# Estradiol increases body weight loss and gut-peptide satiation after Roux-en-Y gastric bypass in ovariectomized rats

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Abstract: Despite the fact that 85% of bariatric operations are performed in women, the effects of the reproductive axis function on outcome of bariatric surgery remain to be determined. Here we developed the first published model of Roux-en-Y gastric bypass (RYGB) in female rats. We show in ovariectomized rats receiving estradiol or control treatment that (1) RYGB-induced body weight loss and (2) the satiating efficacy of endogenous glucagon-like peptide-1 and cholecystokinin satiation were significantly increased in estradiol-treated rats. These data are relevant to the care of obese women, in particular perimenopausal women, undergoing bariatric surgery.

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**Title:** Estradiol increases body-weight loss and gut-peptide satiation after Roux-en-Y gastric bypass in ovariectomized rats

**Short title:** Estradiol improves gastric bypass outcome

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Abbreviations: CCK = cholecystokinin; E2 = estradiol; GLP-1 = Glucagon-like peptide-1; RYGB = Roux-en-Y gastric bypass.

Keywords: bariatric surgery; obesity; CCK; GLP-1; Exendin-9; sex differences

Abstract

Despite the fact that ~85% of bariatric operations are performed in women, the effects of the reproductive-axis function on bariatric surgery outcome remain to be determined. Here we developed the first published model of Roux-en-Y gastric bypass (RYGB) in female rats. We show in ovariectomized rats receiving estradiol or control treatment, that (1) RYGB-induced body-weight loss and (2) the satiating efficacy of endogenous glucagon-like peptide-1 (GLP-1) and cholecystokinin (CCK) satiation were significantly increased in estradiol-treated rats. These data are relevant to the care of obese women, in particular peri-menopausal women undergoing bariatric surgery.

Bariatric surgery is currently the most effective treatment for morbid obesity. Morbid obesity (BMI > 40 m/kg$^2$) is more prevalent in women than in men[4,8], and the adverse effects of obesity on psychological well-being are more pronounced in women[5]. Correspondingly, more than 85% of patients undergoing bariatric surgery are female[9]. Despite the well-known effects of normal reproductive-axis function on eating and body weight[2], the impact of reproductive-axis function on the outcome of bariatric surgery remains to be determined. Here we investigated the effects of Roux-en-Y gastric bypass (RYGB) surgery on body weight and glucagon-like peptide-1 (GLP-1)- and cholecystokinin (CCK)-induced satiation in a rat model of menopause.

Adult Long-Evans female rats were fattened for 3 wk by offering only a high-energy diet (*Phase 1*) before ovariectomy, the standard rodent menopause model, and RYGB- or sham surgery. Rats were then fed high-energy and regular chow ad libitum for 28 d (*Phase 2*). A near-physiological regimen of estradiol treatment (2 μg estradiol-benzoate once each 4$^{th}$ d, s.c.), or oil-vehicle treatment (100 μl sesame oil) was begun on d 12, resulting in four groups: SHAM-E2, SHAM-OIL, RYGB-E2, and RYGB-OIL. Beginning on d 29, rats were fed Ensure Plus liquid diet and chow for 21 d (*Phase 3*). Finally, the acute effects of Exendin (9-39) and Devazepide on Ensure intake were tested (*Phase 4*).

At arrival, rats weighed 185±6 g. During *Phase 1*, rats gained ~60 g, reaching an overall mean preoperative body weight of 247±3 g (Figure 1A). The continued rapid weight gain of SHAM-OIL rats during *Phases 2 & 3* was significantly attenuated in

RYGB-OIL rats (19 vs. 42 g and 30 vs. 67 g in *Phases 2 & 3*, standard errors of the difference, 8 and 7 g, respectively; Figure 1A). The weight-lowering effect of RYGB was significantly greater in estradiol-treated rats than oil-treated rats, by 31 and 49 g in *Phases 2 & 3*, respectively. Overall, SHAM-OIL rats gained 109 g during *Phases 2 & 3*, versus 49 g in RYGB-OIL rats and -1 g in RYGB-E2 rats.

RYGB and estradiol treatment had similar effects on energy intake as on body weight both during *Phase 2*, when rats were fed solid, high-energy diet, and during *Phase 3,* when the rats were fed Ensure, which increased energy intake in all groups, (Figure 1B). RYGB-OIL rats ate less than SHAM-OIL rats in both *Phases*, RYGB-E2 rats ate less than RYGB-OIL in *Phase 2* and tended to eat less in *Phase 3* ($P < 0.06$), and SHAM-E2 rats ate less than SHAM-OIL rats in both *Phases*. RYGB also reduced the rats' selection of both test diets vs. chow (Supplementary Table 1).

We tested the effects of Exendin (9-39) and Devazepide, which are potent and selective receptor antagonists of GLP-1 and CCK, respectively, during 60-min Ensure tests (*Phase 4*). In both sham-operated and RYGB rats, the antagonists' eating-stimulatory effects were significantly greater in estradiol-treated than oil-treated rats (Figure 2). In neither case, however, was the antagonist effect greater in RYGB-E2 rats than in SHAM-E2 rats.

We consider three aspects of our data important. First, to our knowledge, this is the first study investigating the influence of reproductive-axis function on bariatric-surgery outcome. These data are critical because the great majority of gastric bypass operations are performed in women and because the reproductive axis, in particular

estrogens, potently influence the physiology of eating and body-weight control. Second, we showed that estradiol significantly increased RYGB's potency to reduce weight gain and to inhibit eating. Similar effects were obtained when RYGB rats were fed a high-energy solid diet (*Phase 2*) and Ensure, a 57%-energy/sugar liquid diet (*Phase 3*), suggesting that they do not depend on particular dietary forms or components. Our data suggest that RYGB may be more effective in healthy premenopausal women than in postmenopausal women who do not receive hormone-replacement therapy or in women with reduced estrogen levels, such as patients with polycystic ovary syndrome[7]. We are not aware of any clinical studies assessing this question, although we found recently that bariatric surgery was more effective in postmenopausal-age women than premenopausal-age women (Ochner, Geary and Asarian, unpublished). Whether estrogens enhance the eating and body-weight lowering effects of RYGB via the same mechanisms by which they contribute to the normal control of eating and body weight also deserves further research. Furthermore, it is unclear whether estrogens affect the outcome of other types of bariatric surgery procedures, such as gastric banding or gastric sleeve resection. Third, we provide novel evidence that estrogens increase endogenous GLP-1 and CCK satiation in RYGB. In previous reports, estradiol increased CCK satiation in rats with intact intestines[1,3,6]. This is the first report that estradiol also increases GLP-1 satiation in intestine-intact rats and that estradiol increases the satiating effect of CCK and GLP-1 in RYGB rats. We did not, however, detect any difference in the potency of either GLP-1 or CCK in estradiol-treated RYGB vs. estradiol-treated, sham-operated rats, suggesting that increases in the satiating potencies of these peptides alone are not sufficient to account for the decreased total food intake and decreased weight gain in our model of RYGB. Possibly larger doses of the antagonists, chronic antagonist treatment, or modifications of RYGB method (e.g., longer intestinal

bypass) would reveal a surgical effect. Finally, other estrogenic effects, such as metabolic effects, may also have contributed.

Figure Legends:

Figure 1. Body weights (A) and food intakes (B) following RYGB or sham operation in ovariectomized rats that were treated with estradiol (E2) or the oil vehicle (OIL). $^{\#}$SHAM-OIL vs. RYGB-OIL; *RYGB-OIL vs. RYGB-E2; $^{+}$SHAM-OIL vs. SHAM-E2; Ps<0.01 for body-weight gains and Ps<0.05 for food intakes.

Figure 2. Effects of RYGB on the de-satiating actions of GLP-1 antagonism with Exendin (9-39) (A) and of CCK antagonism with Devazepide (B) in ovariectomized rats treated with estradiol (E2) or the oil vehicle (OIL). $^{+}$Estradiol decreased Ensure intake after control injections in same surgery group; *Antagonist increased Ensure intake, same surgery group; Ps<0.05.

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