New objective measures to quantify stress urinary incontinence in a novel durable animal model of intrinsic sphincter deficiency

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Rodríguez, Larissa V., Shinhong Chen, Gregory S. Jack, Fernando de Almeida, Kyo Won Lee, and Rong Zhang. New objective measures to quantify stress urinary incontinence in a novel durable animal model of intrinsic sphincter deficiency. Am J Physiol Regul Integr Comp Physiol 288: R1332–R1338, 2005. First published January 13, 2005; doi:10.1152/ajpregu.00760.2004.—Existing animal models of stress urinary incontinence (SUI) are limited because of the low rate of incontinence seen in the animals and to their relatively low durability. In addition, most methods described to measure incontinence are operator-dependent. The aim of this study was to develop a new durable animal model of SUI and establish objective measures to quantify SUI. We subjected female rats to transabdominal urethrotomy. At baseline and at 1, 4, 8, 12, and 24 wk after intervention, animals underwent cystometry and evaluation with abdominal leak point pressure (ALPP). Urethral resistance was evaluated by retrograde urethral perfusion pressure (RUPP). Tissues were obtained for histology and immunohistochemistry. Normal female rats had an average ALPP of 19.4 cm H2O and RUPP of 22.6 cm H2O at baseline. More than 93% of the animals had significantly decreased ALPP and RUPP after the procedure. The mean ALPP and RUPP decreased to 9.8 cm H2O and 11.2 cm H2O, respectively, by 1 wk after urethropotomy. These changes were maintained for up to 24 wk. Changes seen in urethral resistance and ALPP appear to be mediated by apoptosis, decreased neuronal mass, and smooth muscle atrophy. These results indicate that transabdominal urethrotomy is a reliable method of achieving durable decreased urethral resistance in a SUI model. RUPP and ALPP are objective and reproducible methods of assessing urethral resistance. Changes in continence and urethral resistance appear to be mediated by denervation and smooth muscle atrophy, which are seen in both elderly incontinent patients and in patients with intrinsic sphincter dysfunction.

stress incontinence; abdominal leak point pressure; retrograde perfusion; urethral resistance

URINARY INCONTINENCE AFFECTS 13 million Americans, the vast majority of whom (85%) are women. In 1995, the annual cost of incontinence in the United States was estimated to be $26.3 billion (26). This represents a 92% increase from the 1984 cost, and it is expected to increase due to the aging population (9). Approximately 50% of all incontinent women are classified as having stress incontinence (5, 8). The pathophysiology of stress urinary incontinence (SUI) is multifactorial and poorly understood but appears to be in part due to anatomic changes in urethral support and dysfunction of the intrinsic sphincteric mechanism of the urethra resulting in involuntary urine loss during any activity that causes abdominal straining. Given that the list of such activities includes everyday occurrences like coughing, laughing, and sneezing, SUI has an enormous impact on quality of life. In the elderly, other factors also appear to contribute to the development of an incompetent female urethra. These factors include changes in the skeletal muscle, atrophy of the smooth musculature of the urethra, and loss of neuronal mass among others.

To better study the pathophysiology of SUI and to develop a way to better evaluate the impact of experimental interventions to treat SUI, there is a need for reliable, reproducible, and durable animal models. In the past few years, some useful rat animal models have been proposed such as simulating birth trauma with intravaginal balloon inflation (1, 4, 15), vaginal distension, and ovariectomy (12, 19), pudendal nerve crush (4, 7, 10), spinal cord injury (21, 28), and electrocauterization (2, 27). These important investigations have shed light on the pathogenesis of SUI. However, vaginal trauma models tend to heal with rapid spontaneous resolution of SUI, and neurogenic models do not duplicate the pathophysiology present in most women with SUI. In addition, most available models use subjective determinations of SUI. Our goal is two-fold: 1) to develop a durable and reproducible animal model of SUI, and 2) to develop objective methods to evaluate urethral resistance in these animals.

MATERIALS AND METHODS

All experimental protocols were approved by the Chancellor’s Animal Research Committee of the Office for Protection of Research Subjects at UCLA. A total of 140 female Sprague-Dawley retired breeder rats aged 6 to 9 mo and weighing from 340 to 400 gm were used in this study (Charles River, Wilmington, MA). All animals underwent urodynamic and urethral resistance evaluation preoperatively. They were then divided into five groups and urodynamics performed at 1, 4, 8, 12, or 24 wk postoperatively. Three affected animals were killed at each time point and the bladder and urethral tissues were examined by histology and immunohistochemistry. A group of animals (n = 10) underwent sham surgery where the abdominal cavity was opened and the bladder manipulated with forceps but the urethra and bladder neck were untouched. These animals were evaluated 1 wk postoperatively. In addition, to evaluate the impact of parity on the development of incontinence in this animal model, 10 female virgin rats 6 mo of age and weighing from 270–320 gm were used to evaluate the differences between virgin rats and retired breeders.

Cystometrogram and measurement of abdominal leak point pressure. All animals were anesthetized with an intraperitoneal injection of ketamine (70–90 mg/kg body wt). The urinary bladder was emptied with a 22G transurethral catheter. A 2F microtip transurethral Intraceath was used for bladder filling and recording via a three-way stopcock. The tubing was connected to a pressure transducer (Duet...
logic, Medtronic, Denmark) in line with an infusion pump (Harvard Apparatus, Holliston, MA). Saline at 37°C was infused at the rate of 100 μl/min. A computer with data acquisition software was used for recording the cystometric studies. The infusion volume was defined as maximal bladder capacity when the first urine drop appeared at the urethral meatus in conjunction with a rapid rise of the intravesical pressure. Bladder capacity was determined as the average between three voiding cycles. The bladder was then emptied with a 22G transurethral catheter and filled to half capacity with saline mixed with methylene blue to facilitate determination of urine leakage. The transurethral catheter was then removed and a 3F Fogarty tube with 0.15 ml balloon (120403F Edwards Lifesciences, Germany) was put in the rectum and connected to the pressure transducer to record intra-abdominal pressures (Fig. 1A). Steady suprapubic pressure was applied manually. The pressure was increased at 10 cmH2O intervals and recorded. The lowest intra-abdominal pressure that led to leakage of urine at the urethral meatus was chosen as the abdominal leak point pressure (ALPP). ALPP determination was determined four times for each animal. We report the average ALPP.

Threshold pressure. The measurement of threshold pressure (TP) was determined during cystometric examination as previously described (16, 19). It is defined as the difference between the bladder pressure immediately before micturition and the baseline pressure at the beginning of the saline infusion. The threshold pressure combined with bladder compliance is typically used as a parameter of neurogenic bladder dysfunction.

Retrograde urethral perfusion pressure. The retrograde urethral perfusion pressure (RUPP) estimates urethral resistance by measuring variations of intraurethral pressure to a constant flow. After completion of the ALPP determination, the bladder was emptied. The urethral meatus was catheterized with one 2F microtip transurethral Intracath and another customized 2F silicon infusion tube (SIL-C20 Instech Solomon, PA). These catheters were placed just inside the urethral meatus, and therefore, their tips were within the distal urethra. They were made watertight using a 5–0 polyglactin (Vicryl) suture to close the urethral meatus around the catheters (Fig. 1B). The perfusion pump was zeroed and connected to the silicon tubing; saline was then infused at a rate of 100 μl/min. The transurethral Intracath was connected to a pressure transducer for recording. By infusing at a constant flow and measuring the pressure of the occluded urethra during early filling, we can estimate the urethral resistance to flow. This measurement is done early in filling, before cystometric capacity is reached or increases in bladder pressures are seen. Thus RUPP is the pressure required to achieve and maintain an open urethral sphincter. Three measurements of RUPP were taken at each time point for each animal. We report the average of these measurements.

Interobserver and intraobserver variability. To determine interobserver variability, an analysis was performed on the RUPP of 25 normal rats obtained by three independent investigators (S. Chen, G. S. Jack, F. de Almeida). Three animals were tested in quadruplicate to analyze intraobserver variability. Each quadruplicate study was performed under the same anesthetic. For each of the studies, the catheters were removed, and the bladder was emptied between individual data point acquisitions.

Transabdominal urethrolysis. One week after the baseline urodynamics evaluation, the animals were anesthetized with an intraperitoneal injection of ketamine (60 mg/kg body wt) and xylazine (5mg/kg body wt). They were placed supine on a water-circulating heating pad. The abdomen was prepped and draped in standard surgical fashion. A lower abdominal midline incision was made, and the bladder and urethra were identified. The proximal and distal urethra was detached circumferentially by incising the endopelvic fascia and detaching the urethra from the anterior vaginal wall and pubic bone by sharp dissection (Fig. 2). Care was taken not to injure the ureters or compromise the inferior vesical vasculature. A cotton swab was put into the vagina to aid with the dissection. The rectus fascia and skin were closed with 4–0 polyglactin (Vicryl) and 4–0 Nylon sutures, respectively. The animals were kept under watchful observation in a water-circulating heating pad and turned from side to side until they were able to maintain sternal recumbency before being returned to the vivarium.

Incontinence measurement. We defined a measurable clinically relevant change in urethral competency as ALPP or RUPP values less than 1 SD from the preoperative mean.

Histological studies. Animals were killed with an overdose of intravenous pentobarbital sodium (100 mg/kg). The whole bladder and urethra were harvested by removing the symphysis pubis and thus preserving the entire urethral segment. The specimens were fixed in 10% neutral buffered formalin overnight and embedded in paraffin. Paraffin blocks were cut in 5-μm-thick sections. These were deparaffinized and hydrated with distilled water. Two sequential sections were then stained with Masson’s trichrome to determine the distribution of smooth muscle and extracellular matrix.

Apoptosis. The terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) assay was followed by tetramethylrhodamine deoxyuridine 5’-triphosphate (TMR) staining. An in situ cell death assay with the TMR detection kit (Roche Diagnostic, Indianapolis, IN) was performed on paraffin-embedded
blocks according to the manufacturer’s instructions. Each slide contained a sagittal section of the whole bladder and three cross sections of the urethra (one proximal, one mid, and one distal urethra). Two sequential histological sections from each killed animal were used. Deparaffinized tissue sections were incubated in 20 μg/ml protease K for 15 min at room temperature and incubated in TUNEL reaction mixture for 60 min at 37°C in the dark. After washing in phosphate-buffered saline, the samples were counterstained with 4’, 6-diamidino-2-phenylindole (DAPI; Vector Laboratory, Burlingame, CA). Positive controls were treated for 10 min with 0.5 mg/ml deoxyribonuclease I in sodium cacodylate buffer (pH 7.2). Terminal deoxynucleotidyl transferase was omitted from the nucleotide mixture for the negative controls.

Nerve staining with antiprotein gene product 9.5 antibody PGP 9.5. Protein gene product 9.5 (PGP9.5) is an ubiquitin hydrolase widely expressed in neuronal tissues at all stages of neuronal differentiation. For evaluation of changes in neuronal mass, PGP9.5 immunoreactivity was used as a neural marker. Two sequential sections from each killed animal were deparaffinized, hydrated in distilled water, and washed with PBS (Sigma, St. Louis, MO) at room temperature (3 times × 10 min). The sections were then incubated for 1 h at room temperature with PBS containing 2% (wt/vol) BSA (Sigma, St. Louis, MO) and 0.3% (vol/vol) Triton X-100 (Sigma, St. Louis, MO) to block nonspecific antibody binding, and then incubated with rabbit polyclonal anti-PGP9.5 neuronal marker (DAKO, Carpinteria, CA) at 1:500 and 4°C overnight. The sections were labeled with a fluorescein isothiocyanate-conjugated anti-rabbit secondary antibody (Vector Laboratory, Burlingame, CA).

Quantitative image analysis. The quantitation of the staining obtained by either histochemical or immunohistochecmical techniques was performed by computerized densitometry using the ImagePro 4.01 program (Media Cybernetics, Silver Spring, MD) coupled to an Olympus BHS microscope equipped with a Spot RT digital camera.

Statistical analysis. Data are summarized as means ± SD. Analysis was performed using statistical software (SPSS 11.0, SPSS, Chicago, IL). Normally distributed variables were compared with the Student t-test. The Kruskal-Wallis test was used for nonparametric-independent multiple group comparisons and applied to test for differences in preoperative and postoperative ALPP and RUPP at each time point. Chi-square analysis was performed to test for differences in continence status before and after urethral detachment at each time point. One-way ANOVA was used for multiple-group comparison to test for differences in preoperative and postoperative bladder capacity and threshold pressure at each time point, as well as determination of interobserver variability. Pearson correlation coefficient was applied to the study of the correlation between ALPP and RUPP. Statistical significance was determined at P values less than 0.05.

RESULTS

Urodynamic evaluation. The overall changes in urodynamic parameters are shown in Table 1. Anatomic urethral detachment had no effect on bladder capacity, bladder filling pressures, or compliance. In addition, there were no significant changes between the baseline values of the preoperated normal rats and the sham-operated animals (bladder capacity 1.6 vs. 1.4, threshold pressure 26.3 vs. 25.8, P > 0.05). Statistically significant decreases were seen in ALPP and RUPP after urethralysis at all time points (Table 1). Normal retired breeder female rats had an average baseline RUPP of 22.6 cmH2O and ALPP of 19.4 cmH2O preoperatively. There was no statistically significant difference between the baseline values of the retired breeder female rats and the sham-operated animals (mean RUPP 21.9 cmH2O, P > 0.05; mean ALPP 21.4 cmH2O, P > 0.05). One week after urethralysis, the mean ALPP and RUPP decreased to 9.8 cmH2O and 11.2 cmH2O, respectively. We consider a measurable clinically relevant change as values less than 1 SD from the preoperative mean. We defined this change as the incontinence threshold. With this definition, 1 wk after urethralysis, 100% and 93% of all animals had lower VLPP and RUPP than the defined incontinence threshold. These decreases in ALPP and RUPP were maintained in 75% and 69% of animals, respectively, for up to 4 weeks postoperatively (Table 1).

Table 1. Urodynamic parameters in the animals preoperatively and post-transabdominal urethralysis

<table>
<thead>
<tr>
<th>Duration after surgery</th>
<th>1 week (n = 30)</th>
<th>4 weeks (n = 26)</th>
<th>8 weeks (n = 24)</th>
<th>12 weeks (n = 20)</th>
<th>24 weeks (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder capacity, ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperatively</td>
<td>1.6±0.7</td>
<td>1.7±0.8</td>
<td>1.7±0.8</td>
<td>2.0±0.9</td>
<td>1.6±0.9</td>
</tr>
<tr>
<td>Postoperatively</td>
<td>1.7±0.6</td>
<td>2.0±0.6</td>
<td>1.9±0.7</td>
<td>2.1±0.9</td>
<td>2.2±0.8</td>
</tr>
<tr>
<td>Value</td>
<td></td>
<td>0.30</td>
<td>0.31</td>
<td>0.31</td>
<td>0.61</td>
</tr>
<tr>
<td>Threshold pressure, cmH2O</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperatively</td>
<td>26.2±7.4</td>
<td>25.5±5.4</td>
<td>26.2±7.4</td>
<td>26.4±7.8</td>
<td>26.2±7.4</td>
</tr>
<tr>
<td>Postoperatively</td>
<td>30.3±9.8</td>
<td>28.1±10.5</td>
<td>26.1±8.6</td>
<td>29.9±10.9</td>
<td>22.0±5.3</td>
</tr>
<tr>
<td>Value</td>
<td>0.15</td>
<td>0.30</td>
<td>0.97</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Abdominal leak point</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pressure, cmH2O</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperatively</td>
<td>19.4±3.8</td>
<td>22.4±3.4</td>
<td>20.1±4.3</td>
<td>18.8±3.6</td>
<td>19.4±3.8</td>
</tr>
<tr>
<td>Postoperatively</td>
<td>9.8±3.2</td>
<td>13.4±4.1</td>
<td>12.3±4.3</td>
<td>14.6±5.3</td>
<td>10.6±6.4</td>
</tr>
<tr>
<td>Value</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.005</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RUPP, cmH2O</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperatively</td>
<td>22.6±5.0</td>
<td>22.1±4.4</td>
<td>22.6±3.7</td>
<td>20.4±2.8</td>
<td>22.4±3.9</td>
</tr>
<tr>
<td>Postoperatively</td>
<td>11.2±4.9</td>
<td>12.7±5.9</td>
<td>14.5±5.1</td>
<td>14.2±5.3</td>
<td>12.6±7.9</td>
</tr>
<tr>
<td>Value</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.005</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are presented as means ± SD. *One-way ANOVA for preoperative and postoperative bladder capacity and threshold pressure (P > 0.05).
24-wk postoperatively (Table 2). The Pearson correlation coefficient \( r \) between the individual ALPP and RUPP in all animals was 0.54 \( (P \leq 0.01) \). On cystometry, there was evidence of very low detrusor contractions of 3–4 cmH2O not leading to leakage in 
\( 10–15\% \) of animals after 12 wk with the majority of these presenting at 24 wk postoperatively. No animal suffered from urinary retention or urinary obstruction.

Analysis of the interobserver variability of three independent investigators showed no significant variation in RUPP determination. The average variation from the mean was 8.2%. The average \( (\pm SD) \) RUPP for each investigator was 21.9 \( (\pm 5.3) \), 25.2 \( (\pm 6.8) \), and 19.9 \( (\pm 6.1) \) cm H2O, respectively \( (P = 0.15) \). In addition, there was minimal variability within intraobserver RUPP results with an average RUPP of 21.4 cm H2O and an average SE of 1.1 cmH2O (range 0.7 to 1.4). To evaluate the effect of parity on the SUI model, we evaluated 10 virgin rats for comparison. There were no significant differences in bladder capacity, threshold pressure, ALPP, or urethral resistance between retired breeder and virgin rats (data not shown).

**Urethral smooth muscle distribution.** Masson’s trichrome staining revealed marked smooth muscle atrophy in the urethral wall musculature. The urethral smooth muscle to collagen ratio was 1.2:1 preoperatively. This was not significantly different from the sham-operated group (normalized preoperative ratio of 1 vs. sham ratio of 0.97, \( P > 0.05 \)). Following urethrolysis, the smooth muscle content of the urethra decreased by an average of 65% at 1, 4, and 8 wk (Fig. 3). Some recovery of the urethral smooth muscle was noted by 12 wk post urethrolysis, however, the ratio still remained below baseline \( (P < 0.05, \text{Fig. 3}) \). There was a strong correlation between RUPP and urethral smooth muscle atrophy \( (r = 0.97) \), and a lower correlation between ALPP and smooth muscle atrophy \( (r = 0.85) \).

**Apoptosis.** The results from the in situ analysis of apoptosis in the urethra and bladder tissue specimens are summarized in Fig. 4. The number of apoptotic cells in the urethra and bladder was significantly higher in the urethral detachment groups than in the control animals. At early time points, most apoptotic cells were identified in the epithelium and submucosa. With time, apoptosis was more prominent in the submucosa and muscle layers. These changes were seen throughout the bladder tissue and were not localized to a particular anatomic bladder region. There was no difference in the number of apoptotic

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**Table 2. Percentage of incontinent rats at different time points after transabdominal urethrolysis**

<table>
<thead>
<tr>
<th>Time, wk</th>
<th>Incontinence, ( n )</th>
<th>Continence, ( n )</th>
<th>Total, ( n )</th>
<th>Percentage, %</th>
<th>( P ) Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-op</td>
<td>4</td>
<td>26</td>
<td>30</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>0</td>
<td>30</td>
<td>100</td>
<td>\textless 0.001</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>8</td>
<td>24</td>
<td>66.7</td>
<td>\textless 0.001</td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>4</td>
<td>20</td>
<td>80.0</td>
<td>\textless 0.001</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>6</td>
<td>16</td>
<td>62.5</td>
<td>\textless 0.001</td>
</tr>
<tr>
<td>24</td>
<td>12</td>
<td>4</td>
<td>16</td>
<td>75.0</td>
<td>\textless 0.001</td>
</tr>
</tbody>
</table>

Abdominal leak point pressure (ALPP) for baseline is 19.4 cmH2O, whereas the retrograde urethral perfusion pressure (RUPP) is 22.4 cmH2O. The ALPP for the incontinence measurement is 15.6 cmH2O, whereas the RUPP is 18.5 cmH2O. *Incontinence measurement is defined as the mean preoperative ALPP or RUPP minus one SD; †Chi-square test.

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**Fig. 3.** Comparison of smooth muscle content among treatment groups. Comparison of the histologic findings between preoperative and post transabdominal urethrolysis at 1, 4, 8, and 12 wk. Masson’s trichrome staining \( (\times 40) \) revealed marked mucosal atrophy in urethral wall (both smooth and striated), with an increase in connective tissue content and a decrease in the muscular layer \( (P = 0.03) \). There are no statistically significant differences between the animals that underwent transabdominal urethrolysis at any time points \( (P > 0.05) \).
cells between the preoperative controls and the sham-operated animals.

**Innervation.** Neurons were identified using the neuronal-specific marker PGP 9.5. Fig. 5 shows PGP-9.5 staining within the urethra and bladder after urethral detachment at 1, 4, 8, and 12 wk. Compared with the preoperative control animals, there was a time-dependent loss of neuronal content. There was no difference between the preoperative controls and the sham-operated animals.

**DISCUSSION**

SUI is characterized by involuntary urine loss due to an increase in abdominal pressure. The etiology of SUI is poorly understood but is likely multifactorial. Although loss of anatomic support due to vaginal deliveries appears to play an important role in its development, other changes seen with aging also contribute to the development of an incompetent urethra. Such changes include atrophy of the skeletal rhabdosphincter, atrophy of the smooth muscle of the urethra, loss of neuronal mass, and hormonal changes seen with menopause (3, 6, 11, 24, 25). It has been clear that no single alteration but a combination of factors acting together cause SUI. Therefore, the development of reproducible and durable animal models will allow us to explore the pathophysiology of SUI and to develop new treatments for this condition.

An animal model of SUI was first developed in 1998 (15). This model attempts to simulate one of the known risk factors for the development of SUI, the trauma of vaginal delivery by vaginal balloon dilation of the female rat. Since its first description, a number of investigators have further evaluated and modified this model (1, 4, 12, 19, 22). One of the main problems with this model is the relatively low rate of incontinence and the spontaneous restoration of continence with time (15). In the originally described model, only 46% of animals were deemed incontinent 4 wk after the procedure (15). In an attempt to increase the rate of incontinence, these groups have evaluated the role of ovariectomy and pregnancy on this animal model. When animals were ovariectomized and balloon-dilated immediately after delivery, the authors were able to achieve an incontinence rate of 72% at 8 wk, but this was not durable (22). This same group was unable to demonstrate significant changes in modified leak point pressures in female rats after ovariectomy and vaginal balloon dilation (19). In addition, they reported changes in bladder dynamics with detrusor instability in 64% of their castrated and balloon-distended animals (19). Another group was unable to show evidence of incontinence after 4 wk, at which time the animals showed spontaneous recovery (12). This animal model has been criticized because of the relatively low rate of incontinence, the difficulty of reproducibility shown by different investigators, the high rate of bladder dysfunction, and the relatively short durability. All these factors make this an unreliable model for the long-term evaluation of SUI, especially the effects of intervention in restoration of normal urethral function. An additional concern is that the way by which this model causes incontinence is poorly understood but seems to be related to vaginal and

![Fig. 4. Analysis of apoptosis. The number of apoptotic cells in the urethra and bladder was significantly higher in the urethral detachment groups than in the control animals. Magnification ×100.](image1)

![Fig. 5. Analysis of innervation. The figure shows PGP 9.5 staining within the urethra and bladder after transabdominal urethralysis at 1, 4, 8, and 12 wk. There is a decrease in innervation with time. Magnification ×200.](image2)
overall pelvic ischemia leading to bladder and urethral dysfunction and vaginal ischemia. Therefore, this is not an ideal model recreating the isolated urethral dysfunction that we see in the majority of women who present with SUI.

Other animal models of incontinence have been briefly described. Some of these models use pudendal nerve crush or spinal cord injury (4, 7, 10, 14, 20, 23). Unfortunately, these neurogenic models do not represent the pathophysiology of most of the patients with SUI, and in addition, most have not been evaluated in the long term. Recently, an animal model of intrinsic sphincter dysfunction has been described that involves electrocoagulation of the surrounding tissues of the urethra (2, 27). Although this appears to be a model with potential, the authors describe severe cautery effect and destruction of the skeletal rhabdosphincter. In addition, of the two groups reporting on this technique, one studied only a small number of animals, with only 6 animals per group (2), while the other only followed their animals for a total of 30 days (27). Neither study reports on the rate of incontinence with this procedure.

The transabdominal urethrolysis described in the current study demonstrates measurable significant changes in urethral function in the majority of animals at all time points with more than 93% of animals with significant decreases in urethral resistance at 1 wk. These changes are durable in the majority of animals at 6 mo with ~70% of animals showing altered urethral resistance. A 6-mo end point was chosen since it represents ~100% of the animals’ reproductive life (since at a 6 mo followup, the animals were ~15 mo old) and would translate to adequate followup in the human. Although we assumed that parity would have an effect on incontinence in the rat, we did not see any differences between retired breeders and virgin rats.

Although there is a clear limitation to studying voiding cycles and voiding dynamics in anesthetized animals incapable of voluntary voiding at baseline, these models have been previously validated. Overall bladder dynamics appear to be unchanged in this model. In particular, bladder capacity, compliance, and threshold pressure are unchanged. Although bladder pressures are dependent on overall urethral resistance, we are still able to measure bladder dynamics and pressure because in the rat, the lumen of the urethra is so small that the diameter of the intravesical catheter used for CMG is big enough to provide occlusion of the urethra and allow for bladder measurements and assessment of bladder function. Minimal bladder dysfunction was seen in the animals after urethrolysis in the long term, with 10–15% of animals experiencing intermittent low-pressure bladder contractions not leading to urine leakage prior to voiding. This might be a long-term effect of the urethrolysis procedure, or it might be a result of aging itself, as at the 24-wk followup after urethrolysis, the animals were ~15 mo of age. This age represents ~65% of the animal’s life expectancy.

Research of the existing animal models of incontinence has been further complicated by the lack of objective parameters to measure urethral dysfunction in the rat. The original description involved the subjective determination of urinary leakage when anesthetized rats were made to sneeze by stimulating their nostrils with a whisker (15). Further attempts at finding more subjective measures have looked at modified urethral pressure profiles and modified leak point pressures (19). Unfortunately, both of these procedures involve placing a catheter in the animal’s urethra. Given that the lumen of the rat urethra is so small, the presence of any instrument in the urethra will interfere with adequate evaluation of urethral incontinence. In addition, the modified leak point pressure was really a measure of the bladder capacity at the time of overflow incontinence around the catheter and not a true measure of urethral resistance. Other investigators have modified this technique by placing a suprapubic catheter to measure intravesical pressures at the time of increased abdominal pressure applied to the abdomen by the finger of the investigator (1, 2, 4). Because the rat’s bladder is so fine and small, the presence of a foreign object in the bladder might lead to bladder irritation and involuntary precipitous voiding. Given that these animals are incapable of voluntary voiding, it is difficult to determine urine loss due to bladder irritability vs. that due to urethral dysfunction. We bypass this problem by describing a technique to measure ALPP in the animal without any catheters in the urinary tract by placing a pressure transducer in the rectum of the animal. To our knowledge this is the first description of this method in the rat. In this fashion, we do not interfere with the normal function of the animal’s urethra or bladder. We find that with ALPP determined in this fashion, we are able to demonstrate significant decreases in ALPP after urethrolysis in our animal model. Nevertheless, even this method involves subjective determinations. For one, the investigator has to apply manual pressure in the abdomen, but more importantly, an observer makes the determination of leakage at the time of urine loss through the urethral meatus of the animal. In an attempt to minimize investigator bias and to find a more objective quantifiable model of decreased urethral resistance, we have developed a novel description of retrograde perfusion as a determination of urethral resistance. Retrograde perfusion was used in the past to estimate urethral resistance and overall sphincter function in men, especially after placement of the artificial urinary sphincter (13). In this animal model, we described RUPP as an additional global measure of urethral function and resistance. By placing a suture at the end of the urethral meatus, thus avoiding leakage of fluid and by performing repetitive measurements, we have validated a method to estimate urethral function that is not operator-dependent. In this procedure the measurements are obtained from a pressure transducer, thus eliminating completely the investigators subjective determination of urinary leakage. It also provides a quantifiable method to evaluate possible effects on urethral resistance of different potential therapeutic approaches. Interestingly, when we defined the incontinence threshold as being an ALPP or RUPP greater than 1 SD below the mean of the baseline parameters, we found that a slightly lower number of animals showed dysfunction with the RUPP than with ALPP (93% vs. 100% at 1 wk, and 69% vs. 75% at 6 mo). We believe that this is likely due to the elimination of the investigator’s bias during ALPP determination. In addition, RUPP appeared to have a stronger association with the smooth muscle changes seen at the structural level than ALPP (r = 0.97 vs. r = 0.85).

Lastly, at the structural level, this model appears to nicely reproduce some of the changes seen in the intrinsic structure of the urethra with aging in the human (3, 6). Immunohistochemical analysis demonstrated that urethral dysfunction was accompanied by decreased smooth muscle content and/or increases in connective tissue deposition. In addition, the skeletal muscle content was also diminished. This parallels the results of studies evaluating the urethra and rhabdosphincter of human...
cadavers, which have shown an age-dependent decrease in both smooth and skeletal musculature of the urethra and sphincter (3, 6, 17, 18, 24, 25).

Apoptosis is a highly regulated process of cell death. Studies of human tissues have shown that the age-dependent changes seen in the muscular composition of the urethra and rhabdosphincter are correlated to age-related increases in apoptosis in these tissues and suggest a positive correlation between cellular apoptosis and external and intrinsic urethral sphincteric weakness in older patients (25). Interestingly, our animal model also shows a time-dependent increase in apoptosis that appears to be related to the observed time-dependent smooth muscle and skeletal muscle atrophy seen in these animals. In addition, a concomitant time-dependent decrease in the pan neuronal marker PGP9.5 is seen in the tissues of the incontinent animals after transabdominal urethrolysis. We speculate that these changes represent a neuronal and vascular injury to the urethra at the time of surgical detachment of the urethra. Interestingly, some of these changes were also seen in the bladders of the animals, and it is possible that during the surgical procedure, the vasculature or innervation of the bladder is also altered. Nevertheless, this did not translate to significant bladder dysfunction as measured by urodynamics. Further experiments need to be conducted to better understand the pathophysiology of decreased urethral resistance in this animal model.

In this study, we have evaluated the impact of transabdominal urethrolysis on urethral innervation, smooth muscle content, and urethral and bladder function. Compared to existing models of SUI, transabdominal urethrolysis is reliable and long-lasting. ALPP in the presence of a nonintubated urethra and RUPP are reliable quantifiable measures of urethral dysfunction. RUPP provides a less subjective assessment by the examiner compared to ALPP. The loss of anatomic support, muscle atrophy, and loss of innervation seen in the urethrolysis animal model parallels changes seen in the incompetent aged female urethra.

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