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FACILITATED CENTRAL PAIN MECHANISMS ACROSS THE MENSTRUAL CYCLE IN DYSMENORRHEA AND ENLARGED PAIN DISTRIBUTION IN WOMEN WITH LONGER PAIN HISTORY

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ABSTRACT

Dysmenorrhea, or recurrent menstrual pain, is a highly prevalent pain condition among otherwise healthy women . However, the progression of dysmenorrhea over time and the influence of the menstrual cycle phases need to be better understood. While the location and distribution of pain have been used to assess pain mechanisms in other conditions, it is unexplored in dysmenorrhea. Thirty otherwise healthy women with severe dysmenorrhea and 30 healthy control women (HC) were recruited into three subgroups (n=10) according to the length of their menstrual history (<5, 5-15, or >15 years since menarche). The intensity and distribution of menstrual pain were recorded. Pressure pain thresholds (PPTs) at abdominal, hip, and arm sites, pressure-induced pain distribution, temporal summation of pain, and pain intensity after pressure cessation over the gluteus medius were assessed at 3 menstrual cycle phases. Compared with HC, women with dysmenorrhea showed lower PPTs in every site and menstrual cycle phase ($P<0.05$), enlarged pressure-induced pain areas during menstruations ($P<0.01$), and increased temporal summation and pain intensity after pressure cessation in the overall menstrual cycle ($P<0.05$). Additionally, these manifestations were enhanced during the menstrual and premenstrual phases compared to ovulation in women with dysmenorrhea ($P<0.01$). Women with long-term dysmenorrhea demonstrated enlarged pressure-induced pain distribution, enlarged menstrual pain areas, and more days with severe menstrual pain compared to the short-term dysmenorrhea subgroup ($P<0.01$). Pressure-induced and menstrual pain distributions were strongly correlated ($P<0.001$). These findings suggest that severe dysmenorrhea is a progressive condition underscored by facilitated central pain mechanisms associated with pain recurrence and exacerbation.

PERSPECTIVE

Enlarged pressure-induced pain areas occur in dysmenorrhea, associated with the length of the condition and the distribution of menstrual pain. Generalised hyperalgesia is present throughout the entire menstrual cycle and intensifies during premenstrual and menstrual phases.

INTRODUCTION

Dysmenorrhea is a recurrent pain syndrome characterized by intense menstrual pain. Secondary dysmenorrhea is caused by pelvic pathology,[39, 48] whereas primary dysmenorrhea occurs in women without pelvic findings and otherwise healthy.[10, 15, 40] In these women, increased menstrual prostaglandin synthesis and uterine contractions have been indicated.[10, 25] Dysmenorrhea starts shortly after menarche, affecting 90% of adolescents and more than 50% of menstruating women. Of those, 20% suffer severe dysmenorrhea, which is associated with significant functional impairment.[9, 12, 32] Increasing age and parity are associated with decreasing menstrual pain intensity and prevalence.[9, 32, 59] However, nearly two out of three women continue suffering from dysmenorrhea in adulthood.[14, 17, 59] The number of years presenting menstrual pain is linked with increased pain sensitivity in women with chronic pelvic pain.[31] Whereas greater neuroendocrine and functional brain alterations are associated with the duration of dysmenorrhea in otherwise healthy women,[57, 58, 60] there are no studies clarifying the relationship between pain mechanisms and menstrual pain history.

Regarding the influence of pain mechanisms in dysmenorrhea, otherwise healthy women with dysmenorrhea show hyperalgesia[18, 24, 53] and increased reports of pain intensity in response to noxious stimulation.[2, 21, 29] Thus, central nociceptive mechanisms are considered contributors to the exacerbation of menstrual pain.[28, 44] Conversely, descending pain modulation is not a critical factor differentiating young women with only dysmenorrhea and women without dysmenorrhea, as both groups respond to conditioned pain modulation protocols similarly.[24, 45] However, this is not the case when dysmenorrhea is associated with bladder pain hypersensitivity.[24] Additionally, whether the menstrual cycle influences the processing of nociceptive information, for example, by altering sensitization or modulatory mechanisms, is unclear.[17, 44]

Quantitative sensory testing (QST) and documentation of pain distribution can help to identify differences in nociceptive processing due to altered descending modulation[43, 55] or between conditions such as fibromyalgia[3] or frozen shoulder.[7] Women with dysmenorrhea usually experience pain in the lower abdominal quadrants and often report additional areas of pain extending to the pelvis, lower back, and thighs.[2, 15, 50] With regards to experimentally-induced pain, larger areas of pain following uterine cervix mechanical stimulation have been observed in otherwise healthy women with dysmenorrhea in comparison to asymptomatic controls.[2] As opposed to visceral stimulation, pressure stimulation on muscle tissue is a non-invasive process[13] which has demonstrated expanded pain areas in fibromyalgia[3] and different musculoskeletal contexts.[13, 41] Additionally, a less efficient endogenous analgesia system has been associated with an expansion in pain areas throughout several body regions.[43, 55] Similar results are also found when assessing pain distribution in women with chronic pain. In such cases, relatively larger areas of pain are experienced in women with pre-existing dysmenorrhea.[35] Nevertheless, no focused studies have utilized pressure-induced pain distribution in women with dysmenorrhea to understand the influence of the menstrual cycle phases in the processing of nociception. Investigating pain distribution using pressure-induced pain could clarify if nociceptive processing and/or mechanisms are modulated in dysmenorrhea. Finally, there is an emotional component in dysmenorrhea, as research reports higher levels of depression, anxiety, and stress in these women compared with asymptomatic controls.[6, 17] However, the role of these emotional components across different menstrual cycle phases and life stages also remains understudied in this population.

This study assessed localized and widespread pain sensitivity, temporal summation of pain, distribution of menstrual and pressure-induced pain, and the emotional state throughout the menstrual cycle in otherwise healthy women with dysmenorrhea, considering the length of the pain history. It was hypothesized that women with dysmenorrhea compared with pain-free healthy control women would show widespread hyperalgesia, facilitated temporal summation of pain, and larger pain areas induced by noxious pressure stimulation, as well as higher scores of depression, anxiety and stress, throughout the menstrual cycle and more notably during the painful periods. Moreover, women with a longer history of dysmenorrhea were hypothesized to present facilitated pain mechanisms compared to younger women with a shorter pain history.

MATERIALS AND METHODS

Study design and participants

A prospective observational case-control study was conducted. Ethical clearance was obtained from the local Committee for Research (C.P. – C.I. PI15/0124) according to the Helsinki declaration. All participants signed the written informed consent and completed identical procedures. Participants were recruited in the local community, through informative posters and flyers placed in local universities and primary care consultations, informing about the main inclusion criteria: (i) healthy and regularly cycling women aged 18 to 45, not taking hormonal contraception, pregnant, in postpartum or breastfeeding during the last year; and (ii) presenting severe menstrual pain or absent menstrual pain since menarche. Subjects who volunteered were asked to complete three questionnaires to assess selection criteria. The first collected demographical (birth date, race, height, and weight -used to calculate the body mass index- and educational level) and gynecological data (menarche age, menstrual cycle duration, bleeding duration, hormonal contraception, pregnancies, parity, menstrual pain intensity and duration, and history of pelvic pathologies or dysfunctions). The second questionnaire was extracted from the consensus statement published by the EUROPAIN and NEUROPAIN consortia[19], exhaustively assessing the health status and the pain incidence to ensure the healthy condition of subjects for QST-based studies. In addition, volunteers completed the Spanish version of the Depression, Anxiety and Stress Scale (DASS-21).[11]

The additional inclusion criteria for the group of otherwise healthy women with dysmenorrhea (DYS) were: (i) presenting severe menstrual pain for more than one day each period, above 60 mm on a visual analog scale (VAS) from 0 to 100 mm, with self-reported impact in daily life activities and needing analgesic intake during menstruations, being this the general pattern since menarche;[18, 29] (ii) regular menstrual cycles lasting 24 to 32 days; (ii) pain-free outside menses; and (iv) confirmed ovulation. The exclusion criteria were: (i) history of endometriosis, pelvic pathology, or chronic pelvic pain symptoms such as bladder, bowel, vulvar, vaginal, dyspareunia, defecatory, abdominal, low back, pelvic girdle or pelvic floor pains; (ii) not complying with the cited criteria of being considered as healthy volunteers in QST-based studies,[19] i.e., having a history of any chronic urological, gynecological, gastrointestinal, cardiovascular, respiratory, neurological, metabolic, musculoskeletal, psychological, psychiatric or pain disorders; having suffered any pain or taken analgesics for more than 3 isolated or consecutive days during the last 3 months for any cause -excepting menstrual pain according to the aims of this study-; alcohol abuse, use of illegal drugs or regular medication; and scores above 16/21 in any of the depression, anxiety or stress subscales of the DASS-21, according to the percentile cut-off values considered normal in the scale's manual;[36] and (iii) body mass index above 25 kg/m.[47] The reason for establishing the stringent criterion for body mass index (i.e., excluding not only obese cases but also overweight cases) was the location of the algometric assessment points: both the abdominal and gluteus medius points could be considered areas of increased accumulation of adipose tissue, which has been shown to interfere with sensitivity when assessing QST measurements.[47] The selection criteria for the healthy control (HC) group comprised all the cited for the DYS group. However, HC had to report absent or mild menstrual pain (i.e., under 30 mm on a 100 mm VAS) as a general pattern since menarche, without any daily life limitation or analgesic intake.[18, 29]

Once passing these initial criteria and before enrolling in the study, potential eligible volunteers followed a screening period lasting two menstrual cycles to prospectively confirm compliance with the selection criteria. After individual personal instruction, women were asked to complete a paper-based questionnaire including daily average and maximal pain intensity in a 100 mm VAS, menstrual bleeding (presence/absence), and ovulation test results. These pain recordings were carried out each day in the evening.

To detect ovulation, volunteers completed urinary luteinizing hormone surge ovulation predictor kits (One Step®) once a day from the 10th to the 20th days of each cycle from the start of menstruation, as ovulation is expected over the 14th-16th days, but relevant variability has been found.[44] Women were considered potential eligible volunteers if they presented regular cycles lasting between 24 and 32 days during the two consecutive menstrual cycles evaluated, with confirmed ovulation, menstrual pain intensities according to the values previously exposed for DYS and HC groups, but also pain-free and without bleeding or analgesics intake outside menses. In addition, subjects passing these criteria for the HC group were individually evaluated through QST, following the cited recommendations stated by the EUROPAIN and NEUROPAIN consortia,[19] i.e., volunteers had to present normal findings on pressure pain thresholds assessed in both hands -thenar eminence- as compared to the normative data reported by the German research network on neuropathic pain,[37] which were obtained from Caucasian women with differentiated values set according to age. It was established the exclusion of women presenting abnormal pressure pain thresholds (±50 kPa than the mean-range normative values) to avoid the risk of including subjects with unrecognized sensory dysfunction as healthy control participants.

A priori sample size calculation was performed with G*Power (v3.1.9.2; Heinrich-Heine-University, Dusseldorf, Germany) to conduct a repeated-measures ANOVA between 2 groups (6 subgroups in total) during the 3 menstrual cycle phases. A power of 80% and an alpha level of 0.05 were selected to detect a medium effect size (f=0.3). Based on the requirements, 54 participants (27 per group) were needed. However, 30 DYS and 30 HC participants were intended to achieve a homogenous group distribution of 10 participants by age sub-groups. Accordingly, eligible volunteers in the DYS or HC group allocation were stratified into 3 sub-sets according to the number of years since menarche: up to 5 years since menarche, above 5 and under 15, and from 15 years since menarche. Once stratified, participants were randomly selected to form the study groups and subgroups. Subgroups of women up to 5 years since menarche were called DYS short-term and HC short-term; subgroups of women above 5 and under 15 years since menarche were labeled as DYS medium-term and HC medium-term; and subgroups of women from 15 years after menarche were DYS long-term and HC long-term. Non-selected eligible volunteers comprised a reserve list, from which drop-outs were replaced following the order of the recruiting list.

Protocol

Before starting the study sessions, each participant was involved in an individual training session to get instructed and familiarized with the study procedures. Questions were clarified by the research team, ensuring that participants understood and correctly performed all the questionnaires and experimental pain assessments. Three laboratory sessions were conducted within one menstrual cycle for each participant, set according to three differentiated cycle phases: days 1-2 (menstrual phase, within the first two days of bleeding), days 13-16 (ovulation phase, within 24 hours after the first positive result of the luteinizing test) and days 25-28 (premenstrual phase, within the 48 hours preceding the set of menstrual bleeding).[5, 26] The order of the sessions regarding the menstrual cycle phases was randomized for each group of DYS and HC women, and sessions took place in the same time slot for each woman.

An identical protocol was used in all sessions. Participants started with a 5-minutes habituation period in which they lay comfortably and quietly on a bench before QST recordings. Participants were asked to avoid any medication intake within the 24 hours before the sessions, as well as avoid caffeine, smoking, and sport during the previous 2 hours.[54] All procedures were conducted by the same assessor (RF-R) trained in the protocol and blinded to the group allocation and the menstrual cycle phase.

Pressure pain thresholds

A handheld pressure algometer (*Somedic, Hörby, Sweden*) with a 1 cm diameter probe was used to record pressure pain thresholds (PPTs). The pressure was applied manually at a rate of 30 kPa/s until the subject detected the pressure as being perceived as painful and pressed a stop button. Measurements from 3 test sites were taken bilaterally (Fig. 1): abdominal (above the rectus abdominis muscle, 4 cm lateral to the umbilicus)[5, 18], hip (over the gluteus medius muscle, 3 cm cranial to the tip of the greater trochanter),[30] and arm (over the deltoid muscle, 10 cm under the lateral face of the acromion).[5] The abdominal corresponds to the area where menstrual pain is usually referred and within the uterine viscerotome,[52] whereas the arm was selected as a control site, distant from menstrual pain referral,[5] and the hip also as a control site but close to the typical menstrual pain areas. These locations were marked with semi-permanent ink, lasting until the end of the study. The PPT assessment was repeated three times for each site with a 60-second interval between measures. The mean value of the three measurements for each site was averaged across both sides (right and left) and used for data analysis.[33]

Pain induced by sustained pressure stimulation

A sustained pressure stimulation[42] was applied with the algometer over the right hip assessment site (gluteus medius muscle) (Fig. 1). This muscle was selected as its experimental stimulation has induced referred pain beyond the pelvis [30] and with a partly overlapped pattern with locations of menstrual pain. The pressure was increased at a rate of 30 kPa/s until reaching 120% of the PPT previously obtained at the right hip site and then maintained for 60 seconds.[13] Participants rated their pain intensity on a 100 mm VAS after 30-s (VAS-30s) and 60-s (VAS-60s), and immediately after the stimulation, subjects drew the painful areas on electronic body charts, anterior, posterior, and lateral views (Navigate Pain App®; Aglance Solutions; Aalborg, Denmark).[8] The size of the pressure-induced pain area (from now on, AREA-size of induced pain) was extracted accounting for the total number of pixels. Temporal summation of pain (TSP) was calculated by subtracting the VAS-30s from VAS-60s (VAS-60s – VAS-30s). The use of a tonic stimulation is a method as valid as the use of phasic repetitive stimulation, representing the same physiological phenomenon.[16, 20] In addition, 60 seconds after withdrawing the stimulus, subjects were asked to rate on a VAS the intensity of the possibly remaining pain after stimulus cessation (VAS-Aft) (Fig. 1).

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Questionnaires

During the entire menstrual cycle of study, all women completed a daily questionnaire at home, which was received by a collaborator (ES-S) to blind the principal researcher. Daily maximal and average pain intensities were reported on a VAS, and pain areas on paper-based body charts (anterior and posterior views). Presence of menstrual pain was considered when participants reported any level of menstrual pain (i.e., VAS > 0). The presence/absence of bleeding, analgesic intake (yes/no), possible incidence of injuries or pathologies (yes/no and brief description), and results of ovulation tests (positive/negative) were also evaluated. Based on these daily reports, menstrual phase duration was calculated from the number of bleeding days reported, and overall menstrual pain parameters were obtained: (i) number of days presenting any menstrual pain; (ii) number of days with maximal menstrual pain intensities over 60 on a 100 mm VAS; (iii) maximal menstrual pain intensity, by selecting the higher value of pain intensity reported during the menstruating days; (iv) average menstrual pain intensity, calculated by the mean of the average pain intensity values reported during the first and the second menstruating days (when menstrual pain generally peaks, decreasing or disappearing from that moment on);[9] and (v) the size of the overall menstrual pain areas, accounting for the total pixels. If additional painful areas were reported (i.e., apart from the menstrual pain distribution, such as headaches), these were not counted. For the electronic calculation of the parameters regarding overall menstrual pain areas, an added in-person session was scheduled by the end of menstruation. At that session, participants transferred their daily paper-based body charts to the Navigate Pain App®, drawing all the areas where menstrual pain was felt to a single electronic record. This non-experimental session was carried out by a collaborator (ES-S), to blind the principal investigator. Additionally, on the first menstruating day, participants reported the quality of their menstrual pain by selecting pain descriptors from the Spanish version of the McGill Pain Questionnaire[34, 49]. Women were asked to select only the adjectives fully representing their pain, with a maximum of one word for each group of adjectives.

The daily questionnaire was also used to detect withdrawal criteria, considering if any pain or analgesic intake was reported during days outside the menstrual phase of the cycle, pain was not located in areas corresponding to the typical menstrual symptoms, any injury or pathology emerged during the study, or ovulation was not confirmed.

In addition, participants completed the Spanish version of the Depression, Anxiety and Stress Scale 21-item[11] during the menstrual and ovulation phases, to compare their emotional state during the painful period and in a distant pain-free moment of the cycle. This scale was not assessed during the session conducted in the premenstrual phase as the present study aimed to capture the last 1-2 days immediately preceding menses, whereas DASS-21 retrospectively assesses the last week. The evaluation of this scale corresponding to the non-painful period was conducted immediately after concluding the laboratory protocol at the experimental session carried out in the ovulation phase. The assessment of the DASS-21 corresponding to the painful period was conducted in the non-experimental session scheduled by the end of menstruation.

Statistical analysis

A three-way mixed model analysis of variance (ANOVA), with the 3 menstrual cycle phases (menstrual, ovulation, and premenstrual) as a repeated factor, and groups (DYS, HC) and subgroups (DYS short-term, DYS medium-term, DYS long-term, HC short-term, HC medium-term, HC long-term) as between-group factors, was carried out for each set of parameters corresponding to: (i) PPTs of each body site, (ii) pressure-induced pain, and (iii) emotional state (only menstrual and ovulation phases for the latter). When indicated, post hoc Bonferroni corrections were used for pairwise comparisons between subgroups of DYS and HC women with the same menstrual history (DYS short-term vs. HC short-term; DYS medium-term vs. HC medium-term; DYS long-term vs. HC long-term), between subgroups within DYS women (DYS short-term vs. DYS medium-term vs. DYS long-term), between subgroups within HC (HC short-term vs. HC medium-term vs. HC long-term), and between phases within each group and subgroup (ovulation vs. premenstrual vs. menstrual phases). Furthermore, the Greenhouse-Geisser correction was applied to correct violations of sphericity. The set of variables corresponding to the overall menstrual pain during the studied cycle (e.g., days, maximal VAS, size of the menstrual pain area) were analyzed by one-way ANOVA, with DYS-subgroups as between-group factors (DYS short-term vs. DYS medium-term vs. DYS long-term). For the analysis of the pain areas, a logarithmic transformation was applied for secondary normal distribution.[51] However, raw data (pixels) was presented for an easier interpretation. A P-value under 0.05 was considered significant for all the analyses of variance conducted.

An exploratory study of correlations was carried out for the principal parameters in the group of women with dysmenorrhea in the three phases. Pearson's (r) or Spearman's (rho) correlation coefficients were used according to the distribution of each parameter following the Shapiro-Wilk test. Bonferroni corrections were applied to consider statistically significant correlations by dividing the P-value (0.05) by the number of correlations for a parameter conducted at each phase.

RESULTS

A total of 249 women were contacted by email or phone to enroll in the study, and 73 did not comply with the selection criteria. Therefore, 176 women were invited to follow the two-cycle prospective screening period, after which 84 volunteers were excluded. Ninety-two eligible women were stratified by group and number of years since menarche. From those, a sample of 60 women was randomly selected to comprise the study groups and subgroups. Six women dropped out because of painful gastrointestinal, flu or musculoskeletal complaints emerging during the study and were replaced by volunteers stratified on the reserve list, following the order of the recruiting list. Other minor incidents occurred in 31 participants, preventing the completion of the entire menstrual cycle assessed at that moment. Of these 31 women, 20 completed an entire cycle in a second trial and 11 dropped out, being replaced by volunteers on the reserve list following the order of the recruiting list. Therefore, 60 women formed the final sample completing all the study sessions (supplementary material, figure 1). The demographical characteristics of women who dropped out (n=17) were comparable to women who successfully completed the study (n=60) (supplementary material, table 1).

All participants were white Caucasian women of European origin. No woman reported hormonal contraceptives use for longer than 3 months in the past, nor pregnancies lasting above 3 months (apart from those who had children). DYS group and subgroups were comparable to the HC group and to the respective HC-subgroups in parameters of age, body mass index, educational level, parity, menstrual cycle length, and menstrual phase duration. No differences between groups or subgroups were found in the depression, anxiety and stress scores (DASS-21) assessed during the ovulation and menstrual phases. The DYS group presented earlier menarche as compared to HC ($P<0.001$) (Table 1).

Menstrual pain

All women in the DYS group reported severe menstrual pain for 2 or more days, with maximal intensities over 60/100. "Cramping", "sore", "unbearable", and "spreading", in this order, were the adjectives selected with higher frequency to describe severe menstrual pain (89%, 76%, 72%, and 71% of women with dysmenorrhea, respectively). HC women presented, if any, menstrual pain intensities under 20/100. No participant reported pain or bleeding outside menses in the daily questionnaire.

In the one-way ANOVA comprising women with dysmenorrhea, a *subgroup effect* was found for the size of the menstrual pain area and the days with menstrual pain intensity above 60/100. Post-hoc comparisons showed increased size in the DYS long-term and the DYS medium-term subgroups than the DYS short-term ($P<0.05$) (Fig. 2). Additionally, the DYS long-term subgroup showed more days with menstrual pain above 60/100 than the DYS short-term ($P<0.05$). No differences were found between DYS-subgroups in the maximal and average VAS and days with menstrual pain of any intensity (supplementary material, figure 2).

The distribution of menstrual pain in women with dysmenorrhea affected the lower abdomen in 100% of cases, the low back in 76,7%, followed by 36,7% of cases reporting pain in the pubo-perineal area, and 30% at the groins and inner thighs. Other less prevalent locations were the gluteal region (10% of cases), posterior thighs (6,7%), dorsal spine (6,7%), and anterior thighs (3%). In addition, two women reported pain in the breasts, and three indicated headaches (Fig. 2).

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Pressure pain thresholds

*Group*subgroup*phase* interactions were found in the 3-way ANOVAs for the PPTs of the abdomen ($F[4, 108]=7.56$, $P<0.001$) and arm ($F[4, 108]=3.86$, $P<0.007$) sites. Post-hoc comparisons revealed lower PPTs for the DYS medium-term and DYS long-term subgroups as compared to the HC medium-term and HC long-term, respectively, in every menstrual cycle phase, at the abdominal and arm locations, whereas lower in the DYS short-term compared to HC short-term only during menstruation phase (Bonferroni: $P<0.05$). Additionally, the HC short-term subgroup showed lower abdominal PPTs compared to HC medium-term and HC long-term subgroups during ovulation, and lower than HC long-term subgroup in the menstrual phase (Bonferroni: $P<0.05$). No differences were found between dysmenorrhea subgroups at any location (Fig. 3; Table 2).

Dysmenorrhea subgroups showed lower PPTs in the menstrual and premenstrual phases compared to ovulation at the abdomen, hip and arm sites (Bonferroni: $P<0.01$). The HC long-term subgroup showed lower PPTs in the premenstrual and menstrual phases compared to ovulation only at the abdomen (Bonferroni: $P<0.01$) (Table 2).

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Pain intensities, temporal summation of pain, and pain intensity after pressure stimulus cessation

There was a *group*subgroup*phase* interaction for the VAS-60s ($F[4, 108]=3.0$, $P=0.022$). Post-hoc analysis showed that, in the premenstrual phase, VAS-60s was increased in the DYS medium-term subgroup compared to HC medium-term, as well as higher in the HC long-term subgroup when compared to HC medium-term (Bonferroni: $P<0.05$).

There was a *group*phase* interaction for the VAS-Aft ($F[1.64, 88.71]=6.60$, $P=0.004$). Post-hoc analysis showed that VAS-Aft was increased in the DYS group compared to HC during the menstrual phase. In addition, women with dysmenorrhea presented increased VAS-Aft at menstruations than in ovulation and premenstrual phases, whereas control women displayed no differences across the menstrual cycle phases.

There was a *group* effect for TSP ($F[1, 54]= 9.09$, $P=0.004$). As for the overall values of the menstrual cycle, the DYS group presented higher TSP than HC (Bonferroni: $P<0.05$) (Table 2).

Distribution of pressure-induced pain

A *Group*subgroup*phase* interaction was found for the AREA-size of induced pain ($F[3.55, 95.97]=4.45$, $P=0.003$). In the post-hoc, DYS long-term showed a larger AREA-size than DYS medium-term and DYS short-term during the menstrual phase (Bonferroni: $P<0.01$), as well as larger than the DYS short-term subgroup during the premenstrual phase (Bonferroni: $P<0.05$). As for the comparison between groups, women with dysmenorrhea displayed enlarged AREA-size than controls during menstruation and in the overall values of the cycle (Bonferroni: $P<0.05$). Within the DYS group, AREA-size was more expanded during the menstrual and premenstrual phases as compared to ovulation (Bonferroni: $P<0.01$), whereas it did not differ across the menstrual cycle in the HC group (Fig. 4; Table 2).

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Study of correlations

Supplementary tables 2-4 present correlation coefficients in the DYS group for each menstrual cycle phase.

The PPTs found at the abdomen, arm and hip were strongly associated with each other in women with dysmenorrhea during menstruation (0.69< r <0.73, P<0.001). In addition, the PPTs correlated moderately with age and with the number of years since menarche (-0.57< rho< -0.54, P<0.002). Regarding pressure-induced pain parameters, the correlation between AREA-size and VAS-Aft was very strong during the menstrual and premenstrual phases (0.81< rho <0.83), while moderate in ovulation (rho=0.58) (P<0.001).

The size of the menstrual pain areas was strongly associated with the size of the pressure-induced pain areas during menstruation (r= 0.71), as well as with age and number of years since menarche (0.62< rho <0.65) (P<0.001). Additionally, maximal menstrual pain intensity correlated with the PPTs at the arm during the menstrual phase (r=-0.54) and at the abdominal (r=-0.67) location during the premenstrual phase (P<0.002).

DISCUSSION

The study presents novel findings about menstrual pain in women with dysmenorrhea in relation to their menstrual cycle phases by characterizing the nociceptive system using QST and menstrual and pressure-induced pain distribution. Generally, hyperalgesia was evident in women with dysmenorrhea as compared to control women, within the menstrual pain area and in remote asymptomatic sites, at every stage of the menstrual cycle. Upon stimulation of the hip muscles, women with dysmenorrhea reported enlarged pressure-induced pain areas suggesting facilitated pain mechanisms in the menstrual phase. During menstruation and premenstrual days, women with dysmenorrhea showed greater hyperalgesia and pressure-induced pain areas as compared to ovulation. Collectively, these findings show that women with dysmenorrhea fluctuate considerably in comparison to control women across the menstrual cycle phases. A subgroup comparison, based on pain duration (short-, medium-, or long-term dysmenorrhea) provided evidence that women with a long history of menstrual pain demonstrated more enhanced pain mechanisms.

Widespread hyperalgesia

Widespread hyperalgesia is a consistent finding across studies investigating pain mechanisms in chronic pain populations,[1, 4] although much less investigated in women with dysmenorrhea.[28, 44] While some studies reported pressure hyperalgesia in women with dysmenorrhea compared to control women when assessing a single menstrual cycle phase,[5, 24] the present study extends these results into the ovulation, premenstrual and menstrual phases. The findings of this study show persistent and widespread pressure hyperalgesia compared to controls throughout the entire menstrual cycle, which may contribute to pain recurrence and exacerbation in otherwise healthy women with dysmenorrhea.

Pressure-induced pain

Women with dysmenorrhea reported larger pressure-induced pain areas, increased temporal summation and higher pain intensity after cessation of pressure stimulation compared to controls, in the overall menstrual cycle and more intensively during the menstrual phase. Increased temporal summation is consistent in several chronic pain conditions, suggesting facilitated central pain mechanisms.[46, 55] Women with dysmenorrhea and controls have shown comparable temporal summation of pain in studies applying noxious stimuli at the forearm[45] or knee.[24] However, upon distension of the uterine cervix in an asymptomatic period, increased pain intensities, temporal summation, and pain distribution were observed in women with dysmenorrhea compared to asymptomatic women.[2] In accordance with the results of the present study, these enhancements are within proximity to the uterus and may reflect a more local driver of sustained hypersensitivity to noxious stimulation. Further, the increased pain intensity after cessation of noxious pressure stimulation for women with dysmenorrhea is in line with other studies reporting greater pain duration after saline injection.[29] Overall, this study reinforces the notion that pain mechanisms are facilitated in otherwise healthy women with dysmenorrhea.

Sensitization across the menstrual cycle phases

The present study shows that the nociceptive processing is enhanced perimenstrually in women with dysmenorrhea (i.e., lower pressure pain thresholds, enlarged pressure-induced pain distribution, and higher pain intensity after stimulus cessation) as compared to the ovulation phase, and in contrast with the general homogeneity found in control women across the menstrual cycle. Only two studies previously compared, with adequate sample size, the impact of both painful and pain-free phases of the menstrual cycle on the nociceptive processing of women with dysmenorrhea. These two studies similarly concluded that the sensitivity to noxious heat stimuli remains unaffected by the menstrual cycle phase.[21, 45] However, the results of the present study are more aligned with previous research demonstrating increased hyperalgesia to electrical stimulus perimenstrually compared to the ovulation phase in women with dysmenorrhea.[18] Notably, these findings are reflected by the studies of the deep tissues rather than the superficial layers of the skin, further reinforcing the importance of deep tissue investigations when assessing women with dysmenorrhea.[18, 28]

The hormonal profiles of the premenstrual and menstrual phases (with declining and low estrogen and progesterone levels, respectively) seem to exert a pro-nociceptive effect also in other chronic pain conditions such as irritable bowel syndrome, fibromyalgia, migraine, or temporomandibular pain.[23, 27, 38] Noteworthy, these are conditions with increased prevalence in women and frequently presenting comorbid dysmenorrhea. Therefore, women with facilitated pain mechanisms in the premenstrual and menstrual hormonal context, may be at greater risk for developing dysmenorrhea and other chronic pain syndromes.

Sensitization more pronounced in long-term dysmenorrhea

A novel and relevant finding of this study was that young women with a history of dysmenorrhea under 5 years (short-term) did not present hyperalgesia during the pain-free phases of the cycle. Nor was there any indication of enlarged pressure-induced pain distributions compared to controls, in contrast with the manifestations of enhanced nociception observed in women with long-term dysmenorrhea. The cross-sectional timeline observations open a debate. Do these results reflect an adaptative development[58] or preserved pain modulatory mechanisms[24, 45] in young women with short-term dysmenorrhea? Or do these findings suggest that with time these young women may transition or be on a trajectory towards more and more chronic condition? The strong associations previously reported between neuroendocrine (i.e., lower cortisol levels)[57] and brain alterations (i.e., altered functional connectivity in the second somatosensory area)[60] with an increased duration of the dysmenorrhea, may partially explain the trajectory of facilitated pain mechanisms in long-term dysmenorrhea. Moreover, considering the potential effect of the inhibitory mechanisms in pain distribution,[43, 56] a plausible hypothesis for the present findings regarding the duration of the condition, is that women with dysmenorrhea who present larger pain areas may have impaired endogenous analgesia. Such a hypothesis is supported by strong evidence indicating a natural decline in the endogenous inhibitory function associated with normal aging.[22] These findings are indeed alarming, as they suggest that dysmenorrhea may be progressive, and the condition may intensify with the age or duration of dysmenorrhea for a subgroup of women with more pain severity.

Update in dysmenorrhea mechanisms

Noteworthily, women with long-term dysmenorrhea present enlarged menstrual pain distribution and more days with severe menstrual pain than women with short-term dysmenorrhea. Furthermore, a more expanded distribution of menstrual pain is strongly associated with the enlargement of pressure-induced pain. Hence, the findings of the present study point out pain distribution as relevant information about nociceptive processing in otherwise healthy women with dysmenorrhea.

Although there is strong evidence of the association between dysmenorrhea and depression, anxiety and stress,[6, 17] the present study agreed with those studies reporting no relevant differences between otherwise healthy women with and without dysmenorrhea at the emotional level.[45, 57] These findings suggest that it is still possible to present severe dysmenorrhea without co-occurring emotional factors. Nevertheless, future studies recruiting participants from the clinical and hospital setting with a broader sample would better capture the emotional aspects of this population.

Strengths and limitations

Overall, the assessment protocol conducted throughout the menstrual cycle in the present study allows a comprehensive vision of pain mechanisms in otherwise healthy women with dysmenorrhea. A gynecological assessment was not performed in this study, which is a limitation. Instead, tissue pathology was ruled out by means of the gynecological referral of participants carried out in the clinical setting, in which the gynecologists did not suspect other causes underlying menstrual pain nor recommended a laparoscopic intervention. Thus, secondary dysmenorrhea cannot be discarded in the sample of study despite the selection criteria and screening process intended for approaching that aim being rigorous. On the other hand, the healthy control condition was strictly assured. A notable strength of the present study were the assessment of different and selected moments of the menstrual cycle, with special methodological efforts to capture the premenstrual days. However, hormonal levels were not assessed. Therefore, even if normal levels have been demonstrated in women with dysmenorrhea as compared to asymptomatic controls,[29, 57, 58] if any hormonal disbalance existed in the participants of this study, it may have influenced the results. Additionally, the inclusion of only white Caucasian women of European origin may affect the external validity of the findings. Unfortunately, research on pain mechanisms across the menstrual cycle in women with dysmenorrhea is still sparse, and future studies assessing nociceptive and modulatory mechanisms in this population are warranted.

Conclusion

Widespread hyperalgesia in every phase of the menstrual cycle, and enhanced pressure-induced pain distribution during menstruations are evident and may contribute to pain recurrence and exacerbation in otherwise healthy women with dysmenorrhea. Pressure pain sensitivity and pressure-induced pain distribution varied across the cycle phases, being more pronounced at menstruations but also during the pain-free premenstrual days as compared to ovulation. Furthermore, especially those women with a longer pain history show enhanced pain distribution. Finally, in addition to age, the phase of the menstrual cycle and the severity of menstrual pain are important variables that can significantly impact results in pain studies involving women.

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FIGURE LEGENDS

Figure 1. *Laboratory protocol.*

Figure 2. *Overlays of the pain areas from all digital drawings in the dysmenorrhea (DYS) group, and overlays divided by subgroups, representing the menstrual pain distribution.* ***a****: differences in the size of the menstrual pain area compared to DYS short-term.*

Figure 3. *Mean and standard deviation (SD) of pressure pain thresholds (PPTs) in healthy controls (HC) and dysmenorrhea (DYS) subgroups in the abdomen (A) and the arm (B). Symbols:* ***a****, for differences compared to the corresponding matched HC subgroup;* ***b****, for differences compared to the <5 subgroup, within the same HC or DYS group;* ***d****, for differences compared to ovulation phase, within a subgroup;* ***e****, for differences compared to premenstrual phase, within a subgroup (Bonferroni, $P<0.05$). Abbreviations: HC, healthy controls; DYS, dysmenorrhea; <5, up to 5 years since menarche; 5-15, above 5 and under 15 years since menarche; >15, from 15 years since menarche.*

Figure 4. *Overlays of the drawn pain areas from all digital pressure-induced pain drawings in the healthy controls (HC) and dysmenorrhea (DYS) groups across menstrual cycle phases. Symbols:* ***a*** *for significant differences between groups;* ***d*** *for significant differences with the ovulation phase, within the DYS group ($P<0.01$).*

Figure 1

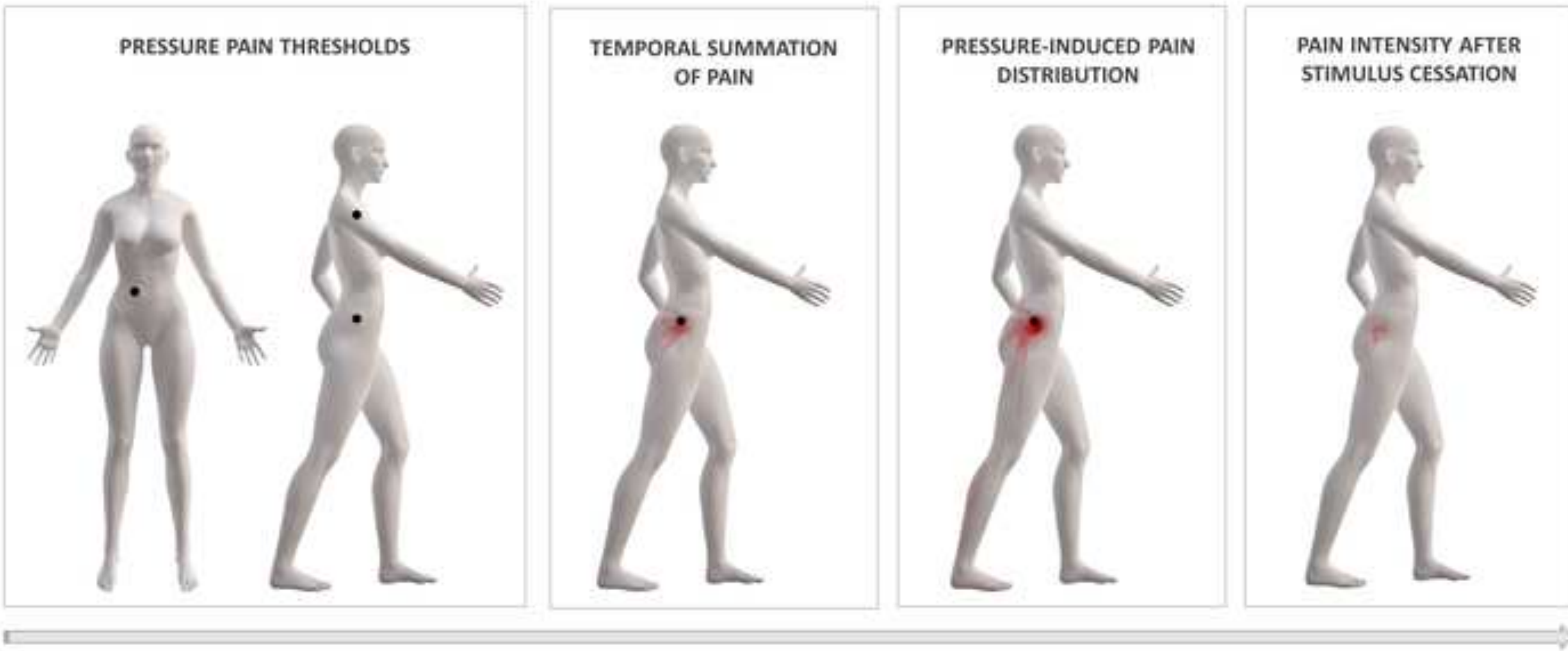
PRESSURE PAIN THRESHOLDS
TEMPORAL SUMMATION OF PAIN
PRESSURE-INDUCED PAIN DISTRIBUTION
PAIN INTENSITY AFTER STIMULUS CESSATION

Figure 2

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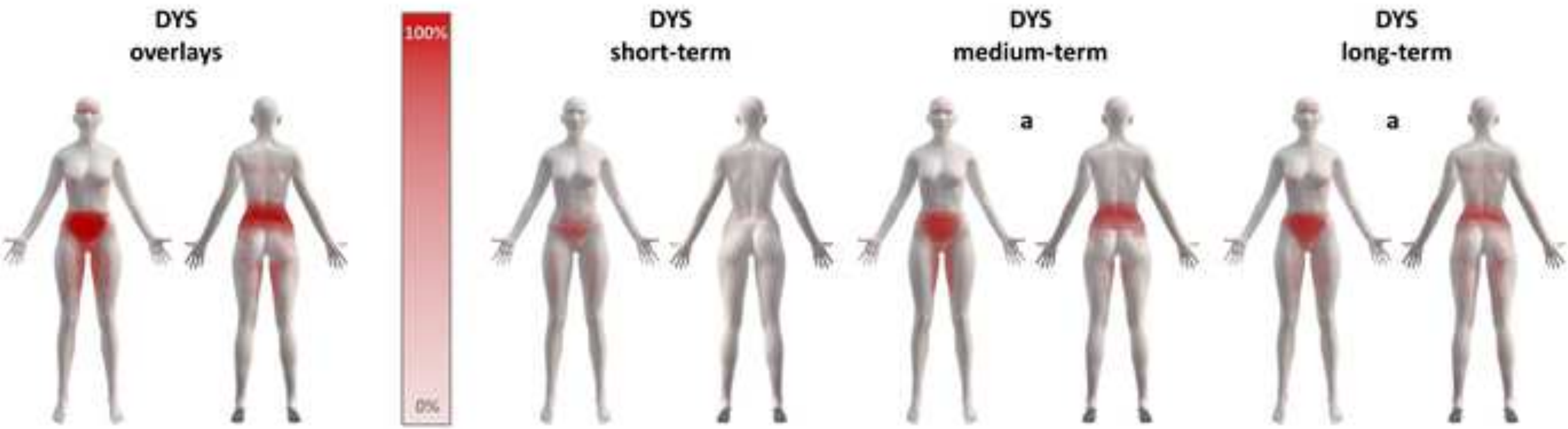

Figure 3

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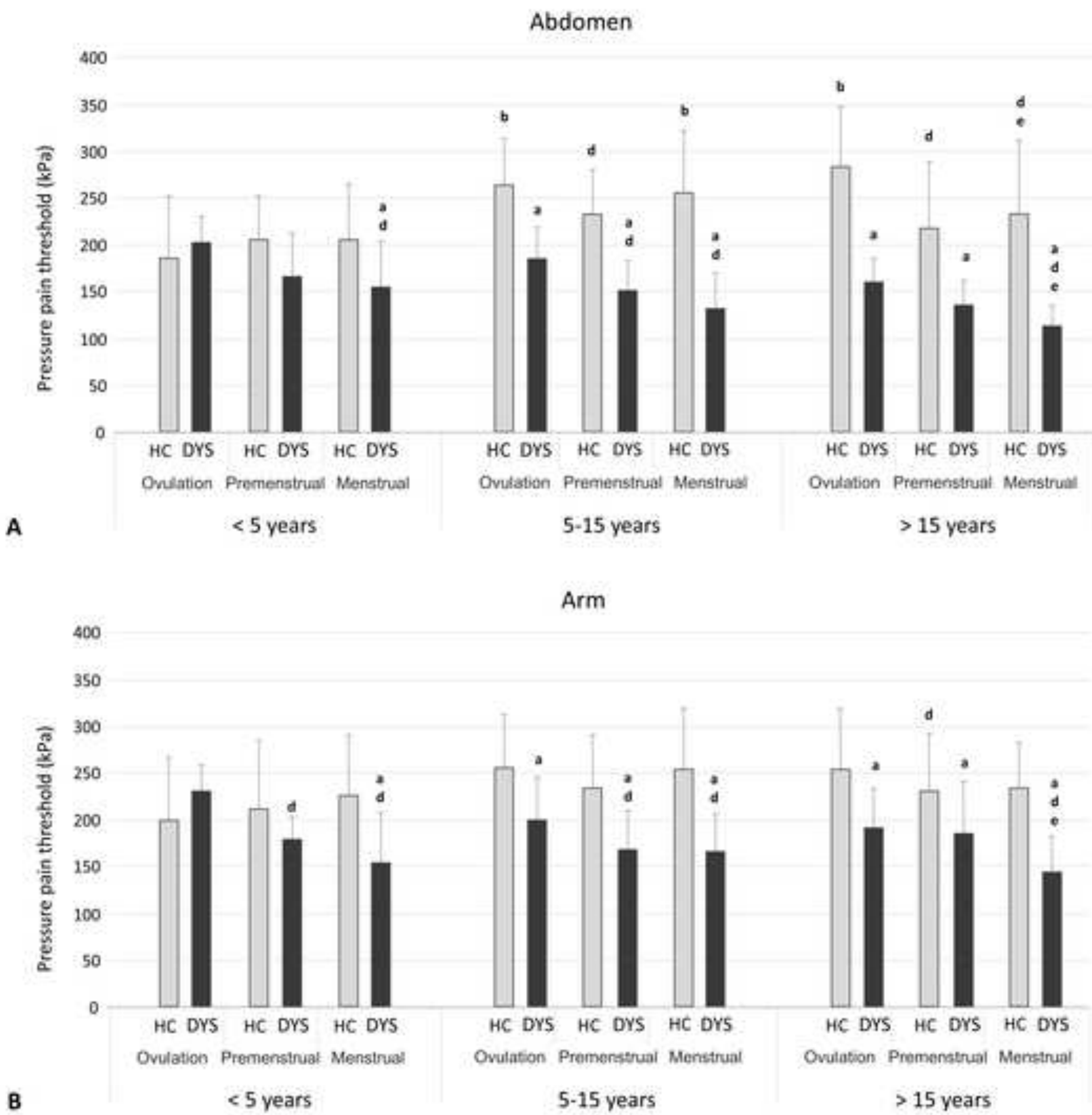

Figure 4

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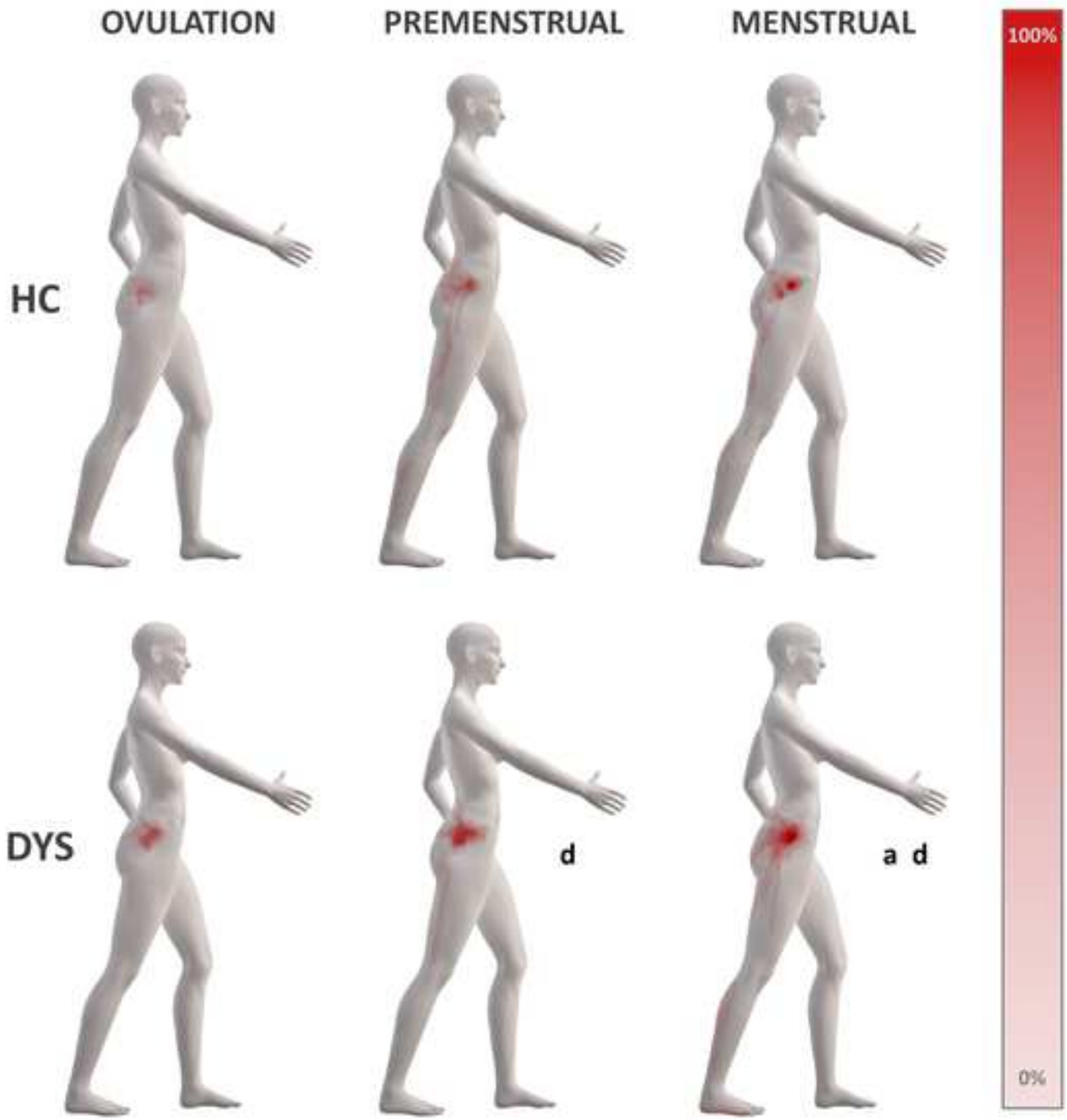


Table 1. Demographics, menstrual pain features, and depression, anxiety and stress scores in DYS and HC groups and subgroups.

	ALL		< 5 years		5-15 years		> 15 years	
	DYS (n=30)	**HC (n=30)**	**DYS (n=10)**	**HC (n=10)**	**DYS (n=10)**	**HC (n=10)**	**DYS (n=10)**	**HC (n=10)**
Age	23.8 ±(6.0)	24.7 ±(6.2)	18.0 ±(0.0)	18.1 ±(0.3)	22.6 ±(3.1)	24.1 ±(2.2)	30.7 ±(3.6)	31.9 ±(3.5)
Menarche age	12.6 ±(0.7) a	13.4 ± (0.6)	13.1 ±(0.3)	13.4 ± (0.5)	12.4 ±(0.7) a	13.3 ± (0.7)	12.4 ±(0.7) a	13.5 ± (0.7)
Years since menarche (lived with MP in DYS women)	11.1 ±(6.2)	11.3 ±(6.1)	4.9±(0.3)	4.7 ±(0.5)	10.2 ±(3.2)	11.8 ±(2.3)	18.3 ±(3.3)	19.4 ±(3.4)
BMI (Kg/m2)	20.9 ±(2.3)	20.9 ±(1.9)	22.1 ±(3.0)	22.0 ±(2.6)	20.4 ±(1.9)	20.6 ±(1.6)	20.2 ±(1.5)	20.2 (1.0)
Children (n)	0 [0-0]	0 [0-0]	0 [0-0]	0 [0-0]	0 [0-0]	0 [0-0]	0 [0-1]	0 [0-2]
Menstrual cycle (n)	27.8 ±(1.5)	27.7 ±(1.6)	26.9 ±(1.4)	26.7 ±(0.8)	28.2 ±(1.6)	28.0 ±(1.8)	28.2 ±(1.2)	28.3 ±(1.6)
Days menstrual phase (n)	5.1 ±(0.8)	4.7 ±(0.7)	5.1 ±(0.7)	5.1 ±(0.7)	4.9 ±(0.7)	4.6 ±(0.7)	5.3 ±(0.8)	4.5 ±(0.5)
Days MP (n)	4 [3-4]	1 [0-1.25]	4 [3-4]	1 [0-2]	4 [3-4]	0.5 [0-1]	4 [3-4]	0 [0-1.25]
Days VAS-max MP>60 (n)	2 [2-3]	0 [0-0]	2 [1.8-2.3]	0 [0-0]	2 [2-3]	0 [0-0]	3 [2-3.3] bc	0 [0-0]
VAS-max MP	71.3 ±(6.5)	6.9 ±(6.9)	70.1 ±(7.8)	8.8 ±(6.6)	70.7 ±(7.3)	6.0 ±(6.3)	73.2 ±(3.9)	6.0 ±(8.1)
VAS average MP	62.9 ±(6.2)	1.9 ±(2.9)	62.1 ±(7.8)	4.1 ±(3.4)	62.8 ±(6.3)	0.9 ±(2.2)	63.8 ±(4.7)	0.7 ±(1.6)
AREA-size MP	17497 ±(13216)	457 ±(549)	7304 ±(3387)	540 ±(521)	16492 ±(10455) b	405 ±(555)	28693 ±(13644) bc	427 ±(616)
DASS-21 – OV	6.70 (5.77)	7.13 (5.63)	7.70 (6.60)	5.90 (5.80)	6.20 (5.31)	6.50 (5.50)	6.20 (5.81)	9.00 (5.68)
DASS-21 - MENS	6.80 (5.77)	6.77 (5.55)	6.40 (5.99)	5.00 (4.67)	7.60 (5.30)	6.60 (5.64)	6.40 (6.50)	8.70 (6.17)

Menstrual pain variables analyzed with one-way ANOVAs between dysmenorrhea subgroups; DASS-21, depression, anxiety and stress, analysed with three-way ANOVAs, for the PD and HC groups and subgroups, in ovulation and menstrual phases; otherwise, two-way ANOVAs for HC and PD groups and subgroups. Values presented as mean (±SD) or median [interquartile range]. Symbols: a, significant differences compared with the HC group and matched HC-subgroups; b, significant differences within the DYS group, as compared to the DYS <5 years subgroup; c significant differences within the DYS group, as compared to the DYS 5-15 years subgroup (Bonferroni: P< 0.05). Abbreviations: DYS, dysmenorrhea; HC, healthy controls; MP, menstrual pain; BMI, biomass index; n, number; VAS, visual analogue scale; AREA-size, size of the pain areas; OV, ovulation phase; MENS, menstrual phase.

Table 2. Pressure pain thresholds and pressure-induced pain over the gluteus medius muscle.

	ALL		< 5 years		5-15 years		> 15 years	
	DYS (n=30)	**HC (n=30)**	**DYS (n=10)**	**HC (n=10)**	**DYS (n=10)**	**HC (n=10)**	**DYS (n=10)**	**HC (n=10)**
PPT abdomen (kPa)	a				a	b	a	b
OV	179 ±(33) a	244 ±(67)	200 ±(28)	182 ±(58)	179 ±(32) a	265 ±(67) b	158 ±(26) a	287 ±(13) b
PREM	149 ±(38) ad	216 ±(49) d	164 ±(49) d	197 ±(45)	150 ±(35) ad	235 ±(65) d	135 ±(24) a	215 ±(24) d
MENS	133 ±(41) ade	228 ±(57) de	153 ±(51) ad	196 ±(57)	131 ±(40) ad	250 ±(68) b	114 ±(21) ade	238 ±(26) de
PPT hip (kPa)	a							
OV	211 ±(62)	241 ±(75)	221 ±(55)	222 ±(70)	226 ±(82)	292 ±(61)	186 ±(34)	208 ±(69)
PREM	178 ±(42) ad	228 ±(68)	189 ±(45)	231 ±(57)	186 ±(52)	265 ±(50)	159 ±(22)	188 ±(76)
MENS	163 ±(54) ad	231 ±(69)	177 ±(61)	244 ±(67)	173 ±(54)	266 ±(69)	138 ±(42)	183 ±(80)
PPT arm (kPa)	a							
OV	209 ±(48) a	239 ±(62)	234 ±(31)	206 ±(69)	205 ±(49) a	256 ±(46)	188 ±(52) a	254 ±(61)
PREM	174 ±(46) ad	227 ±(56)	175 ±(25) d	218 ±(61)	169 ±(49) ad	239 ±(50)	178 ±(61) a	225 ±(61) d
MENS	165 ±(40) ad	238 ±(52)	184 ±(31) ad	226 ±(66)	165 ±(46) ad	247 ±(47)	144 ±(33) ade	240 ±(42)
VAS-60s (mm)	a							
OV	53.2 ±(12.0)	46.7 ±(13.5)	49.8 ±(9.1)	47.2 ±(10.2)	53.0 ±(16.5)	45.2 ±(18.4)	56.8 ±(9.2)	47.7 ±(11.9)
PREM	54.5 ±(14.7)	48.7 ±(17.9)	51.0 ±(14.9)	47.8 ±(19.7)	56.1 ±(16.9) a	38.4 ±(11.8)	56.3 ±(13.0)	60.1 ±(15.2) cd
MENS	60.1 ±(18.2)	53.9 ±(13.4)	55.6 ±(21.1)	47.5 ±(6.8)	55.6 ±(16.7)	57.2 ±(14.3) e	69.1 ±(14.6)	57.2 ±(16.1)
TSP (mm)	a							
OV	28.0 ±(10.7)	19.5 ±(11.6)	23.9 ±(7.5)	21.9 ±(11.8)	28.7 ±(11.1)	18.5 ±(15.0)	31.4 ±(12.3)	18.1 ±(7.6)
PREM	27.3 ±(11.8)	19.9 ±(15.0)	22.3 ±(11.2)	15.5 ±(17.6)	28.2 ±(12.9)	14.4 ±(13.3)	31.3 ±(10.3)	30.0 ±(8.4)
MENS	28.8 ±(15.9)	22.6 ±(14.0)	25.3 ±(18.0)	21.8 ±(9.1)	24.2 ±(14.1)	23.9 ±(15.5)	36.9 ±(13.3)	22.3 ±(17.4)
AREA-size PIP (pixels)	a						a	
OV	382 ±(375)	572 ±(1055)	268 ±(192)	603 ±(714)	508 ±(574)	307 ±(192)	369 ±(233)	806 ±(1704)
PREM	1000 ±(1288) d	811 ±(1630)	252 ±(155)	756 ±(1165)	1348 ±(1771) a	261 ±(148)	1401 ±(1117) b	1418 ±(2537)
MENS	1515 ±(2372) ad	634 ±(1267)	697 ±(752) d	259 ±(256)	750 ±(630)	905 ±(1592)	3098 ±(3604) abcde	740 ±(1524)
VAS-Aft (mm)	a							
OV	0 [0-5.5]	0 [0-0]	0 [0-1.8]	0 [0-9.5]	0 [0-6]	0 [0-0]	0 [0-18]	0 [0-4.8]
PREM	0 [0-11]	0 [0-0]	0 [0-0]	0 [0-6.8]	0 [0-12]	0 [0-0]	5.5 [0-23.3]	0 [0-4.3]
MENS	15.5 [0-23] ade	0 [0-0]	8.5 [0-21]	0 [0-0]	7.5 [0-20]	0 [0-5.8]	21 [9.8-28.3]	0 [0-0]

Analysed with three-way ANOVAs for HC and DYS groups and subgroups. Values presented as mean (±SD) or median [interquartile range]. Symbols: a, significant differences compared with the HC group and HC-subgroup; b, significant differences within the PD or HC group, as compared to the <5 years subgroup; c, significant differences within the DYS or HC group, as compared to the 5-15 years subgroup; d, significant differences within group or subgroup as compared to the ovulation phase after Bonferroni corrections; e, significant differences within group or subgroup as compared to the premenstrual phase (Bonferroni: $P < 0.05$). Abbreviations: DYS, dysmenorrhea; HC, healthy controls; PPT, pressure pain threshold; TSP, temporal summation of pain; PIP, pressure-induced pain; n, number of participants; OV, ovulation phase; PREM, premenstrual phase; MENS, menstrual phase.

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Demographics and menstrual pain features in women who completed the study and those who dropped out, in DYS and HC groups and subgroups.

	< 5 years				**5-15 years**				**>15 years**			
	DYS (n=10)	**dropouts (n=3)**	**HC (n=10)**	**dropouts (n=0)**	**DYS (n=10)**	**dropouts (n=7)**	**HC (n=10)**	**dropouts (n=1)**	**DYS (n=10)**	**dropouts (n=2)**	**HC (n=10)**	**dropouts (n=4)**
Age	18.0 ±(0.0)	18.0 ±(0.1)	18.1 ±(0.3)		22.6 ±(3.1)	23.8 ±(4.0)	24.1 ±(2.2)	25	30.7 ±(3.6)	32.1 ±(2.8)	31.9 ±(3.5)	32.3 ±(2.6)
Menarche age	13.1 ±(0.3)	13.2 ±(0.8)	13.4 ± (0.5)		12.4 ±(0.7)	12.4 ±(0.5)	13.3 ± (0.7)	13	12.4 ±(0.7)	12.6 ±(0.5)	13.5 ± (0.7)	13.4 ± (0.6)
Years since menarche	4.9±(0.3)	4.8±(0.7)	4.7 ±(0.5)		10.2 ±(3.2)	11.4 ±(3.7)	11.8 ±(2.3)	12	18.3 ±(3.3)	19.5 ±(1.8)	19.4 ±(3.4)	18.9 ±(3.3)
BMI (Kg/m2)	22.1 ±(3.0)	21.1 ±(3.2)	22.0 ±(2.6)		20.4 ±(1.9)	21.6 ±(3.0)	20.6 ±(1.6)	21.2	20.2 ±(1.5)	22.1 ±(1.8)	20.2 ±(1.0)	22.3 ±(1.3)
Children (n)	0 [0-0]	0 [0-0]	0 [0-0]		0 [0-0]	0 [0-0]	0 [0-0]	0	0 [0-1]	1 [1-1]	0 [0-2]	1 [1-2]
Menstrual cycle (n)	26.9 ±(1.4)	26.5 ±(2.4)	26.7 ±(0.8)		28.2 ±(1.6)	27.3 ±(1.2)	28.0 ±(1.8)	29	28.2 ±(1.2)	28.9 ±(2.6)	28.3 ±(1.6)	27.2 ±(1.5)
Days menstrual phase (n)	5.1 ±(0.7)	5.6 ±(0.2)	5.1 ±(0.7)		4.9 ±(0.7)	5.1 ±(0.8)	4.6 ±(0.7)	5	5.3 ±(0.8)	5.8 ±(0.5)	4.5 ±(0.5)	5.0 ±(0.8)
Days MP (n)	4 [3-4]	4 [4-4]	1 [0-2]		4 [3-4]	4 [4-4]	0.5 [0-1]	0	4 [3-4]	4 [4-5]	0 [0-1.25]	0.5 [0-1]
Days VAS-max MP>60 (n)	2 [1.8-2.3]	2 [2-3]	0 [0-0]		2 [2-3]	3 [2-3.4]	0 [0-0]	0	3 [2-3.3]	3 [3-3]	0 [0-0]	0 [0-0]
VAS-max MP	70.1 ±(7.8)	71.2 ±(9.9)	8.8 ±(6.6)		70.7 ±(7.3)	74.1 ±(5.8)	6.0 ±(6.3)	13	73.2 ±(3.9)	75.0 ±(7.8)	6.0 ±(8.1)	7.8 ±(5.6)
VAS average MP	62.1 ±(7.8)	69.7 ±(2.5)	4.1 ±(3.4)		62.8 ±(6.3)	71.4 ±(6.5)	0.9 ±(2.2)	0	63.8 ±(4.7)	64.0 ±(5.6)	0.7 ±(1.6)	4.5 ±(6.4)

Abbreviations: DYS, dysmenorrhea; HC, healthy controls; MP, menstrual pain; BMI, biomass index; n, number; VAS, visual analogue scale.

Supplementary table 2. Correlation values (*ρ*) between pain-sensory variables for the ovulation phase in women with dysmenorrhea.

		PPT abdomen	PPT hip	PPT arm	VAS-60s	TSP	VAS-Aft	AREA-size PIP	VAS-max MP	AREA-size MP	DASS-21	Age	Years since menarche
PPT abdomen	ρ	1.000	**0.529 a**	0.457	-0.439	-0.283	0.075 a	-0.039 a	-0.302	-0.372	0.346 a	**-0.548 a**	**-0.541** a
	P value		0.003	0.011	0.015	0.130	0.695	0.837	0.105	0.043	0.061	0.002	0.002
PPT hip	ρ		1.000	0.464 a	-0.048 a	-0.237 a	0.376 a	0.144 a	-0.219 a	-0.277 a	0.325 a	-0.335 a	-0.305 a
	P value			0.010	0.803	0.207	0.041	0.449	0.244	0.138	0.080	0.071	0.101
PPT arm	ρ			1.000	-0.264	-0.181	0.114 a	0.145 a	-0.104	-0.180	0.045 a	-0.530 a	-0.505 a
	P value				0.159	0.340	0.549	0.446	0.586	0.342	0.812	0.003	0.004
VAS-60s	ρ				1.000	**0.694**	-0.027 a	-0.012 a	0.303	0.301	-0.191 a	0.377 a	0.387 a
	P value					0.000	0.888	0.948	0.104	0.106	0.312	0.040	0.035
TSP	ρ					1.000	-0.062 a	0.053 a	0.392	0.432	-0.053 a	0.379 a	0.433 a
	P value						0.744	0.782	0.032	0.017	0.782	0.039	0.017
VAS-Aft	ρ						1.000	**0.576** a	-0.065 a	0.077 a	0.142 a	0.015 a	0.066 a
	P value							0.001	0.734	0.684	0.456	0.939	0.727
AREA-size PIP	ρ							1.000	0.109 a	0.279 a	0.345 a	0.237 a	0.276 a
	P value								0.565	0.135	0.062	0.208	0.140
VAS-max MP	ρ								1.000	0.178	0.149 a	0.298 a	0.312 a
	P value									0.346	0.432	0.110	0.093
AREA-size MP	ρ									1.000	0.159 a	**0.620 a[a]**	**0.653** a
	P value										0.403	0.000	0.000
DASS-21	ρ										1.000	-0.146 a	-0.141 a
	P value											0.442	0.457
Age	ρ											1.000	**0.981 a**
	P value												0.000
Years since menarche	ρ												1.000
	P value												

N=30. ρ expressed as Pearsons's r unless indicated. a Spearman's rho values. Numbers highlighted in bold represent a significant correlation after Bonferroni correction (P=0.05/25: P<0.002 for VAS-max MP, AREA-size MP, **Age** and Years since menarche; P=0.05/10: P<0.005 for the rest of parameters). Abbreviations: PPT, Pressure Pain Threshold; TSP, temporal summation of pain; PIP, pressure-induced pain; MP, menstrual pain.

Supplementary table 3. Correlation values (ρ) between pain-sensory variables for the premenstrual phase in women with dysmenorrhea.

		PPT abdomen	PPT hip	PPT arm	VAS-60s	TSP	VAS-Aft	AREA-size PIP	VAS-max MP	AREA-size MP	Age	Years since menarche
PPT abdomen	ρ	1.000	**0.586 a**	0.327	-0.217	-0.201	-0.263 a	-0.207 a	**-0.676**	-0.143	-0.409 a	-0.401 a
	P value		0.001	0.078	0.249	0.287	0.160	0.272	0.000	0.450	0.025	0.028
PPT hip	ρ		1.000	0.383 a	-0.342 a	-0.391 a	-0.453 a	-0.361 a	-0.499 a	-0.138 a	**-0.574 a**	**-0.551** a
	P value			0.037	0.064	0.032	0.012	0.050	0.005	0.465	0.001	0.002
PPT arm	ρ			1.000	0.097	-0.054	0.093 a	0.103 a	-0.229	0.262	-0.201 a	-0.185 a
	P value				0.611	0.777	0.626	0.589	0.225	0.162	0.287	0.327
VAS-60s	ρ				1.000	**0.694**	**0.499** a	**0.489** a	0.273	0.334	0.323 a	0.312 a
	P value					0.000	0.005	0.006	0.145	0.071	0.082	0.093
TSP	ρ					1.000	0.437 a	0.382 a	0.217	0.495	0.375 a	0.373 a
	P value						0.016	0.037	0.249	0.005	0,.041	0.043
VAS-Aft	ρ						1.000	**0.813** a	0.316 a	0.455 a	0.453 a	0.454 a
	P value							0.000	0.089	0.011	0.012	0.012
AREA-size PIP	ρ							1.000	0.218 a	0.447 a	0.160 a	0.276 a
	P value								0.247	0.013	0.022	0.140
VAS-max MP	ρ								1.000	0.178	0,298 a	0.312 a
	P value									0.346	0.110	0.093
AREA-size MP	ρ									1.000	**0.620 a**	**0.653** a
	P value										0.000	0.000
Age	ρ										1.000	**0.981 a**
	P value											0.000
Years since menarche	ρ											1.000
	P value											

N=30. ρ expressed as Pearsons's r unless indicated. a Spearman's rho values. Numbers highlighted in bold represent a significant correlation after Bonferroni correction (P=0.05/25: P<0.002 for VAS-max MP, AREA-size MP, **Age** and Years since menarche; P=0.05/9: P<0.006 for the rest of parameters). Abbreviations: PPT, Pressure Pain Thresholds; TSP, temporal summation of pain; PIP, pressure-induced pain; MP, menstrual pain.

Supplementary table 4. Correlation values (ρ) between pain-sensory variables for the menstrual phase in women with dysmenorrhea.

		PPT abdomen	PPT hip	PPT arm	VAS-60s	TSP	VAS-Aft	AREA-size PIP	VAS-max MP	AREA-size MP	DASS-21	Age	Years since menarche
PPT abdomen	ρ	1.000	**0.726 a**	**0.713**	-0.127	-0.105	0.190 a	0.099 a	-0.444	-0.100	0.057 a	-0.486 a	-0.470 a
	P value	.	0.000	0.000	0.503	0.579	0.315	0.603	0.014	0.598	0.765	0.007	0.009
PPT hip	ρ		1.000	**0.689 a**	-0.217 a	-0.221 a	-0.022 a	-0.013 a	-0.429 a	-0.147 a	0.010 a	-0.409 a	0.405 a
	P value		.	0.000	0.250	0.240	0.910	0.945	0.018	0.438	0.959	0.025	0.026
PPT arm	ρ			1.000	-0.263	-0.245	0.167 a	0.017 a	**-0.541**	-0.138	-0.013 a	**-0.566 a**	**-0.559 a**
	P value				0.161	0.192	0.377	0.927	0.002	0.467	0.947	0.001	0.001
VAS-60s	ρ				1.000	**0.837**	0.479 a	**0.579 a**	0.332	0.505	0.072 a	0.367 a	0.356 a
	P value					0.000	0.007	0.001	0.074	0.004	0.705	0.046	0.053
TSP	ρ					1.000	0.386 a	0.509 a	0.207	0.511	0.004 a	0.339 a	0.309 a
	P value						0.035	0.004	0.272	0.004	0.981	0.067	0.096
VAS-Aft	ρ						1.000	**0.821 a**	0.199 a	**0.498 a**	0.061 a	0.227 a	0.228 a
	P value							0.000	0.291	0.005	0.747	0.228	0.226
AREA-size PIP	ρ							1.000	0.253 a	**0.705 a**	0.292 a	**0.426 a**	0.456 a
	P value								0.178	0.000	0.118	0.019	0.011
VAS-max MP	ρ								1.000	0.178	0.019 a	0.298 a	0.312 a
	P value									0.346	0.919	0.110	0.093
AREA-size MP	ρ									1.000	0.152 a	**0.620 a**	**0.653 a**
	P value										0.424	0.000	0.000
DASS-21	ρ										1.000	-0.075 a	-0.073 a
	P value											0.693	0.703
Age	ρ											1.000	**0.981 a**
	P value												0.000
Years since menarche	ρ												1.000
	P value												

N=30. ρ expressed as Pearsons's r unless indicated. a Spearman's rho values. Numbers highlighted in bold represent a significant correlation after Bonferroni correction (P=0.05/25: P<0.002 for VAS-max MP, AREA-size MP, **Age** and Years since menarche; P=0.05/10: P<0.005 for the rest of parameters). Abbreviations: PPT, Pressure Pain Thresholds; TSP, temporal summation of pain; PIP, pressure-induced pain; MP, menstrual pain; DASS-21, Depression, Anxiety and Stress Scale.

Supplementary figure 1.

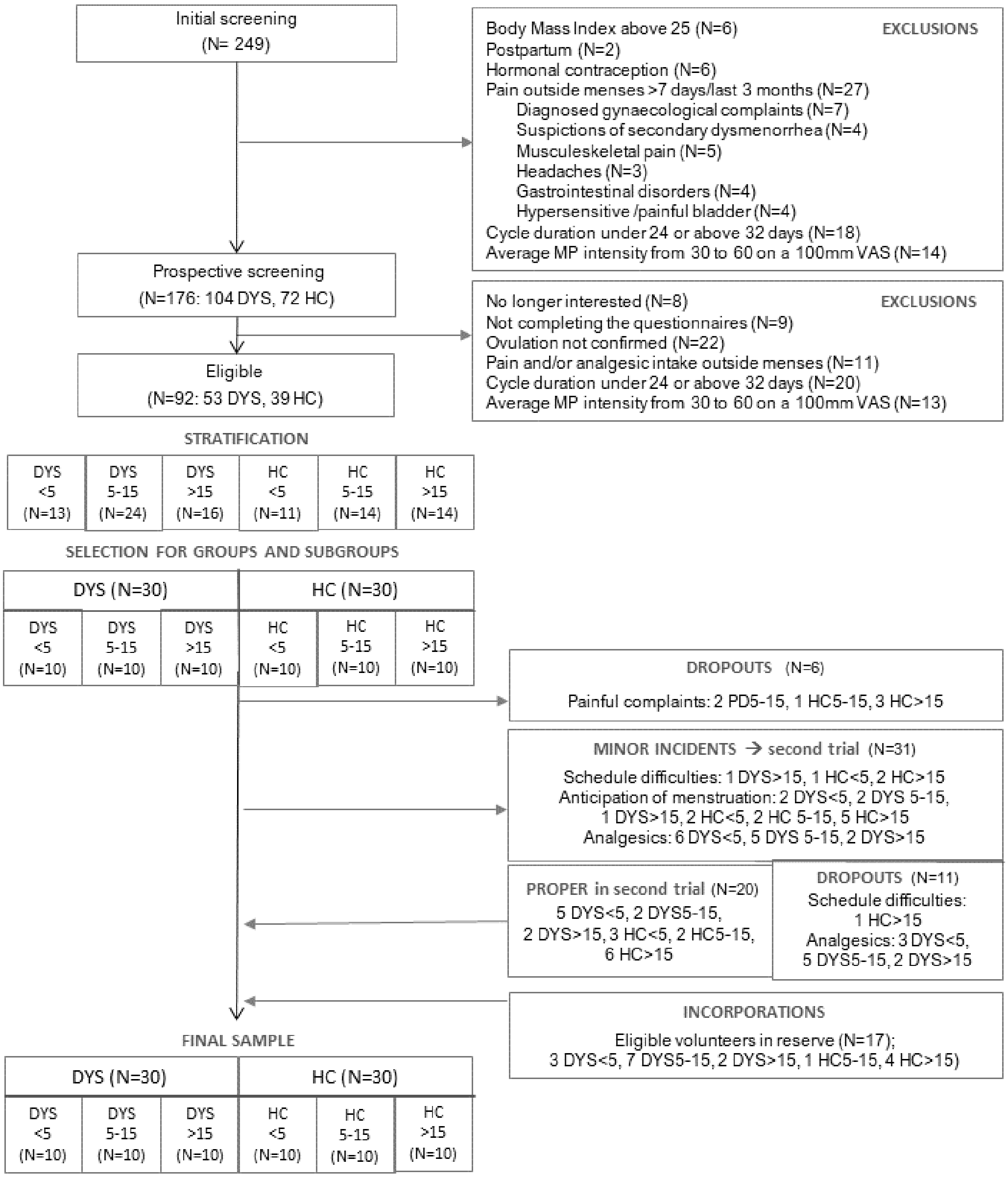
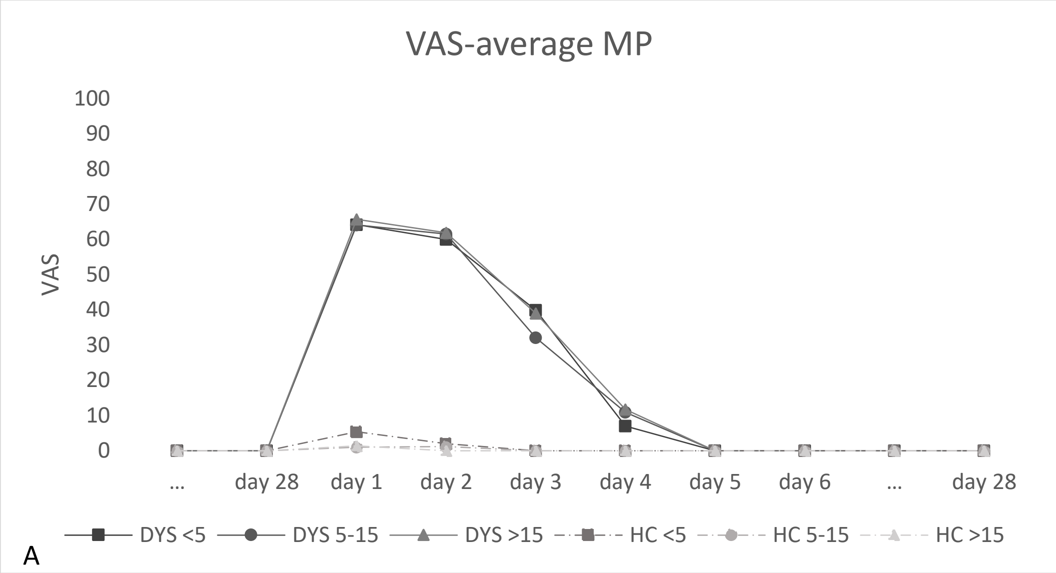


Figure legend 1. *Participants flow chart and study groups. Abbreviations: MP, menstrual pain;* ***DYS****<5, dysmenorrhea up to 5 years since menarche;* ***DYS*** *5-15, dysmenorrhea above 5 and under 15 years since menarche;* ***DYS****>15, dysmenorrhea from 15 years since menarche; HC<5, healthy controls up to 5 years since menarche; HC5-15, healthy controls above 5 and under 15 years since menarche; HC>15, healthy controls from 15 years since menarche.*

Supplementary figure 2.



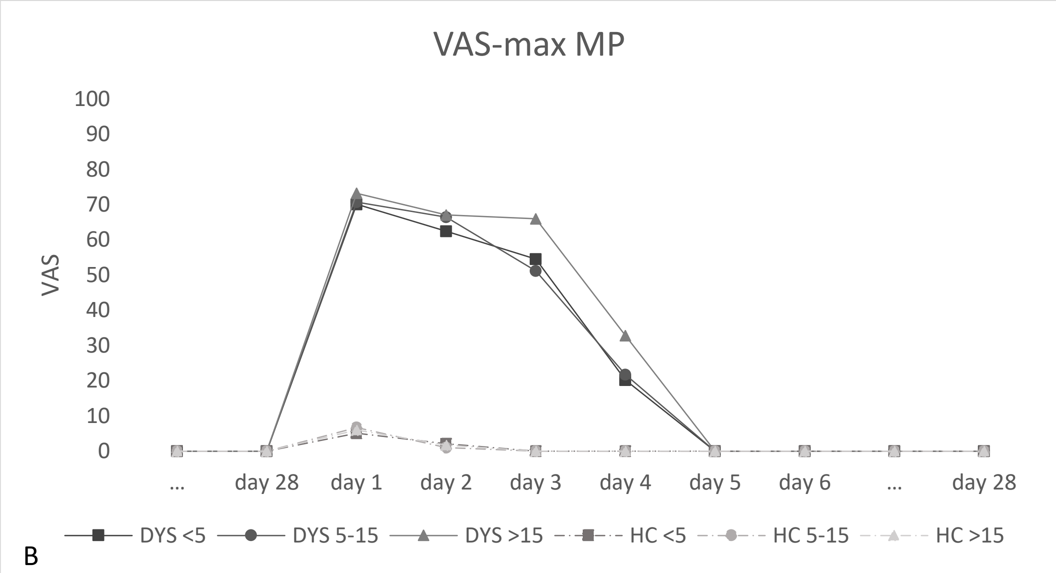


Figure legend 2. *Daily reports of average (A) and maximal (B) menstrual pain intensities during the menstrual phase of the cycle in healthy controls (HC) and dysmenorrhea (DYS) subgroups. Abbreviations: n, number, MP, menstrual pain; DYS <5, dysmenorrhea up to 5 years since menarche; DYS 5-15, dysmenorrhea above 5 and under 15 years since menarche; DYS >15, dysmenorrhea from 15 years since menarche; HC <5, healthy controls up to 5 years since menarche; HC 5-15, healthy controls above 5 and under 15 years since menarche; HC >15, healthy controls from 15 years since menarche.*