Morphometry of the Pelvic Floor Muscles in Women With and Without Provoked Vestibulodynia Using 4D Ultrasound

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ABSTRACT

Introduction. It has been suggested that pelvic floor muscles (PFMs) play an important role in provoked vestibulodynia (PVD) pathophysiology. Controversy in determining their exact contribution may be explained by methodological limitations related to the PFM assessment tools, specifically the pain elicited by the measurement itself, which may trigger a PFM reaction and introduce a strong bias.

Aim. The aim of this study was to compare PFM morphometry in women suffering from PVD to asymptomatic healthy control women using a pain-free methodology, transperineal four-dimensional (4D) ultrasound.

Methods. Fifty-one asymptomatic women and 49 women suffering from PVD were recruited. Diagnosis of PVD was confirmed by a gynecologist following a standardized examination. All the participants were nulliparous and had no other urogynecological conditions. The women were evaluated in a supine position at rest and during PFM maximal contraction.

Main Outcome Measures. Transperineal 4D ultrasound, which consists of a probe applied on the surface of the perineum without any vaginal insertion, was used to assess PFM morphometry. Different parameters were assessed in sagittal and axial planes: anorectal angle, levator plate angle, displacement of the bladder neck, and levator hiatus area. The investigator analyzing the data was blinded to the clinical data.

Results. Women with PVD showed a significantly smaller levator hiatus area, a smaller anorectal angle, and a larger levator plate angle at rest compared with asymptomatic women, suggesting an increase in PFM tone. During PFM maximal contraction, smaller changes in levator hiatus area narrowing, displacement of the bladder neck, and changes of the anorectal and of the levator plate angles were found in women with PVD compared with controls, which may indicate poorer PFM strength and control.

Conclusion. Using a reliable and pain-free methodology, this research provides sound evidence that women with PVD display differences in PFM morphometry suggesting increased tone and reduced strength. Morin M, Bergeron S, Khalifé S, Mayrand M-H, and Binik YM. Morphometry of the pelvic floor muscles in women with and without provoked vestibulodynia using 4D ultrasound. J Sex Med **,**:**–**.

Key Words. Provoked Vestibulodynia; Dyspareunia; Vulvodynia; High Tone Pelvic Floor Dysfunction; Levator Ani; Ultrasound

Introduction

Considered as the leading cause of premenopausal dyspareunia [1], provoked vestibulodynia (PVD), is a chronic burning pain localized at the entry of the vagina when pressure is applied to the area, most frequently during sexual activity [2]. The etiology of PVD is poorly understood. Among the proposed mechanisms, the involvement of the pelvic floor muscles (PFMs) is thought to play an important role [3–6]. It is hypothesized that vestibular pain and inflammation trigger a protective defense mechanism of the PFM in addition to poor muscle
control and hypertonicity [7–12]. PFM hypertonicity may also act as an initiator of vestibular sensory changes and inflammation [3]. However, empirical studies have yielded contradictory results when comparing PFM function in women with PVD vs. controls. Some studies showed that women with PVD had PFM hypertonicity assessed with palpation [9] and an increased electromyographic resting activity [11–13] while others found a nonsignificant difference [9,14]. The same divergent findings were observed in studies of lower maximal strength of the PFM in women with PVD [9–11,14]. Methodological flaws in the current PFM assessment techniques may explain such discrepancies. Digital palpation is a subjective assessment [15] that could be biased by patient fear or pain reactions, which could influence the evaluator toward scoring tone level as higher [10]. Regarding electromyography (EMG), confounding factors such as the contact between the electrodes and the mucosa, the position of the electrodes in relation to the muscle fibers, the degree of vaginal lubrication, and the thickness of the vaginal tissue can all affect signal detection and compromise comparison between participants, especially when assessing the amplitude of the signal at rest and during contraction [16]. In addition, the intravaginal approach (i.e., insertion of a probe or a finger) associated with these procedures can be a key problem in PFM measurements in women with PVD. The pain instigated by the assessment itself might trigger a PFM contraction and an increase in tone. Such protective contractions have already been demonstrated during a painful stimulus to the vestibule area in women with PVD [11]. Therefore, it has not been possible to investigate whether the PFM dysfunctions found in this population are due to a protective reaction to the painful assessment or whether these dysfunctions are present independently of pain. Four-dimensional (4D) transperineal ultrasound, mostly used in women with pelvic organ prolapse to assess PFM function [17,18], can overcome these limitations because it is a pain-free procedure in which a convex probe is gently applied on the perineum without vaginal insertion.

**Aim**

The aim of this case control study was to compare the morphometry of the PFM in women with PVD and asymptomatic women using transperineal 4D ultrasound. The hypotheses were that PFM impairments are part of the pathophysiology of PVD and are not limited to a protective reaction to the assessment-induced pain. Specifically, we expected that the morphometry of the PFM at rest would show hypertonicity in women with PVD compared with controls and that women with PVD would present lower PFM strength during a maximal contraction.

**Methods**

**Participants**

Women suffering from PVD and asymptomatic controls were recruited by means of posters in universities and affiliated hospitals, health professional referrals, university website advertisements, as well as by local newspaper announcements in a large metropolitan area. In the PVD group, the sample included 6% recruited during visits with health professionals (gynecologists and psychologists), 90% recruited through posters and advertisements, and the last 4% were women recruited via another PVD study having expressed interest in participating in similar studies. In the control group, 75% were recruited through posters and advertisements and 25% by word of mouth. Women interested in participating in the study were invited to contact the research assistant and to take part in a screening telephone interview. The eligibility criteria were also verified by the physiotherapist and the gynecologist. In order to confirm the PVD diagnosis, all women reporting pain during intercourse underwent an interview and a physical examination performed by a gynecologist using a standardized protocol [19]. Part of the protocol involved a cotton-swab test, which consisted of pressure applied at the level of the vulvar vestibule (3, 6, and 9 o’clock). The women had to report the intensity of their pain using a numerical rating scale (NRS) from 0 to 10. The test was considered positive when the pain elicited reached an average intensity of 5 or more, indicative of a moderate to severe pain [20–22], and was reported to be similar to the pain perceived during vaginal intercourse. The gynecological assessment was performed on a different occasion than the ultrasound measurements. Women with PVD met the following inclusion criteria: (i) ability to experience complete vaginal penetration, which is painful on at least 80% of the intercourse attempts; (ii) pain during intercourse, which is subjectively distressing and has lasted for at least 6 months; and (iii) pain limited to intercourse and other activities involving vestibular pressure (e.g.,
bicycling). Asymptomatic women had to report no history of vulvovaginal pain and no difficulties with sexual activity, gynecological examinations, or insertion of tampons. They also had to be sexually active, defined as having had vaginal penetration in the last 6 months, in order to assess any potential sexual or pain problem. The exclusion criteria for the two groups were: (i) pelvic or vulvar pain not clearly linked to intercourse; (ii) deep dyspareunia; (iii) postmenopausal status; (iv) current or previous pregnancy that had lasted more than 18 weeks; (v) one of the following urogynecologic symptoms ([a] urinary or anal incontinence; [b] urinary urgency; [c] pelvic organ prolapse [>1 stage at the pelvic organ prolapse quantification [23]], [d] active urinary or vaginal infection [or in the last 3 months]); (vi) previous vulvovaginal surgery; (vii) ongoing treatment for dyspareunia; and (viii) age less than 18 or greater than 45. Of the 133 women who met the eligibility criteria, 12 women with PVD and 21 controls refused to participate, resulting in sample size of 49 women with PVD and 51 controls.

Procedure
Participants were invited to an assessment session performed in a private obstetrics and gynecology clinic. A gynecologist (S.K.) performed all ultrasound assessments, while a physical therapist (M.M.) gave instructions to the participants and supervised the testing procedure. After providing informed consent, participants were asked to empty their bladder prior to the assessment. Information about the pelvic floor anatomy and physiology was given to each participant, as well as instructions about how to contract the PFM. Vaginal palpation was used to ensure that the participants were able to properly contract the PFM, producing a squeeze and inward movement and avoiding straining and other muscle contractions such as gluteal, hip adductor, or abdominal muscle. The women adopted a gynecological position with their feet in the stirrups. The probe, covered with a nitril glove with conducting applied gel above and below, was placed on the perineum in a midsagittal plane oriented cranially. The volume scanned had to include the posteroinferior margin of the pubis symphysis up to the back sling of the puborectalis muscle. The women were first asked to rest and then contract the PFM maximally as if they wanted to stop urinating or prevent the escape of flatus, until the maximal cranioanterior displacement of the anorectal angle was attained. The volume was recorded using 4D ultrasound for two repetitions and the contraction producing the highest anorectal angle displacement was selected for analysis. Participants received a financial compensation ($20) as well as written information about PVD and PFM exercises. The present study was approved by the Institutional Review Boards of the two health centers where diagnostic gynecological examinations were completed.

Main Outcomes Measures
The transperineal ultrasound measurements were recorded with a GE Voluson E8 Expert system (GE Healthcare, Mississauga, ON, Canada) using a three-dimensional/4D convex probe (RAB4-8 NHZ). The volume acquisition angles were set at their maximal of 85° in the sagittal plane and 90° in the coronal plane in order to provide a view of the entire PFM. The frame rate was 2.0–2.8 Hz depending on the depth adjustment, which was set for optimal visualization of the structures in each participant.

Measurements were performed in both midsagittal and axial planes according to the methodology developed by Dietz et al. [17,24] and were cited in several subsequent studies [18,25–29]. The parameters measured have already been studied for their psychometric properties and have demonstrated good test–retest and interobserver reliability [18,24–27,29–31]. Moreover, supporting the validity of the measurement, transperineal ultrasound parameters have shown to be associated with different pelvic floor assessment techniques and diagnostic tools [30–32]. For instance, dimensions of the hiatus and levator displacement assessed with ultrasound have been associated with pressure perineometry [30–32] and magnetic resonance imaging measurement [25], whereas anorectal angle dimensions have been associated with evacuation difficulty revealed with defecography findings [28]. Ultrasound analyses were conducted offline with the 4D View v.7.0 software (GE Healthcare) by an observer blinded to the clinical data of the participants.

Analysis in the Midsagittal Plane
The midsagittal plane was first adjusted and the urethra and the anal canal in the axial plane were used to validate the correct positioning (see Figure 1). The analyses were performed at rest and during maximal PFM contraction. The anorectal angle was defined as the angle between the posterior wall of the rectal ampulla and the anal canal [33]. During PFM contraction, the anorectal angle

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becomes more acute and it moves cranially. The anorectal angle excursion was computed as the angle at rest minus the angle during contraction. The levator plate angle was measured between the horizontal reference line at the level of the pubis symphysis and the line from the inferioposterior margin of the symphysis pubis to the crux of the anorectal angle [18]. PFM contraction results in an increase in the levator plate angle. The levator plate excursion was calculated by subtracting the angle at rest from the angle at contraction. In addition, cranioventral displacement of the bladder neck during contraction was assessed with a vector calculation as described by Dietz et al. [24]. To this end, the positions of the structures were analyzed in a horizontal (x-axis) and vertical (y-axis) positions relative to a horizontal reference line at the level of the inferioposterior margin of the pubis symphysis. The displacements in X (DX) and in Y (DY) were measured by subtracting the rest position from the position at maximal contraction. The cranioventral displacement of the bladder neck was calculated on a spreadsheet using the following formula: cranioventral displacement = SQRT(DX^2 + DY^2).

Analysis in the Axial Plane
The parameters in the axial plane (see Figure 2) were measured in the plane of minimal hiatal dimensions, identified by Dietz et al. [17] as the minimal distance between the hyperechogenic posterior aspect of the symphysis pubis and the hyperechogenic back sling of the puborectalis muscle (see localization in midsagittal plane in Figure 1). The levator hiatus area was delimited by the puborectalis muscle, symphysis pubis, and inferior pubic ramus in the axial plane. Inside these borders, the anteroposterior (AP) distance corresponded to the levator hiatus AP diameter and the transverse distance measured at the widest part of the levator hiatus, defined as the levator hiatus left–right (LR) transverse diameter [17,18,26]. Measurements were taken at rest and during contraction. The hiatus reductions during a PFM contraction (hiatus and diameters) were also

Figure 1  Midsagittal plane produced by transperineal ultrasound at rest. Identifying the anorectal angle (ARA, dotted line), the levator plate angle (LP, full line), bladder neck (BN), pubis symphysis (PS), anal canal (A), rectum (R), and the horizontal reference line (REF).

Figure 2  Axial plane produced by transperineal ultrasound at rest. Measurements were taken in the axial plane of minimal hiatal dimensions. Identifying the pubis symphysis (PS), the urethral (U), the vagina (V), and the anal canal (A). Levator hiatus area (LH area) is marked with lines. The levator hiatus anteroposterior (AP) and left–right (LR) transverse diameters are drawn as a dotted line.
calculated as the percentages of change from baseline (i.e., levator hiatus narrowing = (levator hiatus at rest - levator hiatus at contraction)/levator hiatus at rest).

Statistical Analysis
Statistical analyses were performed using PASW Statistics version 18.0 (SPSS Inc., Chicago, IL, USA). Assumptions of normality of the data were confirmed graphically. The baseline characteristics of women suffering from PVD were compared with those of asymptomatic healthy controls using \( \chi^2 \)- and Student’s \( t \)-tests. One way multivariate analysis of variance (MANOVA) was used to compare several morphometry parameters between the two groups in order to take into account their potential interrelationships. Subsequent univariate analyses (ANOVAs) were used to ascertain which individual parameters differed significantly between the two groups. Significant levels were set at 0.05. To better appreciate the significance of our data, effect sizes were evaluated with eta-squared (\( \eta^2 \)) (where 0.01 indicates a small effect, 0.06 indicates a medium effect, and \( \geq 0.14 \) indicates a large effect) [34].

Results
Baseline Characteristics
As shown in Table 1, women with PVD and asymptomatic controls did not differ statistically on baseline characteristics. All women were able to perform a correct PFM contraction as evaluated with palpation.

Using the NRS, women with PVD had a mean pain intensity during vaginal intercourse (past 6 months) of 6.8/10 and a standard deviation (SD) of 1.9. They had experienced pain for a mean of 5.6 years (SD = 4.6). Among the PVD group, 29 (59%) had primary PVD (i.e., the pain has been present since the first tampon use or intercourse) and 20 (41%) had secondary PVD (i.e., women had had painless tampon insertion or intercourse, with the subsequent development of vestibular pain).

PFM Morphometry
A MANOVA, with participant group as the independent variable and the PFM morphometry parameters at rest and during contractions as the dependent variables, indicated significant differences between the women with and without PVD on one or more of these parameters \( (F(2,83) = 6.068, \ P < 0.001; \text{Wilks’ } \lambda = 0.477) \).

PFM morphometry parameters at rest using univariate ANOVAs are presented in Table 2. In comparison with healthy controls, women with...
PVD showed an elevated levator plate as measured by the larger levator plate angle ($P = 0.013$). They also had a significantly more acute anorectal angle ($P < 0.001$), with an $\eta^2 = 0.235$, indicating a large effect. In the axial plane, the levator hiatus area and AP diameter were significantly smaller in women with PVD ($P \leq 0.028$). A trend for lower LR diameter in women with PVD was observed, but it did not reach the significance level ($P = 0.097$).

Table 3 shows morphometry differences between the two groups during a maximal PFM contraction using ANOVAs. Regarding the maximal absolute values measured during a PFM contraction, the levator plate angle and levator hiatus LR diameter were significantly different when comparing women with PVD and controls ($P < 0.044$). When assessing the changes from baseline, PFM contraction in women with PVD resulted in less displacement of the bladder neck, lower excursion of the levator plate, and anorectal angles comparing with healthy controls ($P < 0.001$). Women with PVD also had less levator hiatus narrowing (area, AP, and LR diameter) as opposed to healthy controls ($P < 0.001$). These parameter differences showed a large effect size with all $\eta^2 \geq 0.143$.

### Discussion

This study represents the first controlled examination of the role of PFM in pathophysiology of PVD using a valid, reliable, and pain-free measurement [18,30–32,35]. Our purpose was to compare PFM morphometry of women with PVD with healthy controls. Confirming our hypotheses, results indicate that women with PVD display differences in PFM morphometry at rest and during maximal contraction, suggesting higher tone, lower strength, and poor control.

Our analysis of the PFM morphometry at rest revealed that women with PVD showed a significantly smaller levator hiatus area and AP diameter, a smaller anorectal angle, and a larger levator plate angle than controls, which is consistent with higher PFM tone. Our findings are in line with those from other studies in women with PVD using vaginal palpation and EMG for evaluating tone or resting activity, respectively [7,9–13]. Our research group also found similar results in men with urological chronic pelvic pain syndrome, who showed an elevated PFM tone assessed with ultrasound [36]. However, 4D transperineal ultrasound offers greater advantages considering its validity and reliability, and most importantly for women with PVD, it is a pain-free procedure without vaginal insertion. These important advantages may allow us to further investigate the role of PFM tone in the pathophysiology of PVD by overcoming the limitations of previous measurements. It has been hypothesized that PFM dysfunctions reported in the literature could be explained by a protective defense provoked by pain during assessment [11]. Despite the fact that this reaction may be present, our results suggest that the PFM impairment is not limited to a reaction to pain during vaginal penetration, but is rather chronic, given that transperineal ultrasound is a pain-free procedure.

Some studies failed to find a significant difference in PFM resting activity between healthy controls and PVD using EMG [9,14]. It was argued that the pathophysiology of PVD may not be explained by an electrogeneric difference between the two groups as assessed with EMG. Simons and
Mense [37] described that general muscle tone in skeletal muscle comprises the measurements of the viscoelastic properties of the muscular tissue, physiological contracture (more commonly defined as trigger point), electrogenic spasm (which include unintentional muscle contraction with or without pain that could be controlled voluntarily), and normal electrogenic contraction (involves resting activity in normally relaxed muscle and also myotatic reflex during stretching). Therefore, the first two components are not recorded by EMG measurement, suggesting that only a portion of general muscle tone is assessed. Also, the role of the electrogenic component in the pathophysiology of PVD cannot be excluded as some studies found a significantly higher PFM resting activity in women with PVD compared with controls [7,11,13]. These inconsistent findings may be explained by the confounding factors affecting EMG measurement. On the contrary, all the components of general muscle tone evaluation are measured when using a subjective scale with palpation or objectively in morphometry analysis with ultrasound, as in the present study. Our results demonstrated PFM morphometric differences at rest in women with PVD, which likely reflects higher general muscle tone altogether.

Gentilcore-Saulnier et al. [11] proposed that superficial and deep layers of the PFM may differ in their involvement in PVD as assessed with EMG external surface electrodes and an intravaginal probe, respectively. They found that women with PVD have significantly higher resting activity in the superficial muscle (bulbocavernous) in comparison with controls. The difference was not significant for the deep layer. However, when assessing both layers together with digital palpation, the difference between the two groups was significant. Frasson et al. [12] showed significantly higher resting activity in both layers using needle EMG in women with PVD. It is suggested that 4D transperineal ultrasound assesses mainly the deep layer of the PFM [38]. Within the deep layer, it is thought that changes in the anorectal angle and hiatus size are caused by the contraction and relaxation of the pubococcygeus, ischiococcygeus, and ischiococcygeus muscles [38]. Bladder neck displacement is likely caused by a combination of all these muscles [39]. Therefore, our findings are in line with those of Frasson et al. [12], indicating that the deep layers of the PFM are involved in PVD pathophysiology.

The contractile ability of the PFM was hypothesized to play a role as important as PFM tone in the pathophysiology of PVD. Measurements of bladder neck displacement, levator plate, and anorectal angle excursions and hiatus narrowing have been used widely as a proxy of PFM strength in women with incontinence and pelvic organ prolapse [26,31]. Maximal strength is commonly defined as the difference between maximal force and resting values in the assessment of skeletal muscle of the upper and lower limbs using dynamometry [40]. Following the same principle for calculating maximal strength, our data demonstrated, with large effect sizes, that women with PVD had smaller levator hiatus narrowing, displacement of the bladder neck, and changes of the anorectal and levator plate angles during maximal contraction. In line with our findings, some studies have showed that women with PVD had an inferior maximal EMG amplitude during maximal contraction [7,9] and lower strength at vaginal palpation using the Oxford Grading Scale [10]. In contrast, other studies found a nonsignificant difference in strength between the two groups using the same measurement procedure [11,14]. Methodological limitations may explain such discrepancies. From the calculation of maximal strength (resting values subtracted from the maximal values) used in these studies, it was not possible to determine whether it was PFM tone or contractile ability that was responsible for the difference between PVD and control participants. For example, when a woman with PVD has a high PFM tone and attempts to contract maximally, a small angle of excursion may be shown. This could be explained by either the high tone or semicontracted state of her muscle or this state combined with PFM weakness. To further investigate if the contractile ability plays a role independently of the resting state, we examined the maximal absolute values. We found that women with PVD had significantly lower levator plate angle and levator hiatus LR diameter during maximal contraction, suggesting that contractile ability contributes to a reduction in angle excursion and narrowing of the hiatus. Considering the effect sizes and the number of significant parameters, our findings suggest that the influence of tone seems to be predominant.

Some authors provided theoretical explanations about the mechanism by which PFM can be
involved in PVD pathophysiology [3–6]. It was proposed that pain during vaginal penetration may trigger a PFM protective defense, an increase in PFM tone and a reduction in strength and lack of control [7,10]. Spano and Lamont [6] proposed a circular cognitive–behavioral model in which pain during intercourse and fear of pain may decreased sexual arousal and result in vaginal dryness and increased PFM tone. Zolnoun et al. [3] also suggested a circular model, whereby PFM hypertonicity might act as an initiator of vestibular sensory changes and inflammation [3]. Our results showed that an increase in muscle tone is not only a reaction to the assessment procedure but is also present even in the absence of pain. This finding is important because it suggests chronic, long-lasting disruptions in PFM function in women with PVD, as opposed to reactive, punctual changes. The PFM morphometry in women with PVD also suggested a reduction in strength and control. Our findings are thus in line with models explaining that PFM dysfunctions may maintain and exacerbate pain. However, because of our cross-sectional design, it is not possible to answer the question of whether PFM dysfunctions are an antecedent to PVD or solely a consequence that would play a role in its maintenance, or both. Future research should include a longitudinal design following asymptomatic women to determine whether PFM dysfunctions are a precursor to the development of PVD.

The strengths of the present study include an innovative, pain-free methodology for measuring PFM function, a control group, and a standardized protocol for diagnosing PVD performed by a gynecologist. Pain-free measurement such as 4D transperineal ultrasound represents an important advantage for women with vulvodynia, potentially improving compliance with the assessment. Moreover, the present study corrected prior limitations in the literature by including a homogeneous sample [7,13]. We targeted nulliparous women to ensure that the PFM morphometry differences were related to PVD and not influenced by pregnancy and delivery [41]. Moreover, we recruited women who never followed PFM physiotherapy or biofeedback. Finally, the blinding of the assessors reviewing the ultrasound images is a further strength of our study, increasing the validity of the findings.

There are some limitations to this study that have to be acknowledged. These include the cross-sectional design and the absence of extensive pain measurement. Moreover, the external validity of our study is limited to nulliparous women suffering from PVD, and hence the findings could not be generalized to other subtypes of dyspareunia.

Conclusion

This research provides sound evidence that women with PVD display PFM impairments at rest and during maximal contraction using an innovative technology. Our results showed that women with PVD have altered morphometry at rest, suggesting increase in muscle tone. As 4D transperineal ultrasound does not require vaginal insertion and is pain-free, we showed that these impairments are not limited to a defense reaction but are rather chronic. Moreover, the morphometry of the PFM during contraction is suggestive of lower PFM strength in women with PVD. This finding has promising clinical implications as it supports the rationale for physical therapy treatment targeting these impairments in women with PVD.

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