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Metabolic responses to exogenous ghrelin in obesity and early after Roux-en-Y gastric bypass in humans

Robyn A. Tamboli¹, Joseph Antoun¹, Reem M. Sidani¹, B. Austin Clements¹, Emily A. Eckert¹, Pam Marks-Shulman¹, Bruce D. Gaylenn², D. Brandon Williams¹, Ronald H. Clements¹, Vance L. Albaugh¹, and Naji N. Abumrad¹

¹Department of Surgery, Vanderbilt University Medical Center, Nashville, TN

²Division of Endocrinology and Metabolism, Department of Medicine, University of Virginia, Charlottesville, VA

Abstract

Aims—Ghrelin is a gastric-derived hormone that stimulates growth hormone (GH) secretion and has a multi-faceted role in the regulation of energy homeostasis, including glucose metabolism. Circulating ghrelin concentrations are modulated in response to nutritional status, but responses to ghrelin in altered metabolic states are poorly understood. We investigated the metabolic effects of ghrelin in obesity and early after Roux-en-Y gastric bypass (RYGB).

Materials and Methods—We assessed central and peripheral metabolic responses to acyl ghrelin infusion ($1 \text{ pmol kg}^{-1} \text{ min}^{-1}$) in healthy, lean subjects ($n=9$) and non-diabetic, obese subjects ($n=9$) before and two weeks after RYGB. Central responses were assessed by GH and pancreatic polypeptide (surrogate for vagal activity) secretion. Peripheral responses were assessed by hepatic and skeletal muscle insulin sensitivity during a hyperinsulinemic-euglycemic clamp.

Results—Ghrelin-stimulated GH secretion was attenuated in obese subjects, but was restored by RYGB to a response similar to lean subjects. The heightened pancreatic polypeptide response to ghrelin infusion in the obese was attenuated after RYGB. Hepatic glucose production and hepatic insulin sensitivity were not altered by ghrelin infusion in the RYGB subjects. Skeletal muscle insulin sensitivity was impaired to a similar degree in the lean, obese, and post-RYGB in response to ghrelin infusion.

Conclusions—These data suggest that obesity is characterized by abnormal central, but not peripheral, responsiveness to ghrelin that can be restored early after RYGB before significant

Correspondence: Robyn A. Tamboli, 1161 21st Ave S, CC2308 MCN, Nashville, TN 37232-2730, Phone: (615)322-8588, Fax: (615)343-6456, robyn.tamboli@vanderbilt.edu.

AUTHOR CONTRIBUTIONS

R.A.T. planned the study design, conducted experiments, reviewed the data, performed data analysis, and wrote the manuscript. J.A. conducted experiments, performed sample analysis, and contributed to the discussion. R.M.S. performed sample analysis. B.A.C. researched data. E.A.E. conducted experiments and provided study support. P. M.S. conducted experiments and provided study support. B.D.G. performed sample analysis. D.B.W. researched data and contributed to discussions. R.H.C. researched data and contributed to discussions. V.L.A. assisted with data analysis, contributed to discussions, and edited the manuscript. N.N.A. planned the study design, reviewed the data, and edited the manuscript. All authors approved submission of the manuscript.

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weight loss. Further work is necessary to fully elucidate the role of ghrelin in the metabolic changes that occur in obesity and after RYGB.

INTRODUCTION

Ghrelin is a gastric hormone that was discovered as an endogenous stimulator of growth hormone (GH) secretion¹ that stimulates the hypothalamic-pituitary axis via the growth hormone secretagogue receptor type 1a (GHSR-1a). An octanoyl group at Ser-3 of the ghrelin peptide (acyl ghrelin, hereafter referred to as ghrelin) is necessary for binding to GHSR-1a; however, unacylated (desacyl) ghrelin is also present in the circulation, and evidence suggests it is biologically active.² Ghrelin is more widely known for its centrally-mediated regulation of short- and long-term energy homeostasis by increasing hunger, food intake, and adiposity,^{3–5} and thus has been proposed as target for anti-obesity therapies. Ghrelin can cross the blood-brain barrier,⁶ but the central effects of peripherally-secreted ghrelin may also be mediated by the vagus nerve.^{4,7–9} Ghrelin also plays a role in the peripheral regulation of energy homeostasis as a negative regulator of glucose metabolism by increasing insulin resistance.^{10,11} Evidence suggests that ghrelin-mediated insulin resistance can occur independent of ghrelin-induced GH secretion.^{12,13}

Although the physiologic functions of ghrelin are closely linked to the metabolic abnormalities in obesity, little is known regarding the metabolic effects of ghrelin in obese humans or in the setting of insulin resistance. This is important knowledge in determining the therapeutic potential of targeting the ghrelin system in obesity and metabolic disorders. Circulating ghrelin concentrations are lower in obese compared to lean individuals^{14,15} and are accompanied by an attenuated GH response to ghrelin administration.^{3,16} The hyperglycemic response to exogenous ghrelin is retained in obese subjects.^{17–19} Ghrelin infusion in lean subjects reduced insulin secretion and impaired glucose tolerance in response to intravenous glucose^{20–22} and also worsened insulin-stimulated glucose uptake (insulin sensitivity) during hyperinsulinemic-euglycemic conditions.¹⁰ The effects of ghrelin on insulin resistance in the obese have not been reported.

Roux-en-Y gastric bypass (RYGB) surgery is the most effective treatment for severe obesity and metabolic comorbidities. The RYGB operation involves dividing the stomach to create a small gastric pouch, which is then anastomosed to the jejunum. The altered gastrointestinal anatomy and nutrient exposure after surgery produces changes in gastrointestinal hormones that regulate nutrient and energy homeostasis that are thought to contribute to the weight loss-independent improvement in glucose metabolism. Plasma ghrelin concentrations were initially reported to substantially decrease after RYGB,²³ however subsequent studies have provided conflicting data.²⁴ This discrepancy has been attributed to small study cohorts, variations in surgical technique, differences in ghrelin measurements, and length of time after surgery.²⁴ Some data support an initial decrease in ghrelin concentrations at early postoperative time points that rebounds later.^{24–26}

The purpose of this study was to perform initial studies to determine if central and peripheral responses to ghrelin were altered in obesity and changed early after RYGB, a time of caloric restriction with minimal weight loss. We examined central ghrelin effects by measuring the

GH and pancreatic polypeptide (PP, a measure of vagal activity) responses to ghrelin infusion. We also assessed the peripheral effects of ghrelin by measuring hepatic and muscle insulin sensitivity with hyperinsulinemic-euglycemic clamps during acyl ghrelin infusion.

MATERIALS AND METHODS

Study Participants

Obese participants approved for RYGB were recruited from the Vanderbilt Center for Surgical Weight Loss and lean participants with a BMI $<30 \text{ kg/m}^2$ from the community. This study included participants without evidence of type 1 or type 2 diabetes, liver or kidney disease, or abnormal cardiac function. Two obese subjects were withdrawn from the study due to inability to maintain venous or arterial access during the first study visit. Nine obese and nine lean subjects completed the study. All study procedures were performed at the Vanderbilt Clinical Research Center (CRC) after obtaining informed, written consent. This study was approved by the Vanderbilt Institutional Review Board and the United States Food and Drug Administration and registered with [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00884494) (NCT00884494).

Study Protocol

Obese participants completed two study visits; within the month prior to RYGB and two weeks after RYGB. Lean participants completed one study visit. Each visit was two consecutive days, with a hyperinsulinemic-euglycemic clamp with glucose tracer ($[3\text{-}^3\text{H}]$ -glucose, PerkinElmer) on each day in combination with either an acyl ghrelin (Clinalfa[®], Bachem) or saline infusion. An acyl ghrelin infusion rate of $1 \text{ pmol kg}^{-1} \text{ min}^{-1}$ was chosen to be less than previously used in hyperinsulinemic-euglycemic clamp studies^{10,11,27} and show differential effects in obesity.³ The duration of infusion coincided with the time needed to reach steady state in the hyperinsulinemic-euglycemic clamps. The infusion order of ghrelin or saline was randomized for each study visit; participants were blinded to the order of infusion.

Upon admission, a history and physical examination, ECG, and safety labs were obtained. Participants were given a standardized meal and snack, and restricted to water after 20:00. The next morning a catheter was inserted in an antecubital vein for infusions and a second catheter for blood drawing was placed either in a radial artery or heated, superficial vein to obtain arterialized-venous blood. After a baseline blood draw at 08:30, infusions of $[3\text{-}^3\text{H}]$ -glucose ($33 \text{ } \mu\text{Ci}$ prime then $0.14 \text{ } \mu\text{Ci min}^{-1}$) and acyl ghrelin ($53.4 \text{ pmol kg}^{-1} \text{ min}^{-1}$ for 10 min, then $1 \text{ pmol kg}^{-1} \text{ min}^{-1}$) or saline were initiated and continued for 270 min. After a 120 min tracer equilibration, blood was sampled every 5 min for glucose and $[3\text{-}^3\text{H}]$ -glucose measurements and every 15 min for measurements of insulin, ghrelin, free fatty acids (FFA), GH, and PP (basal period). Additional GH and PP measurements were obtained at 30, 60, and 90 min to account for the initial rise and subsequent decline in GH during a constant ghrelin infusion.¹⁰ At 150 min, the hyperinsulinemic-euglycemic clamp began with an insulin infusion that continued for 120 min ($160 \text{ mU m}^{-2} \text{ min}^{-1}$ for 8 min, then $80 \text{ mU m}^{-2} \text{ min}^{-1}$). A variable 20% dextrose infusion maintained plasma glucose concentration between 90–100 mg/dl. During the last 30 min of the insulin infusion, blood was sampled every 5 min for glucose and $[3\text{-}^3\text{H}]$ -glucose measurements and every 15 min for insulin and ghrelin

measurements (insulin period). Blood pressure (cuff placed on leg), heart rate, and ECG were monitored (DINAMAP®) throughout the clamp procedure and are reported during the last 30 min of the basal period. After the procedure, subjects were given a meal and remained on the CRC. Participants were given a standardized meal and snack, and restricted to water after 20:00. All study procedures were repeated the second day, except with the alternate infusion (ghrelin or saline). Fat mass and fat-free mass were acquired by dual-energy x-ray absorptiometry as previously described.²⁸

Sample Collection and Analysis

Blood was collected in chilled EDTA tubes and immediately processed and frozen. Glucose was measured by the glucose oxidase method (YSI 2300 STAT Plus). Insulin was measured by RIA (Millipore). Acyl ghrelin was measured in using a two site sandwich assay specific for the full-length peptide as previously characterized²⁹; AEBSF (8mM) was added to blood and plasma was acidified (0.1N HCl). ELISA was used to measure total ghrelin (acyl + desacyl ghrelin, Millipore), GH, and insulin-like growth factor-1 (IGF-1) (R&D systems). Total ghrelin levels in one subject were undetectable after RYGB and were assigned the lowest value on the standard curve (90 pg/ml). PP was measured with the MILLIPLEX® map Human Metabolic Hormone Magnetic Bead Panel immunoassay (Millipore). PP levels were undetectable in some obese subjects and were re-assayed with double volume to increase sensitivity. Still, <10% of samples were still undetectable and assigned a value of the lowest standard (1.04 pg/ml); these values were primarily confined to one subject before and after RYGB. PP and total ghrelin analyses performed without these subjects yielded the same results. FFA were determined by the NEFA-HR(2) enzymatic colorimetric method (Wako Diagnostics). Plasma [3-³H] glucose was measured by liquid scintillation counting of the Somogyi filtrate after removal of radioactive water.

Calculations

Glucose, insulin, FFA, and total ghrelin are the average of three samples during the basal and/or insulin periods; acyl ghrelin was measured once each period. Hepatic glucose production (HGP) was calculated as [3-³H]-glucose infusion rate/[3-³H]-glucose specific activity ([3-³H]-glucose/glucose), averaged from five measurements during the basal period after subtraction of [3-³H]-glucose background. The hepatic insulin sensitivity index (HISI) was calculated as $1000/(\text{HGP mg min}^{-1} \times \text{insulin } \mu\text{U ml}^{-1})$.³⁰ Peripheral (primarily skeletal muscle) insulin sensitivity was measured as the steady-state glucose infusion rate ($\text{mg kg}^{-1} \text{min}^{-1}$) needed to maintain euglycemia under hyperinsulinemic conditions (M) and relative to steady-state insulin levels (M/I) calculated as $1000 \times (\text{mg kg}^{-1} \text{min}^{-1}/\mu\text{U ml}^{-1})$.

Statistical Analyses

Data are expressed as mean $\pm$ SEM. Incremental area under the curve (AUC) was calculated using the trapezoidal rule. Repeated measures two-way ANOVA was used to test for a treatment (ghrelin or saline) by group (lean or obese pre-RYGB) interaction and treatment (ghrelin or saline) by time (pre-RYGB or post-RYGB) interaction; Bonferonni post-hoc tests were used to determine the effect of ghrelin within each group or time point. Unpaired and paired Student's *t*-tests were also used to make comparisons as appropriate; data distributions were examined and non-parametric tests were used for non-normally

distributed data. $P < 0.05$ was considered significant. Analyses were performed in GraphPad Prism[®] version 6. Power and sample size calculations were based on published data in eight lean subjects which demonstrated a $2.9 \text{ mg kg}^{-1} \text{ min}^{-1}$ reduction in peripheral insulin sensitivity with ghrelin infusion.¹⁰ We estimated that a sample size of at least nine subjects per group would allow us to detect a $1.9 \text{ mg kg}^{-1} \text{ min}^{-1}$ within-group difference in peripheral insulin sensitivity with ghrelin infusion ($\alpha = 0.05$, 90% power).

RESULTS

Characteristics of the Study Participants

The study cohorts consisted of nine lean subjects and nine obese subjects of similar age, and were all females without type 2 diabetes (Table 1). At approximately two weeks (14 ± 3 days) after RYGB, obese participants lost $6.1 \pm 0.4 \text{ kg}$ ($P = 0.0001$), amounting to a $5.4 \pm 0.4\%$ body weight loss. Approximately two-thirds of the weight loss was due to decreased fat-free mass (Table 1).

Ghrelin Concentrations

Acyl and total ghrelin levels during the saline and ghrelin infusions within the basal period of the clamp are shown in Figure 1A & 1C. Lean subjects had an endogenous, fasting acyl ghrelin concentration ~60% higher than the obese subjects before RYGB (79.8 ± 19.0 vs. $31.7 \pm 3.6 \text{ pg/ml}$, $P < 0.0001$). At two weeks after RYGB, acyl ghrelin was reduced ~50% ($15.8 \pm 2.8 \text{ pg/ml}$, $P = 0.0002$). Total ghrelin exhibited similar trends (754 ± 77 , 348 ± 40 , $170 \pm 22 \text{ pg/ml}$). Ghrelin infusion increased plasma acyl ghrelin to ~250 pg/ml during the basal period, with significantly higher acyl ghrelin concentrations in the lean compared to obese pre-RYGB ($P = 0.009$) that were not different before versus after RYGB ($P = 0.347$). Acyl ghrelin concentrations increased 2.5-fold in lean, 8.5-fold in obese pre-RYGB, and 15.5-fold post-RYGB. Total ghrelin concentrations reached ~3000 pg/ml with ghrelin infusion and, in contrast to acyl ghrelin, were not different between the lean and obese pre-RYGB ($P = 0.149$). Total ghrelin concentrations increased 4-fold in the lean, 10-fold in obese pre-RYGB, and 19-fold after RYGB.

Acyl and total ghrelin concentrations during the saline and ghrelin infusions within the insulin period of the clamp are shown in Figure 1B & 1D. During the insulin period of the clamp, the endogenous acyl ghrelin concentration in the lean was no longer higher than the obese pre-RYGB ($P = 0.134$), and the post-RYGB remained lower ($P = 0.002$). During ghrelin infusion, acyl ghrelin concentrations were higher in obese than lean ($P = 0.001$) and did not change after RYGB ($P = 0.112$). The values for total ghrelin followed the same trend, although there were differences in the comparisons that reached significance.

Others have observed that insulin infusion decreases circulating endogenous ghrelin levels.^{31,32} During the insulin infusion period on the saline day, endogenous acyl ghrelin concentrations were decreased ~40% in the lean ($P < 0.0001$) but not in the obese ($P = 0.603$; $P = 0.0009$, group $\times$ treatment interaction) or after RYGB ($P = 0.398$). There was no effect of insulin on plasma acyl ghrelin levels during the ghrelin infusion (all $P = 0.678$).

Tolerability and Cardiovascular Effects of Ghrelin Infusion

The ghrelin infusion was well-tolerated by all participants. Hunger and fatigue were each reported only by two subjects. Heart rate and blood pressure were monitored throughout the study (Table 2). Ghrelin infusion did not alter heart rate in the lean nor obese pre-RYGB, but caused a small but significant decrease in heart rate at two weeks after RYGB (-6 ± 2 bpm). Systolic blood pressure was significantly decreased in lean participants with ghrelin infusion (-9 ± 4 mm Hg), but was not altered in the obese pre-RYGB and post-RYGB subjects. Neither diastolic blood pressure nor mean arterial pressure were affected by ghrelin infusion.

Effect of Ghrelin Infusion on GH and PP

Ghrelin infusion stimulated GH secretion in lean, obese pre-RYGB, and post-RYGB subjects (Figure 2A–C). There was no differential effect of ghrelin infusion on the GH AUC between lean and obese (Figure 2G; $P=0.421$ for treatment $\times$ group interaction); however, two lean subjects had spontaneous GH secretion during the saline infusion (as previously observed³³) that likely affected this analysis. When directly comparing the GH AUCs from the ghrelin infusion day only, the lean had a significantly higher GH secretion than the obese ($P=0.003$). The effectiveness of ghrelin to increase GH secretion was increased after RYGB (Figure 2G; $P=0.0002$, time $\times$ treatment interaction). We also measured IGF-1, a mediator of the effects of GH and negative regulator of GH secretion. IGF-1 did not change in response to ghrelin infusion in lean subjects (Figure S1A&B) despite a large increase in GH (Figure 2A). Therefore, we did not measure IGF-1 during ghrelin infusion in the obese pre-RYGB and post-RYGB subjects. The obese had significantly lower fasting IGF-1 levels compared to lean, with no change post-RYGB (Figure S1C).

Ghrelin infusion only stimulated PP secretion in the obese pre-RYGB subjects (Figure 2D–F). There was a differential effect of ghrelin infusion on the PP AUC between lean and obese (Figure 2H; $P=0.015$ for treatment $\times$ group interaction). The effect of ghrelin to increase PP secretion was reduced after RYGB (Figure 2H; $P=0.021$, time $\times$ treatment interaction).

Effect of Ghrelin Infusion on Peripheral Glucose Metabolism

After two hours of ghrelin infusion, plasma glucose levels were significantly increased in the lean and obese pre-RYGB, and the effect was significantly greater in the obese (Table 2). The effect of ghrelin to increase plasma glucose was attenuated after RYGB. Ghrelin infusion did not alter HGP in the lean, obese pre-RYGB, or post-RYGB (Table 2). There was a slight effect of ghrelin to reduce hepatic insulin sensitivity in the lean that was not present in the obese before or after RYGB (Figure 3A).

Ghrelin infusion decreased insulin-stimulated glucose disposal (M value) in the lean, obese pre-RYGB, and post-RYGB (Table 2). However, there was no significant difference in the effect of ghrelin on the M value in lean compared to obese pre-RYGB or obese pre-RYGB to post-RYGB. While ghrelin did not significantly affect steady-state insulin concentrations during the clamp in any of the three groups (Table 2), the insulin concentrations were overall lower in the lean compared to the obese ($P=0.0001$) and after surgery ($P=0.021$). Even when accounting for plasma insulin concentrations, M/I (Figure 3B), there was still no differential

effect of ghrelin on peripheral insulin sensitivity between lean and obese pre-RYGB or obese pre-RYGB and post-RYGB.

DISCUSSION

In this study, we determined if the central and peripheral responses to ghrelin were altered by obesity and after RYGB. The reduced sensitivity to the GH-stimulating effects of ghrelin in the obese was restored at two weeks after RYGB. Conversely, the enhanced vagal stimulation (assessed by PP) by ghrelin in the obese was reduced at two weeks after RYGB. While ghrelin caused a greater elevation in fasting plasma glucose in the obese that was absent after RYGB, this occurred without any detectable effect of ghrelin on HGP. Ghrelin worsened peripheral insulin sensitivity with no differential effect in the obese or after RYGB. Our data point to an abnormal response to the central effects of ghrelin in the obese that is improved after RYGB, while the peripheral effects of ghrelin are unchanged with obesity or early after RYGB, before major weight loss occurs.

Ghrelin has been shown to exert a negative effect on glucose metabolism. Few studies have explored this effect of ghrelin in the context of obesity and insulin resistance. Our data demonstrate that the hyperglycemic response to ghrelin infusion is present in the obese and appears greater than the response observed in lean subjects. Nonetheless, we did not detect an increase in hepatic glucose production with ghrelin infusion that could explain the observed increase in plasma glucose production, in agreement with previous reports.^{10,11} Ghrelin caused similar reductions in skeletal muscle insulin sensitivity in the obese and lean that was of similar degree to those reported for a higher ghrelin infusion rate.^{10,11,27} Tong and colleagues did not find an effect of ghrelin on insulin sensitivity in lean, healthy volunteers despite using a higher ghrelin dose;^{20,22} however, a different methodology was used to evaluate insulin sensitivity (IV glucose tolerance test with minimal model) that may contribute to this discrepancy. The mechanism(s) responsible for ghrelin-induced insulin resistance is unknown. Although GH can cause insulin resistance and glucose intolerance,³⁴ the effects of ghrelin on glucose metabolism appear to be independent of ghrelin-induced GH secretion in humans.^{11,12,35} Our data may support this due to the differential effects of ghrelin on GH secretion and the consistent effects on insulin sensitivity across differing metabolic states. However, we cannot completely rule out the possibility of a differential sensitivity to the peripheral actions of GH. Genetic or pharmacological blockade of the GHSR-1a in rodents results in improved insulin sensitivity, suggesting a receptor-mediated mechanism.^{36,37} Given both insulin-sensitive lean and insulin-resistant obese had similar reductions in insulin-stimulated glucose uptake, it is unlikely that ghrelin infusion directly interferes with insulin signaling. This is in agreement with the lack of an effect of ghrelin infusion in humans on muscle insulin signaling pathways.^{11,38} Local administration of ghrelin to the muscle did not alter glucose uptake, indicating an indirect, systemic effect of ghrelin on muscle insulin sensitivity,^{38,39} which warrants further investigation.

Ghrelin was discovered as a gastric peptide that is a direct, endogenous stimulator of GH secretion, which plays a role in nutrient metabolism and body composition. Consistent with previously reported findings, the data from our study suggest that the central effects of peripherally-secreted ghrelin may be exerted directly,⁶ most likely on the

hypothalamus,^{40–42} or mediated via the vagus nerve.^{4,7,8,43,44} Several lines of evidence support a primarily hypothalamic effect of ghrelin on GH secretion.⁴² For example, ghrelin receptors are present on GHRH neurons, which are stimulated by ghrelin.⁴⁰ Additionally, hypothalamic-pituitary disconnection in humans blunts the GH response to ghrelin, suggesting a hypothalamic site of action of ghrelin.⁴¹ However, we cannot discount that the ghrelin-induced GH secretion in our study is due to direct effects of ghrelin on the pituitary. We observed a blunted GH response to ghrelin in the obese, consistent with two previous reports.^{3,18} At two weeks after RYGB, the responsiveness of GH to ghrelin is nearly normalized. The differential effects of ghrelin on GH do not appear related to IGF-1, as it did not change in response to acute ghrelin infusion despite increased GH, consistent with previous reports.^{27,45} Fasting IGF-1 was not altered at two weeks after RYGB; however, the literature is inconsistent in this regard.^{46–48} While the exact mechanisms for the observed improvement in the central effect of ghrelin after RYGB are unclear, they are likely related to the postoperative caloric restriction concurrent with a 5% weight loss. A body of literature in rodents describes a hypothalamic ghrelin resistance in the obese state that is reversed by diet-induced weight loss.⁴⁹ Another potential contributing factor could be related to the ~50% reduction in plasma ghrelin after RYGB. Lean subjects with long-term total gastrectomy had a ~50% reduction in ghrelin levels and an increased ghrelin-induced GH secretion compared to matched subjects with an intact stomach.⁵⁰ These data raise the possibility that the reduction in ghrelin levels observed after RYGB could lead to hypersensitization of the hypothalamus and contribute to the improvement in ghrelin-induced GH secretion after the operation.

We indirectly assessed the effect of ghrelin infusion on vagal signaling by measuring PP secretion, which is largely under vagal control.⁵¹ Ghrelin infusion did not stimulate PP secretion in the lean subjects. This is consistent with another report showing that a constant ghrelin infusion did not alter PP secretion,⁵² but differs from another study wherein a bolus injection of ghrelin caused PP secretion in lean subjects.⁵³ The vagal hyper-responsiveness to ghrelin in the obese was attenuated following RYGB. While care is taken during the RYGB procedure to preserve the vagal trunks, it is conceivable that nerve branches are compromised during creation of the gastric pouch causing vagal dysfunction.⁵⁴ In rodents, blockade of vagal signaling attenuated ghrelin-induced GH secretion and feeding,^{7,8} although the latter effect was not observed in one study.⁴³ Conversely, ghrelin infusion in human subjects with previous vagotomy retain the GH-stimulating effects of ghrelin^{9,44} but no longer respond to the feeding effect of ghrelin.^{4,9} Taken together, the human studies suggest that the effect of ghrelin to increase GH secretion is primarily mediated by the hypothalamus, and it is likely that the increased vagal activation observed in the obese, which is reversed with RYGB, is not related GH secretion but rather to feeding behaviors. Interestingly, one study reported that obese subjects had increased sensitivity to the appetite-stimulating effects of ghrelin compared to lean.³ Further work is clearly necessary to elucidate the effects of obesity and RYGB on the central responses to ghrelin.

It is important to note that our study has some limitations. This was a single-blind study in a small, homogenous (females without diabetes) cohort, and additional studies are needed with larger cohorts to determine if the results are applicable to other populations. As we were interested in the potential role of ghrelin in the immediate metabolic improvements

after surgery, future studies should explore ghrelin responsiveness in the long-term after RYGB. Our findings were observed with acute elevations of plasma ghrelin to supra-physiologic levels, and therefore such findings cannot be extrapolated to account for metabolic effects that might occur with chronic changes in ghrelin levels. The hyperinsulinemic-euglycemic clamp directly evaluates the effect of ghrelin on tissue insulin sensitivity, and future studies should explore the potential contribution of neurohormonal signals, such as GLP-1^{52,55}, from the gastrointestinal tract or the known effect of ghrelin to reduce glucose-stimulated insulin secretion.^{21,22,52}

In summary, this is the first study to examine the metabolic effects of exogenous ghrelin administration in Class III obese subjects before and after RYGB. The peripheral effects of ghrelin were not altered in the obese or changed after RYGB. While our data do not suggest that changes in ghrelin after RYGB affect peripheral insulin sensitivity, additional studies, as outlined above, are necessary to fully characterize the involvement of ghrelin in the beneficial peripheral metabolic effects of RYGB. Conversely, we observed altered central effects of ghrelin in the obese that were restored to that of the lean at two weeks after RYGB. These data indicate that different pathways may regulate the central and peripheral metabolic effects of ghrelin and that the central metabolic effects of ghrelin are sensitive to metabolic status.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Kojima M, Hosoda H, Date Y, et al. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*. 1999; 402:656–660. [PubMed: 10604470]
2. Delhanty PJ, Neggers SJ, van der Lely AJ. Des-acyl ghrelin: a metabolically active peptide. *Endocr Dev*. 2013; 25:112–121. [PubMed: 23652397]
3. Druce MR, Wren AM, Park AJ, et al. Ghrelin increases food intake in obese as well as lean subjects. *Int J Obes (Lond)*. 2005; 29:1130–1136. [PubMed: 15917842]
4. Huda MS, Dovey T, Wong SP, et al. Ghrelin restores 'lean-type' hunger and energy expenditure profiles in morbidly obese subjects but has no effect on postgastrectomy subjects. *Int J Obes (Lond)*. 2009; 33:317–325. [PubMed: 19188925]
5. Tschöp M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature*. 2000; 407:908–913. [PubMed: 11057670]

6. Banks WA, Tschop M, Robinson SM, Heiman ML. Extent and direction of ghrelin transport across the blood-brain barrier is determined by its unique primary structure. *J Pharmacol Exp Ther.* 2002; 302:822–827. [PubMed: 12130749]
7. Al-Massadi O, Trujillo ML, Senaris R, et al. The vagus nerve as a regulator of growth hormone secretion. *Regul Pept.* 2011; 166:3–8. [PubMed: 21056594]
8. Date Y, Murakami N, Toshinai K, et al. The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology.* 2002; 123:1120–1128. [PubMed: 12360474]
9. le Roux CW, Neary NM, Halsey TJ, et al. Ghrelin does not stimulate food intake in patients with surgical procedures involving vagotomy. *J Clin Endocrinol Metab.* 2005; 90:4521–4524. [PubMed: 15914532]
10. Vestergaard ET, Djurhuus CB, Gjedsted J, et al. Acute effects of ghrelin administration on glucose and lipid metabolism. *J Clin Endocrinol Metab.* 2008; 93:438–444. [PubMed: 18042651]
11. Vestergaard ET, Gormsen LC, Jessen N, et al. Ghrelin infusion in humans induces acute insulin resistance and lipolysis independent of growth hormone signaling. *Diabetes.* 2008; 57:3205–3210. [PubMed: 18776138]
12. Gauna C, Meyler FM, Janssen JA, et al. Administration of acylated ghrelin reduces insulin sensitivity, whereas the combination of acylated plus unacylated ghrelin strongly improves insulin sensitivity. *J Clin Endocrinol Metab.* 2004; 89:5035–5042. [PubMed: 15472202]
13. Date Y, Kojima M, Hosoda H, et al. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology.* 2000; 141:4255–4261. [PubMed: 11089560]
14. Shiya T, Nakazato M, Mizuta M, et al. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *J Clin Endocrinol Metab.* 2002; 87:240–244. [PubMed: 11788653]
15. Tschop M, Weyer C, Tataranni PA, et al. Circulating ghrelin levels are decreased in human obesity. *Diabetes.* 2001; 50:707–709. [PubMed: 11289032]
16. Tassone F, Broglio F, Destefanis S, et al. Neuroendocrine and metabolic effects of acute ghrelin administration in human obesity. *J Clin Endocrinol Metab.* 2003; 88:5478–5483. [PubMed: 14602793]
17. Fusco A, Bianchi A, Mancini A, et al. Effects of ghrelin administration on endocrine and metabolic parameters in obese women with polycystic ovary syndrome. *J Endocrinol Invest.* 2007; 30:948–956. [PubMed: 18250617]
18. Tassone F, Broglio F, Destefanis S, et al. Neuroendocrine and metabolic effects of acute ghrelin administration in human obesity. *J Clin Endocrinol Metab.* 2003; 88:5478–5483. [PubMed: 14602793]
19. Guido M, Romualdi D, De Marinis L, et al. Administration of exogenous ghrelin in obese patients with polycystic ovary syndrome: effects on plasma levels of growth hormone, glucose, and insulin. *Fertil Steril.* 2007; 88:125–130. [PubMed: 17276431]
20. Tong J, Davis HW, Summer S, et al. Acute administration of unacylated ghrelin has no effect on Basal or stimulated insulin secretion in healthy humans. *Diabetes.* 2014; 63:2309–2319. [PubMed: 24550190]
21. Tong J, Prigeon RL, Davis HW, et al. Ghrelin suppresses glucose-stimulated insulin secretion and deteriorates glucose tolerance in healthy humans. *Diabetes.* 2010; 59:2145–2151. [PubMed: 20584998]
22. Tong J, Prigeon RL, Davis HW, et al. Physiologic concentrations of exogenously infused ghrelin reduces insulin secretion without affecting insulin sensitivity in healthy humans. *J Clin Endocrinol Metab.* 2013; 98:2536–2543. [PubMed: 23589527]
23. Cummings DE, Weigle DS, Frayo RS, et al. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *N Engl J Med.* 2002; 346:1623–1630. [PubMed: 12023994]
24. Tymitz K, Engel A, McDonough S, Hendy MP, Kerlakian G. Changes in ghrelin levels following bariatric surgery: review of the literature. *Obes Surg.* 2011; 21:125–130. [PubMed: 21104455]

25. Tamboli RA, Breitman I, Marks-Shulman PA, et al. Early weight regain after gastric bypass does not affect insulin sensitivity but is associated with elevated ghrelin. *Obesity (Silver Spring)*. 2014; 22:1617–1622. [PubMed: 24777992]
26. Sakata I, Sakai T. Ghrelin cells in the gastrointestinal tract. *Int J Pept*. 2010; 2010
27. Damjanovic SS, Lalic NM, Pesko PM, et al. Acute effects of ghrelin on insulin secretion and glucose disposal rate in gastrectomized patients. *J Clin Endocrinol Metab*. 2006; 91:2574–2581. [PubMed: 16621911]
28. Tamboli RA, Hossain HA, Marks PA, et al. Body composition and energy metabolism following Roux-en-Y gastric bypass surgery. *Obesity (Silver Spring)*. 2010; 18:1718–1724. [PubMed: 20414197]
29. Liu J, Prudom CE, Nass R, et al. Novel ghrelin assays provide evidence for independent regulation of ghrelin acylation and secretion in healthy young men. *J Clin Endocrinol Metab*. 2008; 93:1980–1987. [PubMed: 18349056]
30. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care*. 1999; 22:1462–1470. [PubMed: 10480510]
31. Flanagan DE, Evans ML, Monsod TP, et al. The influence of insulin on circulating ghrelin. *Am J Physiol Endocrinol Metab*. 2003; 284:E313–316. [PubMed: 12531744]
32. Saad MF, Bernaba B, Hwu CM, et al. Insulin regulates plasma ghrelin concentration. *J Clin Endocrinol Metab*. 2002; 87:3997–4000. [PubMed: 12161550]
33. DeBell WK, Pezzoli SS, Thorner MO. Growth hormone (GH) secretion during continuous infusion of GH-releasing peptide: partial response attenuation. *J Clin Endocrinol Metab*. 1991; 72:1312–1316. [PubMed: 2026752]
34. Yuen KC, Chong LE, Riddle MC. Influence of glucocorticoids and growth hormone on insulin sensitivity in humans. *Diabet Med*. 2013; 30:651–663. [PubMed: 23510125]
35. Broglio F, Arvat E, Benso A, et al. Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. *J Clin Endocrinol Metab*. 2001; 86:5083–5086. [PubMed: 11600590]
36. Longo KA, Govek EK, Nolan A, et al. Pharmacologic inhibition of ghrelin receptor signaling is insulin sparing and promotes insulin sensitivity. *J Pharmacol Exp Ther*. 2011; 339:115–124. [PubMed: 21775475]
37. Qi Y, Longo KA, Giuliana DJ, et al. Characterization of the insulin sensitivity of ghrelin receptor KO mice using glycemic clamps. *BMC Physiol*. 2011; 11:1. [PubMed: 21211044]
38. Vestergaard ET, Buhl M, Gjedsted J, et al. Acute peripheral metabolic effects of intraarterial ghrelin infusion in healthy young men. *J Clin Endocrinol Metab*. 2011; 96:468–477. [PubMed: 21084392]
39. Vestergaard ET, Moller N, Jorgensen JO. Acute peripheral tissue effects of ghrelin on interstitial levels of glucose, glycerol, and lactate: a microdialysis study in healthy human subjects. *Am J Physiol Endocrinol Metab*. 2013; 304:E1273–1280. [PubMed: 23592479]
40. Osterstock G, Escobar P, Mitutsova V, et al. Ghrelin stimulation of growth hormone-releasing hormone neurons is direct in the arcuate nucleus. *PLoS One*. 2010; 5:e9159. [PubMed: 20161791]
41. Popovic V, Miljic D, Micic D, et al. Ghrelin main action on the regulation of growth hormone release is exerted at hypothalamic level. *J Clin Endocrinol Metab*. 2003; 88:3450–3453. [PubMed: 12843202]
42. van der Lely AJ, Tschop M, Heiman ML, Ghigo E. Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. *Endocr Rev*. 2004; 25:426–457. [PubMed: 15180951]
43. Arnold M, Mura A, Langhans W, Geary N. Gut vagal afferents are not necessary for the eating-stimulatory effect of intraperitoneally injected ghrelin in the rat. *J Neurosci*. 2006; 26:11052–11060. [PubMed: 17065447]
44. Takeno R, Okimura Y, Iguchi G, et al. Intravenous administration of ghrelin stimulates growth hormone secretion in vagotomized patients as well as normal subjects. *Eur J Endocrinol*. 2004; 151:447–450. [PubMed: 15476443]

45. Lucidi P, Murdolo G, Di Loreto C, et al. Metabolic and endocrine effects of physiological increments in plasma ghrelin concentrations. *Nutr Metab Cardiovasc Dis*. 2005; 15:410–417. [PubMed: 16314227]
46. Breitman I, Saraf N, Kakade M, et al. The effects of an amino acid supplement on glucose homeostasis, inflammatory markers, and incretins after laparoscopic gastric bypass. *J Am Coll Surg*. 2011; 212:617–625. discussion 625–617. [PubMed: 21463799]
47. Pardina E, Ferrer R, Baena-Fustegueras JA, et al. The relationships between IGF-1 and CRP, NO, leptin, and adiponectin during weight loss in the morbidly obese. *Obes Surg*. 2010; 20:623–632. [PubMed: 20195789]
48. Rubino F, Gagner M, Gentileschi P, et al. The early effect of the Roux-en-Y gastric bypass on hormones involved in body weight regulation and glucose metabolism. *Ann Surg*. 2004; 240:236–242. [PubMed: 15273546]
49. Zigman JM, Bouret SG, Andrews ZB. Obesity Impairs the Action of the Neuroendocrine Ghrelin System. *Trends Endocrinol Metab*. 2016; 27:54–63. [PubMed: 26542050]
50. Popovic V, Miljic D, Pekic S, et al. Low plasma ghrelin level in gastrectomized patients is accompanied by enhanced sensitivity to the ghrelin-induced growth hormone release. *J Clin Endocrinol Metab*. 2005; 90:2187–2191. [PubMed: 15644398]
51. Schwartz TW. Pancreatic polypeptide: a hormone under vagal control. *Gastroenterology*. 1983; 85:1411–1425. [PubMed: 6138294]
52. Tong J, Davis HW, Gastaldelli A, D'Alessio D. Ghrelin impairs prandial glucose tolerance and insulin secretion in healthy humans despite increasing GLP-1. *J Clin Endocrinol Metab*. 2016 jc20154154.
53. Arosio M, Ronchi CL, Gebbia C, et al. Stimulatory effects of ghrelin on circulating somatostatin and pancreatic polypeptide levels. *J Clin Endocrinol Metab*. 2003; 88:701–704. [PubMed: 12574202]
54. Sundbom M, Holdstock C, Engstrom BE, Karlsson FA. Early changes in ghrelin following Roux-en-Y gastric bypass: influence of vagal nerve functionality? *Obes Surg*. 2007; 17:304–310. [PubMed: 17546836]
55. Gagnon J, Baggio LL, Drucker DJ, Brubaker PL. Ghrelin Is a Novel Regulator of GLP-1 Secretion. *Diabetes*. 2015; 64:1513–1521. [PubMed: 25412624]

Figure 1. Acyl (A,B) and total (C,D) ghrelin levels during the basal (A,C) and insulin (B,D) periods of the hyperinsulinemic-euglycemic clamp procedure with saline or ghrelin infusion. Data are expressed as the mean $\pm$ SEM for $n=9$ per group. ** $P<0.01$, *** $P<0.001$, and **** $P=0.0001$ for Lean vs. Obese and Obese/Pre-RYGB vs. Post-RYGB.

Figure 2. Effect of ghrelin infusion on growth hormone (GH) and pancreatic polypeptide (PP) secretion

A–F: Time courses for GH (A–C) and PP (D–F) responses to ghrelin and saline infusion over 150 minutes in Lean (A,D), Obese Pre-RYGB (B,E), and Post-RYGB (C,F) subjects. Data are mean $\pm$ SEM for 7–9 subjects per time point. G,H: Area under the curves for GH (G) and PP (H) responses to saline and ghrelin infusions for panels A–F. Data are mean $\pm$ SEM for nine lean subjects and nine subjects before and after RYGB. *** $P < 0.001$ and **** $P = 0.0001$ for Saline vs. Ghrelin; # $P < 0.05$ and ### $P < 0.001$ for group $\times$ treatment or time $\times$ treatment interactions.

Figure 3. Liver and muscle insulin sensitivity in response to ghrelin infusion

A: HISI, Hepatic insulin sensitivity index. B: M/I, glucose infusion rate/plasma insulin concentrations during hyperinsulinemic euglycemia as a measure of peripheral (mostly muscle) insulin sensitivity. Data are mean $\pm$ SEM for nine lean subjects and nine subjects before and after RYGB. **P<0.01 and ***P<0.001 for Saline vs. Ghrelin. #P < 0.05 for group $\times$ treatment interaction.

Table 1

Characteristics of the study participants

	Lean	Obese Pre-RYGB	Post-RYGB
n	9	9	9
BMI (kg•m⁻²)	22 ± 1	44 ± 2 ^{****a}	42 ± 2 ^{**b}
Weight (kg)	60 ± 2	115 ± 6 ^{****a}	109 ± 6 ^{****b}
Body Fat %[†]	30 ± 2	53 ± 1 ^{****a}	54 ± 1 ^{*b}
Fat Mass (kg)[†]	18 ± 2	62 ± 5 ^{****a}	60 ± 5 ^{*b}
Fat-Free Mass (kg)[†]	42 ± 1	54 ± 2 ^{****a}	50 ± 2 ^{***b}
Age (years)	36 ± 4	41 ± 4	–
Sex (M/F)	0/9	0/9	–
T2D (Y/N)	0/9	0/9	–

Data are mean ± SEM.

^{**} P<0.01 and^{****} P = 0.0001 for^a Lean vs. Obese and^b Pre-RYGB vs. Post-RYGB.[†] n=8 for Obese Pre-RYGB and Post-RYGB

Table 2

Cardiovascular, metabolic, and hormone measurements with and without ghrelin infusion

	Lean		Obese Pre-RYGB		Post-RYGB		Lean vs. Obese P value Group $\times$ Treatment	Pre-RYGB vs. Post-RYGB P value Time $\times$ Treatment
	Saline	Ghrelin	Saline	Ghrelin	Saline	Ghrelin		
HR (bpm)	69 $\pm$ 4	72 $\pm$ 3	76 $\pm$ 5	77 $\pm$ 3	75 $\pm$ 3	69 $\pm$ 2 *	0.812	0.014
SBP (mm Hg)	124 $\pm$ 4	115 $\pm$ 4 *	128 $\pm$ 4	128 $\pm$ 5	123 $\pm$ 3	122 $\pm$ 7	0.001	0.920
DBP (mm Hg)	67 $\pm$ 2	65 $\pm$ 2	72 $\pm$ 3	68 $\pm$ 2	70 $\pm$ 2	69 $\pm$ 2	0.364	0.394
MAP (mm Hg)	88 $\pm$ 2	86 $\pm$ 2	93 $\pm$ 3	90 $\pm$ 2	91 $\pm$ 2	89 $\pm$ 3	0.912	0.684
Glucose (mg/dl)	91 $\pm$ 2	95 $\pm$ 3 *	94 $\pm$ 2	104 $\pm$ 1 ****	89 $\pm$ 2	92 $\pm$ 1	0.009	0.004
Insulin (μ U/ml)	5.3 $\pm$ 0.6	6.6 $\pm$ 0.8	22.8 $\pm$ 2.9	24.6 $\pm$ 3.5	12.3 $\pm$ 1.3	11.3 $\pm$ 1.5	0.768	0.116
FFA (mM)	0.61 $\pm$ 0.05	0.80 $\pm$ 0.04 **	0.75 $\pm$ 0.05	0.96 $\pm$ 0.04 **	0.80 $\pm$ 0.04	1.11 $\pm$ 0.06 ***	0.788	0.251
HGP ($\text{mg kg}^{-1} \text{ min}^{-1}$)	1.57 $\pm$ 0.07	1.54 $\pm$ 0.06	0.89 $\pm$ 0.03	0.94 $\pm$ 0.05	0.82 $\pm$ 0.03	0.81 $\pm$ 0.02	0.348	0.193
M ($\text{mg kg}^{-1} \text{ min}^{-1}$)	8.9 $\pm$ 0.6	6.9 $\pm$ 0.3	3.2 $\pm$ 0.4	1.7 $\pm$ 0.2	2.8 $\pm$ 0.3	1.8 $\pm$ 0.2	0.374	0.204
Insulin Clamp (μ U/ml)	125 $\pm$ 7	126 $\pm$ 5	195 $\pm$ 14	185 $\pm$ 10	173 $\pm$ 13	160 $\pm$ 7	0.305	0.724

Data are mean $\pm$ SEM for n=9 per group, except for cardiovascular measurements which are n=8 for RYGB subjects.

* P<0.05,

**

P<0.01,

P<0.001, and

P 0.0001 for Saline vs. Ghrelin post-hoc test. HR, heart rate; bpm, beats per minute; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; mm Hg, millimeters of mercury; FFA, free fatty acids; HGP, hepatic glucose production; M, glucose infusion rate during hyperinsulinemic euglycemia.