Ex vivo biomechanical properties of the female urethra in a rat model of birth trauma

Rachelle L. Prantil,1 Ron J. Jankowski,1 Yasuhiro Kaiho,2 William C. de Groat,2 Michael B. Chancellor,3 Naoki Yoshimura,2,3 and David A. Vorp1,4,5

Departments of 1Bioengineering, 2Pharmacology, 3Urology, and 4Surgery, and the 5McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania

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Prantil RL, Jankowski RJ, Kaiho Y, de Groat WC, Chancellor MB, Yoshimura N, Vorp DA. Ex vivo biomechanical properties of the female urethra in a rat model of birth trauma. Am J Physiol Renal Physiol 292: F1229–F1237, 2007. First published December 26, 2006; doi:10.1152/ajprenal.00292.2006.—Stress urinary incontinence (SUI) is the involuntary release of urine during sudden increases in abdominal pressures. SUI is common in women after vaginal delivery or pelvic trauma and may alter the biomechanical properties of the urethra. Thus we hypothesize that injury due to vaginal distension (VD) decreases urethral basal tone and passive stiffness. This study aimed to assess the biomechanical properties of the urethra after VD in the baseline state, where basal muscle tone and extracellular matrix (ECM) are present, and in the passive state, where inactive muscle and ECM are present. Female rat urethras were isolated in a rat model of acute SUI induced by simulated birth trauma. Our established ex vivo system was utilized, wherein we applied intraluminal static pressures ranging from 0 to 20 mmHg. Outer diameter was measured via a laser micrometer. Measurements were recorded via computer. Urethral thickness was assessed histologically. Stress-strain responses of the urethra were altered by VD. Quantification of biomechanical parameters indicated that VD decreased baseline stiffness. The passive peak incremental elastic modulus of the distal segment in VD urethras was less than for controls (1.84 ± 0.67 vs. 1.19 ± 0.70 × 106 dyne/cm², respectively; P = 0.016). An increase was noted in passive low-pressure compliance values in proximal VD urethras compared with controls (9.44 ± 2.43 vs. 4.62 ± 0.60 mmHg-1, respectively; P = 0.04). Biomechanical analyses suggest that VD alters urethral basal tone, proximal urethral compliance, and distal stiffness. Lack of basal smooth muscle tone, in combination with these changes in the proximal and distal urethra, may contribute to SUI induced by VD.

stress urinary incontinence; sphincter; collagen

COSTS FOR URINARY INCONTINENCE exceed $16 billion annually, and stress urinary incontinence (SUI) is the most common type of urinary incontinence among middle-aged women (25, 26). SUI is associated with weakened and overstretched muscles and connective tissues, which lead to reduced muscle tension in the urethral sphincter complex. This results in the inability of the urethra to close tightly during increased abdominal pressure. Particularly in younger women, neuromuscular injury during vaginal birth often causes disorders involving sphincteric mechanisms (30). The incidence of intrinsic sphincter deficiency was recently found to be greater than previously thought (18), and previous clinical studies suggested that impaired urethral closure mechanisms contribute to intrinsic sphincter deficiency in SUI.

Decreased electromyographic activity of the striated urethral sphincter muscle has been detected in middle-aged women with SUI (37). Using a rat model of birth trauma, Resplande et al. (28) demonstrated that maximal urethral closure pressure significantly decreased compared with controls and suggested that this was associated with an increase in cellular apoptosis, altered smooth muscle shape, and degenerated mitochondria in the urethra. VD models of SUI in the female rat have also exhibited changes in the ratio of type I/II striated muscle in the pelvic floor and external urethral sphincter and decreased leak-point pressures (20, 22). Although efforts have been made to assess the correlation between VD and a dysfunctional sphincteric mechanism, some questions still remain to be answered.

The urethra is a complex organ comprising both circumferentially and longitudinally oriented smooth and striated muscle within a connective tissue matrix. The smooth muscle provides an intrinsic, involuntary sphincter mechanism and the striated muscle, which extends distally from the proximal urethra, is known as the extrinsic urethral sphincter and is involved in the voluntary control of continence. Circumferentially arranged smooth and striated urogenital sphincter muscles contribute to the bulk of the permanent luminal closure force, but the role of longitudinal smooth muscle is less clear (3). The smooth and striated muscle components of the urethra function as a sphincter to produce active urethral closure pressure, and the ECM, composed of collagen and elastin, plays a passive role, keeping the urethra from overdistending during increases in bladder pressure and voiding and helping it return to a closed state (3). These active and passive traits can be altered in various disease states or with nerve or mechanical damage induced by birth trauma, leading in turn, to urethral dysfunction. The ability to properly characterize fundamental mechanical tissue properties can be a powerful approach for understanding normal tissue function, as well as elucidating underlying causes of dysfunction and its pathological progression.

The purpose of this study was to determine whether VD in the rat that is known to induce urethral dysfunction alters the biomechanical properties of the urethra in a segmental fashion. The experiments were performed with our previously described ex vivo testing apparatus (4, 16), which allowed pressure-diameter data to be generated and subsequently analyzed to calculate and compare various biomechanical properties of the whole-mount urethra.
MATERIALS AND METHODS

Animals. Adult female Sprague-Dawley rats were used in this study (200–250 g, ~8–12 wk of age; Harlan, Scottsdale, PA) and were housed at the University of Pittsburgh under the supervision of the Department of Laboratory Animal Resources. The policies and procedures of the animal laboratory are in accordance with those detailed in the Guide for the Care and Use of Laboratory Animals, published by the US Department of Health and Human Services. Procedural protocols were approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

Animal model and urethral isolation. For the VD group, rats were anesthetized with halothane, and a 4-ml balloon catheter was inserted into the vagina, filled with saline to maximal capacity, and maintained for 4 h. Four days later, animals were anesthetized with urethane (1.2 g/kg), and the urethra was excised as previously described (16).

Briefly, an intraluminal catheter (PE-50) was inserted into the urethra, an incision was made at the apex of the bladder dome, and the bladder, as well as the distal external meatus, was ligated. The ureters were tied off to prevent leakage and to serve as anatomic landmarks to facilitate maintenance of urethra in vivo length, which is important for the standardization of testing (24). Before transport to the testing system, urethral specimens were placed in media 199 that was bubbled for a minimum of 30 min with a gas mixture (95% O2-5% CO2) to prevent hypoxia.

Biomechanical testing. The methods used for biomechanical testing have been previously reported by our laboratory (16). Briefly, specimens were mounted onto the tees of the ex vivo testing system (Fig. 1), and the hydrostatic pressure reservoir was manually displaced along a calibrated ringstand to apply intraluminal static pressure, while outer diameter measurements were acquired with a helium-neon laser micrometer (Beta LaserMike, Acuscan 1000, Dayton, OH). The tissue was first preconditioned by supplying 10 cycles of intraluminal pressure with each cycle ranging from 0–8 mmHg and applied over 20 s. This was followed by subjecting the urethra to 2-mmHg increments of intraluminal pressure ranging from 0 to 20 mmHg. Each pressure step was maintained for 1 min. Biomechanical testing was performed in baseline (i.e., no chemical agents added to the bath to induce or inhibit a muscular response) and passive (i.e., muscular response inhibited by calcium and magnesium chelation with 3 mM EDTA; E3126, Sigma) conditions.

Fig. 1. Schematic of the modified urethral testing system. Pressure is applied intraluminaly with a media-filled reservoir attached to a calibrated ringstand. Pressure transducers attached to a monitor enable pressure measurements. A laser micrometer measures outer diameter, and a computer continuously records pressure-diameter data (adapted from Ref. 16).

To allow for the possibility that urethral tissue is nonhomogenous, measurements were taken at three positions along the length (L) of each specimen: the proximal (L × 0.3 from the proximal end of the specimen), middle (L × 0.5 from the proximal end of the specimen), and distal (L × 0.7 from the proximal end of the specimen) positions. From pressure and diameter data, we were able to calculate various biomechanical parameters. For example, compliance (C) is the fractional change in volume that occurs in response to a unit change in pressure, and was estimated as follows

\[ C = \frac{(D_{\text{max}} - D_{\text{min}})/D_{\text{inc}}}{(P_{\text{max}} - P_{\text{min}})/P_{\text{inc}}} \]

Here, \( D_{\text{max}} \) and \( D_{\text{min}} \) represent the measured diameters corresponding to the maximum and minimum pressures, \( P_{\text{max}} \) and \( P_{\text{min}} \) used to define the range over which the compliance is calculated. Due to the nonlinearity of the pressure-diameter curve, compliance was evaluated over linear portions of the curve, i.e., for the following pressure ranges: 0–6 mmHg (low), 6–12 mmHg (middle), and 12–20 mmHg (high). This approach permitted correlations of differences in biomechanical behavior with tissue microstructure (27, 36, 40). We also quantified urethral biomechanical properties with beta stiffness (\( \beta \)), which is a dimensionless parameter that may be utilized to describe the full-range, nonlinear pressure-diameter response (11). This is given by

\[ \beta = \frac{\ln(D/D_i)}{P_i - P} \]

where \( P_i \) and \( D_i \) are the standard pressure and paired corresponding diameter, respectively. We chose \( P_i \) to be 10 mmHg, since it is roughly at the midpoint of the physiological range.

The incremental elastic modulus, \( E_{\text{inc}} \), characterizes tissue stiffness by taking into account the changes in tubular geometry induced by incremental increases in pressure while providing a single constant representing a modulus for both the radial and circumferential directions (14). This is defined as

\[ E_{\text{inc}} = \frac{\Delta P}{\Delta R_i} \left( \frac{2R_i^2}{R_i^2 - R_o^2} \right) + \frac{2PR_i^2}{R_o^2 - R_i^2} \]

where \( \Delta P \) and \( \Delta R_i \) are the incremental changes in transmural pressure (2 mmHg) and inner radius, respectively. \( R_o \) and \( R_i \) are the outer and inner radius and the total pressure at the beginning of the increment, respectively.

If we assume that urethral specimens are thick-walled, linearly elastic, isotropic cylinders, the circumferential stress (\( \sigma_{\theta} \)) may be estimated by (6)

\[ \sigma_{\theta} = P \left( \frac{R_i^3}{(R_o^2 - R_i^2)} \right) \left( 1 + \frac{R_o^2}{r^2} \right) \]

where \( r \) is the radial coordinate through the thickness of the wall (i.e., \( r = 0 \) at the center of the lumen, \( r = R_o \) at the inner wall surface, \( r = R_i \) at the outer wall surface, etc.).

Circumferential strain, \( \varepsilon_{\theta} \), is defined by

\[ \varepsilon_{\theta} = \frac{\Delta R}{R_o} \]

where \( \Delta R \) is the change in the mean or midwall radius

\[ \bar{R} = \frac{R_o + R_i}{2} \]

from its unpressurized value, \( R_o \).
**Geometric estimation.** Due to the nonuniform nature of the urethra along its length (10), a thickness must be determined for each segment (i.e., proximal, middle, and distal segments) separately to calculate $R_i$ from the measured $R_o$ for use in Eqs. 1–5. Thus, after the biomechanical ex vivo testing was performed, specimens were fixed under zero transmural pressure in 4% paraformaldehyde overnight, followed by 30% sucrose solution. Specimens were then snap frozen in TBS freezing medium (15-183-13, Fisher Scientific) within a cryomold. Urethral cross sections were cut using a cryostat (Thermo Shandon, Waltham, MA) to 15–20 µm at temperatures of −29 to −31°C and stained with Lillie’s modified Masson’s trichrome stain.

Photographs of the entire urethral cross section were taken at a magnification of ×40 with Magni-Fire Software (DP12, Olympus, Dulles, VA) and a color digital camera (version 2.1C,DP12, Olympus) connected to an Olympus microscope (model BX45). The images of each urethral cross section were then imported using Scion Imaging software (version 0.4.0.3, Scion, Fredrick, MD), the dimensions of urethral cross sections were quantified, and four to five urethral cross section for each segment (proximal, middle, and distal) were averaged for each urethra. Twice the thickness measurement was subtracted from the outer diameter measured by the laser micrometer during the experiment to yield the inner diameter for that section. If we assume that the tissue is incompressible and that no length changes occur, then it can be assumed that cross-sectional area does not change, regardless of increases in intraluminal pressures. The following relationship for cross-sectional area will then hold:

$$\pi(R_o^2 - R_i^2)_{\text{outer}} = \pi(R_o^2 - R_i^2)_{\text{inner}}$$

(6)

Here, $R_o$ and $R_i$ are the outer and inner radii, respectively, at any applied pressure ($P_i$), while $R_o$ and $R_i$ are the respective outer and inner radii measured at $P_o = 0$ mmHg. Note that everything is known in Eq. 6, except for $R_i$, so that for every given measured $R_o$, the corresponding value of $R_i$ can be estimated from

$$R_i = \sqrt{R_o^2 - R_i^2}$$

(7)

**Statistical analyses.** Statistical analyses were performed using a statistical software package (SigmaStat, version 2.0, Jandel, Richmond, CA). For parametric data, a Student’s $t$-test was used to compare compliance and beta stiffness between VD and control groups in both baseline and passive states and between baseline and passive data for controls and VD separately. For nonparametric data, a Mann-Whitney rank sum test was performed. For comparisons within groups but across regions, (i.e., proximal vs. middle, middle vs. distal, etc.), one-factor ANOVA with repeated measures was used. For comparisons involving incremental elastic moduli and circumferential stress values at a given pressure, two-factor ANOVA with repeated measures was used where group (VD vs. control) was one factor and pressure level (0–20 mmHg) was the second factor. Post hoc testing was performed with a Student-Newman-Keuls test. For nonparametric data, Friedman’s repeated-measures ANOVA on ranks was performed with Dunn’s pairwise comparison post hoc test. Statistical significance was indicated by a $P$ value <0.05.

**RESULTS**

**General biomechanical behavior.** Under baseline conditions, nonlinear, sigmoidal shaped pressure-diameter curves were noted for all segments of the control group, and for the middle and distal segments of the VD group (Fig. 2). The proximal segment of the baseline VD specimens exhibited an exponential shape, as did all segments for both VD and control specimens under passive conditions. A proximal-to-distal gradient was noted in the pressure-diameter (Fig. 2) and in the circumferential stress-strain responses (force per unit area in the circumferential direction vs. the normalized change in outer diameter) of control specimens (Fig. 3), where the proximal segment was the most compliant and the distal segment was stiffer, as indicated by the shift to the left of each respective curve. While the proximal segment remained the most compliant for the VD group, there was no clear difference between the middle and distal segments.

The proximal-to-distal gradient observed in control urethras under baseline conditions was abolished in the passive state (Figs. 2 and 3). However, this change in biomechanical response was not observed in VD urethras.

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**Fig. 2.** Average pressure-diameter response curves for baseline and passive controls ($n = 11$) and vaginal distension (VD; $n = 8$) urethral segments. Baseline pressure-diameter curves exhibited a sigmoidal shape, and passive curves were exponential. Baseline control and VD showed major differences in the proximal segment, where the VD proximal segment lacked this sigmoidal shape.
Compliance and beta stiffness. Under baseline conditions, no significant differences in compliance (i.e., lack of stiffness) were noted between segments for the control urethras (Tables 1 and 2). However, VD resulted in a significant increase in low-pressure compliance, from 0 to 6 mmHg, for the proximal segment compared with the middle and distal segments. Similarly, beta stiffness was significantly lower in the proximal segment compared with the middle segment for the VD group. Under passive conditions, a significant proximal-to-distal gradient in low-pressure compliance was observed for both control and VD groups (Table 2). No other segmental differences in compliance or beta stiffness values were noted.

Passive middle pressure compliance was decreased for middle and distal control segments and also for VD proximal segments, compared with their respective baseline values (Tables 1 and 2, Figs. 4 and 5).

Under baseline conditions, no significant differences between control and VD groups were noted in compliance or beta stiffness for any urethral segment (Table 1). However, VD groups exhibited a significantly increased beta stiffness (i.e., lack of distensibility for the entire pressure range) for the middle segment under baseline conditions. Under passive conditions, VD urethras had increased low-pressure compliance but only in the proximal urethral segment; whereas, in the baseline state, this same comparison was not significant ($P = 0.055$). No significant differences in beta stiffness were noted for any segment between control and VD groups under passive conditions (Table 2).

Incremental elastic modulus. Due to spontaneous basal urethral smooth muscle contractions in the baseline state, negative $E_{\text{inc}}$ values were generated for pressures ranging from 0 to 4 mmHg; therefore, $E_{\text{inc}}$ values are reported for 6 to 20 mmHg only. With only a few exceptions, $E_{\text{inc}}$ (i.e., incremental elastic moduli or stiffness values for each step of pressure) for both the control and VD urethras increased with increasing pressure in both baseline and passive states (Fig. 6).

In the baseline state, data trends suggest that the peak value of $E_{\text{inc}}$ for the proximal segment was higher compared with that for middle and distal segments in both control and VD

| Table 1. Baseline compliance and beta stiffness values for control and VD urethras |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                                | Proximal        | Middle          | Distal          | Proximal        | Middle          | Distal          | Proximal        | Middle          | Distal          |
| Stage                                           |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| Low Pressure                                    | $4.62 \pm 0.60$ | $3.24 \pm 0.55$ | $3.19 \pm 0.38$ | $9.44 \pm 2.43^{\dagger}$ | $2.57 \pm 0.29^*$ | $2.69 \pm 0.65^{\dagger}$ | $7.31 \pm 0.90$ | $7.78 \pm 0.80^{\dagger}$ | $7.58 \pm 0.80$ |
| Middle Pressure                                 | $1.15 \pm 0.18$ | $1.64 \pm 0.20$ | $1.34 \pm 0.14$ | $1.77 \pm 0.24$  | $1.26 \pm 0.21$  | $1.67 \pm 0.41$  | $6.80 \pm 0.88^{\dagger}$ | $10.44 \pm 0.87^{\dagger}$ | $9.47 \pm 1.08$ |
| High Pressure                                   | $0.69 \pm 0.17$ | $0.80 \pm 0.15$ | $0.67 \pm 0.10$ | $1.05 \pm 0.41$  | $0.66 \pm 0.09$  | $0.74 \pm 0.41$  |                                 |                 |                 |
| Baseline compliance ($\times 100$)              |                 |                 |                 |                 |                 |                 | $6.80 \pm 0.88^{\dagger}$ | $10.44 \pm 0.87^{\dagger}$ | $9.47 \pm 1.08$ |

Values are means $\pm$ SE. VD, vaginal distension. Student’s $t$-test was used for compliance comparisons and the Mann-Whitney rank sum test was used for beta stiffness comparisons. Significant ($P < 0.05$) differences between control and VD values or proximal, middle, and distal values within groups are indicated as follows: $^{*}$VD proximal vs. VD middle segments; $^{\dagger}$VD proximal vs. VD distal segments; $^{\ddagger}$control middle vs. VD middle segments; and $^{\S}$VD proximal vs. VD middle segments.
urethras, although these differences were not significant (Table 3). A similar trend was noted for the VD urethras in the passive state, but the opposite trend was observed for the control urethras (Fig. 6).

DISCUSSION

In this paper, we have provided for the first time a rigorous analysis of the biomechanical changes that occur in the urethra for a female rat model of SUI induced by VD. Our findings suggest that VD results in increased low-pressure compliance in the proximal urethral segment and increased beta stiffness in the middle segment. To assess the influence of basal tone on the biomechanical properties of the urethra, we also compared both control and VD urethras under baseline and passive conditions. Our results suggest that basal tone influences the general biomechanical response of the urethra (Figs. 2 and 3) and, in particular, increases middle- and high-pressure compliance and decreases beta stiffness in all urethral segments (Figs. 4 and 5, Tables 1 and 2).

Biomechanics is a useful tool for understanding structural changes in healthy and diseased tissue (27, 40). Past research has shown that separate components of soft tissue (i.e., muscle, collagen, and elastin) contribute distinctly to separate components of the pressure-diameter or stress-strain curves (29, 36). Compliance, a linear relationship between pressure and volume, is commonly used in urology for assessment of lower urinary tract dysfunction. In this study, the pressure-diameter curve was separated into linear portions to accommodate the linear characteristics of this measure and also to reveal possible structural changes in the urethra. For example, compliance at low pressure was significantly higher in the proximal urethra after VD than that of control specimens. Since this finding was distinguished in both the baseline (not significant, \( P = 0.055 \)) and passive states, both a lack of muscular tone and an altered ECM are likely related to this change in compliance following VD. Since this finding was true only at low pressures (0–6 mmHg), this could further suggest that VD alters elastic fiber networks, which are known to be dominant at low pressure in biological soft tissues (Fig. 4, top) (8, 29).

Comparisons of compliance between baseline and passive states also indicated changes following VD. Most differences were found in the middle pressure ranges (6–12 mmHg). Middle and distal control urethral segments in the baseline state had significantly higher compliance values compared with that of the passive state. This may be due to the presence of basal tone, providing a muscle contraction that creates a “reserve” of stretch, whereas in the absence of the active tone, the passive tissue is stretched to the maximum with the increasing pressure. For VD specimens, this was only true for the proximal segment, not the middle and distal urethral segments, indicating that the basal tone of the middle and distal urethras may largely be affected.

The concept of beta stiffness was developed to address the issue of a nonlinear pressure-diameter curve (11). Like Young’s incremental elastic modulus for linear materials (e.g., steel), this parameter provides one constant that represents stiffness for biological soft tissues over the entire pressure range, providing an idea of general urethral resistance to pressure. VD middle segments had a significantly higher beta stiffness value than that of control middle segments and VD proximal segments in the baseline state, thus providing evidence for a change in middle urethral stiffness after VD. This indicates that the urethra may have higher stiffness due to the lack of basal tone that provides reserved stretch over the entire pressure range. Comparing baseline and passive states, it is clear that the basal tone was defective after VD. Baseline control urethras had lower beta stiffness values compared with that of the passive state. There were no difference between baseline and passive states in the VD proximal, middle, and distal urethral segments.

\( E_{inc} \) provides a more rigorous measure for urethral stiffness or resistance at each step in pressure ranging from 0 to 20 mmHg. In contrast to low-pressure compliance values, in the control proximal segment, this part of the urethra had the highest \( E_{inc} \) value at maximum pressure, 20 mmHg, indicating that at maximum pressures it is the stiffest of all three segments of the urethra. This may be indicative of the least amount of basal tone present in the proximal urethra compared with that of middle and distal baseline controls. Further studies must be performed to evaluate the importance of orientation and amounts of muscle and extracellular matrix components to the biomechanical measurements.

The biomechanical changes observed in our model for acute SUI may stem from functional, structural, neural, or vascular alterations that result from VD (1, 5, 9, 13). Previous in vivo investigations using a similar model showed that simulated birth trauma produces lower bladder leak-point pressures compared with healthy controls (5). VD has been found to cause neural degeneration, necrosis in both smooth and striated musculature, as well as irregularly shaped muscle fibers (5,
Other studies have revealed an alteration in the amount of urethral collagen in women with genuine stress urinary incontinence (21). The observed biomechanical changes may also stem from damaged lower urinary tract vasculature. Using a rat model similar to the one used in the current work, Damaser et al. (9) studied blood flow to three major pelvic organs: bladder, vagina, and the urethra. The bladder and the urethra experienced periods of hypoxia during VD, which can greatly affect the urothelium and neuromuscular components of this tissue.

Defective nerve-mediated urethral closure mechanisms have been considered an important factor in SUI induced by vaginal parity. Researchers have found that pudendal nerve blockade in healthy women decreases urethral resistance (39) and after transection of the pudendal nerve in rats, the middle urethra loses 80% of its activity (17). Sievert et al. (33) also found that immunoreactivity in proximal and middle urethral segments for neuronal nitric oxide synthase and tyrosine hydroxylase (marker for sympathetic nerves) significantly declined after VD was induced. Neuromedioto mediated urethral closure mechanisms (via somatic nerves) have been proposed by Kamo et al. (7, 17) to mediate increases in urethral pressure before sneeze transmission, followed by an increase in urethral resistance as sneeze-induced abdominal pressure exceeds increased bladder pressure. The involvement of neural mechanisms in urethral resistance is also supported by the fact that, with bilateral pudendal nerve block, there is a significant decrease in urethral closure pressure (39).

Our study was conducted in the absence of neural activity (neither electrical field stimulation nor neurotransmitters were present) and without any stress conditions other than intraluminal pressure application. However, the basal smooth muscle activity may act as a prime contributor to the continual maintenance of continence. The results of our studies have revealed a decrease in basal smooth muscle activity in a given pressure range and a reduction in urethral resistance after VD. Lack of

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**Fig. 4.** Low (top), middle (middle), and high (bottom)-pressure compliance values for baseline vs. passive comparisons of control (n = 11) and VD (n = 8) groups in the proximal, middle, and distal segments. All comparisons were made using Student’s t-test. Low-pressure compliance (0–6 mmHg): VD increased compliance for proximal urethral segments in the passive state (*P < 0.05). Control proximal segments had significantly higher compliance values than middle (*P < 0.05) and distal (+P < 0.05) segments in the passive state. VD proximal segments had significantly higher compliance than middle (###P < 0.05) and (+,##P < 0.05) distal segments in the both baseline and passive states, respectively (via ANOVA with repeated measures). Middle-pressure compliance (6–12 mmHg): control middle and distal segments in the baseline state had increased compliance compared with control middle (###P < 0.05) and (+,##P < 0.05) distal segments in the passive state, respectively (via Student’s t-test). VD proximal urethral compliance was increased in the baseline state compared with VD proximal urethral compliance in the passive state (*P < 0.05). Error bars represent SE.

**Fig. 5.** Beta stiffness values for baseline vs. passive comparisons of control (n = 11) and VD (n = 8) groups in the proximal, middle, and distal segments. In the baseline state, beta stiffness values for control middle segments were lower compared with VD middle segments (###P < 0.05, via Mann-Whitney rank sum test), and VD middle urethral segments had higher beta stiffness values than VD proximal segments (###P < 0.05, Friedman’s repeated-measures ANOVA on ranks). Control proximal, middle, and distal segments in the passive state had increased stiffness values compared with proximal (###P < 0.05), middle (###P < 0.05), and distal (###P < 0.05) segments of controls in the baseline state, respectively (via Mann-Whitney rank sum test), but this was not true for VD passive and baseline comparisons. Error bars represent SE.
basal tone may contribute to the deficiency of neural control found in the previously mentioned studies. Since the basal smooth muscle tone is commonly stretch sensitive (15), it may help to provide an initial resistance to the increase in intravesical pressure during a stress episode. Urethral spontaneous myogenic tone may act as a preexisting and constant tone, preparing the contractile mechanisms to contract more if neurotransmitters are released to prevent leakage (12). Such a basal tone may allow rapid and efficient control of the urethra both as a tight seal and as a controlled conduit.

The VD-induced changes in the biomechanical properties of the proximal urethral segment may have significant implications, as this segment aids in maintaining urinary continence via sympathetic nerve excitatory control of the urethral smooth muscle (34). This has been shown to be a major factor in promoting tonic contraction and maintaining storage (19). The observed increase in proximal urethral low pressure compliance may be due to a change in proximal urethral geometry (open bladder neck) (31), altered intrinsic basal urethral tone, or changes in ECM properties, more specifically, elastin, which is responsible for responding to low pressures (29). An increase in proximal urethral compliance may also contribute to the “funneling” of the proximal urethral neck seen in patients with SUI (13). Indeed, our data support the notion that VD-induced biomechanical changes are associated with changes in basal smooth muscle tone activity. The sigmoidal shape of the proximal urethral pressure-diameter curve in the baseline state was not present after VD; i.e., it is more exponential in shape, similar to that for the urethra in the passive state (Fig. 2).

The middle segment of the urethra also contributes to the urethral continence mechanism, although in a distinct manner from the proximal segment. The middle urethral segment contains the most abundant proportion of smooth and striated muscle (16) and is the site of pudendal nerve innervation, which mediates striated sphincter muscle-storage reflexes. Our results indicated a significant increase in beta stiffness of the middle segment of the urethra under baseline conditions following VD (Table 1). This may be due to structural or neural damage induced by VD or to alterations in basal tone or ECM components within this segment. It is possible that defects in both basal tone and matrix occurred since beta stiffness is a parameter represented over the entire pressure range. Reduced basal tone is consistent with our observation that there are significant differences in biomechanical properties of the healthy middle urethral segment between the passive and baseline states, but not for the VD group (Figs. 4–6). In addition, the middle segment of the VD urethra has a less pronounced sigmoidal shape in its pressure-diameter response under baseline conditions compared with the same segment of the control urethras (Fig. 2). An initial, pressure-induced myogenic contraction in the control urethras may provide a reserve

Table 3. Peak $E_{inc}$ in baseline and passive states

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<th>Baseline</th>
<th>Passive</th>
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<tr>
<td></td>
<td>Proximal</td>
<td>Middle</td>
</tr>
<tr>
<td>Control ($n = 8$)</td>
<td>3.20±1.26</td>
<td>1.04±0.12</td>
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<tr>
<td>VD ($n = 8$)</td>
<td>2.04±0.73</td>
<td>1.24±0.54</td>
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Values are means ± SE expressed as $\times 10^6$ dyne/cm². $E_{inc}$, incremental elastic modulus. In the passive state, peak $E_{inc}$ values for control distal segments were significantly higher than for VD distal segments (*$P < 0.05$) by 2-factor ANOVA with repeated measures.

Fig. 6. Incremental elastic moduli ($E_{inc}$) values for proximal (top), middle (middle), and distal (bottom) urethral segments. Proximal segments showed differences in baseline and passive control comparisons (at 10 and 16 mmHg). The same held for middle control segments (at 6–16 and 20 mmHg). Passive controls were different from passive VD distal urethras (at 10, 14, and 20 mmHg). Error bars represent SE. *Significant difference ($P < 0.05$) between baseline and controls at the indicated pressure. +Significance between control and VD within that particular segment at the indicated pressure. All data were compared with 2-factor ANOVA with repeated measures.
capacity of distensibility so that the middle segment of the control urethra may be distended further in response to pressure than VD urethras.

The role of the distal urethra is unclear (3), although it is thought that its contribution to the continence mechanism increases when the proximal urethral region is damaged and weak (41). As with the other segments, our results suggest VD-induced alterations in basal tone within the distal segment. However, we did not note any significant differences in the biomechanical properties of this segment between VD and control urethras.

To our knowledge, very few studies have been previously performed to assess the influence of SUI on the biomechanical properties of the urethra. Third and Lose (38) studied urethral viscoelasticity in both continent and incontinent women with symptoms of genuine SUI. They concluded that urethral stress relaxation was significantly greater in SUI than for healthy females, with the greatest differences occurring at the bladder neck or proximal urethral segment. The viscoelasticity of muscular tissues is related to the basal tone of the tissue (2, 8, 32). Therefore, our observation that VD leads to a decrease in basal tone in the urethra is consistent with the finding by Third and Lose (38) that urethral viscoelasticity is altered in SUI. Measurements of passive length-tension curves in urethral strips indicated a significant increase in urethral compliance in postpartum rabbits compared with urethras of virgins (19). This is consistent with our current results (Tables 1 and 2).

There are some important limitations to these studies that should be kept in mind. First, the urethra is an organ surrounded by supporting pelvic floor musculature, the pubourethral ligament, and the anterior vaginal wall. As a result, physiological transmural urethral pressure is difficult to measure (23). The pressure range chosen in this study was based on measured maximal voiding pressures for a female rat (35). Second, the urethra consists of musculature arranged in two directions: circumferential and longitudinal. The results of this study were limited to the circumferential biomechanical behavior of the urethra and do not provide insights into longitudinal biomechanical properties/behavior. The maximum urethral closure force is generally attributed to the circumferential musculature (3); however, the longitudinal properties of the urethra could affect its overall biomechanical and functional behavior. Simultaneous analysis of both circumferential and longitudinal properties could lead to important new insights into urethral biomechanics and function. Finally, the design of the current study only assessed the contribution of the biomechanical properties of the urethral basal smooth muscle tone and ECM, and the external urethral striated sphincter remained passive. However, striated muscle does not have stretch-sensitive qualities similar to that of smooth muscle that are responsible for generating basal tone.

In conclusion, we have shown that VD leads to biomechanical changes in the urethra that are potentially relevant to the mechanisms that underlie the development of SUI. While these changes may result from structural, neural, vascular, or functional alterations known to be associated with SUI, we provide evidence that VD leads to altered urethral basal smooth muscle tone. Further biomechanical and structural analyses will need to be explored to gain a more detailed understanding of the devastating effects of VD on the urethra.

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REFERENCES


