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Repeated Gastric Distension Alters Food Intake and Neuroendocrine Profiles in Rats

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Abstract

The consumption of a large food bolus leads to stomach distension. Gastric distension potently signals the termination of a meal by stimulating gastric mechanoreceptors and activating neuroendocrine circuitry. The ability to terminate a meal is altered in disorders such as bulimia nervosa (BN), binge-eating disorder (BED) and certain subtypes of obesity in which large quantities of food are frequently ingested. When a large meal is consumed, the stomach is rapidly stretched. We modeled this rapid distension of the stomach in order to determine if the neuroendocrine abnormalities present in these disorders, including increased gastric capacity, leptin dysregulation, and alterations in neuropeptide Y (NPY), and proopiomelanocortin (POMC) expression, were influenced by the rapid stretch aspect of repeatedly consuming a large meal.

To test the effects of repeated gastric distension (RGD) on neuroendocrine factors involved in energy homeostasis, a permanent intra-gastric balloon was implanted in rats, and briefly inflated daily for 4 weeks. Though body weights and daily food intakes remained equivalent in RGD and control rats, a significant delay in the onset of feeding was present during the first and second, but not the third and fourth weeks of inflations. Despite equivalent body weights and daily caloric consumption, RGD animals had significantly decreased leptin levels ($p < 0.05$), and tended to have increased fasting arcuate NPY levels ($p = 0.08$), which were suppressed more than control animals following food intake (control and RGD decreases from baseline were 184.95% and 257.42%, respectively). NPY expression in the nucleus of the solitary tract followed a similar pattern. These data demonstrate that the act of regularly distending the stomach can have effects on the regulation of energy balance that are independent from those related to caloric consumption, and may be related to disorders such as BN, BED, and certain types of obesity in which meal termination is impaired.

Keywords

Leptin; NPY; Arcuate Nucleus; Nucleus of the Solitary Tract; Gastric Distension; Binge Eating

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1. Introduction

In the developed world, there has been an increase in the number of overweight and obese individuals [1], as well as a high rate of individuals with binge-eating disorder (BED), and bulimia nervosa (BN) [2]. All of these conditions are defined in part by a marked overconsumption of food. Elevated caloric intake can be the result of an increase in either the number of eating episodes, or the volume or energy density of food consumed during each eating episode. There are numerous, well-characterized neuroendocrine factors involved in the onset, as well as the termination, of a meal (for review, see [3-5]).

One mechanism by which meal termination is thought to occur is via the distension of the stomach and subsequent activation of gastric mechanoreceptors. These mechanoreceptors transport their signal along the vagus nerve, and influence the initiation and termination of a meal by communicating the digestive state to the nucleus of the solitary tract (NTS) [6], which then relays the signal to other feeding-related areas of the brain, including the hypothalamus [7], and either activates or inhibits the orexigenic signaling based on the cumulative effect of the brain and gut inputs. This leads to a feeling of either hunger or fullness in the body, and an alteration in food intake [8].

Studies dating back over 50 years have demonstrated that the behavioral response to gastric distension nearly always includes a reduction in food intake [9, 10], whether this distension is due to ingestion of food [11], or acute intra-gastric balloon inflation. Normal weight humans exhibited a marked decrease in food intake after acute balloon inflation [12], and studies of obese humans who received chronic balloons as a weight-loss therapy lost weight during the first three months. The implantation of an intra-gastric balloon lacks clinical application, however, as these patients did not continue to lose weight over the one-year observation period [13]. Similarly, in rats, weight loss effects persisted for approximately six weeks following implantation of a permanently inflated gastric balloon, suggesting that animals do slowly adapt to a permanent intra-gastric bolus [14].

The combined effect of stomach distension and feeding-related hormones has been examined as a factor in the termination of a meal. Chronic balloon distension in obese humans led to a temporary, but marked decrease in levels of the hunger signal, ghrelin [15], and distension of both the fundus and corpus markedly increased c-fos expression of glucagon-like peptide 1/2 neurons in the rat NTS [16]. Gastric distension also lowers the threshold necessary for cholecystokinin (CCK)- [17] and leptin- [18] induced decreases in food intake. Both of these hormones increase activation of the vagus nerve in response to gastric distension [18, 19]; these effects occur more robustly with increased volume [20]. Collectively, these data indicate an important role for gastric distension in the complex network of neuroendocrine factors involved in meal termination and the control of food intake.

Though no differences in gastric accommodation [21] or food consumption in response to balloon inflation [22] have been observed in moderately obese individuals, there was a slight increase in the size of their distal stomach [21]. It is notable that obesity in these subjects was heterogeneous in origin; as obesity is a diverse condition with numerous etiologies, it is important to isolate specific behavioral patterns and physiological abnormalities, and analyze them as they pertain to their sub-type of the condition. Many individuals with obesity exhibit hyperphagia and increased latency to terminate a meal; this has been observed to be particularly acute in clinical populations with mutations to the melanocortin-4 receptor, and untreated leptin deficiency [23]. These problems with regulating meal duration are also present in disorders in which binge-eating, or the uncontrolled consumption of more food than is considered normal in a given situation, is a

diagnostic criteria. Individuals with BED have significantly larger gastric capacities than non-binging individuals [24]. Similarly, women with BN exhibit numerous gastric abnormalities, including increased gastric capacity [25], delayed gastric emptying, and a higher fullness threshold [26]. Compared to control individuals of a similar body weight, individuals with BN also have endocrine abnormalities, including elevated insulin levels [27], increased, yet stagnant ghrelin levels over the course of a meal [28, 29], and significantly decreased leptin levels [30]. Leptin levels in non-bulimic, normal-weight women were also diminished following maintenance on a “binge-style” diet, where the majority of daily calories were consumed in a single meal [31]. Individuals with BN are more likely than control individuals to have auto-antibodies against the proopiomelanocortin (POMC) product, α -melanocyte stimulating hormone (α -MSH), suggesting abnormalities with these neurons are present [32]. Additionally, neuropeptide Y (NPY) levels have also been observed to be high in individuals with BN, as compared to non-binge eating controls of similar weight [33]. Unfortunately, there is a paucity of data related to the change in these neuroendocrine factors over the course of a meal in individuals with BN and BED. There are also few studies of rapid food ingestion in which caloric consumption and stomach distension are investigated separately.

Given the dearth of experimental data surrounding the effects of repeated binge episodes, or the consumption of high-volume meals in general, as well as the interest in understanding the effects of previous gastric loads on future meal consumption, we evaluated the effects of repeated gastric distension (RGD), via permanent intra-gastric balloon, on multiple parameters involved in energy balance, including food intake, body weight, and pre- and post-prandial leptin, NPY, and POMC levels. Balloon distension has been used previously to study factors such as gastric capacity [25] and pain in the GI tract [34, 35], however, to the best of our knowledge, this is the first time that a chronic intra-gastric balloon has been used as a model for the gastric experiences that occur during chronic overeating or repeated cycles of binge eating.

2. Materials and Methods

2.1 Animals, housing, and surgeries

All procedures were conducted according to Purdue Animal Care and Use Committee (PACUC) guidelines. Seventy-five adult (225-249 g) male, Long Evans rats (Harlan, Indianapolis, IN) were maintained in plastic shoebox cages at 25° C on a 12:12 h light/dark cycle. All rats received *ad libitum* access to tap water throughout the duration of the experiment, as well as access to pelleted chow (diet #2018, Harlan Teklad, Indianapolis, IN) except where noted. Rats were handled and weighed daily.

Permanent, indwelling gastric balloons were constructed from a 2 cm long nitrile balloon and a piece of silastic tubing (ID: 0.025 in, OD: 0.047 in), by tying surgical suture around the balloon, and sealing the hole with silicone gel (adapted from [36]). A small nub was created just beyond the balloon so that the balloon would remain secured inside the stomach, and a small piece of surgical mesh was attached to the opposite end of the tubing, in order to secure it to the skin via back mount. The apparatus was thoroughly tested for leaks, and then sterilized with 70% isopropyl alcohol.

Balloon implantation occurred following a 1-2 week period of acclimation to the laboratory. Rats were food, but not water deprived at least 12 h prior to surgery. Animals were anesthetized with 1.2 mg/kg sodium pentobarbital (Nembutal), and abdominal and intra-scapular hair was removed and the area was sterilized with betadine. An anterior/posterior incision (approximately 4 cm in length) exposed the abdominal cavity. A small hole was made in the abdominal wall to access the stomach, after which a small perforation was

created in the fundus. A small volume of the stomach content was aspirated in order to avoid spillage during surgery. The balloon apparatus was inserted into the stomach, positioned so that it would not obstruct gastric function at either the pyloric or lower esophageal sphincter, and secured to the stomach with a purse string suture between the balloon and silicone gel nub (Figure 1). The tubing was then routed through the muscle wall, tunneled subcutaneously, and exteriorized through the back. All incisions were sutured using silk thread. Following surgery, rats received intramuscular injections of buprenorphine (0.01 mg/kg) and 3 mL lactated ringers' injection, and were placed in a tub cage atop an electric blanket. Rats were given access to moistened chow in addition to their regular chow for the day following surgery, and were allowed to recover to their pre-surgical body weight prior to testing. All animals were monitored for infection and weight loss.

Following surgery, rats were weight-matched and placed in either a control group (n = 32) or a RGD group (n = 36). Animals that developed infection over the course of the experiment (3 control and 1 RGD) or damaged their inflation systems (3 RGD) were excluded from data analyses. This attrition resulted in 29 rats in the control group, and 33 in the RGD group. Four additional rats received balloon implants and were used to determine inflationary volumes (INF).

2.2 Inflation of intra-gastric balloons

In order to assure that inflationary volumes were similar to the volume of food rats voluntarily consume after fasting, and to account for the natural growth of the stomach during the experimental period, INF rats were fasted for 18 h and presented with vanilla ensure for 30 minutes. The amount of ensure consumed was recorded, and the weekly averages were rounded to the nearest half mL, such that the weekly inflationary volumes were (from weeks 1-4, respectively) 3.0, 3.5, 4.0, and 5 mL. Thirty minutes prior to the onset of the dark cycle, the balloons of RGD rats were inflated with sterile 0.9% saline measuring approximately 25° C (Figure 2). This timing was chosen because rats consume low levels of food during the hours immediately preceding onset of the dark cycle; as such, the inflation of the balloons was more likely to be consistent between groups, due to decreased food within the stomach. Inflations lasted approximately 15 minutes, after which the saline was removed from the balloon. Control rats received a similar procedure, where the plug attached to their exteriorized tube was removed and replaced, however these rats did not experience any gastric inflation.

2.3 Food Intake Measurements

Food intake and spillage were weighed daily. In order to ensure that all animals were in a similar state of energy balance, food was removed 4 h prior to the onset of the dark cycle, and returned at lights-out (immediately following balloon inflations) on days in which cumulative food intakes were measured. Food and spillage were weighed at baseline, 30, 60, 90, and 120 m, as well as 24 h after the onset of the dark cycle.

2.4 Blood and Tissue Collection

On the day of sacrifice, food was removed from all rats 4 h prior to the onset of the dark cycle. For baseline measurements, 9 control and 11 RGD rats were sacrificed 30 m prior to lights out. At the onset of the dark cycle, food was returned all of the other rats, but no balloon distension occurred. In order to ascertain whether a post-prandial leptin alterations occurred in these rats, another cohort of rats (10 control, 11 RGD) were sacrificed 30 m following to lights out. Changes in NPY and POMC gene expression were analyzed via a third cohort of rats (10 control, 11 RGD), which were sacrificed 2 h following the onset of the dark cycle. Trunk blood was collected in order to measure plasma leptin; this was stored on ice in K⁺EDTA vacutainer tubes during collection, after which it was centrifuged at 2000

rpm for 15 m at 4°C. Plasma was then aspirated and stored at -80°C until further processing. Brains were removed and immediately submerged into iced isopentane for 25 seconds, and stored on dry ice until decapitations were completed. All blood and tissue were stored at -80°C until further processing took place.

After sacrifice, stomachs were observed for tearing, infection, or other deformities; no abnormalities were visible. Balloons were then removed from the stomachs and inflated to determine leakage. All balloons remained inflated during this test period.

2.5 Radioimmunoassay

Leptin was assayed using a commercially available RIA kit (Millipore, Billerica, MA). Duplicate samples of plasma were used, and the assay was performed as indicated by the manufacturers. A standard curve was derived from included tubes containing known quantities of the hormones; levels in plasma were calculated by comparing those percentages to that of the standard curve. The range of sensitivity was 0.5 ng/ml. The inter/intra assay variability for this kit was 3.0-5.7%/2.0-4.6%.

2.6 In situ hybridization

Brains were sectioned coronally at 14 μ m, and mounted onto electrostatically charged Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA), and stored at -80°C. Brain slices were then fixed with 4% paraformaldehyde and dehydrated with an ascending series of alcohols. Samples from each rat containing the arcuate nucleus of the hypothalamus and the NTS were selected and stored at -80°C for future processing. Plasmids of NPY and POMC that were linearized with the appropriate restriction enzymes were used. Antisense riboprobes were labeled with 35S-labeled UTP (Perkin Elmer, Waltham, MA), using in vitro transcription systems with appropriate polymerases (T3, and SP6 respectively), according to protocols provided by the manufacturer (Promega, Madison, WI). Probes were then purified using Quick Spin RNA columns (Roche Diagnostics, Indianapolis, IN).

For processing, slides were warmed and rinsed in triethylamine (TEA) buffer (pH 8.0) and TEA and acetic anhydride. Sections were incubated in hybridization buffer comprised of 50% formamide, 0.3 M NaCl, 10 mM Tris HCl (pH 8.0), 1 mM EDTA (pH 8.0), 1x Denhardt's solution (Eppendorf, Westbury, NY), 10% dextran sulfate, 10 mM DTT, 500 μ g/mL yeast tRNA, and 10⁸ cpm/ μ L 35S-UTP, and incubated overnight in a 56°C humid chamber. After hybridization, sections were washed three times in 56°C 2x SSC, then SSC + DTT, and treated with 20 μ g/mL RNase A (Sigma Aldrich, St. Louis, MO) in buffer containing 5 M NaCl, 5 M EDTA, 1 M Tris, pH 7.5 and ddH₂O. Sections were washed twice in 2XSSC + DTT, and then twice in 0.1XSSC + DTT, and dehydrated in an ascending series of alcohols. Slides were exposed to Kodak Biomax film for 2-3 days, after which autoradiographic images were scanned, and quantified with ImageJ software (National Institutes of Health), utilizing autoradiographic 14C-microscales (Amersham Pharmacia Biotech, Pittsburgh, PA) as a standard. Data for each animal were expressed as means of the product of hybridization area x density, with the background density subtracted from the three sections, reflecting the region-specific levels of gene expression. These levels were then normalized to controls and expressed as mean $\pm$ standard error (SE).

2.7 Statistical Analysis

In order to determine the effects of RGD on both hormone and gene expression, a completely randomized factorial design (CRF-pq) design was used in these experiments, with three sacrifice time points per experimental group. Analysis of factors insensitive to sacrifice time, including food intake and body weight, were collapsed by experimental group. Plasma leptin levels were also unaffected by time, and were therefore collapsed into

RGD and control groups. Daily food intakes, and change in body weights were analyzed via two-way analysis of variance (ANOVA). Post-hoc analyses were conducted via Tukey's Honestly Significant Difference test (Tukey's HSD). Weekly cumulative food intakes were analyzed at individual time points by planned two-tailed student's t-tests. Plasma leptin was analyzed via one-tailed student's t-test based on the *a priori* prediction that RGD rats, like humans engaged in binge eating, would have decreased leptin levels. Analysis of POMC and NPY gene expression were conducted by one-way ANOVA. Post-hoc analyses between groups were conducted using Tukey's HSD.

3. Results

3.1 Effects of repeated gastric distension on food intake and body weight measurements

Effects of RGD and time on body weight change, and daily food intakes are depicted in Figure 3. Two-way ANOVA revealed a significant main effect of time ($F_{[1, 20]} = 812.5$, $p < 0.001$) on body weight change, however no significant differences were detected between treatments. A small but significant main effect of time ($F_{[1, 29]} = 4.681$, $p < 0.001$) was also detected for daily caloric intake, indicating that all animals slowly increased their caloric intake over the course of the experiment. No significant effects of treatment on food intake were detected.

Cumulative food intakes over the course of a day were measured weekly at 0, 30, 60, 90, 120 and 240 minutes are illustrated in Figure 4. Student's t-tests revealed significantly lower cumulative food intake in the RGD group at 30 ($p < 0.0001$), 60 ($p < 0.001$), 90 ($p < 0.0001$), 120 ($p < 0.001$), and 240 ($p < 0.05$) minutes, but not at 24 hours ($p > 0.05$; see Figure 3) during week 1. During week 2, RGD intake was depressed at 30 ($p < 0.001$), 60 ($p < 0.01$), and 90 ($p < 0.05$) minutes, but not at 120 or 240 minutes, or 24 hours ($p > 0.05$). Significant differences between control and RGD groups were not detected at any time point during weeks 3 and 4 ($p > 0.05$ for all time points). No significant differences in food intake were detected on the day of sacrifice ($p > 0.05$).

3.2 Effect of repeated gastric distension on neuroendocrine parameters

Student's t-test revealed a decrease in leptin levels after RGD ($p < 0.05$) (Figure 5A). One-way ANOVA exposed a significant main effect of RGD on POMC expression in the arcuate nucleus (Figure 6A). ($F_{[3, 34]} = 3.078$, $p < 0.05$). Further analysis using Tukey's HSD revealed no significant between-group effects. A significant effect of time ($p < 0.05$) was revealed by combining RGD and control groups at baseline and 2h (Figure 6B). No significant effects on POMC expression in the NTS (data not shown) were found ($F_{[3, 35]} = 0.9258$, $p > 0.05$).

Figure 6C depicts arcuate NPY expression. A significant main effect was detected via one-way ANOVA ($F_{[3, 35]} = 16.47$, $p < 0.0001$). Individual contrasts were examined using Tukey's HSD; no significant differences between control and RGD groups were detected at baseline ($p > 0.05$), however the RGD group showed a trend towards higher NPY levels ($p = 0.0835$). Control animals ($p < 0.01$) and RGD animals ($p < 0.0001$) exhibited significantly depressed NPY following access to food. There were no differences between experimental groups at 2 hours ($p > 0.05$). NTS NPY expression (Figure 6D) follows a similar pattern to that in the arcuate nucleus; one-way ANOVA revealed a significant main effect ($F_{[3, 36]} = 3.411$, $p < 0.05$). Tukey's HSD revealed a significant decrease in NPY after food intake in the RGD group ($p < 0.05$); this effect did not reach significance in the control animals ($p = 0.0975$). No between-group differences were detected at either time point ($p > 0.05$).

4. Discussion

The effects of RGD on pre- and post-prandial POMC and NPY expression in the arcuate nucleus and NTS were examined. In the arcuate nucleus, POMC levels rose predictably following food intake for both control and RGD animals. POMC expression levels were low in the NTS, and no significant differences were detected in either group following food intake. Decreased plasma leptin levels, and trends towards increased baseline arcuate and NTS NPY mRNA expression were observed in animals exposed to RGD. This marginal elevation in baseline NPY following RGD may contribute to the hyperphagia that occurs in BN, BED, and certain types of obesity.

Following food intake, NPY expression declined to normal levels in RGD rats. This would imply that RGD leads to a change in feelings of hunger, but not satiety. It is perplexing that, despite this trend towards elevated NPY, animals do not show any differences in food intake patterns at the time of sacrifice, as compared to control animals. One possibility is that, following RGD, sensitivity to NPY is decreased. Though we have demonstrated no effect of RGD on POMC expression, it is possible that the relative decrease in NPY is leading to less inhibition of CART or MC4r activity, resulting in a greater feeling of satiety. One would expect a longer inter-meal interval if this were the case. As our measurements of food intake lacked the temporal resolution required to test this, further investigation is needed.

RGD animals had lower leptin levels, as compared to controls, but this reduction was unrelated to daily food intakes or body weights. It is difficult to ascertain whether the trend towards decreased leptin resulted from alterations in gastric or peripheral leptin. Since animals weighed the same and did not differ in cumulative daily food intakes, it is unlikely that they had increased adiposity; however, the possibility remains that RGD, or the altered food intake patterns resulting from RGD, led to an alteration in fat mass accumulation. Future studies will investigate this phenomenon via nuclear magnetic resonance to determine if and when overall adiposity is altered in RGD, and fat pads will be weighed upon sacrifice to determine any regional differences in fat accumulation.

If distension contributes to gastric leptin secretion, overstimulation of mechanoreceptors via RGD could lead to a desensitization of the secretory granules, and an elevated threshold for leptin secretion. Gastric leptin exerts at least some of its effects via the vagus nerve, as evidenced by a reduction in food intake in sham-operated but not vagotomized rats following a physiological dose of leptin [45]; it is likely that mechanoreceptors in the gut are one of its targets. If this is the case, it is possible that irregularities present in binge eating disorders, such as the higher threshold for fullness and increased gastric capacity, are due, at least in part, to alterations in gastric leptin. Given the want for knowledge pertaining to gastric leptin, more research is warranted to characterize this important hormone. Irrespective of its source, the RGD-induced leptin suppression despite similar body weights is consistent with the human literature. While it would be premature to suggest a causal role for RGD on plasma leptin levels, a relationship between the two should not be ruled out.

Regardless of treatment group, all rats exhibited similar growth patterns, and consumed equal amounts of food throughout the experimental period. Though unsurprising, given the transient nature of the inflations, this is in contrast to previous findings in rats and humans that have been given permanent, indwelling gastric balloons, in which total daily food intake levels and body weights were reduced for a period of time following implantation [13, 46]. The periodicity of feeding, however, was altered during the first two weeks of RGD, with experimental rats consuming their food at least an hour later than the control group. Many others have observed this phenomenon [47-49]. In the human literature, individuals reported an increased feeling of fullness after balloon distension, as well as a decreased desire to eat.

This effect was transient, and both human subjects and RGD rats consumed equivalent daily calories as controls [50]. Two possible explanations for this delay in meal-initiation are that the mechanoreceptors in the GI tract continue to signal that distension is occurring for a period of time beyond inflation, or that the signal that the stomach is no longer distended has not been relayed to the NTS or hypothalamic nuclei. A third possibility is that the mechanical distension of the stomach increased the efficacy of the chemoreceptors in the GI tract, thereby increasing the effect of even a small amount of food. Hormonal involvement is also likely, as both CCK [51] and leptin [52] are known to amplify the afferent signal resulting from gastric distension.

Interestingly, RGD rats resumed normal patterns of eating by weeks 3 and 4. This phenomenon is less well characterized, however it coincides with the cessation of weight loss in humans and animals with permanently inflated, indwelling gastric balloons [12, 46], as well as the increased fullness threshold, and higher tolerance for large balloon inflations in BN [26]. Given that leptin potentiates gastric distension sensitive neurons in the NTS under normal circumstances [52], it is possible that the leptin levels present in RGD animals are insufficient to amplify the distension-induced vagal afferent signal to threshold levels. This lack of modulation could eliminate the delay in meal initiation observed during the first two weeks of RGD. Studies of the change in neuroendocrine profiles and gene expression at time points when food intake patterns between RGD and control animals were discordant would provide insight into the cause of the meal pattern adaptation.

The vagus nerve is often associated with satiety mechanisms, including those derived from gastric distension. Selective and complete vagotomy led to a reduced or entirely eliminated ability to detect changes in gastric volume, and led to a transient increase in food intake for the first meal after surgery. Somewhat paradoxically, despite their inability to detect distension in the stomach; completely vagotomized animals consume fewer calories after 48 hours recovery [53], and exhibit an increase in arcuate NPY expression, and a decrease in serum leptin, which were not observed following splanchnectomy [54]. While it is extremely unlikely that the balloon implantation surgery drastically damaged the vagus (as control animals who received the same apparatus were unaffected), nor is it likely that the moderate degree of distension inflicted upon these animals led to gross changes to vagal morphology, it is possible that RGD led to a temporary desensitization of mechanoreceptors, and therefore influenced food intake.

Another explanation for this adjustment in meal patterns is that RGD differentially affects subpopulations of mechanoreceptors. For example, intramuscular arrays, which are involved in the gastric accommodation reflex, may be more active in RGD animals, perhaps as an adaptation to the repeated strain incurred during the inflationary periods. Intraganglionic laminar endings, which are responsible for distension-related satiety signaling [55], may exhibit a diminished response as a result of RGD, thereby elevating the threshold for fullness. Neural tracing studies may provide insight into both of these possibilities.

A fourth possibility is that RGD rats learn to dissociate gastric distension from energy consumption. The regulation of food intake is crucial for survival, and therefore remarkably plastic [56, 57]. Redundant systems are in place, and the manipulation of one often leads to a subsequent change in another. It is likely that the feeding-regulatory system has adapted, either via neuroendocrine feedback systems, or learning mechanisms, in order to utilize more reliable cues for caloric intake.

The goal of these experiments was to induce distension related to moderate overeating, rather than hyperdistension that could elicit feelings of malaise. Despite using a conservative volume to inflate the intra-gastric balloons, the possibility persists that the stomachs of RGD

rats were larger or more compliant than those of control rats by the end of the four-week period. Because volumes used to inflate the balloon were based on the amount of a liquid diet consumed by a non-RGD rat it is possible that, despite increases over time, the inflationary volume ceased to provide adequate distension. In order to eliminate this possibility, future inflationary levels will be based on pressure, rather than volume. Furthermore, in order to better represent the extremely large quantities of food typically ingested during binge episodes, and therefore better correlate studies of RGD with disorders such as BN and BED, animals with implanted balloons that have been trained to binge-eat [58] will be used to determine the inflationary volumes and levels of gastrointestinal pressure to be utilized later in the experiment. In addition, future studies will address possible effects of RGD on elements of gastric function, including gastric compliance and rate of emptying.

Since BN is a disorder that includes both binge-eating and compensatory behaviors, including self-induced vomiting, it is possible that the rat, which lacks the emetic reflex, may be an inappropriate model for such a disorder. Interestingly, we found a trend toward altered NPY expression in the caudal brainstem, a region associated with emesis in animals with that ability [59]. NPY itself has been demonstrated to occasionally induce an emetic response [60]; it is possible that NPY contributes to both binge-eating and subsequent purging. As other factors consistent with BN were observed in a non-vomiting animal, it is possible that gastric distension alters the relationship between food consumption and malaise-avoidance. Study of this relationship would be greatly enhanced by modifying the paradigm to work in an animal capable of vomiting, and would provide better insight into BN.

RGD, as implemented in the present set of experiments, induced alterations in food intake patterns, and influenced plasma leptin levels and NPY mRNA expression, without a noticeable impact on body weight, or total daily food intake. Since this paradigm uses a simple mechanism for manipulating the stomach, it avoids many of the confounding factors present in more naturalistic models of eating disorders and overconsumption of food, and may serve as a valuable complement to the human subjects research that is the current standard for the study of disorders in which binge-eating is a component.

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Highlights

- >Brief, daily bouts of gastric distension did not alter daily food intake or body weight.
- >Gastric distension altered food intake patterns for two weeks. >Four weeks of gastric distension led to decreased plasma leptin, and marginally increased NPY response.
- >These data may be related to exacerbation of symptoms in binge-eating disorders and chronic overeating.

Figure 1.

Diagram of the balloon apparatus used throughout this experiment. (1) Flexible nitrile balloon. (2) Silastic tubing tunneled through the skin and exteriorized intrascapularly. (3) Silicone gel bulges secure the balloon in place within the stomach. (4) Holes punctured in the end of the silastic tubing assure that the tubing does not suction to the wall of the balloon.

Figure 2.
Schematic of the balloon within the rat. At (A) the balloon is deflated, and the stomach remains undistended. At (B) the balloon is filled with saline, and the stomach stretches to accommodate the larger volume within it.

Figure 3. Body weight change over the course of the experiments. (A) Mean ($\pm$ SEM) change in body weights (g) in control animals and those subjected to repeated gastric distension (RGD). Surgeries were performed approx. 10 days prior to weight matching. (B) Mean ($\pm$ SEM) food intakes (g) in control animals and those subjected to repeated gastric distension (RGD).

Figure 4.

Food intake during the experiments. Mean ($\pm$ SEM) cumulative food intakes at 30, 60, 90, 120, and 240 minutes during weeks 1-4 (A-D, respectively) of RGD. Data are presented as mean $\pm$ SEM, and * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$, and **** indicates $p < 0.0001$.

Figure 5. Leptin alterations in response to RGD. Mean ($\pm$ SEM) plasma leptin levels were decreased in animals subjected to repeated gastric distension (RGD), as compared to controls. Data are presented as mean $\pm$ SEM, * indicates $p < 0.05$.

Figure 6.

Hypothalamic and NTS gene expression in response to RGD. (A) There was a significant main effect of RGD on POMC mRNA expression, however there were no between group differences. (B) A significant effect of time was revealed by combining RGD and control groups at baseline and 2h (C, D) Mean ($\pm$ SEM) neuropeptide Y (NPY) levels in the arcuate nucleus (C), and NTS (D) as a percentage of baseline values. In the arcuate nucleus, a significant main effect was present between baseline and 2h post food intake time points in RGD rats. NPY mRNA levels were lower at 2h for both control and RGD groups Data are mean $\pm$ SEM, * indicates $p < 0.05$, *** indicates $p < 0.001$.