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Evaluation of menstrual cycle effects on morphine and pentazocine analgesia

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Abstract

Studies have demonstrated menstrual cycle influences on basal pain perception, but direct evidence of menstrual cycle influences on analgesic responses has not been reported in humans. Our aim was to determine whether the magnitude of morphine and pentazocine analgesia varied across the menstrual cycle. Sixty-five healthy women, 35 taking oral contraceptives (OC) and 30 normally cycling (NOC), underwent experimental pain assessment both before and after intravenous administration morphine (0.08 mg/kg) or pentazocine (0.5 mg/kg) compared to saline placebo. Both active drug and placebo were administered once during the follicular phase and once during the luteal phase. Measures of heat, ischemic and pressure pain sensitivity were obtained before and after drug administration. Change scores in pain responses were computed to determine morphine and pentazocine analgesic responses, and medication side effects were recorded. The data were analyzed using mixed-model ANOVAs. NOC women showed slightly greater heat pain sensitivity in the follicular vs. luteal phase, while the reverse pattern emerged for OC women ($p=0.046$). Also, OC women showed lower pressure pain thresholds compared to NOC women ($p < .05$). Regarding analgesic responses, NOC women showed greater morphine analgesia for ischemic pain during the follicular vs. the luteal phase ($p=0.004$). Likewise, side effects for morphine were significantly higher in NOC women in the follicular phase than in the luteal phase ($p=0.02$). These findings suggest that sex hormones may influence opioid responses; however, the effects vary across medications and pain modalities and are likely to be modest in magnitude.

Keywords

Menstrual Cycle; Morphine; Pentazocine; Analgesic Response; Pain Sensitivity; Opioid Side Effect

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There are no conflicts of interest.

1- Introduction

The body is widely responsive to gonadal steroid hormones. Aside from their reproductive functions, gonadal hormones act on nervous system regions involved in higher cognitive functions such as mood, motor behavior, and pain mechanisms [33]. For example, pregnancy-related changes in reproductive hormone levels increase pain thresholds and decrease anesthesia requirements in humans and some non-human animals; a phenomenon that can be re-created by hormonal simulation in non-pregnant animals [22,41]. The influence of gonadal hormones, particularly estrogens, has been implicated as a potential mechanism for explaining sex differences in both basal pain sensitivity and opioid analgesia [15,17].

Barring several exceptions, evidence suggests that male rats exhibit more robust antinociception than females in response to μ -receptor selective (e.g., hydromorphone, buprenorphine) and κ -receptor selective agonists (e.g., U50,488 [7,8,29,38]. Moreover, early hormone manipulation of fetal and neonate rodents (e.g., male castration, female ovariectomy, hormone augmentation) has lasting effects on morphine dose-response curves [3,5]. In contrast to these organizational hormonal influences, research investigating the acute activational effects of gonadal hormones on responses to opioid administration in non-human animals has yielded inconsistent results [9]. For example, several studies have found that morphine and buprenorphine antinociception increased in ovariectomized (OVX) female rats, whereas chronic estrogen (E2) replacement decreased morphine potency to levels similar to those seen in intact females, suggesting that hormone depletion in adulthood might modulate opioid potency [43,44]. Similarly, Ali et al. [1] showed that gonadectomized (GDX) male and female rats showed greater morphine antinociception than their intact counterparts. In contrast, Cicero et al. [5] noted that GDX adult male and female rats displayed no significant changes in the expected sex differences in morphine-induced antinociception.

Estrous phase also appears to modulate the expression of morphine analgesia in reproductively intact rats. Several studies have found that μ opioids were less potent during estrus, when females are sexually active and estrogen is low, relative to other cycle phases (i.e., metestrus/diestrus, proestrus) [6,29,38,47]. Studies using high efficacy opioids such as morphine have yielded relatively small to moderate effect sizes [5,32,47]. In comparison, larger, more statistically robust estrous cycle effects have been found with less-efficacious compounds, such as buprenorphine, suggesting that the effects of gonadal hormones interact with opioid characteristics [48,49].

In contrast to rodent studies, human clinical and experimental pain models show a general trend for women to derive superior analgesia from a variety of opioids compared to men; however, there is substantial variability between studies [4,14,37]. Scant data are available regarding the influence of the human menstrual cycle on responses to exogenous opioid analgesics. Therefore, the present study investigated the effects of human menstrual cycle phase on basal pain responses, morphine and pentazocine analgesia as well as post drug side effects in healthy young women using well-validated experimental pain models. These two opioids were chosen, because morphine represents the prototypical μ -agonist, and at the time the study was designed, evidence of sex differences in pentazocine analgesia had emerged, which had been attributed to its activity at the kappa opioid receptor [18,24].

2 - Methods

2.1 - Participants

Ninety women were initially recruited via posted advertisements, 44 of whom were normally cycling women not taking oral contraceptives (NOC), and 46 were taking oral contraceptives (OC). Participants were healthy nonsmokers, free of clinical pain, psychiatric disturbance, substance abuse, or use of centrally acting medications. Participants refrained from using over-the-counter medication for at least 24 hours before testing. Twenty women (9 NOC, 11 OC) dropped out of the study before completing, eight due to side effects from the medication, and the remainder due to scheduling difficulties or other protocol-related issues (e.g. unable to gain iv access). This resulted in a sample of 70 women (35 NOC, 35 OC) who completed the study. All women reported having regular and predictable menstrual cycles (between 25–35 days) with no notable irregularities or gynecological problems. Menstrual cycle length was confirmed during the study using menstrual cycle calendars. All procedures were approved by the University of Florida's Institutional Review Board. Any clinically significant adverse effects were reported to the Institutional Review Board and tabulated for data analysis. Participants were reimbursed \$25 per hour for their participation.

2.2 - General Experimental Procedures

The study was conducted at the University of Florida's General Clinical Research Center. The study employed a mixed 2 (OC Group, between subjects) $\times$ 2 (Drug, between subjects) $\times$ 2 (Cycle Phase, within subjects) factorial design to investigate responses to morphine and pentazocine across the menstrual cycle in OC versus NOC women. Each participant attended 5 sessions (one introductory session and four experimental sessions involving drug administration). The introductory session was conducted to obtain consent, familiarize participants with testing procedures, and complete a series of health and psychological questionnaires. The health questionnaire asked women to indicate the age at which they experienced first onset of menses (i.e. age of menarche), which was used as a variable in correlational analyses described below. In the experimental sessions participants in both groups (i.e., NOC, OC) were randomized (double blind) to receive either morphine or pentazocine (0.5 mg/kg) during the study. For the morphine group, each individual received a bolus of morphine (0.08 mg/kg) on two different testing days, once during the follicular and once during the luteal phase of her menstrual cycle. Similarly, for the pentazocine group, each individual received a bolus of pentazocine (0.5 mg/kg) once during the follicular and once during the luteal phase. All participants also had two sessions during which saline was administered, one during the follicular and one during the luteal phase. All follicular phase sessions were scheduled between days 4 and 10 after the onset of menses, and all luteal phase sessions were scheduled between 4 and 9 days before the onset of menses (i.e., between days 19 and 23 of a 28 day menstrual cycle). Participants were randomly assigned to one of four drug administration orders (see Table 1), and whether participants started the study in the follicular vs. luteal phase was counterbalanced. After each session the side effects reported by the participant and observed by the research nurse were recorded.

2.2.1 - Menstrual Cycle Phase Staging—For all women, follicular phase timing was determined by having participants call at onset of menses, and the session was scheduled within the next 4–10 days. For luteal phase sessions, NOC women used an ovulation kit to pinpoint the day of ovulation, and the luteal phase sessions were scheduled approximately one week ($\pm$ 2–3 days) from the day of ovulation. Two NOC women whose cycle length exceeded 35 days were excluded from the analysis, because their luteal phase sessions fell outside the window of 4–9 days before onset of next menses. Luteal phase was also confirmed *post hoc* via noting elevated serum progesterone levels in NOC women only. An additional 5 NOC women were excluded, because their progesterone levels were below 2.0

ng/mL, indicating an anovulatory cycle. This resulted in a final sample of 28 NOC and 35 OC women. For OC women, the luteal phase cycle was based on predicted menses onset according to the contraceptive pill packet. Menses onset was subsequently determined by self-report, and the number of days between the luteal phase session and onset of menses was recorded.

2.2.2 - Hormone Assays—Blood serum levels of estradiol-17 β , progesterone (NOC women only), and total testosterone were subsequently confirmed using Immulite®/ Immulite® 1000 (Diagnostic Products Corporation) *in vitro* analyzers and assay procedures. Measurement of progesterone levels was used to confirm ovulation; therefore, progesterone levels were only assayed in NOC women. Progesterone required sequential competitive immunoassay procedures, whereas estradiol-17 β and total testosterone required solid-phase, competitive chemiluminescent enzyme immunoassay. Diagnostic Products Corporation reports intraassay precision coefficients of variation (CV³) values ranging from 6.3% – 15% (pg/mL) for estradiol-17 β and 6.3% – 16% (ng/mL) for progesterone. Interassay CV³ values range from 6.4% – 16% (pg/mL) for estradiol-17 β and 5.8% – 16% (ng/mL) for progesterone. For total testosterone, precision CV values are reported as ranging from 7.7% – 16.4% (ng/dL).

2.3 - Pain Testing Procedures

Two experimenters conducted all sessions (either two females, or one female and one male). One experimenter interacted with participants while the other operated the equipment and recorded data. Experimental pain procedures were conducted before and after drug administration, as reported in Fillingim, et al. [15,16]. Digitally recorded instructions were played for the participant prior to each pain procedure. Pressure and thermal pain were delivered in counterbalanced order, separated by a 5-minute rest period. Ischemic pain always occurred last to reduce the possibility of carryover effects. During all study procedure participants remained in the supine position on hospital beds. Each participant underwent 4 experimental drug sessions: saline follicular phase, saline luteal phase, opioid [pentazocine or morphine] follicular phase and opioid (pentazocine or morphine) luteal phase. All experimental sessions started with insertion of an intravenous cannula for drug administration. Before drug administration, measures of thermal, pressure, and ischemic pain were obtained (pre-drug pain testing). After the pre-drug pain testing, a 15-minute rest period was observed, followed by double-blind intravenous bolus administration of opioid drug (either morphine [0.08 mg/kg] or pentazocine [0.5 mg/kg]) or saline. Fifteen minutes after drug administration, resting cardiovascular measures were re-assessed. Post-drug pain testing was repeated in a manner identical to the pre-drug testing. After post-drug testing, participants completed questionnaires assessing somatic and cognitive/affective side effects.

2.3.1 - Pressure Pain Threshold—Pressure pain threshold (PPTh) was assessed using a handheld algometer (Pain Diagnostics and Therapeutics, Great Neck, NY). Mechanical pressure was applied with a 1-cm² probe. A deliberate application rate of 1 kg/sec was used to minimize reaction time artifacts. Participants reported when the pressure first became painful. PPThs were assessed three times at each anatomical site to obtain mean pressure ratings. Sites tested were: center of the right upper trapezius (posterior to the clavicle); right masseter (approximately midway between the tragus of the ear and the corner of the mouth), and the right ulna (dorsal forearm, approximately 8 cm distal to the elbow). The order of site presentation was counterbalanced.

2.3.2 - Thermal Pain Procedures

2.3.2.1 - Threshold and Tolerance: The first thermal procedure involved assessment of heat pain threshold (HPTh) and heat pain tolerance (HPTo). Contact heat stimuli were

delivered by using a computer controlled Medoc Thermal Sensory Analyzer (TSA-2001; Ramat Yishai, Israel), which is a Peltier-element-based stimulator. Temperature levels were monitored by a contactor-contained thermistor and returned to a preset baseline of 32°C by active cooling at a rate of 8–10°C/sec. The 3 × 3 cm contact probe was applied to the right ventral forearm. In separate series of trials, warmth thresholds, HPTh, and HPTo were assessed by using an ascending method of limits. From a baseline of 32°C, probe temperature increased at a rate of 0.5°C/sec until the subject responded by pressing a button to indicate when they first felt pain and again when they no longer felt able to tolerate the pain. Four trials of HPTh and HPTo were presented to each subject. Although it remained on the right ventral forearm, the position of the thermode was altered slightly between trials to avoid either sensitization or response suppression of cutaneous heat nociceptors. For each measure, the average of all four trials was computed for use in subsequent analyses.

2.3.2.2 - Temporal Summation of Thermal Pain: After a 5-minute rest period, the temporal summation procedure was conducted. This procedure involved administration of brief, repetitive, suprathreshold heat pulses to assess first and second pain and temporal summation of the latter. Participants rated thermal pain intensity of 10 repetitive heat pulses applied to the right dorsal forearm. The target temperatures were delivered for less than 1 second, with a 2.5-second interpulse interval during which the temperature of the contactor returned to a baseline of 40°C. Participants were asked to rate the peak pain for each of the 10 heat pulses. Because participants vary in their responses to heat pain, we examined temporal summation at two different stimulus intensities. This increased the likelihood that at least one set of stimuli would be at least moderately painful yet tolerable for the vast majority of participants. Therefore, two sets of target temperatures, 49°C and 52°C, were used. Participants were instructed to verbally rate the intensity of each thermal pulse by using a numeric rating scale on which zero represented no sensation, 20 represented a barely painful sensation, and 100 represented the most intense pain imaginable. Participants were told that the procedure would be terminated when they reported a rating of 100, when 10 trials had elapsed, or when they wished to stop. The average of all 10 ratings for each temperature was computed and used in subsequent analyses.

2.3.3 - Modified Submaximal Tourniquet Procedure—After the first two pain procedures, a five-minute rest period was observed, after which participants underwent the modified submaximal tourniquet procedure [35,39,46]. The right arm was exsanguinated by elevating it above heart level for 30 seconds, after which the arm was occluded with a standard blood pressure cuff positioned proximal to the elbow and inflated to 240 mm Hg using an E20 Rapid Cuff Inflator (D.E. Hokanson, Inc, Bellevue, WA). Participants then performed 20 handgrip exercises of two-second duration at four-second intervals at 50% of their maximum grip strength. Participants were instructed to report when they first felt pain (ischemic pain threshold [IPTh]) and then to continue until the pain became intolerable (ischemic pain tolerance [IPTo]), and these time points were recorded. Every 30 seconds, participants were prompted to alternately rate either the intensity or unpleasantness of their pain by using joint numeric (0 to 20) and verbal descriptor box scales. An uninformed 15-minute time limit was observed. In addition to IPTh and IPTo, two total pain scores were created, one for pain intensity and one for pain unpleasantness, by summing all ratings obtained during the procedure. To replace missing values created by participants terminating the procedure before the time limit, the last rating provided was carried forward as the value for each subsequent rating that would have occurred.

2.4 – Side Effects

The clinical research nurse recorded side effects that were observed or reported by the participant. Specifically, the nurse recorded whether the participant experienced each of the

following side effects: nausea, emesis, dizziness/lightheadedness, pallor, diaphoresis, headache, pruritis, and fainting. Also, any other less common side effects that occurred were recorded as “other.” For analytic purposes, the total number of side effects experienced was computed for each drug administration.

2.4 - Data Analysis

Descriptive statistics were calculated, which included means for continuous variables (e.g. hormone assay values, basal pain scores, and analgesic difference scores) by group and cycle phase. To reduce the probability of type 1 error, variable reduction was accomplished by computing summary z-scores for each pain modality (e.g. heat pain threshold, ischemic pain threshold, heat pain ratings) for basal pain sensitivity. Z-scores for each pain modality were then averaged to derive an overall mean z-score for each pain modality. The modality z-scores were computed based on the following individual variables: heat z-score = mean of HPTh, HPTo, Ratings at 49, Ratings at 52; pressure z-score = mean of PPT at the masseter, trapezius, and ulna; ischemic z-score = mean of IPTh, IPTo, Summed Intensity Ratings, Summed Unpleasantness Ratings. Variables involving pain ratings were reverse scored such that for all z-scores higher values indicate lower pain sensitivity. Similarly, modality-specific z-scores for analgesic difference scores were also created. That is, each pre-post drug difference score was converted to a z-score, and then modality-based analgesic z-scores were computed. Because ceiling effects for HPTo and IPTo produced significant missingness, these tolerance measures were excluded from the computation of analgesic z-scores. This variable reduction approach is empirically justified, based on our previous factor analyses of both basal pain responses [26] and analgesic responses [30].

A series of 2×2 mixed-model analyses of variance (ANOVA) was used to test for differences on z-scores for each of the three pain modalities for basal pain sensitivity, morphine analgesia, and pentazocine analgesia. Cohen's d is reported for menstrual cycle effects on analgesia. [12]. The independent variables were group (NOC vs. OC) × menstrual cycle phase (follicular vs. luteal phase). Pearson correlations were used to test for significant associations between hormone levels and z-scores for each of the three pain modalities for basal pain sensitivity, morphine analgesia, and pentazocine analgesia. All analyses were performed using SAS statistical software. As this is the first study in humans to systematically examine menstrual cycle effects in analgesia, a critical value of .05 was used.

3 - Results

3.2 - Sample Characteristics

Demographic characteristics with the days when the pain testing was performed during the menstrual cycle are presented in Table 2. Regarding ethnicity, 58.5% of participants self-identified as Caucasian, 20% were Hispanic, 9.2% African American, 9.2% Asian/Pacific Islander, and the remainder identified as “Other” (3.1%). OC women were younger than NOC women [$F(1,61)=4.43$, $p=0.039$], and had a slightly lower body mass index [$F(1,61)=3.32$, $p=0.078$].

3.3 - Hormone Assays

Mean hormone assay values fell within expected reference ranges (Table 3). Follicular phase estradiol-17 β (E2) was similar for OC and NOC women; however, as expected, NOC women had significantly higher E2 than OC women during the luteal phase [$F(1,61)=22.2$, $p < .0001$, $d = 1.21$]. This large effect size is consistent with expected hormone levels for NOC and OC women during luteal phases. Because there was a range of days within each cycle phase, which could influence estrogen levels, correlations between day of cycle phase and hormone values were computed. During the follicular phase for NOC women, cycle day

was positively correlated with E2, such that NOC women tested later in the follicular phase showed higher E2 levels (Cycle 1: $r = 0.35$, $p = .065$; Cycle 2: $r = 0.59$, $p = .001$). E2 was not correlated with day of cycle for OC women. Women (OC and NOC) in luteal phases had significantly lower testosterone (T) levels than during follicular phases ($p < .05$, $d = .40$ and $.36$ respectively). OC women had significantly lower luteal phase T levels than NOC women [$F(1,61) = 10.79$, $p = .002$, $d = .78$]. For NOC women, progesterone (P) levels fell within expected reference ranges for both follicular and mid-luteal phases, confirming that the NOC sample underwent pain testing at appropriate menstrual intervals. Progesterone (P) levels were not assayed for OC women because menses onset was predictably triggered by withdrawal of exogenous progesterone, and was timed based on pill packet days.

3.4 - Basal Pain Sensitivity

Pre-drug pain responses did not significantly differ on active drug days versus saline days (p 's $> .10$); therefore, results were averaged across the two sessions for each cycle phase (Table 4). Analysis of pre-drug z-scores for each pain modality revealed a significant cycle phase X group interaction for heat pain [$F(1,61) = 4.14$, $p = 0.046$]. Analysis of simple effects revealed no significant comparisons [NOC: $F(1,27) = 3.16$, $p = 0.087$; OC: $F(1,34) = 1.51$, $p = 0.228$], thus the interaction was due to the fact that heat pain sensitivity was greater in the follicular vs. luteal phase for NOC women, while the reverse pattern emerged for OC women. A significant main effect of group emerged for pressure pain [$F(1,61) = 4.76$, $p = 0.03$], with NOC women showing lower pain thresholds than OC women. No significant effects emerged for ischemic pain [Group: $F(1,61) = 0.16$, $p = 0.691$; Phase: $F(1,61) = 0.00$, $p = 0.986$; Group X Phase: $F(1,61) = 0.02$, $p = 0.877$].

3.5 - Relationship between Basal Pain Sensitivity and Hormone Levels

Pearson correlations were conducted between basal pain sensitivity Z scores during follicular and luteal phases and corresponding hormone levels. For women taking oral contraceptives, there were no significant correlations between heat, ischemic or pressure pain sensitivity and hormone levels. For NOC women, luteal E2 was positively correlated with ischemic pain sensitivity Z scores ($r = 0.54$, $p = 0.003$), indicating that higher E2 level was associated with lower ischemic pain sensitivity. No other significant correlations between hormone levels and basal pain responses emerged.

3.6 – Morphine and Pentazocine Analgesia

Morphine change scores are presented in Table 5. There were no significant group (i.e., NOC vs. OC) differences in morphine analgesic scores. A Group X menstrual cycle effect emerged for morphine analgesia on ischemic pain [$F(1,29) = 6.18$, $p = 0.019$]. Analysis of simple effects revealed a significant cycle phase effect among NOC women [$F(1,13) = 12.62$, $p = 0.004$], while no cycle phase effect was present for OC women [$F(1,16) = 2.0$, $p = 0.177$]. Inspection of the means reveals that NOC women showed greater morphine analgesia for ischemic pain during the follicular vs. the luteal phase. No significant group or cycle phase effects on pentazocine analgesia were observed (see Table 6).

3.7 - Relationship between Analgesic effect and Hormone Levels

Pearson correlations were used to determine associations of morphine and pentazocine analgesia with hormone levels during follicular and luteal menstrual phases (Tables 7 and 8, respectively). For NOC women higher follicular E2 levels were associated with greater morphine analgesia on pressure pain assays, and higher luteal testosterone predicted lower morphine analgesia for heat pain. Hormone levels were not associated with any measure of pentazocine analgesia among NOC women.

Among OC women, follicular E2 was associated with lower morphine and greater pentazocine analgesia on ischemic pain assays (see Table 7 and 8). Also, higher luteal E2 and luteal testosterone (T) levels were associated with greater pentazocine analgesia on ischemic pain for OC women. Higher follicular T was associated with greater morphine analgesia on pressure pain. (see Table 8).

3.8 - Relationship Between Side effects and Menstrual Cycle

A significant cycle phase by group interaction emerged for total side effects from morphine [$F(1,29)=4.3, p = .035$]. Analysis of simple effects revealed that there was no cycle phase effect on side-effects for OC women [$F(1,16)=0.04, p = 0.848$]; however NOC women showed significantly more morphine side-effects during the follicular phase versus the luteal phase [$F(1,13)=7.0, p = .02$](see Table 9). No significant group or cycle phase effects were observed for pentazocine side effects.

3.9 - Associations Between Age of Menarche, Basal Pain Sensitivity and Analgesic Responses

Correlations between age of menarche and pain and analgesic responses are presented in Table 10. For NOC women, later menarche was associated with lower heat pain sensitivity during both the follicular and luteal phases. There were no significant correlations between age of menarche and basal pain sensitivity for women taking oral contraceptives.

For NOC women during the follicular phase, later age of menarche was associated with lower heat pain sensitivity, greater morphine analgesia on ischemic pain but reduced morphine analgesia for heat pain. Also, later menarche predicted greater pentazocine analgesia for heat pain. During the luteal phase, later menarche was associated with lower heat pain sensitivity and predicted greater morphine analgesia for ischemic pain among NOC women.

For OC women during the follicular phase, later menarche was related to greater morphine analgesia on pressure pain but lower pentazocine analgesia tested against pressure pain. During the luteal phase in OC women, later menarche was correlated with greater morphine analgesia for heat pain.

4 - Discussion

Human and non-human animal studies have revealed considerable, if not consistent, sex differences in response to exogenous analgesics [14,16]. Compared to females, male rats exhibit more robust opioid analgesia [7,13,29,33,42]; however, in human clinical studies, females experience greater analgesia from mixed action opioid agonist-antagonists, such as pentazocine, butorphanol, and nalbuphine [18,19,20,21]. These results have not been replicated in experimental pain models [14,40,49]. For morphine, a recent meta-analysis found that both clinical and experimental studies indicate greater morphine analgesia for women than men, though effects sizes are modest. [40] Gonadal hormones may contribute to sex differences in responses to opioid analgesics, as demonstrated in pre-clinical studies [11,25]. However, limited research has examined the influence of menstrual cycle and gonadal hormones on opioid analgesia in healthy women.

4.1 - Basal Pain Responses: Menstrual cycle and OC status

Minimal menstrual cycle effects emerged for basal pain responses, which is somewhat inconsistent with previous findings of greater sensitivity in the luteal versus the follicular phase. However, effects from previous studies have typically been small and inconsistent [39,41]. Relatively few previous studies have included women taking OC. Goolkasian [23]

reported menstrual cycle influences on heat pain only among NOC women and not in those taking OCs. However, more recent findings have shown no menstrual cycle effects on pressure pain [49] or cold pain [31] among OC or NOC women. In contrast, LeResche and colleagues [34] demonstrated menstrual cycle influences on clinical pain among women with temporomandibular disorders, with both NOC women and those taking OCs reporting greater pain premenstrually and during menses. Regarding exogenous hormone use, our findings revealed greater sensitivity to pressure pain among OC women, which contrasts with recent findings that OC women showed higher pressure pain thresholds [50]. However, others have reported higher cold pain thresholds among NOC women compared to those using OC. [31]. Limited significant associations between circulating hormone levels and pain responses emerged. Specifically, among NOC women higher levels of estrogen during the luteal phase were associated with reduced luteal phase ischemic pain sensitivity. Thus, our findings demonstrate modest hormonal associations with basal pain sensitivity.

4.2 - Hormonal Influences on Morphine and Pentazocine Responses

The present results reveal menstrual cycle effects on morphine but not pentazocine analgesia. Morphine analgesia based on the ischemic pain procedure was greater during the follicular than the luteal phase for NOC women, while no significant cycle effect emerged for OC women. That this menstrual cycle effect emerged for ischemic pain is noteworthy, as the ischemic pain task represents our most sensitive analgesic assay [15,16]. No differences in analgesic responses emerged as a function of OC status. Several associations between circulating hormone levels and analgesic responses were observed, but no clear pattern is discernable. For NOC women, higher follicular E2 was related to greater morphine analgesia for pressure pain. For OC women, higher follicular estrogen predicted lower morphine and pentazocine analgesia on the ischemic task, and greater follicular testosterone was related to greater morphine analgesia for pressure pain. Overall, these findings suggest that if there are hormonal influences on opioid analgesia, they are not consistent across pain assays and are of relatively low magnitude.

Gonadal hormonal influences on opioid analgesia in humans remain poorly understood, but substantial evidence demonstrates interactions between the gonadal axis and the opioid system [2]. An abundant preclinical literature has examined hormonal influences on opioid antinociception [10,14,25]. Craft [11] has recently suggested that chronic estradiol exposure reduces morphine analgesia in females, due in part to estradiol-induced reductions in brain μ -opioid receptor availability. However, findings from primates and humans are more limited and less consistent in revealing sex differences and hormonal influences on opioid analgesia. For example, treatment with luteal phase levels of progesterone plus estradiol in ovariectomized rhesus monkeys enhanced kappa-opioid analgesia (U50,488), but had little effect on analgesic responses to morphine, butorphanol or nalbuphine [36]. Also, Zubieta [53] and colleagues have demonstrated greater pain-induced activation of brain μ -opioid receptors among men than women [45,53], but administration of exogenous estrogen increased pain-related μ -opioid receptor binding among women.

4.2 - Association of Age of Menarche with Pain and Analgesia

One novel finding from our data relates to associations between age of menarche and pain and analgesic responses. Specifically, for NOC women, later menarche predicted lower heat pain sensitivity and more robust morphine analgesia on ischemic pain during the follicular and luteal phases. Also, during the follicular phase later menarche was associated with lower morphine analgesia but greater pentazocine analgesia for heat pain. Among OC women in the follicular phase, later menarche predicted more robust morphine analgesia but poorer pentazocine analgesia tested against pressure pain, and during the luteal phase later menarche predicted greater morphine analgesia for heat pain. One could speculate that a

younger age of menarche is associated with greater cumulative exposure to ovarian hormones which may reflect the organizational effects of estrogen. Consistent with our findings, age of menarche has been associated with menstrual pain, such that women with an earlier age of menarche showed increased risk for menstrual pain [52]. Also, early age of menarche has been associated with chronic upper extremity pain [51] and chronic pelvic pain [33]. Thus, a later menarche age, which may indicate reduced cumulative gonadal hormone exposure, may be protective against pain and may confer increased analgesic sensitivity. Our findings indicate that any influences of age of menarche on basal pain sensitivity and/or opioid analgesia may vary depending on several factors, including OC use, pain assay, and the specific opioid analgesic administered. Regarding basal pain sensitivity, menarche was associated with heat pain sensitivity only among NOC women. We can only speculate as to why this association only emerged in NOC women. However, it seems plausible that OCs represent an exogenous hormonal intervention in adulthood, which may alter the impact of earlier organizational hormonal events (such as age of menarche). Of course, given our limited sample size, additional evidence is needed to confirm and extend these findings.

4.3 - Hormonal Associations with Side Effects

Limited information is available regarding menstrual cycle influences on opioid-related side effects. In the present study, NOC women showed significantly more side effects in response to morphine during the follicular than the luteal phase. While previous studies have not examined menstrual cycle influences on opioid side effects specifically, some information regarding post-operative nausea and vomiting (PONV) is available. Sener et al [43] reported no differences in post-operative opioid requirements as a function of menstrual cycle phase; however, use of anti-emetics and non-opioid analgesics was greater in the luteal phase. Also, nausea and vomiting occurred with greater frequency during luteal and follicular phases compared to perimenstrual and ovulatory phases. Others have reported greater PONV during the luteal [27] or combined ovulatory/luteal phase [28]. In contrast, Ramsay and colleagues [38] found greater PONV during days 9–15 of the menstrual cycle among women using OCs but not in NOC women. Taken together, the evidence suggests that hormonal fluctuations may influence side effects, but the direction and magnitude of these effects are inconsistent across studies.

The results of our study should be interpreted in light of its limitations. Specifically, given the intensity of the experimental procedures, our sample size is modest, which may have prevented detection of significant effects. Also, participants received only one dose of morphine or pentazocine, precluding examination of dose-response functions. Also, while our 17β -estradiol assay was a sensitive measure of endogenous estrogens, it does not detect most estrogens used in OC preparations and may show cross-reactivity with the synthetic estrogens found in OCs; therefore, E2 levels observed in the OC group may not accurately reflect their exposure to estrogens. The use of experimental pain models was necessary in order to systematically study menstrual cycle influences in a repeated measures design; however, these findings may not be relevant for opioid analgesia in the clinical setting. Moreover, for pain tolerance measures, ceiling effects required that these measures be excluded from the computation of analgesic z-scores. Also, while the number of statistical tests was reduced through variable reduction, we still conducted multiple uncorrected statistical tests. Given that this is the first study in humans to systematically examine menstrual cycle effects in analgesia, we believe that Type II errors are of greater concern in this situation, because falsely accepting the null hypothesis may prematurely discourage investigators from further examining the topic. However, it should be acknowledged that the significant findings reported here would not have survived correction for multiple tests.

In conclusion, we report limited influences of menstrual cycle and gonadal hormones on baseline pain responses; however in NOC women, morphine analgesia for ischemic pain and morphine side effects were significantly greater in the follicular versus the luteal phase. Also, later menarche was associated with lower heat pain sensitivity and greater morphine analgesia for ischemic pain in NOC women. These findings suggest that sex hormones may influence opioid responses; however, the effects vary across medications and pain modalities and are likely to be modest in magnitude. Future investigation of hormonal influences on analgesic responses in the clinical setting would be valuable.

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Table 1

Order of Drug Administration Across Experimental Sessions

	Follicular 1	Luteal 1	Follicular 2	Luteal 2
Order 1	Active Drug	Placebo	Placebo	Active Drug
Order 2	Active Drug	Active Drug	Placebo	Placebo
Order 3	Placebo	Placebo	Active Drug	Active Drug
Order 4	Placebo	Active Drug	Active Drug	Placebo

Table 2

Demographic characteristics and days during menstrual cycle when testing occurred, Mean (SD)

	NOC Women (n = 28)	OC Women (n = 35)
Age (yrs)	24.75 (6.73)	22.11 (2.78)
Body mass index	24.89 (4.90)	23.11 (2.91)
Average # days after menses for follicular phase opioid testing	6.71 (2.09)	7.59 (2.03)
Average # days after menses for follicular phase saline testing	6.75 (2.05)	7.65 (1.77)
Average # days before menses for luteal phase opioid testing	7.32 (3.06)	6.76 (2.31)
Average # days before menses for luteal phase saline testing	7.75 (5.02)	6.15 (2.02)
Age of menarche (yrs)	12.61 (1.50)	12.77 (1.46)

In bold significant group difference, $p < .05$

Table 3

Hormone levels by menstrual cycle phase and contraceptive status.

	NOC Women (n = 28)		OC Women (n = 35)	
	Assay Value Mean (SD)	Reference Range*	Assay Value Mean (SD)	Reference Range**
Follicular Estradiol (pg/mL)	45.41 (20.22)	ND - 160 pg/mL	42.47 (34.39)	ND - 102 pg/mL
Luteal Estradiol (pg/mL)	124.81(93.87)	27 – 246 pg/mL	35.85 (54.30)	ND - 102 pg/mL
Follicular Testosterone (ng/dL)	76.48 (29.84) ^a	ND - 81 ng/dL	60.61 (46.62) ^a	ND - 81 ng/dL
Luteal Testosterone (ng/dL)	67.02 (27.54)^a	ND - 81 ng/dL	47.64(19.20)^a	ND - 81 ng/dL
Follicular Progesterone (ng/mL)***	0.43 (0.19) ^a	ND - 0.98 ng/mL	N/A***	N/A***
Luteal Progesterone (ng/mL)***	7.15 (2.81) ^a	6.0 – 24 ng/mL	N/A***	N/A***

In bold significant group difference, $p < .05$.^a indicates significant phase difference, $p < .05$.

NOC women had significantly higher E2 than OC women during the luteal phase [F (1,61)=22.2, $p < .0001$, $d = 1.21$]. During the follicular phase for NOC women, cycle day was positively correlated with E2, such that NOC women tested later in the follicular phase showed higher E2 levels (Cycle 1: $r = 0.35$, $p = .065$; Cycle 2: $r = 0.59$, $p = .001$). E2 was not correlated with day of cycle for OC women. Women (OC and NOC) in luteal phases had significantly lower testosterone (T) levels than during follicular phases ($p < .05$, $d = .40$ and $.36$ respectively). OC women had significantly lower luteal phase T levels than NOC women [F (1,61)=10.79, $p = .002$, $d = .78$]. For NOC women, progesterone (P) levels fell within expected reference ranges for both follicular and mid-luteal phases, confirming that the NOC sample underwent pain testing at appropriate menstrual intervals.

* Central 95% as reported on package insert

** 90% Range as reported on package insert.

*** Progesterone levels were used to confirm luteal phase for NOC women, and were not assayed for OC women.

Table 4Basal Pain Responses^{*}, Mean (SD)

	NOC women (n=28)		OC women (n=35)	
	Follicular	Luteal	Follicular	Luteal
Heat Pain	0.10 (0.97)	0.17 (0.88)	-0.08 (0.75)	-0.14 (0.76)
Pressure Pain	0.24 (0.75)	0.28 (0.73)	-0.19 (0.96)	-0.22 (0.95)
Ischemic Pain	0.05 (0.90)	0.05 (0.90)	-0.04 (0.94)	-0.04 (0.94)

In bold significant group difference, $p < .05$. A significant main effect of group emerged for pressure pain, with NOC women showing lower pain thresholds than OC women [$F(1,61)=4.76$, $p = .03$].

* Score converted to Z scores for analyses. Higher scores reflect lower pain sensitivity.

Table 5

Analgesic Z-Scores* for Morphine, Mean (SD)

	NOC women (n=14)			OC women (n=18)		
	Follicular	Luteal	Cohen's D	Follicular	Luteal	Cohen's D
Heat Pain	-0.24 (0.70)	0.01 (0.60)	-0.38	0.19 (0.78)	-0.006 (0.99)	0.22
Pressure Pain	-0.04 (0.77)	0.16 (0.63)	-0.29	0.03 (0.66)	-0.013 (0.88)	0.06
Ischemic Pain	0.26 (1.29)	-0.05 (1.18)	0.25	-0.20 (0.67)	-0.04 (0.86)	-0.21

A Group X menstrual cycle effect emerged for morphine analgesia on ischemic pain [$F(1,29)=6.18, p=0.019$]. There was a significant cycle phase effect among NOC women [$F(1,13)=12.62, p=0.004$]. Bold indicates significant menstrual cycle phase differences. The significant menstrual cycle effect for morphine ischemic analgesia may be surprising, given the small effect size for this difference. This occurs because the F statistic corrects for correlation between the two repeated measures, and because this correlation is higher for ischemic than heat or pressure pain, a smaller effect is found to be statistically significant (i.e. with higher correlation, there is greater statistical power).

* Higher scores reflect greater analgesia.

Table 6

Analgesic Z-Scores* for Pentazocine, Mean (SD)

	NOC women (n=14)			OC women (n=17)		
	Follicular	Luteal	Cohen's D	Follicular	Luteal	Cohen's D
Heat Pain	0.002 (0.67)	0.02 (0.93)	-0.02	-0.002 (0.98)	-0.01 (0.64)	0.01
Pressure Pain	0.10 (0.93)	0.11 (0.98)	-0.01	-0.08 (0.66)	-0.09 (0.70)	0.01
Ischemic Pain	0.13 (1.06)	0.28 (1.30)	-0.13	-0.11 (0.97)	-0.23 (0.61)	0.15

No significant group or cycle phase effects on pentazocine analgesia were observed.

* Higher scores reflect greater analgesia

Table 7
Correlations Between Morphine Analgesic Z-Scores and Hormone Levels, *r* (*p*)

	NOC Women (<i>n</i> = 14)			OC Women (<i>n</i> = 18)		
	Heat pain	Ischemic pain	Pressure pain	Heat pain	Ischemic pain	Pressure pain
Follicular Estradiol	-0.32 (.260)	-0.06 (.848)	0.78 (.001)	-0.02 (.937)	-0.69 (.002)	-0.09 (.718)
Follicular Testosterone	-0.02(.951)	-0.17 (.573)	-0.131 (.657)	0.26(.294)	0.01 (.961)	0.51 (.030)
Follicular Progesterone	0.05 (.870)	-0.52 (0.058)	-0.20 (.499)	N/A	N/A	N/A
Luteal Estradiol	-0.122 (.677)	-0.13 (0.650)	-0.31 (.287)	0.16 (0.543)	-0.41 (.105)	0.09 (.739)
Luteal Testosterone	-0.62 (.017)	-0.12 (0.672)	0.04 (.886)	-0.08 (0.766)	0.02 (.960)	-0.42 (.091)
Luteal Progesterone	-0.21 (.464)	0.03 (0.928)	-0.40 (.151)	N/A	N/A	N/A

For NOC women higher follicular E2 levels were associated with greater morphine analgesia on pressure pain assays, and higher luteal testosterone predicted lower morphine analgesia for heat pain. For OC women, follicular E2 was associated with lower morphine on ischemic pain and follicular testosterone was associated with greater morphine analgesia on pressure pain. In bold significant $p < 0.05$. Higher scores reflect higher analgesia.

Table 8Correlations Between Pentazocine Analgesic Z-Scores and Hormone Levels, r (p)

	NOC Women ($n = 14$)			OC Women ($n = 17$)		
	Heat pain	Ischemic pain	Pressure pain	Heat pain	Ischemic pain	Pressure pain
Follicular Estradiol	0.24 (.415)	-0.06 (.849)	-0.25 (.384)	0.30 (.247)	0.52 (.032)	0.39 (.123)
Follicular Testosterone	0.50 (.070)	0.19 (.519)	-0.20 (.495)	-0.33 (.199)	-0.05 (.850)	-0.08 (.747)
Follicular Progesterone	0.26 (.367)	0.14 (.641)	0.10 (.733)	N/A	N/A	N/A
Luteal Estradiol	0.29 (.306)	0.19 (.516)	-0.06 (.841)	0.47 (.060)	0.56 (0.02)	0.22 (.397)
Luteal Testosterone	0.17 (.552)	0.12 (.681)	0.10 (0.746)	0.18 (.496)	0.59 (.012)	0.44 (.080)
Luteal Progesterone	-0.22 (.443)	0.18 (.532)	-0.23 (.420)	N/A	N/A	N/A

For OC women, follicular E2, luteal E2 and luteal T was associated with greater morphine on ischemic pain. In bold significant $p < 0.05$. *

Table 9

Total Side Effects for Morphine and Pentazocine as a Function of Cycle Phase*

	NOC women (n=14)		OC women (n=17)	
	Follicular	Luteal	Follicular	Luteal
Morphine ^{a,b,c}	1.79 (1.19)	0.79 (0.89)	0.88 (0.93)	0.94 (1.09)
Pentazocine	1.5 (1.83)	1.36 (1.5)	1.76 (1.71)	1.47 (1.70)

NOC women showed significantly more morphine side-effects during the follicular phase versus the luteal phase [$F(1,13)=7.0$, $p = .02$].

^aPhase $p<0.05$,

^bOral Contraceptive group $p>0.05$,

^cPhase X Oral Contraceptive group $p<0.05$.

Bold shows that the interaction was driven by significantly greater side effects in the follicular vs. luteal phase for NOC but not OC women. $p<0.05$

Table 10

Correlations Between Age of Menarche and Basal Pain Sensitivity and Analgesic Responses

	NOC Women		OC Women	
	Follicular Phase	Luteal Phase	Follicular Phase	Luteal Phase
Basal Pain Responses				
Heat	0.375 (0.049)	0.427 (0.023)	−0.169 (0.331)	−0.116 (0.509)
Pressure	0.187 (0.341)	0.190 (0.333)	−0.002 (0.673)	−0.168 (0.341)
Ischemic	0.113 (0.566)	0.099 (0.617)	−0.199 (0.253)	−0.086 (0.623)
Morphine Analgesia				
Heat	−0.552 (0.041)	−0.437 (0.092)	0.040 (0.876)	0.536 (0.027)
Pressure	−0.129 (0.660)	0.438 (0.117)	0.522 (0.025)	0.321 (0.210)
Ischemic	0.774 (0.001)	0.733 (0.003)	0.158 (0.531)	−0.042 (0.872)
Pentazocine Analgesia				
Heat	0.559 (0.038)	0.277 (0.338)	−0.171 (0.511)	−0.331 (0.194)
Pressure	0.324 (0.259)	0.128 (0.663)	−0.550 (0.022)	−0.260 (0.314)
Ischemic	0.187 (0.522)	0.136 (0.642)	−0.126 (0.629)	−0.278 (0.280)

For NOC women during the follicular phase, later age of menarche was associated with lower heat sensitivity, greater morphine analgesia on ischemic pain but reduced morphine analgesia for heat pain. Also, later menarche predicted greater pentazocine analgesia for heat pain. During the luteal phase, later menarche was associated with lower heat pain sensitivity and predicted greater morphine analgesia for ischemic pain among NOC women. For OC women during the follicular phase, later menarche was related to greater morphine analgesia on pressure pain but lower pentazocine analgesia tested against pressure pain. During the luteal phase in OC women, later menarche was correlated with greater morphine analgesia for heat pain. In bold significant $p < 0.05$.