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Daily Fluctuations of Progesterone and Testosterone Are Associated With Fibromyalgia Pain Severity

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Abstract: The purpose of this longitudinal blood sampling study was to examine relationships between sex hormones and fibromyalgia pain. Eight women meeting case definition criteria for fibromyalgia provided venous blood samples and reported their fibromyalgia pain severity over 25 consecutive days. All women exhibited normal menstrual cycles and were not taking oral contraceptives. Cortisol, and the sex hormones estradiol, progesterone, and testosterone, were assayed from serum. A linear mixed model was used to determine if fluctuations of sex hormones were associated with changes in pain severity. In the entire sample, day to day changes in progesterone ($P = .002$) as well as testosterone ($P = .015$) were significantly and inversely correlated with pain severity. There was no relationship between estradiol and pain ($P = .551$) or cortisol and pain ($P = .633$). These results suggest that progesterone and testosterone play a protective role in fibromyalgia pain severity. Sex and other hormones may serve to increase as well as decrease fibromyalgia pain severity.

Perspective: ***Sex hormones fluctuate normally in women with fibromyalgia, but may still contribute to pain severity.***

Key words: *Fibromyalgia, pain, progesterone, testosterone, longitudinal.*

Fibromyalgia, a chronic pain disorder of unknown etiology, disproportionately affects women over men.[49] The condition is characterized by diffuse musculoskeletal pain, increased sensitivity to pressure at soft tissues, profound fatigue, and self-reported cognitive disruption.[46] One notable feature of fibromyalgia is that the symptom severity can vary markedly over short periods of time, with severity on any particular day being largely unpredictable.[43] The lack of predictability adds to the debilitating nature of the disorder, because planning future activities can be difficult for sufferers.

The higher prevalence of fibromyalgia in women shows biological sex to be an important consideration in the disorder.[30] There is, however, only limited evidence supporting a link between sex hormones and fibromyalgia incidence or severity. The incidence of pediatric fibromyalgia has been shown to be similar in both genders until the onset of puberty, when the incidence rate increases in girls.[26] In addition, it has been posited that the sex hormone testosterone may provide a protective effect against these types of chronic pain disorders,[7] with transdermal testosterone gel significantly reducing fibromyalgia pain in women.[45]

There are several mechanisms by which sex hormones could affect the experience of pain, which have been covered extensively in recent review articles.[34,41] These mechanisms include effects on: peripheral nociceptors,[17] central nociceptive processing,[28] spinal inflammation,[39] central microglia,[35] affective brain systems that modulate pain,[2,47] and opioid systems.[25,40]

Testosterone[5] and progesterone[8] are generally shown to be associated with lower experience of pain. Progesterone in particular appears to have central anti-inflammatory, neuroprotective, and analgesic effects.[11,16,22] Progesterone may also have local effects as it has been shown to be superior to corticosteroids in treating carpal tunnel syndrome.[32]

Estradiol effects on nociception and pain are more complex. Estradiol has been shown in many cases to be

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antinociceptive,[12,44] as well as pronociceptive;[20,31] the latter effect being mediated partially by upregulation of vanilloid-1 and anoctamin-1 in primary sensory neurons.[48] It is likely that estradiol metabolites (estradiol-3-glucuronide and estradiol-17-glucuronide) have complex effects on pain.[21] Also, specific estrogen receptor subtypes may play different roles in the relative pro- and antinociceptive properties of estrogen.[9]

To test if sex hormones affect fibromyalgia pain, we conducted this daily sampling study in 8 normally-cycling women with fibromyalgia. Sex hormones (estradiol, progesterone, and testosterone) and self-reported pain were assessed daily during the 25-day protocol. We tested 3 specific hypotheses in this study: first, progesterone would be negatively associated with pain. Second, testosterone would be negatively associated with pain. Third, estradiol would be positively associated with pain. Convincing cases for either a positive estradiol-pain relationship or negative relationship could be made and supported by the literature. Although we hypothesized a positive relationship between estradiol and pain, all tests were conducted 2-tailed. In addition, because previous literature has shown a positive relationship between cortisol levels and fibromyalgia pain,[15] we also assayed cortisol at all time points. To our knowledge, this is the first study to use daily hormone measurements to test the relationship between sex hormones and fibromyalgia severity.

Methods

Participant Selection

All procedures were approved by the University of Alabama at Birmingham (UAB) institutional review board. This study was completed between June 2015 and February 2017. Potential participants were identified via the laboratory's database of 1,200 local individuals who had expressed an interest in research participation. Individuals from the online database were selected for an additional phone screening if they were female, were within 1 hour driving distance to the laboratory, were between the ages of 18 and 65 years, met the American College of Rheumatology 2010 self-reported fibromyalgia case definition criteria,[46] and indicated an average daily pain of at least 3 of 10. Pain was assessed with a single item, "How would you rate your average daily pain on a scale from 0 (no pain at all) to 10 (worst possible pain)." Individuals did not pass the online screening stage if they indicated a diagnosis or medications for any significant medical conditions (eg, cancer, heart disease, diabetes, liver disease, neurological disorders, or neurodegenerative diseases). Individuals were also not considered if they indicated a diagnosis or medications for any autoimmune or rheumatologic disorder.

Twenty individuals from the online database were contacted via phone for a second screening, conducted by a member of the research team. In the phone screening, individuals were excluded from participation if they indicated any blood clotting disorders or other contraindications for phlebotomy, or if reporting a hysterectomy, current pregnancy, or active plans to become pregnant. Participants must also have indicated having normal and predictable menstrual cycles. Medications were reviewed and individuals were excluded if taking blood thinning, anti-inflammatory, rheumatologic, antibiotic, hormone-based contraceptive, opioid analgesic medications, or any hormone therapies. During the phone screening, the study was also described in depth and individuals were asked whether they were interested in participating.

Nine women who passed the screenings and were interested in participation were invited to a laboratory screening session at the Clinical Research Unit at UAB. At the screening visit, participants provided written, informed consent and provided a blood sample for screening tests. Participants were excluded from the study if they had abnormal renal function, tri-iodothyronine, thyroxine, thyroxin binding globulin, thyroid stimulating hormone, complete blood count with differential, or 25-hydroxy vitamin D. Participants were also excluded if they presented with detectable rheumatoid factor or antinuclear antibody, or had an erythrocyte sedimentation rate >60 mm/h. All screening blood results were reviewed by Dr. Timothy Ness (UAB Department of Anesthesiology and Perioperative Medicine).

During the screening visit, participants also completed several self-report questionnaires that were not used in analyses, but instead used to capture additional exclusionary criteria. Depressed mood and anxiety was measured with the Hospital Anxiety and Depression Scale.[50] The depressed mood and anxiety subscales yield possible scores of 0 to 7 (normal), 8 to 10 (mild), 11 to 14 (moderate), and 15 to 21 (severe), with scores >15 being exclusionary. Pain severity was assessed with the Brief Pain Inventory.[27] The Brief Pain Inventory provides scores for pain intensity (range = 0–10) and pain interference (range = 0–10). A minimum of 3 for pain intensity was required for participation. Participants also completed the Fibromyalgia Assessment Form[46] that yields a widespread pain index (range = 0–19) and a symptom severity score (range = 0–12). Finally, participants completed a clinician-guided Mini International Neuropsychiatric Interview,[37] and were excluded if meeting clinician criteria for major depressive disorder. All selected screening tools are commonly used in the pain literature and have shown acceptable reliability and validity.

Sample Size

Sample size was based on a minimal correlation of interest of r = .3 and desired power of 95%. With a *P* value threshold of .05, 25 repeated measures per individual, and assumed repeated measures correlation of .5, 8 individuals would be needed to achieve 95% power. Although our main analyses (see the Statistical Analyses section) examined correlations in participant-nested data, the power analyses provided an approximation of required sample size. However, some features of the acquired data (for example, autocorrelation) were not accounted for in the sample size calculation, because

power calculators for linear mixed models are not adequately developed at this time.

Study Procedures

The study was a 6-week observational project, including a 2-week baseline period followed by a 25-day daily sample collection period. Immediately after the on-site screening, participants started an observational baseline period of 2 weeks. Daily symptoms were obtained through a questionnaire delivered on Android-based tablets running Qualtrics Survey Software (Qualtrics; Provo, UT). Fibromyalgia pain severity was rated on a 0 to 100 visual and numerical scale with the question, "Overall, how severe has your pain been today?" The far left of the scale was anchored at "no pain" and the far right anchored at "severe pain." The daily questionnaire, completed at the end of the day, also contained items to measure fatigue, mood, stress, sleep quality, physical activity, and gastrointestinal symptoms. Those items were not analyzed in this study. To reduce bias, participants were not informed of the exact blood tests to be conducted, and were not informed of the study hypotheses until their completion of the protocol. During the study period, participants were asked to avoid taking over-the-counter analgesics, and to report any such use on the daily questionnaire.

At the end of the baseline period, participants began the daily blood draws. Laboratory visits were scheduled for 25 consecutive days, including weekends. Appointments were held at the same time each day for the individual, to minimize diurnal effects. Trained phlebotomists or research nurses drew 8 mL of blood with a 21- to 23-gauge butterfly needle into serum separating tubes (BD Vacutainer; Becton, Dickinson and Co, Franklin Lakes, NJ). Using standard processing protocols, the blood samples were kept at room temperature for 30 minutes and then centrifuged at 1,300*g* for 15 minutes. The serum layer was extracted and stored at -80°C. Throughout this phase of the study, participants continued to complete their daily pain reports. Participants were paid $50 for each laboratory visit.

Sample Processing

Sex hormone concentrations were analyzed in serum samples by the Metabolism Core at UAB, under the direction of Dr. Barbara Gower. Total progesterone, estradiol, and testosterone assays were conducted using standard assay manufacturer protocols using an automated immunoassay analyzer (AIA-900; Tosoh Bioscience, South San Francisco, CA), using the fluorescent enzyme immunoassay method. Cortisol was also assayed in sera samples using the same procedures. Laboratory technicians were blinded to the study protocol.

Statistical Analyses

The 3 primary hypotheses were tested in a single, multivariate linear mixed model using SPSS version 24 (IBM Corp, Armonk, NY), a restricted maximum likelihood estimation procedure, and an autoregressive covariance structure. Daily pain was the dependent variable. Daily values of all 3 sex hormones (estradiol, testosterone, progesterone, and cortisol) were entered as independent predictors. Data were nested according to subject, with study day as the repeated-measures index variable. All time-series data were person-centered (z-scored) to remove the influence of between-subject differences.

A Bonferroni adjustment was used for the 3 main predictors to hold the overall chance of error to .05, yielding a *P* value threshold of .017. The decision to use a correction for multiple comparisons was made after data collection and was therefore not accounted for in the sample size calculation performed before data collection.

Results

One individual was excluded at baseline because of abnormally elevated erythrocyte sedimentation rate values. Eight individuals, aged 33 ± 8.6 years, met all criteria for participation. Average depression symptom severity (6.9, SD = 3.9) was in the normal range, as was anxiety (5.6, SD = 2.4). The group showed moderately high pain severity (6.8, SD = 3.8) and moderate pain interference (4.6, SD = 1.5). The Fibromyalgia Assessment Form widespread pain index was 10.3 (SD = 4.6) and the somatosensory scale was 9.6 (SD = 2.5). No participants met criteria for major depressive disorder in the Mini International Neuropsychiatric Interview. One participant took pregabalin for the duration of the study, and 1 took gabapentin (Table 1). No participants reported use of over-the-counter analgesics or anti-inflammatories during the study. Table 1 shows additional demographic information on the final study cohort. On visual review of individual-level sex hormone plots, all participants showed the expected peaks of estrogen and progesterone, suggesting normal menstrual cycles. Across the 25-day study period, average progesterone was 4.11 ± 5.64 ng/mL, estradiol 92.56 ± 65.62 pg/mL, and testosterone 42.36 ± 17.49 ng/mL. The pain level across all days was 56.49 ± 26.54. Average cortisol was 12.92 ± 6.4 μg/dL. Hormone and pain levels across time are shown for the entire sample in Fig 1.

Hormone data were not obtained for 6 of 200 visits (3%) because of missed laboratory visits. These days were treated as missing values and were not imputed. There were no missing pain reports.

The interassay coefficients of variation were: progesterone = 2.26%, estradiol = 1.42%, testosterone = 7.49%, and cortisol = 2.46%. Intra-assay coefficients of variation were 1.04%, 2.31%, 11.05%, and 5.19%, respectively. Minimum detection values were .10 ng/mL, 25.0 pg/mL, 10 ng/mL, and .2 μg/dL, respectively. All samples provided detectable levels of hormones.

There was a significant and inverse relationship between pain severity and progesterone (F = -9.76, *P* = .002) as well as testosterone (F = -6.01, *P* = .015). Serum levels of estradiol were not associated with pain (F = .36, *P* = .551). Because previous research has shown a positive

Table 1. Demographic Characteristics and Screening Blood Test Values

Participant	Age	Race	Pain	Free T3	Free T4	TSH	Vit-D	hsCRP	ESR	RBC	WBC	Analgesics
1	39	Caucasian	9	3.2	1.15	1.23	14.6	1.47	10	4.56	9.08	
2	37	Caucasian	4	86 (Total)	1.3	.99	27.8	.86	6	4.24	8.38	
3	21	Caucasian	6	2.8	1.23	.60	55.9	.9	0	4.42	2.67	Pregabalin
4	46	Caucasian	3	2.1	.77	2.10	29.3	3.66	14	4.55	8.35	
5	27	Asian	7	3.4	11.3 (Total)	3.00	6.3	.32	4	5.11	6.09	
6	24	Caucasian	5	90 (Total)	6.3	.92	16.7	.45	5	4.41	6.84	
7	26	African American	8	3.0	1.03	.73	15.3	<.2	10	4.69	4.41	
8	39	African American	9	88 (Total)	.86	1.59	15.2	34.2	36	4.22	8.22	Gabapentin

Abbreviations: T3, tri-iodothyronine (nanograms per deciliter; free unless indicated as total); T4, thyroxine (nanograms per deciliter; free unless indicated as total); TSH, thyroid stimulating hormone (international units per milliliter); Vit-D, vitamin D (nanograms per milliliter); hsCRP, high sensitivity C-reactive protein (milligrams per liter); ESR, erythrocyte sedimentation rate (millimeters per hour); RBC, red blood cell count (million per microliter); WBC, white blood cell count (thousand per microliter).

NOTE. No participants had detectable levels of antinuclear antibody or rheumatoid factor (not shown). Pain values were collected from a single-item measure, "How would you rate your average daily pain on a scale from 0 (no pain at all) to 10 (worse possible pain)?"

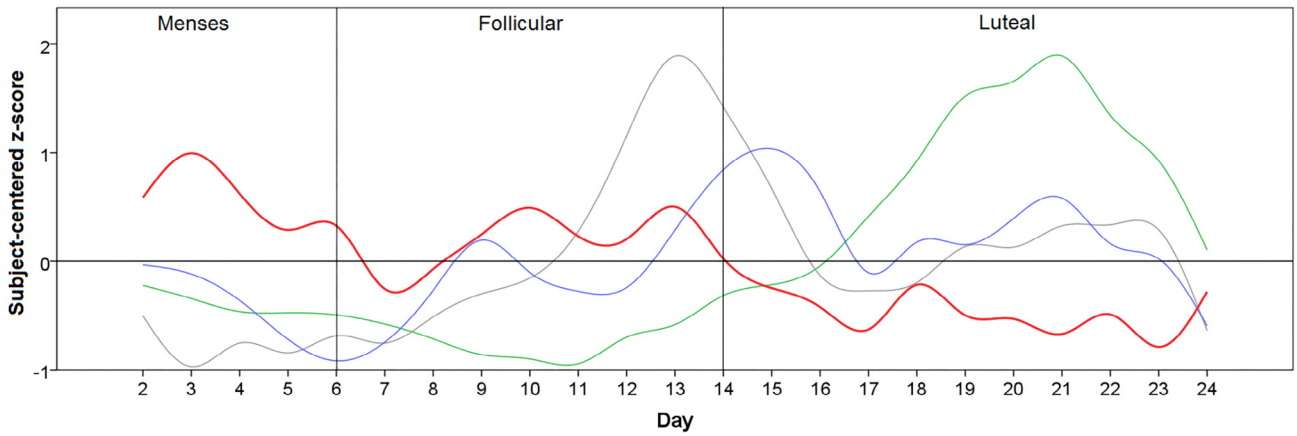

Figure 1. Relationship between progesterone (green), testosterone (blue), estradiol (gray), and pain (red) over 25 days in 8 women with fibromyalgia. Individuals began their participation at different points of their cycle, therefore time courses have been shifted to allow representation of group averages. The "day" index variable was shifted so that the first estradiol peak occurred in the same time period for all participants. The fit was confirmed by observing that the progesterone peak also was temporally aligned for all participants. All y-axis values have been subject-centered (z-scored) to allow variables to be plotted on the same scale, and represent SDs from the subject mean.

relationship between cortisol and fibromyalgia pain,[15] we also included main effects for cortisol, which was not significant (F = .23, *P* = .633). The strongest relationship, on the basis of statistical indices, was found between progesterone and pain, and Fig 2 shows individual plots for each participant.

Post hoc analyses were performed to examine possible interactions between cortisol and sex hormones. Interaction terms were entered into the same linear mixed model. Cortisol interacted with progesterone to influence pain (F = -11.20, *P* = .001). To explore the nature of the interaction, cortisol was split into tertiles (low, medium, and high). Progesterone was not correlated with pain during low cortisol (r = .10, *P* = .454) or during moderate cortisol (r = -.22, *P* = .096), but was during times of high cortisol (r = -.45, *P* = .0004). Fig 3 shows the interaction of cortisol and progesterone on pain. Pain was greatest on days when progesterone was low and cortisol was high. The correlation of progesterone and pain was not related to participants' average cortisol over the 25 days (r = -.33, *P* = .420). Significant interactions were not identified between cortisol and other sex hormones.

As a post hoc analysis, we further tested the relationship between progesterone and pain. A linear mixed model was constructed to determine if pain differences between high and low progesterone phases were statistically and clinically significant. We contrasted average pain in the 3-day progesterone nadir to the 3-day progesterone peak. When progesterone was lowest, average pain was 66.5. When progesterone was highest, pain was rated as 50.4. This 25.6% decrease in pain was statistically significant (F = 9.1, *P* = .005).

Discussion

In this study, we tested if the sex hormones estradiol, progesterone, and testosterone are associated with daily pain severity in women with fibromyalgia. We found that progesterone and testosterone, but not estradiol, were associated with day-to-day changes in self-reported pain severity. Progesterone and testosterone were inversely associated with pain, with peaks of those hormones occurring on days with lower reported pain. Self-reported

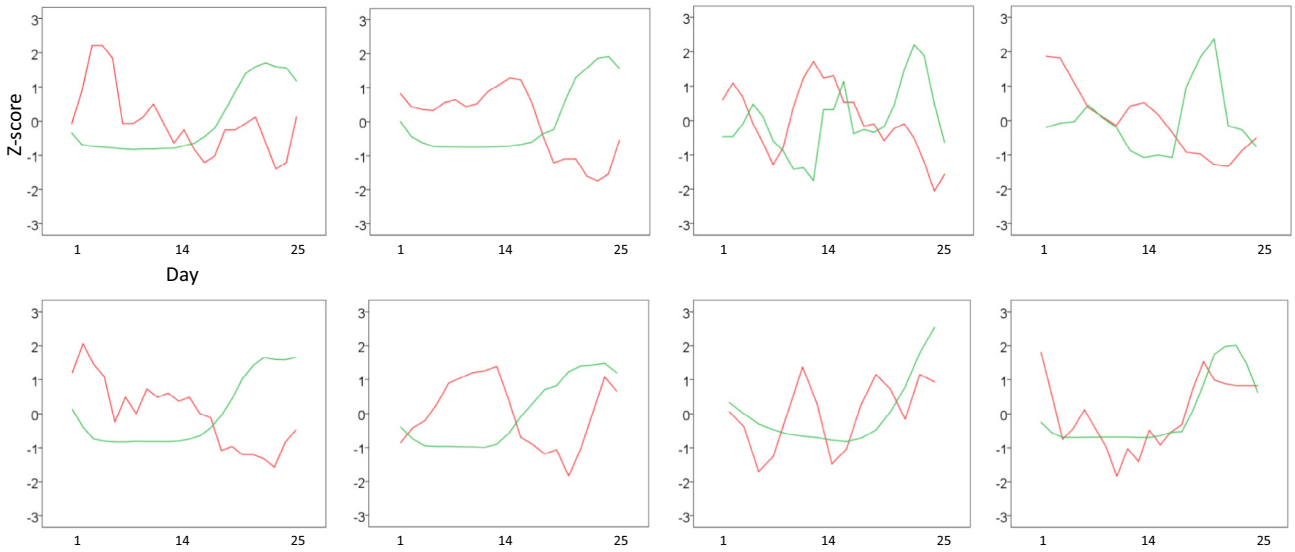

Figure 2. Relationship between progesterone and pain in all 8 participants over 25 days (x-axis). The green line represents progesterone and the blue line represents pain. Progesterone and pain have been subject-centered (z-scored) to allow plotting on the same scale. Time courses have been shifted to reflect the expected menstrual cycle, so that the progesterone peak occurs at the end of each participants' time series.

fibromyalgia pain was lowest in the midluteal phase, corresponding to high progesterone and moderate estradiol and testosterone levels. Pain was highest during the menstrual phase, when all sex hormones are at low levels. These results are consistent with previous reports that women with chronic pain show higher pain sensitivity in the menstrual phase.[18]

Our results largely agree with previous research. Decades of research have shown that sex hormones affect pain processing in animal models.[13,39,41,44] Not only do rodent and other animal models show greater pain sensitivity in the female, but that sensitivity can be reliably affected by experimental manipulation of sex hormones.[23] Large studies of humans have similarly identified relationships between sex hormones and pain. de Kruijf and colleagues reported in a large, population study of 9,717 participants that lower levels of sex hormones (estrogen, testosterone, androstenedione, and 17-hydroxyprogesterone) were associated with the prevalence and incidence of chronic musculoskeletal pain.[10] Another cross-sectional study of 188 women using hormonal contraceptives reported that progestin-only contraceptive users had a higher pain tolerance than participants receiving a combined hormonal contraceptive.[24] Our results

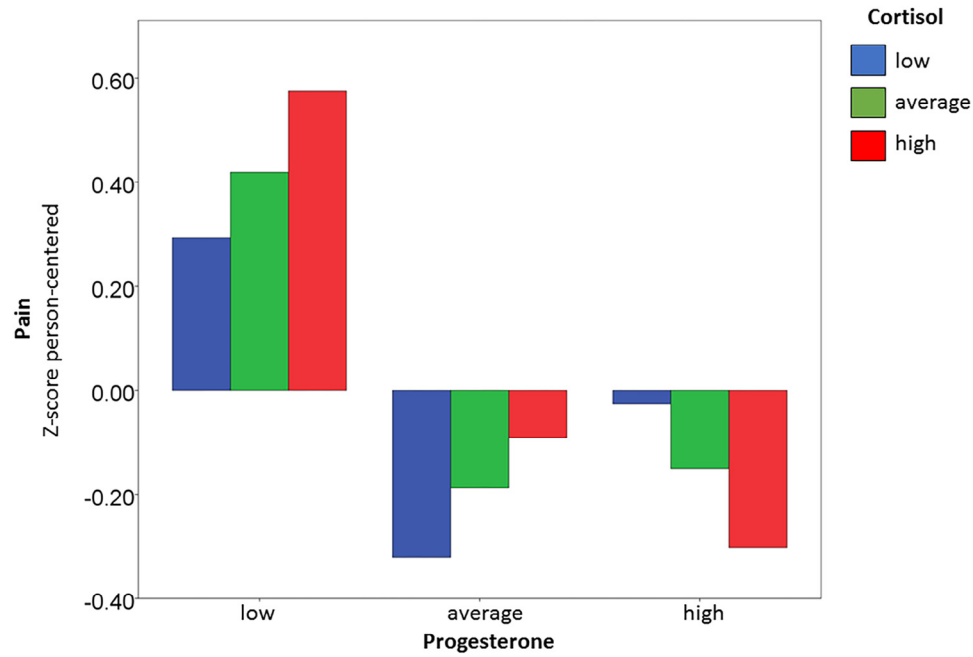

Figure 3. Progesterone and cortisol interaction predicts fibromyalgia pain. Person-centered cortisol (x-axis) and progesterone (x-axis bin) have been split into low, average, and high tertiles. Person-centered pain is on the y-axis. Pain is highest when progesterone is low (left bin) while cortisol is high (red column).

do diverge from a previous study[29] examining sex hormones and fibromyalgia pain in 74 women completing 3 laboratory sessions across a menstrual cycle. In that study, no significant relationship between sex hormones and pain threshold or tolerance was found. It is possible that our use of 25 consecutive days of sampling gave us greater power to detect mild to moderate relationships between hormones and pain.

We also observed that sex hormones may interact with other hormones to influence pain. A low progesterone level was associated with higher pain level, but particularly so during times of high cortisol (Fig 3). There are likely many more interactions of interest that could be further explored with this research model, including adrenal and thyroid hormones, neuropeptides, neurotransmitters, cytokines, and other factors. Using the same daily sampling approach, we have previously reported inflammatory and hormonal drivers of fatigue in chronic fatigue syndrome.[42] Although that study was conducted in chronic fatigue syndrome participants, many such individuals also meet criteria for fibromyalgia.[38] It is possible that an optimized set of analytes could predict most pain fluctuations in fibromyalgia. Comprehensively measuring chemistry related to pain is outside the scope of this pilot study, but is of great interest for future research. Daily sampling, although intensive and potentially burdensome on participants, can provide unique data to explore psychophysiological relationships.

Although we found significant effects for progesterone and testosterone, we did not observe an effect for estradiol. It is possible that the effects of estradiol, which is preceded by an increase of testosterone and co-occurs with an increase of progesterone, cannot easily be temporally separated from the effects of other hormones. As noted in the introductory section, the relationship between estrogens and pain is complex and unresolved, showing proalgesic as well as analgesic properties in the literature. Polymorphisms in estrogen receptors likely need to be closely studied[15] as well as the differential effects of the various estrogen metabolites.[33] It is also possible that the effect of sex hormones on pain will differ between men and women.

The change of pain associated with sex hormones may be clinically significant, because the progesterone peak is associated with a 25.6% lower pain severity. It is unknown if the results from this and other studies indicate that manipulation of sex hormones may be used to modulate fibromyalgia pain. There is little experimental evidence in chronic pain conditions on this topic, and exogenous modulators of sex hormones could act differently than normally cycling hormones. We noted cases in which sex hormones were administered systemically[45] or locally[32] to reduce pain and/or inflammation, and the use of sex hormones in treating autoimmune pain disorders is still a topic of interest.[19] Effects of administered sex hormones on pain have also been studied in the context of transsexual individuals receiving hormone treatment. In Aloisi and colleagues' study[3] assessing varied chronic pain conditions, 55% of female-to-male trans-sexual individuals with chronic pain reported a reduction of pain after testosterone treatment (none reported increased pain). In male-to-female individuals receiving estrogen, 23% reported initiation of chronic pain after estrogen and antiandrogen therapy and another 18% reported a greater sensitivity to pain. This evidence collectively suggests that testosterone can reduce pain severity.

A few limitations should be discussed. One limitation of the study was its observational approach. Although animal studies show that sex hormones have an effect on nociception and pain behavior, very little similar experimental research has been conducted in humans, especially in the context of chronic pain.[14] Because all women showed the canonical sex hormone cycle, it is more likely that sex hormones influenced pain rather than vice-versa, but this study cannot definitively determine causation. It is also important to note that we did not observe any dysregulated sex hormones in this small sample. Consistent with previous studies,[1,6,29,36] sex hormones appear normal in women with fibromyalgia. There is no evidence that sex hormones are part of fibromyalgia pathology. Rather, sex hormones may be a moderator of pain in fibromyalgia. These effects are likely not unique to fibromyalgia, and could be studied in other chronic pain conditions.

The subject size of 8 women is limited. Although the study involves 200 independent laboratory visits, and therefore sufficient statistical power for our tests, using only 8 subjects means we cannot confidentially generalize results to the entire fibromyalgia population. Replication in an independent and larger sample will be required. Collecting samples over 28 days instead of 25 days would also have been preferable, allowing more complete coverage of a full menstrual cycle. Collection was stopped at 25 days to avoid requiring laboratory visits over a fourth weekend, which is particularly burdensome for participants and staff. It is also possible that more sensitive or comprehensive measures of the primary pain outcome could be used, such as the pain intensity scale of the Brief Pain Inventory. Our single-item pain severity marker does not have demonstrated reliability and validity. The study may have also been strengthened by including a healthy control group. However, healthy women who have completed self-report measures in our protocol show insufficient variability of pain (often reporting 0 pain for all days) to allow correlational analyses between sex hormones and pain to be computed. Another limitation is that participant #8 returned a C-reactive protein result of 34.2 mg/L at baseline, which could indicate an inflammatory disorder or infection. The individual did not meet exclusionary criteria for the study and was retained in the analyses. There are likely important individual differences in the relationship between sex hormones and pain, especially because several participants showed no observable relationship between their hormone levels and pain. With a small sample size, we cannot account for those individual differences, and will explore possible fibromyalgia subgroups in future studies.

Conclusions

There are several additional questions regarding sex hormones and pain that could be answered with future

applications of this study design. We are interested to know if sex hormones predict pain levels in postmenopausal women, and women taking oral contraceptives. It would also be interesting to examine other pain syndromes that can exhibit day-to-day fluctuations in severity. We should also determine if sex hormones can drive pain severity in men with fibromyalgia. Future studies may also examine the relationship of sex hormones on other aspects of fibromyalgia, such as fatigue and cognitive symptoms. We conclude that this research adds to the strong literature showing a relationship between sex hormones and pain,[4] and suggest that those relationships have real-world consequences for individuals with chronic pain disorders. Sex hormones may be an important target for successfully managing some chronic pain conditions.

Acknowledgments

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