Objective: Premenstrual dysphoric disorder (PMDD) is associated with increased pain, but there has been a lack of well-controlled research assessing pain responsivity, sex hormones, and their relationships in this group. This study was designed to address this gap in the literature.

Materials and Methods: Healthy, regularly cycling participants (14 PMDD, 14 non-PMDD) attended pain testing sessions during the mid-follicular, ovulatory, and late-luteal phases of the menstrual cycle. Pain sensitivity was measured from electrocutaneous threshold/tolerance, ischemic threshold/tolerance, sensory and affective ratings of electrocutaneous and ischemic stimuli, and the nociceptive flexion reflex threshold (NFR, a measure of spinal nociception).

Results: Women with PMDD had higher sensory pain ratings of electrocutaneous stimuli and trends for lower ischemic thresholds, and the nociceptive flexion reflex threshold/tolerance, sensory and affective ratings of electrocutaneous and ischemic stimuli, and the nociceptive flexion reflex threshold (NFR, a measure of spinal nociception).

Discussion: Overall, women with PMDD may have a phase-independent hyperalgesia, with pain amplification likely occurring at the supraspinal level rather than the spinal level, given the lack of group differences in NFR threshold. Testosterone levels were also lower during the mid-follicular and ovulatory phases in PMDD. Correlations between pain outcomes and estradiol and testosterone indicated that these hormones are hypoalgesic, with estradiol having a greater hypoalgesic effect within the PMDD group.

Key Words: pain sensitivity, sex hormones, premenstrual dysphoric disorder, menstrual cycle, nociceptive flexion reflex

P

remenstrual dysphoric disorder (PMDD) is a cyclical syndrome characterized by debilitating affective, behavioral, and physical symptoms that occur during the late-luteal (premenstrual) phase of the menstrual cycle. Interestingly, pain processing may be disrupted in PMDD. Specifically, laboratory studies suggest PMDD is associated with lower ischemic pain thresholds/tolerances, in addition to greater ischemic, pressure, and cold pressor pain intensity ratings, when compared with healthy control women. Moreover, it appears this PMDD-related hyperalgesia may not be phase-dependent (ie, not during late-luteal phase only); rather, it persists throughout the menstrual cycle. These disturbances of pain processing may explain why clinical pain symptoms (eg, mastalgia, headaches, musculoskeletal pain) are among those that contribute to PMDD-related disability.

Although the specific mechanisms contributing to enhanced pain in PMDD are unknown, abnormalities in sex hormones may play a role. Supporting this is evidence that premenstrual symptoms are: (1) reduced by oophorectomy and use of ovulation inhibitors; and (2) absent during ovulatory cycles. Sex hormones are also related to pain processing in women without PMDD. For instance, a recent study from our laboratory found that testosterone was associated with hypoalgesia on several experimental pain outcomes in healthy women (Bartley et al, unpublished data). Several studies have also shown that testosterone plays a protective role in pain and inflammation in animals, and having androgen levels that are too low (especially testosterone) may promote chronic pain in both men and women. Although estradiol and progesterone appear to also influence pain in healthy women, the results are not consistent across studies. Some find that they are hypoalgesic and others find they are hyperalgesic. Although it is unclear as to why divergence exists across studies, methodological differences may account for these contradictory findings.

To our knowledge, only 1 study has examined the relationship between sex hormones and experimental pain in PMDD. Straneva et al assessed estradiol and ischemia pain thresholds/tolerances during the mid-follicular and late-luteal phases of the menstrual cycle and observed hyperalgesia in PMDD, but found no relationships between estradiol and pain outcomes. However, it is important to point out that their study assessed a single pain modality (ie, ischemia) and only examined estradiol. Further, they did not study pain during the ovulatory phase, which is associated with enhanced pain inhibition in women without PMDD. Thus, it is unclear whether a sex hormone/pain
Prospective Diagnoses of PMDD

The purpose of the current study was to examine the relationships between sex hormones (estradiol, progesterone, testosterone) and measures of experimental pain in women with and without PMDD during the mid-follicular, ovulatory, and late-luteal menstrual cycle phases. No directional hypotheses were made. Ongoing evidence for the effects of estradiol and progesterone, would be associated with phase-independent hyperalgesia sensitization at the spinal level. We predicted that PMDD be used to determine whether hyperalgesia stems from Assessment of NFR in PMDD is innovative because it can be used to determine whether hyperalgesia stems from sensitization at the spinal level. We predicted that PMDD would be associated with phase-independent hyperalgesia and that testosterone would be hypoalgesic. Given equivocal evidence for the effects of estradiol and progesterone, no directional hypotheses were made.

MATERIALS AND METHODS

Participants

Healthy women with PMDD (PMDD; n = 14) and healthy controls without PMDD (HC; n = 14) were recruited from the surrounding community by radio/newspaper advertisements, online advertisements, OB/GYN doctors, flyers, and email distribution. Participants were initially evaluated for exclusion criteria using a brief phone screen; however, a more thorough evaluation/interview was conducted during their initial session. Participants were excluded for: less than 18 years of age; menopausal or postmenopausal; use of hormone preparations in the last 6 months; failure to regularly cycle; hysterecomy; polycystic ovarian syndrome; endometriosis; pregnant or trying to become pregnant; pregnant in the last 6 months or currently breastfeeding; body mass index >35 (due to difficulty getting a nociceptive reflex in persons with high adiposity); history of cardiovascular, neuroendocrine, or neurological disorders; Raynaud disease; hypertension; history of, or current chronic pain; current opioid, anti-depressant, or anxiolytic medication use; apparent cognitive impairment; or current Axis I pathology defined by the DSM-IV-TR1 assessed from the Structured Clinical Interview for DSM-IV Axis I Disorders, Nonpatient Version, SCID-I/NP.21

Prospetive Diagnoses of PMDD

Confirmation of PMDD was based upon DSM-IV-TR1 diagnostic criteria for the disorder, which is defined as having ≥ 5 of the following 11 symptoms present for 2 consecutive symptomatic cycles: (1) depressed mood, hopelessness, or self-deprecating thoughts; (2) anxiety, tension; (3) affective lability; (4) persistent and marked anger or irritability or increased interpersonal conflicts; (5) decreased interest in usual activities; (6) difficulty concentrating; (7) lethargy, easy fatigability, or marked lack of energy; (8) change in appetite, overeating, or specific food cravings; (9) hypersomnia or insomnia; (10) overwhelmed or feeling out of control; or (11) physical symptoms. At least 1 symptom must be mood-related (eg, irritability, depressed mood) and symptoms must interfere with work, school, or relationships/usual social activities.

To provide a preliminary diagnosis of PMDD, a semistructured interview was conducted during the initial session. Diagnosis was then confirmed at the end of the study after reviewing daily symptoms reported on the Prospective Record of the Impact and Severity of Menstrual Symptoms (PRISM) calendar.23 The PRISM calendar was used to record daily affective (eg, depressed), behavioral (eg, insomnia), and physical (eg, breast tenderness) symptoms; lifestyle impact; life events; basal body temperature; and luteinizing hormone (LH) test results. Participants completed calendars daily for 3 consecutive menstrual cycles (during which pain testing occurred) for verification of cycle regularity and to prospectively diagnose PMDD. To discourage retrospective reporting, participants were asked to mail in calendars on a weekly basis.

Women in the PMDD group were required to meet the following criteria: (1) at least a 30% increase in ≥ 5 PMDD symptoms from the follicular phase (days 5 to 10) PRISM ratings to the luteal phase (1 to 6 d before menses) PRISM ratings2; (2) at least one of the symptoms had to be depressed mood, anxiety/tension, mood lability, anger, or irritability, and there had to be a symptom-free period during the follicular phase; (3) a 30% increase in functional impairment in work/school or social activities/relationships from the follicular to the luteal phase PRISM ratings, with lifestyle impact items rated at least “moderate” in intensity. Because of the brief nature of the study requirements (ie, completion of only 3 cycle calendars), participants were said to have PMDD as long as they met criteria for at least 2 of the 3 monitored cycles (even if they were non-consecutive). Women were considered as controls if they: (1) did not meet criteria for PMDD; (2) experienced only mild affective symptoms during the late-luteal phase (premenstrual) days; and (3) experienced only moderate physical symptoms on < 3 premenstrual days.3

Apparatus

A computer running LabVIEW software (National Instruments, Austin, TX) equipped with dual monitors and A/D board (National Instruments, PCI-6036E) controlled all stimuli, questionnaire presentation, and data acquisition. Physiological signals for NFR and experimental timing were monitored by an experimenter in an adjacent room by use of a 17-inch flat panel monitor. Questionnaires were presented by an LCD projector onto a large screen positioned approximately 2 m in front of the participant, and sound attenuating headphones and a video camera allowed the experimenter to communicate with and monitor the participant from the adjoining room. Electrocutaneous stimulations were delivered to the left ankle over the retromalleolar pathway of the sural nerve by use of a Digitimer stimulator (model D57A) and bipolar stimulating electrode (Nicolet, 019-401400, Madison, WI). A computer controlled the timing of the stimulations and the maximum stimulation intensity was set at 50 mA to ensure participant safety. Physiological signals for recording the NFR were amplified and filtered online using Grass Instruments Model 15LT amplifiers (with AC Modules 15A54 and DC Modules 15A12). A Lafayette Instrument Hand Dynamometer (Models 78010 and 78011, Lafayette, IN) and Prestige Medical Cuff (Northridge, CA) were used to assess ischemia pain.

Hormone Assessment

Urinary LH surge tests (Clearblue Easy; Swiss Precision Diagnostic, Bedford, United Kingdom) were conducted at home by participants to verify and identify the
timing of ovulation. The test provides a positive or negative reading for the LH surge and is over 99% accurate. Participants were asked to take a digital photo of each positive test and email it to experimenters immediately for verification of compliance and scheduling of testing sessions.

Saliva was collected on days of pain testing during the mid-follicular, ovulatory, and late-luteal phases. Salivary assessment was chosen over serum-based/plasma-based measurement as it represents a noninvasive and cost-effective medium for measuring sex hormone levels, and is reliable and relatively easy to collect.24-27 To avoid potential salivary contamination that could impact immunoassay, participants were asked to abstain from: (1) alcohol use 12 hours before sample collection; (2) eating a meal 60 minutes before testing; and (3) brushing their teeth 12 hours before sample collection. Approximately 10 minutes before sample collection, participants rinsed their mouths with water to eliminate any potential contaminants in their saliva. Per specifications of Salimetrics LLC (State College, PA),28 participants were initially asked to allow saliva to pool in their mouth. Then, they were required to passively salivate down a 2-inch straw that was placed into a plastic test tube. Samples were refrigerated within 30 minutes after collection and stored in a freezer kept at or below −20°C. Samples were shipped on dry ice to Salimetrics LLC for analysis of estradiol, progesterone, and unbound testosterone levels. On the day the samples were to be assayed, they were thawed to room temperature, vortexed, and then centrifuged for 15 minutes at approximately 3000 rpm (1500g). Samples were tested for estradiol using a high-sensitivity enzyme immunoassay (Cat. No. 1-3702). The test had a lower limit of sensitivity of 0.1 pg/mL, a standard curve range from 1.0 to 32.0 pg/mL, an average intra assay coefficient of variation of 7.1%, and an average interassay coefficient of variation <15% between samples 1 and 2.28

Assessment of Nocticeptive Processing

NFR Threshold Assessment

The NFR is a spinoally mediated withdrawal reflex elicited by activation of A5 fibers following noxious stimulation29,30 and is used as an electrophysiological correlate of spinal noception. Electromyographic (EMG) signals for NFR were sampled at 1000 Hz and recorded using Ag-AgCl electrodes. To apply EMG and stimulating electrodes for delivering electrocutaneous stimulation, the skin was initially cleaned with alcohol, slightly abraded using NuPrep gel to attain impedances below 5 kΩ, and then conductive gel (EC60; Grass Instruments) was applied. The NFR was assessed by attaching 2 electrodes over the biceps femoris muscle of the left leg 10 cm superior to the popliteal fossa, with a common ground electrode placed over the lateral epicondyle of the left femur. The raw biceps femoris signal was amplified, bandpass filtered online (10 to 300 Hz), and rectified.

NFR threshold was determined using validated procedures.31,32 Trains of five 1 ms rectangular wave pulses at 250 Hz were delivered to the sural nerve with a varying intertrain interval of 8 to 12 seconds to reduce stimulus predictability. The first train began at 0 mA (current) and was increased in 2 mA steps until an NFR was detected. The mean biceps femoris EMG activity 90 to 150 ms post-stimulation that exceeds mean EMG activity during the 60 ms prestimulation baseline interval by 1.4 SD was used to define the presence of the NFR.31 Prior research has shown that using the 90 to 150 ms timeframe avoids potential contamination by the non-nociceptive RII reflex, startle responses, and/or voluntary movements.33,34 The stimulus intensity was decreased in 1-mA steps until an NFR was no longer observed. Then, this updown staircase procedure was repeated twice using 1-mA steps. NFR threshold was defined as the average stimulation intensity (mA) of the last 2 peaks and troughs of this updown staircase procedure.

Electrocutaneous Pain Threshold and Tolerance

Electrocutaneous pain was assessed by delivering electrical stimulation to the sural nerve. This method evokes pain through the activation of both A-δ and C fibers (although it also activates A-β fibers), and produces a stinging or prickling sensation.35 Electrocutaneous pain threshold was assessed through 3 ascending/descending staircases of electric stimuli. Stimulus parameters were the same as those used in NFR threshold. Following each electric stimulus, participants rated their sensation using a computer-presented numerical rating scale (NRS) used in numerous prior studies by our laboratory and others.36,37 The scale was labeled: 0 (no sensation), 1 (just noticeable), 25 (uncomfortable), 50 (painful), 75 (very painful), and 100 (maximum tolerable). Starting with 0 mA (current), the current was increased in 4-mA steps, with a variable 8- to 12-second interval, until pain threshold was reached (a rating ≥ 50 on the 0 to 100 NRS). When pain threshold was reached, the intensity was decreased in 2-mA steps until the participant rated a stimulus as 40 on the scale. This process was repeated 2 more times in 2-mA steps. Pain threshold was defined as the average of the 4 stimuli (mA) immediately above and immediately below a rating of 50 on the last 2 ascending/descending staircases.

Electrocutaneous pain tolerance was assessed by a single ascending staircase of electric stimuli delivered over the sural nerve. Stimulus parameters were the same as those used in NFR threshold. Following each electric stimulus, participants rated their sensation on the NRS. Electric stimulations began at 0 mA and increased in 2-mA steps until the participant rated a stimulus as 100 or until the maximum of 50 mA was achieved. Pain tolerance was defined as the stimulus (mA) rated 100 (or 50 mA if the maximum was reached).

Ischemic Pain Threshold and Tolerance

Ischemia pain activates C fibers of the skin and produces a deep, aching sensation similar to some clinical pain.38,39 Initially, hand exercises were conducted using a dynamometer at 50% maximal effort with the right hand for 120 seconds, followed by 15 seconds of arm elevation for exsanguination. Then, a blood pressure cuff was affixed
to the right biceps of the arm and inflated to 220 mm/Hg to occlude blood flow and induce ischemia pain. Participants were then asked to continuously (in real-time) rate the intensity of the blood pressure cuff on the NRS described above. The time (seconds) until they reached pain threshold (rating ≥ 50 on the NRS) and tolerance (rating of 100 on the NRS) was assessed. To ensure participant safety, a maximum cutoff of 25 minutes was used.39

**McGill Pain Questionnaire-Short Form (SF-MPQ)**

The SF-MPQ40 is a reliable and valid pain rating scale commonly used in pain research that allows quantitative, multidimensional pain ratings to be obtained in a brief period. Participants were asked to rate 11 sensory (eg, throbbing, shooting) and 4 affective (eg, sickening, fearful) pain descriptors on a scale ranging from 0 (none) to 3 (severe). The SF-MPQ was administered twice, once immediately following electrocutaneous pain tolerance and once immediately following ischemia pain tolerance. A sum of sensory words and affective words was used to compute sensory and affective pain rating scores, respectively, for each stimulus modality.

**Procedure**

All procedures were approved by the University of Tulsa ethics review board. Before study enrollment, a brief phone health screening was conducted to evaluate inclusion/exclusion criteria; however, a more comprehensive assessment was conducted during an initial laboratory session with those individuals who met inclusion criteria via the phone screen. During this initial laboratory session, participants were given a complete overview of the study and informed consent was obtained. Then, a brief demographics/health status form was used to assess inclusion/exclusion criteria and attain relevant background information. Afterwards, height and weight were assessed to determine body mass index. To evaluate potential PMDD status, a semistructured interview22 was conducted, and then the SCID-I was administered to assess and exclude for the presence of current psychiatric conditions (other than PMDD). If deemed eligible to participate, participants were given instructions on menstrual cycle monitoring and then randomly assigned to one of 6 menstrual phases. Menstrual phase was entered as a nominal within-subjects variable and group (PMDD vs. HC) was entered as a nominal between-subjects variable. Dependent variables were NFR threshold, electrocutaneous pain threshold and tolerance, ischemia pain threshold and tolerance, and McGill sensory and affective pain ratings to electrocutaneous and ischemic stimuli. For analyses of pain outcomes, testing order (ie, session 1 to 3) was entered as a variable to determine whether testing order influenced the pain outcomes independently of the menstrual phases. Controlling for order improves statistical power and improves the validity of the statistical models by removing potential variance due to exposure effects. Follow-up mean comparisons to significant F tests were conducted using Fisher Least Significant Difference tests. The SPSS MIXED procedure uses Satterthwaite estimation for the denominator degrees of freedom (df) which produced noninteger values that vary from analysis to analysis. These dfs were rounded to the nearest integer for ease of reporting. Cohen’s d was reported as the effect size for mean comparisons.

To determine whether hormone levels were associated with pain outcomes, Pearson correlations were conducted separately for each group, and for the combined sample. Fisher r-to-z analyses were conducted to determine whether there were group differences in the correlations.

**RESULTS**

**Participant Characteristics**

Table 1 reports demographic as well as other relevant menstrual cycle-related variables for PMDD and HC participants. Results revealed no significant differences between the 2 groups on any of the variables. Overall, 28
females completed mid-follicular phase testing (1 HC participant completed only partial testing), 27 completed ovulatory phase testing, and 28 completed late-luteal phase testing. Eight females (4 HC, 4 PMDD) attended their first testing session during their mid-follicular phase, 9 females (4 HC, 5 PMDD) attended their first testing session during the ovulatory phase, and 11 females (6 HC, 5 PMDD) attended their first testing session during their late-luteal phase. Of the 28 women who completed the mid-follicular testing session, 27 (96.4%) were tested during days 5 to 8 (M = 7.25, SD = 1.14) and all were tested before ovulation. Of the 28 women who completed the late-luteal testing session, 25 (89.2%) were tested 1 to 6 days preceding menses (M = 4.04, SD = 3.32) and all were tested after ovulation and during an ovulatory cycle. Reasons for not testing within the designated timeframe include inability to predict the onset of menses from prior cycles and LH surges, as well as menses lasting >8 days. All 27 women tested during the ovulatory phase were tested in the 48 hours immediately following a positive ovulation test.

Sex Hormone Levels

Descriptive and inferential statistics for estradiol, progesterone, and testosterone are presented in Table 2. The main effect of menstrual phase for estradiol and progesterone was significant. Specifically, estradiol levels were highest during the ovulatory phase than mid-follicular (P < 0.001, d = 0.66) and late-luteal (P = 0.01, d = 0.39) phases, whereas progesterone levels were lowest during the mid-follicular phase relative to ovulatory (P < 0.001, d = 1.02) and late-luteal (P < 0.001, d = 1.29) phases. The main effect of group and the Menstrual phase x Group interaction for estradiol and progesterone were not significant (Ps > 0.05). The main effect of group was significant for testosterone, but this was qualified by a significant Menstrual phase x Group interaction indicating that the PMDD group had lower testosterone levels than the HC group during the mid-follicular (P = 0.04, d = 0.71) and ovulatory (P = 0.006, d = 1.17) phases, but not the late-luteal phase (P = 0.65, d = 0.17). The main effect of menstrual phase was nonsignificant for testosterone (P > 0.05).

### Measures of Experimental Pain Sensitivity

Descriptive and inferential statistics for measures of experimental pain sensitivity are reported in Table 3. There were no significant main effects of menstrual phase for any of the pain outcomes (Ps > 0.05), although there was a nonsignificant trend for ischemia pain threshold (P = 0.06). Pairwise comparisons indicated that thresholds were lower during the late-luteal phase relative to the mid-follicular phase (P = 0.02, d = 0.41).

For the main effect of group, all measures of pain sensitivity were nonsignificant (Ps > 0.05), with the...
exception of the PMDD group having higher MPQ sensory ratings for electrosensitive stimuli relative to the HC group ($P = 0.005$, $d = 1.00$). Further, there was a non-significant trend for ischemia pain threshold ($P = 0.09$, $d = 0.43$) and MPQ affective electrosensitive ratings ($P = 0.06$, $d = 0.64$) indicating that the PMDD group had lower thresholds and higher affective pain ratings than the HC group. The Menstrual phase × Group interaction was nonsignificant for all of the pain sensitivity outcomes (all $P$s > 0.05).

Results also indicated main effects of testing order (ie, pain varied across the 3 testing sessions, but independent of menstrual phase) for MPQ sensory ischemia ratings ($F_{1,25} = 5.79$, $P = 0.02$) and MPQ affective ischemia ratings ($F_{1,25} = 5.20$, $P = 0.03$). Specifically, sensory ischemia ratings ($P = 0.04$, $d = 0.28$) and affective ischemia ratings ($P = 0.02$, $d = 0.29$) were higher during the participant’s third experimental testing session relative to their first experimental session, suggesting sensitization across testing sessions.

### Relationship Between Sex Hormones and Pain Sensitivity

Correlations between sex hormones and pain outcomes are presented in Table 4. Estradiol was significantly related to electrosensitive pain threshold, electrosensitive pain tolerance, ischemia pain threshold, and ischemia pain tolerance in the combined group (all $P$s < 0.05). However, as can be seen in Table 4 and Figure 1, this was primarily due to medium and large correlations in the PMDD group (all these correlations were significant in PMDD group, but nonsignificant in the HC group). The Fisher $r$-to-$z$ analysis indicated that the groups significantly differed on correlations with ischemia pain threshold and ischemia pain tolerance ($P$s < 0.05). Thus, estradiol appears to have been hypoalgesic in the PMDD group.

No correlations between progesterone and pain outcomes were significant in the combined group, although there was a nonsignificant trend for the relationship with electrosensitive pain threshold ($P < 0.07$). This was primarily because of a medium-sized and significant correlation in the PMDD group, but the Fisher $r$-to-$z$ failed to show a group difference ($P > 0.05$). There was also a nonsignificant trend for progesterone to be related to electrosensitive pain threshold in the PMDD group ($P = 0.10$). Thus, it appears that progesterone has minimal relations to these pain outcomes.

Testosterone was significantly related to electrosensitive pain threshold, electrosensitive pain tolerance, ischemia pain tolerance, and affective pain ratings of electrosensitive stimuli in the combined group (all $P$s < 0.05; Fig. 2). These correlations were of similar magnitude in both the groups, as confirmed by the Fisher $r$-to-$z$ analyses (all $P$s > 0.05). But, when subgroup analyses were conducted, only the relationship between testosterone and electrosensitive pain threshold in the PMDD group was significant ($P < 0.05$). According to the Fisher $r$-to-$z$ analysis, there was

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**TABLE 3. Descriptive and Inferential Statistics for Measures of Experimental Pain Sensitivity Across Menstrual Phase and Group**

<table>
<thead>
<tr>
<th>Pain Outcomes</th>
<th>Mid-Follicular</th>
<th>Ovulation</th>
<th>Late-Luteal</th>
<th>Phase (P)</th>
<th>Group (G)</th>
<th>P×G</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$M$</td>
<td>SD</td>
<td>Range</td>
<td>$M$</td>
<td>SD</td>
<td>Range</td>
</tr>
<tr>
<td>NFR Thr (0-50 mA)</td>
<td>13.7</td>
<td>11.5</td>
<td>2.5-35.8</td>
<td>15.2</td>
<td>12.9</td>
<td>1.5-46.5</td>
</tr>
<tr>
<td>PMDD</td>
<td>15.6</td>
<td>9.3</td>
<td>2.5-30.5</td>
<td>16.0</td>
<td>7.9</td>
<td>3.5-27.3</td>
</tr>
<tr>
<td>HC</td>
<td>13.1</td>
<td>5.9</td>
<td>4.27-5.7</td>
<td>16.0</td>
<td>10.6</td>
<td>3.0-39.5</td>
</tr>
<tr>
<td>Elec Thr (0-50 mA)</td>
<td>16.1</td>
<td>8.4</td>
<td>7-39.5</td>
<td>16.2</td>
<td>10.9</td>
<td>5.5-39.5</td>
</tr>
<tr>
<td>PMDD</td>
<td>22.8</td>
<td>12.8</td>
<td>8-50</td>
<td>23.3</td>
<td>14.5</td>
<td>8-50</td>
</tr>
<tr>
<td>HC</td>
<td>25.8</td>
<td>10.1</td>
<td>10-50</td>
<td>30.7</td>
<td>11.9</td>
<td>14-50</td>
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<tr>
<td>Elec Tol (0-50 mA)</td>
<td>3.1</td>
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<td>0-10</td>
<td>3.6</td>
<td>3.3</td>
<td>0-9</td>
</tr>
<tr>
<td>PMDD</td>
<td>4.4</td>
<td>3.0</td>
<td>0-8</td>
<td>5.4</td>
<td>4.0</td>
<td>0-12</td>
</tr>
<tr>
<td>HC</td>
<td>11.0</td>
<td>6.5</td>
<td>2-23</td>
<td>10.9</td>
<td>5.6</td>
<td>4-23</td>
</tr>
<tr>
<td>MPQ Sens Elec (0-33)</td>
<td>174</td>
<td>5.7</td>
<td>8-24</td>
<td>16.3</td>
<td>5.2</td>
<td>7-24</td>
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<tr>
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<td>11.1</td>
<td>6.5</td>
<td>2-23</td>
<td>10.9</td>
<td>5.6</td>
<td>4-23</td>
</tr>
<tr>
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<td>6.5</td>
<td>2-23</td>
<td>10.9</td>
<td>5.6</td>
<td>4-23</td>
</tr>
<tr>
<td>MPQ Aff Elec (0-12)</td>
<td>3.0</td>
<td>3.6</td>
<td>0-10</td>
<td>2.2</td>
<td>3.2</td>
<td>0-10</td>
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<tr>
<td>PMDD</td>
<td>4.4</td>
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<td>5.4</td>
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<td>4-23</td>
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<tr>
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<td>17.9</td>
<td>7.0</td>
<td>4-28</td>
<td>18.4</td>
<td>7.6</td>
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<td>PMDD</td>
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<td>2.2</td>
<td>3.2</td>
<td>0-10</td>
</tr>
<tr>
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<td>0-11</td>
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<td>4.0</td>
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<tr>
<td>HC</td>
<td>3.1</td>
<td>3.0</td>
<td>0-9</td>
<td>3.6</td>
<td>3.3</td>
<td>0-9</td>
</tr>
</tbody>
</table>

* $P$ ≤ 0.05.
† $P$ ≤ 0.10.

Aff indicates affective; Elec, electrocutaneous; HC, healthy control; Isch, ischemia; MPQ, McGill Pain Questionnaire-Short Form; NFR, nociceptive flexion reflex; PMDD, premenstrual dysphoric disorder; Sens, sensory; Thr, threshold; Tol, tolerance.
a significant group difference in the correlation between testosterone and sensory pain ratings of ischemia stimuli (P < 0.05), but neither of the correlations were significant in the subgroups. There was also a nonsignificant trend for testosterone to be correlated with NFR threshold in the PMDD group (P = 0.10), implying that testosterone might be associated with dampened spinal nociception in this group. Therefore, it appears that testosterone has a small to moderate relationship with some pain outcomes in both groups suggesting it has a hypoalgesic effect.

**TABLE 4. Correlations Between Sex Hormones and Pain Outcomes by Group**

<table>
<thead>
<tr>
<th>Pain Outcomes</th>
<th>Correlations With Estradiol</th>
<th>Correlations With Progesterone</th>
<th>Correlations With Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC</td>
<td>PMDD</td>
<td>ALL</td>
</tr>
<tr>
<td>NFR Thr (0-50 mA)</td>
<td>0.074</td>
<td>0.228</td>
<td>0.105</td>
</tr>
<tr>
<td>Elec Thr (0-50 mA)</td>
<td>0.090</td>
<td>0.373*</td>
<td>0.243*</td>
</tr>
<tr>
<td>Elec Tol (0-1200 s)</td>
<td>0.114</td>
<td>0.339*</td>
<td>0.275*</td>
</tr>
<tr>
<td>Isch Thr (0-1200 s)</td>
<td>0.111</td>
<td>0.532*</td>
<td>0.300*</td>
</tr>
<tr>
<td>Isch Tol (0-1200 s)</td>
<td>0.018</td>
<td>0.503*</td>
<td>0.257*</td>
</tr>
<tr>
<td>MPQ Sens Elec (0-33)</td>
<td>0.165</td>
<td>0.045</td>
<td>0.002</td>
</tr>
<tr>
<td>MPQ Aff Elec (0-12)</td>
<td>0.051</td>
<td>0.237</td>
<td>0.153</td>
</tr>
<tr>
<td>MPQ Sens Isch (0-33)</td>
<td>0.063</td>
<td>0.096</td>
<td>0.063</td>
</tr>
<tr>
<td>MPQ Aff Isch (0-12)</td>
<td>0.030</td>
<td>0.241</td>
<td>0.150</td>
</tr>
</tbody>
</table>

*P < 0.05. †P ≤ 0.10.

ALL indicates all participants combined; HC, healthy control; PMDD, premenstrual dysphoric disorder; z, Fisher r-to-z value that tests the group differences in correlations.

**FIGURE 1.** Significant relationships between estradiol and pain outcomes. Estradiol was significantly associated with electrocutaneous pain threshold (A), electrocutaneous pain tolerance (B), ischemia pain threshold (C), and ischemia pain tolerance (D) in the combined group. The magnitudes of these correlations were larger in the PMDD group, but only the correlations between estradiol and ischemia pain threshold (C) and estradiol and ischemia pain tolerance (D) were significantly different according to Fisher r-to-z analyses. Thus, estradiol was hypoalgesic, primarily so in the PMDD group. HC indicates healthy control; PMDD, premenstrual dysphoric disorder.
Secondary Analyses: Examining Testosterone as a Mediator of Diagnostic Group and Sensory Pain

As testosterone was associated with hypoalgesia and PMDD had lower overall levels of testosterone, it is plausible that sex hormones may contribute to pain in PMDD. This may be especially true for the enhanced sensory pain ratings observed within the PMDD group. To address this, a post hoc mediation analysis was conducted to examine whether testosterone significantly mediated the relationship between-group (PMDD vs. HC) and MPQ sensory ratings for electrocutaneous stimuli. Follow-up analyses of mediation were conducted using a mixed-effects regression model entering group (PMDD vs. HC) as the predictor, sensory pain ratings as the outcome, and testosterone as the hypothesized mediator. To be considered a mediator, the relationship of the predictor to the outcome must be reduced (or become nonsignificant) once the mediator (testosterone) is entered into the model.42

Initial analyses examining the effect of group on MPQ ratings was significant ($F_{1,28} = 9.47, P = 0.005, b = -5.63$). When the analysis was repeated including testosterone as a predictor variable, the group effect was no longer significant ($F_{1,78} = 3.23, P = 0.076, b = -6.26$), suggesting that testosterone may partially mediate group differences in MPQ sensory ratings for electrocutaneous stimuli.

DISCUSSION

PMDD-related Differences in Pain Processing

The current study assessed the relationships between sex hormones (estriadiol, progesterone, testosterone) and experimental pain sensitivity across 3 menstrual phases in women with and without PMDD. The only statistically significant difference that emerged was higher sensory pain ratings for electrocutaneous stimuli relative to healthy women; however, inspection of the means for pain outcomes (Table 3) suggest that, compared with control women, women with PMDD had lower thresholds/tolerances and rated noxious stimuli as more painful. This pattern of results is consistent with 2 prior studies that found severe premenstrual symptomatology was associated with no difference in measures of thresholds or tolerances, but higher pain ratings.5,43 Specifically, Kuczmierczyk et al5 found that women with severe premenstrual syndrome (PMS, a less severe form of PMDD) exhibited higher ratings of pressure pain relative to healthy women even though there were no significant differences for pressure pain thresholds and tolerances. Similarly, Klatzkin et al43 found that women with current PMDD (plus a history of major depressive disorder) experienced greater pain unpleasantness to a cold pressor
task than women without PMDD (but with a history of major depressive disorder). This was noted even though there were no group differences on ischemic or cold pressor pain thresholds and tolerances. By contrast, 2 studies did find PMDD-related hyperalgesia on measures of threshold/tolerance, but the effect sizes for group differences on pain ratings tended to be even larger than the effect sizes for threshold/tolerance measures. This suggests that PMDD may affect the retrospective report of pain more so than threshold/tolerance measures, perhaps due to PMDD-related affect influencing memory for pain more so than real-time evaluation of painful stimuli.

Two additional pain-related observations from the present study are worth noting. First, we found no evidence that enhanced premenstrual pain sensitivity in PMDD was specific to the premenstrual (late-luteal) phase, because all group by phase interactions for pain outcomes were non-significant (Table 3). This is consistent with a prior study that assessed pain across multiple menstrual phases in PMDD and found no changes in PMDD-related hyperalgesia across menstrual phases. Second, NFR threshold, a physiological measure of spinal nociceptive processing, did not differ between groups (and Cohen’s d effect sizes were small, ranging from 0.08 to 0.25 across phases) or across menstrual phases. The current findings corroborate a recent study that found NFR does not vary across the mid-follicular and late-luteal phases of the menstrual cycle in healthy women and extends those findings to show that it also does not vary during ovulation or by PMDD status. Because the spinally mediated NFR was not significantly correlated with estradiol, progesterone, or testosterone, it also appears that these sex hormones may not affect spinal nociceptive processing. Therefore, findings from the current study and others suggest that enhanced pain in PMDD may be (1) most pronounced on measures of pain report; (2) phase-independent; and (3) more likely to stem from amplification of pain at the supraspinal level (eg, corticocortical mechanisms, report bias) rather than the spinal level. However, given our small sample size, caution is warranted in interpreting our null findings.

Hormones and Pain Processing

Estradiol and progesterone levels indicated that testing sessions were conducted during the correct phases. Although the expected phase-related changes in these hormones were noted, there were no group differences. In contrast, unbound (bioavailable) testosterone did not show phase-related changes, but did show group differences. Testosterone was significantly lower in women with PMDD, particularly during the mid-follicular and ovulatory phases. Unfortunately, data on sex hormone levels in women with menstrual cycle-related disorders is equivocal. Three studies found PMS was not associated with changes in total (bound + unbound) testosterone. Two studies found that PMS was not associated with differences in total testosterone but that unbound testosterone was higher during follicular, ovulatory, and luteal phases. And finally, 1 study found that testosterone was associated with lower plasma levels of total and unbound testosterone during the ovulatory phase.

Only 1 study has examined testosterone in women prospectively diagnosed with PMDD with the authors finding a nonsignificant trend for unbound, but not total, testosterone to be lower during the ovulatory phase. If our results can be replicated, this suggests that low testosterone could potentially contribute to PMDD symptoms. Indeed, testosterone appears to exert an antinociceptive effect on pain in both animals and humans, and testosterone therapy in women can improve libido, mood, and well-being. To our knowledge, the use of testosterone as a therapy in women with PMDD-related pain has not been investigated, likely due to the potential for treatment-related side effects (ie, masculinization). Therefore, this may require further research to determine whether testosterone is, in fact, pathophysiologically relevant to PMDD and to assess whether testosterone treatment (eg, transdermal testosterone) might help PMDD-related symptoms.

Interestingly, groups differed in the degree to which sex hormones modulated experimental pain outcomes. For instance, estradiol had a hypoalgesic effect on thresholds and tolerances for electrocutaneous and ischemic stimuli; however, only the associations between estradiol and ischemia thresholds/tolerances varied across groups. This suggests that estradiol had a more robust hypoalgesic effect in women with PMDD. These results are in contrast to a study that failed to observe an association between estradiol and ischemia pain thresholds/tolerances in PMDD. Although it is unclear why our results differ, methodological differences may have contributed (eg, serum vs. salivary assays, assessment during ovulatory phase). Progesterone was not related to any of our pain outcomes, with the exception of electrocutaneous pain tolerances in PMDD. However, this relationship did not differ when comparing across groups. This is interesting as it suggests that progesterone may have minimal influence on experimental pain. Further, as predicted, testosterone appeared to have a hypoalgesic effect on electrocutaneous thresholds/tolerances, ischemia tolerances, and affective ratings for electrocutaneous stimuli. These relationships did not differ across groups, suggesting testosterone was associated with hypoalgesia in both PMDD and HC. Although there have been few investigations of testosterone’s effects on pain in women, this is in line with research suggesting testosterone attenuates pain sensitivity and protects against clinical pain.

Our findings also suggest that sex hormones may contribute to pain in PMDD because testosterone was hypoalgesic, and women with PMDD had lower levels of testosterone. To examine this further, a post hoc mediation analysis was conducted to assess whether testosterone significantly mediated the relationship between diagnostic group and MPQ sensory ratings for electrocutaneous stimuli, given group differences in this variable. When analyses were repeated using testosterone as a predictor, the group effect was no longer significant indicating that testosterone may partially mediate group differences in sensory pain ratings for electrocutaneous stimuli. This suggests testosterone may play a role in group differences in pain and could be a potential target for treatment.

When taken together, results indicate estradiol and progesterone were antinociceptive, but that progesterone had a minimal effect on experimental pain sensitivity. Further, some relationships between sex hormones and pain outcomes varied across groups (particularly with estradiol). Although the current findings suggest sex hormones may contribute to PMDD, it is important to note that some evidence suggests that PMDD may be related to individual sensitivity to normal cyclical fluctuation in sex hormones (rather than absolute hormone levels)—effects which may influence neurotransmitter responsiveness to trigger symptomatology. Regardless of the etiology, our findings highlight the complexity of sex hormone influences.
in the modulation of pain and suggest that sex hormones may impact experimental pain differently in PMDD. Future studies are needed to clarify the specific effect that sex hormones have on pain and PMDD, and to investigate whether testosterone and estradiol therapy, or a combination of both, may be a therapeutic option when treating pain-related symptoms in PMDD.

**Strengths and Limitations**

There are several strengths of this study that merit acknowledgment including the prospective diagnosis of PMDD, measurement of hormones, verification of cycle regularity, and assessment of multiple pain outcomes (including NFR). Despite these strengths, some limitations are worth noting. First, our failure to detect significant group differences (with the exception of sensory ratings for electrotactile stimuli) may have been impacted by low power. Thus, some meaningful group differences may have been missed. For example, post hoc power analyses revealed that group sizes between 22 and 148 would have resulted in power equal to 0.80 for outcomes that were marginally significant (ie, MPQ affective electrotactile ratings, ischemia threshold), but at least 50 or more (up to 43,485 per group) would have been needed for other non-significant outcomes. So, caution is warranted when interpreting the nonsignificant findings given our small sample size. Second, even though several experimental measures were used to assess pain sensitivity in the present study, it is possible that the stimuli used were not adequate to assess the clinical phenomenon of interest in PMDD. For instance, pressure stimulation evokes a natural pain sensation, activating both A and C fibers, as well as nociceptors of the muscles and tendons. Thus pressure may be more suitable for evoking pain similar to PMDD-related symptomatology (eg, mastalgia, headache, musculoskeletal pain). For this reason future studies might consider other pain-induction methods (eg, mechanical pressure pain, heat/cold) and/or symptoms (eg, emotion dysregulation) to study in this population. Third, the women who participated in our study may not be representative of other women with PMDD. The study required significant participant involvement to track menstrual cycles, attend multiple testing sessions, and endure several pain tests. Further, our classification of PMDD was based upon a modified version of the diagnosis (ie, criteria did not have to be met for 2 consecutive cycles). As a result, our sample may have been more resilient or less symptomatic than other women with PMDD. Fourth, we chose to measure hormone levels from saliva rather than blood, because salivary-based assays are noninvasive, inexpensive, and easy to collect. Further, they measure the biologically available fraction of hormones in the body. For this reason, they may provide a more valid estimate of hormones available to affect target tissues (and thus pain processing). Nonetheless, a limitation of salivary-based assays is their difficulty detecting low hormone levels, especially in nonreproductive females. As a result, our study may not accurately characterize the relationships between hormones and pain in women with very low hormone levels. And finally, we chose not to counterbalance the order of our pain tasks within the testing sessions as this could have potentially resulted in carryover effects. We attempted to minimize this problem by building in mandatory rest periods and placing the most intense tolerance tests at the end.

Future studies may consider counterbalancing pain procedures to circumvent this issue.

**Summary**

In sum, the current results indicate that women with PMDD have phase-independent hyperalgesia on sensory pain ratings of electrotactile stimuli. This hyperalgesia is likely to occur at the supraspinal level, because there were no group differences in NFR (and between-group effect sizes were very small). Further, sex hormones may contribute to pain in PMDD because testosterone was hypogonadal and women with PMDD had lower testosterone. In addition, stronger relationships between pain and estradiol were found in women with PMDD. However, future studies are needed to explore whether exogenous administration of sex hormones influence pain in PMDD.

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