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● *Clinical Note*

RELIABILITY OF SUPERFICIAL MALE PELVIC FLOOR STRUCTURAL MEASUREMENTS USING LINEAR-ARRAY TRANSPERINEAL SONOGRAPHY

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Abstract—This study evaluated reliability of measures for superficial structures of the male pelvic floor (PF) obtained *via* transperineal sonography. Two embalmed cadavers were dissected to identify positioning of muscles on and around the bulb of the penis and to confirm the PF protocol. Cross-sectional area (CSA) and linear thickness of the bulb of the penis, urethra, bulbospongiosus (BS) muscles, and ischiocavernosus (IC) muscles were measured on 38 transverse images from 20 male patients by three raters with varied study knowledge and sonographic experience. Intra- and inter-rater reliability were calculated with two-way, mixed effects intra-class correlation coefficients. Measures of the bulb of the penis had the best reliability. CSA of all muscles and sagittal thickness of the BS near the central tendon had good reliability. Reliability varied for rater-identified thickest muscle region and measures of the urethra. Our study suggests that structures of the male PF can be reliably evaluated using a transperineal sonographic approach. (E-mail: sroll@usc.edu) © 2015 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Musculoskeletal imaging, Pelvis, Chronic pain.**

Sonography is routinely used to evaluate the deep internal structures of the male pelvic region, primarily the prostate and bladder. Although disorders of these deep structures are common, there is an increasing clinical focus on the evaluation of superficial pelvic floor structures. Sonography has become the imaging modality of choice for evaluation and treatment of pelvic floor dysfunction in women (Dietz 2010). This has translated to increased use of sonographic imaging to evaluate superficial pelvic structures in men (Davis et al. 2011; Whittaker et al. 2007).

The primary focus of male pelvic evaluation using sonography previously has been on deeper internal structures using a transrectal or transabdominal sonographic approach (Ghai and Toi 2012; Loch et al. 2007; Terris and Klaassen 2013). These approaches have also been used to evaluate superficial structures. Primarily the purpose of these investigations is to relate problems of the superficial structures to pathologic conditions of deeper tissue, such as relating prostate and urologic conditions to pain in the pelvic floor muscles (Dellabella et al. 2006; Khorasani et al. 2012; Nahon et al. 2011). However, patients who have spasms of superficial pelvic muscles often do not have anomalies in the deeper structures that are observed using these approaches (Shoskes et al. 2007). Moreover, because of poor resolution of structures in the far field (*i.e.,* deeper on the image), involvement of the superficial pelvic muscles cannot be effectively evaluated with these techniques.

More recently, transperineal approaches have been used to evaluate the superficial structures in the male pelvic floor. The first report using this approach meant to evaluate the orientation of anal-pelvic structures—that is, anorectal angle and levator plate angle (Davis et al. 2011). A similar transperineal approach has been reported in evaluation of the displacement and dynamic changes of pelvic structures during contraction of pelvic floor and anorectal muscles (Stafford et al. 2012, 2013). These studies used a low-frequency curvilinear probe with data collection in a sagittal plane to evaluate the entire pelvic region. Recently, an alternative protocol has been reported using a higher-frequency linear probe to more carefully investigate the superficial muscles of

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the pelvic floor in both sagittal and transverse planes (Roll and Kutch 2013).

Transperineal sonographic protocols hold potential for advancing research and clinical evaluation of the morphologic and anatomic correlates of male pelvic floor dysfunction. However, despite increasing use of sonographic imaging to evaluate these superficial structures, rigorous reliability and validity testing for various imaging protocols has not been reported. Therefore, the purpose of this study was to evaluate reliability of measures of superficial structures in the male pelvic floor using a transperineal sonographic approach with a linear transducer based on published literature. Findings are presented in accordance with the guidelines for reporting reliability and agreement studies (GRRAS) (Kottner et al. 2011).

MATERIALS AND METHODS

Study design

This study was completed in two phases. In the initial phase, pelvic images were obtained from two volunteers and cadaver dissections were completed to confirm structural positions and to refine the imaging protocol. The refined protocol was then used for cross-sectional, prospective image acquisition from 20 participants to evaluate the reliability of image analysis in the second phase. The university's Institutional Review Board approved the data collection protocol described in this manuscript. All patients provided signed informed consent to participate after the study protocol had been fully explained.

Protocol development

The pelvic regions of two embalmed male cadavers were dissected to verify structural identification in pelvic floor sonographic images obtained using a linear-array transperineal protocol (Roll and Kutch 2013). The university's Anatomic Gift Program approved the use of these specimens for this study. One-to-one quantitative validation of the cadaveric pelvic floor structures using sonographic imaging was unable to be completed because of the embalmed, fixed status of the cadaver tissues. Instead, cine clips and images of the anteroposterior and left-right expanse of the pelvic floor were acquired from two male research team members for qualitative comparison with the dissected specimens.

During dissection, the skin and subcutaneous fat tissue were removed to expose the external pelvic floor fascia. The superficial perineal (Colles') fascia and all small vascular structures were removed to reveal the fiber patterns of the bulbospongiosus, ischiocavernosus and superficial transverse perineal muscles. Care was taken to maintain the deep transverse perineal muscle and urogenital diaphragm to clearly separate the superficial pelvic region from the ischioanal fossae. All fat and loose connective tissue was removed from the fossae and superficial pelvic structures. The superficial pelvic muscles (*i.e.,* bulbospongiosus, ischiocavernosus, superficial transverse perineal), bony landmarks of the innominate bone and bulb of the penis were identified in the completed cadaver dissection.

Two research team members reviewed the two dissected specimens, and sonographic data from the two male volunteers to establish face validity for structural identification. The approximate subcutaneous depth, directionality and relative orientation among the structures identified in the dissections were compared with the sonographic cine clips and static images (Fig. 1). The thin, flat bulbospongiosus muscles were identified as they wrapped obliquely around the superficial-lateral bulb of the penis. The paired cordlike ischiocavernosus muscles were appreciated slightly deep and lateral to the bulb of the penis, running in an oblique angle from the base of the penis to ischial tuberosities. The superficial transverse perineal muscles were difficult to identify in both the cadaver and on the sonograms because they were significantly thin and closely united with the urogenital diaphragm, the deep transverse perianal muscle, and the external anal sphincter. It was determined that conducting the imaging protocol in a transverse plane could accurately identify the bulbospongiosus and ischiocavernosus muscles, as well as the bulb of the penis and urethra, but that the protocol should not be used to evaluate other muscles, nerves or vessels in the perineal region without further adjustments.

Participants

In the second phase, images were obtained from 20 male participants who were prospectively recruited for a larger research study. Ten participants were recruited from patients referred to a physical therapy clinic for treatment of chronic pelvic pain syndrome and 10 age-matched control participants with no history of pelvic pain were recruited. For data collection, participants disrobed below the waist, were covered with a sheet, and rested supine in a modified lithotomy position with feet flat on the table. To expose the perineum, the participant held the penis and scrotum superiorly with a loose grip to avoid causing increased tension or movement of the skin and subcutaneous tissue. The left-right width (cm) of the perineum was measured as the distance between the ischial tuberosities and the anteroposterior length (cm) was measured beginning at the junction of the perineum and the scrotal sac to the anterior edge of the anus. During anteroposterior measurement, skin markings were made at one quarter, one third, one half, two thirds and three quarters the distance from the scrotum to the anus.

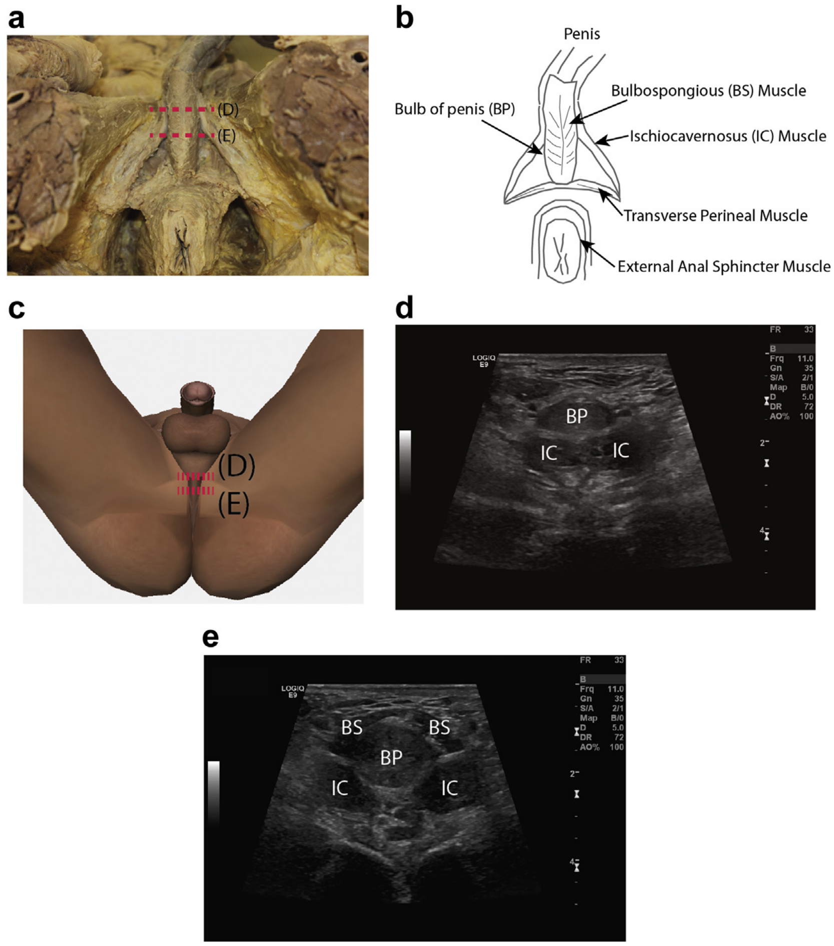

Fig. 1. Cadaver dissection of the superficial male pelvis (a) with superficial structures of interest identified (b) and structural correlates on transverse sonographic images indicated by the *red dashed lines* (c) at one quarter (d) and one half (e) the anteroposterior length of the perineum.

Image acquisition

Using settings described in a previous published protocol (Roll and Kutch 2013), a Logiq E9 (GE Healthcare Ultrasound) with 11-MHz linear transducer was used. Initially, cine clips were obtained by moving the transducer through the anteroposterior length of the perineum in a transverse plane. The cine clips provided a reference for individual morphology and ensured that the sonographer was able to identify and obtain quality images of the structures of interest. Static images were then obtained in the transverse plane at one quarter, one third, one half, two thirds and three quarters of the anteroposterior perineum length, using the skin markings as a guide for transducer placement. Although the use of external skin markings can introduce error, internal sonographically identified landmarks for this protocol have not yet been established. Thus, skin markings provided a means for standardizing intervals from which images were obtained across participants and improved reliability of repeated image acquisition within participants. An investigator registered in musculoskeletal (RMSK) sonography completed all image acquisition. To minimize error caused by inadvertent oblique transducer positioning, the transducer was adjusted to ensure a non-distorted, uniform shape of the bulb of the penis was obtained in each image. Images were acquired twice for each participant, before and after 30–45 min of intermittent contract-relax exercises (*i.e.,* part of the larger study protocol, unpublished data).

Image analysis

To avoid biasing acquisition, image analysis was not initiated until images had been acquired from all participants. To increase the sample size, both a pre-exercise and post-exercise image were selected for each participant from the series of images obtained at each skin marking. Criteria for image selection was where the urethra was the most uniformly circular and fully within the deep boundary of the bulb of the penis. Standardized techniques were developed for a total of 16 measures of

Table 1. Description of standardized techniques for measuring structures of the pelvic floor

Structure	Measurement technique
Bulb of penis and urethra	
Sagittal diameter, mm	Linear vertical measurement (image superficial to deep) with caliper placement at the junction of the echogenic central bulb/lumen and the hyperechoic connective tissue border
Transverse diameter, mm	Linear horizontal measurement (image left to right) with caliper placement at the junction of the echogenic central bulb/lumen and the hyperechoic connective tissue border
Cross-sectional area, mm^2	Multi-point trace of the inner hyperechoic connective tissue border with spline interpolation between points
Bulbospongiosus muscles	
Medial thickness, mm	Linear measurement between the inner boundaries of the hyperechoic connective tissue sheath adjacent to the central tendon and roughly perpendicular to the tangent of the bulb of the penis
Thickest region, mm	Linear measurement between the inner boundaries of the hyperechoic connective tissue sheath roughly perpendicular to the tangent of the bulb of the penis at the thickest point identified by the individual rater
Cross-sectional area, mm^2	Multi-point trace of the inner hyperechoic connective tissue border with spline interpolation between points
Ischiocavernosus muscles	
Thickest region, mm	Linear measurement between the inner boundaries of the hyperechoic connective tissue sheath at the thickest diameter of the ovoid muscle identified by the individual rater
Cross-sectional area, mm^2	Multi-point trace of the inner hyperechoic connective tissue border with spline interpolation between points

the bulb of the penis and the urethra, as well as both left and right bulbospongiosus and ischiocavernosus muscles (Table 1). For the bulb of the penis and the urethra, two orthogonal diameters were measured (mm). Medial thicknesses (mm) of the bulbospongiosus muscles were obtained near the central tendon where the muscle borders were noted to be most parallel to each other. Finally, each rater identified and measured what they perceived to be the thickest region of the left and right bulbospongiosus and ischiocavernosus muscles. Cross-sectional area (mm^2) was obtained for each of the six structures using multi-point trace along the inner hyperechoic connective tissue boundary (Fig. 2).

Three raters independently completed three trials of the 16 measurements on each image. Raters were provided with no immediate feedback other than an indication that each measurement had been successfully recorded; that is, no measurement results were displayed to the raters at any point during image analysis. In addition, none of the raters were aware of any measurement results until all analyses were completed. To minimize repeated measurement bias, no image was measured multiple times in direct succession. Instead, the raters completed measurements of all images within each trial before repeating measurements on any image for successive trials. Once a rater initiated analysis, that rater completed all measurement trials within 1 wk. Images were coded to ensure raters were blinded to patient or control status. All images were manually analyzed using a graphic user interface with MatLab software (2012a, MathWorks, Natick, MA, USA).

Statistical analysis

Averages (SD) were calculated to describe the study sample (*i.e.,* age and perineum measurements) and were compared between patients and controls using *t*-tests to ensure that no significant group differences existed that could influence measurement reliability. Averages (SD) were calculated for each of the 16 image measures by rater and across all raters to describe and characterize structural morphology in the male pelvic floor. Intra-

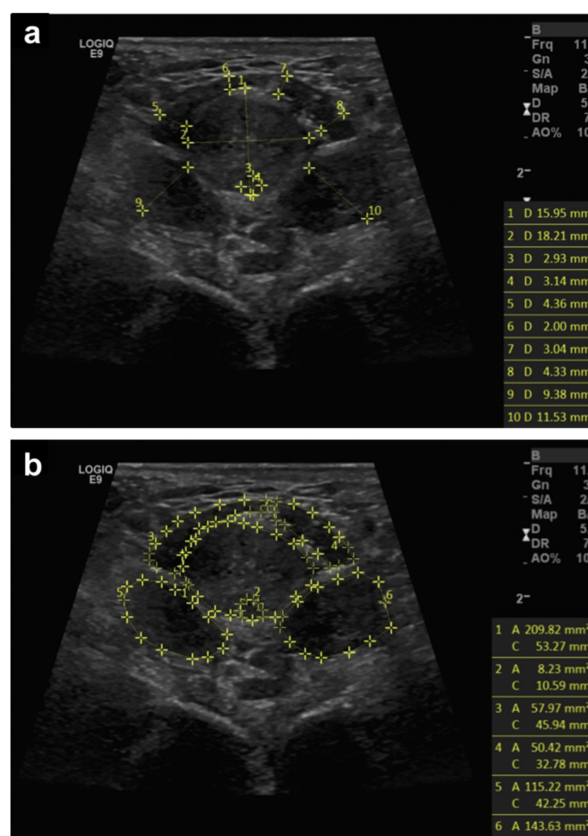

Fig. 2. Example images analysis of (a) 10 linear thickness and (b) 6 cross-sectional area measurements of the pelvic floor structures. D = diameter; A = area; C = circumference.

Table 2. Averages (SD) for each of the 16 pelvic floor structural measures across all images (N = 38) by rater and overall

Structure/measure	Rater 1	Rater 2	Rater 3	Overall
Bulb of penis				
Sagittal diameter, mm	11.76 (2.86)	12.51 (2.73)	11.84 (2.78)	12.04 (2.81)
Transverse diameter, mm	14.05 (4.00)	15.70 (4.00)	14.74 (3.84)	14.83 (4.00)
Cross-sectional area, mm^2	135.27 (66.58)	136.27 (62.00)	112.78 (58.61)	128.66 (63.32)
Urethra				
Sagittal diameter, mm	3.86 (1.00)	4.05 (0.91)	4.11 (0.75)	4.00 (0.90)
Transverse diameter, mm	3.90 (0.99)	4.68 (1.08)	4.81 (0.92)	4.45 (1.08)
Cross-sectional area, mm^2	13.60 (5.08)	15.80 (6.83)	12.43 (3.96)	14.00 (5.62)
Bulbospongiosus (right)				
Medial thickness, mm	2.67 (0.90)	2.96 (1.02)	2.80 (0.93)	2.81 (0.96)
Thickest region, mm	4.55 (1.20)	5.09 (1.50)	5.10 (1.37)	4.91 (1.38)
Cross-sectional area, mm^2	46.47 (15.78)	46.42 (16.33)	41.87 (15.62)	45.03 (16.01)
Bulbospongiosus (left)				
Medial thickness, mm	2.81 (0.88)	3.16 (0.91)	2.83 (0.81)	2.94 (0.88)
Thickest region, mm	4.51 (1.09)	4.91 (1.15)	4.63 (1.00)	4.69 (1.09)
Cross-sectional area, mm^2	42.38 (12.91)	44.17 (12.79)	39.19 (11.23)	42.01 (12.50)
Ischiocavernosus (right)				
Thickest region, mm	8.22 (1.89)	9.28 (2.50)	9.84 (2.17)	9.09 (2.30)
Cross-sectional area, mm^2	110.48 (41.72)	111.36 (38.71)	113.78 (45.40)	111.81 (41.80)
Ischiocavernosus (left)				
Thickest region, mm	8.14 (2.37)	9.40 (2.59)	9.96 (2.06)	9.14 (2.47)
Cross-sectional area, mm^2	105.67 (42.03)	113.00 (41.85)	119.32 (46.76)	112.42 (43.71)

rater and inter-rater reliability was determined for all 16 measurements using two-way, mixed effects intraclass correlation coefficients (ICC 3,1), within repeated trials by rater (intra-rater) and using averages across raters (inter-rater). All ICC coefficients and 95% confidence intervals were descriptively compared across the 16 measures and among the three raters. Minimum acceptable reliability for ICC coefficients and the lower range of 95% confidence intervals (CIs) for this preliminary measurement study was set at 0.60 and significance for statistical tests was set at $p < 0.05$. All statistical analyses were completed with SPSS v. 21 (IBM Corp., Armonk, NY, USA).

RESULTS

Sample characteristics

The 20 male participants had an average age of 32.3 y [19–53 y], average perineum height of 10.5 cm [7.5–15.0 cm] and average perineum width of 7.4 cm [5.5–10.0 cm]. No significant differences were found between the 10 patients and 10 control participants. Pre-exercise image acquisition was successfully completed with all 20 participants; however, imaging was unable to be completed after exercise in two participants (one patient and one control) because of participant time constraints requiring termination before data collection completion. Therefore, a total of 38 images from the 20 participants were identified for analysis. Using the image selection criteria based on the shape and location of the urethra, all selected images were acquired at either one half or two thirds of the anteroposterior perineal length.

Rater characteristics

Images were independently analyzed in three repeated trials by the three raters. The raters included the RMSK-certified investigator who acquired the images (rater 1), an investigator with 5 y of experience obtaining and analyzing musculoskeletal sonographic images (rater 2), and a sonographer with more than 30 y of experience (rater 3). The first two raters were study investigators who conceptualized the analysis techniques, whereas the third rater had no prior knowledge of the study design or pelvic floor sonography.

Structural morphology

Averages within and across the three raters for each of the 16 measurements are presented in Table 2. Measures by rater 1 were consistently more conservative than the other raters. Rater 3 was more conservative than rater 2 for nearly all measures with exception of ischiocavernosus measures. Heterogeneity in tissue morphology across the study sample is indicated by large SDs (*i.e.,* 37%–49% of the average) in the cross-sectional area measures. This is consistent with anthropometric heterogeneity indicated by the range of perineum measurements across the study sample.

Reliability estimates

Inter-rater estimates for each measure were calculated for patient and control groups separately. Reliability for measuring the urethra transverse diameter urethra in controls was 0.73 compared with 0.42 for patients. Conversely, inter-rater reliability for measuring the thickest region of the right ischiocavernosus in patients was

Table 3. Inter-rater and intra-rater reliability for the 16 pelvic floor structural measures using two-way, mixed-effects ICC

				Intra rater reliability					
		Inter rater reliability		Rater 1		Rater 2		Rater 3	
Structure	Measure	ICC	95% CI	ICC	95% CI	ICC	95% CI	ICC	95% CI
BOP	Sagittal diameter	0.93	0.81–0.97	0.96	0.93–0.98	0.95	0.91–0.97	0.94	0.87–0.98
BOP	Cross-sectional area	0.92	*0.55–0.98*	0.98	0.96–0.99	0.97	0.96–0.99	0.97	0.94–0.99
BOP	Transverse diameter	0.92	0.67–0.97	0.96	0.93–0.98	0.96	0.94–0.98	0.95	0.90–0.98
BS (Right)	Cross-sectional area	0.89	0.80–0.94	0.85	0.75–0.92	0.82	0.72–0.90	0.92	0.84–0.96
BS (Left)	Medial thickness	0.87	0.68–0.94	0.80	0.69–0.88	0.78	0.65–0.87	0.86	0.76–0.93
BS (Left)	Cross-sectional area	0.84	0.69–0.91	0.82	0.70–0.89	0.77	0.62–0.87	0.74	*0.57–0.86*
IC (Left)	Cross-sectional area	0.82	0.70–0.90	0.78	0.66–0.87	0.79	0.65–0.88	0.72	*0.33–0.88*
IC (Right)	Cross-sectional area	0.81	0.70–0.89	0.76	0.62–0.86	0.69	*0.49–0.82*	0.74	*0.36–0.89*
BS (Right)	Medial thickness	0.80	0.69–0.89	0.85	0.76–0.92	0.81	0.70–0.89	0.85	0.74–0.92
Urethra	Sagittal diameter	0.80	0.68–0.88	*0.56*	*0.37–0.72*	0.64	*0.47–0.77*	0.74	*0.57–0.86*
BS (Right)	Thickest region	0.77	0.61–0.87	0.82	0.71–0.89	0.85	0.76–0.91	0.77	*0.57–0.89*
BS (Left)	Thickest region	0.77	0.63–0.87	0.70	*0.55–0.82*	0.72	*0.57–0.83*	*0.56*	*0.34–0.75*
Urethra	Cross-sectional area	0.69	*0.42–0.84*	0.81	0.69–0.89	0.75	0.62–0.85	0.67	*0.47–0.82*
IC (Left)	Thickest region	0.68	*0.33–0.85*	0.68	*0.52–0.80*	0.65	*0.48–0.79*	0.70	*0.44–0.76*
IC (Right)	Thickest region	0.65	*0.39–0.81*	0.61	*0.43–0.75*	*0.55*	*0.36–0.71*	0.62	*0.25–0.83*
Urethra	Transverse diameter	0.61	*0.21–0.82*	0.71	*0.56–0.83*	0.66	*0.48–0.79*	*0.50*	*0.28–0.71*

BOP = bulb of penis; BS = bulbospongiosus muscle; CI = confidence interval; IC = ischiocavernosus muscle; ICC = inter-class correlation coefficients.

NOTE: Results are sorted by inter-rater reliability, and measures with poor reliability coefficients (<0.60) are italicized.

0.73 versus 0.59 in controls. For all other measures, differences in reliability estimates between the two groups were less than 0.10. With no substantial data suggesting reliability estimates were higher in one group versus the other, evaluation and interpretation of reliability estimates were completed across the aggregate of all participants.

Across the 16 measures, inter-rater reliability coefficients ranged from 0.61 to 0.93 and intra-rater reliability coefficients ranged from 0.50 to 0.98 (Table 3). All inter-rater reliability coefficients exceeded the minimum threshold of 0.60, whereas intra-rater reliability coefficients of four measures fell below the threshold. These included urethra sagittal diameter, urethra transverse diameter, thickest region of the left bulbospongiosus, and thickest region of the right ischiocavernosus. Lower boundaries of statistical confidence were noted to consistently fall below the *a priori* threshold of 0.60 for measures of the urethra and measurement of the rater-identified thickest regions of the four muscles.

Measures of the bulb of the penis were consistently the most reliable both within and across raters with all reliability coefficients greater than 0.92. The only measure of this structure with a lower boundary of statistical confidence below acceptable standards was the area (ICC 3, 1 = 0.92; 95% CI: 0.55–098). Inter-rater reliability was greater than 0.80 for muscle area measures and the medial thickness measure of the bulbospongiosus muscles. Intra-rater reliability varied between 0.69–0.92, with four lower boundaries of statistical confidence falling below 0.60 for these measures. Inter- and intra-rater reliability for measurement of the rater-identified thickest region of the bulbospongiosus and ischiocavernosus muscles ranged from 0.65 to 0.77 and 0.55 to 0.85, respectively; however, 75% (12/16) of the lower boundaries of confidence for these measures were less than 0.60. Reliability for measures of the urethra had the widest range (0.50–0.81) and lowest 95% CI boundaries of all reliability coefficients, as low as 0.21.

Across all measures, intra-rater reliability coefficients were similar, ranging from 0.56 to 0.98 for rater 1, from 0.55 and 0.97 for rater 2, and from 0.50 to 0.97 for rater 3. Rater 1 was most consistent with lower bounds falling below 0.60 for 5 of the 16 measures. More variability was noted for raters 2 and 3 with the lower bounds of 7 and 10 CIs falling below 0.60, respectively.

DISCUSSION

This study describes reliability for a transperineal sonographic image acquisition and analysis technique for evaluating superficial structures of the male pelvic floor given raters with varied experience and knowledge of the male pelvic region. Across all participants, measures of the bulb of the penis have very good reliability, bulbospongiosus and ischiocavernosus muscle cross-sectional area measures have good inter-rater reliability and fair-to-good intra-rater reliability, and measurement of bulbospongiosus sagittal thickness near the central tendon has good reliability. Measurement of the rater-perceived thickest portion of the pelvic floor muscles has varied reliability, supporting the need for protocols using anatomic landmarks to minimize rater bias. Measures of the urethra had the poorest reliability, indicating that this transperineal approach may not be the best protocol for evaluating the urethra.

The most consistent rater (rater 1) was an experienced sonographer who directly participated in the cadaveric dissection, developed the scanning protocol, and acquired the images. The rater with the least sonographic experience (rater 2) tended to obtain larger structural measures than the two expert raters. The impartial sonographer who was not a member of the research team and had no previous knowledge of superficial structures in the male pelvic floor (rater 3) had slightly more variability compared with the two raters who developed the measurement protocol. Despite these differences, measurements were stable across raters as indicated by all inter-rater reliability coefficients falling within an acceptable range. These data provide confidence for the reliable implementation of pelvic floor structural measurement by individuals with different levels of sonography experience and varying familiarity with pelvic floor anatomy using the standardized measurement protocols described in Table 1.

Individuals with dysfunction of the pelvic region may have altered muscle characteristics, such as muscle spasm or trigger points, which could change the morphologic presentation of structures investigated in this study. Although the purpose of this data analysis was not to compare the measures between patients and controls, data from both groups were included in the reliability analysis to ensure that measurement reliability would not be affected by potential structural differences between the groups. The results of this study indicate that similar measurement reliability can be achieved across both patients and controls. With the ability to clearly identify and measure the superficial muscles in both groups, this transperineal protocol provides a valid means for studying and differentiating morphologic characteristics of pathologic conditions. Valid identification and measurement of these structures may enhance intervention by improved targeting of pathologic structures. Furthermore, using a reliable protocol will provide a means for obtaining baseline and outcome measures to validate intervention efficacy in patients.

The stability of measures across raters with varied experience gives impetus for point-of-care use by non-traditional sonography professionals, such as urologists and physical therapists, who provide direct care to patients with pelvic disorders. Specifically, this approach may be most useful in the evaluation and treatment of chronic pelvic pain syndrome (CPPS), the most common urologic diagnosis for men $<$50 y (Allsop et al. 2011). Although up to 95% of cases are idiopathic (Habermacher et al. 2006), one potential mechanism for CPPS is involuntary, localized neuromuscular activity in the superficial pelvic floor muscles evaluated in this study (Hetrick et al. 2006). The primary intervention used to treat presumably high resting tension in these pelvic floor muscles is manual stretching (Anderson et al. 2005; Cornel et al. 2005). Although this has been shown to be more effective than placebo (FitzGerald et al. 2009), up to 41% of patients in another study reported little to no improvement or a worsening of symptoms after treatment (Anderson et al. 2005).

The lack of understanding of mechanisms and inconsistent intervention outcomes indicate a need for a viable clinical screening technique. The protocol described in this study may be useful in exploring anatomic or morphologic differences in the muscles (*e.g.,* size, shape, orientation, fascicular patterning) between patients and controls to inform diagnosis. Furthermore, this protocol may be useful in differentiating between therapy responders and non-responders to inform intervention planning and enhance targeting of interventions to those patients who would most benefit. Combining this transperineal approach with other sonographic investigations of the pelvic floor, such as those evaluating structure orientation and dynamic movements (Davis et al. 2011; Stafford et al. 2012, 2013), may lead to a robust evaluation protocol for individuals with CPPS.

The quality aspects of the image analysis reported in this study are limited to face validity and reliability. First, although sonographic examination of the pelvic region for the embalmed cadavers was attempted, the embalming process reduced tissue perfusion in the small superficial structures of the perineal region. This resulted in highly echogenic sonographic images that lacked adequate contrast among the structures necessary to complete image analyses. Instead, the structural identification process comparing cadaveric dissection to images from male research team members provides face validity of the image acquisition protocol, which is an improvement over previously reported expert consensus. When combined with good measurement reliability, this face validity provides a foundation on which to build clinical and research investigations. Second, one sonographer acquired all images in this study; therefore, inter-rater reliability of the image acquisition protocol was not evaluated. Future studies might consider use of fresh specimens, *in vivo* measurement during surgery, and comparison MRI for one-to-one measurement validation, as well as evaluation of the reliability and validity of image acquisition and measurement by multiple sonographers.

The imaging protocol used in this study was specifically selected to evaluate a standardized method for measuring the superficial muscles of the male pelvic floor; therefore, any interpretation is limited to the structures discussed. Using a high-frequency, linear transducer restricted the ability to clearly identify and measure deeper pelvic and abdominal structures. Additionally, because it would be most clinically efficient, the measurement of all structures was completed on one image. Although all structures of interest were clearly captured within each image, when necessary, images were optimized to most clearly delineate the borders of the bulbospongiosus muscle and bulb of the penis, which were in the central, superficial scan field. Because of these methodology choices, reduced reliability of urethra and ischiocavernosus measurements may have been a result of decreased optimization of the hyperechoic borders in the deep and lateral fields, respectively. Furthermore, the orthogonal directionality between the various structures may have resulted in increased anisotropy in some individuals, effectively reducing reliability for structures that were not optimized in the central focal zone. Although it would be less efficient and decrease the ability to evaluate each structure relative to another, future adaptations to this protocol that obtain multiple images optimized for each individual structure may improve reliability.

CONCLUSIONS

Using this transperineal sonographic approach, superficial male pelvic floor structures can be captured, identified and reliably measured. An internal landmark for the standardization of image acquisition was established at a point where the urethra was fully within the deep border of the bulb of the penis, which is located between one half and two thirds of the anteroposterior length of the perineum in all participants. Using this protocol and internal landmark, raters with different levels of experience and familiarity with the region achieved similar results when measuring superficial structures in both patients and controls. Deeper structures and rater-identified thickest regions of a muscle had varied reliability and may not be appropriate clinical measures using this approach. Further investigations using this protocol are warranted to explore differences in muscles between patients with pelvic pain or muscle dysfunction and healthy controls, as well as between responders and non-responders to interventions, to advance diagnosis and clinical practice.

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