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Development of a Device to Standardize Leak Point Pressure Experiments in Rats

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Abstract

Aims—The objective of this study was to test a custom device aimed at increasing repeatability of abdominal leak point pressure (LPP) measurements in rodents.

Methods—The device consisted of a soft-tipped force applicator with a force sensor which was moved in the vertical direction by a linear actuator, laser crosshairs for accurate positioning, and a hand-held wired remote control system. One expert and two novice experimenters acquired LPP measurements using manual and device methods by applying a gradually increasing force with the finger or the device, respectively, until a leak was visually observed at the urethral meatus. The device was tested at fast, medium, and slow speeds. A leak sensor was also tested to determine presence of a leak and reaction time of the user. The change in bladder pressure due to the externally applied force (LPP) was the primary outcome.

Results—There were no significant differences in mean LPP value when compared across experiments (expert, novice), method (manual, device), or speeds. The pooled variance of LPP was significantly decreased ($P < 0.05$) compared to the manual method when using the device at medium speed; however the slower speed showed no further improvement over the medium speed. The wet sensor detected leaks 385 ± 187 msec earlier than users' reaction.

Conclusions—Use of the device can decrease variability of the experiment but does not alter mean values. Reaction time, inherent in both the manual and device methods, has the potential for a significant impact on LPP outcomes.

Keywords

bladder; force; rate; reaction time; rodent; urethra; urodynamics; variability

INTRODUCTION

Stress urinary incontinence (SUI), defined as the complaint of involuntary leakage of urine on effort or exertion, or on sneezing or coughing,¹ is a common disorder, affecting 15% of women by age 35 and 28% by age 55.² Clinically, SUI is objectively demonstrated as decreased urethral resistance to leakage by asking the patient to either cough or perform a Valsalva maneuver to increase abdominal pressure until leakage occurs.³ Abdominal leak point pressure

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(LPP) is defined as the bladder pressure at which urine leaks from the urethral meatus in the absence of a detrusor contraction.¹

Several animal models have been developed, mostly in rats, to study the mechanisms of SUI development.^{4–6} Simultaneously, several techniques have been developed to increase abdominal pressure, thereby inducing leakage to obtain LPP in rats. These include, but are not limited to, a Crede technique, a sneeze-response, a tilt table method, and retrograde urethral perfusion pressure (RUPP) measurement.^{4,6,7}

The Crede technique of determining LPP is performed manually on an anesthetized rat by applying an increasing external force over the bladder with the experimenter's index finger until a leak is visually observed at the urethral meatus.⁸ Though this technique consistently demonstrates changes in urethral resistance with different interventions^{5,9} and is considered among the most reliable of the methods in animals,⁷ it has the potential for high levels of variability and a steep learning curve due to its manual nature. Possible sources of variability include differences in reaction times, position-of-force application, and speed-of-force application. The objective of this experiment was to test a custom device designed to make the Crede LPP technique in rodents more repeatable, efficient, and easier to learn and master. We also investigated the effect of speed of the device and reaction time of the user on LPP measurement.

MATERIALS AND METHODS

LPP Device

The device consisted of a force applicator with a soft tip attached, a mechanical disengage safety to prevent excessive forces at 2N (approximately 200 g), a Series 43000 hybrid linear actuator (Haydon Switch and Instrument, Inc., Waterbury, CT) for vertical (Z) positioning controlled by a micro-stepping motor board with a programmable control interface (1024i, Applied Motion Products, Watsonville, CA), a strut system (80/20, Inc., Columbus City, IN) that allowed for X–Y adjustments, laser crosshairs (Quatron USA, Walnut, CA) for easy tip positioning, and a washable platform resting on top of a Z-adjustable lab-jack (Fig. 1). A force transducer (LCFA-250G, Omega Engineering, Stamford, CT) provided force measurements of tip application and a linear displacement transducer (potentiometer; LP803-03, Omega Engineering) provided relative position information. These sensors fed into an amplifier system (model P122, Grass-Telefactor, West Warwick, RI) for output on a chart recorder (Dash 8Xe, Grass-Telefactor) and analog-to-digital conversion with data recording at 50 Hz. The device was controller-operated, allowing a user to adjust the height of the tip, save a position, and return to that saved position during an experiment. To use the device, an experimenter positioned the tip of the device directly over the bladder (position 0 for excursion measurements), pressed and held-down the “use” button which moved the soft tip down at constant speed. Upon observation of a leak, the user released the “use” button, which immediately and rapidly retracted the tip to the saved position (Fig. 1).

Catheter Implantation

In each experiment, we tested 4 normal female rats (virgin, Sprague-Dawley, 180–200 g) with suprapubic bladder catheters implanted 2 days prior to testing, according to methods used previously.⁸ For catheter implantation, the rats were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg, i.p.), and a small incision was made in the center of the bladder. A catheter (PE 50 tubing) was then implanted in the bladder, secured with a purse-string suture until water-tight, and tunneled subcutaneously to the back of the neck where it exited the skin. The abdominal incision was closed, and the catheter was plugged until used.

Experiment I

Two days after catheter implantation, three users (one expert and two novices) performed both manual and device LPPs to test if the device improved consistency. The expert had performed LPP on rats manually thousands of times over 7 years. The two novices had never before performed the technique and were given verbal instructions only. The orders of users and methods were randomized to avoid creating methodological artifacts.

The bladder catheter was connected via a stopcock to both a flow pump (Model 200, KD Scientific, Holliston, MA) and a pressure transducer (Model PT300, Grass-Telefactor). The pressure transducer was connected to an amplifier (Model P122, Grass-Telefactor) and data were collected both on the chart recorder and was digitized at 50 Hz. Manual LPP tests were performed as previously reported.⁸ In brief, rats were anesthetized with urethane (1.2 g/kg, i.p.) and the user applied a gradually increasing force with their index finger, directly over the bladder until a leak was visually observed at the urethral meatus. The finger and all force was then immediately and rapidly removed. Device LPP tests were performed as outlined above. Both for manual and device trials, the primary test outcome was the change (baseline subtracted from peak) in bladder pressure due to the externally applied force (LPP). In the first experiment, each user tested two device speeds: a fast speed (1.0 mm/sec) and a medium speed (0.5 mm/sec). Additional data obtained in the device trials were force of tip application and device excursion.

The rats were accommodated to bladder filling with saline, via the bladder catheter (5 ml/hr), prior to conducting LPP tests by repeating a fill-void cycle three times. We did not measure residual volume since normal rats have previously been shown to void fully and without residual urine.¹⁰ Subsequently, bladders were filled to approximately half-capacity (0.5 ml) and either a manual or device LPP was performed. We have previously shown that LPP in rats is not highly volume dependent.⁸ Between tests, the bladder was allowed to refill for 30 sec to replace the drop that leaked. After four LPP tests, the bladder was manually emptied by palpation and allowed to refill to half-capacity for continued testing. In the rare case of a spontaneous contraction or void triggered by the LPP test, the data was not used and the bladder was emptied and refilled. We were able to obtain a complete set of LPP data on each rat in the absence of bladder contractions. At the end of testing, rats were euthanized with an overdose of pentobarbital (100 mg/kg, i.p.).

Experiment II

The results of the first experiment indicated that identification of response time to leakage could be important to LPP results. Therefore, a wet sensor was designed to automatically detect leakage at the urethral meatus. It consisted of a probe with two electrically separate, counterwound wires at the tip. A voltage potential (approx. 1.2 V DC) was applied across the two leads via a high resistance bridge circuit, and the circuit was tested to ensure discontinuity of the probe. The probe was held in position at the urethral meatus with a gimble system. When the probe came in contact with fluid, bridging the gap in the circuit, there was a dramatic change in conductance across the two leads. This change was measured using a Wheatstone bridge circuit and recorded at 50 Hz. The probe was then dried by hand, and was ready to use again for further testing. The maximum current that could be conducted by the probe was resistor-limited to 2 μ A for safety.

A second experiment was then conducted in which one expert and one novice user performed LPP tests both manually and with the device at the medium speed (0.5 mm/sec) and a slow speed (0.25 mm/sec). These measurements were taken both with and without the wet sensor placed at the urethral meatus to determine if the wet sensor obstructed observation of a leak. The user was blinded at all times during the experiment to wet sensor results. The experimental

protocol was otherwise unchanged from that of experiment I (i.e., randomization of user-method-speed orders, etc.).

Data Analysis

Points of initial rise and peaks in pressure, force, and height data were automatically detected and recorded by a data analysis program (Matlab). Baseline pressure was calculated by automatically averaging all sample values 5 sec prior to the initial rise point. The program selected and graphed the candidate data points, prompting the user to accept each one.

For pressure, force, and excursion data, the baseline and peak data points were recorded, and differences were calculated. The mean of each outcome variable was taken across all rats to examine mean differences due to operator and method (manual/device/device speed). To assess the precision of the different test methods (device and manual) across a series of test animals, it was necessary to remove those components of the variance estimates due only to animal-to-animal variation. Consequently, the variability within a given investigator/test method was separately determined for each animal. These measures of variation were then pooled to provide an estimate of measurement precision, which was a function only of investigator and test method. A repeated measures mixed model was used to determine statistically significant differences in mean value. The Foster–Burr test was used to determine if there were differences in the variance between each group. $P < 0.05$ was taken to indicate a significant difference between groups in both cases.

RESULTS

Experiment I

As force is applied to the abdomen, pressure rises until leakage occurs (Fig. 2). Device trials with constant speed of excursion induced smoother pressure curves than manual trials, although mean values were not different. Both pressure and force are exponentially related to excursion. Pressure demonstrates hysteresis when plotted against excursion or force in a phase plot. In contrast, force shows little hysteresis when plotted against excursion (Fig. 2). Although the peak forces were higher at fast speeds (99.2 ± 15.5 g) than at medium speeds (89.3 ± 22.2 g), there was no significant difference between them ($P = 0.27$). There were no significant differences in mean LPP when compared across experimenters (expert, novice), methods (device, manual) or speeds. When variance was pooled across all experimenters for the manual method and the two different device speeds, the pooled variances of the device at medium speed was significantly less ($P < 0.05$) than the pooled variance of the manual method, indicating higher variability with the manual method (Fig. 3). When variances of all test methods were pooled, the expert had significantly less variation in performance than either of the novices ($P < 0.05$).

Experiment II

To mimic the statistical analysis performed in experiment I, only data collected in the absence of the wet sensor was used to compare methods (manual and device at medium and slow speeds). The experiment produced no significant differences between methods, in mean or variance for LPP data, suggesting the slower speed did not improve upon the medium speed (Fig. 4). However, between experimenters, across test methods, novice variability was significantly greater ($P < 0.05$) than that of the expert. Although the peak forces were higher at medium speeds (91.2 ± 5.7 g) than at slow speeds (74.1 ± 5.7 g), there was no significant difference between them ($P = 0.08$). We believe the lower standard deviation in the 2nd experiment has to do with practice using the device and the slower speeds.

The wet sensor prototype properly detected leaks approximately 80% of the time. The 20% failure rate was due to positioning difficulties with the probe: a leak would occur but the probe was not properly placed on the meatus to detect it. There was no significant difference in LPP results between trials with (34.4 ± 1.6 cm H₂O) and without (37.5 ± 1.6 cm H₂O) the wet sensor in place ($P = 0.26$). There was no dependence of this result on user or speed, implying that the wet sensor did not obstruct the experimenter's ability to detect urine.

When successfully used, the wet sensor detected a leak 385 ± 187 msec ($n = 96$) earlier than the response time recorded by the user. Bladder pressures at wet sensor leakage detection were therefore lower than peak bladder pressures. To determine the significance of these differences, we defined LPP wet as baseline pressure subtracted from the bladder pressure at wet sensor leakage detection (32.2 ± 1.4 cm H₂O) and compared it to LPP, determined from peak bladder pressures (34.3 ± 1.5 cm H₂O). When compared, these differences were not significant ($P = 0.22$). As above, there was no dependence of this result on user or speed, implying that earlier detection of leakage by the wet sensor would not lead to significant changes in experimental results.

DISCUSSION

The rat model for SUI is relatively new and various strategies of inducing SUI have been developed.^{4–6} Simultaneously, urethral resistance testing for these models has been evolving, with a variety of techniques being tested.^{4,6,7} The Crede method has been used extensively^{7,8,11–15} although it has the potential for high variability due to its manual nature. Other methods for measuring urethral resistance to leakage include the sneeze-response, the tilt table method, as well as RUPP measurement.^{4,6,7}

Three studies have compared the Crede method to other methods that have been developed simultaneously. Conway et al.⁷ compared the sneeze, Crede LPP test, and tilt-table techniques and found that all three techniques are reliable for assessing urethral function, although the variability in the sneeze test was larger than the other two methods. Phull et al.¹² used both the Crede LPP test and RUPP measurement to determine the role of angiotensin II in maintenance of urethral tone. As with the Conway study, both methods of measuring urethral resistance to leakage gave similar results and similar variability. Rodriguez et al.,⁶ when they first introduced the RUPP method in rats, tested it against the Crede LPP technique. As with the above studies, they found similar results and similar variability between the two methods. In all cases, when new methods are introduced, the Crede LPP is used as the gold standard for comparison.

The aim of developing our device was to standardize the Crede technique. The device could have the following advantages: it regulates the speed of the maneuver, it maintains a consistent position of force application, and it has a consistent force applicator (shape, size, softness). We hypothesized this standardization would reduce LPP data variability and would improve reliability and sensitivity of the LPP method.

The device properly functioned in that speed, position, and tip shape were all consistent, but it illuminated a variety of other difficulties with the method. From the phase curves, we observed that urine leakage occurs in the latter portion of the graph, where pressure and force curves are steepest. The large impact of a small change in excursion or time on pressure, suggests that reaction time could significantly impact LPP outcomes, potentially leading to higher variability of data. This steep incline was greater at higher device speeds, suggesting that variations in reaction time impact LPP to a greater extent at high speeds, and demonstrating the importance of maintaining a slow consistent speed during LPP testing.

These observations are supported by the first experiment which demonstrated that a device speed of 1.0 mm/sec has similar variability to the manual method, but a device speed of 0.5 mm/sec significantly reduces LPP variance compared to the manual method. Experiment II demonstrated that reducing the speed even further did not further reduce the variability.

In both experiments, the expert achieved lower variability of data than the novices, suggesting that, regardless of the technique, training and practice are crucial to obtaining consistent LPP results. The novice in Experiment II was one of the novices in Experiment I and he received no experience or training between the two experiments. Therefore, although he had practiced the method in Experiment I, he still had higher variability of data than the expert in Experiment II. It is the novice using the manual method that accounts for the high variability in Experiment II (Fig. 4). This suggests that more than four rats are needed to fully train on the LPP technique.

The wet-sensor was developed to investigate user response time, which, at 385 msec on average, would not be expected to significantly alter pressure results. This was borne out by our statistical analysis of pressure results which indicated that earlier detection of leakage by the wet sensor would not lead to significant changes in experimental results. The wet sensor could be further developed to create a closed loop system with a redundant trigger, in which either the device or the experimenter could automatically trigger the tip to retract upon detection of a leak. This could reduce variability created due to reaction time, but is not likely to change the mean value of results. However, before the loop is closed, improved methods of positioning and drying the probe are needed.

Since it was outfitted with a force sensor and potentiometer for excursion measurements, the device enabled investigation of bladder biomechanics. The force-excursion relationship is nonlinear and exponentially rising, suggesting the bladder wall is under tension at half capacity. Using the manual method, we have previously shown that leak point pressure does not vary significantly with volume in the bladder, except at very low volumes.⁸ Assuming that pressure is a function of force and excursion, this suggests that the bladder is under tension at all but very low volumes. Increasing speed of the device increased peak forces, but not significantly.

Pressure-force and pressure-excursion phase plots consistently demonstrated nonlinear relationships and hysteresis loops, suggesting that the area of bladder displaced by the probe tip varies with excursion and was not the same when being depressed as retracted. Alternatively, hysteresis could be a result of time delay for the pressure signal to be transmitted through the water filled tube to the pressure transducer. However, exploratory tests on the biomechanics of the pressure measurement system (data not shown) indicate that this cannot account for the hysteresis loops in their entirety.

Consistent positioning of the tip directly over the bladder is important for accurate and repeatable LPP measurements. The device has the advantage of having a tip that remains in the identical position in the X-Y plane between trials and iterations. However, the bladder sometimes shifts position between trials and the point-of-force application needs to be changed accordingly. This is easily and instinctively done using fingers in the manual method but is presently cumbersome with the device.

The aim of developing this device was to reduce LPP data variability. A reduction in variability would improve this model's reliability and sensitivity, reducing the number of animals needed to produce statistically significant results in an experiment. This study suggests that the device improves LPP consistency over the manual Crede method if a constant medium speed (less than 0.5 mm/sec) is used. Regulating speed to a medium or slow rate minimizes the impact of a slow reaction time. However, the device also had the following drawbacks: difficulty in positioning the tip, loss of tactile feedback, relatively high cost of materials, and reduced efficiency of LPP testing since only a single animal at a time can be tested with the device.

Therefore, the manual method may suffice in most situations unless very small variability is required for a specific study or few animals can be utilized for a study due to expense or availability.

Alternatively, if a wet sensor that reliably detects leakage can be developed, perhaps the use of a device will be made unnecessary, and an experimenter would be able to combine the manual method of LPP testing with an automatic method of leak detection. The LPP could then be collected post-hoc by determining the pressure at the time the wet sensor triggered.

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Fig. 1.

A: Photograph of device used for LPP experiments, with important features labeled. A pen is shown for size comparison. **B:** A photo demonstrating the crosshair positioning system. **C:** The remote controller for the device.

Fig. 2.

Example LPP trials with pressure (a), force (b), and excursion (c) for the manual method (dashed line, pressure only) and device speeds of 1.0 mm/sec (dotted line) and 0.5 mm/sec (solid line). Time of initial rise and peak values are marked by an asterisk and circle respectively. The peaks were superimposed so that peak pressures coincide. Phase plots for the same LPP device trials (dotted line = 1.0 mm/sec; solid line = 0.5 mm/sec) with force (d) and pressure (e) plotted against excursion. Pressure is also plotted against force (f). In the phase plots, the number 1 and a solid line denote tip excursion into the rat's abdomen; the number 2 and a dotted line denote the return upwards.

Fig. 3.

Leak point pressure (LPP) data in Experiment I normalized to remove rat-to-rat variation and (A) pooled across experimenters to display variability in device method and speed and (B) pooled across device method and speed to display variability between experimenters. Normalized mean is represented by a square; raw data are represented by circles; the upper and lower limits of the box indicate a single standard deviation from the normalized mean; and the error bars indicate two standard deviations from the mean. * in A denotes a significant difference in pooled standard deviation compared to the manual method. * in B denotes a significant difference in pooled standard deviation compared to the expert.

Fig. 4. Leak point pressure (LPP) data in Experiment II normalized to remove rat-to-rat variation and (A) pooled across experimenters to display variability in device method and speed and (B) pooled across device method and speed to display variability between experimenters. Normalized mean is represented by a square; raw data are represented by circles; the upper and lower limits of the box indicate a single standard deviation from the normalized mean; and the error bars indicate two standard deviations from the mean. * in B denotes a significant difference in pooled standard deviation compared to the expert.