Published in final edited form as:

Obes Surg. 2013 July ; 23(7): 920–930. doi:10.1007/s11695-013-0884-4.

Roux en Y Gastric Bypass Increases Ethanol Intake in the Rat

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Abstract

Roux en Y gastric bypass (RYGB) surgery is currently the most effective therapy employed to treat obesity and its associated complications. In addition to weight loss and resolution of metabolic syndromes, such as diabetes, the RYGB procedure has been reported to increase alcohol consumption in humans. Using an outbred rodent model, we demonstrate that RYGB increases postsurgical ethanol consumption, that this effect cannot be explained solely by postsurgical weight loss and that it is independent of presurgical body weight or dietary composition. Altered ethanol metabolism and postsurgical shifts in release of ghrelin were also unable to account for changes in alcohol intake. Further investigation of the potential physiological factors underlying this behavioral effect identified altered patterns of gene expression in brain regions associated with reward following RYGB surgery. These findings have important clinical implications as they demonstrate that RYGB surgery leads directly to increased alcohol intake in otherwise alcohol nonpreferring rat and induces neurobiological changes in brain circuits that mediate a variety of appetitive behaviors.

Keywords

Roux en Y gastric bypass; Ethanol; Orexin; Dopamine

Introduction

Roux-en-Y gastric bypass (RYGB) is a surgical method used to treat obesity and the numerous secondary effects of this chronic condition. It is currently the most effective treatment to yield lasting weight loss and the resolution of a number of comorbidities [1]. In addition to these desirable outcomes, the RYGB procedure has been reported to lead to an increase in the rate of alcohol consumption, craving, and abuse following surgery [2, 3]. However, due to the inability to conduct randomized, carefully controlled trials in human patients, researchers have been unable to draw definitive, causal conclusions with respect to the role of RYGB surgeries in alcohol intake.

It is well-established that weight loss and food restriction contribute to increased reward-seeking behaviors, as well as increases in the subjective reinforcement value and ingestion of rewarding substances [4]. Thus, observed increases in alcohol intake following RYGB may simply be a consequence of the reduced caloric intake and/or weight loss that occur as a result of this procedure, independent of the surgery itself, a possibility that has been difficult to assess in human clinical trials.

Patterns of alcohol use following RYGB surgery are likely influenced by biological substrates underlying reinforcing properties of alcohol. These reinforcing effects are mediated by brain reward pathways that are also responsive to caloric intake and metabolic status, including the lateral hypothalamic (LH) orexin system and the mesoaccumbens dopamine (MADA) pathway [5, 6]. Prior reports indicate that bariatric surgery reduces striatal dopamine receptor availability in humans suggesting that this procedure induces postsurgical effects within brain reward circuitry [7]. Importantly, it has been suggested that decreases in brain dopamine function may drive increased alcohol consumption [8]. Further, significant changes in gut hormones, including ghrelin, are associated with the RYGB procedure [9, 10]. In addition to affecting food intake, ghrelin has also been implicated in reward-related behaviors and, specifically, in the modulation of alcohol intake [11, 12].

Finally, pre-existing behavioral differences have also been proposed to contribute to enhanced alcohol consumption following surgery. A recent study indicates that patients undergoing the RYGB procedure have a higher lifetime prevalence of alcohol use disorders; this study also reported that weight loss following surgery was not associated with increased alcohol abuse, concluding that a history of alcohol abuse is a stronger predictor of postsurgical alcohol use disorders [13]. In addition, chronic overeating and obesity are associated with alterations in brain dopamine function [14], which may persist following surgery and contribute to increases in alcohol ingestion. Thus, it is unclear if the RYGB procedure itself or pre-existing psychological or physiological attributes regulate the initiation and maintenance of post-surgical alcohol intake.

Therefore, employing a rodent model, the present studies aimed to (1) assess whether the RYGB procedure contributes directly to increased alcohol intake, independent of prior obesity and/or subsequent weight loss and (2) determine possible biological mechanisms that may contribute to this behavioral outcome. In particular, we hypothesize that postsurgical changes in brain reward mechanisms or neuro-endocrine pathways implicated in the regulation of both ingestive and reward-related behaviors may contribute to increased alcohol use following surgery.

Materials and Method

Subjects

Subjects were male, Long–Evans rats housed individually in plastic tub cages and weighed 250–300 g upon arrival to the laboratory. Prior to surgery, rats were maintained on a high-fat diet (Research Diets, New Brunswick, NJ, USA; 4.41 and 1.71 kcal/g from fat) for a period of 8 weeks to induce obesity. Rats then underwent RYGB ($n=6$), sham surgery ($n=10$), or no surgery ($n=5$). In order to test the effects of the RYGB surgery on ethanol consumption independent of dietary composition and presurgical metabolic status, a separate group of rats was maintained on standard rodent chow (Teklad; 3.41 and 0.51 kcal/g from fat) prior to either RYGB ($n=7$), sham surgery ($n=8$), or no surgery ($n=8$). A final group of rats ($n=7$) was maintained on ad lib high fat diet (HFD) for a period of 8 weeks, then food-restricted for approximately 20 days to mimic postsurgical weight loss in the obese RYGB group. These animals will be referred to as the weight loss (WL) control group.

Surgical Procedure

The RYGB surgery consists of (1) a completely separated gastric pouch of roughly 20 % of the total stomach volume created by a cutting stapler (Ethicon, Ithaca, NY, USA) with two straight triple-staple lines between the lesser and greater curvature, (2) a jejunal transection 40 cm from the ileocecal valve, (3) an end-to-side gastrojejunostomy, and (4) an end-to-side jejuno-jejunostomy 25 cm from the ileocecal valve, creating a 15-cm long Roux limb, a 25-cm long common limb, and a roughly 40-cm long biliopancreatic limb. Rats were fasted 24 h prior to surgery and were given access to a liquid diet only (Osmolite OneCal) for the first 3 days after surgery, after which animals were returned to the presurgical diet. For the remainder of the experimental period, rats had ad lib access to either the HFD or standard chow and water. All animal procedures were carried out in accordance with National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee at the University of Cincinnati.

Nuclear Magnetic Resonance

Body composition was assessed using a whole-body nuclear magnetic resonance instrument (Echo-MRI, Waco, TX, USA) to determine percent fat, lean, and water content for each animal. To determine body composition, each animal was immobilized in a clear Plexiglas tube and scanned for 45 s. Body composition was assessed prior to surgery and on day 35 following surgery.

Quantitative PCR

Obese RYGB ($n=6$), obese–sham ($n=10$), and WL rats ($n=7$) were sacrificed via CO₂ asphyxiation and brains were rapidly removed, frozen, and stored at -80°C until processing. The LH, nucleus accumbens (NAcc) and medial prefrontal cortex were microdissected using an AHP-1200CPV freezing plane (Thermoelectric Cooling America, Chicago, IL, USA) which maintained a constant temperature of 12°C throughout the dissection process. Total RNA from microdissected tissue was isolated by Trizol reagent (Invitrogen, Carlsbad, CA, USA) and purified using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to manufacturer's instructions. The total RNA was treated to remove any potential genomic DNA contamination using RNase free DNase (Promega, Madison, WI, USA), and was quantified using a NanoVue spectrophotometer (GE Healthcare, Cambridge, UK). RNA quality was confirmed by standard agarose gel electrophoresis. Complementary DNA (cDNA) was then retrotranscribed (RT) from 1 to 2 μg of total RNA by a mixture of random hexamers and oligo DT priming using the SuperScript III First Strand Synthesis Kit (Invitrogen, Carlsbad, CA, USA). Nonretrotranscribed (no RT) reactions were also prepared

from each sample to control for potential genomic DNA contamination. The cDNA and no RT controls were diluted and 5–10 ng of template cDNA from each sample was used to measure mRNA expression of selected genes by real-time quantitative PCR utilizing the MyIQ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). Triplicate measurements for each sample were run on standard iCycler 96-well plates, along with no template controls to detect potential cross contamination, in 20 μ l reaction volumes consisting of 10 μ l 2 $\times$ iQ Sybr Green Supermix (Bio-Rad, Hercules, CA, USA), 1 μ l of 0.2–0.5 μ M each primer, 3 μ l DEPC water, and 5 μ l of template. All quantitative PCR reactions included a melt curve analysis to ensure specificity of signal. Relative expression for each gene of interest was calculated by extrapolation to a standard curve individually run on each plate and derived from serial dilutions of a pooled sample of reference cDNA, and normalized to relative expression of reference genes (acidic ribosomal phosphoprotein 36B4 for gene expression in hypothalamic tissue, and mitochondrial ribosomal protein L32 for expression in the nucleus accumbens).

Primers

The following primer sequences (IDT, San Diego, CA, USA) were used to amplify rat preproorexin (Acc.# NM_013179), orexin-1 receptor (Acc.# NM_013064), orexin-2 receptor (Acc.# NM_013074), dopamine receptor D1A (Acc.# NM_012546), dopamine receptor D2 (Acc.# NM_012547), 36B4-Arbp (Acc.# NM_007475), and mrpL32 (Acc.# NM_001106116), respectively: *Preproorexin*: 5'-TTC CTT CTA CAA AGG TTC CCT-3', 5'-GCAACAGTTCGTAGAGACGGCAG-3'; *Orexin receptor 1*: 5'-CTCTGGTCTGCCTGGCTGTGTG-3', 5'-AAGGCATGGCCGAAGAGCCATGA-3'; *Orexin receptor 2* 5'-G T G C T T G T G A C C A T C A C C T G - 3', 5'-TGGCTGTGCTCTTGAACATC-3'; *Dopamine Receptor D1A*: 5'-CGAGGCTCCATCTCCAAGGACTG-3', 5'-CGGTGTCATAGTCCAATATGACCG-3'; *Dopamine Receptor 2*: 5'-ACCTCCCTTAAGACGATGAGCCGC-3', 5'-CAGCCAGCAGATGATGAACACACC-3'; *36B4-Arbp*: 5'-ATC CCT GAC GCA CCG CCG TG-3', 5'-GCG CAT CAT GGT GTT CTT GC-3'; and *mrpL32*: 5'-C A G A C G C A C C A T C G A A G T T A - 3', 5'-AGCCACAAAGGACGTGTTTC-3'.

Ethanol Intake

Rats were given access to a 10 % (v/v) ethanol solution and water in a two-bottle choice paradigm. Ethanol intake tests were conducted at 36 days (lean RYGB) or 90 days (obese RYGB). Rats received 24 h ethanol access on alternating days for 7–18 days. The access period for each rat began at the same time each day, always within 4 h of dark onset. Intake of ethanol and water was recorded at 15, 30, 60, 120 min, and 24 h.

Blood Alcohol

Thirty minutes following a 5-ml oral gavage of 10 % (v/v) ethanol, tail blood samples were collected into heparinized capillary tubes (Drummond Scientific Company, Broomall, PA, USA) and transferred to 2-ml gas chromatographic vials containing 200 μ l of an internal standard solution (0.6 N perchloric acid and 4 mM n-propanol in double distilled water). Vials were septum-sealed and stored at room temperature until analyzed by head space gas chromatography (Varian model 3900, Varian Medical Systems, Palo Alto, CA, USA) to determine blood alcohol content (BAC). Blood alcohol was measured from obese RYGB rats ($n=6$), sham surgical ($n=10$), and non-operated control rats ($n=5$) 110 days following surgery.

Plasma Ghrelin

Plasma acylated ghrelin levels were measured at 30 and 110 days in obese RYGB ($n=6$) and obese sham ($n=10$) rats following a 6-h fast. Rats for these experiments did not receive ethanol exposure prior to ghrelin measurement. Samples for the acyl-ghrelin assay were acidified with 1 N HCl at 100 μ l/ml, to confer further protection to this molecule. Plasma samples were assayed for acylated ghrelin using a commercially available ELISA (Linco Research, St. Charles, MO, USA; assay limit for acyl-ghrelin: 8 pg/ml, intra-assay coefficient of variance is 0.88–7.5 % with inter-assay variability of 7.5–12.9 %).

Statistics

Data were analyzed using STATISTICA 6.0 for Windows (StatSoft; Tulsa, OK, USA). The effects of RYGB surgery on metabolic parameters, ethanol exposure, blood alcohol, and transcriptional profiles were analyzed using analysis of variance (ANOVA) in combination with least significant differences post hoc comparisons to assess the source of significant main effects. The α level was set at 0.05 for all statistical comparisons.

Results

Body Weight

Consistent with previous reports [15], RYGB surgery produced significant decreases in body weight and body fat compared to animals receiving the sham surgery ($p<0.01$, Fig. 1a and b). Obese rats maintained on high fat diet lost an average of 134.3 g, or 23.1 %, of their initial body weight during the first 3 weeks following RYGB surgery (580.00 ± 14.28 presurgery; 446.00 ± 17.73 postsurgery) and maintained reduced weight levels relative to control animals throughout the remainder of the study, demonstrating the power of this procedure to elicit sustained alterations in body weight. During that same 3-week period, animals receiving sham surgery lost an average of 60.7 g or only 10.1 % of their initial body weight (601.7 ± 12.2 presurgery, 540.97 ± 15.0 postsurgery). Rats in the no-surgery condition did not significantly change their body weight during this period (600.5 ± 17.4 “pre-surgery”, 585.5 ± 20.8 “post-surgery”). One-way ANOVAs confirmed that there was no significant difference in initial body weight between these three conditions ($p=0.50$), but the post-surgical body weights of RYGB-treated rats were significantly lower than rats given either sham surgery or no surgery at the time of ethanol testing ($p<0.01$), which did not differ significantly from each other ($p=0.871$). Rats in the WL control group, which were compared to the lean-RYGB rats at the time of ethanol testing lost an average of 149.5 g, or 24.1 % of initial body weight, (620.1 ± 17.1 prerestriction, 470.5 ± 12.1 postrestriction) following the weight restriction regimen. Importantly, the WL rats lost similar amounts of body weight relative to RYGB-treated rats following surgery or the food restriction manipulation. In fact, the WL rats weighed significantly less than lean RYGB rats at the time that ethanol intake testing began ($p=0.002$). Lean rats maintained on the low-fat chow diet all gained weight during the 3-week postsurgical period ($F=19.3$, $p<0.01$), but the amount of weight gain did not differ based on surgical condition ($F=2.5$, $p=0.106$).

Ethanol Intake

Upon initial exposure to ethanol, obese rats that underwent the RYGB procedure consumed significantly more of a 10 % ethanol solution during 24-h access compared to control rats that received either a sham surgical procedure or no surgery ($p<0.01$, Fig. 2a). This effect occurred 90 days following surgery and was apparent during the first four exposures to the ethanol solution. Body weight loss subsequent to food restriction is a potent modulator of a variety of appetitive behaviors including food and drug intake [4, 16]. Thus, it is possible that the augmenting effects of RYGB surgery on ethanol consumption may be due to

changes in metabolic status associated with body weight loss. To test this possibility, we examined ethanol consumption in (1) WL control rats which experienced equivalent weight loss to the RYGB group without undergoing the surgical procedure and (2) rats that were lean prior to the RYGB procedure. In this experiment, all rats received ethanol 36 days following surgery. There was no effect of weight loss on ethanol intake in the WL group, while lean rats displayed increased ethanol intake following the RYGB procedure, but in the absence of weight loss ($p < 0.01$, Fig. 2b). Collectively, these results suggest that the effect of RYGB to increase ethanol intake occurs independent of negative metabolic status or other changes due to body weight loss. Furthermore, the impact of RYGB on ethanol consumption appears to be independent of dietary or metabolic factors prior to surgery.

Blood Alcohol Concentration

One possible, and simple, explanation for an increase in alcohol intake is that the surgical procedure may alter alcohol metabolism. Indeed, studies have demonstrated that blood/breath alcohol concentrations are elevated in RYGB patients following alcohol intake, suggesting that the procedure may indeed alter the clearance of blood alcohol [17, 18]. To determine if increased ethanol consumption following RYGB surgery was due to altered ethanol absorption in the present study, we measured BAC 30 min following oral gavage of ethanol. Rats that had undergone RYGB surgery (obese RYGB) displayed similar BAC compared to sham surgery and nonsurgery control rats (Fig. 3a) indicating that neither the surgical procedure nor the subsequent weight loss significantly influenced ethanol absorption.

Plasma Ghrelin

Pharmacological administration of the gut hormone ghrelin regulates ethanol intake, while disruption of endogenous ghrelin signaling interferes with ethanol reward in rodents [11] and changes in ghrelin secretion have been observed following bariatric surgery [9, 19, 20]. Thus, we assessed whether increased ethanol consumption observed following RYGB surgery may be associated with increased plasma ghrelin levels. Obese RYGB rats displayed increased fasting acyl-ghrelin levels 30 days following surgery relative to sham-operated rats (Fig. 3b). However, 110 days following surgery, acyl-ghrelin levels were decreased relative to the 30-day measurement and equivalent across groups (Fig. 3c). These data indicate that postsurgical alterations in ghrelin may be time-dependent and do not suggest a direct relationship between ghrelin levels and ethanol consumption.

Gene Expression

To investigate the molecular events in the central nervous system (CNS) that may regulate the effects of RYGB surgery on ethanol consumption, we profiled gene expression changes in the LH and nucleus accumbens of obese rats that received RYGB or sham surgery and weight-matched controls.

Lateral Hypothalamus

In the LH, RYGB surgery significantly increased prepro-orexin expression while orexin-2 receptor expression was increased in the WL condition relative to both RYGB and sham surgery conditions ($p < 0.05$, Fig. 4a and c). No significant effect of surgery or weight loss was observed on orexin-1 receptor, dopamine 1 (D1) receptor, or D2 receptor in the LH (Fig. 2b, d, and e). Recent evidence indicates that orexin peptides increase ethanol consumption in rodents [6]; thus, it is possible that the increased intake observed following RYGB may be regulated in part by increased central orexin tone.

Mesoaccumbens Dopamine Pathway (Nucleus Accumbens)

In the present study, RYGB surgery altered the transcription of genes that regulate dopamine signaling in the nucleus accumbens. Alcohol stimulates dopamine release in the nucleus accumbens of human subjects [21] and aberrant dopamine signaling within this region has been associated with the regulation of ethanol reward and dependence [22]. In the present study, RYGB surgery, but not weight loss induced in the absence of this surgery, led to decreases in dopamine type 1 receptor expression in the NAcc ($p < 0.001$, Fig. 5c), while no differences were seen in expression of the dopamine type 2 receptor (Fig. 2d). In the NAcc, increased levels of orexin 1, but not orexin 2, receptor mRNA were observed in WL animals relative to both sham and RYGB surgery groups ($p < 0.001$, Fig. 2a and b). Interestingly, dopamine signaling in the NAcc attenuates ethanol consumption [23] and recent reports indicate that orexins are capable of targeting NAcc neurons directly to modulate mesolimbic dopamine activity [24]. Together, these data suggest that RYGB induces transcriptional alterations within brain circuits known to regulate DA function, alcohol intake, and reward processes.

Discussion

The present work provides several insights into the effect of RYGB surgery on ethanol consumption. First, our data indicate that RYGB directly induces postsurgical increases in ethanol intake. Importantly, these results show that the ability of RYGB to modulate alcohol intake behavior is not merely a consequence of the significant weight loss induced in obese individuals following RYGB, nor is this effect dependent on pre-existing experience with a highly palatable food. Second, these data demonstrate that the RYGB procedure is associated with transcriptional alterations within metabolically responsive brain regions that regulate alcohol intake, and these expression patterns suggest possible roles for DA and orexin in mediating the observed changes in ethanol intake. Increases in the release of the gastric hormone ghrelin were seen, though ghrelin levels appear to fluctuate in a time-dependent fashion favoring high ghrelin levels at acute time point and low ghrelin levels at more distal time points following the RYGB procedure. Because we observed increased ethanol consumption at both acute and distal time points after surgery, the present data cannot specifically delineate the effects on plasma ghrelin on ETOH consumption. Collectively, these data demonstrate that RYGB is capable of yielding postsurgical effects on behavioral, neuroendocrine, and neurobiological endpoints related to voluntary alcohol intake.

Our behavioral results are consistent with clinical reports indicating that bariatric procedures increase the risk of alcohol consumption in human patients. In particular, RYGB surgery has been reported to lead to increases in alcohol intake, craving, and abuse following surgery [2, 3]. Two previous studies reported that RYGB patients display increased postconsumption blood or breath alcohol levels relative to control subjects, presumably yielding increased pharmacological effects of the drug, which may contribute to increased volume or frequency of intake [17, 18]. In our studies, however, the BAC of RYGB-treated rats did not differ from sham- or non-operated control rats 30 min following oral administration of an equal dose of ethanol. Notably, the studies in human subjects found that there were differences in rate of ethanol absorption, peak BAC, and recovery time [17, 18], while our studies were unable to assess these measures due to collecting blood for BAC at only a single time point. Although RYGB rats appeared to display elevated BAC 30 min following ethanol ingestion, this finding did not reach statistical significance. Thus, it is possible that measuring BAC at additional time points may identify more subtle alterations in alcohol processing following RYGB. In addition, it is also possible that recovery from surgery includes dynamic changes in the absorption of ETOH which cannot be assessed in the present studies.

Food restriction and weight loss are potent modulators of appetitive behaviors related to a variety of rewards, including drugs of abuse [16, 25], thus raising the possibility that increased alcohol consumption following bariatric surgery may be an effect of weight reduction. Our data suggest that weight loss alone cannot account for the increase in ethanol consumption. In the present study, rats that were obese prior to surgery and weight loss controls both lost significant amounts of body weight; however, only rats undergoing RYGB surgery consumed larger amounts of ethanol compared to rats that received sham surgery. Although rats were first exposed to ethanol following stabilization of body weight postsurgery and not during the active weight loss phase, considerable weight loss could have induced prolonged changes in neuroendocrine or other CNS systems which regulate ingestive or reward-related behaviors. Again, however, long-term changes due to weight loss alone would be expected to have an equivalent effect in both the obese rats that underwent RYGB surgery and their weight loss controls, yet changes in alcohol intake were observed only in the RYGB-treated animals. Procedurally, the weight-loss control group was tested at their weight nadir and at the end of the weight loss phase, while the obese RYGB group had undergone a period of weight regain prior to ethanol intake tests, resulting in lower body weights in the nonsurgical weight loss group. If weight loss alone was the causal factor increasing ethanol intake, this would predict a stronger effect in the weight loss control group than the surgical condition. However, our results indicate the opposite—no effect of dietary-induced weight loss on ethanol intake, but a significant increase following surgically induced weight loss, strongly implicating the RYGB procedure as the cause. Further support for this conclusion comes from the lean RYGB group, which consumed significantly more ethanol postsurgically in spite of having little to no change in body weight at the time of ethanol exposure. It is important to note here that we observe increased ethanol intake at two separate time points following surgery. Specifically, we observe increased ethanol intake at 36 days in our lean RYGB group and at 90 days in our obese RYGB group relative to sham control rats. Interestingly, clinical studies have only examined ethanol intake at more distal time points following recovery from the RYGB procedure. Nonetheless, these results support the contention that the RYGB procedure itself is capable of inducing changes in alcohol intake that are independent of the significant body weight loss which is the primary feature of this surgery.

It has been hypothesized that the beneficial effects of RYGB on body weight are dependent on bypassing hormonally active regions of the gut [9]. The gastric hormone ghrelin has received particular attention, given its ability to increase food intake and promote adiposity [26]. Initially, it was believed that dramatic and rapid postsurgical reductions in ghrelin were responsible for the observed weight loss, as well as decreases in reported hunger levels and food intake [9]. However, more recent results have been rather inconsistent with respect to the effect of RYGB on ghrelin secretion, with some indicating little change and others reporting decreased or increased plasma ghrelin levels [9, 10, 19, 20]. These discrepant findings could be attributed to the type of ghrelin measured (acyl vs. desacyl), or the time at which the measurement occurred following surgery. The assay we used here measured acyl-ghrelin which we validated using a variety of transgenic ghrelin and *GOAT null* animals. Acylated ghrelin is the active form of the molecule and is, therefore, a more meaningful indicator of ghrelin function than desacyl-ghrelin. Though it is difficult to directly compare postsurgical time points across species, it should be apparent that the type of ghrelin being measured and postoperative time of measurement may be critical variables to consider in regards to RYGB-induced alterations in plasma ghrelin. Notably, ghrelin has been shown to regulate ethanol consumption in rodents. Specifically, exogenous administration of ghrelin enhances alcohol intake, while pharmacological or genetic disruption of ghrelin signaling attenuates alcohol reward [12]. Thus, we hypothesized that postsurgical effect on ethanol consumption in RYGB rats may be mediated by changes in ghrelin activity.

We detected increased levels of acyl-ghrelin at 30 days postsurgery. However, 110 days following surgery, the time which closely approximated the observed increases in ethanol intake (90 days), acyl-ghrelin levels were decreased relative to the 30-day measurement and equivalent across surgical groups (Fig. 3c). The previously reported decreases in active ghrelin in rodents occurred at 3–4 months and approximately 9 months following the RYGB procedure [9, 10]. However, more recent data indicate that acyl-ghrelin levels are significantly increased at 9–12 months following RYGB and that the unacylated form of ghrelin may mask detection of acyl-ghrelin in some assays [27], while some report no change at all [28]. Our data indicate that RYGB surgery lead to acute increases in acyl-ghrelin which decrease at more distal time points following surgery. Taken together, these results indicate that ghrelin levels appear to fluctuate following the RYGB procedure, and the observed increase in ethanol consumption following RYGB, cannot solely be accounted for by altered ghrelin levels. However, given the variation in postsurgical ghrelin response reported here and in the literature, it is possible that ghrelin levels and/or ghrelin sensitivity may still mediate alcohol intake in some patient populations.

While examining gene expression patterns in the hypothalamus, we observed that RYGB rats, but not sham- or weight-matched controls, displayed increases in orexin expression in the LH. Although LH orexin neurons become activated following caloric restriction [29], the fact that expression was not changed in weight-matched, food-restricted animals in the absence of surgery, suggests that this is not solely a response to a caloric deficit, but rather a specific response to RYGB. The suggestion that increases in LH prepro-orexin expression may be playing a direct role in postsurgical ethanol consumption in the current study is consistent with previous demonstrations that administration of orexin directly to the LH increase alcohol drinking in rats [30]. Additional studies have further demonstrated the role of orexinergic signaling in alcohol-seeking behaviors and in reinstatement of alcohol drinking following extinction or abstinence [6, 31], which is in line with the current understanding of the role LH orexin plays in other drug-related behaviors [32]. However, systemic orexin antagonists did not affect ethanol-conditioned place preference in mice [33], indicating that this peptide may play a more significant role in the motivation to consume alcohol than in the rewarding effects of the drug.

In contrast to the effect of RYGB on prepro-orexin expression, no changes were observed in expression of either orexin 1 or orexin 2 receptor in response to the surgical manipulation. This supports the potential role of the orexin system in driving the increase in alcohol intake in these animals, as no compensation in the form of receptor downregulation occurred, indicating an overall increase in LH orexin tone in the LH relative to the sham surgery condition. Orexin 2 receptor expression in the LH was significantly increased in response to weight loss in the WL group, but not following RYGB surgery. Pharmacological antagonism of this receptor has been shown to attenuate alcohol drinking [34], yet these animals did not alter their consumption of ethanol in this experiment. It is possible that, while blockade of orexin 2 receptor activity reduces intake, the reverse, an increase in intake due to a greater receptor activity, does not occur. It may also be that a compensatory mechanism not measured here developed in these animals and that this counteracted any effect of increased receptor expression on alcohol intake.

Though human studies have yielded inconsistent results with respect to changes in striatal DA availability postsurgically [7, 35], we observed significant reductions in D1 receptor expression in the nucleus accumbens, part of the MADA pathway, in animals subjected to RYGB relative to sham surgery or weight-matched controls. These data also yielded a trend toward reduced D2 receptors in this region, although this did not reach significance. There is an established pathway between LH orexin neurons and the MADA pathway, and it is further known that orexins stimulate DA release in these regions [24, 36]. Based on this, we

speculate that increased levels of prepro-orexin in the LH following RYGB may contribute to increases in NAcc DA levels, which in turn may induce compensatory downregulation of D1 receptors. Behaviorally, reduced mesolimbic DA function is associated with increased subjective pleasure from novel drugs, increased levels of cue-induced craving for alcohol and other drugs, and increased alcohol intake in humans and nonhuman animals [37–39]. D2/D3 receptor levels are decreased in alcoholic subjects and studies demonstrating a lack of increase in receptor availability following dextoxification, along with familial studies assessing genetic variability associated with alcoholism, suggest that this is a predisposing factor for alcohol abuse [40–42]. This evidence supports the notion that the reduction of DA receptors in this region following RYGB surgery may play a substantial role in elevated ethanol intake.

It is known that highly palatable foods are capable of inducing acute neurobiological effects similar to those of drugs of abuse, including dopamine release and activation of brain regions in the MADA pathway [43, 44]. Accumulating evidence is providing support for the notion that chronic overeating has long-term effects in common with substance abuse as well [43, 44]. Specifically, both chronic intake of palatable food and long-term drug use lead to reductions in mesolimbic dopamine activity [42, 45]. As discussed above, this has been strongly associated with increased motivation for and intake of rewarding substances, including palatable foods and alcohol [38, 46, 47] and potentially contributing to a vicious cycle of reward-seeking and appetitive behaviors. Further, binge eating and alcohol abuse share a high comorbidity [48] and patients that display disordered feeding behavior share many common behavioral features with individuals that abuse drugs [49]. Based on this reasoning, it seems logical to propose that patients who were obese and likely overconsumed palatable foods prior to surgery may display excess alcohol intake following RYGB due to impairments in DA function induced by previous chronic overeating. This is supported by the alteration in D1 receptor expression in RYGB-treated rats that persisted weeks beyond the surgical manipulation and following significant weight loss and weight stabilization. However, other data presented here do not support this contention. First, lean animals that consumed, but did not overconsume, a low-fat, less preferred diet prior to RYGB also increased ethanol intake post-surgically. Second, WL controls, which also overconsumed the palatable diet, did not show reductions in dopamine receptor expression, nor did these animals increase ethanol intake. We interpret these findings to indicate that the surgical procedure itself is capable of altering both ethanol consumption and the MADA pathway, and that these effects are independent of a prior history of overeating or the diet composition. However, this interpretation does not exclude the potential for disordered feeding behavior to contribute to excess ethanol consumption following surgery.

In human patients, social factors may also mediate increased ethanol consumption following RYGB. For example, psychosocial characteristics such as depression, anxiety, quality of life, and self-esteem improve dramatically in the first year following RYGB surgery [50–53]. Importantly, social environment has been identified as a critical factor influencing alcohol consumption in adults [54]; thus, it is possible that changes in social milieu following surgery may initiate post-operative drinking behavior. In terms of continued alcohol use or abuse, it is reasonable to assume that individuals undergoing the RYGB procedure consume ethanol for its reinforcing properties. Both the MADA and orexin pathways appear to mediate the motivation to consume alcohol [55, 56]. Importantly, consumption and expectation of alcohol stimulate dopamine release in the MADA pathway and disrupted dopamine signaling is associated with withdrawal symptoms and relapse behavior [8, 22, 55, 57]. In the present study, RYGB surgery led to decreases in transcription of the D1 receptor. Decreases in dopamine signaling in the MADA pathway have been linked to increases in appetitive behaviors, as well as increased subjective pleasure from the use of DA-enhancing substances [37, 39, 47]. Specifically in relation to

alcohol ingestion, it has been suggested that decreased MADA dopamine may drive consumption of excess alcohol to stimulate and restore dopamine levels in abstinent alcoholic patients [8]. This reasoning leads us to the suggestion that there may be a variety of factors that initiate post-surgical alcohol use in human RYGB patients, but that that continued increases in drinking behaviors are likely due to a reduction in NAcc DA tone and an increase in LH orexin tone, which contribute to increased alcohol reward and alcohol seeking.

The present data demonstrate that the RYGB procedure can elicit postsurgical increases in alcohol consumption, and importantly, that this increased alcohol intake is dependent on the surgery itself, rather than postsurgical weight loss or presurgical diet history. Further, the current findings address the possibility that pronounced changes in brain regions that mediate both appetitive and consummatory behaviors may influence postsurgical ethanol consumption. These data are the first to experimentally demonstrate that enhanced ethanol consumption occurs following RYGB surgery and that this behavioral change is associated with altered neurobiology. Given the prevalence of obesity and the increase in the use of surgical treatment strategies, data which characterize the psychological outcomes of such a procedure are critical, as the identification of behavioral alterations which may place patients at further risk is essential for maximizing treatment efficacy and recovery following RYGB surgery.

Acknowledgments

This work was funded by a private research grant from Ethicon Endo-Surgery Inc.

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Fig 1. RYGB surgery decreases body weight and body fat. **a** RYGB surgery produced significant body weight loss and sustained reduction in body weight. **b** RYGB surgery also significantly reduced body adiposity. * $p < 0.05$

Fig 2.

RYGB surgery increases ethanol consumption. **a** High fat diet-induced obese rats that received RYGB surgery (obese RYGB) displayed augmented intake of a 10 % ethanol solution compared to obese sham or unoperated controls. **b** This effect was also apparent in rats that were lean and maintained on a low-fat diet prior to RYGB surgery (lean RYGB) who also consumed significantly more ethanol than sham or unoperated controls. WL rats that lost weight due to food restriction drank significantly less ethanol than lean RYGB rats but did not differ from controls in their level of intake

Fig 3.

RYGB surgery and plasma ghrelin levels. **a** Blood alcohol concentration was not significantly different in RYGB-treated compared to sham or unoperated LE rats 30 min following oral gavage of 10 % ethanol. **b** RYGB surgery led to increased plasma levels of acyl-ghrelin 30 days following surgery. **c** 110 days following RYGB surgery, plasma levels of acyl-ghrelin were decreased relative to the 30-day measurement and equivalent in RYGB and sham rats. * = $p < 0.01$ relative to sham-operated rats

Fig 4.

Post-RYGB expression of orexinergic and dopaminergic transcripts in the hypothalamus. **a** RYGB surgery led to increased hypothalamic expression of prepro-orexin. **b** There were no observable differences in expression of the orexin-1 receptor subtype, **c** however the orexin-2 receptor subtype was elevated in the WL animals compared to those receiving either sham or RYGB surgery. **d**, **e** RYGB surgery did not alter transcription of the dopamine-1 or dopamine-2 receptor in the hypothalamus. [#] $p < 0.01$ relative to sham-operated rats, ^{*} $p < 0.01$ relative to WL rats

Fig 5.

Post-RYGB expression of orexinergic and dopaminergic transcripts in the nucleus accumbens. **a** WL controls displayed elevated expression of the orexin-1 receptor in the nucleus accumbens, **b** but there were no effects on orexin-2 receptor expression. **c, d** The RYGB procedure led to a significant reduction in expression of the dopamine-1 receptor but no change in the level of the dopamine-2 receptor transcript in the nucleus accumbens.

* $p < 0.01$ relative to WL control rats