BRIEF REPORTS

The Effects of Vertical Sleeve Gastrectomy in Rodents Are Ghrelin Independent

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Reductions in levels of the hunger-stimulating hormone ghrelin have been proposed to mediate part of the effects of vertical sleeve gastrectomy (VSG) and Roux-en-Y gastric bypass surgeries for obesity. We studied circulating levels of acyl and desacyl ghrelin in rats after these surgeries. We found that blood levels of ghrelin were reduced after VSG, but not after Roux-en-Y gastric bypass, based on enzyme-linked immunosorbent assay and mass-spectrometry analyses. We compared the effects of VSG in ghrelin-deficient mice and wild-type mice on food intake, body weight, dietary fat preference, and glucose tolerance. We found that VSG produced comparable outcomes in each strain. Reduced ghrelin signaling therefore does not appear to be required for these effects of VSG.

Keywords: Weight-Loss Surgery; Obesity; Treatment; Bariatric Surgery.

Bariatric surgery is currently the most effective treatment for obesity and several related metabolic diseases.[1] In one such procedure termed *vertical sleeve gastrectomy* (VSG), the stomach is resected to approximately 10%–20% of its original size.[2] Although VSG is termed a restrictive procedure, it also involves the removal of the gastric epithelium, which is the primary source of ghrelin, a 28–amino acid peptide that circulates in biologically active (acyl) and inactive (desacyl) forms, and is the endogenous ligand for the growth hormone secretagogue receptor.[3]

Given pharmacologically, acyl ghrelin increases food intake in human beings and rodents, increases ratings for palatable foods, and the neural responses to food cues.[4,5] In addition to effects on energy balance, a wide range of data link acyl ghrelin to reduced hepatic insulin sensitivity and glucose-induced insulin secretion.[6] Given that major effects of VSG and Roux-en-Y gastric bypass (RYGB) include reduced food intake, body weight, and increased hepatic insulin sensitivity and insulin secretion,[2] it is plausible to hypothesize that reduced secretion of acyl ghrelin could contribute to the potent effects of bariatric surgery.

However, despite the obvious nature of this hypothesis, the effect of various bariatric procedures on ghrelin levels has remained controversial (Supplementary Table 1). To that end, we used rat models of both VSG and RYGB (Supplementary Figure 1) in which we could carefully control the conditions under which we collected blood samples and used both enzyme-linked immunosorbent assay and mass spectrometry to determine if acyl and desacyl ghrelin were altered after these surgeries. Acyl and desacyl ghrelin levels from VSG and RYGB rats are depicted in Figure 1. The first sample was taken in ad libitum–fed rats. In the subsequent sample, blood was collected from 6-hour–fasted rats, just before the onset of the dark cycle, at which time acyl and desacyl ghrelin levels were increased in sham, RYGB, and pair-fed rats, but were not increased in VSG rats ($P < .05$). The ratio of acyl:desacyl ghrelin was increased in VSG (2.0 ± 0.2) rats compared with pair-fed (1.3 ± 0.1), bypass (0.8 ± 0.1), and ad libitum– (1.3 ± 0.1) fed rats at this time, but absolute levels of the peptide were reduced. Re-feeding rapidly suppressed acyl ghrelin levels in sham and RYGB rats within 30 minutes ($P < .05$).

Given the controversy over methodologies to measure multiple circulating forms of ghrelin (Supplementary Table 1), we measured the same samples using mass spectrometry. Not surprisingly, the absolute values from this assay were not the same as those from the enzyme-linked immunosorbent assay. However, the pattern of changes between time points and among surgical groups was nearly identical. Fasting levels of acyl ghrelin were reduced significantly in VSG (471 ± 61 pg/mL) compared with the other treatment groups (sham, 799 ± 81.7 pg/mL; RYGB, 1130 ± 562 pg/mL; pair-fed, 716 ± 82 pg/mL), as were circulating levels of desacyl ghrelin (VSG, 315 ± 25.6 pg/mL; sham, 719 ± 83.5 pg/mL; RYGB, 816 ± 192.4 pg/mL; and pair-fed, 797 ± 72.3 pg/mL; $P < .05$). There were no significant differences among groups after refeeding.

Abbreviations used in this paper: KO, knockout; RYGB, Roux-en-Y gastric bypass; VSG, vertical sleeve gastrectomy.

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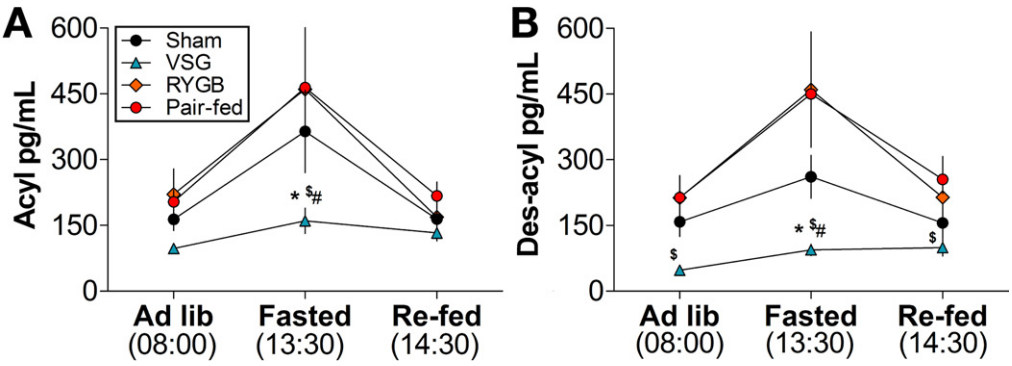

Figure 1. A significant effect of surgery was observed in (*A*) acyl and (*B*) desacyl ($P < .001$) ghrelin levels collected from VSG (n = 7), RYGB (n = 4), pair-fed (n = 10), and sham-operated rats (n = 10) at different times during postoperative days 68–72 ($P < .05$). Circulating levels of acyl and desacyl ghrelin were reduced significantly compared with values in sham-operated rats (*$P < .05$), pair-fed ($^{\$}P < .05$), and RYGB ($^{\#}P < .05$) animals (Bonferroni multiple comparisons tests). Ad lib, ad libitum.

Thus, results from 2 different yet complementary methodologic approaches, clearly showed that circulating levels of acyl or desacyl ghrelin were unaffected by RYGB, but were reduced in VSG. Because we failed to see changes in ghrelin levels after RYGB, we then went on to directly test the necessity of reduced ghrelin signaling for bariatric surgery's benefits by measuring a number of effects of VSG in a mouse with targeted ghrelin gene disruption. We reasoned that if these reductions contributed to the profound effects of VSG on food intake, food preference,[7] body weight, and glucose tolerance, that VSG would be less effective in mice with targeted genetic disruption of the ghrelin gene.

We therefore assigned wild-type and ghrelin-deficient (knockout [KO]) mice to sham or VSG surgical groups, which were matched for lean and fat tissue mass. Geno-

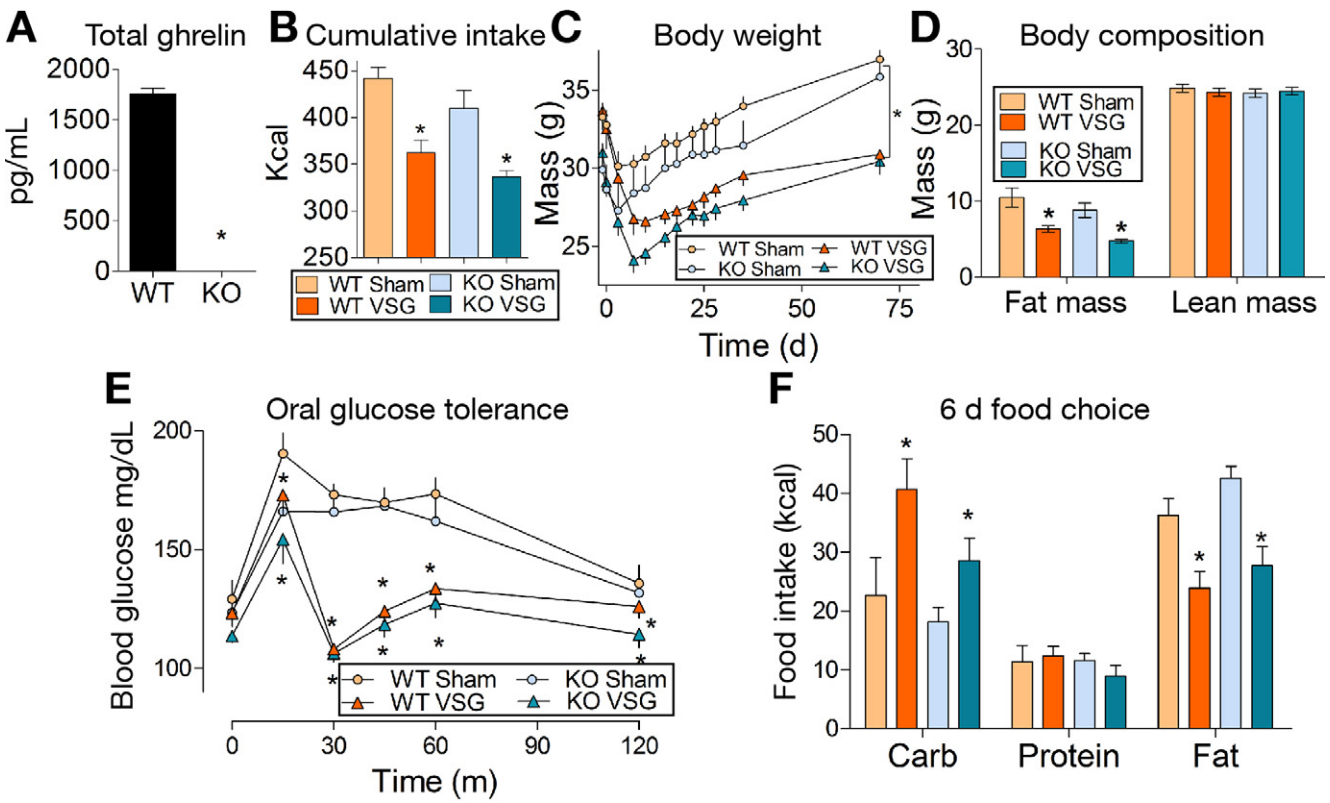

Figure 2. Metabolic effects of VSG in ghrelin KO (n = 10) and wild-type (WT) (n = 9) mice relative to sham-operated mice (n = 7 of 7). (*A*) Ghrelin deficiency was confirmed in KO mice using a commercially available enzyme-linked immunosorbent assay for total ghrelin (*$P < .05$). (*B*) Cumulative food intake 45 days after surgery and (*C*) body weight were reduced by VSG relative to sham-operated mice in both genotypes (*$P < .05$). (*D*) Body composition data measured on postoperative day 42 show reductions in fat mass in VSG compared with sham mice (*$P < .05$), with no effect on lean mass ($P > .05$). (*E*) A mixed-meal tolerance test on postoperative day 28 shows improvements in oral glucose tolerance in VSG mice relative to sham-operated mice (*$P < .05$). (*F*) Food selection test shows VSG mice ate more calories in the form of carbohydrates and fewer calories from fat than sham-operated mice (*$P < .05$) (Bonferroni multiple comparisons tests).