again.

Second, how did we conclude the absence of correlation between prouroguanylin levels and body mass index? Any data supporting that?

The third one, where do we go from here? And how do you envision the future of interesting studies like this one in our understanding and possibly treating obesity?

Although there was negative correlation between hormone levels and the hunger VAS score, performing a pilot study and increasing number of these patients may give different results, which may make the relationship between these hormones, appetite, and energy status more understandable.

Finally, the role of microbiota in the pathophysiology of obesity and GC-C receptor dysregulation could be another promising arena in our fight against obesity.

Thank you so much.

**Dr Alfonso Torquati:** Thank you, Ahmed, for your comments.

Regarding BMI, I think it was important in the paper—I forgot to mention during the presentation—but we found no correlation between the body mass index and body mass change after gastric bypass and the prouroguanylin levels.

In regard to the linear relationship, I am not the sole person on this team, and I will ask that question and report to you later. We have no explanation of why the increase occurred after 90 minutes. We are just talking probably about gastric bypass patients sometimes, but there is some fluid retention that maybe is going to empty later that can maybe justify some of that effect. But definitely, we have no explanation.