

# Ovarian hormones and binge eating in bulimia nervosa

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## ABSTRACT

**Background.** Symptom fluctuation in bulimia nervosa (BN) is related to menstrual cycle phase. However, the relationship between bulimic symptoms and ovarian hormones (estrogens and progesterone) has not been examined directly in women with BN.

**Method.** Regularly menstruating women with DSM-IV BN ( $n=9$ ) and regularly menstruating controls ( $n=8$ ) collected hormone samples and recorded mood and bulimic symptoms daily for 35 consecutive days. Estradiol and progesterone were measured by radioimmunoassay. Within-subject analyses examined prospective longitudinal associations between changes in ovarian hormones and changes in binge frequency in women with BN. Analyses controlled for the possible influence of negative affect on binge frequency as well as the influence of progesterone when examining estradiol associations and the influence of estradiol when examining progesterone associations. Between-subject analyses examined whether women with BN were more likely to have disrupted hormonal profiles than controls.

**Results.** Increases in binge eating were significantly associated with both decreases in estradiol and increases in progesterone in BN women with intact menstrual cycles. Although BN women were more likely to have disrupted hormone profiles than controls, this difference did not reach statistical significance, and mean estradiol and progesterone levels did not differ between bulimic and control groups.

**Conclusions.** The results are consistent with those from experimental animal studies and suggest that decreases in estradiol and increases in progesterone may contribute to increases in binge eating. Ovarian hormone function represents a promising candidate for unraveling the neurobiological mechanisms of binge eating.

## INTRODUCTION

Bulimia nervosa (BN) is defined by episodes of binge eating and inappropriate compensatory behavior that occur, on average, twice a week over a 3-month period (APA, 2000). However, many women with BN report significant fluctuations in symptom frequency in which they alternate between 'good' periods, when they are free of binge/purge episodes, and 'bad' periods of high symptom frequency (Crowther &

Sherwood, 1997). Understanding factors that are related to symptom fluctuation within the course of BN may provide important leads for identifying etiological or maintenance factors for the disorder.

Previous research suggests that symptom fluctuation in BN is related to menstrual cycle phase. Specifically, studies have shown increased binge eating during the mid-luteal and premenstrual phases compared to the follicular and ovulatory phases (Gladis & Walsh, 1987; Price *et al.* 1987; Lester *et al.* 2003). Increased dysphoric mood represents one possible explanation for premenstrual exacerbation of bulimic

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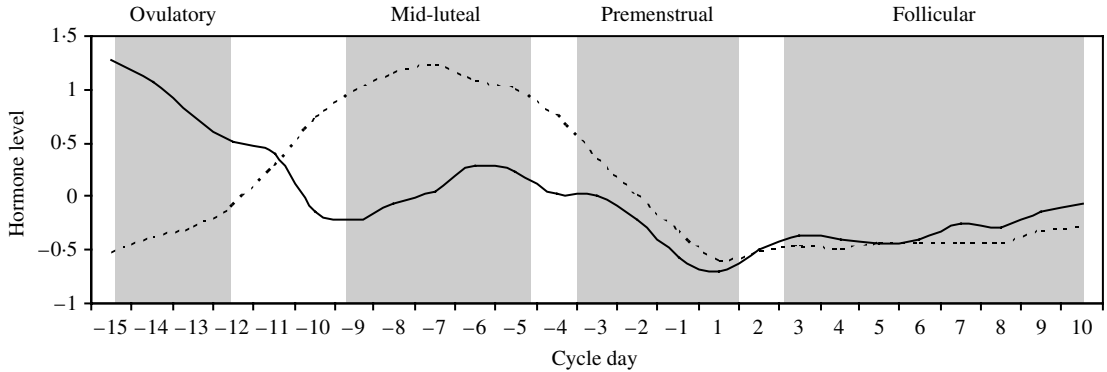


FIG. 1. Schematic diagram of relative changes in estradiol (—) and progesterone (---) levels across the menstrual cycle.

symptoms (Abraham & Beumont, 1982). However, studies have failed to support an association between changes in negative mood and mid-luteal/premenstrual exacerbations of binge eating (Leon *et al.* 1986; Gladis & Walsh, 1987; Lester *et al.* 2003). If changes in bulimic symptoms over the menstrual cycle are independent of mood, then symptom exacerbation may be directly related to cyclic changes in ovarian hormones.

The menstrual cycle is regulated by changes in ovarian hormones to prepare for conception, and each menstrual cycle phase is characterized by different levels of ovarian hormones (see Fig. 1). During the ovulatory phase (days -15 to -12, relative to the first day of menstruation, designated as day +1), a peak in estradiol leads to ovulation while progesterone remains low. During the mid-luteal phase (days -9 to -5), estradiol reaches a smaller, secondary peak and progesterone rises to its highest level. During the premenstrual phase (days -3 to +1), both estradiol and progesterone reach their nadir. During the follicular phase (days +3 to +10), estradiol begins to increase and progesterone remains low and constant. Exacerbation of binge eating seems to occur either when estradiol levels are low (i.e. the premenstrual phase) or when progesterone levels are high (i.e. the mid-luteal phase).

Experimental animal studies suggest that ovarian hormones regulate food intake. Food intake increases dramatically after bilateral ovariectomy, which depletes circulating estradiol and progesterone, and then decreases following

exogenous estradiol administration (Geary, 1997). This effect has been replicated in several species, including rhesus monkeys (Czaja & Goy, 1975). By contrast, progesterone has been shown to increase food intake in studies of non-human animals (Roberts *et al.* 1972; Ross & Zucker, 1974) and appears to influence food intake, in part, by antagonizing estradiol (Geary *et al.* 1994). Mirroring results from experimental animal studies, cyclic increases in food intake have been observed during the estrous and menstrual cycles of intact animals when estradiol levels are low or progesterone levels are high (Drewett, 1974; Kemnitz *et al.* 1989). Thus, menstrual cycle fluctuations in binge eating may reflect the influence of estrogen and progesterone on food intake in women with BN. Although previous studies have demonstrated clear associations between menstrual cycle phase and bulimic symptoms (Gladis & Walsh, 1987; Price *et al.* 1987; Lester *et al.* 2003), no previous study has directly assessed the association between changes in ovarian hormones and changes in binge eating over the course of the menstrual cycle in women with BN.

The current study examined relationships among ovarian hormones, estradiol and progesterone, and bulimic symptoms over the course of the menstrual cycle in women with BN reporting regular menses. In regularly menstruating women, fluctuations in ovarian hormones, specifically increases in estrogen before ovulation and increases in progesterone after ovulation, are dictated by the human reproductive system to prepare for conception.

Thus, changes in ovarian hormones that precede changes in binge eating probably reflect the impact of ovarian hormones on binge eating rather than the reverse. As such, a within-subject examination of these longitudinal associations represents a quasi-experimental design. Given the potential impact of disordered eating on menstrual function (Rock *et al.* 1996; Crow *et al.* 2002), we also compared ovarian hormone function in women with BN to non-eating disordered controls. This allowed us to assess the degree of hidden ovarian hormone disruption associated with BN. Hormone disruption was defined as the absence of a peak in estradiol 12–15 days prior to the onset of menstruation.

We formed three central hypotheses regarding the relationship between ovarian hormones and bulimic symptoms. First, decreases in estradiol would be associated with increases in binge eating. Second, increases in progesterone would be associated with increases in binge eating. Third, these associations would not be explained by concurrent changes in mood. Finally, we expected to find increased hormone disruption in women with BN compared to controls.

## METHOD

### Participants

Women with DSM-IV BN (APA, 2000), purging subtype ( $n=11$ ) and non-eating disordered control women ( $n=15$ ) were recruited from the community. Inclusion criteria were: age between 18 and 45 years, body mass index (BMI) between 19 and 25 kg/m<sup>2</sup>, menstruation every 22–35 days, and no use of psychotropic medication, hormonal contraceptives or hormonal supplements for 8 weeks prior to participation. Exclusion criteria were current pregnancy or lactation, or medical conditions/treatments that could influence appetite, weight or ability to complete study procedures. BN participants met DSM-IV criteria for BN determined by a structured clinical interview. The mean (s.d.) duration of illness in the BN group was 3.4 (2.9) years. Controls were required to have no lifetime history of eating disorder symptoms and no dietary restriction in the 8 weeks prior to study participation. Inclusion/exclusion criteria were assessed at the first study visit and during the course of data collection. Three control subjects met at least one exclusion criterion over

the course of data collection and were excluded from participation.† Four additional control participants collected saliva samples but did not collect blood spot samples. As a result, their data were included in the assessment of protocol feasibility but were excluded from comparisons to the BN group in order to match groups on assessment methods. In addition, two BN participants were not included in hormone analyses because one discontinued participation during data collection and the other did not adhere to the study protocol. The final number of subjects included nine women with BN and eight controls.

### Procedures

Written informed consent was obtained from all subjects before study participation. Participation involved two study visits separated by 5 weeks of at-home daily data collection. Study visits were conducted either in the laboratory or in participants' homes.

During the first study visit, participants completed questionnaires and an interview containing demographic questions and the Structured Clinical Interview for DSM-IV Axis I (SCID-I) eating disorders module (First *et al.* 1995). Participants' height and weight were measured with a wall-mounted tape measure and a digital scale. Participants removed their shoes, outerwear, and heavy objects from their pockets prior to weighing, but otherwise remained clothed for height and weight assessments. Participants were provided materials and training for hormone sample collection, a cooler for transporting samples, and a logbook for recording daily mood and eating behaviors.

We sought to compare the predictive ability of two types of hormone collection methods, saliva and blood spot collection. Shirtcliff *et al.* (2000) reported that hormone-behavior correlations were more robust when estradiol was measured in blood spots rather than saliva. However, participants in that study collected samples on only one occasion, and no information was provided regarding the type of behavior assessed. Salivary measurement of estradiol and progesterone specifically detects

† Of these participants, one reported experiencing three binge episodes; one did not menstruate during the course of data collection; and one reported an episode of vomiting associated with a migraine headache.

the unbound, biologically active form of the steroid, whereas blood spot measurement detects both unbound and bound steroid molecules (Shirtcliff *et al.* 2000). This distinction should favor salivary measurement for detecting hormone-behavior relationships because biologically active hormones are more likely to influence behavior, including food intake. Participants in this study were given a choice between collecting only saliva samples or both saliva and blood spot samples in order to determine the comparative predictive ability and feasibility of the two sample collection methods. Of the 11 eligible BN participants, nine collected saliva, and eight collected blood spot samples. Of the 12 eligible control participants, 12 collected saliva, and seven collected blood spot samples. Thus, feasibility was higher for saliva assessments compared to blood spot assessments (91% *v.* 65%; Wilcoxon ranked sign test  $Z = 2.45$ ,  $p = 0.014$ ).

Participants were instructed to collect 4 ml of saliva (indicated by a line on collection tubes) each morning for 35 days within 30 minutes of waking and before brushing teeth, eating, drinking, chewing or smoking. Participants also collected four blood spot samples each morning, obtained by finger prick with an automatic lancing device. Blood spot samples were stored in a freezer in participants' homes and were transported to the laboratory in a cooler. Each evening, participants recorded several mood and behavioral symptoms in a daily logbook. Biweekly telephone calls were used to ensure compliance with procedures, to answer questions, and to assess continued eligibility. Although research suggests no reliable diurnal variation in ovarian hormones between puberty and menopause (Choe *et al.* 1983), participants were asked to collect hormone samples in the morning so that hormones were sampled prior to that day's mood/behavior.

At the second study visit, participants returned completed materials and completed a second BULIT-R to provide a stable measure of eating pathology.

### Measures

The Beck Depression Inventory (BDI; Beck *et al.* 1961) is a 21-item measure with excellent internal consistency and good concurrent and discriminant validity in community and

psychiatric samples (Beck *et al.* 1961; Richter *et al.* 1998). The Barratt Impulsiveness Scale-11 (BIS-11; Patton *et al.* 1995) is a 30-item measure with good criterion validity and internal consistency across populations (Patton *et al.* 1995). The Bulimia Test-Revised (BULIT-R; Thelen & Farmer, 1991) is a 28-item measure that has been validated in clinical and non-clinical populations and has demonstrated high internal and test-retest reliability (Thelen & Farmer, 1991). The Three-Factor Eating Questionnaire (TFEQ; Stunkard & Messick, 1985) measures three dimensions of eating behavior: cognitive dietary restraint, disinhibition and hunger. The three factors have high test-retest reliability, internal consistency and criterion validity (Stunkard & Messick, 1985). The daily logbook (Lester *et al.* 2003) is a modified version of the premenstrual syndrome (PMS) diary (Thys-Jacobs *et al.* 1995) that includes questions about binge eating, purging, control over eating, and body dissatisfaction. The PMS diary negative affect scale has high internal consistency and good concurrent validity with the Menstrual Distress Questionnaire and Daily Rating Form (Thys-Jacobs *et al.* 1995), and the amended daily logbook demonstrated sensitivity to cyclic changes in bulimic symptoms (Lester *et al.* 2003).

### Assay protocol

Hormone assays were conducted by S.F.L. and included samples from one BN participant and one control participant in each assay. The apparatus used in the assay procedures allowed inclusion of up to 60 samples per assay. In order to include samples from one BN and one control cycle in each assay, five of the 35 samples from each participant were strategically eliminated based on self-reported onset of menstrual bleeding to ensure inclusion, if possible, of samples spanning an entire menstrual cycle.

Saliva samples were assayed for estradiol and progesterone using previously described methods (Jasienska *et al.* 2004). For the estradiol assay, the average intra- and interassay coefficients of variation (CVs) were 9% and 12% respectively. Estradiol assay sensitivity was 4 pmol/l. For the progesterone assay, the average intra- and interassay CVs were 10% and 12% respectively. Progesterone assay sensitivity was 13 pmol/l.

Blood spot samples were assayed using a modification of the protocol developed by Shirtcliff *et al.* (2000). Assay samples were prepared by eluting punches taken from two of the subject's blood spot samples in 0.5 ml assay buffer overnight. Estradiol measurements were made using an I-125 based radioimmunoassay kit (#39100, Diagnostic Systems Laboratories, Webster, TX, USA) with the following modifications to the manufacturer's protocol: tubes received either 20  $\mu$ l of standards or controls plus 180  $\mu$ l of buffer, or 200  $\mu$ l of sample eluate. Estradiol antiserum and I-125 reagent were diluted 1:4 with assay buffer and added to each tube in 100  $\mu$ l amounts to yield a total reaction volume of 400  $\mu$ l per tube. After overnight incubation, 0.5 ml of precipitating reagent was added to each tube. Tubes were subsequently centrifuged for 30 min; after aspiration of the supernatant, tubes were counted in a gamma counter for 2 min. Assay sensitivity was 1.5 pg/ml. Average intra- and interassay CVs were 6% and 15% respectively. Similarly, progesterone measurements were made using an I-125 based radioimmunoassay kit (#3400, Diagnostic Systems Laboratories) with the following modifications to the manufacturer's protocol: tubes received either 10  $\mu$ l of standards or controls plus 190  $\mu$ l of buffer, or 200  $\mu$ l of sample eluate. Progesterone antiserum (50  $\mu$ l) and I-125 reagent (150  $\mu$ l) were added to each tube to yield a total reaction volume of 400  $\mu$ l per tube. After overnight incubation, 0.5 ml of precipitating reagent was added to each tube. Tubes were subsequently centrifuged for 20 min; after aspiration of the supernatant, tubes were counted in a gamma counter for 2 min. Assay sensitivity was 0.3 ng/ml. Average intra- and inter-assay CVs were 4% and 7% respectively. Associations between salivary and blood spot assays for estradiol ( $r=0.28$ ) and progesterone ( $r=0.77$ ) were significant ( $p<0.001$ ), suggesting agreement between methods.

### Data analyses

Menstrual cycle days were assigned according to the self-reported first day of menstrual bleeding, as detailed by Lipson and Ellison (1994). The first day of menstrual bleeding was designated 'day +1' and the previous day was 'day -1'. Consistent with the methods of Lester *et al.* (2003), cycle-day analyses include

the 15 days before and 10 days after the onset of menstrual bleeding, a total of 25 days.

Four cycle phases were identified for each participant based on methods used by Lester *et al.* (2003) with two modifications. First, the follicular phase was truncated at day +7 to keep cycle phases approximately equal in length and to minimize the impact of cycle length variability among participants. Second, cycle phase designations were confirmed with estradiol and progesterone profiles for each participant. Hormone profiles were examined for a peak and subsequent drop in estradiol 12–18 days prior to the onset of menses and a peak in progesterone 5–9 days prior to onset of menses. This pattern indicates ovulation, a characteristic of a regular ovulatory cycle (Lipson & Ellison, 1996). Participants who displayed this pattern were considered to have an expected hormone profile. Those who did not display this pattern were considered to have a disrupted hormone profile, potentially indicative of an anovulatory cycle.

The daily logbook was used to determine the frequency of binge eating and purging in addition to the level of negative affect experienced on a given day. Negative affect was measured as a composite of the following symptoms: irritability, aggressiveness, impulsiveness, sad/depressed, stressed out, and mood instability. Mood symptoms were assessed on a Likert-type scale ranging from 0='none' to 3='severe'. Negative affect was computed as the sum of mood symptom scores. The average internal consistency of this composite score was  $\alpha=0.72$ .

Data analyses for this study replicated methods described by Lester *et al.* (2003). First, cycle-phase analyses were used to examine associations between bulimic symptoms and menstrual cycle phases. Mean symptom frequency was calculated for the days included in each cycle phase and then converted to a Z score based on the participant's overall mean and standard deviation. Second, we examined the association between ovarian hormone levels and binge eating using cycle-day analyses. Cycle-day analyses of binge eating and hormones were based on 5-day rolling averages, as described in Lester *et al.* (2003), that were converted to Z scores based on each participant's overall mean and standard deviation. Averaging symptoms over 5 days minimized random variation

Table 1. *Psychological and ovarian hormone characteristics*

|  | Control mean (s.d.)<br>( <i>n</i> = 8) | BN mean (s.d.)<br>( <i>n</i> = 9) | <i>t</i> , <i>df</i> | <i>p</i> | <i>d</i> |
|--|--|-----------------------------------|----------------------|----------|----------|
| Psychological measures                 |  |                                   |                      |          |          |
| BDI                                    | 1.00 (1.19)                            | 14.11 (6.73)                      | -5.74, 8.57          | <0.001   | -3.92    |
| BIS-11                                 | 51.09 (6.04)                           | 67.82 (9.61)                      | -4.35, 13.07         | 0.001    | -2.41    |
| BULIT-R <sup>a</sup>                   | 41.31 (5.35)                           | 124.35 (7.99)                     | -24.81, 15           | <0.001   | -12.81   |
| TFEQ total                             | 11.63 (5.83)                           | 39.75 (4.63)                      | -11.57, 15           | <0.001   | -5.97    |
| Cognitive restraint                    | 4.50 (3.16)                            | 16.56 (2.18)                      | -9.23, 15            | <0.001   | -4.77    |
| Disinhibition                          | 3.50 (1.69)                            | 13.52 (1.54)                      | -12.78, 15           | <0.001   | -6.60    |
| Hunger                                 | 3.63 (2.88)                            | 9.67 (3.00)                       | -4.23, 14            | 0.001    | -2.26    |
| Salivary hormone levels <sup>b</sup>   |  |                                   |                      |          |          |
| Estradiol (pmol/l)                     | 13.41 (8.77)                           | 7.64 (5.37)                       | 1.60, 11.34          | 0.14     | 0.95     |
| Progesterone (pmol/l)                  | 89.13 (26.9)                           | 74.08 (28.0)                      | 1.13, 15             | 0.28     | 0.58     |
| Blood spot hormone levels <sup>b</sup> |  |                                   |                      |          |          |
| Estradiol (pg/ml)                      | 23.88 (10.1)                           | 22.96 (7.76)                      | 0.20, 13             | 0.85     | 0.11     |
| Progesterone (ng/ml)                   | 3.51 (1.76)                            | 3.90 (2.44)                       | -0.35, 13            | 0.73     | -0.19    |

BN, bulimia nervosa; BDI, Beck Depression Inventory; BIS-11, Barratt Impulsiveness Scale-11; BULIT-R, Bulimia Test-Revised; TFEQ, Three-Factor Eating Questionnaire.

<sup>a</sup> BULIT-R scores represent the average taken from participants' first and second study visits. All other psychological measures reflect levels measured at baseline.

<sup>b</sup> Comparisons of hormone levels include *n* = 8 in each group.

due to environmental circumstances (Gladis & Walsh, 1987), reduced the influence of hormone pulsatility, and smoothed the pattern of hormone variability. Correlations between daily hormone levels and symptom frequency were calculated separately for each participant using Pearson's *r* over the 25-day collection period. Within-subject Pearson's *r* values were converted to Fisher *Z* scores, then averaged across subjects. The average Fisher *Z* score was then converted back to a Pearson's *r* value to yield an overall effect size (Rosenthal & Rosnow, 1991). One-tailed significance levels were converted to *Z* scores, and then assigned negative values if the direction of effect was opposite to the predicted direction. Adjusted *Z* scores were then summed and divided by the square root of the total number of observations. The resulting *Z* score was then converted to a *p* value to yield an overall significance level. Thus, the cycle-day results represent combined correlation effect sizes and significance levels from within-subject analyses. This approach has the advantage of providing readily interpretable effect sizes for associations among variables of interest. We confirmed results of cycle-day analyses by using linear mixed models in SPSS version 14.0 (SPSS Inc., Chicago, IL, USA) to run a multi-level model analysis (also known as hierarchical linear model analysis) (Singer & Willett, 2003).

Binge eating was used as an indicator of bulimic symptom severity in analyses both because the association between binge eating and purging was significant [ $r(9) = 0.96$ ,  $p < 0.001$ ] and because study hypotheses were informed by studies of food intake in non-human animals.

## RESULTS

There were no significant differences between BN and control participants in age, BMI, ethnicity, educational background, or marital status. The mean (s.d.) age of participants was 21.3 (4.5) years, and the mean (s.d.) BMI was 21.5 (1.8) kg/m<sup>2</sup>. The racial/ethnic composition of participants was 82.4% Caucasian and 17.6% Asian. Across participants, 5.9% had a high school education, 70.6% were enrolled in college, 11.8% had completed a 4-year college, and 11.7% had completed graduate school.

Mean scores on psychological measures and mean estradiol and progesterone levels measured in saliva and blood spot samples are presented in Table 1. As expected, BN participants showed significantly more depression, impulsivity and eating pathology than controls. Over the 5 weeks of data collection, the mean (s.d.) frequency of binge eating was 4.0 (2.3) episodes per week, and the mean (s.d.) frequency of purging was 5.2 (3.6) episodes per

week in BN participants. Although women with BN had lower mean levels of estradiol compared to controls, these levels did not differ significantly between groups. Examination of both saliva and blood spot data revealed that two of the nine BN participants (22%) and none of the controls had hidden hormone disruption; however, this did not reach statistical significance [ $\chi^2(1)=2.02$ ,  $p=0.16$ ]. BN participants ( $n=2$ ) who showed disrupted hormone patterns were excluded from cycle phase and cycle-day analyses because of an inability to confirm the presence of distinct cycle phases and to reduce the likelihood that hormone-behavior associations reflected the impact of disordered eating on ovarian hormone function.

Contrast analyses replicated findings of Lester *et al.* (2003) by supporting symptom exacerbation in both the mid-luteal and premenstrual phases compared to the follicular and ovulatory phases [ $F(3, 16)=9.87$ ,  $p=0.001$ ]. Mean (S.D.) Z scores for the average binge frequency across cycle phases were: ovulatory,  $-0.37$  (0.21); mid-luteal,  $0.61$  (0.33); premenstrual,  $-0.08$  (0.35); and follicular,  $-0.30$  (0.35). Frequency of purging showed the same pattern of results [ $F(3, 16)=14.04$ ,  $p<0.001$ ]. Negative affect did not differ across cycle phases in BN participants [ $F(3, 16)=0.92$ ,  $p=0.45$ ]. Thus, symptom fluctuation across cycle phases is not a result of premenstrual or cycle-related mood symptoms.

Within-subject cycle-day analyses showed significant positive correlations between negative affect and binge frequency ( $0.24$ ,  $p<0.01$ ). Thus, all analyses of ovarian hormones and binge frequency control for negative affect. In addition, analyses of estradiol control for progesterone and vice versa (see Table 2). Increases in binge frequency were associated with decreases in estradiol (see Fig. 2a) and increases in progesterone (see Fig. 2b). The results of cycle-day analyses in saliva were confirmed by in a linear mixed effect model in which negative affect [parameter estimate (S.E.)= $0.07$  (0.07),  $t(103.25)=3.24$ ,  $p=0.002$ ], estradiol [ $-0.13$  (0.05),  $t(142.89)=-2.82$ ,  $p=0.005$ ] and progesterone [ $0.15$  (0.04),  $t(142.55)=3.49$ ,  $p<0.001$ ] were all significant predictors of binge frequency. Similar results emerged for hormone values determined by blood spot, estradiol [ $-0.11$  (0.05),  $t(120.82)=-2.34$ ,  $p=0.02$ ] and progesterone [ $0.09$  (0.05),  $t(120.87)=2.04$ ,  $p=0.04$ ].

Table 2. Correlations between binge frequency and ovarian hormones in bulimia nervosa

|                       | df             | r value                |                           |
|-----------------------|----------------|------------------------|---------------------------|
|                       |                | Estradiol <sup>a</sup> | Progesterone <sup>b</sup> |
| Saliva – combined     | 6 <sup>c</sup> | $-0.25^{**}$           | $0.37^{***}$              |
| Subject 5             |                | $-0.45$                | $0.22$                    |
| Subject 11            |                | $-0.39$                | $0.63$                    |
| Subject 17            |                | $-0.56$                | $0.65$                    |
| Subject 28            |                | $-0.05$                | $-0.03$                   |
| Subject 41            |                | $0.31$                 | $0.34$                    |
| Subject 24            |                | $-0.25$                | $0.25$                    |
| Blood spot – combined | 6 <sup>c</sup> | $-0.18^*$              | $0.36^{***}$              |
| Subject 5             |                | $-0.60$                | $0.33$                    |
| Subject 11            |                | $-0.21$                | $0.72$                    |
| Subject 17            |                | $-0.004$               | $0.55$                    |
| Subject 28            |                | $-0.19$                | $0.12$                    |
| Subject 41            |                | $0.36$                 | $0.48$                    |
| Subject 36            |                | $-0.21$                | $0.07$                    |

<sup>a</sup> Values represent partial correlations between binge frequency and estradiol, controlling for progesterone and negative affect.

<sup>b</sup> Values represent partial correlations between binge frequency and progesterone, controlling for estradiol and negative affect.

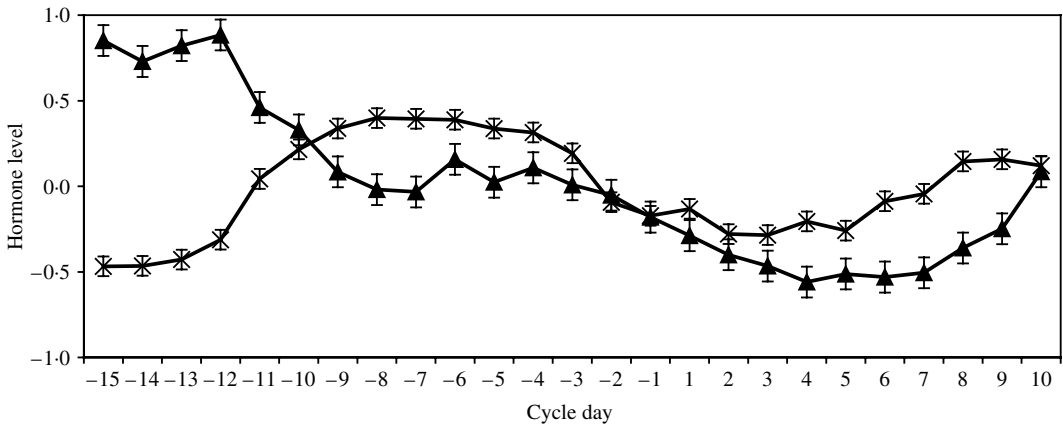
<sup>c</sup> While the number of participants is the same for saliva and blood spot samples, values are based on different participants. One participant (subject 24) elected not to collect blood spots. One participant (subject 36) demonstrated expected hormone values on blood spot but not saliva data. Results were the same when analyses were restricted to participants who collected both saliva and blood spot samples and showed expected menstrual function for both methods.

\*  $p<0.05$ , \*\*  $p<0.01$ , \*\*\*  $p<0.001$ .

Furthermore, direct comparison of correlation coefficients from cycle-day analyses indicated that the association between estradiol and binge frequency was stronger when estradiol was measured in saliva than in blood spots ( $p=0.008$ ), although no difference was found for progesterone.

To estimate the amount of variance in binge frequency accounted for by ovarian hormones, we conducted an exploratory hierarchical regression analysis for each participant. Negative affect was entered in the first block, and estradiol and progesterone were entered in the second block. Across participants, the average  $R^2$  for negative affect was  $0.10$  ( $p<0.01$ ), indicating that negative affect accounted for 10% of the variance in binge eating. By contrast, the average  $\Delta R^2$  associated with the addition of ovarian hormones to the model was  $0.24$  ( $p<0.001$ ). Thus, ovarian hormones accounted for 24% of the variance in binge eating after controlling for negative affect.

(a) Estradiol (▲) and binge frequency (×)



(b) Progesterone (○) and binge frequency (×)

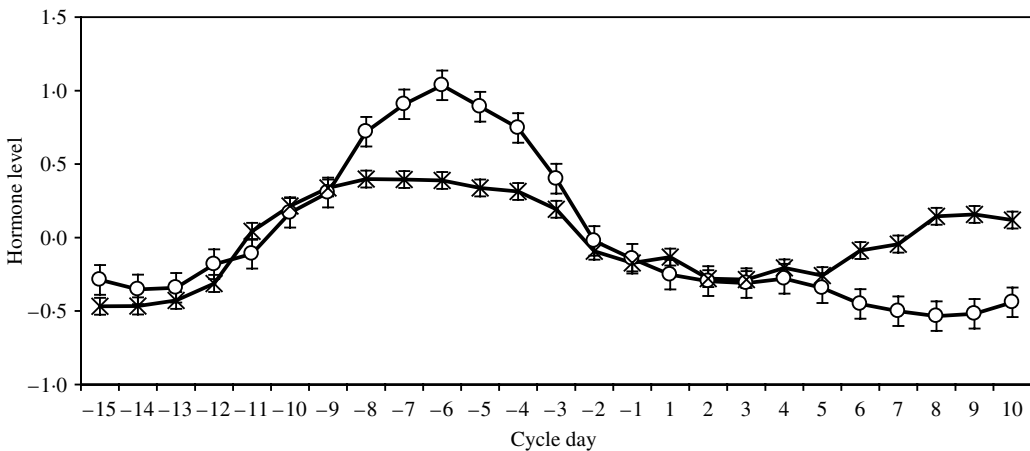


FIG. 2. Salivary ovarian hormones and binge frequency in BN participants with expected hormone profiles. Daily values represent 5-day rolling averages calculated within subjects, then averaged across subjects.

**DISCUSSION**

A significant negative association between estradiol and binge eating was found in bulimic participants with expected ovarian hormone patterns. Results also supported significant positive associations between progesterone and binge eating. Hormone effects were independent of changes in negative affect. Results confirm our a priori hypotheses and explain premenstrual and mid-luteal increases in binge frequency found in this and previous studies (Gladis & Walsh, 1987; Price *et al.* 1987; Lester *et al.* 2003). The significant positive association between negative mood and binge frequency is

consistent with previous research (Johnson & Larson, 1982). Across participants, negative mood accounted for 10% of the variance seen in binge frequency over the menstrual cycle. When estradiol and progesterone were added to regression models, the proportion of variance in binge frequency accounted for by the model increased substantially. To our knowledge, this is the first study to directly examine the association between ovarian hormones and bulimic symptoms over the menstrual cycle. The results are well supported by animals studies, which show that cyclic increases in food intake occur when estradiol decreases and when progesterone increases.



Ovarian hormones may influence binge eating by several mechanisms (Eckel, 2004), including cholecystokinin (CCK) and serotonin (5-HT) systems. Blunted CCK and 5-HT function have been widely studied as biological correlates and risk factors underlying bulimic symptoms (Blundell, 1986; Devlin *et al.* 1997; Steiger, 2004). Experimental animal studies show that the satiety effect of CCK is enhanced by cyclic increases in estradiol and is diminished when estradiol levels are low (Eckel & Geary, 1999). Estradiol has also been shown to decrease 5-HT reuptake (Koldzic-Zivanovic *et al.* 2004). Thus, estradiol has been shown to influence CCK and 5-HT function, and both have been shown to fluctuate over the menstrual cycle (Frick *et al.* 1990; Bethea *et al.* 1998). Future studies should examine both estradiol and progesterone as neurobiological mechanisms that contribute to binge eating.

Contrary to expectations, the proportion of participants experiencing hidden hormone disruption did not differ significantly between BN and control groups, and average ovarian hormone levels did not differ between groups. This probably reflects two design features. First, women in this study were required to be regularly menstruating. Second, the study was underpowered to detect between-group differences of medium and small effect sizes.

Results supplement findings of Shirtcliff *et al.* (2000) regarding the measurement of ovarian hormones in saliva *versus* blood. In the current study, participants preferred collecting saliva samples, and hormone-behavior correlations were more robust for estradiol when measured in salivary samples. Of note, the magnitude of associations between progesterone and binge eating did not differ meaningfully between salivary and blood spot assays, potentially because the ratio of bound *versus* free hormone levels in blood is much lower for progesterone than estradiol (Evans & Stewart, 1980; Choe *et al.* 1983). Thus, our findings support the use of salivary assessment for examining within-subject associations between ovarian hormones and disordered eating behaviors.

This study has several notable strengths. It is the first study to assess the association between changes in ovarian hormones and changes in binge frequency over the course of the menstrual cycle. This enabled us to confirm ovulation in

cycle phase analyses and examine whether mid-luteal/premenstrual exacerbations in bulimic symptoms observed by us and others (Gladis & Walsh, 1987; Price *et al.* 1987; Lester *et al.* 2003) can be explained by ovarian hormone levels. Analyses tested for the distinct effects of estradiol and progesterone as well as examining their combined influence in explaining changes in binge eating. This study also used and compared two different methods for assaying ovarian hormones, which represents a methodological contribution to research in this area. Finally, this study had a high retention rate; 91% of eligible participants completed data collection.

This study also has certain limitations. First, the sample size was small, and this limited our ability to test for differences in hormone disruption between controls and BN participants and warrants concern regarding generalizability of findings. Of note, a second study examining associations between ovarian hormones and a continuous measure of binge eating over the menstrual cycle in a non-clinical sample produced nearly identical results to those presented in the current paper (Culbert *et al.* 2006). The generalizability of findings may also be limited by inclusion criteria. Previous studies suggest that approximately 45–62% of women with BN experience irregular menses (Gendall *et al.* 2000; Crow *et al.* 2002). Thus, our findings may not generalize to women with BN with oligomenorrhea or amenorrhea. Nevertheless, the inclusion criterion of regular menstruation was necessary to ensure adequate variability of ovarian hormones within the 35-day collection period. In addition, comparisons between BN participants and controls on psychological measures indicate that our BN participants were representative on clinical variables. Finally, this study used a quasi-experimental, longitudinal design to test hypotheses about the influence of ovarian hormones on bulimic symptoms. True causality can only be established in an experimental study.

In conclusion, bulimic symptom fluctuation is related at least in part to the hormonal milieu of the female reproductive system. Previous research suggests that gonadal hormones may have both organizing and activating effects on the etiology of disordered eating behaviors (Klump *et al.* 2005). Our results further suggest that ovarian hormones represent a promising

candidate for examining the neurobiological bases of bulimic symptoms. One interesting question is whether absolute levels of circulating hormones or the change in levels over time is the crucial variable for understanding propensity to binge. Future studies could collect data that included at least two instances of non-linearity (i.e. ovulations) so that dynamical systems model analyses could be used to address this question (Boker & Nesselroade, 2002). Finally, increased knowledge of predictable increases in bulimic symptoms over the menstrual cycle may be used to enhance cognitive-behavioral therapy for BN by helping patients to identify biological as well as psychosocial triggers of binge eating.

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## DECLARATION OF INTEREST

None.

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