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Disturbance of Gut Satiety Peptide in Purging Disorder

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Abstract

OBJECTIVE: Little is known about biological factors that contribute to purging after normal amounts of food – the central feature of Purging Disorder (PD). This study comes from a series of nested studies examining ingestive behaviors in bulimic syndromes and specifically evaluated the satiety peptide YY (PYY) and the hunger peptide ghrelin in women with PD (n=25), bulimia nervosa-purging (BNp) (n=26), and controls (n=26). Based on distinct subjective responses to a fixed meal in PD (Keel, Wolfe, Liddle, DeYoung, & Jimerson, 2007), we tested whether postprandial PYY response was significantly greater and ghrelin levels significantly lower in women with PD compared to controls and women with BNp. **METHOD:** Participants completed structured clinical interviews, self-report questionnaires, and laboratory assessments of gut peptide and subjective responses to a fixed meal. **RESULTS:** Women with PD demonstrated a significantly greater postprandial PYY response compared to women with BNp and controls, who did not differ significantly. PD women also endorsed significantly greater gastrointestinal distress, and PYY predicted gastrointestinal intestinal distress. Ghrelin levels were significantly greater in PD and BNp compared to controls, but did not differ significantly between eating disorders. Women with BNp endorsed significantly greater postprandial hunger, and ghrelin predicted hunger. **DISCUSSION:** PD is associated with a unique disturbance in PYY response. Findings contribute to growing evidence of physiological distinctions between PD and BNp. Future research should examine whether these distinctions account for differences in clinical presentation as this could inform the development of specific interventions for patients with PD.

KEY WORDS: Purging Disorder, Bulimia Nervosa, Gut Peptides, Physiology, Classification

Purging Disorder (PD) was added to the DSM-5 within Other Specified Feeding and Eating Disorder to account for a substantial number of women who fail to meet criteria for Bulimia Nervosa (BN) because they purge following normal amounts of food (Allen, Byrne, Oddy, & Crosby, 2013; Field et al., 2012; Stice, Marti, & Rohde, 2013; Swanson, Brown, Crosby, & Keel, 2014). Prevalence estimates indicate PD affects 0.5-0.9% of adolescent girls currently (Allen et al., 2013; Field et al., 2012; Stice et al., 2013) and 1%-5% of women over their lifetimes (Keel & Striegel-Moore, 2009). PD has been characterized as a “partial eating disorder” (Stice, Marti, Shaw, & Jaconis, 2009), and the ICD workgroup proposed revisions that could absorb PD into a broader definition of BN (Uher & Rutter, 2012) given that many individuals with PD experience a loss of control (LOC) after eating normal amounts of food (Keel, Haedt, & Edler, 2005). However, recent findings suggest that PD may be more than a subthreshold variant of BN. In the only longer-term follow-up study to date, PD demonstrated greater diagnostic perseverance compared to purging anorexia nervosa (AN) and higher mortality compared to BN purging (BNp) (Koch, Quadflieg, & Fichter, 2013). This underscores the need to identify factors that contribute to the distinct clinical presentation of this prevalent and serious eating disorder.

Previously, we identified lower postprandial cholecystokinin (CCK) and total glucagon-like peptide 1 (GLP-1) levels in BNp compared to PD and controls, who did not differ from each other (Dossat, Bodell, Williams, Eckel, & Keel, 2015; Keel et al., 2007). While findings validated distinguishing between BNp and PD, they failed to elucidate why individuals with PD after consuming normal quantities of food. Importantly, subjective responses to food intake provided clues to disturbances that might be unique to PD. Women with PD endorsed significantly greater postprandial fullness and gastrointestinal distress compared to women with BNp and controls 30 min after meal completion (Keel et al., 2007). Given the prior study’s focus on CCK, this represented the final post-meal assessment, leaving unclear whether biological

1 contributions to purging in PD may be better captured by factors that contribute to *satiety* rather
2 than *satiation*.

3 Although often used interchangeably, satiation and satiety are distinguished by function,
4 underlying physiology, and time course as demonstrated through experimental studies across
5 species (Blundell, 2006). Specifically, satiation contributes to meal termination while satiety
6 contributes to the delay before the onset of the next meal. Satiation factors, such as CCK and
7 GLP-1, peak 15-20 min postprandially and impact intra-meal interval whereas satiety factors,
8 such as peptide YY (PYY), peak 50-90 min postprandially and impact inter-meal interval
9 (Steinert et al., 2017). Given the timing of differences in fullness and gastrointestinal distress
10 (Keel et al., 2007) and effect of experimentally manipulating PYY on nausea (le Roux et al.,
11 2008), we hypothesized that postprandial PYY response would be exaggerated in PD compared
12 to BNp and controls.

13 PD participants also reported lower hunger compared to controls and women with BNp
14 pre- and postprandially (Keel et al., 2007), suggesting that ghrelin might be disrupted in PD.
15 Ghrelin is released from the stomach in fasting states and stimulates hunger and food intake. It
16 peaks after an overnight fast, falls precipitously after a meal, and then increases 2-3 hours
17 postprandially (Steinert et al., 2017). Experimental studies in animals and humans have
18 demonstrated that the active (acylated) form of ghrelin is responsible for its role in hunger and
19 food intake (Kojima et al., 1999; Kojima & Kangawa, 2010). Prior comparisons of BN and
20 healthy controls have found higher ghrelin concentrations in BN and have posited that this
21 contributes to excessive food intake during binge episodes (Kojima et al., 2005; Monteleone et
22 al., 2005). Based on subject reports of lower hunger in PD (Keel et al., 2007), we predicted PD
23 participants would have lower active (acylated) ghrelin levels compared to controls and women
24 with BNp. Further, we predicted that women with BNp would have higher ghrelin levels
25 compared to controls.

To test these hypotheses, we extended our gut peptide assessments to 120 minutes postprandially in PD, BNp, and control participants. In addition, we explored how gut peptides contributed to subjective responses and whether group differences remained in fullness, hunger, and gastrointestinal distress after accounting for gut peptide responses. Identification of factors uniquely linked to purging in the absence of binge eating may contribute to the development of novel interventions specific for PD. Such advances would both help those suffering from this disorder and support inclusion of PD as a full-threshold eating disorder in future editions of the DSM and ICD.

Methods

Data in this paper were obtained during a series of probes of ingestive behavior to distinguish factors that contribute to binge eating versus purging behaviors in bulimic syndromes.

Participants

Women (N=77), ages 18 to 45 years, were recruited from the community to participate in a four-visit study that was initiated in Iowa City, IA and then transferred after one year to Tallahassee, FL, where recruitment continued for four years. Reflecting this, 13 participants (17%) came from Iowa and 64 (83%) from Florida. The current paper focuses on women who completed all four study visits, representing 77 (81%) women who initiated the study. There was no significant association between study group and recruitment site ($\chi^2(2)=0.80$, $p=.67$) or study completion ($\chi^2(2)=0.27$, $p=.87$). Demographic and clinical variables did not differ significantly by recruitment site or study completion (see on-line supplement). Inclusion and exclusion criteria matched those used previously for controls (n=26), PD (n=25), and BNp (n=26) (Keel et al., 2007), with the following exceptions: 1) we set minimum behavioral symptom frequency at 1/week over the prior 3 months, reflecting DSM-5 BN criteria and PD research criteria (Keel & Striegel-Moore, 2009), and 2) PD and BNp were required to use self-induced vomiting and could not have histories of AN.

Inclusion criteria for all participants were body mass index (BMI) between 18.5 and 26.5 kg/m². In addition, participants had to be free of psychotropic medications for 8 weeks as well as medical conditions or treatments that might influence weight or appetite, could not be pregnant or nursing, and could not have a current major depressive episode or current substance dependence due to their potential influence on weight and appetite. With the exception of hormonal contraceptives, which were permitted for contraceptive purposes only, women had to be free of all medications, including over-the-counter medications, for 72 hours before their third and fourth study visits. Thirty-five (45%) women were on hormonal contraceptives, which showed no significant association with study group ($\chi^2(2)=1.37$, $p=.51$), or PYY ($p=.76$) or ghrelin ($p=.81$) values. Groups did not differ significantly on age (mean[SD] = 20.8[2.9] years; $F[2,74]=0.13$, $p=.88$), BMI (mean[SD] = 22.7 [2.0] kg/m²; $F[2, 74]=0.15$, $p=.86$), or ethnic/racial background ($\chi^2[6]=4.31$, $p=.63$). Racial/ethnic composition was 78% white, non-Hispanic, 10% African American, 5% Hispanic, and 7% Asian.

This study was reviewed and approved by the Human Subjects Committee of the IRB at both sites, and participants provided written informed consent prior to study participation. The informed consent document provided a description of all study risks. Participants were paid \$75, \$50, \$100, and \$100 across the 4 visits.

Procedure

The study protocol occurred over 4 days, with at least 48 hours between days to prevent carry over effects (Albibi & McCallum, 1983; Sanaka, Kuyama, & Yamanaka, 1998). During visit 1, participants completed a brief medical exam, the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) (First, Gibbons, Spitzer, Williams, & Benjamin, 1997), the Eating Disorders Examination (EDE) (Fairburn & Cooper, 1993), and self-report questionnaires (see Table 1) to establish eligibility and clinical characteristics. Interrater reliability was high in the current study ($\kappa=.90$ for EDE eating disorder diagnosis, $r>.97$ across EDE subscales, and $\kappa>.90$

for current and lifetime SCID diagnoses). Visit 2 established a behavioral indicator of satiation through an *ad lib* meal.

During visits 3 and 4, participants came to the lab between 7:30-8:00 a.m. after an overnight fast to complete the fixed meal assessment (Keel et al., 2007). An intravenous catheter was placed in their arm, they rested for 5 min and then were given a pill to consume with a small amount of water. The pill was either a placebo or 10 mg of metoclopramide, a medication which influences gastric emptying rate (Saleh & Lebowitz, 1980). In addition to examining PYY and ghrelin responses across groups, these visits evaluated gastric emptying as another mechanism linked to satiation. Data for the current report come from the placebo visit as this is the first study of PYY and ghrelin response in PD. There were no significant differences across groups in condition order (placebo vs. medication first), with 13 control (50%), 14 PD (56%), and 14 BNp (54%) completing the placebo condition during visit 3 ($\chi^2(2)=0.19$, $p=.91$), and no association between condition order and PYY ($p=.45$) or ghrelin values ($p=.15$). One hour after taking the pill, two fasting blood samples (5 mL) were drawn 10 min apart and averaged to measure preprandial gut-peptide concentrations. After the second fasting sample, participants consumed 900 kCal of Ensure Plus® (609 mL) over 5 minutes. In addition to replicating our prior methods, this test meal reliably produced lower CCK response in BNp compared to controls (Devlin et al., 1997). Moreover, 900 kCal is below the threshold for defining an objective binge episode (OBE) because it represents the upper limit of what controls consume in feeding lab studies (Mitchell, Crow, Peterson, Wonderlich, & Crosby, 1998). Postprandial blood draws occurred 10, 20, 30, 50, 90, and 120 min postprandially. After each sample, participants completed Visual Analog Scales (VAS) to capture momentary changes in feeling "Full," "Hungry," "Nausea," "Stomach Ache," "Urge to Vomit," and "Urge to Binge." We averaged nausea and stomach ache scores to create a composite gastrointestinal distress score ($\alpha=.85$)

Gut Peptide Assessment

Plasma samples for total PYY were collected and stored following instructions for a commercially available radioimmunoassay (RIA) kit (Millipore, St. Charles, MO; PYYT-66HK). Samples collected at the University of Iowa were assayed in the Iowa Clinical Translational Science Core Laboratory. Samples collected at FSU were assayed in a neuroscience laboratory directed by one of the authors (LAE). Plasma was separated from whole blood in a refrigerated centrifuge at 4°C and immediately pipetted into chilled tubes (2 mL aliquots) and then transferred to a -80°C freezer until assayed in batches. This procedure was updated when the study was moved to FSU to separate plasma into multiple microcentrifuge tubes (1.5 µL aliquots) prior to freezing. Samples were batched to include all of a participant's samples from study visits 3 and 4 in the same assay and to balance representation of diagnostic groups in each assay. PYY assay sensitivity was 10 pg/mL, and intra-assay and interassay coefficients of variation (CVs) were 5.3% and 7.0%, respectively.

Plasma samples for acylated ghrelin were collected and stored following instructions for a commercially available RIA kit (Millipore; GHRA-88HK), similar to methods described above with two exceptions. First, 150 µL of aprotinin (Sigma #A6279) was added to 3 mL of whole blood in chilled EDTA tubes to maximize stability of the analyte prior to centrifuging. Second, 50 µL of HCl (1.0 N solution) was immediately added to 1 mL of plasma after centrifuging. As with PYY, plasma samples at FSU were separated into 1.5 µL aliquots, and all samples were immediately transferred in dry ice to the -80° freezer. Due to poor performance of the commercially available RIA for acylated ghrelin at labs in Iowa and Florida, RIAs were discontinued and results are not used in analyses. Back-up samples collected at FSU which had never been thawed (N=64 participants; 83% of the total sample) were sent to the Ghrelin Assay Core Laboratory at the University of Virginia for analysis by two-site sandwich assay (Liu et al., 2008), with sensitivity of 6.7 pg/mL, intra-assay and interassay CVs of 9.1% and 17.8% for total

ghrelin. Given that samples were prepared solely for acylated ghrelin assay, we report acylated ghrelin results.

Data Analyses

Multi-level models captured within-subject changes in postprandial gut peptides (Level 1) and whether group (Level 2) predicted levels or moderated changes (Keel et al., 2007; Singer & Willett, 2003). Bayesian Information Criteria (BIC) were used to identify the best-fitting model. Our primary analyses focused on two outcomes: a group X time effect for PYY response and a group effect for ghrelin levels. Subsequent analyses of how gut peptides contributed to subjective responses were exploratory and hypothesis generating (Chmura Kraemer, 2017). Analyses were conducted in IBM SPSS Version 22.

Results

Clinical Characteristics

BNp participants consumed over 3,000 kCal whereas women with PD consumed less than 500 kcal, on average, during self-reported LOC eating (Table 1). BNp participants reported approximately 6 LOC episodes/week, including 3 OBEs. PD participants endorsed one LOC episode/week and no OBEs. On average, BNp and PD participants self-induced vomiting 5 times/week, fasted or excessively exercised 3-4 times/week, and used laxatives or diuretics once/week. There was a large, positive association between frequency of vomiting and LOC eating in BNp ($r(26)=.71$, $p<.001$) that was not observed in PD ($r(25)=-.13$, $p=.55$), and these associations differed significantly between groups ($Z=3.42$, $p<.001$). On both questionnaires and interviews, participants with PD and BNp demonstrated significant elevations on measures of eating pathology compared to controls, and participants with BNp endorsed significantly greater disinhibition, hunger, eating, weight, and shape concerns, and had significantly higher total EDE scores compared to PD participants. In contrast, PD and BNp participants did not differ significantly on dietary restraint. Finally, PD and BNp participants did not differ significantly on illness duration.

1 *Gut Peptide Responses*

2 Unconditional means and growth models indicated significant variability in PYY levels
3 and within-person change in PYY, respectively (Table 2). A full model was fit in which
4 recruitment site, RIA assay kit, and group were included as between-subject effects to
5 determine their influence on the intercept, slope (time), and rate of change (time-squared). The
6 final best-fitting model included a main effect of time, supporting a significant increase in PYY
7 values following consumption of the test meal, time-squared, indicating levels initially increased
8 and then decreased, and a significant group X time interaction. Women with PD demonstrated a
9 significantly greater postprandial increase in PYY compared to BNp and control participants,
10 who did not differ significantly from one another (see Figure 1a).

11 The best-fitting model for ghrelin (BIC=4705.69) showed a significant postprandial
12 decrease (Time: $b(SE)=-1.86(0.21)$, 95%CI=-2.28–-1.44, $p<.001$) followed by an increase
13 (Time-squared: $b(SE)=0.01(0.002)$, 95%CI=.008–.02, $p<.001$). The final model supported
14 significant variability across the assay runs in which acylated ghrelin concentrations were
15 measured ($F(8, 357.40)=7.51$, $p<.001$) and group differences ($F(2, 359.27)=8.69$, $p<.001$).
16 Compared to controls, PD and BNp participants demonstrated significantly greater ghrelin levels
17 over the assessment (PD: $b(SE)=29.89 (7.39)$, 95%CI=15.35–44.42, $p<.001$; BN: $b(SE)=20.43$
18 (7.14) , 95%CI=6.39–34.46, $p=.004$) but did not differ from one another ($b(SE)=9.46 (7.35)$,
19 95%CI=-4.98–23.91, $p=.20$) (see Figure 1b).

20 *Gut Peptides and Subject Responses Related to Purging*

21 Fullness increased significantly postprandially (Time: $b(SE)=1.03(.11)$, 95%CI=.81–1.25,
22 $p<.001$) and then decreased (Time-squared: $b(SE)=-.008(.001)$, 95%CI=-.001–-.006, $p<.001$).
23 The addition of PYY improved prediction of fullness (BIC=4046.06 vs. 4055.18) whereas ghrelin
24 did not. Higher PYY concentrations predicted greater fullness ($b(SE)=.11 (.03)$, 95%CI=.06–
25 .17, $p<.001$). Time ($b(SE)=.84(.12)$, 95%CI=.61–1.07, $p<.001$) and time-squared ($b(SE)=$

$-.007(.001)$, $95\%CI = -.008-.005$, $p < .001$) remained significant predictors. No other effects improved model fit.

Gastrointestinal distress increased following the meal (Time: $b(SE) = .17(.06)$, $95\%CI = .05-.29$, $p = .006$) and then subsided (Time-squared: $b(SE) = -.002(.0005)$, $95\%CI = -.003-.001$, $p < .001$). The addition of PYY improved prediction of gastrointestinal distress ($BIC = 3612.82$ vs. 3630.67), but ghrelin did not. Once PYY levels were included in the model, time was no longer significant, supporting PYY as a mediator (Bauer, Preacher, & Gil, 2006). The final model included the effects of PYY, group, time-squared, and the group X time-squared interaction. Higher levels of PYY predicted greater gastrointestinal distress ($b(SE) = .08(.02)$, $95\%CI = .05-.11$, $p < .001$), which abated with time (Time-squared: $b(SE) = -.001(.0002)$, $95\%CI = -.002-.0009$, $p < .001$). Post-hoc analyses revealed that PD women endorsed greater gastrointestinal distress compared to controls ($b(SE) = 30.12(5.55)$, $95\%CI = 19.06-41.18$, $p < .001$) and BNp ($b(SE) = 11.05(5.41)$, $95\%CI = .26-21.85$, $p = .04$). BNp women endorsed greater gastrointestinal distress than controls ($b(SE) = 19.06(5.33)$, $95\%CI = 8.43-29.70$, $p = .001$). Post-hoc analyses of the group X time-squared effect showed that controls experienced less fluctuation in gastrointestinal distress compared to women with PD ($b(SE) = .001(.0003)$, $95\%CI = .0002-.0007$, $p < .001$) and women with BNp ($b(SE) = .0008(.0003)$, $95\%CI = .0002-.001$, $p = .006$), who did not differ significantly ($b(SE) = -.0005(.0003)$, $95\%CI = -.0001-.001$, $p = .09$) (see Figure 2a).

Urge to vomit increased significantly following the test meal (time: $b(SE) = .44(.15)$, $95\%CI = .15-.73$, $p = .003$), and then decreased (time-squared: $b(SE) = -.004(.001)$, $95\%CI = -.007-.002$, $p < .001$). The addition of PYY improved model fit ($BIC = 3859.76$ vs. 3878.47), but ghrelin did not. In the final best-fitting model, there were significant effects for PYY ($b(SE) = .12(.02)$, $95\%CI = .07-.16$, $p < .001$) and time-squared ($b(SE) = -.004(.001)$, $95\%CI = -.005-.0004$, $p = .02$), and time was no longer significant ($b(SE) = .18(.15)$, $95\%CI = -.11-.48$, $p = .22$), suggesting that

changes in PYY statistically accounted for increases in urge to vomit following the test meal. In addition, there were significant effects for group, group X time, and group X time-squared. Women with PD had higher urges to vomit over the course of the test meal procedure (Group effect: $b(SE)=32.26(8.08)$, $95\%CI=16.23-48.28$, $p<.001$) and they demonstrated a greater change in rate (Group x time-squared effect: $b(SE)=-.004(.002)$, $95\%CI=-.001--.007$) compared to controls. In addition, women with BNp differed from controls on urge to vomit (Group: $b(SE)=25.07(7.71)$, $95\%CI=9.75-40.38$, $p=.002$), change in urge to vomit (Group x time: $b(SE)=.54(.18)$, $95\%CI=.17-.90$, $p=.004$), and rate of change in urge to vomit (Group x time-squared: $b(SE)=-.005(.001)$, $95\%CI=-.008--.002$, $p=.001$). With PYY included in the model, PD did not differ significantly from BNp on levels ($p=.36$), change in levels ($p=.34$), or rate of change in urges to vomit ($p=.56$).

Gut Peptides and Subjective Responses Related to Binge Eating

Hunger decreased significantly postprandially (Time: $b(SE)= -.92(.15)$, $95\%CI=-1.21--.64$, $p<.001$) and then increased over the two-hour period following the test meal (Time-squared: $b(SE)=.007(.001)$, $95\%CI=.0005-.009$, $p<.001$). The addition of both PYY and ghrelin improved model fit ($BIC=3767.75$ vs. 3788.95), with higher PYY contributing to lower hunger ($b(SE)=-.09(.02)$, $95\%CI=-.13--.05$, $p<.001$) and higher ghrelin contributing to higher hunger ($b(SE)=.06(.02)$, $95\%CI=.01-.10$, $p=.01$). Significant effects remained for time ($b(SE)=-.33(.12)$, $95\%CI=-.57--.10$, $p=.005$) and time squared ($b(SE)=.002(.001)$, $95\%CI=.0005-.004$, $p=.01$). Non-overlap in $95\%CIs$ for time effects between models, suggests gut peptides partially mediated time-related changes in hunger (Bauer et al., 2006). A significant group X time effect indicated that diagnostic group moderated changes in hunger, with BNp reporting less robust decreases in hunger compared to controls ($b(SE)=.45(.15)$, $95\%CI=.16-.75$, $p=.003$) but not PD ($b(SE)=.27(.15)$, $95\%CI=-.02-.57$, $p=.07$), who did not differ significantly from controls ($b(SE)=.17(.15)$, $95\%CI=-.49-.13$, $p=.26$) (see Figure 2b). Hunger demonstrated a flatter

trajectory over time in BNp compared to controls ($b(SE)=-.003(.001)$, $95\%CI=-.006--.0006$, $p=.016$) and PD ($b(SE)=-.003(.001)$, $95\%CI=-.005--.0001$, $p=.03$), who did not differ significantly ($b(SE)=.0004(.001)$, $95\%CI=-.002-.003$, $p=.75$) (Figure 2b).

For urge to binge, the best-fitting model ($BIC=4153.58$) included a significant effect of group. Women with BNp endorsed a significantly greater desire to binge throughout the test meal procedure compared to control women ($b(SE)=15.74(5.56)$, $95\%CI=4.63-26.84$, $p=.006$). PD did not differ significantly from BNp ($-6.54(5.64)$, $95\%CI=-17.81-4.72$, $p=.25$) or controls ($b(SE)=9.19(5.78)$, $95\%CI=2.35-20.73$, $p=.12$). No other variable improved model fit.

Discussion

Consistent with our hypothesis, women with PD demonstrated an exaggerated postprandial PYY response. Replicating prior results (Keel et al., 2007), they also experienced greater gastrointestinal distress compared to other groups. PYY predicted increased fullness, gastrointestinal distress, and urge to vomit. Prior studies of BN and BNp employing a similar test meal have found either lower (Kojima et al., 2005; Monteleone et al., 2005; Rigamonti et al., 2014) or no significant alteration in postprandial PYY response compared to healthy controls (Devlin et al., 2012), suggesting that exaggerated PYY response distinguishes PD from BNp. This is the first study to identify a specific biological dysregulation in PD. Future research should examine how these differences might contribute to urge to vomit in the absence of OBEs in PD. Importantly, given that group differences in postprandial gastrointestinal distress were not fully eliminated by controlling for PYY response, future work should expand the range of psychobiological factors evaluated. Findings challenge the ICD proposition that amount of food intake is unimportant among those who purge (Uher & Rutter, 2012) because biologically-based differences may indicate the need for different interventions.

Also consistent with one of our hypotheses, ghrelin was elevated in BNp compared to controls. However, contrary to hypotheses, ghrelin was also elevated in PD. Both ghrelin and

PYY contributed to hunger, and only BNp evidenced disruption in hunger responses. Previous studies have found higher ghrelin levels in BN compared to controls (Kojima et al., 2005; Monteleone et al., 2005), similar to our findings, or no significant difference (Devlin et al., 2012), potentially due to assay of total vs. active ghrelin following a smaller (600 kcal vs. 900 kcal) test meal in that study (Devlin et al., 2012). In this study, women with BNp maintained greater postprandial hunger compared to PD and control participants, consistent with their higher postprandial ghrelin. PD participants did not differ from controls on hunger, potentially reflecting the competing influences of elevated ghrelin and PYY in this group, as both contributed to prediction of hunger.

All groups demonstrated robust postprandial increases in fullness that were predicted by changes in PYY. Given that individual differences in PYY response contributed to individual differences in fullness and group differences in PYY response, the lack of group differences in fullness is somewhat surprising. It is possible that our reliance on a single-item assessment of fullness contributed to lower measurement reliability. In addition, fullness following ingestion of a large meal is influenced by factors beyond PYY, such as CCK, GLP-1, and gastric stretch receptor responses (Strader & Woods, 2005).

Integrating gut peptide findings across our studies of PD, BNp, and control women suggests a unique pattern of disruptions associated with different symptom configurations of BNp and PD. Compared to controls and a group who purges but does not binge, BNp was associated with blunted CCK and GLP-1 responses (Dossat et al., 2015; Keel et al., 2007). In addition, compared to controls, BNp demonstrated elevated ghrelin. Future research should examine whether a combination of satiation deficits and sustained hunger in BNp could drive large out-of-control binge episodes, which then trigger purging in this group. In contrast, PD was associated with intact CCK (Keel et al., 2007) and GLP-1 function (Dossat et al., 2015) but exaggerated PYY response which predicted greater postprandial distress and urge to vomit. Future work could test whether a combination of intact satiation but excessive satiety function in

1 PD explains normal food consumption that is experienced as excessive and then triggers
2 purging in this group.

3 Data in the current study are cross-sectional, precluding conclusions about direction of
4 associations. If excessive PYY response reflects a predisposing factor for purging in PD, then
5 pharmacological interventions to address the disruption of this peptide (Pucci et al., 2015) may
6 be useful for PD. If excessive PYY response is a consequence of purging that maintains this
7 behavior in PD, PYY may normalize with normalization of eating patterns prescribed in
8 cognitive-behavior therapy (CBT) (Fairburn et al., 2009). In CBT for BN, the therapist prescribes
9 a pattern of eating 3 meals and 2-3 snacks per day with the expectation that this will reduce
10 likelihood of binges and, as a consequence, purging. In PD, there are no binges to prevent.
11 Instead, supervised meals may help PD patients reinterpret stomach discomfort as a digestive
12 process that subsides over time without purging.

13 Findings should be interpreted in light of study limitations and strengths. Our sample is
14 less severe with regard to medical and psychological morbidity than patients presenting for
15 treatment due to our exclusion criteria. We excluded children, adults over the age of 45, men,
16 individuals outside of a healthy weight range, and with medical or other psychological conditions
17 that could influence results, which limit generalizability of findings. Our sample was not
18 completely medication free. We provided no specific instructions regarding use of laxatives or
19 diuretics on days prior to gut peptide assessment and do not know what effects such use may
20 have. We permitted use of hormonal contraceptives, and we did not control for menstrual cycle
21 day in analyses, which should be considered in future studies. The test meal was not adjusted
22 for BMI; however, participants were within a healthy weight range, and groups did not differ on
23 BMI. Moreover, the test meal we employed allowed us to replicate and extend findings from
24 prior studies using the same test meal (Devlin et al., 1997; Keel et al., 2007). Importantly, future
25 research is needed to examine differences between PD and AN, and such work should examine
26 the influences of BMI and weight history. Analyses of the influence of gut peptides and groups

on subjective responses were exploratory and not fully powered to test mediation. Finally, observed differences across groups cannot be interpreted as causal factors because disrupted physiological and subjective responses may be consequences of eating pathology. However, the nature of differences may serve as important maintenance factors for purging in PD and binge eating in BN. While identifying *causal* factors is crucial for identifying who will develop an illness, preventing illness, and developing a true cure, identifying *maintenance* factors is crucial for successfully treating a disorder after it has developed. Thus, findings remain relevant in pursuing a greater understanding of PD and its treatment.

Among study strengths, we utilized a design that eliminated or controlled common confounds. We had high retention, resulting in a final sample that was representative of those originally enrolled. We used both interview and self-report assessments with strong psychometric properties, ensured that our assays demonstrated accuracy and reliability, and employed sophisticated analytic methods. The use of multi-level models allowed us to both examine and account for variability across assay runs of peptides, and both this and balanced representation of study groups across assays minimized the influence of interassay variability on findings. *A priori* power analyses indicated that we would need 25 participants per group to test our primary hypotheses, and we have taken care to avoid over interpretation of findings from exploratory analyses (Chmura Kraemer, 2017). These strengths contributed to our ability to successfully identify differences among groups and increase likelihood of independent replication in future studies. Among group differences, we observed greater severity of illness in BNp compared to PD on measures of body image concerns, eating concerns, disinhibition and hunger. Yet disruptions in PYY response and GI distress were uniquely elevated in PD, suggesting that findings did not simply point to greater severity in one group compared to another.

In conclusion, a specific dysregulation in PYY response was found in PD. Importantly, data from clinical samples suggest that PD is the presenting problem for up to 1 in 10 adult

1 eating disorder patients (Rockert, Kaplan, & Olmsted, 2007) and 1 in 4 adolescent patients
2 (Binford & le Grange, 2005). Yet, no evidence-based treatments specific to PD exist. Future
3 studies are needed to replicate and extend current findings as well as examine their clinical
4 applications to help those seeking treatment for this serious condition (Keel & Striegel-Moore,
5 2009; Koch et al., 2013).

References

- Albib, R., & McCallum, R. W. (1983). Metoclopramide: pharmacology and clinical application. *Ann Intern Med*, 98(1), 86-95.
- Allen, K. L., Byrne, S. M., Oddy, W. H., & Crosby, R. D. (2013). DSM-IV-TR and DSM-5 eating disorders in adolescents: prevalence, stability, and psychosocial correlates in a population-based sample of male and female adolescents. *J Abnorm Psychol*, 122(3), 720-732. doi:10.1037/a0034004 2013-30852-009 [pii]
- Bauer, D. J., Preacher, K. J., & Gil, K. M. (2006). Conceptualizing and testing random indirect effects and moderated mediation in multilevel models: new procedures and recommendations. *Psychol Methods*, 11(2), 142-163. doi:2006-07641-002 [pii] 10.1037/1082-989X.11.2.142
- Binford, R. B., & le Grange, D. (2005). Adolescents with bulimia nervosa and eating disorder not otherwise specified-purging only. *Int J Eat Disord*, 38(2), 157-161.
- Blundell, J. E. (2006). Perspective on the central control of appetite. *Obesity (Silver Spring)*, 14 Suppl 4, 160S-163S.
- Chmura Kraemer, H. (2017). "Evidence"-based medicine in eating disorders research: The problem of "confetti p values". *Int J Eat Disord*. doi:10.1002/eat.22667
- Cooper, P., Taylor, M. J., Cooper, Z., & Fairburn, C. G. (1987). The development and validation of the body shape questionnaire. *Int J Eat Disord*, 6(4), 485-494.
- Devlin, M. J., Kissileff, H. R., Zimmerli, E. J., Samuels, F., Chen, B. E., Brown, A. J., . . . Walsh, B. T. (2012). Gastric emptying and symptoms of bulimia nervosa: effect of a prokinetic agent. *Physiol Behav*, 106(2), 238-242. doi:10.1016/j.physbeh.2012.02.009 S0031-9384(12)00076-5 [pii]
- Devlin, M. J., Walsh, B. T., Guss, J. L., Kissileff, H. R., Liddle, R. A., & Petkova, E. (1997). Postprandial cholecystokinin release and gastric emptying in patients with bulimia nervosa. *Am J Clin Nutr*, 65(1), 114-120.

- 1 Dossat, A. M., Bodell, L. P., Williams, D. L., Eckel, L. A., & Keel, P. K. (2015). Preliminary
2 examination of glucagon-like peptide-1 levels in women with purging disorder and
3 bulimia nervosa. *Int J Eat Disord*, 48(2), 199-205. doi:10.1002/eat.22264
- 4 Fairburn, C. G., & Cooper, Z. (1993). The Eating Disorder Examination. In C. Fairburn & G. T.
5 Wilson (Eds.), *Binge eating: Nature, assessment and treatment*. (12th ed., pp. 317-331).
6 New York: Guilford Press.
- 7 Fairburn, C. G., Cooper, Z., Doll, H. A., O'Connor, M. E., Bohn, K., Hawker, D. M., . . . Palmer,
8 R. L. (2009). Transdiagnostic cognitive-behavioral therapy for patients with eating
9 disorders: a two-site trial with 60-week follow-up. *Am J Psychiatry*, 166(3), 311-319.
10 doi:appi.ajp.2008.08040608 [pii] 10.1176/appi.ajp.2008.08040608
- 11 Field, A. E., Sonnevile, K. R., Micali, N., Crosby, R. D., Swanson, S. A., Laird, N. M., . . .
12 Horton, N. J. (2012). Prospective association of common eating disorders and adverse
13 outcomes. *Pediatrics*, 130(2), e289-295. doi:10.1542/peds.2011-3663 peds.2011-3663
14 [pii]
- 15 First, M., Gibbons, M., Spitzer, R. L., Williams, J. B. W., & Benjamin, L. S. (1997). *Structured*
16 *Clinical Interview for DSM-IV Axis II Personality Disorders (SCID-II)*. Washington, DC:
17 American Psychiatric Press.
- 18 Keel, P. K., Haedt, A., & Edler, C. (2005). Purging disorder: an ominous variant of bulimia
19 nervosa? *Int J Eat Disord*, 38(3), 191-199.
- 20 Keel, P. K., & Striegel-Moore, R. H. (2009). The validity and clinical utility of purging disorder. *Int*
21 *J Eat Disord*, 42(8), 706-719. doi:10.1002/eat.20718
- 22 Keel, P. K., Wolfe, B. E., Liddle, R. A., DeYoung, K. P., & Jimerson, D. C. (2007). Clinical
23 features and physiological response to a test meal in purging disorder and bulimia
24 nervosa. *Arch Gen Psychiatry*, 64, 1058-1066.

- 1 Koch, S., Quadflieg, N., & Fichter, M. (2013). Purging disorder: a comparison to established
2 eating disorders with purging behaviour. *Eur Eat Disord Rev*, 21(4), 265-275.
3 doi:10.1002/erv.2231
- 4 Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., & Kangawa, K. (1999). Ghrelin is a
5 growth-hormone-releasing acylated peptide from stomach. *Nature*, 402(6762), 656-660.
- 6 Kojima, M., & Kangawa, K. (2010). Ghrelin: more than endogenous growth hormone
7 secretagogue. *Ann N Y Acad Sci*, 1200, 140-148. doi:10.1111/j.1749-6632.2010.05516.x
8 NYAS5516 [pii]
- 9 Kojima, S., Nakahara, T., Nagai, N., Muranaga, T., Tanaka, M., Yasuhara, D., . . . Naruo, T.
10 (2005). Altered ghrelin and peptide YY responses to meals in bulimia nervosa. *Clin*
11 *Endocrinol (Oxf)*, 62(1), 74-78.
- 12 le Roux, C. W., Borg, C. M., Murphy, K. G., Vincent, R. P., Ghattei, M. A., & Bloom, S. R. (2008).
13 Supraphysiological doses of intravenous PYY3-36 cause nausea, but no additional
14 reduction in food intake. *Ann Clin Biochem*, 45(Pt 1), 93-95.
15 doi:10.1258/acb.2007.007068
- 16 Liu, J., Prudom, C. E., Nass, R., Pezzoli, S. S., Oliveri, M. C., Johnson, M. L., . . . Thorner, M.
17 O. (2008). Novel ghrelin assays provide evidence for independent regulation of ghrelin
18 acylation and secretion in healthy young men. *J Clin Endocrinol Metab*, 93(5), 1980-
19 1987. doi:10.1210/jc.2007-2235 jc.2007-2235 [pii]
- 20 Mitchell, J. E., Crow, S., Peterson, C. B., Wonderlich, S., & Crosby, R. D. (1998). Feeding
21 laboratory studies in patients with eating disorders: a review. *Int J Eat Disord*, 24(2),
22 115-124.
- 23 Monteleone, P., Martiadis, V., Rigamonti, A. E., Fabrazzo, M., Giordani, C., Muller, E. E., & Maj,
24 M. (2005). Investigation of peptide YY and ghrelin responses to a test meal in bulimia
25 nervosa. *Biol Psychiatry*, 57(8), 926-931.

- 1 Pucci, A., Cheung, W. H., Jones, J., Manning, S., Kingett, H., Adamo, M., . . . Batterham, R. L.
2 (2015). A case of severe anorexia, excessive weight loss and high peptide YY levels
3 after sleeve gastrectomy. *Endocrinol Diabetes Metab Case Rep*, 2015, 150020.
4 doi:10.1530/EDM-15-0020 EDM150020 [pii]
- 5 Rigamonti, A. E., Sartorio, A., Scognamiglio, P., Bini, S., Monteleone, A. M., Mastromo, D., . . .
6 Monteleone, P. (2014). Different effects of cholestyramine on postprandial secretions of
7 cholecystokinin and peptide YY in women with bulimia nervosa. *Neuropsychobiology*,
8 70(4), 228-234. doi:10.1159/000368160 000368160 [pii]
- 9 Rockert, W., Kaplan, A. S., & Olmsted, M. (2007). Eating disorder not otherwise specified: The
10 view from a tertiary care treatment center. *Int J Eat Disord*, 40, S99-S103.
- 11 Saleh, J. W., & Lebowitz, P. (1980). Metoclopramide-induced gastric emptying in patients with
12 anorexia nervosa. *Am J Gastroenterol*, 74(2), 127-132.
- 13 Sanaka, M., Kuyama, Y., & Yamanaka, M. (1998). Guide for judicious use of the paracetamol
14 absorption technique in a study of gastric emptying rate of liquids. *J Gastroenterol*,
15 33(6), 785-791.
- 16 Singer, J. D., & Willett, J. B. (2003). *Applied longitudinal data analysis: Modeling change and*
17 *event occurrence*. New York Oxford University Press.
- 18 Steinert, R. E., Feinle-Bisset, C., Asarian, L., Horowitz, M., Beglinger, C., & Geary, N. (2017).
19 Ghrelin, CCK, GLP-1, and PYY(3-36): Secretory Controls and Physiological Roles in
20 Eating and Glycemia in Health, Obesity, and After RYGB. *Physiol Rev*, 97(1), 411-463.
21 doi:10.1152/physrev.00031.2014 97/1/411 [pii]
- 22 Stice, E., Marti, C. N., & Rohde, P. (2013). Prevalence, incidence, impairment, and course of
23 the proposed DSM-5 eating disorder diagnoses in an 8-year prospective community
24 study of young women. *J Abnorm Psychol*, 122(2), 445-457. doi:10.1037/a0030679
25 2012-30031-001 [pii]

- 1 Stice, E., Marti, C. N., Shaw, H., & Jaconis, M. (2009). An 8-year longitudinal study of the
2 natural history of threshold, subthreshold, and partial eating disorders from a community
3 sample of adolescents. *J Abnorm Psychol*, 118(3), 587-597. doi:10.1037/a0016481
4 2009-12104-014 [pii]
- 5 Strader, A. D., & Woods, S. C. (2005). Gastrointestinal hormones and food intake.
6 *Gastroenterology*, 128(1), 175-191. doi:S0016508504019936 [pii]
- 7 Stunkard, A. J., & Messick, S. (1985). The three-factor eating questionnaire to measure dietary
8 restraint, disinhibition and hunger. *J Psychosom Res*, 29(1), 71-83.
- 9 Swanson, S. A., Brown, T. A., Crosby, R. D., & Keel, P. K. (2014). What are we missing? The
10 costs versus benefits of skip rule designs. *Int J Methods Psychiatr Res*, 23(4), 474-485.
11 doi:10.1002/mpr.1396
- 12 Uher, R., & Rutter, M. (2012). Classification of feeding and eating disorders: review of evidence
13 and proposals for ICD-11. *World Psychiatry*, 11(2), 80-92.

1 Table 1. Comparison of Groups on Eating Disorder Features

Measures (α reliability)	Control (n=26) Mean (SE)	PD (n=25) Mean (SE)	BNp (n=26) Mean (SE)	Mean (SE) Difference	95% CI	p
EDE Total (.96)	0.07 (0.14)	3.3 (0.14)	4.06 (0.14)			
Control vs. PD				-3.20 (.20)	-3.69, -2.71	<.001
Control vs. BNp				-3.99 (.20)	-4.47, -3.50	<.001
PD vs. BNp				-.79 (.20)	-1.28, -.30	.001
EDE Restraint (.88)	0.05 (0.21)	3.79 (0.22)	4.13 (0.21)			
Control vs. PD				-3.75 (.31)	-4.50, -3.00	<.001
Control vs. BNp				-4.08 (.30)	-4.83, -3.34	<.001
PD vs. BNp				-.34 (.31)	-1.09, .41	.81
EDE Eating Concern (.77)	0.00 (0.19)	1.37 (0.20)	2.73 (0.19)			
Control vs. PD				-1.37 (.27)	-2.04, -.70	<.001
Control vs. BNp				-2.73 (.27)	-3.40, -2.07	<.001
PD vs. BNp				-1.36 (.27)	-2.03, -.69	<.001
EDE Weight Concern (.89)	0.12 (0.17)	3.62 (0.17)	4.62 (0.17)			
Control vs. PD				-3.51 (.24)	-4.09, -2.92	<.001
Control vs. BNp				-4.50 (.24)	-5.07, -3.92	<.001
PD vs. BNp				-.99 (.24)	-1.58, -.41	<.001
EDE Shape Concern (.92)	0.10 (0.16)	3.62 (0.16)	4.35 (0.16)			
Control vs. PD				-3.52 (.22)	-4.06, -2.97	<.001
Control vs. BNp				-4.25 (.22)	-4.79, -3.71	<.001
PD vs. BNp				-.73 (.22)	-1.28, -.19	.005
TFEQ Restraint (.94)	3.43 (0.72)	16.42 (0.73)	16.62 (0.72)			
Control vs. PD				-12.99 (1.03)	-15.50, -10.47	<.001
Control vs. BNp				-13.18 (1.02)	-15.67, -10.70	<.001
PD vs. BNp				-.20 (1.03)	-2.71, 2.32	>.99
TFEQ Disinhibition (.90)	2.12 (0.53)	7.59 (0.54)	12.27 (0.53)			
Control vs. PD				-5.48 (.76)	-7.34, -3.62	<.001
Control vs. BNp				-10.15 (.75)	-12.00, -8.31	<.001
PD vs. BNp				-4.68 (.76)	-6.54, -2.82	<.001
TFEQ Hunger (.89)	2.62 (0.64)	5.73 (0.65)	9.14 (0.64)			
Control vs. PD				-3.11 (.91)	-5.35, -.88	.003
Control vs. BNp				-6.52 (.90)	-8.74, -4.31	<.001
PD vs. BNp				-3.41 (.91)	-5.64, -1.17	.001
BSQ (.99)	39.85 (4.21)	132.88 (4.29)	155.80 (4.21)			
Control vs. PD				-93.03 (6.01)	-107.75, -78.32	<.001
Control vs. BNp				-115.95 (5.95)	-130.52, -101.38	<.001
PD vs. BNp				-22.92 (6.01)	-37.63, -8.20	.001

Eating Disorder Symptoms		PD (n=25) Mean (SE)	BNp (n=26) Mean (SE)	Mean (SE) Difference	95% CI	p
Calories in LOC episode	-	486.84 (100.75)	3068.19 (265.21)	-2581.35 (283.71)	-3159.22, -2003.49	<.001
LOC episodes/week	-	1.13 (0.34)	6.39 (0.98)	-5.25 (1.03)	-7.36, -3.15	<.001
Vomiting/week	-	5.85 (0.94)	4.77 (0.65)	1.08 (1.14)	-1.21, 3.36	.35
Purging/week	-	6.04 (0.94)	5.56 (0.63)	.48 (1.12)	-1.78, 2.74	.67
Non-purging ICB/week	-	3.06 (0.67)	3.78 (0.61)	-.72 (.91)	-2.54, 1.10	.43
Duration of Illness (mos)	-	37.08 (4.91)	49.04 (8.82)	-11.96 (10.20)	-32.46, 8.55	.25

- 1 Note: EDE – Eating Disorders Examination; TFEQ – Three Factor Eating Questionnaire(Stunkard & Messick, 1985); BSQ – Body
2 Shape Questionnaire(Cooper, Taylor, Cooper, & Fairburn, 1987); LOC – Loss of Control; ICB – Inappropriate Compensatory
3 Behavior

1 Table 2. PYY Response to the Standardized Test Meal

Parameter	Unconditional Means Model		Unconditional Growth Model		Final Model	
<i>Conditional Likelihood</i>	b (SE)	95% CI	b (SE)	95% CI	b (SE)	95% CI
Intercept	154.44 (5.38)***	143.72–165.14	138.79 (5.08)***	128.69–148.89	119.35 (5.26)***	108.92–129.78
Time (min)			0.35 (.05)***	.24-.45	1.91 (0.15)***	1.61–2.20
Time Squared					-.01 (0.001)***	-.013–.0009
Group X Time					Control: -.029 (.12)** BNp: -.027 (.12)* PD: 0.00 (.00) ^a	-.53–.048 -.51–.03 -
<i>Random Effects</i>	b (SE)		b (SE)		b (SE)	
Residual	1609.57 (107.31)***	1412.41–1834.24	1171.92 (83.86)***	1018.57–1348.36	885.38 (63.87)***	768.65–1019.85
Intercept	1990.46 (359.22)***	1397.45–2835.13	1597.41 (308.40)***	1094.16–2332.11	1608.98 (298.24)***	1118.85–2313.83
Time			0.12 (.04)***	.07–.22	0.12 (0.03)***	.06–.20
BIC	5578.50		5495.56		5396.24	
Deviance (Parameters)	5559.70 (3)		5464.23 (5)		5346.10 (8)	
Model Fit Change			$\chi^2(2)=95.47$ ***		$\chi^2(3)=118.13$ ***	

2 ***p<.001, **p<.01, *p<.05. ^aThe model sets this parameter to 0 to avoid redundancy. Group X Time: BN vs. Controls: b(SE)=.02(.12),
3 95%CI= -.22–.26, p=.88.
4

VAS Gastrointestinal Distress Ratings