



RYGB progressively increases avidity for a low-energy, artificially sweetened diet in female rats



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ARTICLE INFO

Article history:

Received 30 August 2015

Received in revised form

18 November 2015

Accepted 24 November 2015

Available online 17 December 2015

Keywords:

Estradiol

Sweet cravings

Meal-related activity

Behavioral sequence of postprandial satiety

Hunger

Leptin

ABSTRACT

Weight re-gain within 2 y after Roux-en-Y gastric bypass (RYGB) is significantly associated with increased intake of and cravings for sweet foods. Here we describe a novel model of this late increase in sweet appetite. Ovariectomized RYGB and Sham-operated rats, with or without estradiol treatment, were maintained on Ensure liquid diet and offered a low-energy, artificially sweetened diet (ASD) 2 h/d. First, we tested rats more than six months after RYGB. ASD meals were larger in RYGB than Sham rats, whereas Ensure meals were smaller. General physical activity increased during ASD meals in RYGB rats, but not during Ensure meals. Second, new rats were adapted to ASD before surgery, and were then offered ASD again during 4–10 wk following surgery. Estradiol-treated RYGB rats lost the most weight and progressively increased ASD intake to >20 g/2 h in wk 9–10 vs. ~3 g/2 h in Sham rats. Finally, the same rats were then treated with leptin or saline for 8 d. Leptin did not affect body weight, Ensure intake, or activity during meals, but slightly reduced ASD intake in estradiol-treated RYGB rats. Food-anticipatory activity was increased in estradiol-treated RYGB rats during the saline-injection tests. Because increased meal-related physical activity together with larger meals is evidence of hunger in rats, these data suggest that (1) RYGB can increase hunger for a low-energy sweet food in rats and (2) low leptin levels contribute to this hunger, but are not its only cause. This provides a unique rat model for the increased avidity for sweets that is significantly associated with weight recidivism late after RYGB.

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1. Introduction

In most patients, the initial dramatic effects of Roux-en-Y gastric bypass surgery (RYGB) and other bariatric surgeries on weight loss and amelioration of metabolic disease plateau or deteriorate beginning ~1–2 y postoperatively (Brethauer et al., 2013; Courcoulas et al., 2013; Karmali et al., 2013; Magro et al., 2008; Schauer et al., 2014; Sjöström et al., 2014; Still et al., 2014). Although patients endorse lower preferences for sweet foods in the initial two years after RYGB (Pepino et al., 2014; Pepino, Stein, Eagon & Klein, 2014; Ullrich, Ernst, Wilms, Thurnheer, & Schultes, 2013), weight recidivism later after RYGB is associated with increased urges to eat sweet foods, increased intake of sweet foods

and increased total energy intake over the postsurgical nadirs (Brolin, Robertson, Kenler, & Cody, 1994; Sarwer et al., 2008; Yanos, Saules, Schuh, & Sogg, 2015). In the course of studying the physiological mechanisms underlying the appetite-lowering effects of RYGB in rats, we serendipitously discovered a parallel to such late increases in sweet appetite in RYGB patients. That is, we found that RYGB rats maintained on Ensure liquid diet and offered a low-energy, artificially sweetened diet (ASD) 2 h/d progressively increased ASD intake. This paper describes our workup of this phenomenon.

Late increases in RYGB patients' eating may reflect a regulatory response to reduced adiposity. Adipose-tissue loss, which is usually approximated as body-weight loss, beyond a certain level provokes anabolic responses, including decreases in physical activity and resting energy expenditure (beyond what is appropriate for the lower weight) and increases in food intake, hunger and flavor hedonics (Cameron, Goldfield, Cyr, & Doucet, 2008; Franklin, Schiele, Brozek, & Keys, 1948; Johanssen et al., 2012; Ravussin, Leibel, & Ferrante, 2014; Rosenbaum, Sy, Pavlovich, Leibel, & Hirsch, 2008;

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Rosenbaum & Leibel, 2014; Speakman et al., 2011; Stice, Burger, & Yokum, 2013). Because the threshold weight that elicits these responses is higher in obese persons than in never-obese persons, they occur in obese patients who are losing weight well before they reach healthy weights (Ferrannini, Rosenbaum, & Leibel, 2014; Rosenbaum & Leibel, 2014). At present, leptin is the only peripheral signal known to contribute to regulatory responses to weight loss (weight gain also leads to regulatory responses, but no peripheral signals controlling these have yet been clearly demonstrated) (Gloy, Lutz, Langhans, Geary, & Hillebrand, 2010; Ravussin et al., 2014; Rosenbaum & Leibel, 2014; Speakman et al., 2011). In several tests, leptin treatment reversed the anabolic responses stimulated by experimental weight loss (Rosenbaum & Leibel, 2014). But 16 wk of leptin treatment failed to reinstate weight loss in a group of female RYGB patients. These patients were tested at least 18 mo post-operatively, when they were weight stable following loss of a mean of 31% of their body weight and were hypoleptinemic compared to non-operated women of similar body weight (Korner et al., 2013). Leptin treatment did, however, reduce sweet cravings (Conroy et al., 2014). In view of this, we tested whether leptin treatment would reduce ASD intake.

Although obesity increases the risk of many diseases and increases mortality in both men and women (Guh et al., 2009; Pi-Sunyer, 2009), morbid obesity and RYGB are special issues for women's health. In 2010 in the USA, 60% of adults with BMI (weight/height²) ≥ 35 kg/m² were women and 65% of adults with BMI ≥ 40 kg/m² were women (Flegal, Carroll, Kit, & Ogden, 2012). Even more strikingly, ~80% of persons electing RYGB in the USA are women (DeMaria, Pate, Warthen, & Winegar, 2010). There are many likely explanations for the disproportionate number of women RYGB patients. Obesity increases the risk for female-specific health disorders, such as endometrial and breast cancer (Pi-Sunyer, 2009) and disturbed reproductive function and infertility (Klenov & Jungheim, 2014). Obesity also increases the risks of anxiety disorder and of major depression more in women than in men (Anderson, Cohen, Naumova, Jacques, & Must, 2007; Pickering et al., 2011). In addition, more obese women than obese men are subject to weight discrimination, i.e., negative, unequal treatment (Puhl, Andreyeva, & Brownell, 2008). Finally, although it is not widely recognized, overweight and obesity increase the risk of type-2 diabetes mellitus twice as much in women as in men (Guh et al., 2009). Therefore, to increase our work's translational relevance we used ovariectomized female rats, which were either chronically treated with estradiol (E2) in order to model premenopausal women, or were not E2 treated in order to model postmenopausal women (Asarian & Geary, 2002; 2013).

2. Experiment 1

We first noticed that RYGB increases ASD intake in a group of rats that were used in unreported pilot experiments. Because the reduction in food intake caused by RYGB is expressed as a decrease in meal size in patients (Laurenus et al., 2012) and rats (Lutz & Bueter, 2014; Shin, Zheng, Pistell, & Berthoud, 2011; Zheng et al., 2009), we compared the sizes of test meals of ASD and of Ensure in these rats. In addition, because increased food-associated general physical activity is a sign of hunger in rats (Richter, 1922; Patton & Mistlberger, 2013; Sheffield & Campbell, 1954; Siegel & Steinberg, 1949), we determined if scheduled ASD meals were accompanied by increased general activity. The Veterinary Office of the Canton of Zürich approved all procedures.

2.1. Method

Twenty female Long–Evans rats (Centre d'Elevage R. Janvier, Le

Genest-Saint-Isle, France) were housed individually in stainless-steel wire-mesh cages (48 × 25 × 18 cm) in a room with an average temperature of 21–25 °C and a 12 h light/12 h dark cycle (lights off at 1600 h). Rats were offered Ensure Plus Chocolate liquid diet (1.5 kcal/mL, 57% energy from sugar, 28% from fat; Abbott, Baar, Switzerland) and water ad libitum, except as described below, for 5 wk. RYGB or sham-RYGB (Sham) (Bueter, Abegg, Seyfried, Lutz, & le Roux, 2012), ovariectomy (Asarian & Geary, 2002), and intra-junal catheterization (Bächler and Asarian, unpublished) were then done. In RYGB rats, the biliopancreatic limb was ~10 cm in length, the Roux (alimentary) limb was ~60–80 cm, and the common channel was ~25–30 cm.

A near-physiological cyclic regimen of E2 treatment (Asarian & Geary, 2002, 2013) was begun 2 wk after surgery. Half the Sham and half the RYGB rats received, every fourth day, between 1100 and 1130 h (i.e. around the mid-light phase), interscapular subcutaneous injections of 2 µg E2 (17β-estradiol-3-benzoate, #E8515; Sigma–Aldrich) in 100 µL sesame oil (#S3547; Sigma–Aldrich); the other rats received injections of oil alone (Oil).

For the first 6 mo, rats were used in other experiments (unpublished). During these experiments, on 5–7 d/wk Ensure was removed at 1215 h, at 1600 h rats received intrajeunal infusions (10 min, 0.44 mL/min) of 0.9% saline or various nutrients, at 1615 h a low-energy, artificially sweetened diet (ASD, 20 g gelatin [Blatt Gelatine, Migros, Zurich, Switzerland] and 1 g saccharine [Sigma–Aldrich, Buchs, Switzerland] dissolved in 1 L hot water and cooled; ~0.08 kcal/mL) was presented, and at 1815 h, ASD was removed and Ensure was returned.

During these tests we noticed that, in the control conditions, RYGB rats ate more ASD than Sham rats did. To characterize this in terms of meal intake, five Sham and eight RYGB rats were adapted for 8–10 d to the above daily ASD feeding schedule with no other interventions (i.e., no intrajeunal infusions). On the test day (the second day after E2 or oil injections; in E2 rats this models the day of estrus), ASD intake was measured at 15 min intervals. In addition, behaviors were observed for 60 min after ASD presentation using a time-sampling method used previously (e.g., Gibbs, Young, & Smith, 1973; Asarian, Corp, Hrupka, & Geary, 1998). In those experiments, observations were done by an experimenter who had been trained to glance just long enough at each rat to take a mental snapshot of its behavior once per minute, cued by 0.6 s tones presented once each 2 s. The same method was applied here, except without the tone cues. The observer was blind to the rats' experimental groups. Behaviors were classified as eating, locomotion, rearing, sniffing, grooming, resting, etc., as previously described (Gibbs et al., 1973; Asarian et al., 1998; Hinton, Esguerra, Farhody, Granger, & Geary, 1987; Rosofsky & Geary, 1989). General physical activity was defined as observations of locomotion, rearing, or sniffing. Following this, Ensure meals were characterized. Rats were adapted for 2 wk to presentation of Ensure instead of ASD after the deprivation period. One rat developed signs of illness during this period and was euthanized. Behaviors and Ensure intake were then measured as previously.

Meal end was identified by three consecutive observations of resting (i.e., the rat's weight rested on the cage floor and no movement or other behavior, such as sniffing, was observed), which is the terminal behavior in rats' normal post-eating display (Gibbs et al., 1973). The period from meal onset to the first of these three observations of resting was the latency to rest and defined as meal duration. Meal size was the amount of ASD or Ensure consumed up to this point. In two RYGB rats that did not rest within 60 min, latency to rest was scored as 60 min. Latency to rest, meal size and frequency of behaviors of interest during the meal were analyzed with Wilcoxon–Mann–Whitney tests, computed with Social Science Statistics (www.socscistatistics.com). Graphs were

prepared with Prism (graphpad.com/scientific-software/prism). Because of the small sample size and the lack of apparent effect of E2 in this study, data from E2 and Oil rats were collapsed.

2.2. Results

Sham rats were significantly heavier than RYGB rats at the onset of these tests (~6 mo after surgery), 371 ± 20 g vs. 284 ± 9 g (mean \pm standard error of the mean, SEM; $t(10) = 4.37$, $P < 0.01$). This difference was maintained throughout.

As expected, Ensure test meals were significantly shorter ($U = 3.5$, $P = 0.02$) and smaller ($U = 5$, $P = 0.04$) in RYGB rats (Fig. 1, left). Intra-meal activity counts were similar in RYGB and Sham rats ($U = 15.5$, $P = 0.81$), but grooming was observed less frequently in the RYGB rats ($U = 4.5$, $P = 0.04$). In contrast to their smaller Ensure meals, RYGB rats took significantly longer.

($U = 2$, $P = 0.008$) and larger ($U = 2$, $P = 0.008$) ASD meals (Fig. 1, right). This was accompanied by a significant increase in activity counts ($U = 5.5$, $P = 0.03$). Grooming counts were higher than with Ensure, but did not differ between RYGB and Sham rats ($U = 24.5$, $P = 0.51$).

3. Experiment 2

A new group of rats was followed for 10 wk after surgery to characterize the development of the avidity for ASD in RYGB rats.

3.1. Method

Thirty six rats weighing ~180 g were switched from chow to Ensure as their maintenance diet and operated 10 wk later, when they weighed ~300 g. Previous body weight and microCT data collected under similar circumstances (Gloy, Langhans, Hillebrand, Geary, & Asarian, 2011) suggest that this ~300 g represented ~75 g excess body weight and ~55 g excess adipose-tissue weight in comparison with age-matched chow-fed rats.

Beginning 3 wk preoperatively, ASD was presented to all rats daily, as in Experiment 1. The week of surgery was designated wk 1. During wk 1–3, ASD was not presented to reduce the likelihood of formation of conditioned taste aversions. E2 or Oil treatment was begun after 3 wk, resulting in four groups: RYGB-E2 ($n = 9$), RYGB-Oil ($n = 8$), Sham-E2 ($n = 7$) and Sham-Oil ($n = 6$) (six rats were lost in surgery or became ill and were euthanized shortly afterwards). ASD presentation was resumed in wk 4. Body weight, Ensure intake and ASD intake were measured daily.

Weekly data were averaged (mean or median as described below) for analysis. Weekly mean body weights, weekly mean Ensure intakes, and maximum post-operative weight losses were analyzed with planned-comparison t -tests, using standard errors of the difference (SED) computed from analyses of variance using Prism; these data are presented as mean [SEM]. ASD intakes and week of maximum weight loss were not normally distributed, so they were analyzed with Wilcoxon-Mann-Whitney tests as in Experiment 1; these are presented as median [1st – 3rd quartile]. For both nonparametric and parametric tests, four contrasts were tested: [1], RYGB-E2 vs. Sham-E2; [2], RYGB-Oil vs. Sham-Oil; [3], RYGB-E2 vs. RYGB-Oil; and [4], [1] vs. [2]. Analysis-wide α levels were protected at $P = 0.05$ using the Bonferroni–Hochberg method (Hochberg, 1988). Exact P levels were computed with Surfstat (eswf.uni-koeln.de/allg/surfstat). Graphs were prepared with Prism.

3.2. Results

RYGB and E2 treatment each led to weight loss (Fig. 2A). Both

RYGB groups lost weight for 2 wk, and then began to gain weight. After hormone treatment began during wk 4, RYGB-Oil rats continued to gain, whereas RYGB-E2 rats again lost weight. As a result, RYGB-Oil and RYGB-E2 rats weighed less than Sham-Oil and Sham-E2 rats, respectively, during wk 1–10, and RYGB-E2 rats weighed less than RYGB-Oil rats during wk 4–10. The RYGB effect, i.e., the Sham–RYGB differences in each hormone group, tended to be smaller in E2-treated rats than Oil-treated rats, but this may have been due to a floor effect in the RYGB-E2 rats, which weighed least. Maximum body weight loss was larger in RYGB-E2 than RYGB-Oil rats (Fig. 3) and occurred later in RYGB-E2 rats than in RYGB-Oil rats (Table 1). The mean rates of weight gain in the following weeks of the test were not significantly different in RYGB and Sham rats (Table 1).

Mean Ensure intakes roughly paralleled mean body weight trajectories, especially during wk 2–5, when weights diverged most (Fig. 2B). The return of Sham-Oil rats' intakes to about the level of Sham-E2 rats' intakes is typical of ovariectomized rats; i.e., once untreated ovariectomized rats reach 30–50 g overweight in comparison with E2-treated ovariectomized rats or intact female rats, they no longer over-eat (Asarian & Geary, 2013).

Sham-Oil and Sham-E2 rats' median weekly ASD intakes were less than 2 g throughout. In contrast, RYGB-E2 rats progressively increased ASD intake, from 0.8 [0.6–0.9] g during wk 4 to 22.4 [19.6–25.4] g during wk 10 (median [1st–3rd quartile]; Fig. 2C). RYGB-Oil rats as a group did not reliably increase ASD intake, although many individuals did beginning in wk 6: in 5 of the 9 RYGB-Oil rats, ASD intake during wk 6–10 was 8.6 [5.6–16.2] g vs. 0.6 [0.5–0.7] g in the remaining four rats. These five RYGB-Oil rats also had larger maximum weight losses than the remaining four (19.6 [2.4] vs. 13.5 [1.9] g, mean [SEM]) and had poorer weight gains during wk 6–10 (1.6 [0.4] vs. 4.2 [1.4] g/wk).

4. Experiment 3

Low plasma leptin levels signal underweight (Ravussin et al., 2014; Rosenbaum & Leibel, 2014; Speakman et al., 2011), and leptin replacement reduced sweet cravings in a group of women tested at least 18 months after RYGB (Conroy et al., 2014). Therefore, we tested whether leptin replacement would reduce ASD intake or meal-related general activity.

4.1. Method

The rats used in Experiment 2 were re-tested in a within-subject crossover test of twice-daily (1000 and 1800 h) subcutaneous injections of 100 μ g/kg leptin (Preprotech, Hamburg, Germany) or saline for eight consecutive days each. This leptin dose was chosen as a pharmacological replacement treatment because it increased plasma leptin levels 3–4-fold when infused continuously over one day in male rats (Unniappan & Kieffer, 2008). The two eight-day arms of the crossover test were arranged so that the fourth and eighth (last) days of saline/leptin treatment were the day of the E2 treatment cycle that models estrus, when E2's eating-inhibitory effects are largest (Asarian & Geary, 2002, 2013). On the final day of each arm, behaviors were rated as previously for 1 h before and 1 h after ASD presentation. An 8 d washout period separated the two arms. Body-weight gains, Ensure intakes, ASD intakes and behavior counts were transformed to difference scores (saline value – leptin value) and analyzed as above.

After this test and a 3 wk washout period, rats were anesthetized with 50 mg/kg pentobarbital after a 3 h fast in the light phase, heart blood samples were taken, and plasma leptin levels were assayed (rat leptin kit, Meso Scale Discovery, Gaithersburg MD, USA). These were analyzed parametrically as above.

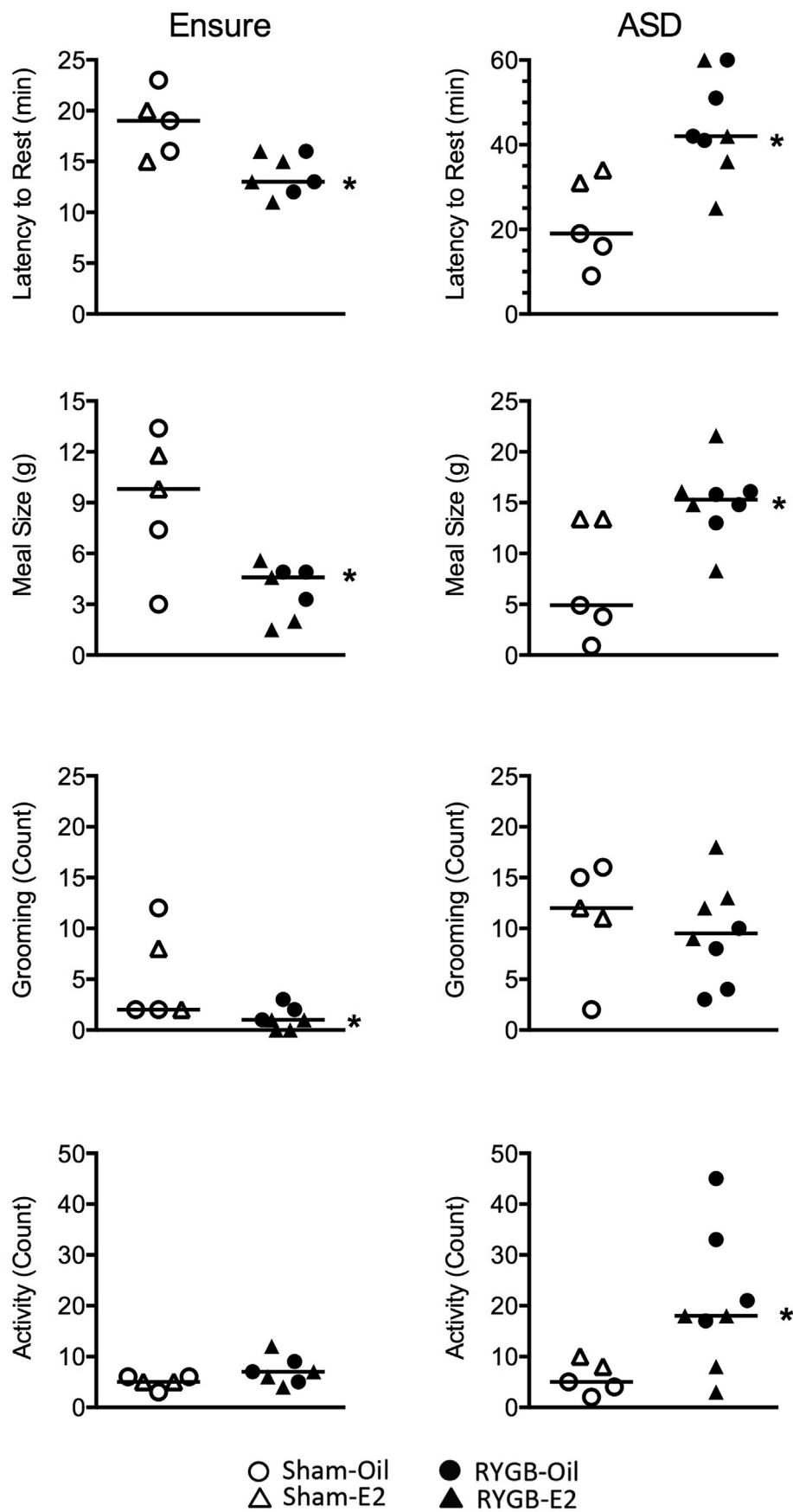


Fig. 1. Latency to rest (meal duration), meal size and intra-meal counts of grooming and general activity in RYGB and Sham rats offered either Ensure (left) or ASD (right) after 4 h of deprivation of the Ensure maintenance diet; individual data, with medians indicated by horizontal lines. *RYGB different from Sham, $P < 0.05$.

4.2. Results

Leptin treatment failed to detectably affect body weight or mean daily Ensure intake in any group (Fig. 4A–C). Leptin did slightly, but reliably, decrease median ASD intake in RYGB–E2 rats (Table 2). Analysis of the data by day of E2-treatment cycle failed to reveal any further effects of leptin (data not shown). Leptin treatment also failed to affect meal-related behaviors or meal size either before or during ASD meals on d 8 of the trials (data not shown). RYGB–E2 rats displayed increased food–anticipatory activity, i.e., increased general activity in the hour before ASD presentation, but did not reliably increase activity during the meal (Table 2). Leptin did not affect either of these activity measures (data not shown). Plasma leptin measurements confirmed the overall inverse association between leptin levels and body weight in the four groups (Fig. 4D).

5. Discussion

The most important finding in these studies is that RYGB led to a progressive increase in the intake of an energy-dilute sweet diet in female rats. This occurred in Experiment 1 in E2-treated and untreated ovariectomized RYGB rats tested six months post-operatively and in Experiment 2 in E2-treated RYGB rats tested 6–10 wk post-operatively, with a trend for an increase in untreated RYGB rats. This provides a novel platform for investigating an important clinical problem, the reduced efficacy of RYGB on eating, weight loss, and metabolic disease that begins 1–2 y post-operatively and in some patients completely reverses RYGB's weight-lowering effects (Brethauer et al., 2013; Courcoulas et al., 2013; Karmali et al., 2013; Magro et al., 2008; Schauer et al., 2014; Sjöström et al., 2014; Still et al., 2014) and that has been linked to increased urges to eat sweets and increased intake of sweets (Brolin et al., 1994; Sarwer et al., 2008; Yanos et al., 2015).

Two aspects of the data support the interpretation that increased ASD intake represents a regulatory increase in hunger in response to weight loss after RYGB. First, low body weight after RYGB appeared to elicit the late increase in ASD intake. In Experiment 2, Sham rats did not lose weight and did not increase ASD intake during the 10 wk experiment, RYGB–E2 rats lost the most weight and had the largest ASD intakes, and RYGB–Oil rats were intermediate in both weight loss and ASD intake. But factors unrelated to RYGB or weight loss must explain why Sham–Oil and Sham–E2 rats eventually increase ASD intake, albeit not to the same degree as RYGB rats (Experiment 1; Bächler et al., unpublished data). Second, the increased ASD intake of RYGB rats appeared to reflect increased hunger. Behavioral observations indicated that rats ate large, normal meals of ASD, i.e., periods of eating followed by grooming and resting (Gibbs et al., 1973; Richter, 1922). These large meals were accompanied by increased activity during the meal in Experiment 1 and by increased food–anticipatory activity in Experiment 3. Increased meal-related general activity is a classical sign of hunger in rats (Richter, 1922; Patton & Mistlberger, 2013; Sheffield & Campbell, 1954; Siegel & Steinberg, 1949). Therefore, we interpret the increased ASD intake in RYGB rats to reflect tonically increased hunger.

Our interpretation of tonically increased hunger late after RYGB is consistent with reports that RYGB rats increase rates of licking of some concentrations of sucrose, increase orofacial acceptance reactions to some sweets, run faster for food reward in alleyways, increase progressive-ratio responding, and increase the number of brief-access sucrose licking trials initiated (Mathes et al., 2012; 2015; Shin et al., 2011), all indicative of increased hunger. The hunger interpretation is also consistent with reports that RYGB rats ate larger meals than control rats under certain circumstances, i.e., when tested during the light phase, when control rats ate small

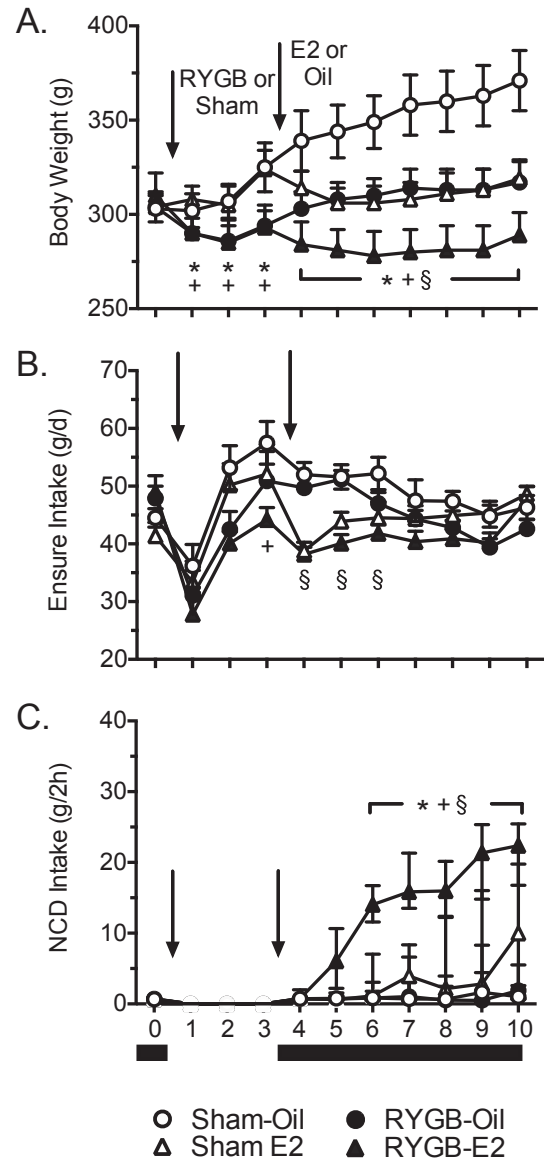


Fig. 2. Evolution of average (A.) body weight (mean \pm SEM), (B.) daily 24 h Ensure intake (mean \pm SEM), and (C.) daily 2 h ASD intake (median, 1st–3rd quartile range) in Sham–Oil, Sham–E2, RYGB–Oil and RYGB–E2 rats during 10 post-operative weeks. Filled bar indicates weeks during which ASD was offered 2 h/d, after 4 h of deprivation of the Ensure maintenance diet. *RYGB–Oil < Sham–Oil; †RYGB–E2 < Sham–E2; §RYGB–E2 < RYGB–Oil; $P < 0.05$.

meals (Lutz & Bueter, 2014), and 4–6 d after a shift from a choice of Ensure, a high-fat diet and chow to chow alone, when control rats ate little (Zheng et al., 2009). Finally, RYGB patients significantly increased sweet cravings (Brolin et al., 1994; Sarwer et al., 2008; Yanos et al., 2015) and hedonic hunger (Cushing et al., 2014) late after RYGB, again suggestive of increased hunger. Neither our data nor the studies described above, however, indicate whether the RYGB specifically increased hunger or more generally increased reward function. For example, RYGB rats also increase intravenous self-administration of ethanol (Polston et al., 2013) and morphine (Biegler, Freet, Horvath, Rogers, & Hajnal, 2015), and RYGB increases alcohol-use disorders in some patients (Steffen, Engel, Wonderlich, Pollert, & Sondag, 2015). Delineating the specific functional processes through which RYGB affects motivation is an important issue for future research.

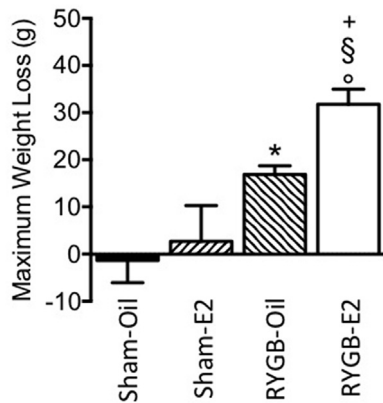


Fig. 3. Maximum body-weight (BW) loss, compared with pre-operative weight in Sham-Oil, Sham-E2, RYGB-Oil and RYGB-E2 rats during 10 post-operative weeks, mean \pm SEM. *RYGB-Oil > Sham-Oil, $P < 0.05$; +RYGB-E2 > Sham-E2, $P < 0.01$; §RYGB-E2 > RYGB-Oil, $P < 0.05$; ○[(RYGB-E2) – (Sham-E2)] > [(RYGB-Oil) – (Sham-Oil)], $P < 0.01$.

Table 2
Selected data from Experiment 3.

	Group			
	Sham-oil	Sham-E2	RYGB-Oil	RYGB-E2
ASD Intake				
Saline	0.6 [0.5–2.0]	1.7 [0.5–4.2]	8.1 [0.9–10.0]	14.6 [12.9–17.1]
Leptin	0.5 [0.5–3.0]	1.5 [0.6–6.0]	8.1 [0.7–9.0]	13.3 [11.2–15.2]
FAA (Saline)	2 [1–3]	1 [1–3]	1 [0–8]	7 [4–16]*
LR (Saline)	3 [2–8]	10 [3–15]	12 [7–21]	15 [11–24]
ADM (saline)	1 [0–2]	1 [1–3]	3 [0–5]	5 [3–6]

ASD intakes are g/2h averaged over the 8 d crossover tests of saline or leptin treatment; FAA (food–anticipatory activity) are counts of general activity observed once per minute during the 60 min before ASD presentation on d 8 of the saline arm of the crossover test; LR (latency to rest) are min elapsed before resting after ASD presentation on d 8 of the saline arm of the crossover test; and ADM (activity during meal) are counts of general activity observed before resting after ASD presentation on d 8 of the saline arm of the crossover test; all data are median [1st–3rd quartile]. *RYGB-E2 > Sham-E2, $P < 0.05$.

Table 1
Body weight (BW) loss and regain in RYGB rats in Experiment 2.

	Group			
	Sham-oil	Sham-E2	RYGB-Oil	RYGB-E2
Week of maximum BW loss	–	–	2 [2–2]	6 [5–7]*
Subsequent rate of BW gain (g/wk)	3.8 [1.0]	3.0 [1.1]	4.8 [0.7]	5.8 [2.3]

Week of maximum BW loss is week after RYGB and ovariectomy during which minimum body weight was recorded (median [1st–3rd quartile]); *Different from RYGB-Oil rats, $U = 14.5$, $P = 0.04$. Rate of subsequent BW gain is weight gain (g/wk) during the following weeks for RYGB rats and weight gain during weeks 7–10 for Sham rats (mean [SEM]); no significant differences.

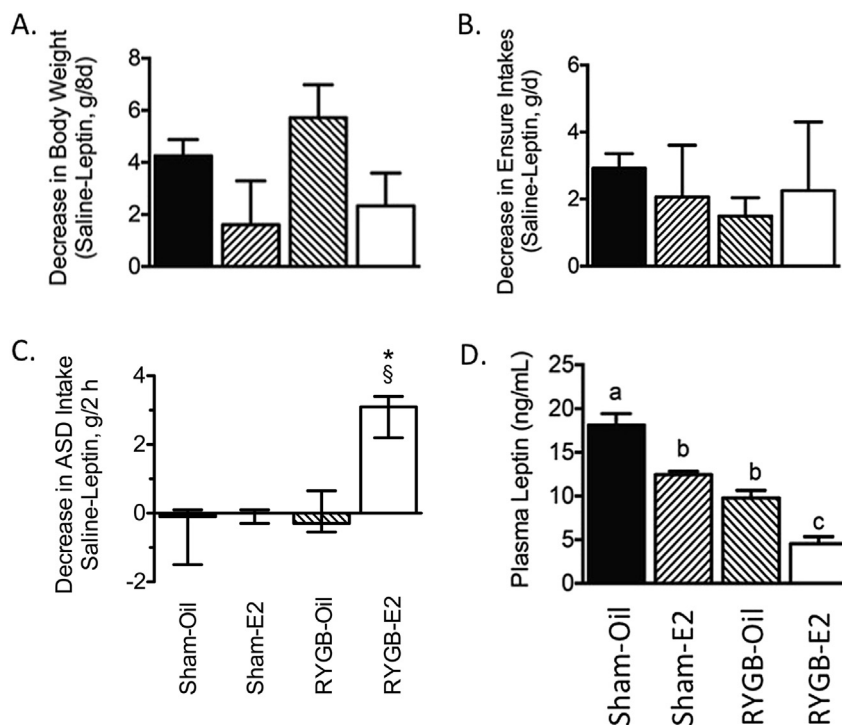


Fig. 4. Effects of twice-daily subcutaneous injections of 100 μ g/kg leptin for 8 d on (A.) body weight, (B.) daily Ensure intake, and (C.) 2 h ASD intake in Sham-Oil, Sham-E2, RYGB-Oil and RYGB-E2 rats. Data are difference scores (saline value – leptin value), mean \pm SEM, except ASD intakes are median, 1st–3rd quartile; *RYGB-E2 > Sham-E2; §RYGB-E2 > RYGB-Oil; $P < 0.05$. (D.) Plasma leptin levels measured after the end of the leptin-injection experiment. Data are mean \pm SEM; groups with different letters differ, $P < 0.05$.

The conclusion that RYGB increased hunger in rats also raises questions. First, if RYGB rats were hungrier than Sham rats, why did they not eat larger Ensure test meals in Experiment 1? We assume

that the exaggerated satiating effects of nutrient-rich Ensure after RYGB overwhelmed their hunger and reduced meal size. This also may explain the smaller spontaneous meals of RYGB rats fed Ensure

or high-fat diet described by Zheng et al. (2009). Increased satiation signaling is a frequently offered hypothesis for the small meals after RYGB. This is supported by the many reports of increased postprandial levels of CCK, GLP-1 and other meal-related hormones after RYGB, although in fact neither these nor any other hypothesized satiation signal has yet been shown to tonically reduce meal size in RYGB mice, rats or patients (Harvey et al., 2010; Lutz & Bueter, 2014; Ye et al., 2014).

A second question facing our hunger interpretation is its relationship to hunger ratings in RYGB patients. RYGB patients endorsed less pre-meal hunger in comparison with their pre-surgical ratings in three studies (Borg et al., 2006; le Roux et al., 2007; Morinigo et al., 2006), which appears inconsistent with our hunger interpretation. There are several plausible explanations for this apparent discrepancy. First, in two similar studies (Bryant et al., 2013; Laurenus et al., 2012), RYGB did not change patients' pre-meal hunger ratings, so that the phenomenon seems more variable than initially thought. Second, all five studies cited above were done within two years of surgery, so it is possible that hunger might increase more reliably later. Third, the concept "hunger" encompasses both negative-reinforcement components related to acute or chronic nutrient depletion as well as positive-reinforcement components independent of such depletion (Betley et al., 2015; Geary & Moran, 2015). Whether the increase in hunger elicited by low body weight is predominately the former or the latter is unknown. This raises the possibility that ASD intake in rats and pre-meal hunger ratings in humans reflect different hunger processes. Fourth, subjective hunger in humans is sensitive to a variety of cognitive influences that may be weaker or absent in rats (e.g., Bryant et al., 2013). For example, if in the studies cited the patients knew that they would receive a satiating, energy-dense meal after the ratings, their ratings may have been more determined by the amount of food that they planned to eat ("expected satiation"; Brunstrom, 2014) than by hunger related to nutrient depletion. It would be illuminating to test RYGB patients in experiments appropriately designed to parse such different facets of hunger.

Leptin is the only peripheral signal known to contribute to regulatory responses to weight loss (Ravussin et al., 2014; Rosenbaum & Leibel, 2014; Speakman et al., 2011), and plasma leptin levels roughly reflected body-weight status in our rats; i.e., RYGB-E2 and RYGB-Oil rats were hypoleptinemic in comparison with Sham-E2 and Sham-Oil rats, respectively. Thus, if hypoleptinemia elicits anabolic responses in RYGB rats, then leptin replacement should reverse them. Our test of this prediction in Experiment 3, however, led to the same disappointing results as reported by Korner and her colleagues in patients (Korner et al., 2013; Conroy et al., 2014). That is, there was no effect on body weight in either rats or women, but a small decrease in ASD intake in rats, which may parallel the decrease in sweet craving in women (Conroy et al., 2014). As pointed out by Korner et al. (2013), it is possible that a regimen of twice daily leptin injections, which we both used, was ineffective because it did not mimic the normal pulsatile and circadian patterns of leptin secretion. This issue warrants further research. It is also important to note that leptin may affect taste-receptor function (Glendinning et al., 2015; Kawai, Sugimoto, Nakashima, Miura, & Ninomiya, 2000; Meredith, Corcoran, & Roper, 2015; Yoshida et al., 2015), and a contribution of this to our data cannot be excluded.

Interestingly, RYGB-E2 rats maintained significantly lower body weights than RYGB-Oil rats. This also occurred in a previous experiment in which ovariectomy and RYGB were done simultaneously (Asarian et al., 2012). This suggests that RYGB weight outcome might be better in premenopausal women than postmenopausal women. This possibility has not been tested directly, and one indirect test provided only weak support for it

(Ochner, Teixeira, Geary, & Asarian, 2013).

This weight difference between RYGB-E2 and RYGB-Oil rats also indicates that RYGB does not completely overwhelm other controls of eating and body-weight regulation. Others reported that RYGB rats lost additional weight when treated with serotonin 2C-receptor agonists (Carmody, Ahmad, Machineni, Lajoie, & Kaplan, 2015), and ate more and gained weight when treated with a melanocortin 3- or 4-receptor antagonist (Mumphy et al., 2014) and temporarily ate more after a period of food restriction during which they lost additional weight (Lutz & Bueter, 2014). Thus, a variety of eating-control mechanisms remain functional after RYGB in rats and mice. These may provide bases for supplementary treatment of RYGB patients with sub-optimal weight-loss outcomes.

The current data together with previous literature in rodents and humans suggest a two-process model of body-weight control following RYGB (Fig. 5). The first process, beginning immediately after surgery, limits food intake and alters dietary choices, which together produce negative energy balance and weight loss. Although not all causes of this are known (Lutz & Bueter, 2014), two likely contributors are early satiation, due to the altered gastrointestinal handling of food, and re-learning of food habits, due to both satiation and gastrointestinal malaise (illness may result from ingesting too much food or food that is no longer well digested, such as fat or whole meat [Graham, Murty, & Bowrey, 2014; Tichansky, Boughter, & Madan, 2006; le Roux et al., 2011]). As body-weight loss continues, the weight threshold to trigger a second process is reached. This second process increases hunger, increases flavor hedonics and alters dietary choices in ways that increase food intake and produce positive energy balance and weight gain. This is similar to the regulatory response to body-weight loss in persons who have not undergone RYGB, as described in the introduction. In RYGB patients, however, there appears to be a specific increase in the selection and intake of sweet foods (Brolin et al., 1994; Sarwer et al., 2008; Yanos et al., 2015). This may be due in part to the innate preference for sweet flavors. But it is likely that flavor-nutrient conditioning also contributes importantly, with sweet intake more than usually reinforcing after RYGB because mono- and disaccharides do not require pancreatic

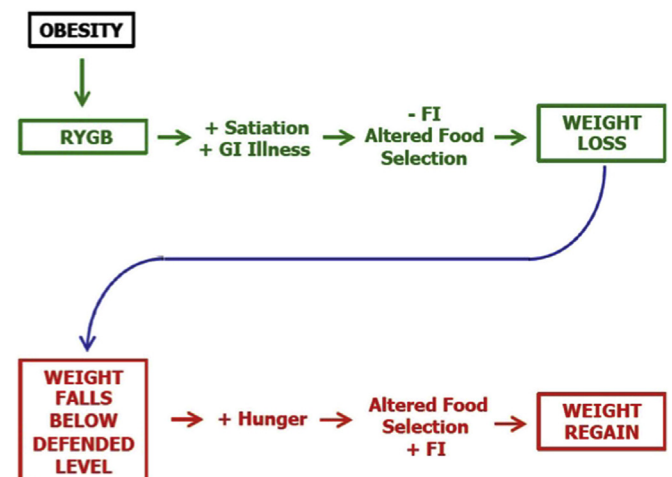


Fig. 5. Schematic diagram of two functional processes hypothesized to produce, first, weight loss (upper row, green) and, subsequently, weight regain (lower row, red) after RYGB in humans or rats. Symbols, +, increase; –, decrease; FI, food intake. Note that "+ hunger" in the weight-regain process refers to an increase in the drive to eat, whether produced by increased hunger *per se*, increased flavor hedonics, or decreased satiation or satiety. Please see text for further explanation of the model.

enzymes for digestion (after RYGB pancreatic juices mix with chyme only in the common channel) and are better tolerated than proteins or fats. The increased intake of nearly non-nutritive ASD here suggests that another factor may also contribute, perhaps the contrast between the relative safety of ingesting ASD vs. less well tolerated Ensure. This second process begins ~2 y post-operatively in humans (Courcoulas et al., 2013). Here it appeared to begin ~6 wk post-operatively in RYGB-E2 rats. After 6 wk, RYGB-E2 rats began to ingest large amount of ASD and returned to a relatively normal rate of weight gain. It is likely that they could not gain more weight because they did not have access to foods that they could tolerate in large amounts. In a previous experiment, RYGB-E2 rats offered both Ensure and chow ate 23% chow, whereas Sham-E2 rats ate only 2% chow (Asarian et al., 2012), suggesting that there may have been a limit to the amount of Ensure that the RYGB-E2 rats could tolerate. Finally, it is important to emphasize that weight regain may continue well past the threshold value that initiated the process. Sweet intake alone could be sufficient for such weight gain because sweets are a self-perpetuating stimulatory control of eating (Davis & Smith, 1990; Sclafani, 2006).

In conclusion, the striking parallel between our animal data and the clinical studies of Korner and her colleagues (Korner et al., 2013; Conroy et al., 2014) supports the likely translational relevance of our model of increased avidity for sweets late after RYGB. Therefore, we suggest it as a useful means to identify novel strategies to combat weight recidivism after RYGB (Brethauer et al., 2013; Courcoulas et al., 2013; Karmali et al., 2013; Magro et al., 2008; Schauer et al., 2014; Sjöström et al., 2014; Still et al., 2014), especially strategies aimed at curbing problematic intake of sweets late after RYGB (Brolin et al., 1994; Sarwer et al., 2008; Yanos et al., 2015). This may be especially difficult for women because there appears to be a physiological sex difference in avidity for sweets (Asarian & Geary, 2013).

Conflicts of interest

The authors disclose no conflicts.

Funding

Supported by National Institutes of Health grant R01 DK092608 (L.A.).

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