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CINE MRI *During* Spontaneous Cramps in Women with Menstrual Pain

Kevin M. HELLMAN[1, 2*] PhD, Caroline S. KUHN[2*] MD, Frank F. TU[1,2]MD MPH, Ms. Katlyn E. DILLANE[1] BS, Mr. Nathan A. SHLOBIN[1,2] MS2, Sangeeta SENAPATI[1,2]MD MPH, Xiaojie ZHOU[1,2] MD, Wei LI[3] MD, Pottumarthi V. PRASAD [3,4] PhD

[1]Dept. of Ob/Gyn, Northshore University HealthSystem, Evanston, IL 60201 USA

[2]Dept. of Ob/Gyn, Pritzker School of Medicine, University of Chicago, Chicago, IL 60637 USA

[3]Dept. of Radiology, Northshore University HealthSystem, Evanston, IL 60201 USA

[4]Dept. of Radiology, Pritzker School of Medicine, University of Chicago, Chicago, IL 60637 USA

* The authors request that the first two authors should be regarded as joint first authors

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Corresponding Author Contact Information: Dr. Kevin M. Hellman

Walgreens Suite 1507, 2650 Ridge Ave. Evanston, IL 60201

telephone: 872-226-7124, fax: 847-570-1846

email: khellman@northshore.org

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Condensation: MRI of uterine contractions during spontaneous cramps demonstrates a method to evaluate myometrial dysfunction and its role in menstrual pain.

Short version of title: MRI During Spontaneous Cramps

Implications and Contributions

A. Cine MRI of women with dysmenorrhea was performed to evaluate whether MRI can detect real-time changes in uterine physiology while women experience menstrual cramping pain.
B. Spontaneous progressive decreases in myometrial signal intensity were associated with cramping pain onset before or 32-70s afterwards.
C. These results show the temporal relationship between myometrial physiology and pain, providing a new paradigm to further characterize the mechanisms underlying dysmenorrhea.

Abstract

Background:

The lack of non-invasive methods to study dysmenorrhea has resulted in poor understanding of the mechanisms underlying pain, insufficient diagnostic tests, and limited treatment options. To address this knowledge gap, we have developed an MRI-based strategy for continuously monitoring the uterus in relation to participants' spontaneous pain perception.

Objective:

The study objective was to evaluate whether MRI can detect real-time changes in myometrial activity during cramping episodes in women with dysmenorrhea, with a hand-held squeeze bulb for pain reporting.

Study design:

Sixteen women with dysmenorrhea and ten healthy control women both on and off their menses were evaluated with MRI while not taking analgesic medication. Continuous MRI was acquired using single-shot HASTE sequence along with simultaneous reporting of pain severity with a squeeze bulb. Pearson's coefficient was used to compare results between reviewers. Proportional differences between women with dysmenorrhea and controls on/off menses were evaluated with Fisher's exact test. The temporal relationships between signal changes were evaluated with Monte Carlo simulations.

Results:

Spontaneous progressive decreases in myometrial signal intensity were more frequently observed in women on their menses than in the absence of pain in the same women off their menses or participants without dysmenorrhea (p's < 0.01). Women without reductions in myometrial signal intensity on their menses either had a history of endometriosis or were not in pain. Observations of myometrial events were consistently reported between two raters blinded to menstrual pain or day status (r=0.97, p<0.001). Episodes of cramping occurred either immediately before or 32-70s after myometrial signal change onset (p's <0.05).

Conclusions:

Transient decreases in myometrial uterine T2-weighted signal intensity can be reliably measured in women with menstrual pain. The directionality of signal change and temporal relationship to pain onset suggest that cramping pain may be caused by a combination of uterine pressure and hemodynamic dysfunction.

Keywords

dysmenorrhea, endometriosis, MRI, pain, uterus

Introduction

Dysmenorrhea, or menstrual "cramps", is a leading reason for missed school or work among women.[1,2] Some women with severe dysmenorrhea, refractory to nonsteroidal anti-inflammatory drugs (NSAIDs), undergo repeated surgeries in search of secondary causes such as endometriosis or ovarian cysts, yet many still do not achieve pain relief.[3,4] While refractory menstrual pain is commonly attributed to uterine contractions and ischemia, non-invasive methods have not been developed to confirm these or other factors to further direct treatment strategies.[2]

Cine MRI, which involves obtaining a continuous series of MR images and is a common feature available on clinical MRI scanners, may be a useful non-invasive method to establish the role of uterine contractions in pain. Cine MRI has been useful for evaluating time-dependent restricted anatomical contributions to pathophysiology in cardiac dysfunction,[5] airway obstruction,[6] and infertility.[7] Studies utilizing cine MR on days in which women experience menstrual pain have observed increased uterine distortion artifact suggestive of myometrial movement.[8,9] Previous reports of combined hormonal contraceptive pills relieving primary dysmenorrhea also found a decrease in distortion artifact, supporting the hypothesis that dysfunctional myometrial activity may be responsible for pain.[10] Studies using ultrasound [11] or intrauterine pressure probes[12,13] have also suggested differences in myometrial activity across the menstrual cycle in women with and without primary dysmenorrhea. However, a primary limitation of all prior research was that the temporal relationship between myometrial activity and pain was not characterized. Overcoming this limitation in study design could expand our

mechanistic understanding of uterine pain, as spontaneous cramps are the primary complaint of women who experience dysmenorrhea. The evaluation of the temporal relationship between the perception of pain and uterine physiological changes is essential for establishing causality. Thus, the current study uses MRI to evaluate the relationship between uterine pain perception and changes in myometrial signal in real time.

Materials and Methods

Patients

Written informed consent was obtained prior to participation in this IRB-approved study. Participants with dysmenorrhea and healthy controls (ages 18-45) were prospectively recruited from physician referral or from participation in a separate study between January 2015 and February 2017. Participants were asked to report their typical menstrual pain using the numeric rating scale (NRS, 0: “no pain at all”; 10: “worst pain imaginable”)[14] during telephone screening. For this study, eligible dysmenorrhea participants were required to report having menstrual pain greater than 5 on an NRS when not taking pain relievers. Menstrual pain was confirmed with an online version of menstrual diaries.[15] Participants reported in their diary their daily pain level, analgesic use and heaviness of their menstrual flow (0-None, 1-Spotting - vaginal blood loss that is not sufficient to require protection, 2-Light bleeding, 3-Normal bleeding, 4-Heavy bleeding).

The study group of participants included women with either primary or secondary dysmenorrhea (adenomyosis, endometriosis, cysts, leiomyomata). Healthy controls were required to report an average pain of less than 3 on an NRS without medication on menses.

Exclusion criteria for the study included history of pelvic or abdominal malignancies, irregular menses (>45 days between periods), pregnancy within prior 6 months, breastfeeding, active genitourinary infection in the previous 4 weeks, body mass index > 40, unwillingness to stop taking NSAIDs on the day of the study visit, unwillingness to have a withdrawal bleed on continuous oral contraceptives, inability to read/comprehend a consent form in English, or standard MRI contraindications.

Study Visits

Participants were scheduled for MRI evaluation during the first 48 hours of menstrual bleeding onset. Although participants were also scheduled to participate during the peri-ovulatory phase of their menstrual cycle, some participants were not able to complete their non-menses visit before study completion (Table 1).

Participants filled out questionnaires to obtain complete medical, surgical, psychological, gynecological, and obstetrical history. Participants were instructed to abstain from taking short-acting analgesic medications at least 8 hours before the visit, or 12 hours for longer acting analgesics.

Upon arrival, participants were asked to rate their baseline pain on an NRS scale. Before entering the scanner, participants practiced using a hand-held squeeze-bulb at 50% and 100% maximal levels to reflect pain intensity graphically presented to them on a computer screen. Since 50% corresponded to 50% compression of the 6 cm diameter squeeze-bulb and 100% corresponded to complete displacement of the squeeze-bulb, usage of this system was intuitive for participants. The squeeze bulb measurements were recorded using a pressure transducer with a data acquisition system with a 100 sample per second rate allowing for rapid scoring of

pain-state in relationship to the obtained images. Bulb squeeze data was synchronized to the MRI timestamp in order to measure the association between self-reported menstrual pain and myometrial signal intensity.

Dysmenorrhea participants were instructed to proportionally squeeze the bulb to indicate their increased pain during a menstrual cramp. Participants were instructed to squeeze the bulb only when they experienced pain above their baseline. During scanning, participants were periodically reminded to squeeze the bulb to match their perceived menstrual pain to provide "real time" self-reported pain intensity. Healthy control participants did not have pain and were instructed to randomly squeeze the bulb every 2-5 minutes to control for potential movement artifact.

MRI data were acquired on a 3.0 T whole-body scanner (Magnetom Verio, Siemens Healthcare, Erlangen, Germany) using a high-performance body coil. Before scanning, preliminary HASTE (Half-fourier Acquisition Single-shot Turbo spin Echo imaging) sequences were performed to identify a pelvic cross-section adjacent to the midline that included the endometrial stripe. CINE MRI data was acquired in the sagittal plane with a HASTE acquisition every 2 seconds for 10 minutes with the following parameters: FOV = 206 x 300 mm, No. of Slice = 1, Slice thickness = 5.0mm, Matrix = 256 x 139, Echo time= 80 ms, Train length= 96 ms.

Image Analyses

Image processing was performed using *Image J* (http://imagej.nih.gov/ij). Assessment of uterine activity was performed by drawing a region of interest over the uterine corpus in a direction perpendicular to the axis of the cervix. The contrast was adjusted to visualize the minimum and maximum uterine signal. In the time-mode graphs, myometrial event frequency

was identified by counting the episodes of changes in myometrial signal intensity over time. These episodes resembled "sustained uterine contractions" as reported by other investigators. [8] As they may not represent actual contractions, these episodes were defined as myometrial "events." Two reviewers with experience interpreting uterine MRI performed the same image processing methods independently blinded to participant and group identity. A third reviewer was consulted whenever there was a discrepancy between the two reviewers. The number of uterine events for each participant was compared with inter-rater agreement analysis. During scanning and image analysis, MRI images were reviewed for adenomyosis, cysts and leiomyomata by fellowship trained gynecologists (>10 years experience). Radiologists (>10 years MR experience) confirmed cases of adenomyosis, cysts and leiomyomata. Leiomyomata were observed in two participants and cysts were observed in two other participants.

Pain Analyses

All dysmenorrhea participants, except one experiencing temporary remission, squeezed the bulb for periods of 10-20 seconds throughout MRI scans. Episodes from participants were visually identified, and the average squeeze pressure was calculated relative to cramp onset in 2-second bins. Cramp onset was defined as the first time point in which the squeeze pressure was elevated, suggesting the very beginning of a self-reported cramp. There was considerable variability in baseline and maximal menstrual pain across participants (Fig 1a). Since a prior study used a histogram to establish criteria for pain levels with a visual analog scale[16], we performed a similar analysis to discriminate a threshold for cramping pain. Scaled bulb pressure was analyzed across quartiles (Fig 1b). Approximately 75% of identified cramping symptom events exceeded 40% of the maximal squeeze pressure (Fig 1b). Therefore, we employed an

algorithm to detect periods of 40% maximal squeeze pressure capable of detecting 75% of all cramping activity.

Statistics

Fisher's exact test was used to determine differences in proportions of subjects with or with myometrial events between the groups.

The significance of temporal relationship was evaluated with Monte Carlo simulations. For computing the time-locked average signal during myometrial events, signal intensity for each image series was obtained over the region of interest in *ImageJ*. The number of ≥40% squeeze threshold events was computed for each time point relative to the nearest myometrial event. Since the threshold for cramping was arbitrary, we also calculated the median squeeze pressure across all myometrial events. The threshold for significance at each time point was determined by the predicted probability using 1000 permutations of the data set with random time shifts.

Since this was a feasibility study, we aimed for the minimally recommended sample size of n=10 per group for estimating variance.[17] Additional participants were added to the dysmenorrhea group to provide a minimum of 15 participants on their menses accounting for 1 drop-out to provide a sufficient number of participants (n>10) that have signal changes for time-locked analyses.

Results

Imaging features of dysmenorrhea

Women with dysmenorrhea (*n* = 16) and healthy controls (*n* = 10) participated in MRI scanning experiments (Table 1).

We first analyzed the reliability of two reviewers, blinded to participant status, at identifying dynamic changes in myometrial signal. We readily observed episodes of focal reduction of myometrial signal progressing from the fundus to cervix (See Video, Supplemental Digital Content 1). An example of a series of 3 myometrial events is shown in Figure 2. Changes in signal intensity were specific to the myometrial layer within the uterus, but not observed in other tissue locations including leiomyomata (Figure 2). One other participant had a leiomyoma, but signal changes were also only observed in the myometrium, not the leiomyoma. Only one participant had adenomyosis, and she also had signal changes within the myometrium.

Myometrial events were distinct from rapid (<5 s) peristalsis because they involved a prolonged (>20 s) 14 ± 3% reduction in signal intensity. There was a high agreement between reviewers for identifying myometrial events in each participant on each menses scan (Kappa agreement = 97% , $p < 0.001$). There was also a high correlation between the number of myometrial events identified between the two reviewers (r=0.97, $p < 0.001$).

Next, we investigated the relationship between myometrial events and menstrual status. Women with dysmenorrhea were more likely to have myometrial events during their menses (11/15) than women with dysmenorrhea off their menses (1/11, $p = 0.0017$) or healthy participants on their menses (1/10, $p = 0.003$; Figure 3). The single dysmenorrhea participant experiencing spontaneous and temporary remission from menstrual pain when lying down in

the scanner (no bulb squeezes) did not have any myometrial events during her menses scan. Three dysmenorrhea participants did not have myometrial events during their menses despite having severe menstrual pain. Review of medical histories found that these three women had a prior history of surgically confirmed endometriosis; albeit, three additional participants with a prior history of surgically confirmed endometriosis also had myometrial events. On the non-menses visit for this cohort (Table 2), only one participant with dysmenorrhea had myometrial events during a non-menses scan. Intriguingly, she reported significant "bowel" pain during her non-menses scan suggesting that the pain may have been of uterine origin.

Two healthy participants and one dysmenorrhea participant had been taking birth control pills during this study. However, among the participants taking birth control pills, only the dysmenorrhea participant had myometrial events.

Temporal relationship between myometrial events and pain

To examine the relationship between myometrial events and participant pain report, we generated a histogram time-locked to myometrial events across all participants experiencing menstrual pain (Figure 4). A similar relationship between signal changes and pain report was obtained by either analyzing the median squeeze-bulb pressure or a pre-defined threshold for cramping pain. Participants reported significantly more cramping pain at time points proximal to the start of the myometrial events and 32-70 seconds afterward (Monte Carlo p's <0.05).

Comment

We demonstrate an original paradigm for assessing visceral pain mechanisms, utilizing an indicator for spontaneous cramping that is time-locked with continuous MRI acquisition. The spontaneous, prolonged, progressive decreases in myometrial signal intensity observed in participants with dysmenorrhea have characteristics resembling the "sustained uterine contractions" previously identified with cine MRI.[9] Our quantitative analysis demonstrates that these myometrial signal changes are temporally related to spontaneous pain report in women with dysmenorrhea.

This paradigm detected a high proportion of subjects with menstrual pain exhibiting myometrial events, comparable to the proportion of participants with endometrial distortion and severe pain (7/9) in a prior study.[9] Conversely, since our study included 10 participants without a history of any menstrual pain, we were able to additionally confirm that myometrial events rarely occur in women without dysmenorrhea, even on their menses. The decrease in signal change during myometrial events is consistent with either blood volume or water content changes, rather than the muscle contracting. Further characterization of these signal changes in women with dysmenorrhea could be used to identify vascular contributions to menstrual cramping pain by experimentally manipulating perfusion or contractility.

These myometrial events, also known as sustained uterine contractions, were originally found in pregnant women.[18] Notably, these events are distinct from uterine peristalsis that involve rapid, subtle rhythmic movement in the sub-endometrial myometrium.[8] Although it remains to be demonstrated how smooth muscle contractions affect MRI, computer models show that with a skeletal muscle contraction, there is a decrease in signal intensity attributed to increases in oxygen extraction. [19] Indeed, uterine contractions have high metabolic demand

potentially creating transient hypoxia.[20] The consistent report of pain immediately prior to these signal changes, and subsequent pain 32-70 seconds afterward, is consistent with the early response of uterine afferents to mechanical pain and delayed response to hypoxemic pain.[21] Recent work in a mouse model demonstrates that molecules associated with dysmenorrhea trigger uterine hypercontractility leading to episodes of transient hypoxemia.[22] Thus, MRI *during* spontaneous pain is a promising approach to confirm a causative role of vascular or metabolic dysfunction in menstrual pain.

Key strengths of our research design include utilization of the real-time monitoring during menses and control for analgesic use. The inclusion of participants with primary dysmenorrhea, adenomyosis, endometriosis, cysts and leiomyoma was also useful for demonstrating the generalizability of these results. Although all of the participants with primary dysmenorrhea, leiomyoma or adenomyosis had myometrial events, three of the participants with endometriosis did not.

A key limitation of all pain research including this study is that pain is a subjective experience.[23] Despite this limitation, we are able to provide evidence that subjective experience of pain in women is temporally related to episodes of myometrial activity. This is of particular importance given that dysmenorrhea has been historically considered a psychosomatic disorder.[24–27] Given that the usage of an arbitrary pain threshold could have affected results, data was analyzed using a second method to reduce potential bias. Nevertheless, potential bias could have occurred with imaging data, since the menstrual status of participants on MRI was obvious. However, it was not possible to identify differences between dysmenorrhea and healthy participants—except by the presence of myometrial

events as reported here. To address the limitation of our sample size and use of a 3T scanner, larger studies should be performed on more commonly available 1.5T scanners to ensure generalizability.

Future application of our methods to evaluate other poorly responsive visceral pain conditions, including irritable bowel syndrome and pancreatitis, could expand the identification of more general mechanical, vascular, and metabolic visceral pain mechanisms. Although, abdominal pain is one of the most frequent reasons for visits to the emergency room, the true cause remains unknown greater than 25% of the time.[28] Refinement of our methods and validation for other organs hopefully will spur further study and novel treatments for chronic pelvic pain conditions.

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302 # References

303 1. Andersch B, Milsom I. An epidemiologic study of young women with dysmenorrhea. *Am J*
304 *Obstet Gynecol*. 1982;144(6):655-660.

305 2. Dawood MY. Primary dysmenorrhea: advances in pathogenesis and management. *Obstet*
306 *Gynecol*. 2006;108(2):428-441. doi:10.1097/01.AOG.0000230214.26638.0c.

307 3. Vercellini P, Trespidi L, De Giorgi O, Cortesi I, Parazzini F, Crosignani PG. Endometriosis and
308 pelvic pain: relation to disease stage and localization. *Fertil Steril*. 1996;65(2):299-304.

309 4. Abbott JA, Hawe J, Clayton RD, Garry R. The effects and effectiveness of laparoscopic
310 excision of endometriosis: a prospective study with 2-5 year follow-up. *Hum Reprod*.
311 2003;18(9):1922-1927.

312 5. Francone M, Dymarkowski S, Kalantzi M, Rademakers FE, Bogaert J. Assessment of
313 ventricular coupling with real-time cine MRI and its value to differentiate constrictive
314 pericarditis from restrictive cardiomyopathy. *European radiology*. 2006;16(4):944–951.

315 6. Donnelly LF, Shott SR, LaRose CR, Chini BA, Amin RS. Causes of persistent obstructive sleep
316 apnea despite previous tonsillectomy and adenoidectomy in children with Down
317 syndrome as depicted on static and dynamic cine MRI. *American Journal of*
318 *Roentgenology*. 2004;183(1):175–181.

319 7. Yoshino O, Hayashi T, Osuga Y, et al. Decreased pregnancy rate is linked to abnormal
320 uterine peristalsis caused by intramural fibroids. *Human reproduction*. 2010;25(10):2475–
321 2479.

322 8. Togashi K. Uterine contractility evaluated on cine magnetic resonance imaging. *Ann N Y*
323 *Acad Sci*. 2007;1101:62-71. doi:10.1196/annals.1389.030.

324 9. Kataoka M, Togashi K, Kido A, et al. Dysmenorrhea: evaluation with cine-mode-display MR
325 imaging--initial experience. *Radiology*. 2005;235(1):124-131.
326 doi:10.1148/radiol.2351031283.

327 10. Kido A, Togashi K, Kataoka M, et al. The effect of oral contraceptives on uterine
328 contractility and menstrual pain: an assessment with cine MR imaging. *Hum Reprod*.
329 2007;22(7):2066-2071. doi:10.1093/humrep/dem122.

330 11. Bulletti C, DE Ziegler D, Setti PL, Cicinelli E, Polli V, Flamigni C. The patterns of uterine
331 contractility in normal menstruating women: from physiology to pathology. *Ann N Y Acad*
332 *Sci*. 2004;1034:64-83. doi:10.1196/annals.1335.007.

12. Woodbury RA, Torpin R. Myometrial physiology and its relation to pelvic pain. *J Am Med Assoc*. 1947;134(13):1081-1085.

13. Milsom I, Hedner N, Mannheimer C. A comparative study of the effect of high-intensity transcutaneous nerve stimulation and oral naproxen on intrauterine pressure and menstrual pain in patients with primary dysmenorrhea. *Am J Obstet Gynecol*. 1994;170(1 Pt 1):123-129.

14. Hjermstad MJ, Fayers PM, Haugen DF, et al. Studies comparing Numerical Rating Scales, Verbal Rating Scales, and Visual Analogue Scales for assessment of pain intensity in adults: a systematic literature review. *J Pain Symptom Manage*. 2011;41(6):1073-1093. doi:10.1016/j.jpainsymman.2010.08.016.

15. Casper RF, Powell AM. Premenstrual syndrome: documentation by a linear analog scale compared with two descriptive scales. *Am J Obstet Gynecol*. 1986;155(4):862-867.

16. Collins SL, Moore RA, McQuay HJ. The visual analogue pain intensity scale: what is moderate pain in millimetres? *Pain*. 1997;72(1-2):95-97.

17. Birkett MA, Day SJ. Internal pilot studies for estimating sample size. *Statistics in medicine*. 1994;13(23-24):2455–2463.

18. Togashi K, Kawakami S, Kimura I, et al. Sustained uterine contractions: a cause of hypointense myometrial bulging. *Radiology*. 1993;187(3):707-710. doi:10.1148/radiology.187.3.8497617.

19. Damon BM, Gore JC. Physiological basis of muscle functional MRI: predictions using a computer model. *J Appl Physiol*. 2005;98(1):264-273. doi:10.1152/japplphysiol.00369.2004.

20. Larcombe-McDouall J, Buttell N, Harrison N, Wray S. In vivo pH and metabolite changes during a single contraction in rat uterine smooth muscle. *J Physiol (Lond)*. 1999;518 (Pt 3):783-790.

21. Berkley KJ, Robbins A, Sato Y. Afferent fibers supplying the uterus in the rat. *J Neurophysiol*. 1988;59(1):142-163.

22. Hellman KM, Yu P, Oladosu FA, et al. The effects of platelet-activating factor on uterine contractility, perfusion, hypoxia and pain in mice. *Reproductive Sciences*. 2017:in press.

23. Katz J, Melzack R. Measurement of pain. *Surgical Clinics of North America*. 1999;79(2):231–252.

24. Kroger W, Freed S. The psychosomatic treatment of functional dysmenorrhea by hypnosis: A preliminary report. *American Journal of Obstetrics and Gynecology*. 1943;46(6):817–822.

25. Hunter WE, Rolf BB. The psychosomatic aspect of dysmenorrhea; a sensory conditioning process. *Am J Obstet Gynecol*. 1947;53(1):123-131.

26. Goldstein-Ferber S, Granot M. The association between somatization and perceived ability: roles in dysmenorrhea among Israeli Arab adolescents. *Psychosom Med*. 2006;68(1):136-142. doi:10.1097/01.psy.0000197644.95292.00.

27. Sigmon ST, Dorhofer DM, Rohan KJ, Boulard NE. The impact of anxiety sensitivity, bodily expectations, and cultural beliefs on menstrual symptom reporting: a test of the menstrual reactivity hypothesis. *Journal of anxiety disorders*. 2000;14(6):615–633.

28. Powers RD, Guertler AT. Abdominal pain in the ED: stability and change over 20 years. *Am J Emerg Med*. 1995;13(3):301-303. doi:10.1016/0735-6757(95)90204-X.

377 **Tables:**

Variable	Dysmenorrhea (n=16)	Control (n=10)
Age	31 [21-37]	31 [20-40]
Body mass index	24 [22-26]	22 [21-25]
Prior pregnancy	6 (38%)	2 (20%)
Parous	3 (19%)	1 (10%)
Race		
African American	5 (31%)	1 (10%)
Caucasian / Other	11 (69%)	9 (90%)
Age of menstrual pain onset	11 [11-14]	N/A
Menstrual pain without NSAIDS [0-100] VAS	78 [70-87]	5[4-9]
Menstrual pain with NSAIDS [0-100] VAS	50 [25-60]	3 [2-5]
# of day of school or work missed in last 3 months due to pain	3 [1-5]	0 [0-0]
Number of bleeding days per cycle	6 [5-6]	6 [5-6]
Bleeding heaviness (0 -4)	2.5 [1.9-2.5]	2.3 [2.1-2.5]
Diary Data (0-10 NRS)		
Average menstrual pain while bleeding	4 [4-5]	0 [0-1]
Maximum menstrual pain while bleeding	7 [5-9]	0 [0-0]
McGill Revised Pain Inventory		
Continuous	4 [5-7]	0 [0 – 0]
Intermittent	3 [2-5]	0 [0 – 0]
Affective	3 [0-4]	0 [0 – 0]
Neuropathic	1 [0-1]	0 [0 – 0]
Scans Performed		
Menses	15	10
Non-menses	12	6

378

379 Table 1: **Demographic characteristics of the recruited population.** Counts (percentage) or
380 medians [with 25 to 75% quartiles] are shown for participants with and without dysmenorrhea.
381 Participants were asked to enter the severity of their menstrual pain on a visual analog scale
382 (VAS, 0 – No pain, 100 – worst pain imaginable) with and without taking NSAIDS. Participants
383 completed menstrual pain diaries to verify eligibility. The average and maximum menstrual pain

384 during menses were calculated for participants. Participants also filled out the McGill Revised
385 Pain Inventory questionnaire to inform the level of menstrual pain on a 0-10 scale for the
386 McGill subscales on continuous, intermittent, affective and neuropathic pain. The number of
387 participants scanned on either their menses or non-menses visit are shown. Some participants
388 were not able to participate in both scans due to scheduling challenges.

389

390

391

	Menses scan	Non-menses scan
Dysmenorrhea participants: cramping episodes during scanning		
Number of participants	14	1
Number of participants with myometrial events	11	1
Dysmenorrhea participants: no cramping episodes during scanning		
Number of participants	1	11
Number of participants with myometrial events	0	0
Healthy controls: no cramping episodes during scanning		
Number of participants	10	6
Number of participants with myometrial events	1	0

392

393 Table 2: **Characterization of myometrial events in participants across conditions.** Women

394 during their menses in pain were more likely to have myometrial events than during their non-

395 menses or than healthy controls.

396

Figure Legends:

Figure 1: Temporal profile of spontaneous cramps. The 25th, 50th, and 75th percentile pain scores are shown for visually identified cramps (a). The reported level of pain (0-10) was determined by self-report of baseline pain and the scaled level of squeeze-bulb pressure. Consecutive cramps were profiled relative to visually identified onset (gray dashed line). The profiles of squeeze-bulb pressure were generated similarly (b) suggesting a threshold of 40% could detect 75% of all spontaneous cramps.

Figure 2: Example measurements of myometrial signal. Shown is a representative image from a continuous series of HASTE scans (a). The red line indicates the cross section defined for the continuous assessment. An enlarged cross section (b) is shown over 10 minutes to demonstrate the stability and specificity of change to the myometrial layer (black arrow). Dynamic changes in myometrial signal were charted (beneath in blue) by the change in average signal intensity in the myometrial layer of the uterus. Bulb-squeezing indicative of cramping pain is indicated by a red line beneath the cross section.

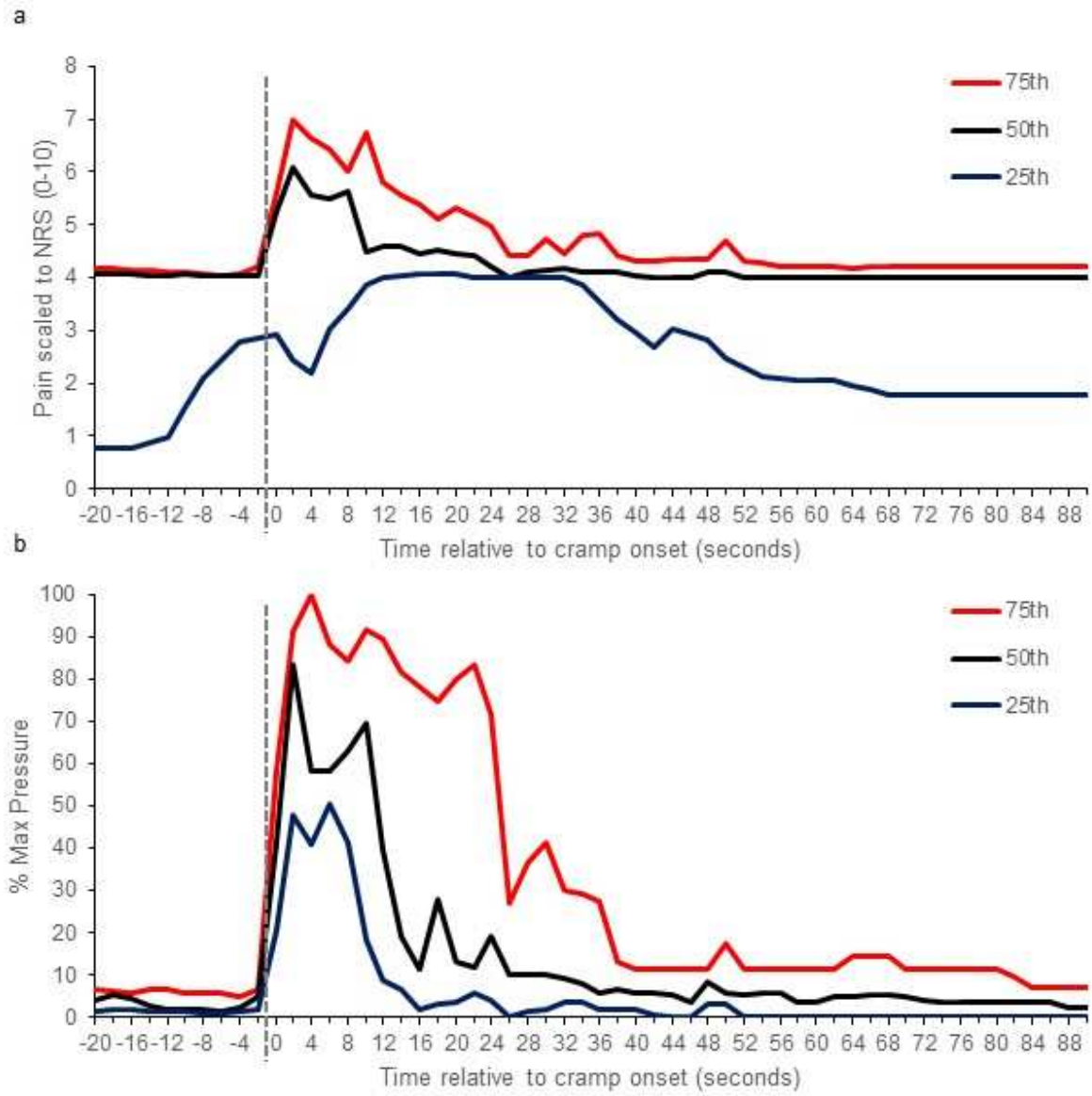
Figure 3: Example images of a healthy participant without menstrual pain on her menses. The red line indicates the position of the cross section defined for the continuous assessment (a). Unlike the participant with dysmenorrhea (Figure 2), the cross-sectional signal was stable over a ten-minute period (b).

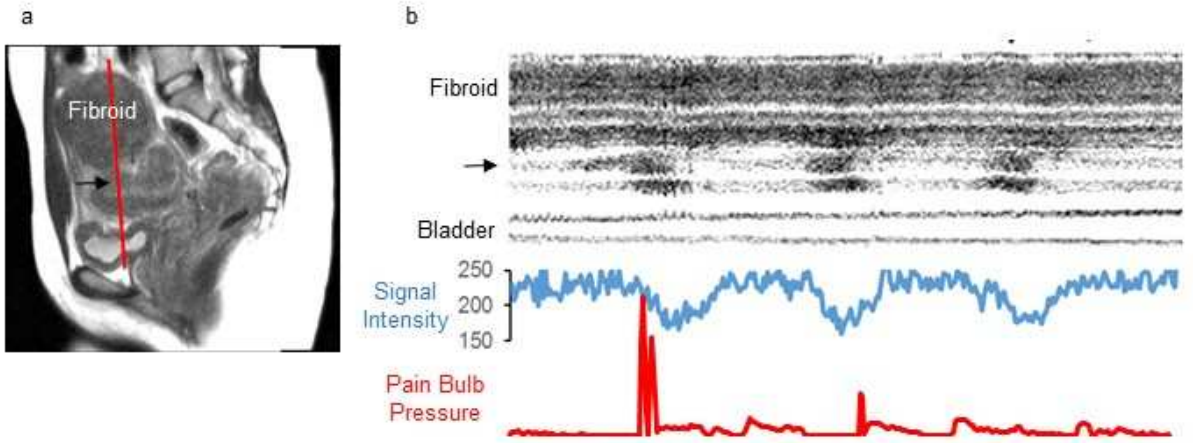
Figure 4: Menstrual cramps occurred more frequently immediately before, and up to 40 seconds after, a myometrial event. The average HASTE signal intensity in the myometrial layer over time was determined across all dysmenorrhea participants with myometrial events (a).

418 The blue line indicates the median signal intensity across all images in all women with
419 myometrial events. Error bars indicate the standard error of the mean. The median bulb-
420 squeeze pressure (b) is highest immediately before and 32-70 seconds after the myometrial
421 event onset. The dotted line indicates the upper 95% confidence interval for above average
422 pressure. Thus, pressure levels above the dotted line indicate levels of pain more than
423 anticipated by chance ($p < 0.05$). The frequency of cramping (# of episodes with >40% pressure)
424 for each time point relative to a myometrial event was calculated (c). Whereas pressure levels
425 indicate pain severity (b), frequency of cramping indicates likelihood of cramping at each time
426 point (c). The dotted line indicates the upper 95% confidence interval for the number of
427 spontaneous cramping episodes expected by chance.

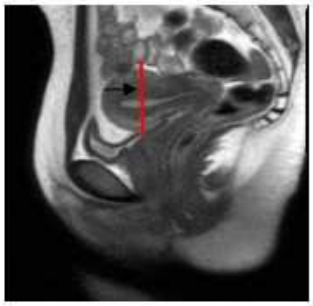
428 **Supplemental Digital Content 1:** Continuous HASTE sequence of a participant with
429 dysmenorrhea on her menses at 4 x speed. The video shows a period of stable signal within the
430 myometrial layer followed by an episode of myometrial signal change. After a decrease in
431 myometrial signal, the participant reported pain.

432

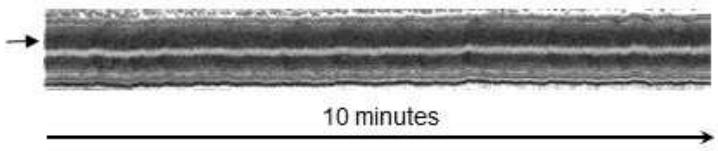

a
Pain scaled to NRS (0-10)
0 1 2 3 4 5 6 7 8
75th
50th
25th
-20 -16 -12 -8 -4 0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84 88
Time relative to cramp onset (seconds)
b
% Max Pressure
0 10 20 30 40 50 60 70 80 90 100
75th
50th
25th
-20 -16 -12 -8 -4 0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84 88
Time relative to cramp onset (seconds)

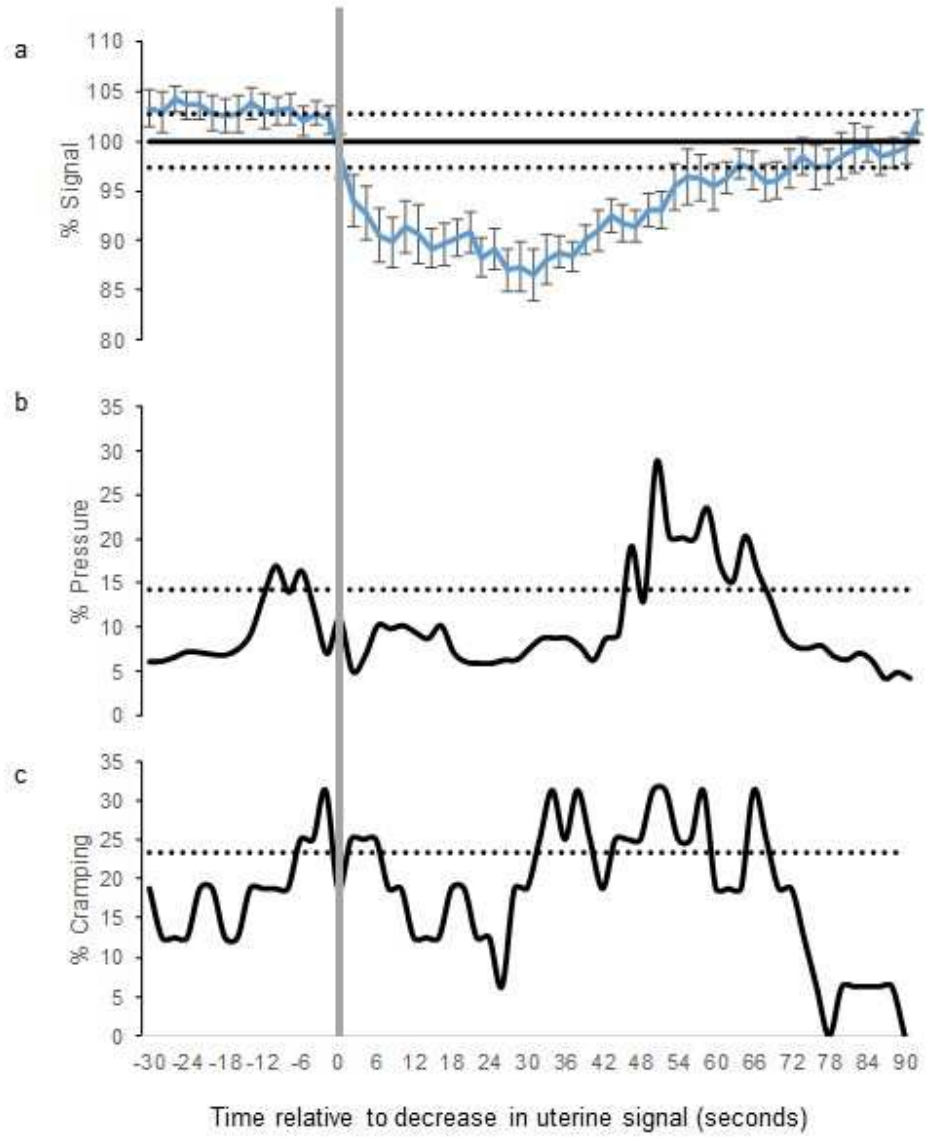


a



b




a
% Signal
110
105
100
95
90
85
80
b
% Pressure
35
30
25
20
15
10
5
0
c
% Cramping
35
30
25
20
15
10
5
0
-30 -24 -18 -12 -6 0 6 12 18 24 30 36 42 48 54 60 66 72 78 84 90
Time relative to decrease in uterine signal (seconds)