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Ghrelin suppression is associated with weight loss and insulin action following gastric bypass surgery at 12 months in obese adults with type 2 diabetes

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Abstract

Roux-en-Y gastric bypass (RYGB) surgery reverses diabetes (T2DM) in ~80% of patients. Ghrelin regulates glucose homeostasis, but its role in T2DM remission after RYGB surgery is unclear. Nine obese T2DM subjects underwent a mixed meal tolerance test before and at 1 and 12 months after RYGB. Changes in ghrelin, body weight, GLP-1, glucose tolerance, and insulin sensitivity were measured. At 1 month, body weight, glycemia and insulin sensitivity were improved, while ghrelin concentrations were reduced ($p<0.05$). After 12 months, body weight and fasting glucose were reduced (30% and 16% respectively; $p<0.05$) and insulin sensitivity was enhanced (3-fold; $p<0.05$). Ghrelin suppression improved by 32% at 12 months ($p<0.05$), and this was associated with weight loss ($R=0.72$, $p=0.03$), enhanced insulin sensitivity ($R=-0.78$, $p=0.01$) and peak post-prandial GLP-1 ($R=-0.73$, $p=0.03$). These data suggest that post-prandial ghrelin suppression may be part of the mechanism that contributes to diabetes remission after RYGB surgery.

Keywords

Gut hormones; weight loss; insulin action; glycemic control

Introduction

Roux-en-Y gastric bypass (RYGB) surgery reverses T2DM in a majority of patients before clinically significant weight loss, suggesting that endocrine-related mechanisms are involved in regulating this outcome (1,2). Ghrelin is a gastrointestinal hormone that has been reported to stimulate appetite, promote adiposity, and impair insulin sensitivity/secretion (2,3). Ghrelin was originally identified as a potentially important link between bypass surgery weight loss and glucose homeostasis (2,4). Obese patients with T2DM often have low fasting ghrelin levels and an inability to suppress ghrelin (5,6). This latter point is clinically relevant as ghrelin suppression may have more direct effects on post-prandial metabolism than fasting values per se (2,4). Although low fasting ghrelin levels after RYGB have been reported in relation to metabolic health gains in adults with T2DM (7), it is unclear if RYGB enhances this suppression (8). Further examination of the effect of RYGB on ghrelin suppression is needed in order to resolve the controversy surrounding ghrelin and weight regulation (4). Therefore, we tested the hypothesis that RYGB surgery would improve ghrelin suppression and this change would be associated with weight loss and higher insulin sensitivity.

Methods

Subjects

Data were obtained on nine obese adults (Table 1) with T2DM (duration: 3.8 ± 1.6 yr, hemoglobin A1c $7.7 \pm 0.7\%$) who underwent 12 month follow up from RYGB surgery. Some data was previously reported as part of a larger study on insulin secretion after RYGB (9). Two subjects received diet therapy alone, while others received: metformin (n=7), sulfonylureas (n=2), or a combination of sulfonylurea, insulin, pioglitazone, metformin or byetta (n=1). Participants were excluded if they had creatinine levels >1.8 mg/dl, elevated liver enzymes >3 times the upper limit of normal, or greater than class 2 (NYHA) heart failure. Subjects were verbally briefed and signed informed consent approved by our Institutional Review Board.

Metabolic testing and RYGB—Subjects refrained from medication 24-hours prior to testing to minimize potential drug effects on study outcomes. A mixed-meal tolerance test (MMTT, Ensure plus; 175 kcal) was performed after a 12-hour overnight fast prior to surgery. Blood samples for glucose, insulin, and free fatty acid (FFA) determination were collected at 0, 30, 60, 90 and 120 minutes. Ghrelin, GIP (gastric inhibitory peptide) and GLP-1 (glucagon-like polypeptide-1) were obtained at 0, 30 and 60 minutes only, as these hormones tend to peak/nadir within this timeframe. RYGB was preformed as described previously (9). After surgery, all subjects repeated the MMTT at 1 and 12 months.

Calculations and Analysis—Insulin sensitivity was estimated from the MMTT using the Matsuda Index (10). First-phase and total glucose-stimulated insulin release was calculated as insulin area under the curve (AUC) divided by glucose AUC during 0-30 and 0-120 minutes of the MMTT (i.e. Ins_{0-30}/Glc_{0-30} and Ins_{0-120}/Glc_{0-120}). The first-phase and total oral disposition index, which provides a measure of β -cell insulin release adjusted for insulin sensitivity (IS), was estimated as $Ins_{0-30}/Glc_{0-30} \times IS$ and $Ins_{0-120}/Glc_{0-120} \times IS$. AUC was

calculated using the trapezoidal method. The highest value obtained at 30 and 60 minutes during the MTTT was defined as peak post-prandial GIP and GLP-1. Percent ghrelin suppression was calculated as: $[(\text{post-prandial nadir} - \text{fasting ghrelin}) \div \text{fasting ghrelin levels}] \times 100$. Blood glucose was measured immediately using the glucose oxidase method. Plasma samples were stored in a -70°C freezer. Plasma insulin was assayed by radioimmunoassay, and total ghrelin, GIP and GLP-1 levels were measured using an enzyme-linked immunosorbent assay (Millipore). FFA were measured by colorimetric assay (Wako Chemicals).

Statistical Analysis

Data were compared using R (Version 2.4.0, The R Foundation). Non-normally distributed data were log-transformed prior to analysis. Two-way (meal (i.e. fasted vs. post-prandial) $\times$ time) repeated measures analysis of variance (ANOVA) was used to determine differences before and after surgery for ghrelin, GIP and GLP-1. A one-way repeated measures ANOVA was used for all other comparisons. In the event of a significant time or interaction effect, pairwise comparisons were conducted. Spearman rank correlations were used to examine associations. Significance was accepted as $\alpha = 0.05$. Data are mean $\pm$ standard error of the mean (SEM).

Results

Compared to baseline, RYGB decreased body weight by 20% and 30% at 1 and 12 months (135.0 ± 8.3 vs. 105.6 ± 6.1 vs. 98.5 ± 5.8 kg; $p < 0.0001$). Fasting glucose levels were decreased approximately 15% ($p < 0.05$) at 12 months in parallel with FFA levels ($p < 0.05$; Table 1). At 1 month, 6 of 9 patients were on metformin, with 1 of these subjects also on glipizide. At 12 months, 2 subjects were still on metformin. RYGB decreased fasting insulin by 38 and 64% at 1 and 12 months, and insulin sensitivity increased ~ 3 -fold at 12 months (Table 1). β -cell function estimated as either first phase or total response was higher at 1 and 12 months, compared to baseline (Table 1). Consistent with these changes, GLP-1 peak post-prandial levels were higher at 1 and 12 months compared to fasting and pre-surgery values. Peak GLP-1 correlated with elevated first-phase and total insulin response after 12 months ($r = 0.73$ and $r = 0.77$, each $p < 0.03$).

One month after RYGB, fasting and post-prandial nadir ghrelin concentrations were lower than either pre-surgery or 12 month levels ($p < 0.05$, Table 1). However, fasting ghrelin was significantly higher at 12 compared to 1 month ($p < 0.05$; Table 1). Ghrelin suppression increased by 18% and 35%, respectively (Figure 1A), and this change correlated with weight loss (Figure 1B), enhanced insulin sensitivity (Figure 1C), and elevated peak post-prandial GLP-1 levels (Figure 1D) at 12 months.

Conclusions

These data indicate that RYGB improved meal-induced ghrelin suppression in adults with T2DM (Figure 1A). In contrast to previous work (7), we did not observe lower fasting ghrelin levels at 12 months after RYGB. Since ghrelin levels rose from 1 month to above baseline at 12 months, our findings suggest a disconnect between low fasting ghrelin

concentrations per se and metabolic health gains after RYGB surgery (4,7). These data are not entirely surprising however, since elevated fasting ghrelin levels in healthy individuals is inversely associated with hemoglobin A1c and BMI (2). Despite controversy regarding ghrelin and bariatric surgery (4), our data suggest that enhanced post-prandial ghrelin suppression may be important for weight loss (Figure 1B) and glucose homeostasis up to 1 year after surgery (2).

The mechanism responsible for improving glucose regulation after RYGB surgery is likely related to enhanced peripheral or hepatic insulin sensitivity and/or enhanced pancreatic function. In this study, ghrelin suppression was strongly correlated with insulin sensitivity (Figure 1C). Although ghrelin was not directly related to β -cell function as suggested by previous work (3), peak post-prandial GLP-1 levels correlated with β -cell function and ghrelin suppression (Figure 1D). In accordance with this observation, GLP-1 infusion in healthy adults reduces ghrelin levels in parallel with enhanced insulin secretion (11). Thus, our findings suggest that enhanced ghrelin suppression after RYGB contributed to improved glycemic control. We recognize, however, that the exact cause for diabetes remission likely involves the interaction of several hormones and substrates.

In summary, ghrelin suppression is related to weight loss and glucose regulation 12 months after RYGB. Although acylated ghrelin is suggested to be more biologically important than total ghrelin, reductions in FFA levels at 12 months (Table 1) would be expected to decrease ghrelin O-acyl transferase, which is an important enzyme that converts total ghrelin to the acylated form (12). Thus, total ghrelin likely represents the biologically active form in this study. Whether enhanced ghrelin suppression at 12 months contributes to improved glycemic control in the subsequent years requires future attention.

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A.S. and S.R.K developed the study hypothesis. P.R.S performed all surgical procedures. S.K.M was primarily responsible for data analysis and statistical integrity. H.H. was responsible for biochemical assays. A.S., S.K.M, H.H., J.P.K, P.R.S., and S.R.K assisted with data analysis, editing, and study organization. A.S., S.K.M, J.P.K., and S.R.K wrote the manuscript. We thank the nursing staff and participants for their efforts. This research was supported by National Institutes of Health Grants RO1 AG-12834 (J.P.K.), and National Institutes of Health National Center for Research Resources, 1UL1RR024989, Cleveland, OH. S.K.M was supported by a T32 DK007319 grant.

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Figure 1. The effects of RYGB on ghrelin (A) in relation to weight loss (B), insulin sensitivity (C) and GLP-1 (D) at 12 months. Data are mean $\pm$ SEM. Significant compared to PRE: * $p < 0.05$.

Table 1

Effects of RYGB on anthropometrics, metabolites and hormones.

	PRE	1 Month	12 Month	P-value [#]
Age (years)	42 ± 6	-	-	-
BMI (kg/m ²) [†]	46.0 ± 1.8	40.6 ± 1.6 *	32.6 ± 1.2 * [^]	0.001
Fasting FFA (mM) [†]	0.80 ± 0.16	0.85 ± 0.07	0.55 ± 0.07	0.09
FFA AUC (mM)	75.0 ± 15.5	68.9 ± 6.8	26.6 ± 3.2 * [^]	0.006
Fasting Glucose (mM)	7.3 ± 0.7	6.0 ± 0.3	5.8 ± 0.5	0.04
Glucose AUC (mM)	986.5 ± 104.8	797.0 ± 49.4	704.0 ± 37.9 *	0.01
Fasting Insulin (pM)	136.6 ± 25.6	72.4 ± 13.2 *	39.5 ± 9.3 * [^]	0.001
Insulin AUC (pM × 10 ⁻³)	2.5 ± 0.3	2.3 ± 0.3	1.2 ± 0.3 * [^]	0.001
Matsuda Index	3.5 ± 0.7	5.5 ± 0.8 *	13.3 ± 2.8 * [^]	0.001
Ins ₀₋₃₀ /Glc ₀₋₃₀	0.18 ± 0.02	0.23 ± 0.03	0.14 ± 0.03 *	0.05
Ins ₀₋₁₂₀ /Glc ₀₋₁₂₀	1.46 ± 0.18	1.62 ± 0.18 *	0.98 ± 0.18 * [^]	0.01
β-cell function ₀₋₃₀ [†]	0.6 ± 0.1	1.2 ± 0.2 *	1.6 ± 0.3 * [†]	0.01
β-cell function ₀₋₁₂₀	4.8 ± 1.0	8.0 ± 0.9 *	10.1 ± 1.3 *	0.004
Fasting GIP (pg/ml)	22.0 ± 5.2	19.9 ± 7.0	21.0 ± 7.6	0.13
GIP peak post-prandial (pg/ml)	110.2 ± 23.2 [^]	134.3 ± 21.5 [^]	90.0 ± 15.7 [^]	-
Fasting GLP-1 (pg/ml)	9.0 ± 2.1	11.6 ± 3.8	6.2 ± 2.4	0.05
GLP-1 peak post-prandial (pg/ml)	11.0 ± 2.3	29.0 ± 6.3 [†]	29.2 ± 6.7 * [†]	-
Fasting Ghrelin (pg/ml)	333.6 ± 66.7	260.6 ± 46.8 [#]	434.9 ± 47.5 [^]	0.05
Ghrelin Nadir (pg/ml)	304.3 ± 45.8	204.7 ± 38.7 [#]	288.6 ± 43.7	-

Data are mean ± standard error of the mean.

[#]P-value = Reflects two-way repeated measures ANOVA.

Time effect (ANOVA) p<0.05.

* Significant compared to PRE: p<0.05,

** p=0.09.

[^] Significant compared to 1 month: p<0.05,
Meal effect (ANOVA) p<0.001.^{^^} p=0.06,[†] p=0.07.

log-transformed data.

[†] Significant compared to baseline: p<0.05.

BMI = body mass index. Free Fatty Acid (FFA). AUC – area under the curve. Glc = Glucose, GLP-1 = glucagon-like peptide and GIP = gastric inhibitory polypeptide.